

ARTIFICIAL INSEMINATION MANUAL FOR CATTLE

1 9 9 2

**Association
of Livestock Technology**

L I S T O F T H E W R I T E R

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PART I BASIC OF A.I.

1. Anatomy
2. Hormones in Farm Animal
Reproduction
3. Reproductive Function of
Male Livestock and Semen
Physiology
4. Theory of Spermatozoal
Freezing

I. Basics of A. I.

1. Anatomy

1-1 The Female Reproductive System

Anatomy is the "Science or study of the structure of the animals body and the relationship of its parts". Physiology is the science "that treats the parts and functions of living organisms".

The female reproductive system of the cow consists of two ovaries and the female duct system. The duct system includes the oviducts, uterus, cervix, vagina and vulva. The embryonic origin of the ovary is the secondary sex cords of the genital ridge. The genital ridges are first seen in the embryos as a slight thickening near the kidneys. The duct system originates from the Mullerian ducts, a pair of ducts which appear during early embryonic development (Fig. 1-1 & Fig. 1-2).

(1) Ovaries

The ovaries are the primary reproductive organs because they produce the female gamete (the ovum) and the female sex hormones (estrogens and progestines).

The ovaries are located at the end of each uterine tube. They are described as almond shaped but the shape is altered when follicles or corpora lutea start to develop. The average size is about 35 x 25 x 15 mm. The size will vary among cows, and active ovaries are larger than inactive ovaries.

It is accepted that all primary follicles are formed during the prenatal period of the female. It is estimated that approximately 75,000 primary follicles are found in the ovaries of a young calf.

When the antrum is formed, the follicle is classified as a tertiary follicle. The mature tertiary follicle which appears as a fluctuant,

fluid-filled, blister-like on the surface of the ovary is also called a Graafian follicle. The fluid in the Graafian follicle is rich in estrogens and is called liquor folliculi. Both theca interna and granulosa cells are involved in the production of estrogen. Granulosa cells are the progesterone producing cells in the corpus luteum. When ovulation occurs, the follicle ruptures about 30 hrs after the beginning of heat or about 12 hrs after the end of estrous (ovulation).

With the rupture of the follicle, bleeding occurs and a blood clot forms at the ovulation site. The ruptured follicle with its blood filled cavity is called a corpus hemorrhagicum. The corpus hemorrhagicum is replaced by the corpus luteum, which forms rapidly by the proliferation of theca externa, theca interna and granulosa cells. The corpus luteum is a solid, yellowish body which produces progesterone and other progestins. In a Holstein heifer, between 1 and 4 days of the cycle the average diameter of the corpus luteum is 8 mm. Between 5 and 9 days, it has grown to an average of 15 mm. The average maximum size of 20.5 mm is reached in 15 to 16 days in a heifer that is not pregnant, then it regresses in size with an average diameter of 12.5 mm at 18 to 21 days. If the animal is pregnant, the corpus luteum will not regress until late pregnancy.

(2) Oviducts

The oviducts (also called fallopian tubes) are a pair of convoluted tubes extending from near the ovaries to and becoming continuous with the tips of the uterine horns. Their functions include transport of ova and spermatozoa which are conveyed in opposite directions. In addition they are the site of fertilization in the early cell divisions of the embryo.

An oviduct, which is from 20 to 30 cm long is divided into three segments (infundibulum, ampulla, isthmus). The funnel-shaped opening near the ovaries is the infundibulum. Fertilization occurs at this junction. The isthmus joins the tip of the uterine horn at

the uterotubal junction. In general oviductal activity is stimulated by estrogens and inhibited by progestins.

(3) Uterus

The uterus extends from the uterotubal junctions to the cervix. The length may range from 35 to 50 cm. The major function of the uterus is to retain and nourish the embryo, or fetus. Before the embryo becomes attached to the uterus, nourishment comes from yolk within the embryo or from uterine milk which is secreted by glands in the mucosal layer of the uterus. After attachment to the uterus, nutrients and waste products are conveyed between maternal and embryonic or fetal blood by way of the placenta.

The uterine horns are held in place in the pelvic cavity by the broad ligaments which are attached to the outside walls of the uterus on one side and the pelvic wall on the other. Estrogens increase the tone of the myometrium, giving the uterus an "erect" feel. Progestins decrease the tone of the myometrium, causing the uterus to feel more flacid.

Cows have cotyledonary placental attachments. Chorionic villi from the extraembryonic membranes penetrate into caruncles which are button-like projections on the endometrium. This union, chorionic villi and caruncle, forms the placentome (also called the cotyledon or caruncles). There are 70 to 150 such cotyledonary attachments in late pregnancy. The placental attachment of the cow is classified as epithelio chorial.

(4) Cervix

While technically a part of the uterus, the cervix will be discussed as a distinct organ. It is thick walled and inelastic, the anterior end being continuous with the body of the uterus (internalos) while the posterior end protrudes into the vagina (externalos). The length will range from 5 to 10 cm with an outside

diameter of 2 to 5 cm.

Estrogens cause the epithelial cells of the cervix to secrete mucus that has antibacterial properties, thus protecting the uterus. During pregnancy the mucus thickens into a gel-like plug, which seals and protects the uterus during the pregnancy. Removal of the mucous plug will increase the chance of abortion.

(5) Vagina

The vagina is tubular in shape, thin-walled and quite elastic. It is from 25 to 30 cm in length. Semen is deposited into the anterior end of the vagina, near the opening to the cervix, during natural mating.

The fornix of the vagina is a blind pocket that encircles the projecting caudal part of the cervix in the cranial vaginal cavity. This pocket may be troublesome for the inexperienced technician trying to introduce the pipet into the cervix.

(6) Vulva

The vulva or external genitalia, consists of the vestibule and related parts of the labia. The vestibule is that portion of the female duct system that is common to both the reproductive and urinary systems. It is from 10 to 12 cm in length. The labia consists of the labia minora, inner folds or lips of the vulva and labia majora, outer folds or lips of the vulva.

The labia majora is covered with fine hair up to the mucosa. The clitoris, homologous to glans penis in the male, is located ventrally, well supplied with sensory nerves and is erect during estrous.

1-2 The Male Reproductive System

The male reproductive system consists of the scrotum, spermatic cords, testes, accessory glands (vesicular gland, prostate gland, bulbourethral gland), penis, prepuce and the male duct system. The embryonic origin of the testes is the primary sex cords of the genital ridges, whereas the male duct system originates from the Wolffian ducts (Fig.2).

(1) Scrotum

The scrotum is an out pouching of skin in the inguinal region where the testes are located. The bovine scrotum should be long and pendulous with well defined neck and medial groove when not contracted. A short-necked or tapered scrotum is less desirable.

(2) Testes

The testes are the primary organs for reproduction in males, just as the ovaries of the female reproductive organ. Testes are considered primary because they produce male gametes (spermatozoa) and male sex hormones (androgens).

Both gonadotrophic hormone and androgens regulate descent of the testes. If only one testis descends, it is a unilateral cryptorchid.

(2-1) Functional morphology: The testis of the bull is 10 to 13 cm long, 5 to 6.5 cm wide and weighs 300 to 400 g. Seminiferous tubules, which spermatozoa are produced within are formed from primary sex cords. They contain germ cells (spermatogonia) and nurse cell (Sertoli cells). With stimulation by FSH, Sertoli cells produce both androgen binding protein and inhibin.

Seminiferous tubules are approximately $200\mu\text{m}$ in diameter, and it has been estimated that from a pair of bull testes,

stretched out and laid end to end, approach 5 km in length. Seminiferous tubules join a network of tubules, the rete testis, which connects 12 to 15 small ducts, the vasa deferentia, which converges into the head of the epididymis.

Leydig (interstitial) cells are found in the parenchyma of the testes between the seminiferous tubules. LH stimulates Leydig cells to produce testosterone and small quantities of other androgens. Testosterone is needed for normal mating behavior, for development of secondary sex characteristics, for the function of the accessory glands, production of spermatozoa and maintenance of the male duct system.

(2-2) Temperature control: High temperature causes degeneration of cells lining the wall of the seminiferous tubules. In cattle, when ambient temperatures range from 5 to 21 °C, temperatures inside the testes will be 4 to 7 °C below body temperature.

The role of the scrotum and spermatic cord in temperature control of the testes involves drawing the testes closer to the body as ambient temperature falls and letting the testes swing further away from the body as ambient temperature rises. Two smooth muscles are involved.

(3) Ductus deferens

The ductus deferens (plural=ductuli deferentia) serve to carry sperm from the tail of the epididymis to the urethra within the pelvis. The terminal portion of the ductus deferens has a thickened wall and is termed the ampulla. The paired ampullae open, together with the excretory ducts from the vesicular glands into the urethra just to the rear of the neck of the bladder.

(4) Spermatic cord

The spermatic cord extends from the inguinal ring to the testis. It

includes the testicular artery, testicular veins, lymphatic vessels, nerves, the ducts deferens, the external cremaster muscle and several layers of tunic. The spermatic cord can be palpated in the neck of the scrotum. The testicular artery is highly coiled just above the testis, in an area termed the pampiniform plexus, continues down to the lower rear aspect of the testis and then branches to vascularize the testis.

(5) Epididymis

The epididymis, the first external duct leading from the testis is fused longitudinally to the surface of the testis and is encased in the tunica vaginalis with the testis. The capus (head) of the epididymis is a flattened area at the apex of the testis where 12 to 15 small ducts, the vasa efferentia merge into a single duct. The corpus (body) extending along the longitudinal axis of the testis, is a single duct which becomes continuous with the cauda (tail). The total length of this convoluted duct is about 34 meters in the bull.

(5-1) Transport: As a duct leading from the testes, the epididymis serves to transport spermatozoa. In sexually active males, the time involved to transport is 9 to 11 days in bulls. Frequent ejaculation has been reported to speed transport by 10 to 20%.

During ejaculation, peristaltic contractions involving the smooth muscle layer of the epididymis and a slight negative pressure (sucking action) created by peristaltic contractions of the vas deferens and urethra actively move spermatozoa from the epididymis into the vas dererens and urethra.

(5-2) Concentration: Spermatozoa entering the epididymis from the testis of the bull, are relatively dilute (approximately 10×10^6 spermatozoa/cc). In the epididymis, they concentrate to about 4×10^9 (4 billion) spermatozoa per cc.

(5-3) Storage: The epididymis of a mature bull may contain 50 to 74 billion sperm. Conditions are optimum in the cauda for preserving the viability of spermatozoa for an extended period. It is the low pH, high viscosity, high carbon dioxide concentration, high potassium-to-sodium ratio, the influence of testosterone, and probably other factors that combine to contribute to a lower metabolic rate and extended life. Spermatozoa may remain alive and fertile for about 60 days. However, after a long period of sexual rest, the first few ejaculates may contain a high percentage of non fertile spermatozoa.

(5-4) Maturation: When recently formed spermatozoa enter the duct from the vasa efferentia they have the ability of motility or fertility. As they pass through the epididymis they gain the ability to be both motile and fertile for up to 25 days. If a high percentage of spermatozoa in freshly ejaculated semen has cytoplasmic droplets, they are considered immature and have low fertilizing capacity.

(6) Urethra

The urethra is a single duct which extends from the junction of the ampullae to the end of the penis. It serves as an excretory duct for both urine and semen. During ejaculation in the bull, there is a complete mixing of spermatozoa concentrate from the vas deferens and epididymis with fluids from the accessory glands in the pelvic part of the urethra to form semen.

(7) Accessory glands

The accessory glands are located along the pelvic portion of the urethra, with ducts which empty their secretions into the urethra. They include the vesicular glands, prostate glands, and the bulbourethral glands. They contribute greatly to the fluid volume

of semen. In addition, their secretions are a solution of buffers, nutrients, and other substances needed to assure optimum motility and fertility of semen.

(7-1) Vesicular glands

The vesicular glands (sometimes called seminal vesicles) are a pair of lobular glands that are easily identified because of their knobby appearance. They have been described as having the appearance of a "cluster of grapes". Two of the compounds in secretions (fructose and sorbitol), are major sources of energy to bull spermatozoa. Both phosphate and carbonate buffers are found in these secretions and are important in that they protect against shifts in the pH of semen.

(7-2) Prostate gland

The prostate is a single gland located around and along the urethra just posterior to the excretory ducts of the vesicular glands. A prostate body is visible in excised tracts and can be palpated in bulls. The secretions of the prostate are high in inorganic ions with sodium, chlorine, calcium and magnesium all in solution.

(7-3) Bulbourethral glands

The bulbourethral (Cowpers) glands are a pair of glands located along the urethra near the point where it exits from the pelvis. They are about the size and shape of walnuts. They contribute very little to the fluid volume of semen.

(8) Penis

The penis is the organ of copulation in males. Bulls have a sigmoid flexure, an S-shaped bend in the penis which permits it to be retracted completely into the body.

The glands penis which is the free end of the penis, is well supplied with sensory nerves and is homologous to the clitoris in the female.

Erectile tissue is cavernous (spongy) tissue located in two regions of the penis. The corpus spongiosum penis is the cavernous tissue around the urethra. These cavernous areas engorge with blood during sexual excitement causing extension of the penis (erection) and facilitating the final ejection of semen during ejaculation.

(9) Prepuce

The prepuce (sheath) is an invagination of skin which completely encloses the free end of the penis. It has the same embryonic origin as the labia minora in the female. It can be divided into a prepenile portion which is the outer fold and the penile portion or inner folds. The orifice of the prepuce is surrounded by long and tough preputial hairs.

(10) Reproductive capacity of the bull

The bull produces billions of sperm daily and stores several day's production of sperm in the distal excurrent ducts available for ejaculation.

(10-1) Definition: (a) Daily sperm production (DSP) --- the total number of sperm produced per day by the two testes. (b) Efficiency of sperm production --- the number of sperm produced per day per gram of testicular tissue. (c) Daily sperm output (DSO) --- the total number of ejaculated sperm collected over an interval of >7 days expressed on a per day basis. (d) Extragonadal sperm reserves (EGR) --- the total number of sperm in the excurrent duct system (epididymides, ampullae and ductuli deferential).

(10-2) Sperm production: Normal males of a given breed have a uniform efficiency of sperm production during most of their reproductive life. Consequently, DSP is highly correlated with testicular weight. However, the efficiency of sperm production is influenced by age, season, environmental factors, hormonal status, and drugs. The size of a bull's testes can be estimated accurately by measuring the scrotal circumference. A plastic measuring tape works well. The larger the scrotal circumference, the more sperm the bull should produce, and the greater his value as a sperm factory.

Following ejaculation, sperm reserves in the tail of the epididymis are restored towards the sexually rested level at a rate equal to the bull's DSP. As the number of sperm in the tail approaches the sexually rested level, sperm losses during urination (or by masturbation) increase. In this way, old sperm are continuously removed from the duct system to make way for newer sperm.

(10-3) Factors influencing the total number of sperm in an ejaculate: (a) The influence of age and season usually are beyond our control. But these factors have a definite influence on sperm production and seminal characteristics. (b) Ejaculation frequency or the interval since the last ejaculate profoundly influences both spermatozoa concentration and total sperm per ejaculate. As frequency of ejaculation is increased (e.g., from once weekly to four times weekly), fewer sperm are obtained in each ejaculate, but more sperm are obtained per week. Depending on the exact procedure used during semen collection, the weekly harvest of sperm usually can be maximized by collection of 2 or 3 ejaculates each Monday and Thursday or Tuesday and Friday.

(10-4) Factors affecting male reproduction: (a) A number of factors may affect spermatogenesis and sperm production. Nutrition is under the direct control of the rancher. Severe underfeeding

will delay puberty. Ideally, a bull calf selected for use as a herd sire or for use in artificial insemination should receive sufficient energy in the form of milk and concentrate to maximize growth from birth through one year of age. Overfeeding a yearling bull will cause excessive fatness, but sufficient energy intake should be provided to allow continued growth to obtain the maximum stature at a relatively young age. A breeding male should be kept relatively thin. (b) Disease or environmental factors causing an elevated testicular temperature will disturb spermatogenesis. A drop in the number of sperm ejaculators in the percentage of normal sperm may not be detected for 4 - 6 weeks after the testicular temperature was initially elevated. (c) Hypogonadism (= small testes) --- some bulls have relatively small testes and are considered to be hypogonad. Such bulls may be fertile, but should not be used as a sire since this condition is heritable. Scrotal circumference of any male considered for use as a sire should be measured and the resulting value compared with norms for bulls of the same breed and of a similar age or body weight. (d) Cryptorchidism --- A cryptorchid bull is one in which one or both testes were retained in the abdominal cavity or inguinal ring. The retained testis will not produce sperm, but will produce hormones. The descended testis will produce sperm and a unilateral cryptorchid bull will be fertile. However, the condition is heritable and a unilateral cryptorchid bull should never be used for breeding. A bilateral cryptorchid is sterile and cryptorchid bulls should be killed as young calves.

1-3 The Estrous Cycle

(1) Puberty

Puberty in females is defined as the age at which it first expressed estrous with ovulation. It should not be considered sexual maturity. If animals are bred at puberty, a high percentage will have difficulty with parturition.

Since puberty is controlled by certain physiologic mechanisms involving the gonads and the anterior pituitary gland, it must also be influenced by several factors of both a hereditary and an environmental nature (season, temperature, nutrition, genetic factors, etc.) working through these organs.

Age and weight at puberty are affected by genetic factors. Average age at puberty is 8 to 11 months for European cows. Jerseys reach puberty at about 8 months and 160 kg while for Holsteins 11 months and 270 kg is the average.

A Holstein heifer receiving the recommended nutrition will reach puberty at about 11 months of age. However, if raised from birth on 62% of the recommended level of energy she will be over 20 months of age at puberty. High environmental temperature delays puberty. Beef heifers, when reared at 10 °C, reached puberty at 10.5 months of age, but similar heifers reared at 27 °C were over 13 months of age at puberty. Other environmental factors that might delay puberty include poor health and poor sanitation in rearing facilities.

Heifers on a low plane of nutrition were 84% older but only 7% smaller at puberty than well fed heifers. Feeding at recommended levels will result in earlier puberty. Holstein heifers fed at 146% of the recommended level reached puberty at an average of 9.2 months of age as compared to 11 months for controls receiving the recommended diet. Both problems associated with overconditioning

and the extra cost of such a diet made overfeeding undesirable.

(2) The estrous

Understanding factors regulating the estrous cycle of the cow is an essential component of reproductive management on dairy farms. Increased veterinary involvement and use of artificial insemination (AI), estrous cycle synchronization and embryo transfer by dairy producers accentuates this need.

The estrous cycle is the elapsed time between two periods of estrous or heat. Estrous cycles are normally from 18-24 days with 21 days considered average. Shorter cycles are "abnormal" while long cycles, especially those that are multiples of 18-24 days, are probably due to failure to detect estrous. Cycle lengths of approximately 30-35 days may be "false heats" that occur after a breeding or reflect early death of the embryo.

(2-1) Periods of the estrous cycle: The estrous cycle is divided into four distinct but continuous phases; proestrous, estrous, metestrus and diestrus. Proestrous is the period between regression of the corpus luteum of the previous cycle and estrous. It is the period of follicular development. Estrous lasts 8-30 hrs and is the period of sexual receptivity. Final maturation of the egg and follicle also occurs. During early metestrus the follicle ovulates and the wall of the ruptured follicle develops into a corpus luteum during the next 3 days. Diestrus lasts 12-15 days and is the period of active progesterone production by the corpus luteum. The endocrine events and ovarian changes associated with these phases of the estrous cycle are discussed in the other sections.

(2-2) Estrous : Estrous is defined as the period of time when the female is receptive to the male and will stand for mating. The length of the period of estrous varies. Estrous lasts

for 12 to 18 hrs in the cow. In the estrous cycle, considerable variation is seen between individuals. Also, cows in hot environments have shorter periods of estrous (10 to 12 hrs) than the average 18 hrs period for cows in cool climates. Ovulation is associated with estrous occurring 10 to 12 hrs after the end of estrous. The day of estrous is the first day of the estrous cycle in the cow.

(2-3) Metestrus: The period of metestrus begins with the cessation of estrous and lasts for about 3 days. Ovulation occurs during this period in cows. Also, a phenomenon known as meterstrus bleeding occurs in cows, appearing in about 90% of all metestrous periods in heifers and 45% in mature cows. The small loss of blood will be noticed as a patch of blood on the tail approximately 35 to 45 hrs after the end of estrous.

It is not an indication of conception or of a failure to conceive. Also, it should not be confused with menstrual bleeding which occurs in humans.

(2-4) Diestrus : Diestrus is characterized as the period in the cycle when the corpus luteum is fully functional. In the cow it starts about day 5 of the cycle, when an increase in blood concentration of progesterone can first be detected and ends with regression of the corpus luteum on day 16 or 17. It has been called the period of preparation of the uterus for pregnancy.

(2-5) Proestrus: Proestrus begins with the regression of the corpus luteum and drop in progesterone and extends to the start of estrous. Late during this period the effects of estrogen on the duct system and behavioral symptoms of approaching estrous can be observed.

(3) Mating behavior

Estrous is defined as the period when the female is receptive to the male. For controlled natural mating or for use of artificial

insemination it is necessary that animal managers be able to recognize the signs of estrus and be aware of factors which contribute to normal estrual behavior.

High levels of estrogens have been associated with the behavioral signs of estrous and are of paramount importance. However, evidence exists for the interaction of estrogens with certain senses in electing the full behavioral response.

Cows are unique in that they display rather strong homosexual tendencies, making estrous detection comparatively easy even when bulls are not present. Cows in estrus will solicit mounts and attempt to mount other cows. They may smell the vulva of other cows. Frequently, they raise and switch their tail and may leave the herd in search of a bull. They will have a congested vulva and clear mucous often seen streaming from the vulva. Cows in other periods of the estrous cycle will mount cows in the period of estrous, but will not stand to be mounted. Therefore, standing for mounting is the strongest single behavioral indication of estrous .

1-4 Pregnancy Diagnosis

The economic value of an early diagnosis of pregnancy in the cow is quite clear. Whether one is dealing with dairy cattle or beef cattle, the ultimate goal is an average 12-month calving interval for the herd. Every management practice which contributes to attain this goal is well worth consideration. Pregnancy diagnosis is one of these tools. Most cows that fail to conceive will return to estrus in approximately 21 days post-breeding (Table 1).

(1) Rectal palpation

In herds where AI is used, the first palpation should be made between 35 and 42 days after insemination. Many cows that are open at this time probably were in estrus 21 days post-breeding but were not observed carefully for estrus. All cows that have not returned to estrus by 60 days post-breeding should be palpated for a final time. A few pregnancies (1-2%) at 30 to 35 days will result in embryonic mortality and can be picked up as open at the 60-days check.

Many practitioners having developed successful palpation techniques are already competent palpators. However, many mistakes are still made in palpation for pregnancy and incompetent palpation continuous to be a problem area in veterinary practice (Table 2).

(2) Structures to be palpated

Most of the diagnosis is based on the uterus and its contents. The size of the uterus influences its position in relation to the pelvis and should be noted. The thickness and tone of the uterine wall are important.

The chorionic membrane can be detected by gently grasping the uterine wall between the thumb and forefinger and lifting slightly. The term "slipping the membrane" has been used to describe this

procedure (Fig. 3). By 120 days the placentomes are large enough to palpate through the uterine wall.

Pregnancy is always accompanied by a corpus luteum. However, one must remember that a corpus luteum is not always accompanied by a pregnancy. In the pregnant cow, the arteria uterine media begins to enlarge when the conceptus is large enough to require an increased blood supply and may reach a diameter of 1 to 1.5 cm by the end of gestation.

(3) Alternative methods of examination

Accurate pregnancy diagnosis is difficult to obtain in the rectal palpation. The most likely reason for making a false-positive diagnosis is subsequent embryonic or fetal death is impossible to exclude. Other reasons for false-positives are incomplete uterine involution, pyometra, mucometra, hydrometra and failure to retract the uterus.

(3-1) Laparoscopy: The reproductive tract can be directly visualized by laparoscopy or endoscopy.

(3-2) Ultrasonographic examination: The uterus and ovaries can be indirectly examined by ultrasonographic techniques. A level of accuracy of 85-95% has been achieved in positively identifying pregnant cows and a large percentage of nonpregnant cows (57-87%) were incorrectly diagnosed as being pregnant. The linear array B-mode ultrasound has been used to daignose pregnancy with a rectal transucer probe. The embryo is first identified between 26 to 29 days when it was 10 mm in length and appeared to be growing in length at 1.1 mm per day. The technique can be used to estimate fetal age up to 140 days of gestation following the measurement of the crown-rump length.

(3-3) Fetal electrocardiography: Fetal electrocardiography has been

noted as a method of pregnancy diagnosis. It is not applicable before five months of gestation, but it might have application for the diagnosis of multiple pregnancies.

(4) Differential diagnosis

Pregnancy is by far the greatest cause of uterine enlargement, but by no means is it the only cause. At each of the stages described, pregnancy must be differentiated from one or more of the other causes of uterine enlargement.

(4-1) Pyometra: Pyometra is a condition characterized by an accumulation of pus in a sealed uterus. The condition occurs when an infectious organism enters the uterus at the time of or prior to the onset of pregnancy. In this case, the contents of the uterus prevent the release of PGF₂ α , and in turn the corpus luteum remains functional. The condition may remain status quo for an extended period.

(4-2) Endometritis: Endometritis is a nonspecific infection of the endometrium. It is characterized by the absence of pus and a uterine wall which feels thickened and spongy. This condition may be confused with pregnancy at the 35 to 40 days stage.

(4-3) Metritis: Metritis is a nonspecific infection of the uterus characterized by the presence of visible pus. The pus may be seen on the lips of the vulva and on the tail where it rubs across the vulva. The pus may also be seen in estrous mucus as cloudiness, yellow or white flakes. The uterine wall is thickened and feels spongy. This condition might be confused with the 35 to 40 day stage of pregnancy.

(4-4) Mummified fetus

Mummified fetus is a condition in which the fetus dies and the fluids and soft tissues are reabsorbed. Depending on the

stage at which the condition is detected, the mass may range from a semi solid to a solid ball. It is not difficult to differentiate between a mummified fetus and normal pregnancy at the 90 to 120 day period. However, with a casual palpation one might feel the mummified fetus and misinterpret it for a normal fetus. Additional palpation would reveal the absence of fluid surrounding the mass and provide the differential diagnosis.

(5) Assay of progesterone in milk or blood

Milk progesterone levels have been found to parallel blood when samples were taken periodically throughout the estrous cycle. The levels correspond to a pattern set by the corpus luteum. That is, high during midcycle and low just before, during and just after estrus (Fig. 4).

The prediction of pregnancy using this test is based on the progesterone level in a single sample at 21 to 24 days post-breeding. For cows producing positive samples (high levels) 80% have been pregnant 40 to 60 days post-breeding as determined by rectal palpation. Essentially all the cows showing negative test results (low levels) have been open.

The greatest shortcoming of the progesterone assay method is that it employs radioisotope assay techniques which must be conducted in a central laboratory having expensive isotope counting equipment.

1-5 Gestation

Gestation is the period of pregnancy. It starts with fertilization, which has been described, and ends with parturition (the birth process). The average length of the gestation period is 281 days for the cow. Both individual and breed differences exist. Gestation is a little longer when cows are carrying a male than when carrying a female. With twins, gestation is shorter.

During the early part of gestation, the embryo floats free, first in the oviduct and then the uterus. The fertilized ovum usually reaches the uterus on day 5-6 postconception. Implantation occurred at about day 30, and the placenta attaches as predesignated to a caruncle in the maternal endometrium. In the cow between 30 to 35 days after fertilization, there will be 3 or 4 fragile cotyledonar attachments in the pregnant horn. Before 40 days fragile attachments will be present in both horns. There will be 40 to 50 cotyledonary attachments in both horns by 70 days and this increases to approximately 150 by the middle of the pregnancy. Before the embryo can derive benefit from placentation, organ development will have to occur to the point that the embryo's circulatory system is functional.

There are three distinctive periods in the development of the conceptus. These are: (1) cleavage, (2) differentiation and (3) growth.

- (1) Cleavage: After fertilization the zygote will divide and redi-
vide many times without any increase in cytoplasm. The
first cleavage will result in a 2-cell embryo (1 day). This
is followed by additional cleavages resulting in the oviduct
into the uterus, a ball of 16 to 32 cells (4-7 days) will be
contained within the zona pellucida. This structure, with
cells too numerous to accurately count, is called a morula.
During the next few days, fluid collecting in the inter
cellular spaces will push to the center forming the
blastocyst, a structure with a fluid filled cavity (the
blastocoele) surrounded by a layer of cells.

(2) Differentiation

(2-1) Differentiation of organ: Is a period when the cells are in the process of forming specific organs in the body of the embryo. Notable events during differentiation include the formation of the germ layers, extraembryonic membrane, and organs. In addition, rapid changes in relative size occur during differentiation.

(2-2) Germ layers: The first evidence that differentiation has begun is the appearance of the germ layers. The endoderm, the inner most germ layer, first appears when a single layer of cells push out from the inner cell mass and grows around the blastocoele. The endoderm is the origin of the digestive system, liver, lungs and most other internal organs. The mesoderm, the middle germ layer, arises from the inner cell mass, pushing between the endoderm and ectoderm. The mesoderm is the origin of the skeletal system, muscles, circulatory system and reproductive system. The ectoderm, the outer germ layer, is the origin of the nervous system, sense organs, hair, skin, mammary glands and hooves.

(2-3) Extraembryonic membranes: Soon after the appearance of the germ layers, formation of the extraembryonic membranes will begin. Two extraembryonic membranes, the amnion and allanto-chorion, will form during this period and function throughout the remainder of gestation. A third extraembryonic membrane, the yolk sac, is seen early during differentiation but disappears by the end of this stage of development. The yolk sac contains an early source of nutrients for the developing embryo. As the yolk is depleted, the yolk sac regresses. A portion of the yolk sac is folded into the embryo, forming its primitive gut.

(2-4) Organ formation: As formation of the extraembryonic membranes progresses, cells within the inner cell mass are differentiating. A neural plate forms from ectodermal cells

as the beginning of the central nervous system. The primitive brain and spinal cord are quickly discernible. The circulatory system develops rapidly from mesodermal cells and by day 22 in the cow an embryonic heartbeat can be detected. The liver, pancreas, lungs and digestive system can be identified as they differentiate from endodermal cells. Within a few days, limb buds, which will form legs, a tail bud, and the lenses of the eyes can be identified.

(3) Fetal growth

The pattern of growth in the fetus is interesting. Relative growth rate is more rapid during early gestation than late gestation. Between 45 and 75 days in the fetal calf, there will be over 1,000% increase in size (e.g., 6 to 72 g). During the last 2 months of gestation a relative change of 18 to 40 kg is seen .

The weight of the fetal fluids, fetal membranes and the maternal uterus increase as gestation progresses. Just before parturition in the cow, the fetal fluids weigh about 15.5 kg and the fetal membranes weigh about 3.8 kg. The uterus will increase from about 1.0 kg to 10 kg during the course of pregnancy. Even with the ten-fold increase in the size of the uterus, the fetus and its associated fluids and membranes will account for about 85% of the total weight of the uterus and its contents.

(4) Twinning

Twinning sometimes occurs in monotocous species. Twinning rates in cattle are relatively low, ranging from 0.5 to 4% for different breeds. In some herds, rates of 8 to 10% have been reported. Twinning rates for Brown Swiss and Holsteins are higher than for Jersey, Guernsey and dairy cows, which have higher twinning rates than beef cows. The twinning rate in most beef breeds is less than 1%. Twinning has not been considered desirable in cattle because of increased incidence of retained placentae, reduction in future

reproductive efficiency, weaker calves that are more difficult to raise and reduced milk production by cows after twinning.

The heritability of twinning is low. The incidence of twinning increases with age for the next several years then drops off again. Most twins are of the dizygous type. That is, they result from ovulation of two oocytes during the same estrous cycle.

Some twins are monozygous, resulting from fertilization of a single oocyte. Monozygous twins are always the same sex and are genetically and phenotypically identical, except that one is frequently larger than the other. The means by which a single zygote can result in twins is not known. About 8 to 10% of all twins births in cattle are monozygous. Embryo loss and abortion may account for the lower percentage of monozygous twins in cattle.

1-6 Parturition and Postpartum Recovery

(1) Parturition

The onset of parturition can be recognized by certain physical changes in the cow. Externally, the udder will become increasingly turgid and may be edematous. These changes can be present for a few days in mature cows or for 1-2 weeks in primiparous females. Parturition is the birth process. It begins with softening and initial dilation of the cervix along with the start of uterine contractions. It ends when the fetus and its associated placental membranes are expelled (Fig. 5).

(1-1) Approaching parturition: Signs of approaching parturition can be seen during the last month of gestation. Careful evaluation of these signs indicate when the female will need to be closely observed.

(1-2) Rotation to birth position: In the cow, the first sign of approaching parturition may start with the rotation of the fetus into the birth position. During most of the gestation period, the fetus will be on its back with its feet pointing up. After rotation into the birth position, it will be resting on its thorax or abdomen with its forefeet positioned at the uterine end of the cervix and its nose resting between the forefeet.

The incidence of abnormal presentations in cattle is about 5%, with a variety of abnormalities possible. This may range from one leg to both legs or the head turned back to several breach positions with the tail pointed toward the cervix.

(1-3) Mammary gland changes: Growth of the mammary glands can be seen during the latter part of gestation. This is likely caused by the synergistic actions of estrogens and progestin, which stimulate development of both ducts and secretory tissue

in the mammary glands. As parturition approaches the mammary glands will enlarge as they fill with milk. Synthesis of milk is a function of prolactin in synergy with other hormones. As oxytocin is released during labor, milk letdown will occur frequently causing milk to leak from the teats.

- (1-4) Other changes: With parturition imminent, relaxin synergizing with estrogen will cause further expansion of the pelvis, enlarging the birth canal to facilitate passage of the fetus.

A sinking of the ligaments around the tailhead will make the tailhead appear more prominent. The vulva will soften and become swollen. Mucus may be seen stringing from the vulva as estrogen causes the epithelial cells of the cervix to secrete new mucus.

Parturition can be divided into three stages. The first stage of parturition ends with complete dilation of the cervix and entry of the fetus into the cervix. This stage usually takes from 2 to 6 hrs. At the end of stage 1 fluids from the allantoic cavity may also be expelled. The second stage ends with expulsion of the fetus. The time varies with the size of the litter. Expulsion of the placenta occurs during the third stage. This may occur within 30 minutes of the expulsion of the fetus, but is more likely to occur 3 to 5 hrs later.

- (1-5) Regulation of the physiological events: Approximately 2 days before the start of parturition a rapid sequence of changes in hormone levels involving both the fetus and mother can be observed.

The main physiological events in parturition are (a) dilation of the cervix which permits the fetus to pass and (b) uterine contractions which forcefully expel the fetus and placental membranes. The initial dilation of the cervix is caused by relaxin as it synergizes with rising levels of estrogen.

The initial uterine contractions are probably caused by PGF_{2a} as it is released from the endometrium by rising estrogen

levels. These early contractions are weak and irregular, occurring at about 15 minute intervals. Just before expulsion of the fetus, uterine contraction will be regular, strong, and frequent, occurring at about 2-minute intervals and lasting for approximately 1 minute. Contraction of abdominal muscles will aid final expulsion. Following expulsion of the fetus the strong uterine contractions subside. However, diminished contractions will continue for another 1 to 2 days. The continued contractions are responsible for expulsion of the placental membranes as well as fluids and fragments of placental tissues that may remain in the uterus.

(2) Care of the newborn

Reproduction is not successfully completed unless the neonate (newborn) survives. The first few hours after expulsion from the uterus is critical to its survival. It has been transferred from a germ-free environment in which its temperature regulation, nutrient, and oxygen requirements have been dependent on the maternal system to an environment where it must survive independently.

The neonate's system has not been challenged. Therefore, it does not have circulating antibodies to ward off diseases. Maternal antibodies do not pass through the placental barrier to fetal blood in cattle. Maternal antibodies can be absorbed through the intestines if the newborn receives a meal of colostrum (the first milk) from its mother soon after parturition. There is evidence that the newborn loses the ability to absorb maternal antibodies through the intestines as early as 6 hrs after birth.

In cold weather, the newborn should be dried and if possible placed in a clean, dry shelter. In the summer, a shade will be beneficial to survival. If the newborn has not nursed within the first hour, it should be assisted in obtaining a meal of colostrum, to provide both nutrients and antibodies. If the mother dies during parturition, colostrum from another cow should be provided in a pail or bottle with a nipple. Normal milk will provide most needed nutrients, but will not contain the needed antibodies.

(3) Retained placentae

During normal parturition in the cow, placental membranes become separated from the endometrium during fetal expulsion.

Retained placenta (also called retained afterbirths) will occur in from 5 to 15% of the parturition in healthy herds cows. They occur when separation of the chorionic villi from the caruncles is delayed. Many of these can be associated with short gestations (270 to 275 days) and twin births which result in shorter gestation periods. They are more likely to occur in high milk producers than low producers, which may account for the higher incidence in dairy cows than is seen in beef cows. Also, retained placentae are more common after difficult births (dystocia) than after uncomplicated births.

In herds where the incidence is over 15 to 20% a problem is indicated. Nutritional deficiencies of either selenium or vitamin A have caused a much higher rate of placental retention. Assistance from a veterinarian will be needed in identifying and treating disease problems. If the placenta of a cow has not been expelled 24 hrs after expulsion of the fetus, it will likely be retained another 5 to 6 days. Uterine infections occur in conjunction with most retained placentae. Some infections may not be eliminated for several weeks. Lower fertility after retention is a problem, with some cows never conceiving again. The stress associated with this problem will reduce milk production below anticipated levels. Another alternative is manual removal 48 to 72 hrs after parturition, followed by intrauterine treatment with antibiotics.

Two approaches are now being recommended. One involves placing antibiotic boluses into the uterus daily until the placenta is expelled. The other involves a single large volume (4-liter) infusion of an antibiotic solution into the uterus 24 to 36 hrs after parturition. Both methods give satisfactory results.

Treatment with synthetic estrogens and/or oxytocin may be of benefit if the placental membranes have been loosened from the endometrium but have not been expelled because uterine contractions have stopped.

(4) Postpartum recovery

Following parturition, the next successful gestation depends on both return to normal estrus and return of the uterine environment to a state that will support another pregnancy.

(4-1) Postpartum ovulation and estrus: After parturition, many dairy cows will ovulate in 20 to 30 days. However, a high percentage of these are frequently called "silent heats" or "quiet ovulations". When these cows ovulate again at 40 to 50 days, the majority will show signs of estrus. Such cows are less likely to have reproductive problems than those that have an extended period of anestrus (absence of cycling). Several factors can extend postpartum anestrus.

Low nutrition, either during gestation or after parturition, will delay return to estrus. Quiet ovulations are also more frequent for cows on a low plane of nutrition. Cows most affected seem to be those in thin condition at time of calving. A combination of low nutrition and suckling will compound the problem. Many beef cows which calve in thin condition and nurse their calves will stay in anestrus beyond 100 days. Some may not have a calf the next year because of failure to show estrus and ovulate while running with bulls during the next breeding season.

Other factors which may extend postpartum anestrus include infectious diseases, metabolic disorders, uterine infections and other health problems.

(4-2) Involution of the uterus: Involution of the uterus implies the return to the normal nonpregnant state.

Uterine involution has been studied more extensively in cows than in other species. This is partly because it is easy to follow the course of involutaion by rectal palpation. Criteria for involution include (a) return of the uterus to the pelvic area; (b) return to the nonpregnant size; and (c) recovery of normal uterine tone. Using these criteria, uterine involution in cows following uncomplicated parturitions requires about 45 days. Histological studies have shown that another 15 days may be required before the endomtrium is histologically normal.

The uterine of cows return to the nonpregnant size in about 30 days. The tone of the nonpregnant horn may be normal at that time. However, another 2 weeks may be needed for normal tone to return to the pregnant horn. Periods of estrus seem to speed the return of normal tone. It should be noted that one uterine horn may be larger than the other after complete involutiaon. Also, in older cows, uteri may not return to the pelvic area. Therefore, tone as determined by rectal palpation is the more precise indicator of involution. Following placental retention and/or uterine infection, involution of the uterus may be delayed by several weeks.

1-7 Male Mating Behavior

The primary purpose of mating behavior is copulation, thus bringing male and female gametes together to insure propagation of the species. For males, the events in mating behavior, listed sequentially, are sexual arousal, courtship (sexual display), erection, mounting, intromission (insertion of penis) and ejaculation. If the female is in estrus, this sequence of events takes only a few minutes.

Vocalization occurs by the bull. Sniffing of the female's genitalia and urine is seen. In cattle, the male will extend his neck and curl his upper lip. Various tactile stimuli, including licking, bunting, and biting, are part of the courtship pattern in most males.

(1) Regulation of mating behavior

Increased aggressiveness and sexual desire can be correlated with increased production of testosterone as a male approaches puberty. In addition, the reduction in sexual activity associated with castration can be restored by injections or infusions of testosterone. Reductions in testosterone and sexual activity during summer in cattle are probably related to reduced thyroid activity, resulting in lower basal metabolic rates rather than to a direct effect of summer temperatures on the Leydig cells.

(2) Social and sexual interaction

Sexual experience during development before puberty is important to the attainment of full sexual activity. It has been demonstrated that males raised in complete isolation never develop as high a level of sexual activity as males raised in social groups.

Lack of early sexual experience has resulted in neither serious nor permanent problems in attaining full sexual activity. In artificial insemination centers, sexually inexperienced bulls are sometimes hesitant, spend a long time exploring the genitalia of female teaser

animals and frequently have weak erections with incomplete ejaculation. However, after repeated use under the same conditions, they adjust to the surroundings and copulate vigorously.

(3) Senses

Certain senses are important to the mating response of males. The sense of smell may be the most important, as in females. Pheromones found in the urine of estrous females are stimulatory to males. Bulls can be trained to mount dummies and serve artificial vaginas when collecting semen for use in artificial insemination. When males do not respond to the dummy mount, sprinkling urine from an estrous female on the mouth will frequently elicit the desired response. Sight of an estrous female, a castrate, a padded dummy mount, or an object that can be mounted will usually stimulate mounting behavior in bulls. Sexually mature bulls wearing blind folds will copulate normally. On the other hand, males blind from birth reach sexual maturity at a later date than do normal males.

The tactile sense is important to normal copulatory behavior in most males. Males may be sensitive to both temperature and pressure. Bulls are sensitive to the temperature of the water in the artificial vagina. Most bulls respond best to the artificial vaginal if the temperature is approximately 45°C. If the temperature of the artificial vagina is too hot or too cold, he will not ejaculate and may become uncooperative in future attempts to collect semen.

(4) Erection and ejaculation

Erection is under control of the autonomic nervous system. With sexual excitement, blood is pumped into and temporarily trapped in the corpus cavernosum penis and corpus spongiosum penis. The corpus cavernosum penis is larger and more important in attaining an erection than the corpus spongiosum penis. Pressure in the corpus cavernosum penis just before ejaculation may exceed 15,000 mm

mercury in bulls.

The energy for this pressure come from the ischio cavernosum muscle, which contracts to pump blood into and traps blood in the corpus cavernosum penis. The corpus cavernosum penis is a closed system with no venous outlet to bleed off the pressure.

(5) Ejaculation

Ejaculation is defined as the ejection of semen from the body. The ejaculate includes spermatozoa from the vas deferens, epididymis and fluids from the accessory glands. Ejaculation is initiated by stimulation of sensory nerves in the glans penis, which triggers a series of peristaltic contractions involving smooth muscles in the epididymis, vas deferens, and urethra. In addition, fluids from the accessory glands are pumped into the urethra. Peristaltic contractions move spermatozoa concentrate and accessory gland fluids through the ducts leading to the external urethral orifice.

Ejaculation varies among species in a number of aspects. Ejaculation occurs almost instantaneously with the thrust of the penis in bulls. Volumes of ejaculates vary in bulls (7cc). Concentration of spermatozoa are 1.2 billion per cc in bulls.

(6) Maintaining libido

Maintaining libido (sex drive) in males is of concern in both range (or farm) conditions and with artificial insemination organizations.

Providing a balanced diet is highly important. Bulls should neither underfeed nor overfeed. As has been mentioned underfeeding will delay puberty. It has also been demonstrated that underfeeding young bulls between the age of puberty (10 to 12 months) and the end of their growth period (30 to 36 months), will reduce libido and total semen production. In mature bulls overfeeding is more likely

to be a problem. If mature bulls are overweight, increase of foot, leg, and joint problems may shorten their reproductive life.

Diseases and injuries will reduce libido. Neither of these conditions has to be serious to reduce sexual activity. A seemingly minor problem, such as a swollen joint or mild respiratory infection may reduce the overall vigor of the bull as well as his libido. When bulls are confined to small areas, special attention must be given to hoof care (trimming, etc.) and moderate exercise to help maintain a state of good health and prevent conditions that make copulation unpleasant. If copulation is unpleasant or causes pain, voluntary retirement from sexual activity is not uncommon.

(7) Sexual exhaustion

Sexual exhaustion can be a problem when males run with females for a prescribed breeding season. A primary effect of sexual exhaustion is loss of libido. This is more likely to happen with a dominant male during the heavy part of the breeding season under range conditions. Subsequent gestations have been delayed because dominant, but sexually exhausted males have prevented subordinate males from copulating. If a number of females are in estrous, or if at least two other males are running with the herd, the dominant but sexually exhausted male will be less able to prevent breeding of estrous females. After adequate rest, males that have been sexually exhausted will regain their libido.

(8) Sexual satiety

Whereas sexual exhaustion is a physical problem that can be cured with rest, sexual satiety is a mental problem. It is a term used to describe a sexual indifference when a bull has copulated with the same cow under the same conditions for an extended period. It is a problem which has been encountered by organizations where bulls are maintained exclusively for the purpose of providing semen for artificial insemination. In most cases sexual satiety can be cured

by providing variety in the collection procedure.

Technicians who collect semen from bulls using an artificial vagina must be careful to make it a pleasant experience for bull. Having too hot or too cold water in the artificial vagina, and excessive bending or of grasping of the penis, may result in bulls becoming uncooperative in future collection attempts.

1-8 Reproductive Management

Management plays an important role in the reproductive efficiency obtained from both females and males. Unfortunately, reproductive efficiency approaching 100% is not possible even with the very best management; however, poor management can result in drastic decreases in reproductive efficiency.

(1) Measurements of reproductive efficiency

In order to determine the effect of management on reproductive efficiency, it is necessary to establish some guidelines for measuring reproductive efficiency.

(1-1) Services per conception: This measurement is determined on a herd basis by dividing total services by the number of pregnancies. On a herd basis, unidentified sterile females will make the calculation less meaningful.

(1-2) Calving rate: Calving rate is calculated by dividing the total number of cows bred by the number that calved. It is also expressed as percent calf crop.

(1-3) Nonreturn rates: With the advent of AI, a means of evaluating the fertility level of semen in the shortest possible time was recognized as a serious need. The nonreturn rate (NR) is the percentage of females that do not return to estrous or receive a second service within a designated time interval. The time intervals most commonly used are 28-35 days, 60-90 days, and 151-180 days. Obviously, the shorter intervals provide earlier information but the longer intervals provide more accurate information. The difference between 28-35 day NR rate and 60-90 day NR rate ranges from 10 to 15 percentage units. The 150-180 day NR rate is only 1 to 2 percentage units lower than the 60-90 day NR rate. For this reason it is not often used.

(2) Management related to the female

The main concern related to when young females should be inseminated or bred for the first time is the body size. The body size at the time of breeding is important because it influences the body size of the animal at first parturition.

(2-1) Size and age at first insemination: The level of nutrition greatly affects the age at which puberty occurs, but once the female reaches puberty, neither size nor conception rate affect within acceptable ranges of management. Heifers bred for the first time between 4 and 5 years of age experience a significant increase in reproductive problems. Heifers calving when older than 36 months will produce slightly more milk during first and possibly second lactations than heifers calving earlier. Heifers that calve at 24 months on the average will have a higher lifetime production than those calving later.

Managers need to take a look at the total cost of carrying the heifer to 3 years of age for first calving versus the cost of growing them fast enough so that they will be large enough to calve at 2 years of age. In addition to an earlier economic return, there is the added advantage of a shorter generation interval.

(2-2) Detection of estrous: The most important management practice is detecting estrous so that insemination can be performed at the proper time. Estrous synchronization, as has been described, may relieve the problems in the dairy heifer and beef cattle industry. Using twice daily checks at 6:00 AM and 6:00 PM as a basis for comparison, an additional check at 12:00 noon increased efficiency by 10%. A fourth observation at 12:00 midnight gave an increase in efficiency of 19.9% over the twice daily checks. The early morning check for estrous

(as soon after daylight as possible) is the most important. More cows estrous between 2:00 AM and 5:00 AM than any other similar period of time during the day.

Checking cows for estrous should not be combined with other chores. It needs the full attention of the individual doing the checking. If feeding is done in connection with checking for estrous the cows will have their mind on eating rather than demonstrating symptoms of estrous. The individual observing cows for estrous should be thoroughly familiar with all of the physiological and psychological symptoms of estrous.

There are procedures for altering males for use in detecting estrous. The penile block and redirected prepuce are recommended for use with cattle. A chin-ball-marker should be attached to the bull so that he will identify the estrous females. Dye preparations that can be painted onto the brisket are available for this purpose. Also, cows treated with androgens have been used to help diagnose estrus in cows.

Some dairymen make a mark on the tail head with a grease-type cattle marker (Marking paint). When cows stand for mounting the mark becomes smeared. Heat check patches are available that can be glued to the tail head of the cow which will greatly increase the chances of detecting estrous (Heat mount detector). The patch has a small capsule of red dye contained inside the larger white capsule. When the animal is in estrous and stands to be mounted, the pressure applied by the mounting animal will release the red dye causing the large capsule to turn bright red. A disadvantage of the heat check patch is that some cows will lose them or rub them off before coming into estrous. The number lost is not great and with daily observations the lost patches can be replaced with a minimum of effort.

Table 1 Methods of pregnancy diagnosis in the cow

Method	Earliest time
Failure to return to oestrus and persistence of corpus luteum	21 days
Progesterone concentration in plasma and milk	21-24 days
Real-time ultrasound (direct imaging)	28 days
Palpation of the allantochorion (membrane slip)	33 days
Unilateral cornual enlargement and disparity in size, thinning of the uterine wall, fluid-filled fluctuation of enlarged horns	35 days
Palpation of the early fetus when the amnion loses its turgidity	45-60 days
Palpation of the caruncles/cotyledons	80 days
Hypertrophy of the middle uterine artery until presence of fremitus	85 days
Oestrone sulphate in blood or milk	105 days
Palpation of the fetus	120 days

(Arthur, G. H., D. E. Noakes & H. Pearson, 1989)

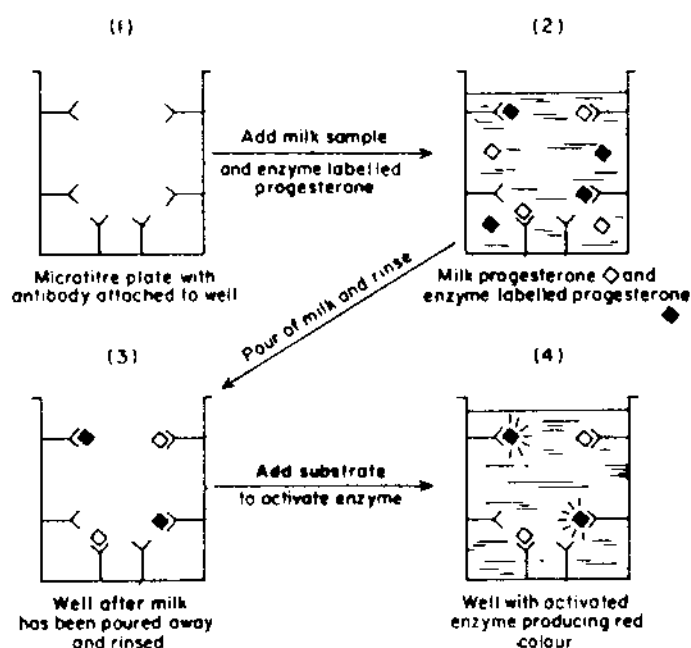


Fig.4 Enzyme linked immunoassay (ELISA) using microtitre plate.

(Arthur, G. H., D. E. Noakes & H. Pearson, 1989)

Table 2 Clinical Diagnosis of Pregnancy in the Cow by Palpation per Rectum

<i>State of Pregnancy (months)</i>	<i>Major Characteristics</i>
Non-pregnant	reproductive tract lies between the rectum and pelvis; two horns of equal size; detectable groove between uterine horns; vaginal mucus is thin and watery during estrus.
Pregnant	
1 month	one uterine horn larger than other; fetal membranes feel like a shirt under sleeves of coat; vagina dry and sticky; thick mucus around cervix; corpus luteum present on same ovary that had ovulated 21 days previously (errors may occur if estrus was unobserved and second ovulation occurred on same side).
2	pregnant horn enlarged and bulging; pregnant horn has tense or resilient feeling; detectable double walls of uterus and placenta; "fetal membranes slip"; gelatinous adhesive and tenacious secretion from cervix; tense kidney-shaped amnion identified close to external bifurcation of horn unilateral with corpus luteum; amnion increases from size of marble (2 cm) at 5 weeks, to hen's egg (5 cm) at 7 weeks.
3	enlarged uterus drops to abdomen; fetus bounces against palm of hand or fingertips; middle uterine artery on pregnant side slightly larger than non-pregnant side; "fremitus" in middle uterine artery of gravid horn identified; "fetal membranes slip"; very small placentomes palpable in uterine wall.
4	fremitus felt in uterine arteries; fetus and caruncles palpable;
5	external os located on pelvic rim; presented extremities lie within reach in front of and below pelvic brim (fetus may be felt, then it sinks into uterus beyond reach)
6	fetus may drop out of reach of hand; fetus is lying in right flank; placentomes (like cuff links under the sleeve of a shirt) and limbs of fetus may be felt; mammary gland in heifers enlarged
7	mammary glands hypertrophied
8	fetus palpable
9	vulvae labiae swollen, prominent and resilient; mammary glands glossy, enlarged and edematous and appear waxy and tumified; mucoid vulval discharge
after 9: prior to parturition	sacrosciatic ligaments soften; apex of sacrum displaced upwards due to relaxation of the sacroiliac ligaments; hindquarters have sunken appearance; tail-base carried high; cervical plug liquefies; cervix relaxed

(Hafez, E. S. E., 1968)

Fig.1 The female reproductive system

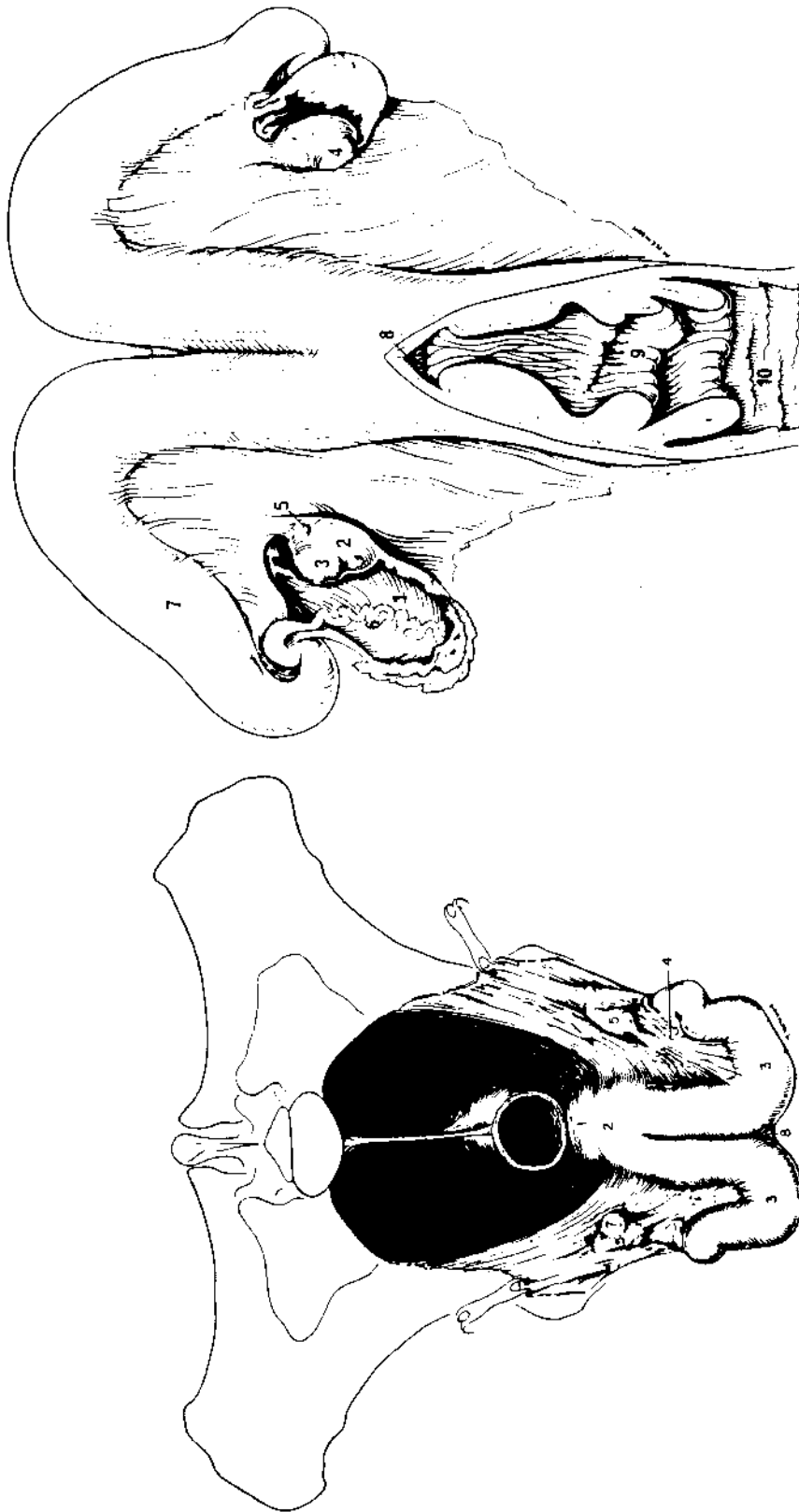


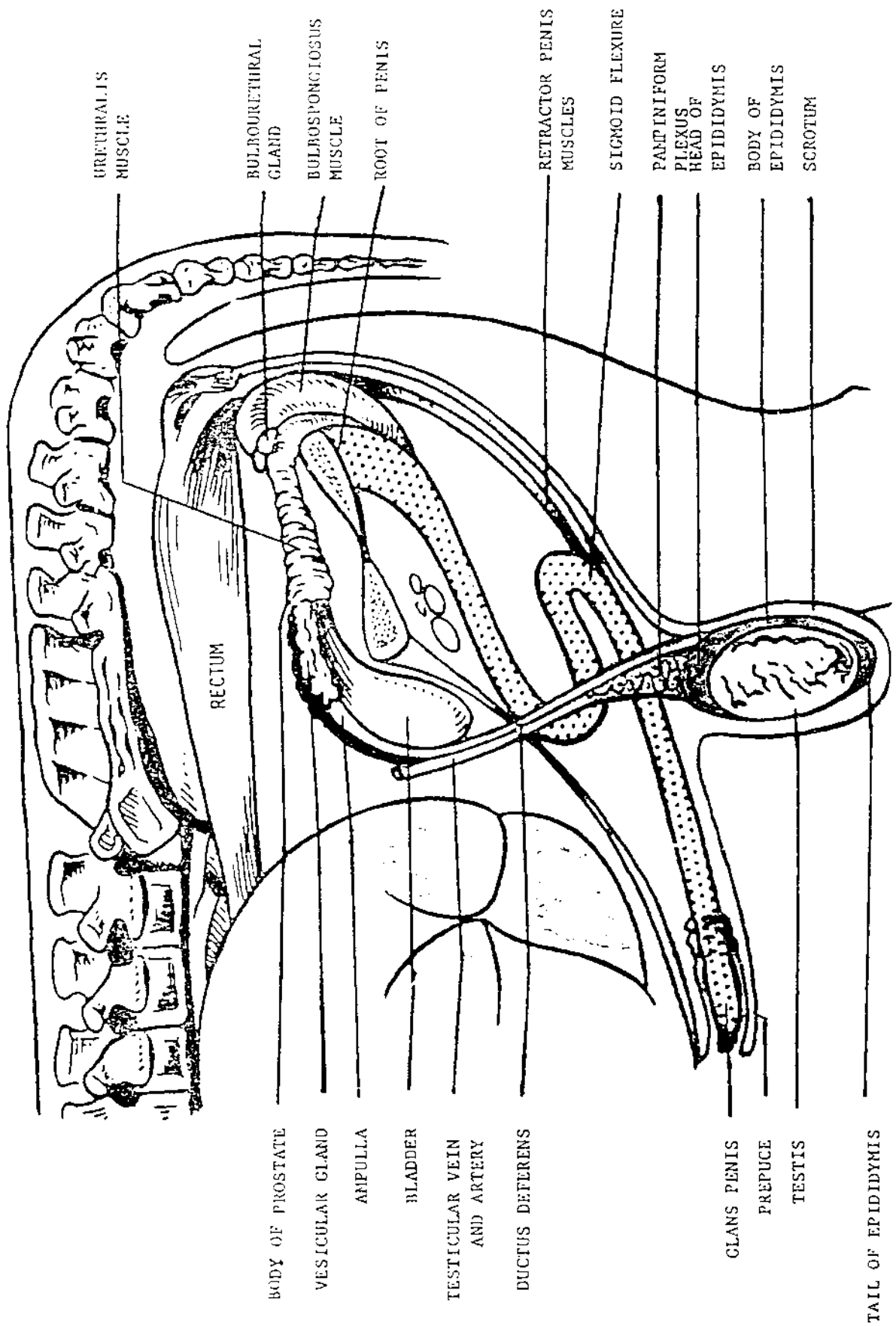
Fig.1-1 Nonpregnant uterus of the cow, cranial aspect. 1. Cervix, 2. Body of uterus, 3. Horn of uterus, 4. Oviduct, 5. Ovaries, 6. Ovarian ventricle, 7. Intercornual ligament, 9. Rectum.

(Roberts, S.J., 1971)

Fig.1-2 Uterus of cow, dorsal aspect. 1. Ovarian bursa, 2. Ovary, 3. Corpus luteum, 4. Follicle, 5. Corpus albicans, 6. Oviduct, 7. Uterine horn, 8. Uterine body, 9. Cervix, 10. Vagina.

(Roberts, S.J., 1971)

Fig. 2 The Genital Organs of the Bull
(Bearden, H. J. & J. W. Fuquay, 1980)



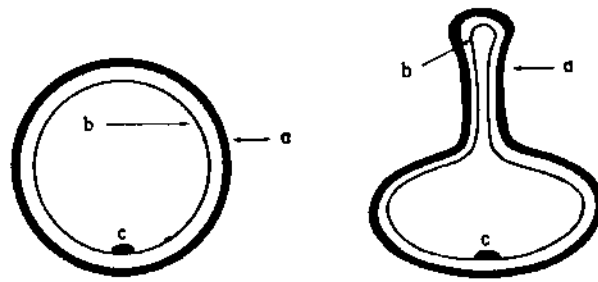


Fig. 3 Schematic drawing of a cross section of the bovine pregnant uterine horn showing pregnancy diagnosis by the "fetal membrane slip." Note the double fold formed by the uterine wall (a) and the chorioallantoic membrane. The thickened segment (b) represents a cross section of the connective tissue band, where blood vessels are imbedded.

(Hafez, E. S. E., 1968)

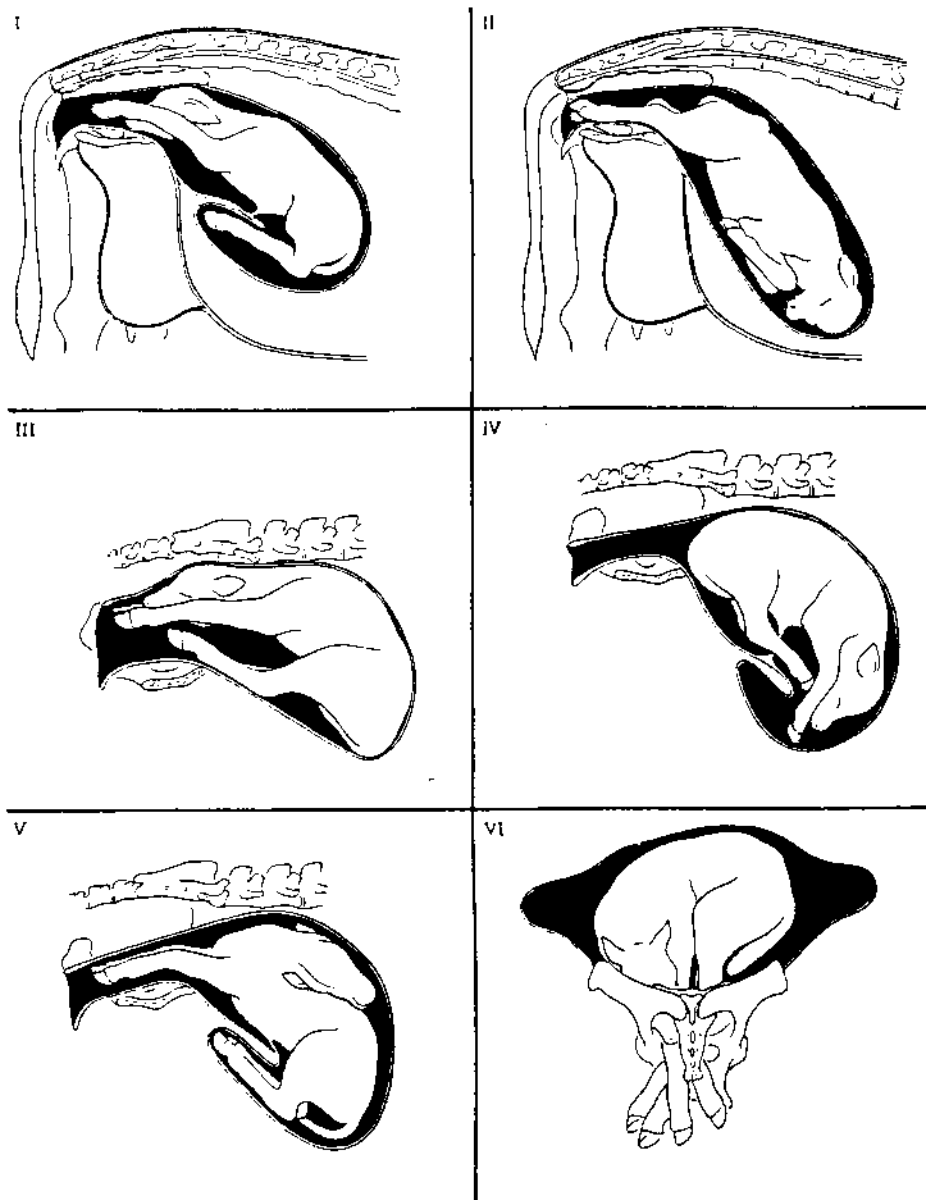


Fig. 5 Normal and abnormal presentations, positions and posture in bovine and equine births. I Normal anterior presentation, II Posterior presentation, III Anterior presentation with the rear legs extended beneath the fetus ("dngsitting" posture), IV Posterior presentation with the rear limbs extended beneath the fetus (breech presentation), V Anterior presentation with the head and neck extended over the fetus, VI Transverse ventral presentation of a foal. (Roberts, S. J., 1971)

2. Hormones in Farm Animal Reproduction

Various hormones take part in the expression of reproductive functions of farm animals, which are due to interactions between hormones.

Recently, marked progress has been made in the following areas: methods of measuring hormones, structure analysis of hormones; development of analogs and antagonists, and development of research methods based on molecular biology. Consequently, mechanisms of control by hormones of the expression of reproductive functions are now being rapidly clarified. In addition, there are continuous and marked changes in practical applications of hormones in artificial control of reproductive functions.

In this report, the main studies performed recently on hormones in the field of farm animal reproduction are introduced with priority given to cows, and a description is also given of mechanisms of control by hormones over reproductive functions as we understand them. In recent years, there have been very many research papers on various areas in this field. It is thus impossible to include all such papers in this report which is limited in its number of pages. The author wishes the readers to remember that papers used for discussion in this report are just a small part of the many research papers published.

2-1 Hormones Taking Part in Reproduction

(1) Outline of Control by Hormones of Reproductive Functions

The hormones shown in Table1-2-1 are considered to take part in the hypothalamo-pituitary-gonadal axis and lead to expression of reproductive functions (Photos 1 and 2). These hormones work through the axis, as

follows: gonadotropic hormone-releasing hormone (GnRH) is secreted from the hypothalamus, GnRH causes the pituitary to secrete follicle stimulating hormone (FSH) and luteinizing hormone (LH), and finally FSH and LH induce the ovaries and testes to synthesize and release sex steroid hormones, i.e. estrogen (E), progesterone (P) and testosterone (T).

These sex steroid hormones have been considered to regulate the functions of accessory reproductive organs such as the oviduct, uterus or vagina in females, and epididymis, accessory reproductive glands or penis in males. In addition, they have been considered to show a feedback effect, that is, to work again on the higher organs, meaning the pituitary and hypothalamus. It has also been confirmed in females that prostaglandin $F_{2\alpha}$ is secreted from the uterus and that it promotes luteolysis in the ovaries. There are still few changes to this concept, but recently the existence of many other hormones and gonad function-regulating substances has been observed.

(2) Discovery of Inhibin, a New Hormone

In 1932, McCullagh suggested the existence of inhibin, but existence of the substance itself was not confirmed for many years after that. In recent years, however, rapid progress has been made into research of the structure and action of inhibin, shedding light upon the details of its physiological actions and its role in the expression of reproductive functions. As shown in Fig.1-2-1, inhibin administered to animals inhibits the secretion of FSH from the pituitary causing a rapid decrease in concentration of FSH in the blood. Inhibin is produced by granular layer cells of the ovaries and Sertoli cells in the testes (Fig.1-2-2) and production is promoted gonadotropic hormones such as FSH and human chorionic gonadotropin (hCG). However, in in vitro experiments, LH has been confirmed to cause no changes in production of inhibin in small quantities but promotes its production in extremely large quantities.

Figure 1-2-3 shows the correlation between the production of inhibin and the expression of reproductive function with the growth of ovarian

follicles in females. In the early stage of growth, when the follicle is small in size, the concentration of inhibin is low and FSH is secreted from the pituitary, leading to the promotion of follicle growth. As the follicle matures, the secretion of inhibin from the follicle is accelerated, resulting in inhibition of FSH secretion. This mature follicle is then released (ovulation), and, at the same time the source of inhibin disappears. This causes a rapid decrease in concentration of inhibin in the blood which results in massive secretion of FSH from the pituitary.

FSH and LH in blood during estrus and immediately before or after ovulation have been considered to show a similar unimodal ovulation surge caused by action on the pituitary of temporarily-increased GnRH. With respect to FSH, however, it was confirmed that the second surge appears after ovulation due to a rapid decrease in secretion of inhibin from the ovary.

From these findings, hormones in the estrous cycle are considered to change as seen in Fig.1-2-4 . However, Fig.1-2-5 shows the correlation between secretory organs and target organs for these hormones. As can be seen from the figures, FSH is considered to be controlled and regulated by a second regulatory factor together with GnRH.

Practically, FSH and LH often show an incompatibility in blood kinetics. The secretion of FSH and LH, however, has conventionally been considered to be controlled and regulated by only GnRH and the reasons for the incompatibility have been explained by the following differences between FSH and LH; a difference in modification by various steroid hormones of the secretion of FSH and LH from the anterior pituitary, and a difference in half-life between FSH and LH. It has been shown that FSH is controlled by a different regulatory mechanism including a second regulatory factor.

There are large differences in the appearance of the second surge of FSH between animal species. In sheep and rats, for example, the surge appears rather distinctly, while in cows it appears rather indistinctly

and may be difficult to recognize in individual measurements. Hasegawa et al. examined the kinetics of inhibin in cow blood, and reported that inhibin showed high concentrations in blood even after ovulation in many cows.

(3) Action of Activin Having a Structure Similar to that of Inhibin

The structure of inhibin was demonstrated to be a dimer consisting of an α -subunit and a β -subunit, shown in Fig.1-2-6, virtually simultaneously in several laboratories in the world including the Department of Obstetrics and Gynecology, School of Medicine of Gunma University in Japan.

In the meantime, a homodimer formed by combination of two β -subunits was found in ovarian follicles of pigs, and was named activin. Activin, in contrast to inhibin, is known from in vitro experiments to promote the secretion of FSH by working on anterior pituitary cells. In addition, activin shows a local action on ovarian follicles, that is, a paracrine or autocrine action, which induces and increases FSH receptors in granular layer cells. FSH induces and increases LH receptors, promoting the process of differentiation of granular layer cells. This inducing action is also found to be promoted by activin. In this manner, activin amplifies the action of the gonadotropic hormone (GTH) by increasing GTH receptors at each stage of growth of the ovarian follicle.

Activin is considered to play an important role in the maturation of ovarian follicles (Fig.1-2-3). In a growing follicle, activin and FSH mutually induce the appearance of receptors and, as a result, a mature follicle shows a marked increase in receptors for both activin and FSH. In addition, activin amplifies the action of LH by promoting the induction of FSH on LH receptors.

(4) Other New Hormones

Hormones other than inhibin and activin are produced by the gonads.

These hormones include oocyte maturation inhibitor (OMI), granular layer cell luteinization inhibiting factor, and LH combination suppressing factor in luteal cells, and all these have peptide bonds in the chemical structure. Many of these hormones show paracrine actions on the target tissues adjacent to the site of the cell.

(5) Recent Change in the Concept of Hormones

Classically, hormones have been considered to be transported mainly through the bloodstream from the endocrine glands at the site of production to their target tissues and to accelerate reactions in cells. Recent progress in endocrinology, however, has revealed that some hormones such as hypothalamic hormones are secreted from nervous tissue and that some hormones such as gut hormones exist in a large quantity even in the central nervous system. The conventional concept of hormones is thus markedly changing. Hormones are understood as being flexible, i.e. target tissues of some hormones are cells very close to the site of production in the same organs, and hormonal actions include not only the promotion of reaction in cells but also inducing or delaying such reactions. Therefore, many neurotransmitters are also considered to be kinds of hormones released into a specific site known as the synaptic cleft. Much research has been performed not only on endocrine actions but also paracrine and autocrine actions.

(6) Neuroendocrinological Regulation of Secretion of Gonadotropic Hormones

The secretion of GnRH, FSH and LH, which regulate reproductive functions, are controlled and regulated by neurotransmitters such as dopamine, norepinephrine and β -endorphine or neuroregulators such as prostaglandins. Dopamine suppresses release of GnRH from a GnRH neuron. Although no norepinephrine secretory cells exist in the hypothalamus, such nerve fibers run to the anterior optic nerve inside the midline protuberance to promote the secretion of GnRH. Dopamine inhibits and norepinephrine promotes the pulse-like secretion of GnRH and β -endorphine

acts directly on GnRH neurons to inhibit the pulse-like secretion of GnRH.

Neuroregulators include many substances which regulate the stimulatory or inhibitory mechanism via neurotransmitters. Prostaglandin E₂ is among these and is produced inside the brain. It regulates nerve conduction at various sites to promote the release of GnRH which accelerates the secretion of LH and FSH.

(7) Regulation of Reproductive Functions by Growth Factors

Marked progress in recent research on hormones has revealed the existence of growth factors which were found to affect the actions of hormones. The term growth factor is given to substances which exist in various cells in the body and promote the proliferation and differentiation of cells. These growth factors include EGF, FGF, IGF, NGF, PDGF and TGF as shown in Table1-2-2

All these factors increase or decrease LH receptors on ovarian follicles and promote production of progesterone (Table1-2-3). They are also considered to play an important part in the growth of follicles and further progress in the studies of these substances is anticipated.

2-2 Hormonal Actions and Receptors

(1) Mechanisms of Action and Hormone Receptors

Peptide hormones first bind with receptors on the cell surface, and then exhibit their actions on the cells according to their own mechanisms of action. In contrast, steroid hormones bind to intracellular receptors and exhibit actions in a similar manner. Consequently, hormonal actions are not expected to be observed in the absence of receptors regardless of the hormone concentration (Fig.1-2-7).

The number of receptors and hormone binding affinity to them differs according to the endocrine environment. For example, single

administration of hormone at high dose causes a decrease in the number of receptors due to "down regulation" while at low doses it causes an increase in receptor number due to "up regulation". In this way, biological functions are conducted smoothly.

(2) Hormone Receptor Dysfunctions

Recently, it has been understood that there are many endocrine dysfunctions caused by receptor abnormalities. Table 1-2-4 shows the classification of human receptor diseases. Human thyroid dysfunctions among these diseases were found to include Basedow's disease associated with the production of autoantibody against receptors.

Some receptor diseases are related to abnormalities of the receptors themselves, and some are due to abnormalities caused by other reasons. Receptor abnormalities include congenital receptor defects and a decrease in affinity hormone receptors. A typical example of a human receptor defect is testicular feminization syndrome which is a subtype of male sex hormone resistance syndrome. Men with this syndrome have normal sex chromosomes of the male type, XY, and show normal secretion of androgen from interstitial testes, though they have female phenotypes, resulting in feminine characteristics, constitution and character without pubic hair, uterus or menses. These abnormalities showing female phenotypes are considered to be caused by disorders of testosterone associated with receptor defects in dihydrotestosterone, an active form of testosterone.

Little progress has been made in such kind of research in livestock, though livestock is also considered to develop endocrine diseases caused by receptor defects and abnormal affinities of hormones to receptors. Accordingly, future developments in this field are greatly anticipated.

2-3 Hormone Secretion Patterns

(1) Pulsatile Secretion of Hormones

Recent progress in methods of measuring hormones made it possible to measure hormones in extremely small quantities. As a result, hormone secretion kinetics have been clarified in detail. In other words, a detailed examination of hormone secretion kinetics revealed that hormones are secreted not continuously but at constant time intervals in a pulse-like manner. This secretion is called pulsatile secretion, rhythmic secretion or throbbing secretion.

A special device, a push-pull pump, was implanted in the brain of two sheep, on which ovariectomy had previously been performed, and samples were collected frequently at short time intervals to measure GnRH. The concentrations of GnRH were compared with those of LH in jugular venous blood. The results are shown in Fig.1-2-8. In both animals, as seen in the figure, GnRH and LH were secreted in a pulse-like manner, and the pulses of these two hormones synchronized well with each other.

Fig.1-2-9 shows changes in the concentrations of FSH, LH, progesterone and estrogen in the posterior vena cava blood of a cow in the early and middle stages of the luteal phase of the estrous cycle. As can be seen from the figure, all hormones showed pulse-like changes, indicating the pulsatile secretion of these hormones.

Pulse-like changes in estrogen and progesterone reflect those in FSH and LH, hormones from a higher organ. However, the changes in FSH and LH reflect those in GnRH, a hormone from an even higher organ.

(2) GnRH Pulse Generator in the Hypothalamus

As described above, the pulsatile secretion of gonadotropic hormones (GTH) reflects that of GnRH from the hypothalamus. The pulsatile secretion of GnRH is considered to be controlled through nerves by a GnRH pulse generator existing in the hypothalamus. Mori et al. implanted electrodes in the hypothalamus of shibayagi (a breed of goat) to record action potentials of nerves electrophysiologically and to confirm the

correlation between neuronal activity and LH in peripheral blood. The results are shown in Fig.1-2-10 . As can be seen from the figure, the neuronal activity and LH synchronize well.

2-4 Methods of Measuring Hormones

(1) Progress in Methods of Measuring Hormones

In recent years, the relationship between the expression of reproductive functions and hormone secretion kinetics has been clarified in detail, as described previously. This is largely due to radioimmunoassay (RIA), a method of measuring hormones developed by Berson & Yalow. Yalow won the Nobel Prize for this achievement in 1977.

In the past, hormones were measured by bioassay. That is, the quantity of a hormone was estimated from the biological effects caused by its administration to animals. For LH, for example, a decrease in the content of ascorbic acid in the corpus luteum was measured in rats using a method called the ascorbic acid depletion method (OAAD). For FSH, and increase in ovarian weight was measured in rats according to the Steelman-Pholay method. For estrogen, cornification of epithelial cells of the vagina or an increase in uterine weight was measured in rats in which ovariectomy had previously been performed. In addition, pregnancy was diagnosed by bioassays such as in the Friedman test using rabbit ovulation.

These bioassays were performed on animals, and naturally showed the following disadvantages; these assays require a group of many well-controlled laboratory animals, it takes a long time after administration of the sample for reactions to occur, the specificity is low, the accuracy is low due to scattering of result, and a large amount of sample is required because of low sensitivity. The last disadvantage is the greatest of all.

Following this, chemical measurement methods were also developed. The

sensitivity was slightly improved but was not satisfactory and the application of these methods was limited to some hormones such as steroids.

Immunoassays solved all these disadvantages dramatically. Immunoassays, in general, show high specificity and sensitivity because immune responses are used, and RIA is now the best method of all.

The advantages of RIA are, the sensitivity and specificity are high, the measuring time is short because the procedure is simple, and many samples can be treated simultaneously. However, RIA has the following disadvantages because radioactive substances are used; special facilities are required, there are problems in the disposal of waste matter, and special knowledge and training are required for personnel. In RIA, samples are measured through immune reactions, and the results obtained are values measured not from bioreactivity but from immunoreactivity. This should be taken into consideration although it is not usually a problem. The development of this method made it possible to measure hormones in an extremely small quantity, which was an historic event in the progress of endocrinology.

Following RIA, enzyme immunoassay and fluorescence immunoassay were conceived as methods which use no radioisotopes and show high sensitivity, and they have been widely put into practical use.

Kits for measuring hormones by enzyme immunoassay with enzymes as the markers were also developed and are now commercially available.

(2) History of Discovery of Gonadotropic Hormone releasing Hormone

It is now widely known that the secretion of FSH and LH from the pituitary is controlled and regulated by GnRH, a hypothalamic hormone, though the process of discovery of GnRH was certainly not that easy. All Hypothalamic hormones including GnRH exist in extremely small quantities and it was difficult to monitor the activities of hormones in the process of separation and refinement by conventional methods due their low

sensitivity. Finally, this problem was solved by the development of radioimmunoassay as described above. One interesting story in the course of discovery of GnRH was Schally's group (Veterans Administration Hospital, USA) and Guillemin's group (Soak Research Institute, USA) severely competing with each other with the aim of leading the way.

In 1950, the existence of GnRH was first predicted by Harris, an English anatomist. For 20 years after that, however, its identity was not clarified despite all the challenges made, because the quantity of GnRH is extremely low. Schally and Guillemin succeeded in determining the structure of GnRH at almost the same time in 1971 and they received the Nobel Prize in 1977. It is well-known that Schally's group was indebted to three Japanese scientists, Arimura, Matsuo and Baba, for the achievements.

Shally is said to have used 165,000 pigs to obtain 0.25 ng (1/1,000,000 mg) of refined GnRH standard sample. This clearly shows how low the content of GnRH in the hypothalamus is. In contrast, Guillemin is said to have used a larger number of sheep to obtain a sample from the hypothalamus. GnRH was finally found to be a peptide consisting of 10 amino acids, and an analog, fertirelin acetate (Conceral), has been developed. This artificial hormone has a higher activity than the natural hormone, and has been widely used for the treatment of reproductive disorders and to promote ovulation and conception.

2-5 Estrous Cycle and Hormones

(1) Growth of Ovarian Follicle and Hormones

As shown in Fig.1-2-11, the process of oogenesis is as follows. The primary follicle (primary oocyte) grows to form a secondary oocyte; the secondary oocyte forms a follicular space and this oocyte becomes a vesicular follicle (tertiary oocyte), and the vesicular follicle matures to form a Graafian follicle. The effects of gonadotropic hormones are unnecessary in the early stage before the formation of the vesicular

follicle in this process, while they are necessary in the latter stage. The FSH-estrogen system, LH alone, and the LH-androgen system play leading roles in the growth of ovarian follicles, in ovulation and in follicular atresia, respectively. In addition, it has recently been found that the process may follow paracrine or autocrine control and regulation by biogenic substances produced in the ovary or follicle such as activin, prostaglandins and growth factors (EGF, FGF, and IGF₁). EGF promotes the proliferation of granular layer cells, while activin promotes the differentiation of function via the emergence of receptors for FSH and LH.

These functions seem to be particularly significant in the early processes.

(2) Ovulation and Hormones

Ovulation is triggered by an "ovulation surge", a large quantity of gonadotropic hormones is released from the pituitary (Fig.1-2-12). In the ovarian follicle immediately after this ovulation surge, there is an increase in secretion of progesterone and related progestins followed by estradiol and prostaglandin F₂ α (PGF_{2 α}). The increase in progesterone causes a rapid rise in the activity of collagenase, and PGF_{2 α} promotes the production of plasminogen activator resulting in an increase in plasmin activity. Biogenic substances such as histamine increase rapidly in the follicle, which leads to a rise in permeability in vessels, the luteinization of granular layer cells and the fusion of collagen in the basement membrane and interstitium at the top of the follicle. In addition, PGF_{2 α} and oxytocin are considered to cause contraction of smooth muscle-like fibers in the follicle wall, resulting in eventual follicular rupture.

(3) Secretion of Oxytocin from the Corpus Luteum

Oxytocin had been considered to be a neurohormone secreted from the posterior pituitary. Recently, however, it has been discovered that oxytocin may be secreted from corpora lutea and that it may play an

important role in the expression of reproductive functions.

Changes in oxytocin in peripheral blood, shown in Fig.1-2-13, show a tendency similar to that for progesterone. In other words, oxytocin increases with the formation of the corpora lutea, shows the highest value in the functional stage of corpora lutea, decreases with luteolysis, and finally shows the lowest value immediately before or after estrous.

In experiments in cows and goats, estrous cycles were shortened by administration of oxytocin in the early stage of the cycle. This shortening was considered to be related to luteolysis caused by stimulation of production of $\text{PGF}_{2\alpha}$ in the endometrium, because administration of oxytocin had already been shown to induce secretion of $\text{PGF}_{2\alpha}$ from the endometrium of sheep and goats.

Secretion of oxytocin from the ovaries is known to be rapidly increased by administration of $\text{PGF}_{2\alpha}$, and a positive feedback mechanism is considered to operate in luteal regression, as follows; a pulsating increase occurs in the oxytocin secretion from the ovaries, the increased oxytocin influences the endometrium to increase the secretion of $\text{PGF}_{2\alpha}$, and this increased $\text{PGF}_{2\alpha}$ further increases the secretion of oxytocin from corpora lutea.

Fig.1-2-14 shows the production of $\text{PGF}_{2\alpha}$ from endometrial cells in luteal regression. In this stage, oxytocin receptors are produced in endometrial cells by the action of estrogen from ovarian follicles, and oxytocin reacts with receptors, resulting in the production of $\text{PGF}_{2\alpha}$.

(4) Large Cells and Small Cells in Corpora Lutea

It has been confirmed by recent research that there are two kinds of cells, large cells and small cells in corpora lutea (Fig.1-2-15). The existence of these cells is as follows; the actual number of small cells is about 6 times that of large cells, the diameter of large cells is almost twice that of small cells, and the volume of large cells is around

6 times that of small cells. Table 1-25 shows the derivation and properties of these cells. As seen in this table, large cells are derived from granular layer cells, while small cells come from theca cells. Large cells are superior to small cells in progesterone production without stimulation by LH, and plays a leading part in continuous secretion of progesterone. Progesterone production of small cells in progesterone production without stimulation by LH, and plays a leading part in continuous secretion of progesterone. Progesterone production of small cells is 22 % or less compared with large cells.

Small cells are superior to large cells in reactivity to LH and secrete a large quantity of progesterone in response to it. Small cells have many receptors for LH while large cells have few receptors, which is the reason for this difference in reactivity to LH between these cells. Large cells produce oxytocin in response to the actions of prostaglandins, and this oxytocin lowers the reactivity of LH on small cells. In addition, $\text{PGF}_2\alpha$ with a luteolytic action combines mainly with large cells. During luteinization, small cells change into large cells, resulting in an increased ratio of large cells to small cells.

Relaxin is secreted only by large cells.

(5) Luteolysis Due to Prostaglandins

It is well known that prostaglandin (PG) $\text{F}_2\alpha$ has a luteolytic action.

It has been shown in sheep, cows, pigs and shibayagi that the concentration of PG in blood increases markedly with a decrease in progesterone during luteal regression during the latter part of the estrus cycle.

Quantitative measurements of $\text{PGF}_2\alpha$ in uterine venous blood are the most favorable for an examination into the kinetics of PG. At the present time, however, such kinetics are estimated from measurements of 15-keto-13, 14-dihydro-prostaglandin $\text{F}_2\alpha$, a metabolite of $\text{PGF}_2\alpha$, or 11-ketotetranor PGF metabolite in jugular venous blood or urine since

catheterization into the uterine vein is complicated.

In sheep, the following changes in $\text{PGF}_{2\alpha}$ in blood have been demonstrated; the low concentration in blood for the first 12 days of the estrous cycle and an increase in concentration from the 13th to 17th day of the cycle, and intermittent secretion of $\text{PGF}_{2\alpha}$. In cows, the baseline level shows low values until the last day of the estrous cycle, but pulses of intermittent secretion of short duration appear as estrus approaches. Luteolysis begins as the amplitude of the pulse markedly increases, and the concentration of progesterone drastically decreases. This marked increase in amplitude continues for about 2 days and estrus appears 1 - 4 days after the amplitude starts to increase.

It has been demonstrated by experimental hysterectomy in cows, sheep, pigs and goats that PG is produced in the uterus and that the endometrium is the site of production. PG secreted from the uterus is transferred by a counter-current mechanism from the uterine vein to the ovarian artery, and then to the corpus luteum where it exhibits its effects, as shown in Fig.1-2-16 .

The mechanisms of action $\text{PGF}_{2\alpha}$ have not yet been clarified in detail.

It is now considered that $\text{PGF}_{2\alpha}$ combines with $\text{PGF}_{2\alpha}$ receptors which exist on large cells, as described previously, and the bound $\text{PGF}_{2\alpha}$ exhibits cytotoxicity, leading to luteolysis.

2-6 Pregnancy and Hormones

(1) Hormones Supporting Pregnancy

When livestock become pregnant, corpora lutea of estrus do not regress but change into corpora lutea of pregnancy, which continue to secrete progesterone. In order to inhibit the regression of corpora lutea of estrus and to make them secrete progesterone in cows, horses, sheep and pigs, embryos must exist in the uterus on the 16 - 17th day, the 14 - 16th day, the 12 - 13th day and the 12th day after mating, respectively.

These times are earlier compared with the times for the beginning of adhesion of embryos to the endometrium, that is, the 18th - 22nd day, the 36th - 38th day, the 16th day and the 18th day after mating, respectively. Accordingly, the pregnant animal perceives pregnancy not through implantation but by a signal from embryos, and lengthens the life of the corpus luteum for the purpose of supporting pregnancy.

Martal et al. used the name "trophoblastin" for a substance which exists in blastocysts and inhibits luteolysis in pregnant sheep. This substance, which exists in sheep blastocysts on days 14 - 15 of pregnancy is said to show species specificity, to be a water-soluble and thermally-labile acidic protein, and to have a molecular weight of about 70,000. This substance is considered to inhibit luteolysis in the following manner, it inhibits the production in the endometrium of PG which takes part in luteolysis or it inhibits the transfer to the ovary of PG produced in the endometrium, or it inhibits the expression of action of PG in corpora lutea. The mechanism of action, however, has not yet been elucidated.

Estrogen, unlike progesterone, does not play a leading role in pregnancy-supporting mechanisms, but is considered to cooperate with progesterone to maintain pregnancy. In the case of pigs, conceptuses secrete estrone sulfate in the early stage of pregnancy, leading to a marked increase in estrogen in the blood of sows. In cows, the concentration of estrogen in blood on the 6th - 18th days of pregnancy is higher than that during the non-pregnancy period. The mechanisms of action of estrogen in maintaining pregnancy is still unknown. From experimental results in rats, however, it has been reported that estrogen makes a marked contribution to an increase in plasticity of the pregnant uterus.

(2) Application of Hormone Measurement to Pregnancy Diagnosis

Robertson and Sarda found that hormone measurements in peripheral blood can be applied to determination of pregnancy and this method has

been widely put into practical use since diagnosis is possible using milk instead of blood. Originally, radioimmunoassay (RIA) was employed as a hormone measurement method but later Nakao et al. developed a method using enzyme immunoassay (EIA), which led to a wide range of applications in diagnosis.

In cows who conceived, the corpus luteum regresses immediately before or after the next estrous cycle, causing a decrease in concentration of progesterone in the blood, while in cows who conceived, the corpus luteum in this stage change into a corpus luteum of pregnancy which continue to secrete progesterone. Such a difference in progesterone concentration is utilized in this diagnosis of pregnancy. Fig.1-2-17 shows the results of diagnosis in milk performed in the author's laboratory. Correct diagnosis using this method accounted for 84 % and 100 % for cows diagnosed as pregnant and non-pregnant, respectively.

Measuring methods have been improved, and the absence of pregnancy can be judged in an extremely short time by colorimetry on a plate using a commercially available kit.

2-7 Parturition and Hormones

(1) Mechanisms of Parturition

An outline of the mechanisms related to the commencement of parturition was shown by experiments in sheep performed by Liggins et al. The fetal pituitary-adrenal gland system plays an important role in the commencement of parturition. Initially, the concentration of adrenocorticotrophic hormone (ACTH) in fetal blood increases rapidly which causes a rapid increase in adrenocortical hormones. These increased cortical hormones stimulate enzymes in placenta, which convert the progesterone produced in placenta into estrogen. In addition to this change, the concentration of progesterone in mother's blood starts to increase, and at the same time the concentration of estrogen starts to increase. This increased estrogen affects the uterus to promote the

synthesis of PG and production of PG starts to increase rapidly 1 - 2 days before parturition.

During pregnancy, uterine muscle contraction is inhibited by the action of progesterone, and this inhibition is negated by a decrease in the concentration of progesterone. Increased estrogen enhances the sensitivity of the uterus to oxytocin, inducing uterine contraction. In addition, the birth canal, i.e., the cervical canal and vagina, is softened and extended by the increased estrogen. In the latter half of parturition, mechanical stimulation by the fetus on the uterine mass further promotes the secretion of oxytocin from the posterior pituitary. PG and oxytocin thus secreted then enhance uterine contraction causing expulsion of the fetus. These mechanisms were studied mainly in sheep.

Parturition in cows is considered to proceed through almost the same mechanisms as those in sheep. However, in cows the concentration of progesterone in blood decreases gradually for 60 days in the latter stage of pregnancy, while estrogen increases gradually and reaches a peak 1 - 4 days before parturition. The concentration of PGF₂ starts to increase rapidly 24 - 48 hours before parturition and the level of prolactin also increases rapidly on the day before delivery, which finally leads to parturition. Cows, unlike sheep, show no conversion of progesterone to estrogen in the placenta, and these two hormones are produced through independent mechanisms.

2-8 Application of Hormones to Improvement in Breeding Efficiency

(1) Rise in Conception Rate

Breeding management and climate have a significant effect on expression of gonadal functions. That is, these factors greatly control the secretion of gonadotropic hormones and the response of gonads to hormones and conception rate is reported to increase by an improvement in the feed supply and method of management. According to Drew, conception

Failure to conceive may be related to anovulation, a delay of ovulation and insufficient luteinization caused by insufficient secretion of gonadotropic hormones, LH in particular, during estrus. In such cases, conception rate is considered to be improved by using LH supplements at the appropriate time.

Wangner et al. administered hCG to cows during estrus on the basis of the above rationale, and examined its effects on conception rate. According to their report, conception was improved by administration of hCG. In recent years, GnRH and its analogs (fertiirelin acetate) have been developed and put into practical use making it possible to improve conception rates. Administration of GnRH or its analogs instead of hCG leads to an improvement in gonadotropic hormone deficiency by endogenous LH or FSH, which is the most favorable method to use in this situation. In addition, GnRH and analogs have a low molecular weight and there is little chance of antibody formation, indicating the possibility of repeated use. Consequently, we studied an intramuscular injection of 100 μ g of the GnRH analog in cows at fertilization, and obtained a high mean conception rate compared with the control group. Nakao et al. later performed an extensive experiment using many cows, and reported that administration of the analog at the same dose is effective for a rise in conception rate and that the effect is particularly marked in areas showing low conception rate.

(2) Increase in Ovulation Numbers and Offspring

(2-1) Application of Immunity to Androstenedione

Attempts have been made to increase the number of offspring by active immunity or passive immunity using steroid hormones. In one example, sheep were immunized with androstenedione-11-BSA, resulting in a mean number of ovulations of 2.13 compared with 1.11 for the control group.

Mating of these sheep caused polyembryony, and the number of offspring increased as a result. The same result was observed in passive immunity in field tests. In other words, 114 and 135 lambs

rate at the first fertilization was improved from 50 % to 69 % by increasing feed immediately before or after fertilization in cows when synchronized with the estrous cycle. Similarly in sheep, ovarian functions are enhanced by improvement in nutrition and this effect is known as flushing. In flushing, the ovulation rate is increased by adding protein-rich feed before mating. In sheep, addition of lupins as a form of high-protein concentrated feed is said to lead to a higher FSH value on the 5th day before estrous compared with the control.

Recently, Lotthammer et al. reported that a deficiency of β -carotin in feed leads to a susceptibility to suffer from reproductive function disorders and β -carotin is a precursor of vitamin A. However, it is said that β -carotin itself exhibits physiologically important actions and that deficiency leads to the following unfavorable states; a deficiency causes insufficient secretion of estradiol-17 β from the ovary, this insufficiency causes a secretory abnormality of LH, this abnormality leads to weak estrus or delayed ovulation, and a follicular cyst may easily develop. β -carotin may be insufficient if silage is supplied throughout the year. Consequently, the composition of feed should be taken into consideration in order to prevent a lowering of breeding efficiency.

Various stresses such as humidity, heat and other unfavorable environments have some effects on gonadal functions. In a high-temperatures environment the following problems are known to occur; secretion of FSH and LH is lowered, females show a prolonged estrous cycle, weak estrous, a shortened estrous cycle, and a decrease in ovulation surge during estrous. Therefore, the environment in cow sheds should be taken into consideration in order to improve conception.

It is necessary to clearly understand estrous especially when animals enter this state in order to perform fertilization at the appropriate time. When fertilization does not lead to conception, the cause should be examined at an early stage. If reproductive disorders are then observed, counter measures or treatment should be considered.

were born to 100 mother sheep in the control group and the passive immunity group, respectively, indicating an increase of 21 in the number of offspring. In the past, the mechanisms of these increases in ovulation numbers were considered to be as follows; negative feedback on the pituitary by steroid hormones produced in the ovaries decreases in immunized sheep, this decrease in negative feedback leads to an increase in secretion of FSH, and the ovulation number increases as a result. Experimental results obtained subsequently, however, showed no increase in FSH and the true causes are considered to be changes in the following actions of steroid hormones on the growth of ovarian follicles; paracrine or autocrine actions, actions via growth factors, and interactions with gonadotropic hormones. These actions have recently attracted the attention of researchers.

(2-2) Application of Steroid Hormone Synthesis Inhibitors

The synthesis of progesterone is inhibited by administration of 3β -hydroxysteroid dehydrogenase (3β -HSD: Epostane), an enzyme which is known to inhibit synthesis of progesterone, resulting in increases in ovulation number and offspring through a feedback mechanism in the hypothalamo-pituitary-gonadal axis. In sheep experiments, for example, administration of 3β -HSD resulted in increases in ovulation number and offspring from 1.27 to 1.75 and 1.19 to 1.55, respectively. This result was mainly due to an increase in twins.

Similar experiments have been performed in cows and pigs, and resulted in an increase in ovulation number and a rise in the concentration of LH, respectively. The increase in the number of offspring, however, is a problem which remains to be solved.

(2-3) Application of Immunity to Inhibin

Very recently, trials have been conducted by increasing the ovulation number by immunity to inhibin in sheep, cows and pigs. In pigs, the ovulation rate was reported to show a rise of 35 % and the increase in ovulation number was in proportion to the production of antibody.

Accordingly, it is considered that the concentration of FSH in blood was increased by inhibiting the activity of inhibin in blood using an antibody, resulting in an increase in ovulation number. The increase in the number of offspring, however, is still a problem to be overcome.

(2-4) Application of Low-dose Gonadotropic Hormones

For a long time, attempts have been made to change monoembryony to polyembryony in cows by the administration of gonadotropic hormones with the aim of improving breeding efficiencies. In these attempts, pregnant mare serum gonadotropin (PMSG) was mainly used to induce mild superovulation which may lead to an increase in the number of offspring. In this method, however, the ovulation number is unstable, resulting in not only 2 ovulations but also 3, 4 or 5 ovulations. Recently, FSH preparation has become available as a gonadotropic hormone in addition to PMSG, and is now under investigation. The results described below were obtained in the study conducted in association with Iwate National Livestock Breeding Station and Hidaka National Livestock Breeding Station, Ministry of Agriculture, Forestry and Fisheries, participating as central stations.

An investigation was thus made into the application of gonadotropic hormones to Holstein seed cows which may require no successors.

In these examinations, a refined FSH preparation (Antorin) was administered in divided doses to cows. In practice, the preparation was administered once, twice and 4 times at doses of 6, 9, 10, 12 and 13 mg as FSH. The results were compared, and combined administration with PGF α was also examined with respect to the timing of administration. As a result, it was shown that an increase in dose to 12 - 15 mg leads to increases in triplets and disadvantages such as miscarriage (Table 2-6).

Consequently, the research group recommends the method shown in Fig. 1-2 18. In this method, 4 - 6 mg FSH is administered to Holstein cows in the functional corpus luteal stage, and, on the second day (after 24

hrs), 2 - 4 mg FSH and 500 μ g PGF₂ α -A (Estramete) are administered. Estrus occurs on the 4th or 5th day or by not later than the 12th day when fertilization is attempted.

The practical merits were simulated as shown in Fig.1-2-19 . According to this simulation, an increase of 21 calves is expected in 100 cows due to mild superovulation.

2-9 Adjustment of Parturition

Parturition starts after a fixed gestation period following fertilization, but the time of parturition cannot be specified in advance because there are differences in the gestation period between individuals. Artificial changes in the time of parturition may exert marked effects on the management of livestock for example fetal damage is prevented by prevention of dystocia associated with excessively large fetuses, the time of parturition is adjusted so it is uniform and nocturnal parturition is shifted to daytime delivery, which leads to labor saving by avoiding a vigil over parturition.

The application of adrenocortical hormones and PGF₂ α has been under investigation as a main method to induce delivery by hormones. In addition, the application of sympathomimetic agents, which inhibit the expression of hormone actions, has been investigated as a method to delay parturition.

(1) Induction of Parturition

(1-1) Adrenocortical Hormones

The following synthetic adrenocortical hormones, which show few adverse effects and high efficacy, are now used; dexamethasone, betamethasone, and flumethasone. They show weak effects on cows until 235 days of pregnancy, but are expected to show suitable effects

following single administration after 255 days of pregnancy.

Synthetic adrenocortical hormones include fast-acting and slow-acting preparations. The effects appear after 2 days and within 2 weeks of administration of the fast-acting and slow-acting preparations.

Originally, in the early stages of hormone administration methods, a slow-acting preparation was injected in a single dose but later a double injection method was employed. In the latter method, a slow-acting preparation is first administered, and 7 - 12 days later a fast-acting synthetic adrenocortical hormone or a substance analogous to $\text{PGF}_{2\alpha}$ (cloprostenol) is administered, which exhibits its effects 2 or 3 days later. These methods have widely been put into practical use in New Zealand but have the following disadvantages; death of calves or mother in some cases, onset of diseases in mothers, low rates in the subsequent conception due to frequent retention of placenta, and a low milk yield.

Frequent retention of placenta is a significant disadvantage in administration of synthetic adrenocortical hormones but is effectively prevented by combined use with estradiol- 17β .

(1-2) Prostaglandin $\text{F}_{2\alpha}$

In cows, the production of progesterone is dependent on the corpus luteum until day 200 of pregnancy and after day 260 of pregnancy, and the removal of corpora lutea causes abortion. Therefore, the parturition-inducing effect of a method using $\text{PGF}_{2\alpha}$ is expected to be observed during these periods. When parturition is close, $\text{PGF}_{2\alpha}$ is administered by intravenous injection (20 mg) or by intrauterine injection (5 mg or more), causing induction of parturition 2 or 3 days later. Similar results were reported from experiments using a substance analogous to $\text{PGF}_{2\alpha}$ (cloprostenol).

The dose of $\text{PGF}_{2\alpha}$ required to induce parturition decreases and the time until the start of parturition shortens as the time of treatment

approaches the expected date of birth. Such birth-inducing effects of PGF₂ α are more marked in multiparae than in heifers.

(1-3) Relaxin and RU486

Recently, it has been reported that relaxin was successfully applied in a trial for early induction of delivery, which led to a decrease in the incidence of dystocia, as described below.

An early start to parturition was caused by intramuscular administration of purified swine relaxin on day 276 or 277 of pregnancy. Delivery was induced at the following times; 64 hrs after the first administration when it was administered twice at a dose of 3000U/mg at intervals of 12 hrs, 80 hrs after single administration at a dose of 3000U/mg, and 125 hrs after administration of phosphate buffered saline (PBS) as a control. Fig.1-2-20 shows the changes in concentration of progesterone in blood. As seen in the figure, a decrease in the concentration of progesterone commenced earlier in the relaxin group. Relaxin is thus assumed to induce parturition by promoting luteolysis and it is considered to lower the incidence of dystocia by taking part in the reconstruction of collagen in tissues and promoting the expansion of the cervical canal.

Recently, it has also been reported that a delivery-inducing effect was exhibited by RU486, an anti-progesterone agent, administered alone or in combination with relaxin.

(2) Delay of Parturition

Attempts have been made to move nocturnal parturition in cows to daytime delivery by adjusting the parturition time, which leads to labor saving in the vigil over delivery and suitable treatment for dystocia. In practice a β -sympathomimetic agent (clenbuterol) is used to delay delivery by inhibiting uterine contraction induced by oxytocin and PG. We administered clenbuterol to rats and observed favorable delivery-delaying effects. Greene (1981) applies this method to cows and reported good results.

2-10 Reproductive Disorders

(1) Application of Low dose GnRH for Promotion of Conception in Cows Showing Retention of Placenta

Retention of placenta is a disorder which frequently occurs following parturition in cows. In cows showing retention of placenta, recurrence of the first estrous after removal of the placenta is delayed, and conception rates are low even if fertilization occurred after the recurrence of estrus. A low-dose analog of GnRH (fertirelin acetate, 20 ug) was administered to such cows 10 - 12 days after delivery. As shown in Table 2-7, the duration between delivery and conception was shortened, and the duration until the first estrous cycle after delivery tended to be shortened. Additionally, the conception rate within three fertilizations was higher in the GnRH analog-treated group, as shown in Table 2-8.

From the above results, administration of an GnRH analog at a low dose is considered to be effective for promotion of conception in cows showing retention of placenta.

(2) Existence of Follicular Cyst with Partial Luteinization of the Follicular Wall

Ovarian cysts have been generally classified into follicular cysts (Photo 3) and corpus luteal cyst according to clinical findings. Recent progress in methods of measuring hormones made it possible to measure the concentration of progesterone in blood of cows with follicular cysts. The concentration was not found to be low but to suggest the existence of corpora lutea. In ovarian follicles of such cows, a portion of the cyst wall is luteinized or the whole cyst wall is luteinized very slightly although it is unclear with the naked eye. Ovarian cystoma may therefore be classified into the following three cysts; corpus luteal cyst with clear luteinization, follicular cyst without luteinization; and another follicular cyst with partial luteinization of the follicular wall.

(3) Possibility of Onset of Follicular Cyst by Stress

A follicular cyst in cows has been considered to be caused by secretory abnormalities of the gonadotropic hormones from the pituitary such as an excessive secretion of FSH and insufficient secretion of LH. Experimental results fully supporting this concept have not yet been obtained so the details of this disease are not clearly understood.

In a recent experiment, estrogen and progesterone were continuously administered to artificially induce a follicular cyst, and the following results were obtained. The concentrations of FSH showed no high values at the time of onset of the cyst and the frequency and amplitude of the LH pulse increased indicating an increase in the concentration of LH in blood.

In our laboratory the following items were examined in cows with follicular cysts; hormone receptors and secretory conditions of hormones from the hypothalamus, pituitary and ovaries, and the response to administered hormones. The results showed that follicles with cysts show a decrease in the number of FSH and LH receptors in the granular layer cells and that follicular fluid shows a low concentration of estradiol-17 β .

Furthermore it was found that continuous administration of adrenocorticotrophic hormone (ACTH) causes the onset of a follicular cyst and that the LH surge disappears before the formation of cysts. Fig.1-2-21 shows one example of hormonal changes in test cows used in this experiment of artificial onset. ACTH was administered for 14 days from the 14th day of the estrous cycle to Cow-3 which showed an LH/FSH surge at the time of estrous in the normal estrous cycle (the day of ovulation was day 0). This cow showed onset of a follicular cyst and the disappearance of the LH/FSH surge which was expected to occur at the next estrous before the onset.

Fig.1-2-22 shows the mechanism of onset of the follicular cyst explained on the basis of the above results. As seen in the figure, the

onset is considered to be induced in the following manner. Under stress, large quantities of ACTH and an adrenocortical hormone (cortisol) are secreted from the pituitary and adrenal cortex, while FSH receptors, LH receptors and estrogen decrease in follicles bearing cysts. In addition, progesterone secretion increases from the adrenal gland and inhibits the secretion of GnRH, LH and FSH from hypothalamus and pituitary and lowers their concentrations in blood. An ovulation surge of LH and FSH at the expected time of the next estrus disappears, leading to onset of a follicular cyst.

2-11 Embryo Transfer and Hormones

(1) Hormonal Treatment of Donor Cow in Embryo Transfer

Transfer of a fertilized ovum (embryo transfer) includes the following processes; induction of superovulation and artificial insemination of a donor cow, collection of fertilized ova and embryos, examination, freezing and storage of embryos, and transfer of embryos to recipient cows. Hormones take part in many aspects of this process such as the induction of superovulation in a donor cow and in the endocrine environment in recipient cows. In this report, recent research trends are described with respect to the induction of superovulation in a donor cow (Photo 4).

Pregnant mare serum gonadotropin (PMSG), FSH and human chorionic gonadotropin (hCG) are used to promote the growth of ovarian follicles with the aim of inducing superovulation. PMSG is a placenta-derived hormone and has been used for a long time due to its availability and price. It has such a long half-life that a single administration is enough leading to a saving in labor. It is considered, however, to have the following disadvantages; it shows irregular reactions and it induces growth of follicle after ovulation.

Recently, purified FSH preparation derived from the pituitary has been marketed and widely used. FSH has a short half-life and the

induction of superovulation requires multiple administration of FSH in divided doses. This means that treatment is troublesome. However, FSH has the following advantages, it shows a rather small variation in reactions, an improvement in properties of embryos is possible.

PMSG with a long half-life causes the growth of follicles even after ovulation. Estrogen secreted from the growing follicle is considered to exert a harmful effect on the properties of embryos. A method of administering an antibody against PMSG has been devised with the aim of eliminating such effects after ovulation.

For a method of using FSH, Wilson of Granada Genetics in the USA, recently reported the results of superovulation-induction tests performed over many years. He also reported the results of superovulation-induction tests using FSH preparation prepared from cows by a recently developed techniques in genetic engineering.

Tablel-2-9 shows a method of inducing superovulation by FSH-P preparation (FSH preparation extracted and purified from swine pituitary Antrin) used by Granada Genetics Co. The total amount of FSH was 18.0 - 37.0 mg.

Tablel-2-10 shows the properties of the FSH-P preparation used. As can be seen from the table, there were differences in FSH or LH contents and the ratio (FSH/LH) between the lots, which led to variation of results of superovulation by the respective treatments.

Tablel 2-11 and 12 shows the results of superovulation induced by an FSH-P preparation and a BFSH preparation which were prepared by genetic engineering. The BFSH preparation was superior to results for non-fertilized ova, degenerated embryos and transferable embryos and the BFSH preparation lead to higher grade embryos.

This result for BFSH may be related to the fact that it is 100 % FSH without any trace of LH. It has also been reported by Donaldson et al. that a trace of LH exerts detrimental effects upon the induction of

superovulation, particularly upon the properties of embryos (Table 1-2-13). In this case, however, the difference in origin of FSH (pigs or cows) should be taken into consideration.

Hormonal treatment of donor cows in embryo transfer are not described in this report, though PGF₂ α in donor cows and the hormonal environment in recipient cows have been examined in various ways. Recently, an investigation has been made into the effects of addition of hormones to culture fluid in in vitro fertilization. These hormone treatments are important problems and will be the subjects of future developments.

Conclusion

Recent research into hormones in the field of farm animal reproduction has made marked advances along with broadening of this topic and an increase in the number of reports.

In this report, an introduction was given into the present use of hormones in the field of farm animal reproduction. A detailed description was not made of research concerning molecular biology or neurophysiology and new fields. Progress in these areas is considered not only to be of academic significance but practically applicable with a marked effect in the field of farm animal reproduction.

Table 1-2-1 Hormones of Reproduction

Source	Hormone	Function
Hypothalamus	Releasing hormones	
	LH-RH (GnRH)	Stimulates release of FSH, LH
	TRH	Stimulates release of TSH and prolactin
	Prolactin inhibiting factor (PIF)	Inhibits prolactin release
	Oxytocin (stored in posterior pituitary, also produced in ovary)	Stimulates uterine contractions, parturition, sperm and egg transport, milk ejection, possible luteolytic action
Anterior pituitary	Follicle stimulating hormone (FSH)	Stimulates follicular growth, spermatogenesis, estrogen secretion
	Luteinizing hormone (LH)	Stimulates ovulation, corpus luteum function, stimulates secretion of progesterone, estrogen and androgen
	Prolactin	Promotes lactation, stimulates corpus luteum function and progesterone secretion in some species, promotes maternal behavior
		Promotes tissue and bone growth
Placenta	Human chorionic gonadotrophin (HCG) (primates only)	Demonstrates LH activity, maintains CL of pregnancy in primate
	Pregnant mare serum gonadotrophin (PMSC)	Demonstrates FSH activity, stimulates formation of accessory CL in mare
	Placental lactogen	Regulation of maternal nutrients to fetus
	Protein B	Unknown
Gonads	Estrogen	Promotes female sex behavior, stimulates secondary sex characteristics, growth of reproductive tract, uterine contractions, mammary duct growth; controls gonadotrophin release, stimulates calcium uptake in bones, has anabolic effects
	Progesterone	Acts synergistically with estrogen in promoting estrous behavior and preparing reproductive tract for implantation, stimulates endometrial secretion, maintains pregnancy, stimulates mammary alveolar growth; controls gonadotrophin secretion
	Androgen	Develops and maintains accessory sex glands, stimulates secondary sexual characteristics, sexual behavior, spermatogenesis; has anabolic effects
	Inhibin	Inhibits FSH release
Uterus	Relaxin	Dilates cervix
	PGF _{2α}	Causes uterine contractions and is luteolytic

(Leaves, 1987, Cited from Reproduction in Farm Animals, 5th ed., ed. Hafez)

Table 1-2-2 Types of Growth Factor

Name of Growth Factor	Abbreviation
Epidermal Growth Factor	EGF
Fibroblast Growth Factor	FGF
Insulin-like Growth Factor	IGF
Nerve Growth Factor	NGF
Platelet Drived Growth Factor	PDGF
Transforming Growth Factor	TGF

Table 1-2-3

Physiological Function of Growth Factor

	Cell Growth	cAMP	Aromatase	LH Receptor	Progesterone	source
EGF	↑	↓	↓	↓	↑	Theca Cell
bFGF	↑		→	↓		(Granulosa Cell Lutein Cell)
IGF I	↑		↑	↑	↑	Granulosa Cell
TGF-β	↓		↑	↑	↑	Theca Cell
PDGF		↑	↑	↑	↑	

Table 1-2-4 Types of Hormone Receptor Disease (Human)

1. Receptor Abnormality	2. Receptor Abnormality and/or Abnormality after Receptor
Testicular Feminization	Pseudohypoparathyroidism
Vitamin D Dependent Rickets II	Renal Diabetes Insipidus
Glucocorticoid Response Disorder	Insulin Resistant Diabetes C
Insulin Resistant Diabetes A	Vitamin D Dependent Rickets I
Insulin Resistant Diabetes B*	Adrenal Cortex ACTH Response Disorder
Basedow Disease*	

*by insulin receptor antibody

(Inada)

Table 1-2-5 Source and Characteristics of Large and Small Luteal Cells

Luteal Cells	Source	Progesterone Productivity	Responsiveness to L H	Oxytocin Productivity	Relaxin Productivity
Large	Granulosa Cell	+++	+	+	+
Small	Theca Cell	+	+++	---	—

Table1-2-6 Rete of Estrus, estimated number of ovulation, conception rate and rate of abortion by different method of FSH and PGF α -A injection in cows

比較項目	区分	Number of cows	Estrus (%)	Conception (%)	Abortion (%)	Twin (%) (Triplet)	Estimated number of ovulation (Mean $\pm$ SD)
Total dose of FSH (more than two times injection)	6mg	113	91 (80.5)	50 (54.9)	1 (2.0)	110 (22.4)	1.6 $\pm$ 0.8
	9mg	49	46 (93.9)	25 (54.3)	1 (4.0)	20 (8.3)	1.6 $\pm$ 0.9
	10mg	68	57 (83.8)	37 (64.9)	2 (5.9)	9 (25.7)	1.4 $\pm$ 0.6
	12mg	40	32 (80.0)	20 (62.5)	3 (40.0)	4 (33.3)	2.2 $\pm$ 1.2
	15mg	25	21 (84.0)	11 (52.4)	1 (9.1)	4 (40.0)	2.0 $\pm$ 1.1
Times of FSH injection (FSH 6mg)	4times	20	19 (95.0)	11 (57.9)	0	2 (18.2)	1.8 $\pm$ 0.9
	2times	20	18 (90.0)	9 (50.0)	0	2 (22.2)	1.8 $\pm$ 0.8
	1times	19	18 (94.7)	11 (61.1)	0	1 (9.1)	1.5 $\pm$ 0.7
Time of PGF α -A injection (FSH 6, 9, 15mg)	Day2 of estrous cycle	44	41 (93.2)	23 (56.1)	1 (4.3)	5 (22.7)	2.1 $\pm$ 1.0
	Day3 "	46	39 (84.8)	18 (46.2)	0	3 (16.7)	1.7 $\pm$ 0.8
FSH Preparation (FSH 6, 9, 10mg)	FSH-S*	63	52 (82.5)	27 (51.9)	1 (3.7)	60 (23.1)	1.4 $\pm$ 0.6
	FSH-R	81	74 (91.4)	54 (73.0)	2 (3.7)	11 (21.2)	1.4 $\pm$ 0.6

* FSH-S: Antrin (National Iwate Livetok Farm Isogai et al.)
 FSH-R: Refined preparation of Antrin

Table1-2-7 Effect of a GnRH-A injection on reproductive performance parameters in cows with retained fetal membranes

Injection		Number of cows	Day from calving to first observed estrus	Days from calving to conception	Services per conception
Saline	Mean $\pm$ sem	48	76.1 $\pm$ 3.0	120.0 $\pm$ 6.5	2.1 $\pm$ 0.2
GnRH-A	Mean $\pm$ sem	64	68.2 $\pm$ 3.4	105.0 $\pm$ 3.5*	2.0 $\pm$ 0.1

*P<0.05

(Mori et al.)

Table1-2-8 Effect of a GnRH Injection on Overall Conception rates (per cent) Between First and Third Service in Cows with Retained Fetal Membranes

Injection	Conception rates*
Saline	49/100 (49)
GnRH A	68/113 (60)

*Overall conception rates between first and third service
 (Mori et al.)

Table 1-2-9 Stimulation Programs Utilized for
Superovulation of Bovine Females

Program	Day of Treatment	A. M. / P. M
A/A1	1	6. 5mg FSH
	2	5. 0mg FSH
	3	4. 0mg FSH+35mg PGF a. m or 25mg PGF a. m and p. m
	4	3. 0mg FSH
B/B1	1	5. 0mg FSH
	2	4. 0mg FSH
	3	3. 0mg FSH+35mg PGF a. m or 25mg PGF a. m and p. m
	4	2. 0mg FSH
C/C1	1	4. 0mg FSH
	2	4. 0mg FSH
	3	2. 0mg FSH+35mg PGF a. m or 25mg PGF a. m and p. m
	4	1. 0mg FSH
D/D1	1	3. 0mg FSH
	2	3. 0mg FSH
	3	2. 0mg FSH+35mg PGF a. m or 25mg PGF a. m and p. m
	4	1. 0mg FSH

(Wilson)

Table 1-2-10 Analysis of Different Lots of FSH-P
Utilized for Superovulation in
Commercial Embryo Transfer Programs

Lot No.	Activity	Dates in Use		FSH	L H
		Start	End	mg/ml	mg/ml
535K80	NA	NA	NA	7. 65	0. 070
512E85	55. 2	9. 24. 85	1. 12. 86	9. 95	0. 056
520H85	60. 0	1. 13. 86	5. 7. 86	3. 00	0. 044
524J85	47. 0	5. 8. 86	8. 23. 86	4. 00	0. 030
534M85	48. 3	8. 29. 86	1. 13. 87	8. 20	0. 040
540A86	55. 3	1. 14. 87	4. 6. 87	9. 10	0. 051
544B86	49. 4	4. 7. 87	6. 2. 87	8. 60	0. 058
548B86	56. 7	6. 3. 87	7. 20. 87	8. 20	0. 045
550D86	NA	7. 21. 87	12. 31. 87	7. 50	0. 063

(Wilson)

Table1-2-11 Embryo Production from Donor Cows Stimulated with FSH-P (1983~July 1988)

Number of Stimulations	Total Ova	Unfertilized Ova	Degenerate Embryos	Viable Embryos
10783	111280	29653	18546	63080
$\bar{X}$	10.32	2.75	1.72	5.85
%		26	17	57

(Wilson)

Table1-2-12 Embryo Production from Donor Cows Stimulated with BFSH (5/87~5/88)

Number of Stimulations	Total Ova	Unfertilized Ova	Degenerate Embryos	Viable Embryos
205	2267	162*	185	1921
$\bar{X}$	11.1	0.8	0.9	9.4
%		7.2	8.1	85.0

*Four cows accounted for 60% of the total unfertilized ova. Fertilization rate:93.1% (Wilson)

Table1-2-13 Comparison of FSH-W and FSH-P for the superovulation of cattle

Embryos Parameters	Treatment		P
	FSH-P Mean $\pm$ SD	FSH-W Mean $\pm$ SD	
Number transbarable	2.9 $\pm$ 4.0	6.3 $\pm$ 6.7	0.001
Total recovered	11.1 $\pm$ 10.0	12.1 $\pm$ 9.6	0.591
Percent transfarable (%)	30 $\pm$ 33	47 $\pm$ 35	0.007
Number fertilized	5.8 $\pm$ 6.7	9.0 $\pm$ 8.2	0.019
Number degenerate	2.4 $\pm$ 3.6	2.5 $\pm$ 3.0	0.819

FSH-P:Antrin
FSH-W:Refined FSH-P (Donaldson et al.)



Photo 1 Cross-sectional photo of the head of a monkey.
The pituitary is at the tip of the needle and the hypothalamus
is on the upper right.

(Original photo: Mori)

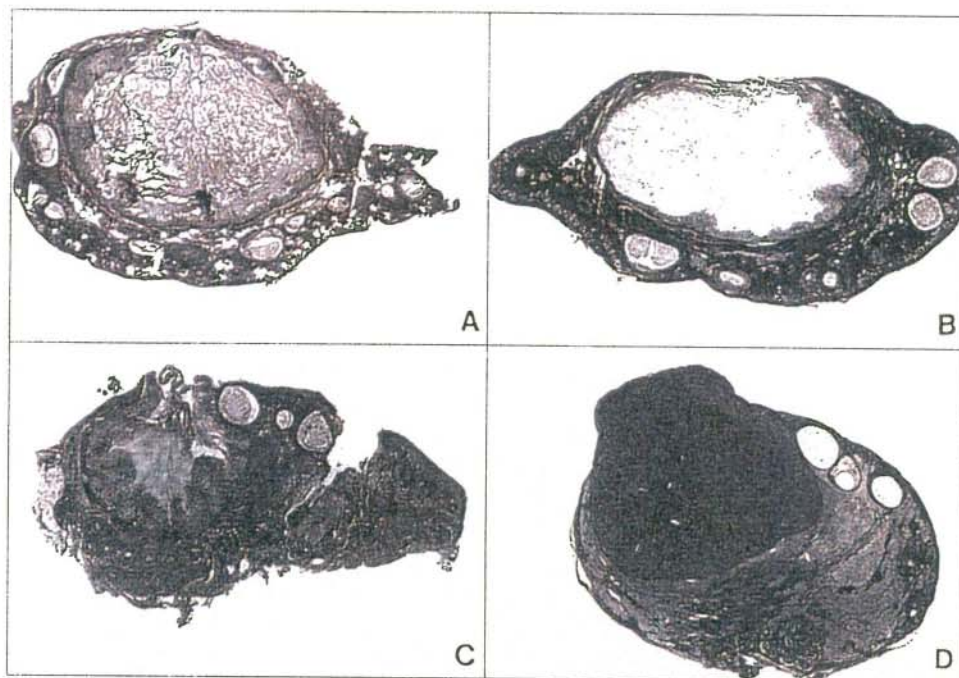


Photo 2 Changes in the ovary according to estrus cycle (monkey).

A: Graafian follicle with stigma on the top

B: After ovulation

C: Early stage of corpus luteum

D: Functional stage of corpus luteum

.(Original photo: Mori)

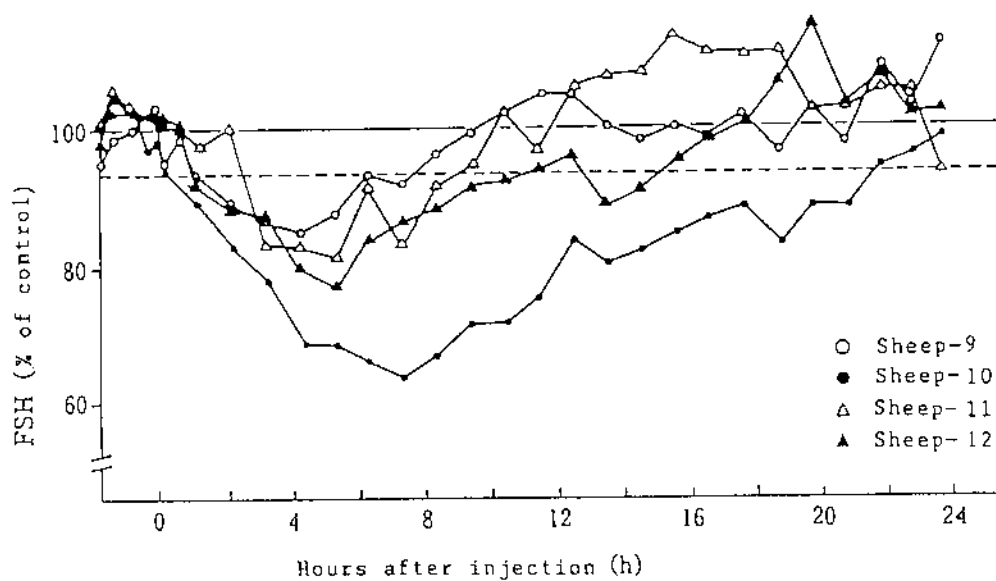


Fig.1-2-1 Changes in concentration of FSH in peripheral blood after injection of purified bovine inhibin into the carotid artery (sheep)

(Findlay and Clarke)

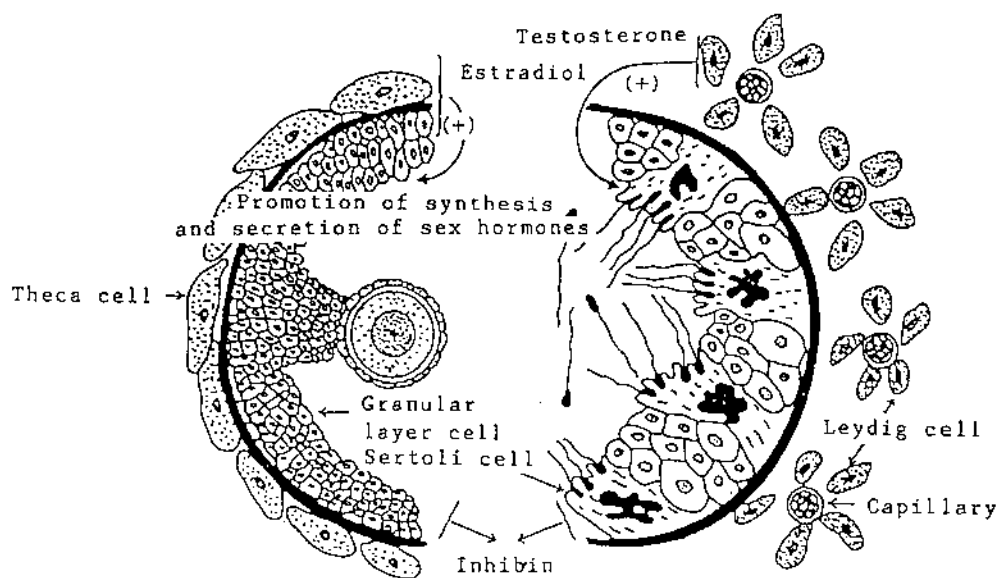


Fig.1-2-2 Secretion of inhibin from granular layer cells and sertoli cells

(Pineda)

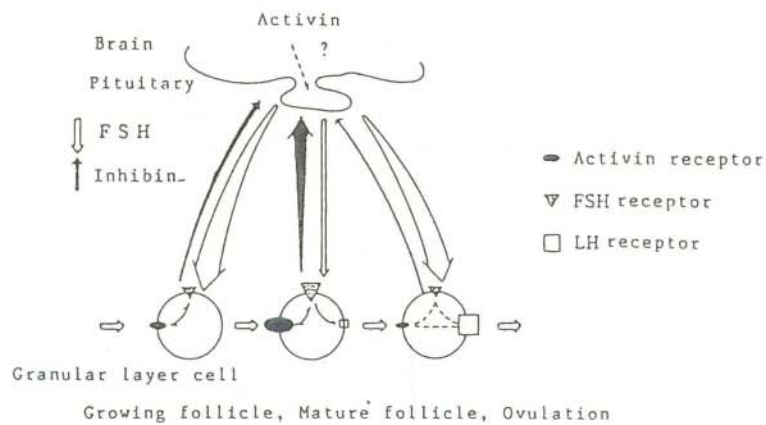


Fig.1-2-3 Diagram showing correlation between changes in inhibin and FSH in estrus cycle and those in activin, FSH and LH receptors in granular layer cells in growth of follicle

(Findlay and Clarke)

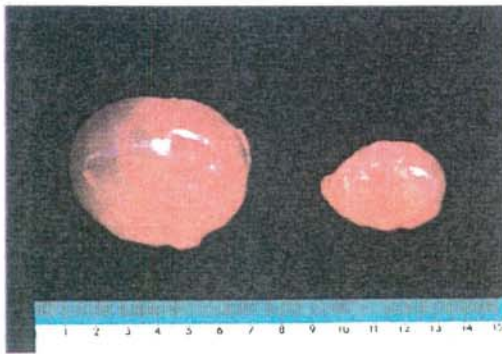


Photo 3 Ovary with follicular cyst (Cow)
(Original photo: Mori)



Photo 4 Ovary of donor cow in which superovulation was induced
(Original photo: Mori)

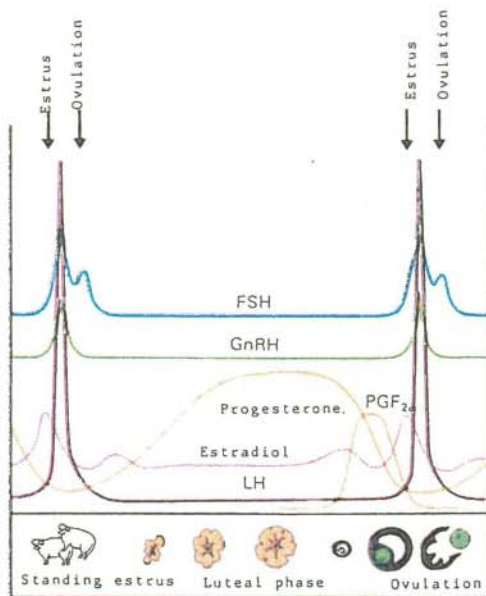


Fig.1-2-4 Diagram showing changes in concentration in blood of GnRH, FSH, LH, estradiol, progesterone and PGF_{2α} in the estrus cycle
GnRH: Gonadotrophic hormone-releasing hormone
FSH: Follicle-stimulating hormone
LH: Luteinizing hormone
PGF_{2α}: Prostaglandin F_{2α}

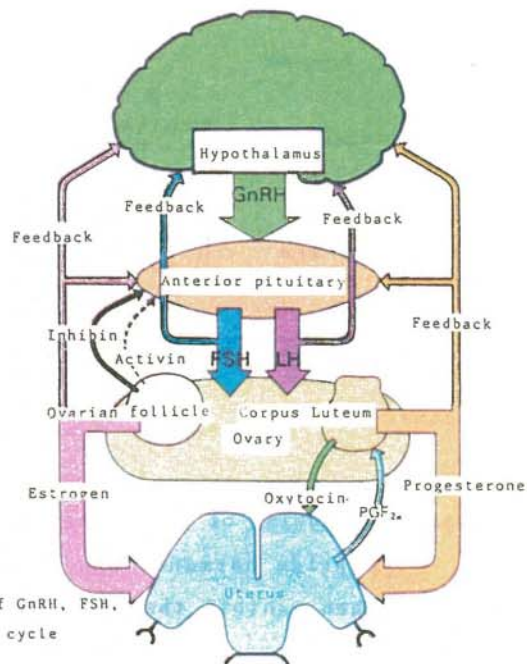


Fig.1-2-5 Diagram showing correlation between secretory organs and target organs of main hormones in reproduction system
(Original figure: Mori)

(Original figure: Mori)

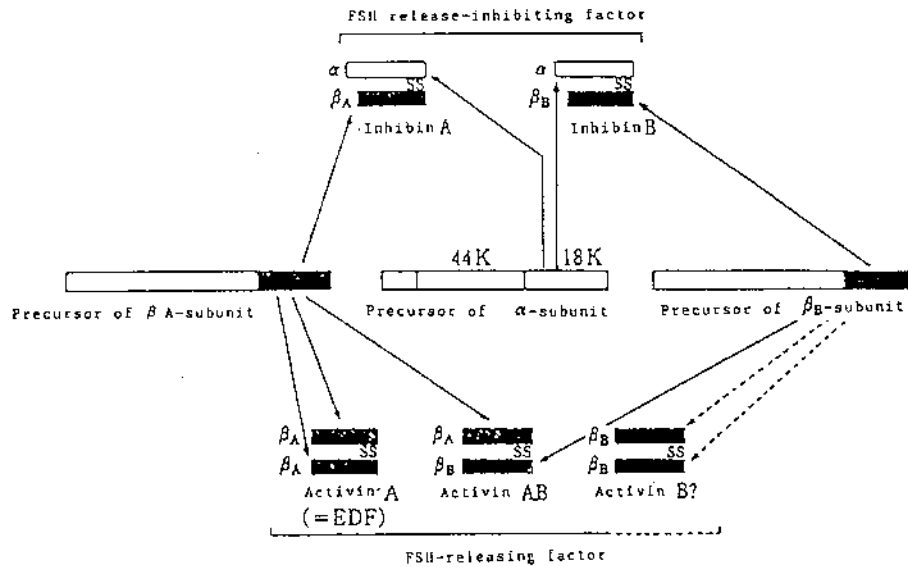


Fig.1-2-6 Diagram showing the formation of subunits comprising inhibin and activin and combination of their dimers

(Ying)

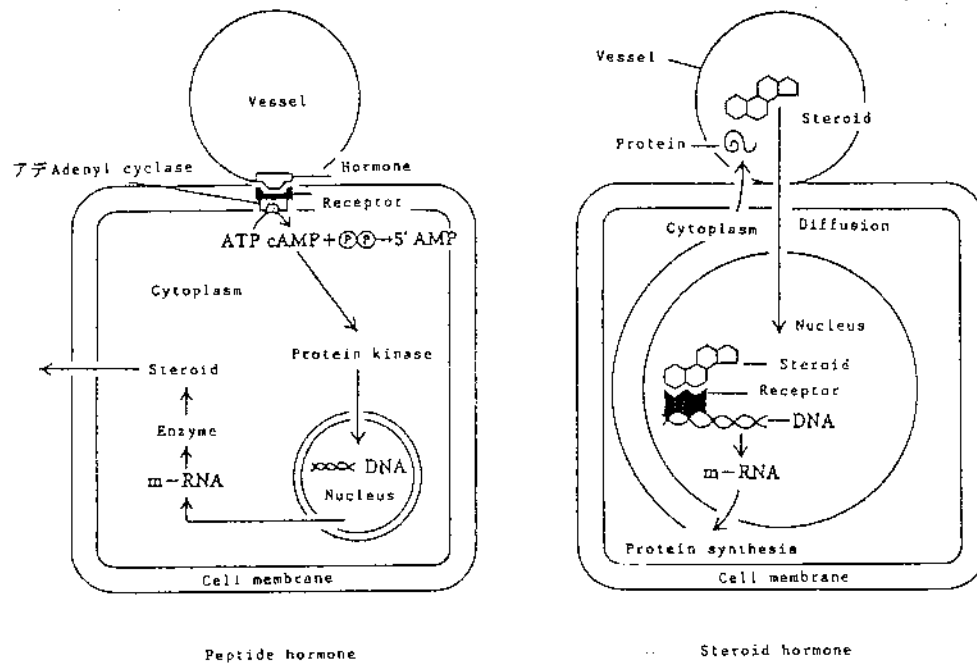


Fig.1-2-7 Mechanism of action of hormone in cells

Peptide hormones bind with receptors on the cell surface, and then exhibit their actions according to their own mechanism of action.

Steroid hormones bind with intracellular receptors and exhibit their actions in a similar manner.

(Reeves)

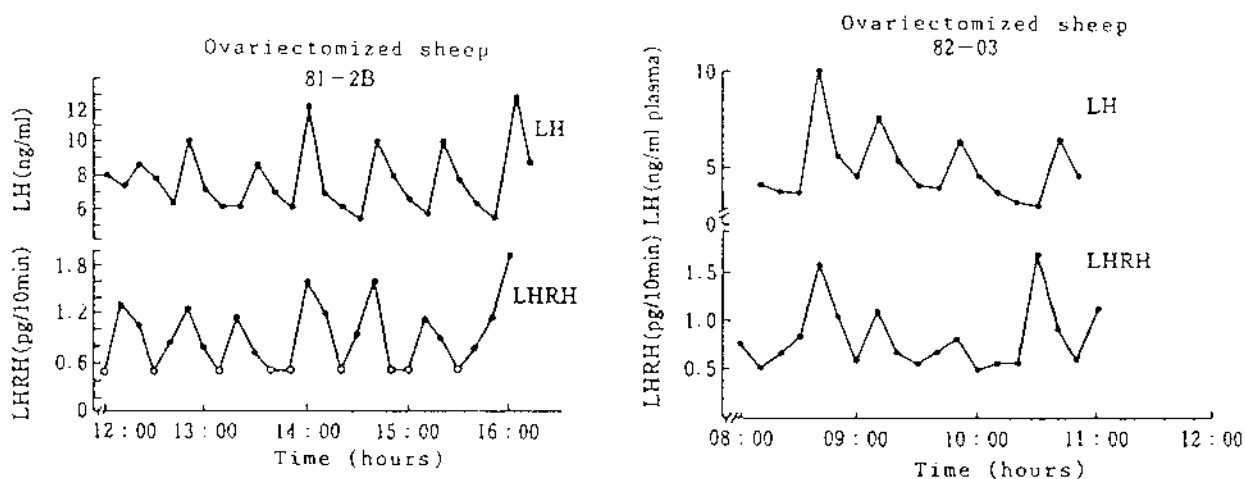


Fig.1-2-8 Pulsation changes LHRH (GnRH) and LH in two individual sheep in which ovariectomy had been previously performed
Pulsating changes in LHRH were observed in samples collected with a push-pull pump, and for LH in plasma samples.
(Levine et al)

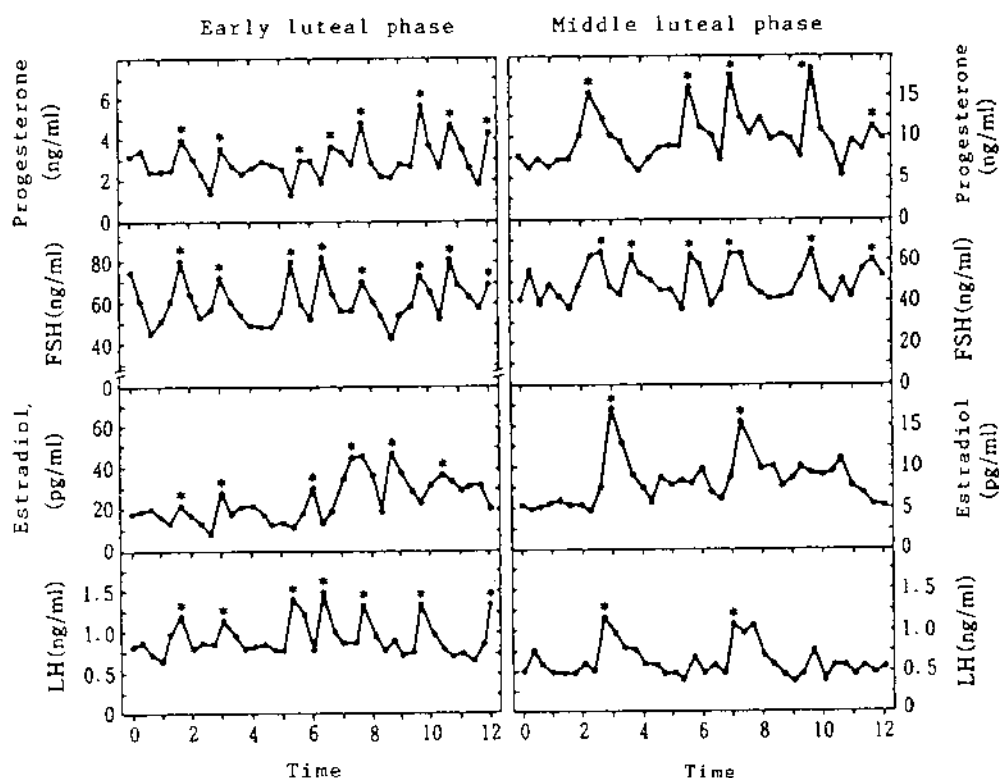


Fig.1-2-9 Changes in various hormones in posterior vena cava blood of a cow in early and middle stages of the luteal phase in the estrus cycle
Samples were collected for 12 hrs at intervals of 20 min from a typical cow.
Pulses are expressed by *

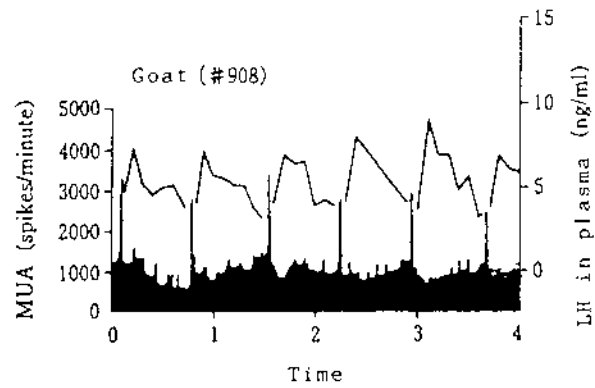


Fig.1-2-10 Relation between LH in blood and MUA in hypothalamus of shibayagi goat (typical example)
A rise in MUA is observed before the pulsation secretion of LH.
MUA: Multi-neuron transmitting action in hypothalamus

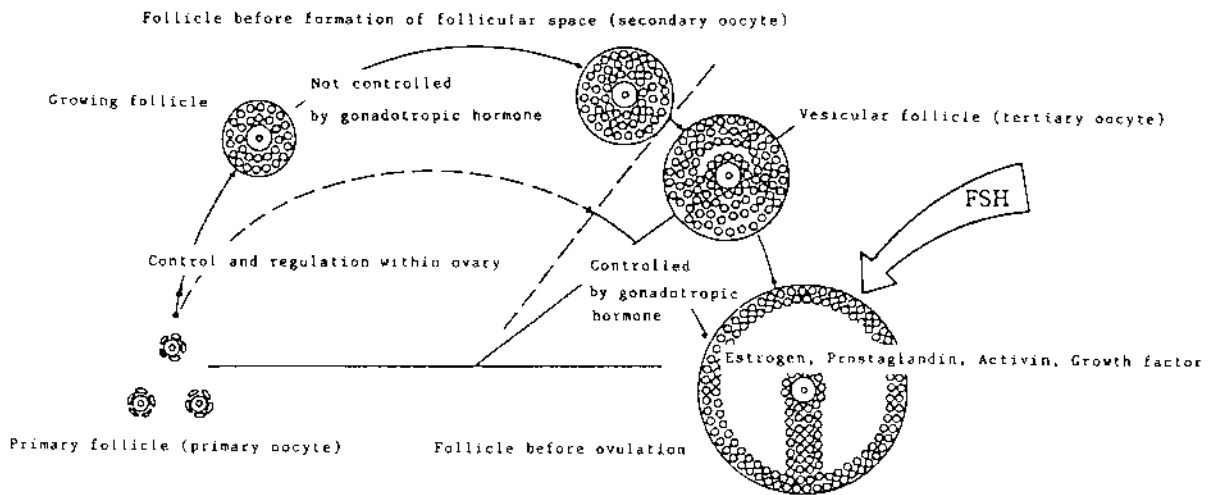


Fig.1-2-11 Participation in control by hormones in each stage of growth of the follicle
(Original figure by Thibault and Levasseur was partly modified by Mori)

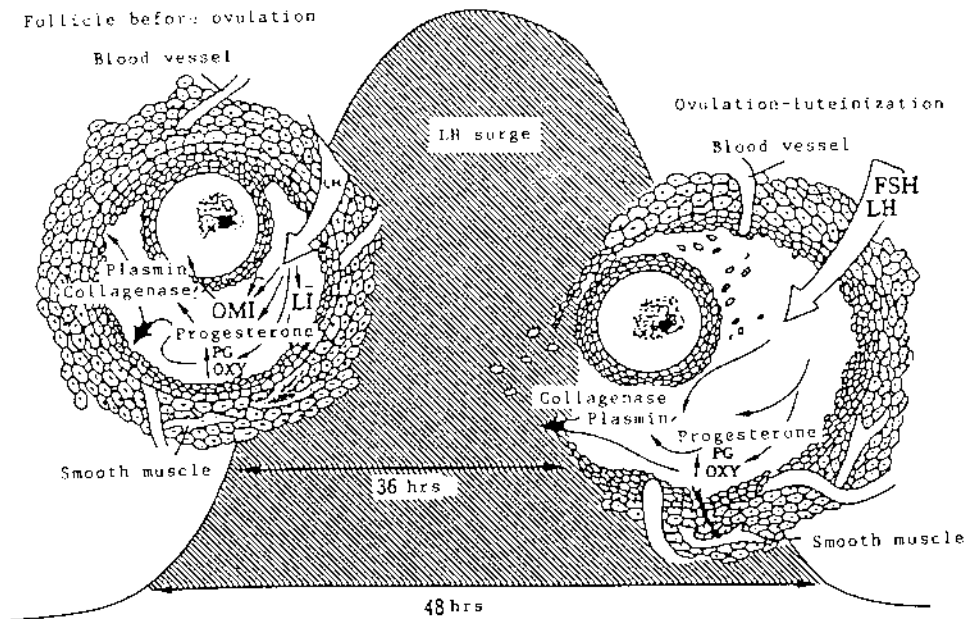


Fig.1-2-12 Relation between biochemical and hormonal changes and LH/FSH surge in follicle before and immediately before or after ovulation (human)

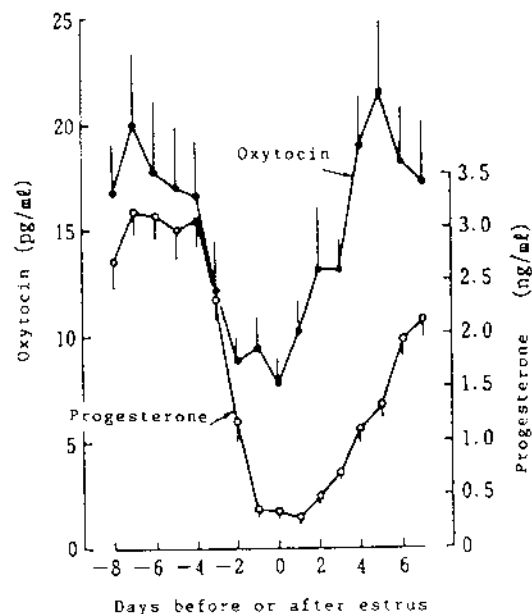


Fig.1-2-13 Changes in concentration of oxytocin and progesterone in peripheral blood of sheep in the estrus cycle
Blood was collected once daily between 14:00 and 16:00.
Each value expresses the mean $\pm$ standard error obtained from results of measurements in two cycles in 17 sheep.

(Flint and Sheldrick)

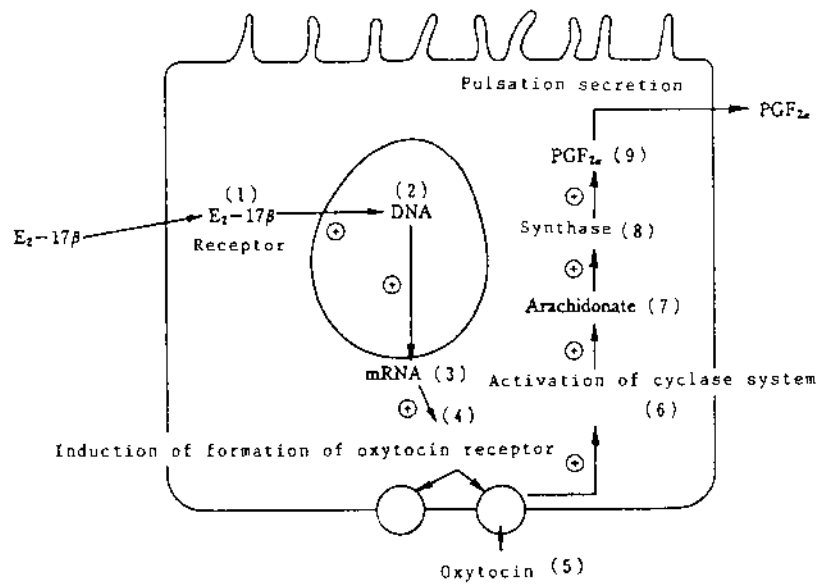


Fig.1-2-14 Participation of oxytocin receptors in secretion of $PGF_{2\alpha}$ from uterus in latter stage of corpus luteum

(McCracken)

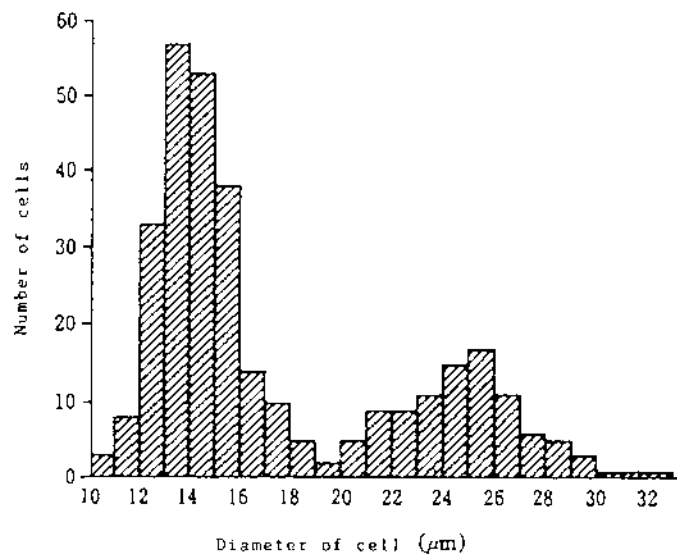


Fig.1-2-15 Distribution of lutein cells of different sizes in corpora lutea of cows

Corpora lutea of cows contain large cells and small cells, the diameter of which is large and small. (Webley)

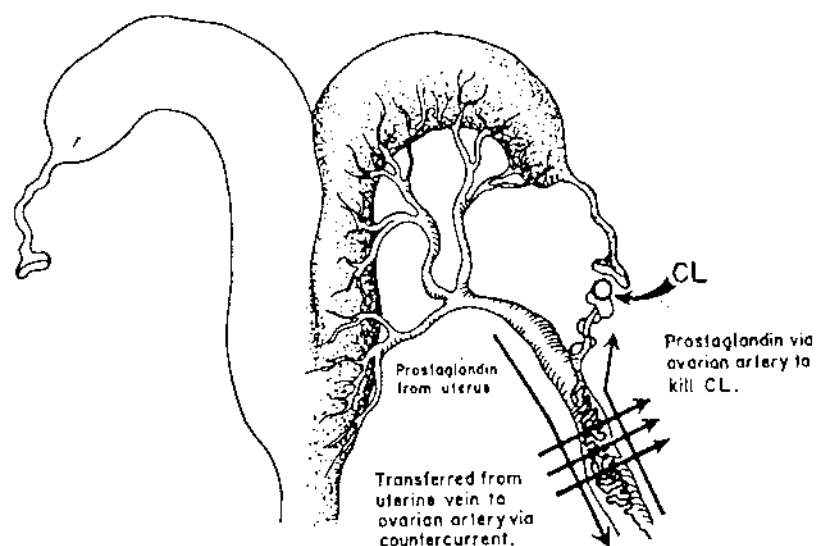


Fig.1-2-16 Prostaglandins transfer from uterus to uterine vein, then countercurrent transfer to ovarian artery, and thence to the CL in the ewe and possibly other species.
(Pineda, cited from Veterinary Endocrinology, 4th ed.; ed Mc.Donald)

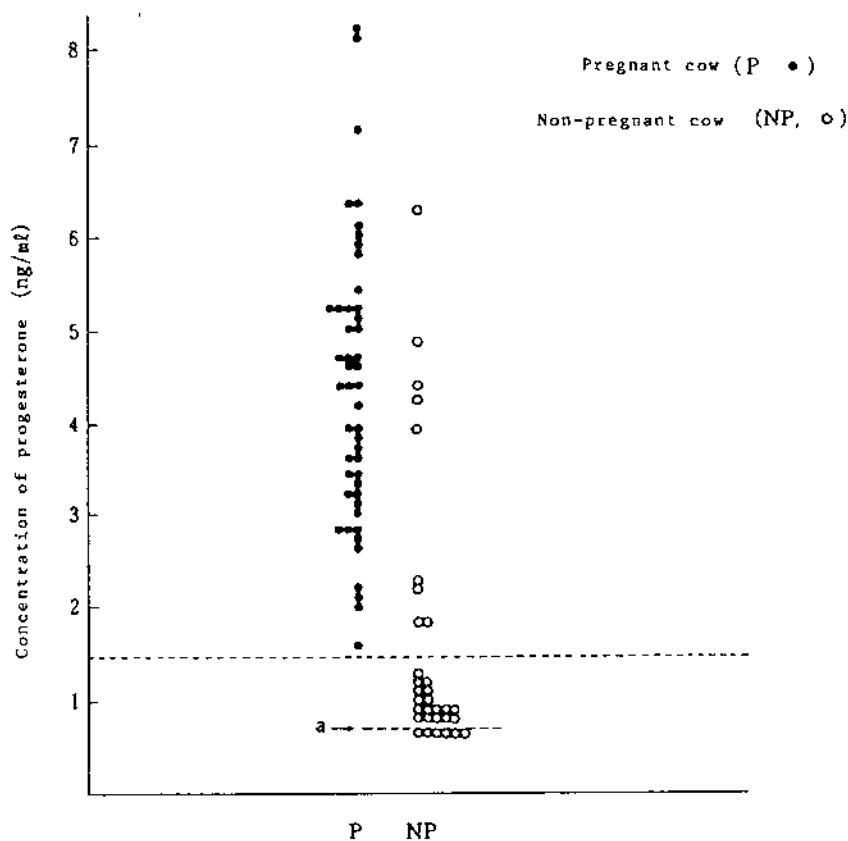


Fig.1-2-17 Diagnosis of pregnancy in cows by measuring the concentration of progesterone in defatted milk using the micro-plate method of EIA

Milk samples were collected 21-24 days after fertilization.

Pregnancy was confirmed by rectal palpation 45-60 days after fertilization.

Concentration of progesterone judged as pregnancy:

1.5 ng/ml or more

Concentration of progesterone not judged as pregnancy:

Less than 1.5 ng/ml

a: Limit of measurement

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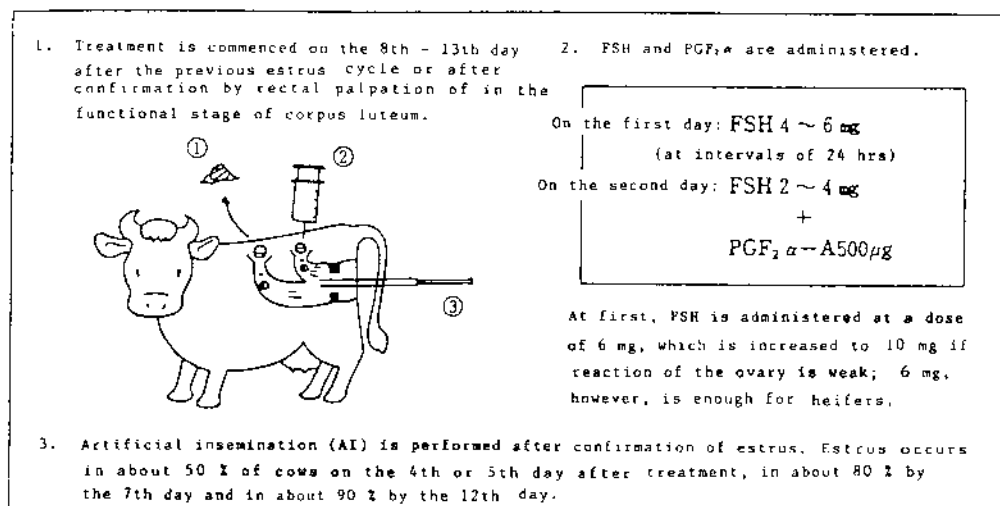


Fig.1-2-18 Method of treatment by administration of low-dose gonadotropic hormones and artificial insemination for the purpose of increasing the number of offspring

(Iwate National Livestock Breeding Station,
Ministry of Agriculture, Forestry and Fisheries)

	AI	IMP	
• Subject	100	100	
• Mating (estrus)	75 (75%)	90 (90%)	
• Conception	45 (60%)	59 (65%)	⇒ Conception number
• Miscarriage	Δ1 (2%)	Δ2 (4%)	+ 14 offspring
• Parturition	44 { Single 42 (95%) Twins 2 (5%)	57 { Single 46 (80%) Twins 10 (18%) Triplets 1 (2%)	Number of offspring
• Offspring	46 { Single 42 Twins 4 (2 × 2)	69 { Single 46 Twins 20 (10 × 2) Triplets 3 (1 × 3)	+ 23 offspring (per one delivery +17%)
• Decrease (e.g. stillbirth)	Δ3 { Single 2 (5%) Twins 1 (19%)	Δ5 { Single 2 (5%) Twins 3 (11%) Triplets	Number of live births
• Live birth	43	64	⇒ + 21 offspring (per one delivery +15%)

Fig.1-2-19 Comparison between AI-group and IMP-group in Trial to Increase the number of offspring by administration of low-dose gonadotropic hormones

AI-group: Control group in which AI was performed at the time of ordinary estrus

IMP-group: Induced multiple-pregnancy group in which low-dose gonadotropic hormone was administered with the aim of increasing the ovulation number

(Iwate National Livestock Breeding Station, Ministry of Agriculture, Forestry and Fisheries)

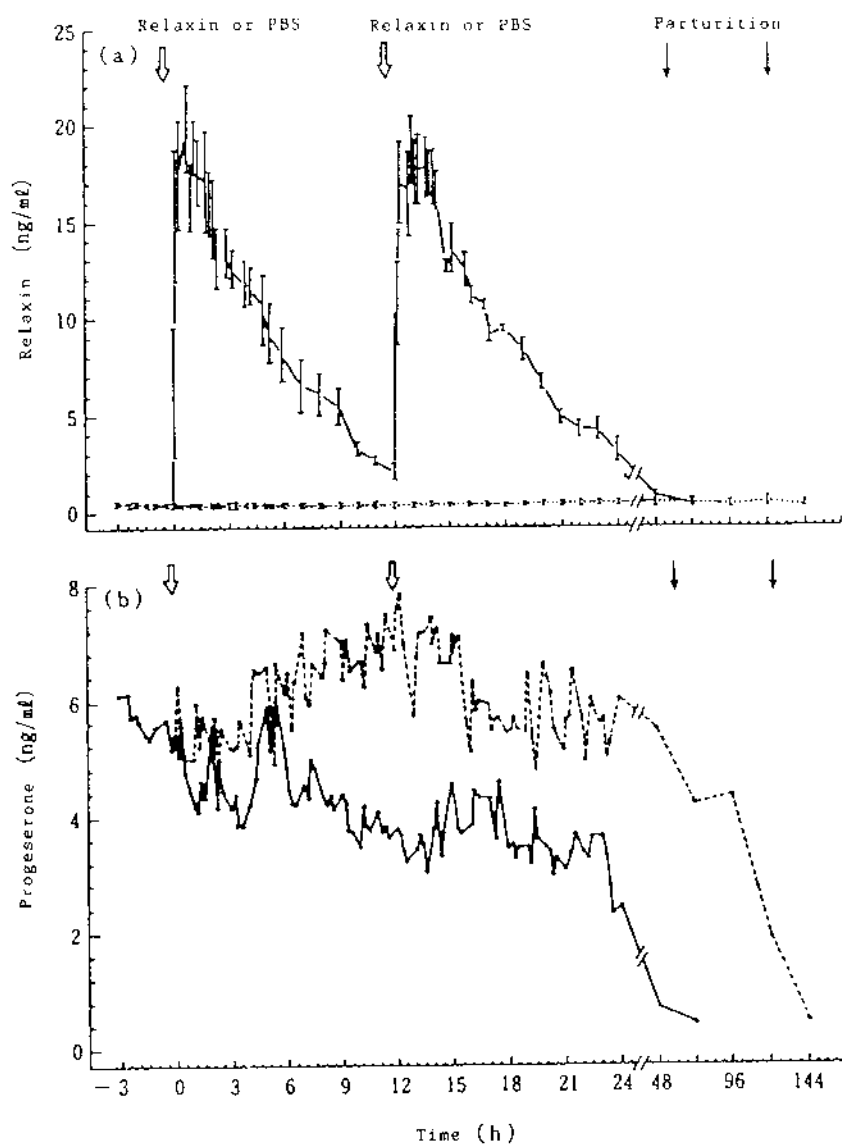


Fig.1-2 20 Changes in concentration of relaxin (a) and progesterone (b) in peripgeral blood of cows when relaxin (3000 U/mg) or phosphate-buffered saline (PBS) is administered twice at intervals of 12 hrs
Solid and broken lines show the relaxin group and the PBS group, respectively.

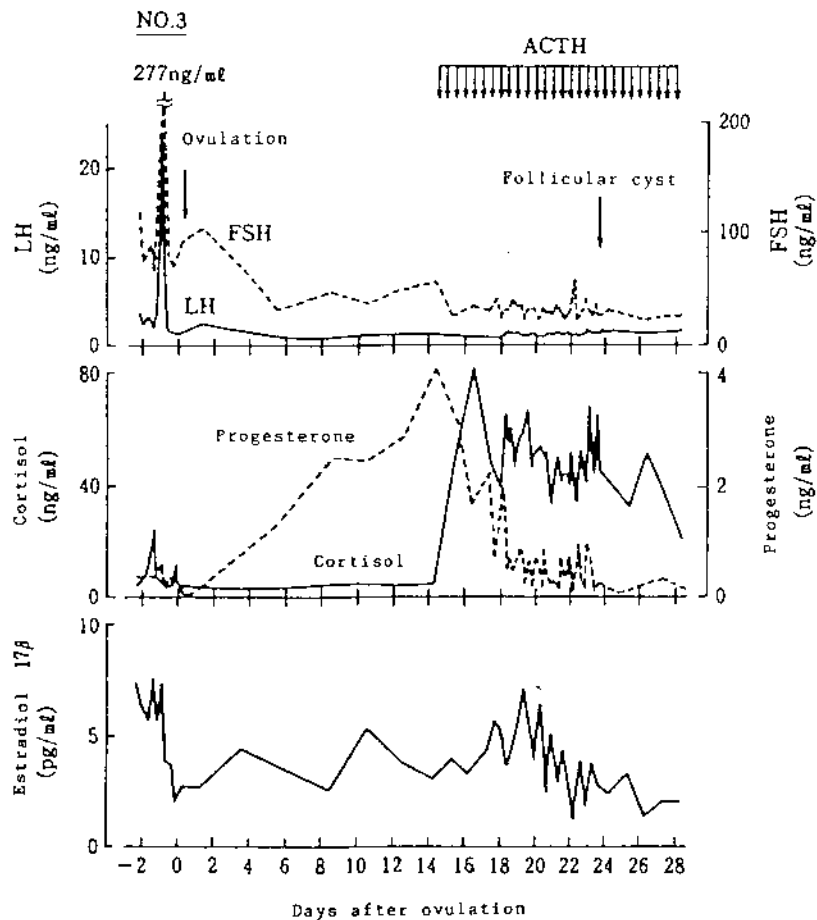


Fig.1-2-21 Changes in blood concentration of LH, FSH, cortisol, progesterone and estradiol-17β in onset of follicular cyst caused by continuous administration of ACTH (typical animal, Cow-3)

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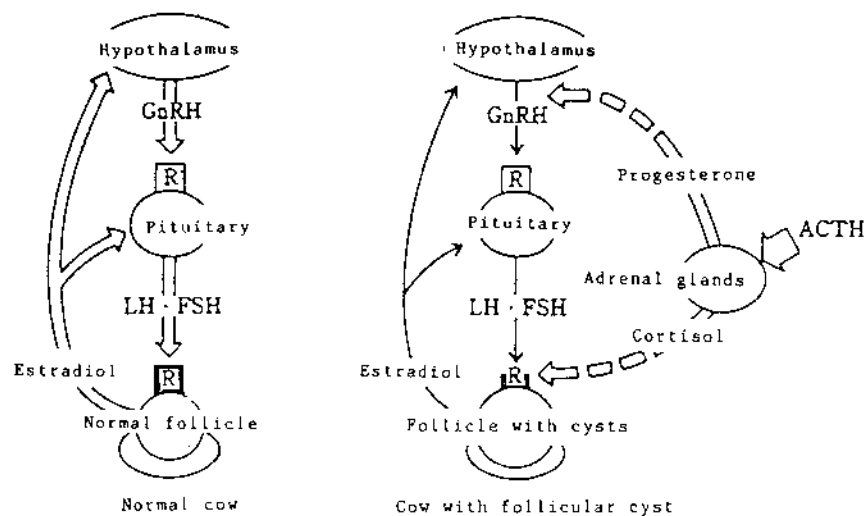


Fig.1-2-22 Diagram showing possible mechanism of onset of follicular cyst in cows

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3. Reproductive Function of Male Livestock and Semen Physiology

3-1 Sexual Maturity and Appropriate Age to Begin Breeding

For male livestock, sexual maturity indicates the time when the testes and accessory reproductive organs are mature enough to produce and ejaculate semen. At this time, males can make females pregnant. Sexual maturity is related to the age in days and the weight of the animal. It is also affected by genetic factors, environmental factors, nutrition, and climate.

The tissue of bull testes begins to differentiate at the age of 3 - 4 month when the interstitial cells start forming. Primary spermatocytes can be observed in bull seminiferous tubules at the age of 2 - 4 months and mature spermatozoa are found at the age of 6 months. A large number of free spermatozoa are found in the spermatic duct at the age of 8 - 10 months when the semen may be collected for artificial insemination. The volume of semen per ejaculation of a Holstein at the age of 11 months is about 3 ml and the number of spermatozoa is about 2.5 billion which is about half the number in a mature bull. The semen volume gradually increases until the 17 month of life from when it shows a more obvious increase. The freezability of spermatozoa is unstable for about 10 months after the male reaches the stage at which semen can be collected but freezability becomes stable by the age of 18 - 20 months.

3-2 Spermatozoa

(1) Formation and Maturation of Spermatozoa

The formation of spermatozoa continues constantly in the seminiferous tubules from the time a male reaches sexual maturity until spermatogenesis ceases due to aging. Spermatogonia, which become spermatozoa, and Sertoli's cells, which supply nutrients to spermatozoa, are the only cells

found in the seminiferous tube before sexual maturity of the animal. Spermatogonia commence division when the animal reaches sexual maturity and the formation of spermatozoa is divided into spermatocytogenesis and spermiogenesis. Spermatocytogenesis includes division of spermatogonia to form primary spermatocytes, and meiosis and mitosis of primary spermatocytes to form secondary spermatocytes and eventually spermatids. Spermiogenesis includes contact between spermatids and Sertoli's cells and their subsequent metamorphosis to spermatozoa with long tails.

(1-1) Spermatocytogenesis

A spermatogonium (A type) will divide into two spermatogonia one of which will diapause for a while. The active spermatogonium (A2 type) repeats mitosis to form intermediate-type cells and later to form B-type cells which comprise 16 primary spermatocytes. The primary spermatocytes soon form secondary spermatocytes through meiosis. This process is referred to as maturation division. One primary spermatocyte forms two secondary spermatocytes (i.e., maturation division I) and then the secondary spermatocytes form four spermatids (i.e., maturation division II). Thus, one spermatogonium forms 64 spermatids. The time required to form spermatids is, for example, 32 - 45 days in a bull.

The diapaused spermatogonium will start dividing about 10 days after it was formed. It will then start repeating division to form spermatids in the same way as described above. Subsequently, the formation of spermatozoa proceeds both cyclically and constantly.

(1-2) Spermiogenesis

A spermatid will form a spermatozoon that has characteristics typical to the animal species while it undergoes morphological transformation. This process is called spermiogenesis. Spermatozoa that have completed metamorphosis will gather together on the Sertoli's cells.

They will soon separate and will exist freely in the seminiferous lumen. The spermatozoa will then pass thorough the straight seminiferous tubule, the rete testis, the head of the epididymis, and eventually reach the tail of the epididymis where they remain prior to ejaculation. The entire process from spermatocytogenesis and spermiogenesis is called spermatogenesis.

(1-3) Maturation of Spermatozoa

The spermatozoa formed in the seminiferous tubule will mature while it is passing down the epididymis by changing its quality and function. The ability to move forward will be acquired while the cell is moving toward the tail of the epididymis. Spermatozoa do not have the ability to move forward while at the head of the epididymis. A cytoplasmic droplet found on the front part of the middle piece of a spermatozoon will move to the end and then disappear. Very little of the droplet will exist on the spermatozoon prior to ejaculation. The ability of spermatozoa to fertilize will be obtained in the lower half of the epididymis and is not seen at the head of the epididymis. This ability to fertilize and spermatozoal motility are maintained while in the tail of the epididymis for a certain period which, in a bull, is about 30 days.

(2) Morphology and Structure of Spermatozoa

A spermatozoon from livestock forms like a tadpole. Its structure can be divided into four parts; the head, the neck, the middle piece and the tail.

Head: A spermatozoon from domestic animals is flat and has an egg shape. A spermatozoon of a rat and a mouse is sickle shaped, and that of chickens has a thread shape. The head of a spermatozoon forms the nucleus of the cell. It mainly contains DNA bound to protein. Both the fore and rear halves are covered with membranes referred to as the acrosomal cap and post-nuclear cap. The acrosomal cap contains enzymes, such as hyaluronidase and acrosin, which are essential for impregnation of an ovum.

Neck: This section is joined to the head, can easily be cut off and contains a centrosome. An axial filament extends out from this section to the end of the tail.

Middle piece: This part is joined to the neck and is thicker than the tail. In the center is an axial fibrillar complex, and around the fibrillar complex is a spiral mitochondrial sheath and cytoplasmic membrane. At the end of the middle piece is an annulus. The middle piece is rich in phospholipid, contains many kinds of oxidases, and supplies energy to the spermatozoon.

Tail: This section is joined to the middle piece and contains an axial fibrillar complex comprising a bundle of nine fibers which is joined to the annulus. Covering the fibrillar complex is a fibrous sheath known as the protoplasm. The tail tapers away from the middle piece, at the end of the tail, there is no fibrous sheath or dense fibers. The axial fibrillar complex is exposed at the end.

(3) Functions of Spermatozoa

Spermatozoa ejaculated into the female reproductive organs will pass upward due to the vigorous movement of their tails until they reach the upper part of the oviduct or the site of fertilization where they wait for an ovum. When an ovum comes along, a spermatozoon enters the ovum and fertilization takes place. Spermatozoa must possess vigorous forward motion although their upward migration in the female mainly depends on the peristaltic motion in the reproductive tract.

While the cells are moving upward through the tract, they must undergo a functional change called capacitation (fertilization ability). The head section of a spermatozoon (namely the acrosome), the nucleus and the membrane must function normally in order to complete this process and this is supported by various metabolic functions.

(3-1) Functional Motility of Spermatozoa

The vigorous motion of spermatozoa soon after ejaculation shows a whirling pattern. In this mass activity, each spermatozoon moves forward by spirally rotating its head while waving the tail. Generally speaking, most spermatozoa soon after ejaculation show vigorous movement as they are moving forward. As time passes this movement gets slower and the spiral movement of the cell changes to a pendulum-like movement until they completely stop moving. Spermatozoa frantically whip their tails after capacitation. The forward velocity is about $50 - 120 \mu\text{m}$ per second at 38°C . This velocity depends on factors such as animal species, temperature and the nature of the solvent.

Spermatozoa run against a current (i. e., rheotaxis). Their heads adhere and gather on the surface of a bubble

or foreign substance and are likely to combine with certain chemical substances such as mucus in the cervical canal and liquor folliculi.

(3-2) Survivability and Fertilization Ability of Spermatozoa

Livestock semen containing spermatozoa with varying motility and a high survival rate shows high fertilization ability. To maintain the vigorous movement and high fertilization ability of spermatozoa outside the male body, it is essential to prevent their loss of energy. In order to maintain these cells, motility and metabolism of spermatozoa must be reversibly decreased. Generally, fertilization ability decreases faster than motility. In particular, in chickens spermatozoa start losing their fertilization ability after one hour after leaving the chicken body if they are not treated though they may still be motile.

(3-3) Metabolic Function of Spermatozoa

Typical metabolism in a spermatozoon comprises glycolysis and respiration.

① Glycolysis

The pH value of semen preserved in vitro decreases in time. This occurs because spermatozoa degrade fructose in the seminal plasma to form lactic acid. Livestock semen utilizes fructose, glucose, and mannose in aerobic conditions, but in anaerobic conditions it uses glucose and mannose before fructose. In both cases, lactic acid is produced in the Embden-Meyerhof pathway. Under aerobic conditions, some of the resulting lactic acid enters the tricarboxylic acid cycle (TCA cycle) and is degraded into carbon

dioxide and water. The fructose degradation index (i. e., the amount of fructose in milligrams degraded by one billion spermatozoa at 37°C /hr) is used to evaluate semen quality. The ability of spermatozoa to degrade fructose is high in cattle, sheep, and goats, and is low in horses and pigs.

② Respiration

Spermatozoa obtain energy mainly through respiration in aerobic conditions. The respiratory pathway comprises the TCA cycle and oxidative phosphorylation linked with the TCA cycle. The extent of respiration is measured by the amount of oxygen consumed (mm³) by one hundred million spermatozoa at 37°C. The ZO₂ value is used as the unit for oxygen consumption and active spermatozoa have a high ZO₂ value.

(4) Factors That Effect the In Vitro Function of Spermatozoa

Motility and survival of spermatozoa when outside the animal body are affected by many factors.

(4-1) Temperature

Normal livestock spermatozoa make normal forward movements at 37 - 38 °C and make much more vigorous movements at higher temperature. They will almost instantly die above 54 - 56°C and a temperature lower than 35°C will result in slower movement. Movement will stop and metabolism will be very slow at certain temperatures depending on the species: 15°C for a pig, about 5 °C for a bull, sheep, and goats. Spermatozoa will die below 0°C because the water inside and outside the cell freezes and the delicate intracellular structure is damaged. However, in the presence of glycerin, which acts as an antifreeze,

the cell may survive at -196°C or lower. A rapid change in temperature is harmful to spermatozoa, in particular, ejaculated spermatozoa undergo irreversible damage by rapid cooling to around freezing point. This is known as temperature shock.

(4-2) Dilution

Diluting is commonly used to preserve semen although adding a large volume of diluent at one time, or making an extremely high dilution, is harmful to semen. Generally, spermatozoal motility is decreased by dilution but improves after a while.

(4-3) Osmotic pressure

Spermatozoa maintain their motility for a long period in solutions that are approximately isotonic to semen (280 - 300 mOs/kg). Many spermatozoa with curved tails are seen in hypotonic solutions since hypotonic conditions have a less beneficial effect on semen than hypertonic conditions. Since spermatozoa can tolerate hypertonic conditions, a slightly hypertonic (approximately 350 mOs/kg) solution is used in cryopreservation.

(4-4) pH

Livestock semen is usually neutral or weakly acidic. Spermatozoal motility is decreased in strongly acidic conditions and is increased in alkaline conditions. Spermatozoa are active within the range pH 4 - 8 (approx). Spermatozoal activity is halted in acid conditions and can be reversed provided the acidity is changed quickly to alkali conditions which activates spermatozoal motility but shortens their lives.

(4-5) Sunlight, Ultraviolet Rays and Radiation

Direct sunlight temporarily activates spermatozoa but has a harmful influence on their life. Ultraviolet light is also harmful. Radiation may affect the DNA although to an extent that may have no influence on spermatozoal activity. They may, however, give rise to abnormal development of embryos.

(4-6) Gases

Oxygen will activate the activity and metabolism of spermatozoa although an excess of oxygen will shorten cell life.

Carbon dioxide gas will reversibly reduce the motility of spermatozoa but a low concentration is essential to maintain smooth motility and metabolism.

(4-7) Chemical Substances and Other Factors

Substances such as acetylcholine, adrenaline, and strychnine increase the activity of spermatozoa at low concentrations. Vitamins such as B₁, C and K are effective in maintaining spermatozoal life. Steroid hormones, especially testosterone and progesterone decrease respiration although they enhance the effects of glucose and pyruvic acid. Although antibiotics at high concentration may have a bad effect on spermatozoa, sulfanilamide, penicillin, and streptomycin are widely used practically.

(4-8) Ions

The effect of ions on spermatozoa differs markedly according to their concentration and structures and the animal species from which the spermatozoa are collected.

A high concentration of potassium will suppress spermatozoal motility while sodium shows the opposite effect. Consequently, the ratio of potassium to sodium is important in maintaining normal motility of spermatozoa. Calcium is essential to fertilization but is also harmful in the maintenance of spermatozoal motility. Copper and zinc are assumed to suppress the effects of substances such as cadmium and mercury. This effect is due to the fact that copper and zinc, combine with the sulphhydryl groups in structural protein. Many heavy metals have a detrimental effect on the motility and of spermatozoa.

3-3 Semen

Semen contains spermatozoa and seminal plasma. The seminal plasma consists of liquid secreted from the epididymis, ductus deferens, seminal vesicle, prostate and bulbourethral gland (Cowper's gland).

(1) Physical and Chemical Characteristics

Color, Odor, and Viscosity: The color of semen is milk white to gray white but sometimes is yellow or yellow green. Semen has almost no odor but sometimes has the characteristic smell of the animal species from which it came. Viscosity and whiteness increase in proportion to the concentration of sperm. Often goat semen is yellow to green yellow.

Hydrogen Ion Concentration (pH): The pH of fresh semen from animals such as a cattle, sheep and goats is weakly acidic ranging from 6.5 to 6.8. Pig semen is weakly alkaline ranging from 7.0 to 7.7. Since the pH is affected by liquids secreted from accessory reproductive glands, an excessive pH value may indicate abnormal functions of these organs or it may indicate mixing of foreign substances, for example urine, into the semen. Highly diluted semen often shows a high pH

value.

Osmotic Pressure: The osmotic pressure of semen ranges from 280 to 300 mOs/kg regardless of the animal it is collected from. If the pressure is low many spermatozoa with malformed tails can be observed.

Viscosity: The viscosity of semen differs depending on the concentration of sperm and the composition of seminal plasma. Viscosity is highest in sheep and goat semen and is the lowest in horses. Cattle semen shows an intermediate value. Pig semen is a little more viscous than semen from a horse.

Semen Volume and Number of Spermatozoa: The semen volume and the number of spermatozoa per ejaculation differ markedly according to factors such as animal species, race, the individual, and age of the animal they are collected from. Other factors such as the collection method, frequency of ejaculation, and the season in which semen is collected is likely to affect these values for the same animal.

Semen from a horse and pig contains a lot of gelatinous material that other animals do not have. In a horse, this material originates from the seminal vesicle and forms a semitransparent milky-white sticky liquid which can be liquefied by warming. A large quantity of this substance can be seen in the semen during the breeding season but little is seen in the non-breeding season. In a pig, this gelatinous material originates from the bulbourethral gland and accounts for about 30 % of the volume of semen. It expands in the presence of water due to its considerable water-absorbing nature. This gelatinous substance is assumed to help fertilization take place by preventing a backflow of semen by forming a vaginal plug at the outer uterine orifice.

(2) Chemical Composition

(2-1) Spermatozoa

The head of a spermatozoon contains mainly DNA and basic proteins such as protamine and histone that combine with DNA. The middle piece and tail contain phospholipids, which are a source of energy, and enzymes, that are essential in metabolism.

(2-2) Seminal Plasma

Seminal plasma contains reducing sugar, that is, mainly fructose, polyols such as sorbitol and inositol, organic acids such as ascorbic acid and lactic acid, lipids such as lecithin, amino acids such as glutamic acid, nitrogen containing bases such as glycerylphosphorylcholine and ergothioneine, prostaglandins, minerals and enzymes.

Fructose: A large proportion of sugar in livestock semen is fructose which originates from the seminal vesicle. The amount of fructose in semen is related to the level of androgens and, therefore, fructose level in semen can be used to measure the activity of androgen. Fructose is the main source of energy for spermatozoa soon after ejaculation and the pH of semen falls after ejaculation and in vitro if it is not treated since lactic acid accumulates due to the consumption of fructose.

Protein and Amino Acid: Semen contains a large amount of protein and free amino acids. They combine with heavy metals to protect semen from their harmful effects.

Citric Acid: Citric acid in semen originates from seminal vesicles and bovine semen has the largest amount of citric acid compared to other livestock. Citric acid is

controlled by androgen and this can be used to indicate sexual function. Citric acid is largely unused by spermatozoa and is assumed to have the role of maintaining osmotic pressure and act as a buffer in semen.

Phosphatase: Semen shows strong phosphatase activity. Acid phosphatase comes from the prostate gland and alkali phosphatase comes from the seminal vesicle. Alkali phosphatase activity is high in livestock semen.

Hyaluronidase: This enzyme plays an important role in fertilization and is present in spermatozoa. Hyaluronidase activity in seminal plasma is higher in semen containing a high number of spermatozoa. This enzyme leaks from spermatozoa if semen is frozen then thawed and the amount of leaked hyaluronidase is an indicator of the the degree of damage to spermatozoa. This enzyme helps spermatozoa impregnate the ovum by removing the granulosa cells around the ovum.

(3) Factors That Affect the Properties of Semen

(3-1) Age: The volume of semen and number of spermatozoa in old male animals is usually greater and more stable than in young animals. Bulls, for example, produce good semen very stably at about 3 - 6 years of age. Semen collected from much older males shows features such as a decrease in the ratio of living spermatozoa, an increase in the ratio of malformed spermatozoa, and decreased freezability.

(3-2) Nutrition markedly affects the properties of semen.: Poor nutrition may cause effects such as a delay of sexual maturity, decreased sexual drive, decreased spermatogenesis, and an increase in malformed spermatozoa.

Good nutrition, or high calories, may cause effects such as early sexual maturity, decreased sexual drive due to

obesity, and an increase in malformed spermatozoa. A high protein diet causes an increase in the volume of semen and in the ratio of living spermatozoa. A deficiency of vitamin A or carotene may have a bad effect on spermatogenesis, but sufficient feed containing appropriate roughage can prevent this. The addition of substances such as blood meal, bone meal and minerals to feed is beneficial in health management of livestock. Feeding bull with silage is not beneficial.

(3-3) Season

Some female livestock, such as horses, sheep and goats, have a non-breeding season. In males at this time a decrease in the volume and number of spermatozoa in semen, as well as spermatozoal activity are observed. The semen of bulls and pigs, which can breed throughout the year, is of poor quality in summer to early fall. The rate of conception for the animals may be low at these times of year. This is known as summer sterility and if this is extreme, it may cause aspermia in a pig. This is caused by the effect of heat on spermatogenesis so a cool environment is the key factor in raising male livestock.

(3-4) Exercise and Exposure to Sunshine

Exercise and exposure to sunshine increase livestock metabolism and improve circulation of blood throughout the whole body. These factors enhance spermatogenesis. Exercise and exposure to sunshine is also good for general health management by, for example, preventing obesity.

(3.5) Frequency of Ejaculation

The frequency of ejaculation affects the volume of semen, the number of spermatozoa, spermatozoal activity, and the ratio of malformed spermatozoa. Frequent ejaculation adversely affects these properties. Horses and pigs, which ejaculate a large volume of semen each time, are easily affected by these factors. Bulls and sheep are not so strongly affected since they accumulate semen every day for a week. For the effective use of male livestock such as maintaining reproductive ability for a long time the most appropriate frequency of semen collection for each animal species is as follows;

Bull: 2 ejaculations per day, every 2 - 4 days.

Pig: 1 ejaculation per day, every 3 - 4 days.

Sheep and goats: 2 - 3 ejaculations per day with 1 or 2 days rest per week.

Horse: 1 - 2 ejaculations per day with 1 or 2 days rest per week.

Table 1-3-1 Size of Spermatozoa of Livestock (μm)

(mainly, Bishop & Walton 1960)

Species	Head		Middle part		Tail		Total length
	Length	Width	Length	Diameter	Length	Diameter	
Bull	8.0-9.2	3.3-4.6	14.8	0.7-1.0	45.0-50.0	0.3-0.7	68.0-74.0
Sheep	7.5-8.5	3.5-4.3	14.0	0.8	40.0-45.0		70.0-75.0
Goat	7.0-8.6	3.0-4.8		0.8	40.0-45.0	0.5	70.0-75.0
Pig	7.2-9.6	3.6-4.3	10		30		49.2-62.4
Horse	6.0-8.1	3.3-4.6	8.0-10	0.5	30.0-43.0	0.5	55.0-63.6
Dog	6.5	3.5-4.5					55.0-66.0

Table 1-3-2 Properties of Semen of Livestock

	Bull		Pig		Horse	
Volume of semen in ml	2~10	(5)	150~500	(200)	20~300	(130)
Concentration of Sperm, $\times 10^6/\text{ml}$	3~20	(20)	0.5~8	(2.5)	0.3~8	(3)
Total number of spermatozoa per ejaculation, $\times 10^6$	30~100	(50)	400~1,000	(500)	40~200	(100)
Motile Spermatozoa, %	60~90	(80)	75~95	(85)	60~90	(80)
Malformed Spermatozoa, %	1~20	(10)	0.2~16.5	(5.6)	6~27	(16)
pH	6.4~6.8	(6.6)	6.7~7.3	(7.1)	6.9~7.7	(7.3)
Specific Gravity	1.034~1.036	(1.035)	1.007~1.025	(1.018)	1.011~1.019	(1.014)
Freezing point depression	0.53~0.65	(0.609)	0.59~0.65	(0.616)	0.58~0.60	(0.595)

() are average values

Table 1-3-3 Chemical Composition of Livestock Semen (Mann 1964, 1969)

	Bull	Sheep	Pig	Horse
Total N	440 - 1,170	900	200	150 - 300
Na	150 - 370	103 - 180	280 - 800	257
K	50 - 380	71 - 90	280 - 830	103
Ca	24 - 60	9	2 - 6	26
Mg	8	3 - 6	5 - 14	9
Cl	150 - 390	87 - 180	240 - 430	80 - 400
Fructose	300 - 1,000	150 - 660	3 - 48	< 1
Sorbitol	10 - 136	26 - 120	8	20 - 60
Inositol	24 - 46	10 - 15	380 - 625	19 - 47
Ergothioneine	< 1	< 1	6 - 30	4 - 16
Glycerolphosphoryl choline	110 - 500	1,600 - 2,000	110 - 240	38 - 113
Citric acid	350 - 1,000	300 - 800	36 - 325	10 - 50
Lactic acid	20 - 50	35	20	9 - 25
Zinc	-	-	4	-

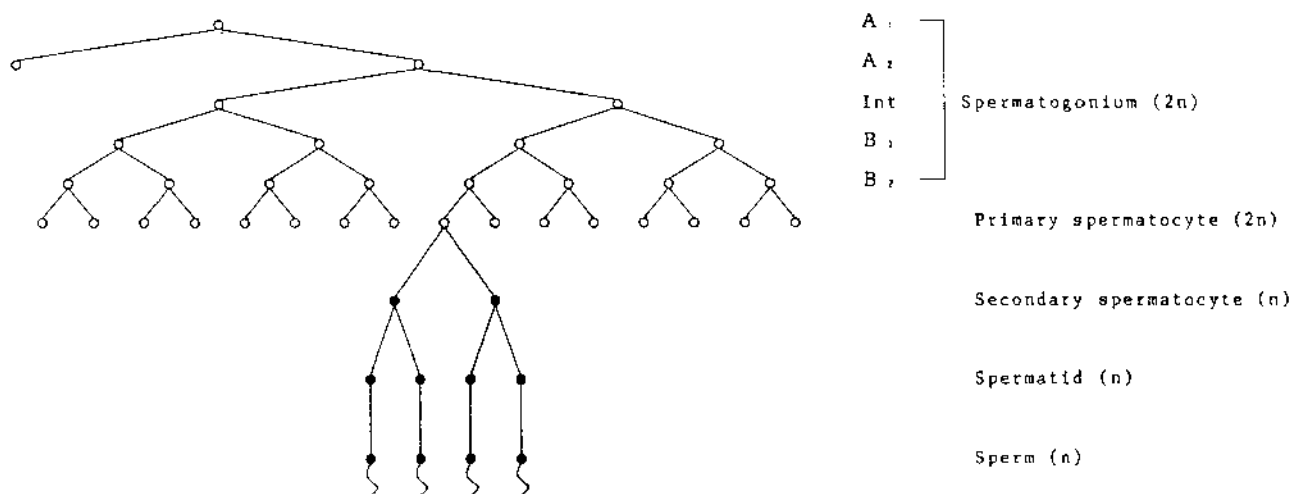


Figure 1-3-1 Diagram of Formation of Spermatozoa

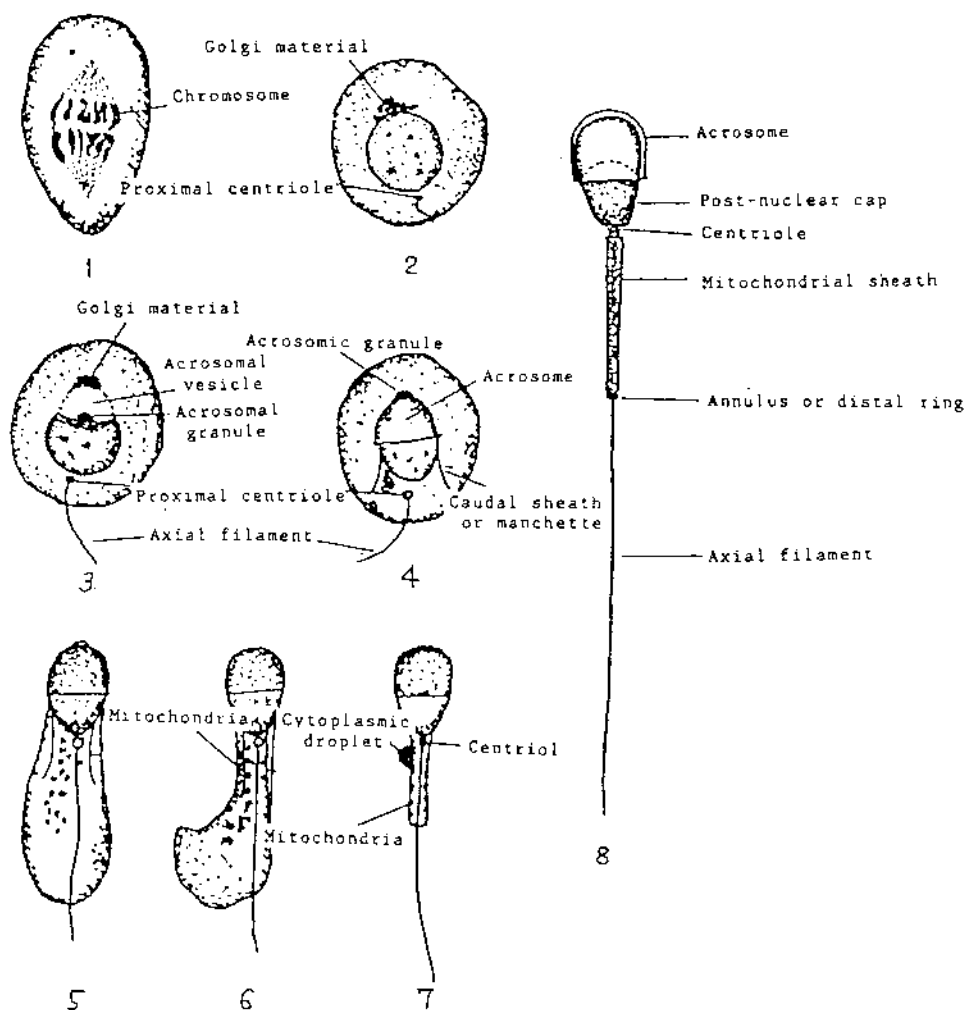


Fig 1-3-2 Process of Spermiogenesis (Gresson & Zlotnik 1948, parthy modified)

1. Primary spermatocyte	2. Granule phase	3. 4. Cap phase
5. Acrosome phase	6. 7. Maturation phase	8. Sperm

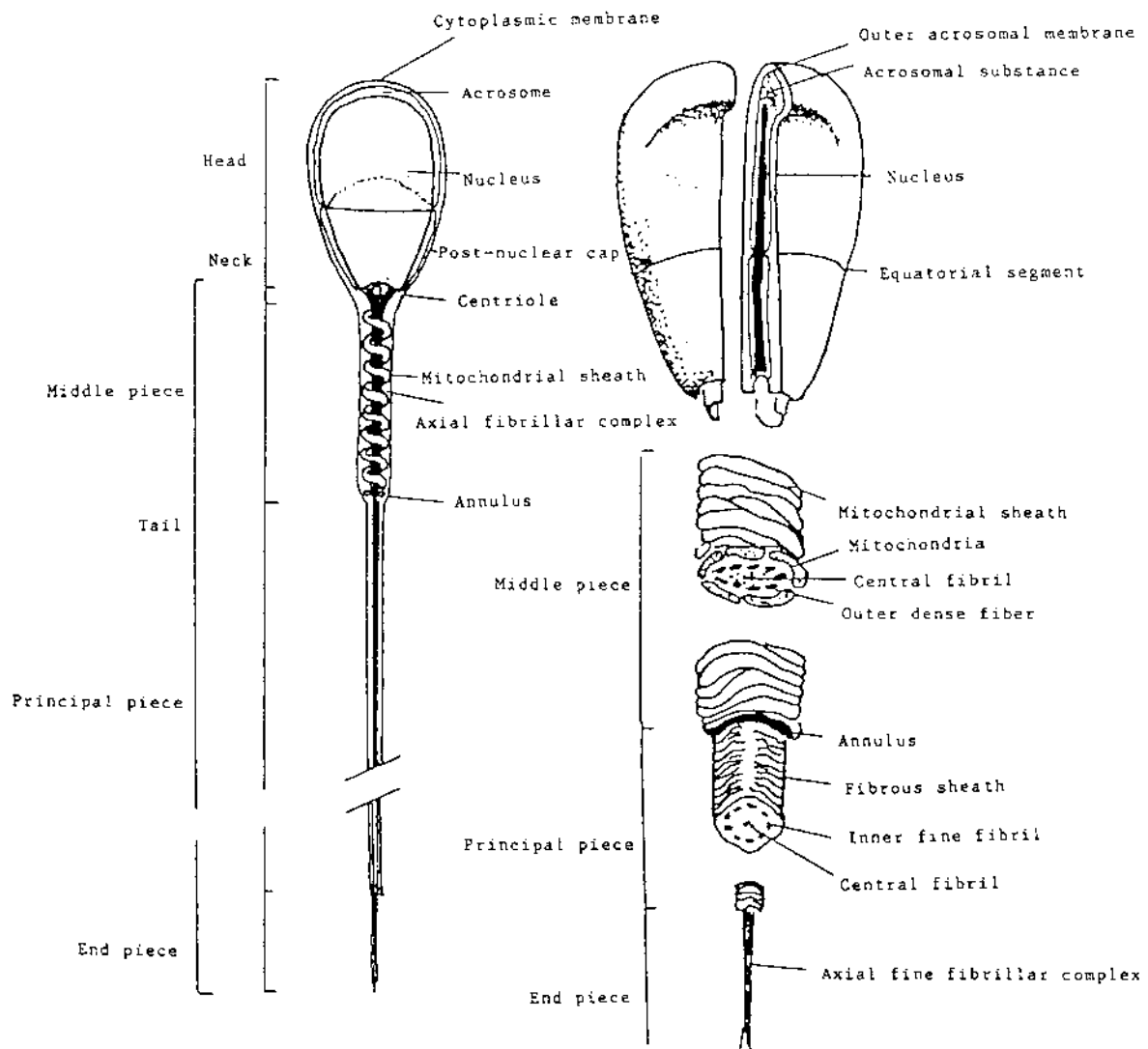


Fig 1-3-3 Structure of Spermatozoa

(Left, Mann, 1963, partly modified, right, Saacke & Almquist, 1964, partly modified)

4. Theory of Spermatozoal Freezing

4-1 Freezing Process

The freezing process includes steps such as supercooling, nucleation, growth of ice crystals, and crystallization at the eutectic point (Figure 1-4-1). When ultra-rapid cooling occurs, the growth of ice crystals is completely restrained and vitrification occurs in the solution.

(1) Supercooling

Gentle cooling decreases the temperature of the solution to freezing point or below while maintaining the liquid state and without freezing. This is called supercooling. This state is very unstable and slight stimulation can initiate nucleation. Contact between an ice strip or a cooled metal strip and the supercooled liquid can destroy the supercooled state. This is known as ice seeding.

(2) Formation of Ice Crystal Nuclei

An ice crystal nucleus may be formed by two processes. One process involves pure water nucleation and occurs at low temperature. The other process depends on a foreign substance in the solution. In this process, a nucleus is formed around the substance and this occurs at a higher temperature than the first process.

(3) Growth of Ice Crystals

Ice crystals grow after nucleation. As growth starts the solution temperature rises steeply to the freezing point because latent heat in the solution is released as they grow.

The length of the plateau differs according to the cooling velocity and rapid cooling yields a short plateau. The

growth rate is maximum at about -20°C then the rate is immediately arrested. When the solution is rapidly cooled, the rate of ice crystal growth is markedly inhibited and a large number of small crystals are formed.

(4) Crystallization at the Eutectic Point

Cooling a solution increases its ice content while the liquid phase decreases thus concentrating the solution. The solution then separates into ice crystals and solute crystals, and, as it is being cooled, the liquid phase disappears at a certain temperature. This temperature is called the eutectic point of the solution. The eutectic point differs largely according to the solutes; -4°C for a glucose solution, -46.5°C for a glycerin solution and -132°C for a DMSO solution.

Taking the freezing of physiological saline ($0.85\% = 0.15\text{M}$ in Figure 1-4-1) as an example, the freezing process can be clearly explained. In this figure, the solution at point ℓ was cooled and the solution commenced supercooling at the freezing point p ($p = -0.55^{\circ}\text{C}$). The temperature then rises to freezing point p' due to latent heat. Continuous cooling separates water from the solution as ice and this increases its concentration. The concentration reaches the maximum value, $22.4\text{g}\%$ (3.8M) at point q (-21.2°C). This is the eutectic point of the saline solution and is the lowest temperature at which liquid water and solid saline can exist at the same time in the solution. The temperature is constant at -21.2°C until all the salt is deposited and all the liquid water has turned to ice. Consequently, the liquid phase completely disappears after which the temperature drops again. At the eutectic point O , a constant temperature and concentration of the solution is observed despite the commencement of a concentration process. The concentration of the saline solution is 22.4% at this point.

4-2 The Cause of Cellular Death by Freezing

(1) Intracellular Freezing

Cells in general, including spermatozoa, die or lose their function following destruction of the intracellular structure due to crystallization of intracellular water. In contrast, cells in physiological saline do not lose their activity if they are surrounded by liquid water, though the extracellular solution will start freezing at -2 to -5°C . Cooling is not deleterious to the cell until the intracellular water freezes despite the surrounding solution being frozen.

(2) Contraction of Cells by Dehydration

Ordinary cells are damaged by contraction caused by dehydration at low temperature although a cell may survive at lower temperatures, for example, -196°C . Intracellular water is gradually removed while the cell suspension is slowly cooled in the extracellular freezing state. About 80% of intracellular water which can be frozen, leaves the cell at about -15°C provided intracellular freezing is prevented. Almost all intracellular water leaves the cell at about -30°C . Therefore, it is assumed that a cell that is resistant to cooling to -30°C can survive at a lower temperature such as -196°C although an ordinary cell may be damaged. Possible biochemical changes in damaged cell at such low temperature include changes in intracellular structure due to changes in hydrogen bonding in polymers, irreversible solidification and precipitation of proteins due to dehydration of the protoplasm.

(3) Extracellular freezing

During extracellular freezing, concentration of the solute is accompanied by an increase in osmotic pressure and changes in pH occur.

The electrolytes sodium and potassium are the most abundant in seminal plasma and are thought to exist in the form of sodium chloride and potassium chloride. The eutectic points at which the concentrations of these salts become highest are -21.2°C for sodium chloride and -11.1°C for potassium chloride and this temperature range is known to be harmful to spermatozoa. Since the increase in the solute concentration accompanied by an increase in osmotic pressure as well as changes in pH all occur within this temperature range, it is not clear which has the most damaging effect on spermatozoa.

(4) Mechanical Damage by Movement and Growth of Ice Crystals

Thawing, like freezing, may damage cells. The movement and recrystallization of ice crystals in the solution in which the cells are suspended may damage the cells during the thawing process. Gas remaining in the ice crystals in bubble form may damage cells in the same process.

This mechanical damage may be prevented by decreasing the size of ice crystals and increasing the number of smaller crystals. Rapid cooling can give rise to such smaller crystals during freezing. In other words, rapid cooling suppresses the growth of ice crystals in the solution and creates freezing conditions similar to vitrification. However, vitreous ice including ice crystals is unstable above -129°C , and their movement and recrystallization damages cells. Movement is enhanced above -40°C and easily damages cells especially at about -20°C .

4-3 Effects of Glycerol as a Cryoprotectant

Glycerol is an essential cryoprotectant for the cryopreservation of spermatozoa.

(1) Characteristics of Glycerol

Glycerol is a trihydric alcohol with a molecular weight of 92.

It is only weakly toxic to cells and permeability across the cytoplasmic membrane is high. It is a substrate for the glycolytic pathway in spermatozoa and is very soluble in water and alcohol. The glycerol molecule itself can act as a solvent for electrolytes and organic compounds and its boiling and freezing points markedly change if it is dissolved in water or alcohol. A high glycerol concentration results in a low freezing point and a glycerol solution shows marked supercooling. Spermatozoal stress is low in a solution with a high concentration of glycerol since it decreases the amount of ice in the solution.

(2) Glycerol changes the Freezing and Thawing of a Cell Suspension

Glycerol affects cells in a cell suspension which is frozen then thawed. The following is an example. If a suspension of spermatozoa in Ringer's solution is spreaded thinly on a glass slide it starts freezing at 0 to -8°C when cooled. Ice crystals are of a flat stick-like shape, a pentagonal shape, or sometimes a blunt arrow-like shape in this temperature range and grow rapidly. At about -20°C , spermatozoa are pushed away by the growth of ice crystals and are forced into the spaces (now very few) between other ice crystals. Warming rapidly decreases the amount of these crystals and stimulates a rapid increase in the number of bubbles and cracks that push on the ice crystals. When the ice thaws, spermatozoa remain along the edge of the crystals. Spermatozoa suspended in Ringer's solution containing 15-20% glycerol start freezing at -10 to -20°C . Ice crystals exhibit a feather-like shape or a fern-like shape as they grow. Even at -40°C , obvious narrow spaces can be seen between the crystals and spermatozoa are suspended in these spaces. When the solution is heated the ice crystals melt slowly but do not show the rapid changes seen previously. In this way glycerin serves as a

diluent for salts and lessens the physical changes accompanying freezing and thawing. (Figure 1-4-3)

(3) Glycerol Penetrates Cells to Prevent Freezing and Lessens Salt Damage

Glycerol affects extracellular freezing, as described above, and decreases the ice crystal content. It also decreases the concentration of salts in solution at 0 to -30°C which is a harmful temperature range. In addition, by permeating into cells it prevents cellular contraction and intracellular freezing by displacing intracellular free water. Glycerol also prevents denaturation of proteins that make up the cell which would normally occur due to the concentration of intracellular salts.

4-4 Disorders of Spermatozoa Caused by Freezing and Thawing

(1) Morphological Changes

Freezing and thawing may cause morphological changes in spermatozoa. Most of the changes that can be readily observed through a light microscope are those to the acrosome and these changes can be classified into three stages according to the degree of damage to the acrosome, i.e. swelling, partial-disconnection and total disconnection (Figure 1-4-4 (2)). The occurrence of acrosomal disorders and immobilized spermatozoa are marked between -10 to -40°C (Figure 1-4-4 (1)).

(2) Leakage of Lipids From Spermatozoa

Many kinds of spermatozoal phospholipids leak due to freezing and thawing. In the case of bull spermatozoa, leakage of choline plasmalogen, lecithin and sphingomyelin are marked. A large volume of those lipids are present in bull spermatozoa under normal circumstances. This leakage causes disorders

such as damage to the cytoplasmic membrane and a reduction in the source of energy for the cell.

(3) Passage of Inorganic Compounds

Intracellular inorganic compounds pass in and out of the spermatozoon during freezing and thawing and this differs according to the animal species. In bull spermatozoa, for example, leakage of potassium and magnesium from the cells and storage of sodium and calcium are observed.

(4) Leakage of Polymers From Spermatozoa

Enzymes leak from spermatozoa during freezing and thawing and these include hyaluronidase, lactic dehydrogenase (LDH), glutamic-oxaloacetic transaminase (GOT) and alkaline phosphatase. Leakage of GOT can be used as an index of the degree of damage to spermatozoa since its leakage is larger than for other enzymes and is related to both survivability and fertilizing ability of spermatozoon.

(5) Decrease in Survivability, Motility and Metabolism

Some 10 - 50% of spermatozoa in semen die despite being diluted in a solvent containing glycerol. However, surviving cells include those which have poor motility and metabolism. The decrease in metabolism is much more obvious for glycolysis than for respiration.

4-5 Factors That Affect The Survivability of Spermatozoa During Freezing and Thawing

(1) Spermatozoal Resistance to Freezing

The ability of spermatozoa to resist freezing is called freezability and is generally measured by the rate of recovery

of spermatozoa after freezing and thawing. In a bull, the freezability of spermatozoa differs according to the individuals they are collected from which in turn differs according to the condition at the time of collecting semen and the animals age.

(1-1) Breed, Individual and Age

Differences in freezability of spermatozoa are obvious between individual bulls but are not clear between breeds although differences can be seen. The breeds that have a high inbreeding factor may exhibit low spermatozoal freezability. The age of a bull affects freezability and this is low in bulls aged 1 - 1.5 yrs but is as follows; is high in a bull aged 2 years or more. Such freezability gradually decreases from about 6 years old.

(1-2) Season

Bulls poorly tolerate summer heat and freezability of spermatozoa decreases at this time of year. This decrease differs according to the individual bull and its age and an older bull is more easily affected by heat. The rate of conception using affected spermatozoa frozen in summer may be lower than that frozen in winter.

(1-3) Consecutive Collection of Semen

In consecutive collection of semen spermatozoa collected from the second ejaculation show better freezability than those collected from the first ejaculation. Spermatozoa collected from the third and fourth ejaculations will retain good freezability, but those collected from the fifth or subsequent ejaculations may

show lower freezability.

(2) Diluent Composition

The principal components of the diluent are saccharide, buffer and egg yolk. The survivability of spermatozoa during freezing and thawing differs according to these components.

The optimum concentration of egg yolk is 15 - 20%. Excessively low or high concentrations (e.g. 32%) are not good for the spermatozoa even though egg yolk protects spermatozoa from freezing damage. This function is mainly due to the effect of lipoprotein and lecithin in the yolk.

Saccharide plays an important role in the diluent by affecting osmotic pressure, exerting a cryoprotective effect, and acting as a source of energy for the spermatozoa. Saccharides with higher molecular weights cause good spermatozoal motility after freezing and thawing (Figure 1-4-5). Saccharides of high molecular weight include, in order of decreasing molecular weight trisaccharides, disaccharides, hexoses, and pentoses. Glucose shows the greatest effect among the hexoses whereas polysaccharides show little effect. Cryoprotection by saccharide is due to the numerous hydroxy groups in the structure which tend to form hydrogen bonds.

The buffer plays an the important roles of maintaining the cytoplasmic membrane during freezing and thawing, of stimulating smooth metabolism in spermatozoa after thawing, and of maintaining their survivability at the same time. A buffer that is suitable as a diluent in freezing should have the following properties; 1) damage to spermatozoa from salts is kept to the minimum; 2) it should be water soluble with an electrolytic dissociation constant of 6 - 8; 3) Permeability across the cytoplasmic membrane should be low and should be highly resistant to enzymes. Zwitter ion buffers

with such properties are obviously better than a phosphate buffer or a sodium citrate buffer. The best zwitter ion buffers are trihydroxymethylaminomethane (Tris) and N-hydroxymethylmethyl-2-aminoethanesulfonic acid (TES).

(3) Storage at 5°C Before Freezing

Storage at 5°C before freezing reinforces freezability of bull spermatozoa.

Conventional treatment of bull spermatozoa includes, 1) primary dilution of semen at 30 °C, 2) gradual cooling to 5°C over a period of 1.5 - 2 hours, 3) secondary dilution with a diluent containing glycerol, then 4) freezing after 2 - 3 hours equilibration.

Storage of cooled spermatozoa at 5°C before secondary dilution markedly improves survival rate after freezing and thawing. One variation of this method is to store the cooled spermatozoa at 5°C overnight (20 - 22 hours) before secondary dilution. The survivability of spermatozoa that have undergone this treatment is obviously better than that of cells treated in the usual manner. A second variation of the conventional method is to keep spermatozoa overnight (equilibrated with glycerol for 20 - 22 hours) at 5°C after secondary dilution. The first variation is far better than the second variation (Table 1-4-1) and this indicates that the freezability of ejaculated spermatozoa differs according to the period of low temperature sensitization.

(4) Concentration of Added Glycerol and Equilibration Time

The optimum concentration of glycerol in the final dilution for freezing bull spermatozoa is about 7%, but this differs slightly according to the components of the diluent. The optimum concentrations that confer good survivability on

spermatozoa are 11% for skimmed milk and 7 - 9.5% for egg yolk. Under condition in which the highest spermatozoal motility and the highest normal acrosome rate are observed, the optimum concentration of glycerol shows a significant correlation with the rate of thawing which indicates that a high concentration of glycerol in the diluent is necessary for a high rate of thawing.

The time between adding glycerol to the diluent (secondary dilution) and the beginning of freezing is called the glycerol equilibration time. There are varying opinions concerning the relationship between the equilibration time and motility of spermatozoa or rate of conception. For example, some workers consider 10 seconds to be optimum and other say 2 hour is best. Some workers says there is no difference between 1 minute and 6 hour, and other say an equilibration time of more than 4 hours is of no use. We believe that storage at 5°C before freezing is a far more important factor for spermatozoa than the length of equilibration time.

(5) Cooling Rate

An extra high cooling rate is detrimental to cells since it causes supercooling of the cell suspension and the water remaining in the cell. This results in extracellular freezing and then intracellular freezing. A slow cooling rate will cause concentration of both intracellular and extracellular solutions and will result in cellular disorders and this is known as the solution effect. The optimum cooling rate is a rate which minimizes both intracellular freezing and the solution effect (Figure 1-4-6). The optimum rate differs according to not only the type of cell but also to such factors as components of the cell suspension and the type of cryoprotectant. For example, saccharide solutions show higher spermatozoal motility with rapid freezing (2 - 4 minutes freezing from 5°C to -79 °C) than with slow freezing

(45 minutes freezing from 5 °C to -79 °C) since the solution effect is restrained. A 5 - 7% glycerol solution shows higher spermatozoal motility with rapid freezing (3 - 5 minutes freezing from 5°C to -130°C) than slow freezing (20 - 40 minutes freezing from 5 °C to -130°C).

(6) Thawing Rate

The method of thawing frozen semen greatly affects the survivability, motility, normal acrosome rate and metabolism of the spermatozoa. Higher survivability can be attained by thawing 0.5 ml straw frozen semen in water at 35°C rather than at 4 or 20 °C. A higher normal acrosome rate also can be attained by thawing in water at 35 - 75°C rather than at 5 - 20°C. However, thawing in water at a much higher temperature, such as 90 °C, will not improve the survivability. If spermatozoa is stored at 37 °C after thawing, cells which have been rapidly thawed at a higher temperature maintain higher survivability.

The oxygen uptake and lactic acid production of thawed spermatozoa are shown in the table 1-4-2. The 0.5 ml straw frozen semen was stored at -196°C and thawed in water at 5 - 50°C. The parameters were measured after thawing them again after 2 hours storage at 37°C. The oxygen uptake shows no obvious difference between thawing temperatures but lactic acid production shows a higher value at higher thawing temperatures over the range 5 - 40°C. These results suggest the thawing rate affects the metabolism of spermatozoa especially glycolysis. Motility and survivability of spermatozoa show a slight difference between thawing temperatures immediately after thawing. However, they show higher values in spermatozoa thawed at 30 - 50 °C after two hours of storage at 37 °C than those thawed at 10 - 15 °C. These results suggest that the effect on spermatozoa of the freezing and thawing process hardly affects the motility

immediately after thawing, but these are affected as time passes. Changes in the plasmalogen content in spermatozoa according to the thawing temperature is shown in Table 1-4-3 and plasmalogen is the main phospholipid in spermatozoa. The extent of the decrease just after thawing was about 10% at all temperatures but had a higher value when thawed at 5°C than at 40 °C . This decreased during three days storage at 4 - 5°C after thawing showed a 4 - 5 % higher value when thawed at 5 °C than at 40 °C .

4-6 Period on Storage of Frozen Semen

There have been several reports on the survivability and fertilizing ability of spermatozoa stored for a long time. It has been recorded that semen stored at -196°C for 20 years was used in Switzerland to produce a calf who was born on July 28, 1975. In Japan, Kyoto University has been leading in experimental conception using semen stored for many years. In 1974, conception using semen which had been stored for 4 - 13 years was reported and a change in average motility index was not observed during long-term storage. In these experiments, the average survival index of 244 straws of frozen semen was 43.9 immediately after freezing and was 44.4 after 4 - 13 years of frozen storage. In both cases, fertilization ability was not lost. These experiments also showed a rate of conception of 45 - 55% for spermatozoa after long-term storage. In 1980, an experiment in which insemination, using semen that had been stored for 16 years, of 100 cows was reported and 54% conceived. In 1986, the rate of conception for semen having been stored for 20 years was reported as 69.8% (60/86). This experiment demonstrated that semen stored frozen for 20 years does not lose its fertilization ability.

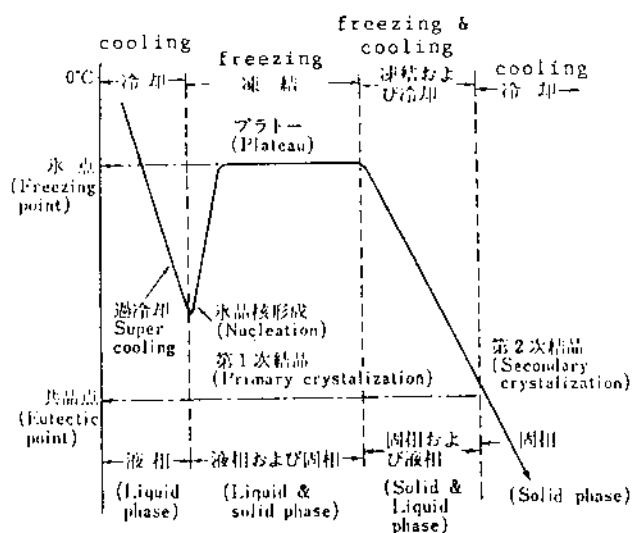


Figure 1-4-1 Solution freezing process

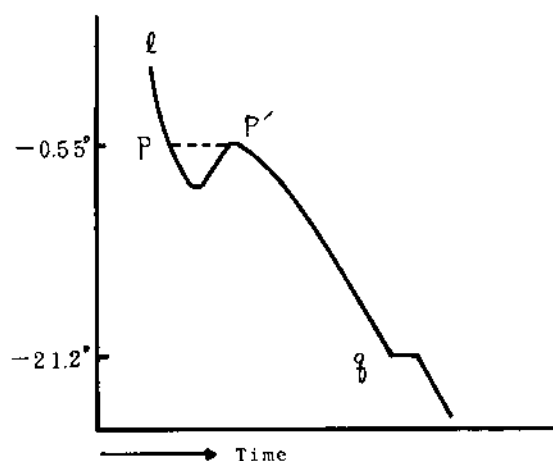


Figure 1-4-2 Freezing of physiological saline solution (Smith)

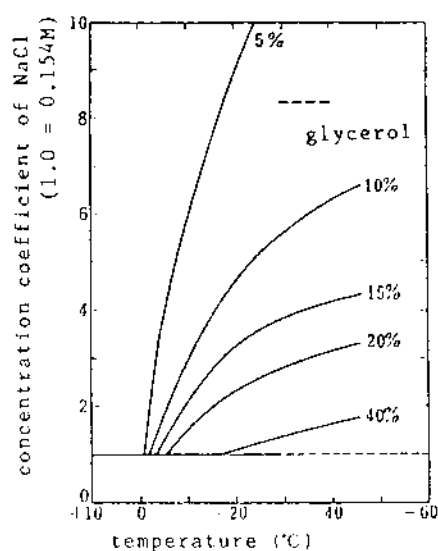
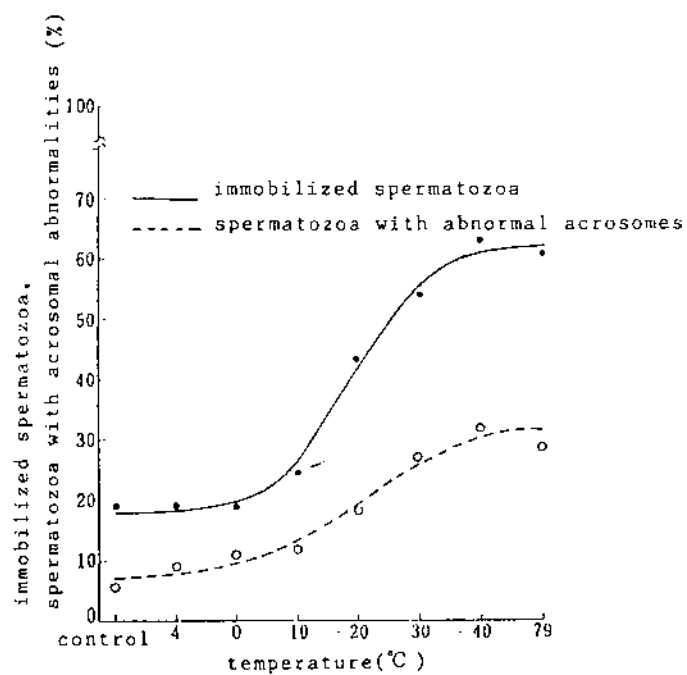
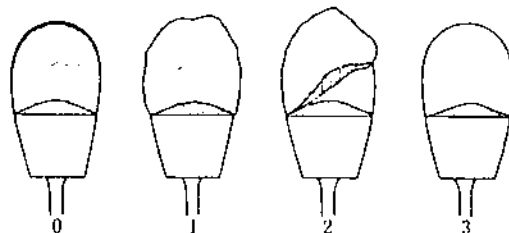


Figure 1-4-3 Effects of glycerol in NaCl solution compared to the NaCl concentration in the remaining solution when the NaCl solution (0.154M) is frozen (Farrant, 1970)



(1) Relationship between immobilized spermatozoa and ratio of acrosomal abnormalities during freezing (bull) (Shiroyama, 1970)



(2) Degree and grade of acrosomal change (Watson & Martin, 1972)

Figure 1-4-4 Changes in the acrosome caused by freezing damage and acrosomal abnormalities caused by freezing

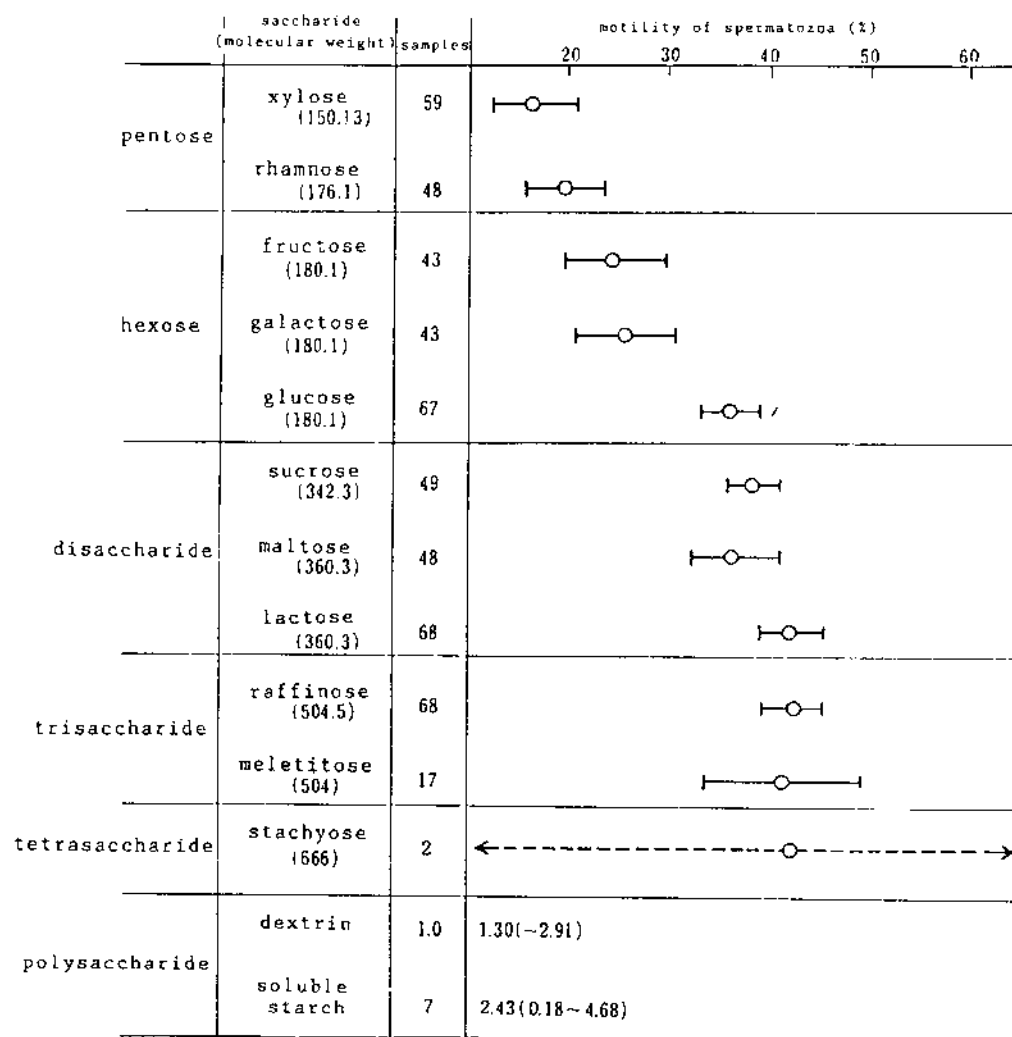


Figure 1-4-5 Effect of saccharide on freeze damage in bull spermatozoa
(tablet freezing method) (Nagase, 1968)

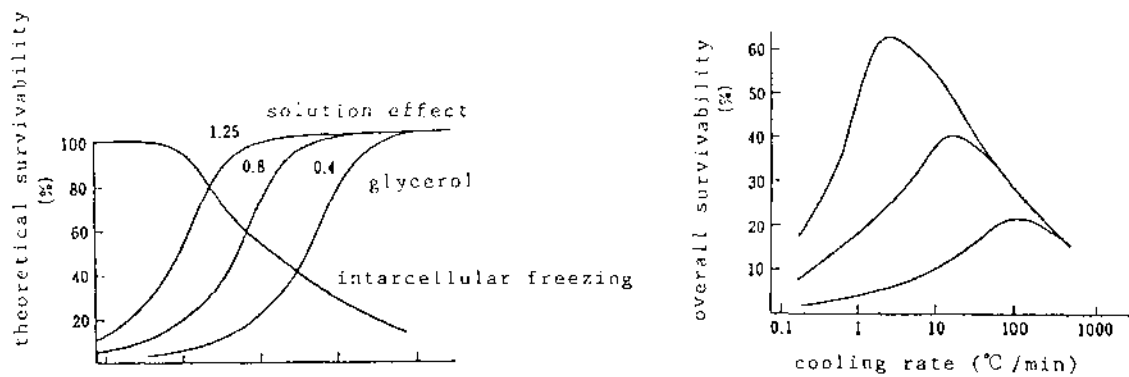


Figure 1-4-6 Optimum cooling curve for intracellular freezing of suspended cell and solution effect (Mazur, 1970)

A high cooling rate results in intracellular freezing while a low cooling rate causes the solution effect and results in cellular disorders. The optimum cooling rate is the rate which minimizes both intracellular freezing and the solution effect.

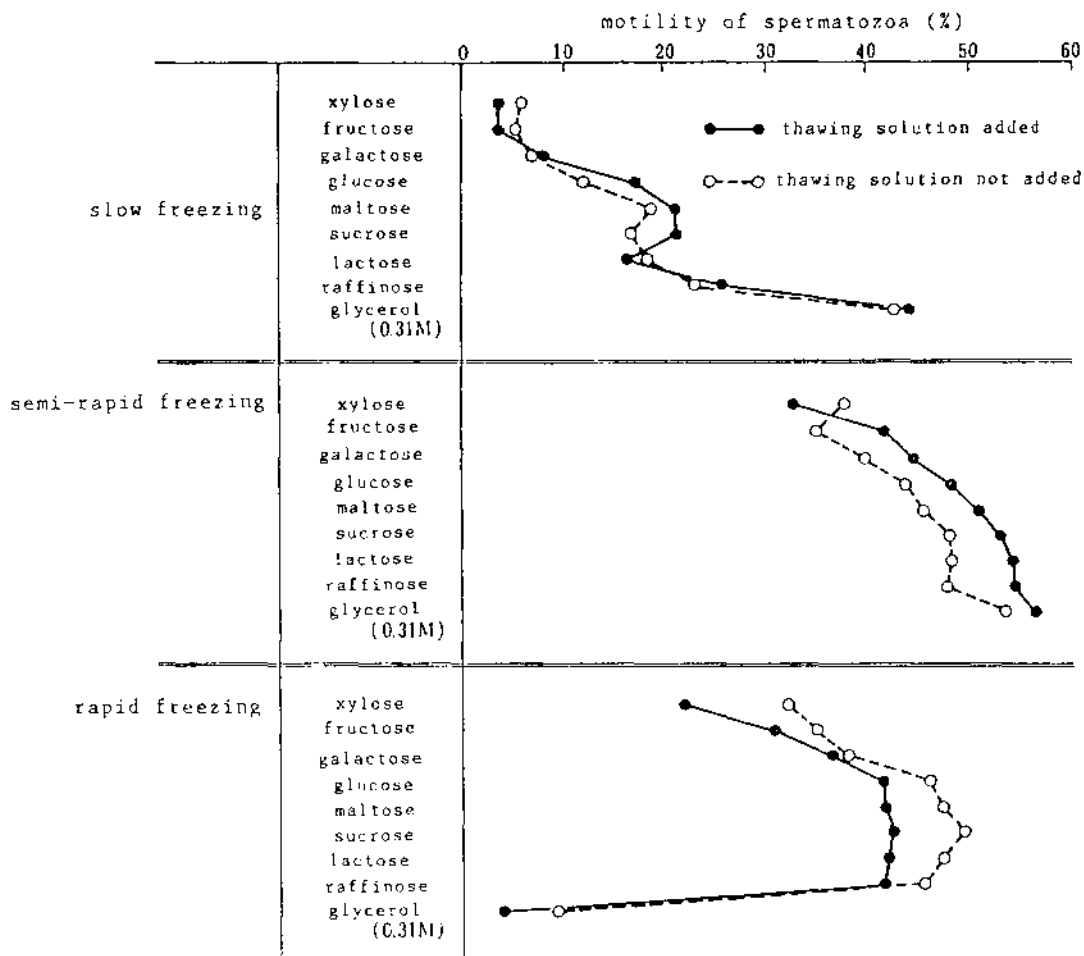


Figure 1-4-7 Freezing damage prevention by saccharide and freezing rate (bull) (Nagase, 1968)

PART II PRACTICE OF A. I.

1. Collection, Processing, Freezing Semen
2. Straw Insemination Procedures
3. Maintaining Insemination Records
4. Reproductive Disorders

II. Practice of A. I.

1. Collecting, Processing, Freezing Semen

1-1 Collecting Semen

The first step in artificial insemination is to collect semen by making bulls ejaculate using an aid such as an artificial vagina.

When collecting semen, it is desirable to make the bulls ejaculate under conditions that are as comfortable and natural as possible.

Semen collected in this way is used for fertilization after several steps, such as diluting, freezing, storing, thawing and injecting. Since the rate of conception is affected by the vitality of the semen at collection, it should be collected with the greatest possible care to maintain its vitality.

(1) Method of collecting semen

Various methods for collecting semen have been attempted since studies on artificial insemination began, and many improvements have been made in this field. The most common method at present is using an artificial vagina - by this method, semen can easily be collected in close to natural conditions.

(1-1) Method of using artificial vagina

The artificial vagina is used as follows: A stud bull is made to mount a dummy cow or a 'tether bull' for mounting, its penis is inserted into the artificial vagina for ejaculation, and the semen is collected.

① Artificial vagina

There are two types of artificial bovine vagina: a triple-walled type and a double-walled type. The artificial vagina is used for collecting semen to one

side of a dummy cow. The structures of the two types of artificial vagina are shown in Figures 1 and 2. The concept of using lukewarm water in the space between the inner and outer tubes during ejaculation is a common feature of these two types.

Figure 2-1-1 shows the triple-walled artificial vagina, which consists of 1) a plastic outer tube, 2) inner rubber liner A, and 3) inner rubber liner B. Inner rubber line B is fixed to the inside of inner rubber liner A, which is fitted to the outer tube, and a semen collecting bottle is placed at the end of inner rubber liner B. The bottle is inside the artificial vagina so semen is protected from the shock of temperature changes.

Figure 2-1-2 shows the double-walled artificial vagina, which is easy to handle since it is shorter than the triple-walled type.

② Dummy cow

Collecting semen using a dummy cow is easier than using a 'tether bull' for mounting. Therefore, bulls should be trained to use dummy cows as often as possible. (Insert Fig. 2-1-3.)

When bulls become old enough to mount and ejaculate, they are showexperienced bulls mounting dummy cows, and the young bulls experience mounting dummy cows and ejaculating into an artificial vagina in order to find out that the act is agreeable. Young bulls are easy to handle, and can be trained easily since they do not have experience of natural mating. In the initial training, it is useful to apply cow urine or rut mucus to the dummy cow. Sometimes, mounting and

ejaculating becomes easier when two to three bulls that have grown enough to mount and ejaculate compete for the dummy cow. Once bulls learn that ejaculation is pleasurable, they mount and ejaculate readily thereafter.

Another method is to collect semen from a young bull in the field by letting him mount a good-tempered 'bull' that is made to walk slowly near the bull. In this case, sufficient care (for example, 2 ropes should be used to hold the bull, one on the left, and one on the right, these being held by two people) should be taken to prevent accidents.

③ 'Tether bull' for mounting

Cows, oxen, or good-tempered bulls can be used as 'tether bull' for mounting to collect semen.

If the bulls for semen collection do not like or mount the 'tether bull', changing the 'tether bull' sometimes leads to good results.

④ Semen collection room

It is desirable that the place for semen collection be kept clean and quiet. In many cases, a cattle squeezer for washing the prepuce and a stand-by room for bulls are prepared near the dummy cow or a stanchion for tether bull for mounting. (Insert Figs. 2-1-4, and 2-1-5.)

⑤ Preparing for semen collection

- 1) Confirm that the dummy cow is adjusted to an appropriate height and that there are no

abnormalities or damage. In order to prevent accidents, place non-slippery material, such as a hemp-palm mat, behind the dummy cow or 'tether bull' for mounting so that the hind limbs of the bull do not slip when he stands up to mount. Further, sprinkle the mat with a small amount of water before the start of semen collection to prevent scattering of dust.

- 2) Wash the inside of the semen collection bull's prepuce. Since there is bacteria on the inside of the prepuce, bacterial contamination of semen can occur if semen is collected without washing. Therefore, always wash the inside of the prepuce with clean warm water before the start of semen collection, using a prepuce douche. Change the nipple and the plastic cup fixed on the end of the prepuce douche nozzle to a new sterilized one after each collection to prevent infectious diseases of the reproductive organs. (Insert Fig. 2-1-6.)
- 3) Wash the rear half of 'tether bull' for mounting, and restrict then to the cattle squeezer. The reason for washing is to prevent dirt contamination of semen, by removing dirt from the rear half of tether bull. Bind the tail of the 'tether bull' for mounting with a thin rope and tie the other end to the neck through the space between the 2 hind limbs and beneath the body so that the tail is no hindrance to semen collection.
- 4) Preparing the artificial vagina
 - a) Use an undamaged artificial vagina tube and an inner rubber liner. The inner rubber liner

should be one which has been washed well, dried sufficiently, and stored under hygienic conditions. These rubber products are usually stored in a box that has a sterilizing light. This light sterilizes and dries the rubber products which have been washed, and sterilized by bouling. (Insert Fig. 2-1-7.)

- b) In the case of the triple-walled artificial vagina: i) Attach inner rubber liner A to the outer tube of the artificial vagina and fold back both ends of the liner. Be careful that the liner does not twist. ii) Fix a semen collection bottle to inner rubber liner B on the side which the semen is to be collected, and bind it tightly with a piece of thin elastic cord. iii) Insert inner rubber liner B (to which the semen collecting bottle is fixed) into the artificial vagina (to which liner A is attached). Fold back the side on which the penis is inserted. Be careful that liner B does not twist. iv) Tightly bind both edges of the artificial vagina, to which liners A and B are attached with a piece of thin elastic cord.
- c) In the case of the double-walled artificial vagina: i) Attach a rubber liner to the outer tube of the artificial vagina and fold back both ends. Be careful that the rubber liner does not twist. ii) Attach the semen collecting rubber to the outer tube at the side on which the semen is being collected, fix a semen collection tube to the end of the cone, and bind it tightly with a piece of thin elastic cord. iii) Tightly bind both

edges of the artificial vagina, to which liner and cone are attached, with a thin piece of elastic cord.

- d) Pour water with a temperature of 42 ° - 43°C from the mouth of the outer tube to maintain the temperature of the inner liner at approximately 40°C . Be careful that the temperature is not too high or it may adversely affect the semen. Also, the temperature should not be too high or too low as it may inhibit ejaculation.

If excess warm water is poured into the artificial vagina, the inner rubber liner might break or fall from the outer tube because of physical pressure at the time of semen collection. Therefore, an appropriate amount of warm water, depending on the penis size, is necessary.

- e) Immediately before semen collection, apply an appropriate amount of white vaseline or mucilaginous jelly, to the inner rubber liner into which the penis is to be inserted, between the entrance and 1/3 of the way into the tube, using a sterilized glass stick. Be careful not to apply too much or it may contaminate the semen.

It is preferable that the artificial vagina be exchanged for a new one after each bull ejaculation. When the number of the tubes of the artificial vagina present is smaller than the number of bulls for collecting semen, it is convenient to use the triple-walled type, since

this type only requires changing the water and inner rubber liner B.

At some artificial insemination center, the artificial vagina, with all its attachments such as the inner rubber liner, is placed in an incubator at 40 ° - 42°C until it is used. (Insert Fig. 2-1-8.)

- 5) Lead the bull to the collection room but keep him from mounting the near by dummy cow or 'tether bull' for mounting (pretreatment).

To inhibit mounting (pretreatment), if the bull has already mounted the dummy cow or 'tether bull' for mounting pull him back before ejaculation, or prevent him from mounting in the first place. Repeat this two or three times. The bull will start to drip seminal vesicula juice from the preputial oritice, and some bulls will expose the penis from the preputial oritice. Before the bull is allowed to mount for semen collection, confirm that a discharge from the preputial oritice or the top of the penis is seen, which shows that the pretreatment has been adequate. Semen collected without adequate pretreatment contains a great deal of secretion from the accessory genital glands, which is to be avoided.

In particular, in young bulls, it is necessary to carry out adequate inhibition of mounting, since they tend to ejaculate hurriedly.

⑥ Method of collecting semen

- 1) The person who collects the semen approaches the bull at the same time as the bull mounts. It is

possible that the bull's hind hooves will step on the collector's toes, so he should wear safety shoes to avoid such accidents. It is preferable that the collector touch his left shoulder to the body of the bull (if the semen is to be collected from the bull's right side) and draw his left leg to the rear in order to prevent the bull stepping on his foot.

- 2) Hold the artificial vagina in the right (or left) hand, stand at the right side of the dummy cow or 'tether bull' for mounting, and keep the artificial vagina at an angle of approximately 35° , with the orifice (into which the penis is inserted) facing downward. (Insert Fig. 2-1-9.)
- 3) After completion of mounting, lead the penis to the entrance of the artificial vagina by holding the prepuce with left hand and pulling gently to the side (do not touch the penis). When the penis is inserted into the entrance of the artificial vagina with good timing, the bull ejaculates at the moment he thrusts. The practice of inserting the penis into the artificial vagina with good timing requires a certain degree of experience. At the moment of ejaculation, the tube of the artificial vagina is pushed forward by the bull. Do not oppose this movement.
- 4) Immediately after ejaculation, hold the orifice of the artificial vagina upward, and gently draw the bull away from the dummy cow or 'tether bull' for mounting. The bull sometimes charges towards the collector so, not only should the collector take care regarding this, but the person who controls the 'tether bull' for mounting should restrain

the bull.

- 5) Drain the water from the tube and take the semen collection bottle to the laboratory, taking care that bottle is not contaminated with water.
- 6) Occasionally, there is an "empty thrust"; that is ejaculation does not occur when the penis is inserted into the tube of the artificial vagina. This is often seen when the timing of inserting the penis into the tube of the artificial vagina is not good, when the temperature of the water in the tube is too low, or when the amount of water in the tube is inadequate to sustain an appropriate pressure.

1-2 Examination of Semen and Spermatozoa

The semen should be examined as soon as possible during the period between collecting and processing. Macroscopic then microscopic examination is performed. Generally, the following items are looked at.

Macroscopic examination

Characteristics of the semen, including volume, color, and smell, and pH

Microscopic examination

Concentration, Motility, Percentage of motile Spermatozoa, and percentage of abnormal Spermatozoa rate

(1) Macroscopic examination

(1-1) Volume of semen

Semen consists of spermatozoa and semen 'plasma' (accessory genital gland secretions). The volume of semen differs according to the individual, age, season, frequency of ejaculation and the technique of semen collection.

The volume of semen is measured using a semen collecting bottle with a graded scale. Normal ranges are shown in Table 2-2-1.

Table 2-2-1 Volume of semen, concentration of spermatozoa,
and number of spermatozoa per ejaculation

Volume of semen (cc)		Concentration of spermatozoa (million/cc)		Number of spermatozoa per ejaculation (million)	
Average	Range	Average	Range	Average	Range
5.0	2-10	1,000	300-2,000	5,000	3,000-7,000

(1-2) Color

The color is usually milky-white, but it becomes whiter as the concentration of spermatozoa increases. Sometimes though, the color is greenish as a result of carotene in the feed. When blood is mixed with the semen, the color becomes amber. Grayish white semen is often caused by contamination by soil or sand on the penis at the time of collection. Occasionally there is soil or sand sediment at the bottom of the semen collecting bottle.

If the collected semen is thick, observe it in a transparent test tube in good light. If the semen is usable, active movement of the spermatozoa and collective vortex streams are seen. The spermatozoa of such semen show good motility.

(1-3) Smell

Fresh semen usually has little smell, but it sometimes carries the smell of the bull. It is considered that the odor specific to semen comes from protein and phospholipid in prostetic secretions. When urine is mixed with the semen, the semen smells of urine. Semen stored for a long time has a putrid smell because of decomposition products produced by bacterial growth.

(1-4) pH (hydrogen ion concentration)

An electric pH-meter can be used for accurate measurement of pH, but measurement by filter paper is generally used. This method is simple: apply a small amount of semen to the filter paper, leave the filter paper to stand until color change is complete, and compare the resulting color with that of the standard to determine the pH. Generally, small square pieces of paper with B.T.B. (bromothymol blue; measurable range: pH 6.2 - 7.8) or B.C.P. (bromocresol purple; measurable range: pH 5.2 - 6.8) are used. The pH range of normal bovine semen is 6.2 - 6.8.

(2) Microscopic examination

(2-1) Motility of spermatozoa

① Examination of Motility

The Motility of spermatozoa is usually examined microscopically using a semen examination plate. Take put one droplet of semen using a flame-sterilized platinum wire loop (do not use while still hot - rather, wait for it to cool) or a thin glass stick, place it in the central ring of the examination plate, and place a cover slip on it. Examination using the examination plate is easy since the liquid layer has a constant thickness in each viewing field. (Insert Fig. 2-1-10.)

The percentage motile spermatozoa and motility are significantly affected by temperature. It is useful to carry out microscopic examination using a heat-retaining device which maintains the temperature at

approximately 38°C . A sliding heater is used in most heat-retaining devices. (Insert Fig. 2-1 11.)

② Expressing the motility

The motility of spermatozoa is expressed using both the percentage of motile spermatozoa and motility. The percentage of motile spermatozoa (%) is estimated by visual measurement, with the number of spermatozoa in all viewing fields taken as 100, and the motility classified as follows:

- +++ Vigorous movement: the greatest most movement; in some cases, the movement looks like a vortex or stream of water
- ++ Active forward movement
- + Weak movement
- ± Very weak movement: moves like a swinging pendulum
- No movement

For example, "80+++" means that 80% of the spermatozoa in the viewing fields have survived and are moving vigorously; "50+++, 20++" means that 70% have survived, with 50% of the total number moving vigorously and 20% moving actively.

The motility of the spermatozoa immediately after collection is usually 80-95+++.

③ Measurement of percentage of motile spermatozoa using aniline blue

This method makes use of the fact that living and dead spermatozoa show different color permeability or staining. From the results of staining, a

spermatozoon can be judged to be alive or dead.

a) Aniline blue solution

Phosphate buffer solution (pH 7.2)	100 cc
(M/8 Na ₂ HPO ₄ 80.4 cc + M/8 K ₂ PO ₄ 19.6 cc)	
Eosin	1.0 g
Aniline blue	1.0 g

b) Mix the semen and aniline blue solution on a glass slide in a ratio of 1: 10-20, smear the mixture following the usual semen-smearing practice (see 3 - 1)-a)), and heat to dry.

c) Examine 500 of the spermatozoa and calculate the percentage of unstained (dead) spermatozoa. The survival rate is obtained by subtracting the percentage of dead spermatozoa from 100.

(2-2) Concentration

① Hemocytometer method

Thoma's hemocytometer is used. Semen employed for counting should be well-mixed to prepare a homogenized solution.

a) Method of semen processing: Usually use a RBC melangeur. Gently touch the tip of the pipette to the surface of well homogenized semen and suck. If the number of the spermatozoa is large, suck to the 0.5 mark; if it is small, suck to the 1.0 mark. Remove any semen adhering to the tip of the pipette using the tip of a finger straight away. Then, suck the diluent (3% saline solution) accurately to the 101 mark, and shake

well with both ends of the pipette covered (with the thumb and middle finger). Semen and the diluent can be uniformly homogenized by moving the glass balls in the bulb of the pipette. When semen is sucked to the 0.5 or 1.0 marks, a 200-fold or 100-fold dilution is obtained, respectively. (Insert Fig. 2-1-12.)

- b) Counting chamber: As shown in Fig. 2-2-4, there is a square section in the center of the hemocytometer which the counting chamber. Each side of the chamber is 1 mm in length, and this 1 mm is divided evenly into 20 parts to create 400 tiny squares, each with an area of $1/400 \text{ mm}^2$. To make 16 easily detectable lattices, lines (E lines) are drawn in the center of every fifth lattice both lengthwise and breadthwise. (Insert Fig. 2-1-13.)

There are elevated areas to the right and left of the counting chamber. These areas form a space $1/10 \text{ mm}$ in height when a deck glass is placed over them. Diluted semen for counting the number of spermatozoa is dropped from the pipette into this space.

- c) Calculation: Drain two to three drops of well homogenized diluted semen from the pipette. Then let one drop fall on site A, the outside of the part in which the chamber and a deck glass overlap each other. In this way, the space is filled with the dilution.

Leave the hemocytometer horizontal for two to three minutes and wait for the spermatozoa precipitate. Then place the hemocytometer on the

microscope, find the chamber at low magnification, and count the number of spermatozoa at higher magnification. Include the number of spermatozoa found in two of the margins (for example, in the upper and left-hand margins) together, and do not count those found in the other two margins (in this example, the lower and right-hand side margins). The number of spermatozoa can be calculated as follows (assuming the total number of spermatozoa in all the lattices is 200):

	Number of spermatozoa in 1 mm ³ dilution	Number of spermatozoa in 1cm ³ dilution
With semen up to the mark of 0.5	200 x 10 x 200 = 400,000	400,000,000
With semen up to the mark of 1.0	200 x 10 x 100 = 200,000	200,000,000

The method described above is the method for counting the total number of spermatozoa in all of the lattices (400 lattices) in the counting chamber. There is another, simpler method: count the number of spermatozoa in five random blocks (1 block [16 lattices] x 5 = 80 lattices) and multiply the number by five to obtain the total number. Generally, the number found in four diagonal blocks (64 lattices) and in another random block (16 lattices) - a total of five blocks (80 lattices) - is counted, and the figure thus obtained is multiplied by five. In any case, repeat the counting at least three times and calculate the average.

② Measuring instrument method

In recent years, at large artificial insemination center or laboratories, a method for counting the number of the spermatozoa which uses a spectrophotometer or cell counter, have been employed.

Measurement using a spectrophotometer is performed as follows: add a small amount of testing semen (0.1 cc) into 100-times the amount of the diluent dilution (for example, 3% solution of sodium citrate), measure the light permeability using a spectrophotometer, and calculate the number of spermatozoa, referring to a standard curve or values previously prepared. (Insert Fig. 2-1-14.)

(2-3) Examination of morphology

Examination of the morphology of spermatozoa can be performed using either stained or unstained specimens. The method of staining with carbol fuchsin is as follows:

- 1) Smear: let a tiny droplet of semen fall on one end of the cover glass; touch this end of the cover glass (with the side of the droplet downward) on a glass slide, at an angle; let the semen spread uniformly; and let the cover glass slide down onto the slide to prepare as thin a smear as possible.
- 2) Air-dry
- 3) Fix with methanol for two to three minutes
- 4) Wash with water
- 5) Stain with carbol fuchsin solution for several minutes (5% phenol and fuchsin-saturated ethanol - 11-12 g

Fuchsin in 100 cc ethanol - are mixed at a ratio of 9:1).

- 6) Examine microscopically after washing with water and drying

Although specimens obtained in this way can be stored without any additional steps, they can also be used as permanent specimens if sealed with balsam. The morphology of 500 - 1,000 spermatozoa (magnified 400 - 600 times) is examined and the rate of deformity is calculated.

(2-4) Abnormal spermatozoa

Abnormal spermatozoa are broadly classified into 2 categories: deformed and premature. Deformities include deformity of the head, abnormality of the head cap, a double head, an abnormally large or small head, enlargement or shrinking of the neck, deformity or absence of the middle piece, a coiled or double tail, and absence of a tail; whereas in premature spermatozoa, a drop of protoplasm is seen adhering to the middle piece.

The proportion of abnormal spermatozoa in normal semen is generally 10% or less. If the ratio exceeds 20 - 30%, the rate of conception is usually low. An increase in the number of premature spermatozoa is seen when semen is collected at frequent intervals. (Insert Fig. 2-1-15.)

1-3 Freezing of Semen

For bull semen to be frozen, it is necessary that the semen have good characteristics and satisfactory resistance to freezing, in addition to an excellent genetic nature. The semen's resistance to freezing should be examined beforehand. In practice, semen with 35 - 40% or more motility moving spermatozoa after thawing is regarded as semen with good resistance to freezing. The straw method is used worldwide to freeze bovine semen. This is a method in which semen in a straw is rapidly frozen with liquid nitrogen gas.

(1) Diluent

● Glycerin and Egg Yolk Citrate Solution

This is the solution used by Polge & Rowson when they succeeded in freezing bovine semen. It is a basic diluent used for freezing semen. The egg yolk and concentration of glycerin were studied subsequently, and solutions with the following compositions are used at present.

● Solution A (the first diluent)

To 5.6 g sodium citrate dihydrate, add sterilized distilled water to prepare 200 cc of solution. After sterilizing by boiling with a double boiler, or by direct boiling, cool the solution, add 40 cc of fresh egg yolk to 160 cc of the solution, and mix well by stirring.

● Solution B (the second diluent)

To 86 cc of solution A, add 14 cc of glycerin (special grade), and mix well by stirring. To solutions A and B, add penicillin and streptomycin at concentrations of 500 - 1,000 U/cc and 500 - 1,000 μ g/cc, respectively.

As well as the solutions described above, other diluents are used by other scientists or artificial insemination center. These solutions are: a modified sodium and egg yolk citrate solution (the amount of sodium citrate is reduced a little, approximately 1 g of glucose is added, and the amount of egg yolk is reduced to 25%); citrate buffer tris-hydroxymethyl-aminomethane and citric acid are used instead of sodium citrate); and the supernatant of a mixture of raffinose, lactose and egg yolk (raffinose, lactose and 20% egg yolk are added and mixed, the mixture is left to stand at 4 °C, and the supernatant is used). In all cases, glycerin is added to solution B.

● Egg Yolk-Tris-Suggr Solution

● Solution A (the first diluent, per 1,000 cc)

Dissolve tris-aminomethane (17.25 g), citric acid (9.65 g), lactose (15.55 g) and raffinose (27.95 g) in sterilized distilled water to prepare 880 cc of solution. After sterilizing by boiling with a double boiler, or by direct boiling, cool the solution, and add 220 cc of fresh egg yolk, and mix well by stirring. Add penicillin and streptomycin to the solution at concentrations of 1,000 U/cc and 1,000 μ g/cc, respectively, mix well, and use the supernatant.

● Solution B (the second diluent, per 500 cc)

To 500 cc of solution A, add 74.7 cc of glycerin (special grade), and mix well by stirring.

(2) Glycerin equilibration

The semen dilution prepared by dilution with solution B containing glycerin (the second solution for dilution) is

subjected to freezing after being left to stand at 4 - 5°C for a certain period of time. The time from immediately following the second dilution to the start of freezing is called the glycerin equilibration time. During this time, the spermatozoa acquire the ability to survive freezing because of the glycerin-induced dehydration and the permeation of glycerin into the cell (dehydration is necessary before freezing). Generally, bovine semen is subjected to freezing after 2-16 hours' glycerin equilibration time.

(3) Diluting and freezing of semen

(3-1) First dilution

Carry out the first dilution (up to five-fold) using solution A at approximately 30°C (near the temperature of semen) after motility testing has been completed. Place the diluted semen in an incubator at 5 °C , and gradually cool to 5 °C over 60-90 minutes.

(3-2) Second dilution

Carry out the second dilution in an incubator after the temperature of the first semen dilution is reaches 5°C . First, dilute with solution A (at 5°C) again to prepare half the amount of the final dilution; then, dilute this solution with an equal amount of solution B (at 5°C). Since solution B has high osmotic pressure, repeat the dilution approximately four times with a quarter of the diluent each time at intervals of 10 - 15 minutes, or carry out dilution by dripping in diluent. These methods serve to make the osmotic pressure of the dilution rise gradually. (Insert Fig. 2-1-16.)

(3-3) Printing on straw

To tell the sealed straws containing diluted semen a part, write or print information, such as the identification code of bulls, their name and the date of semen collection, on the straw. In facilities which handle only a small number of straws, a step-type printer is often used, whereas in facilities handling a large number of straws, often an automatic straw printer is used.

(3-4) Filling and sealing

Filling the diluted semen (after completion of the second dilution) to straws in an incubator at 5°C, and seal the straws. Leave a space of approximately 1.8 cm between the bottom of the seal and the surface of the semen dilution. When an automatic filling and sealing machine is used, the time taken for this process can be shortened.

(3-5) Glycerin equilibration

After 2-16 hours of glycerin equilibration, start preparation for freezing. When a simple rapid freezer is used, stand the straws in the wire basket. In some artificial insemination center which handle a large number of straws, the straws are placed horizontally on a straw rack.

(3-6) Preparation for and the process of freezing

① Simple rapid freezer

- 1) Pour liquid nitrogen into the freezing tub to a level a little higher than the stated level, cover the tub, and cool the inside of the tub for approximately 10 - 15 minutes beforehand.
- 2) After preliminary cooling, confirm that the liquid

nitrogen reaches the stated level. If not, add further liquid nitrogen till it reaches the stated level.

- 3) Hang the wire basket (in which the straws are standing) on its holder in the freezer, and gradually let the wire basket down using the coarse and fine adjustments. It takes approximately 2 minutes to lower the basket to the 0 mark using the fine adjustment.
- 4) Leave the basket in this position in the nitrogen vapor for approximately 5 minutes (making a total of 7 minutes); then, let the wire basket down further by operating the fine adjustment, until the basket is completely submerged beneath the liquid nitrogen. (Insert Fig. 2-1-17.)

② Freezing with horizontal placement

- 1) Fill the freezer with liquid nitrogen up to a certain level, and cool the freezer beforehand to fill the vacant space of the freezer with nitrogen vapor. Check the level of liquid nitrogen in the freezer before the start of freezing. If the level is low, add nitrogen up to the stated level.
- 2) Place the sealed straws horizontally on the straw-freezing rack.
- 3) Insert the automatic temperature recorder sensor into one of the straws containing the diluted semen, and place this on the straw rack as described in 2).
- 4) Place the straw rack described in 3) on the

punching plate in a freezer filled with liquid nitrogen vapor to begin freezing of the semen dilution in the straw.

- 5) When the semen in the straw has frozen to some extent, the temperature of the semen increases temporarily. Immediately switch the heater for producing liquid nitrogen vapor, on.
- 6) The temperature of the semen rapidly decreases as the liquid nitrogen vapor is produced by the heat from the heater. In this way, freezing is completed. (Insert Figs. 2-1-18 and 2-1-19.)

The temperature curve for semen in the straw during processing should bear a close resemblance to that shown in Fig. 2-1-20.

(4) Storage of frozen semen

Store the frozen semen in a cannister at -196°C in a frozen-semen storage tank filled with liquid nitrogen. Add further liquid nitrogen if the level in the storage tank is lowered by $1/3$, to maintain the storage temperature at -196°C .

In many cases, frozen semen is transported in straws in frozen-semen storage tank that have a volume of 20 - 30 l and are filled with liquid nitrogen. The person who conducts artificial insemination often uses a frozen-semen storage tank with a volume of 10 - 17 l.

When removing the frozen semen, keep the top of the cannister at a level 10 cm below the mouth of the storage tank, and remove the required number of straws within a few seconds. Pay particular attention that the semen temperature does not increase beyond -130°C , in order to maintain adequate vitality of the semen after thawing.

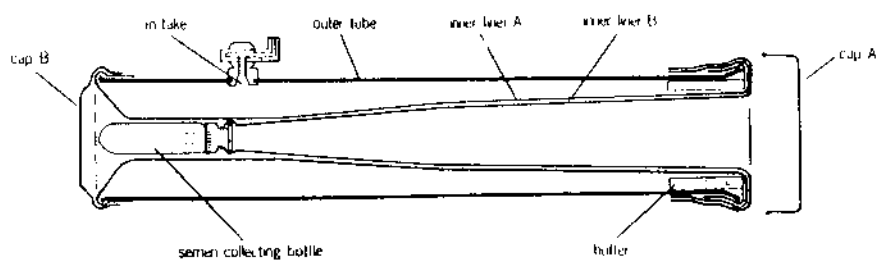


Fig. 2-1-1 Triple-walled artificial vagina

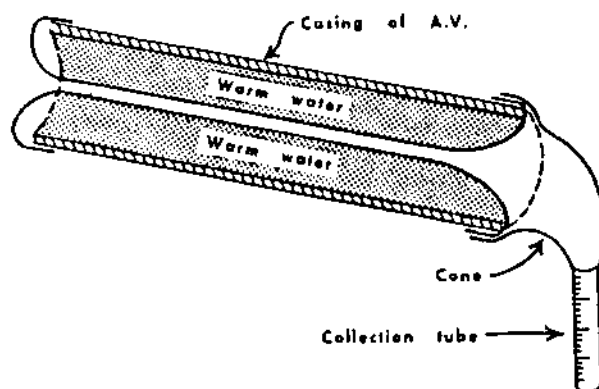


Fig. 2-1-2 Double-walled artificial vagina

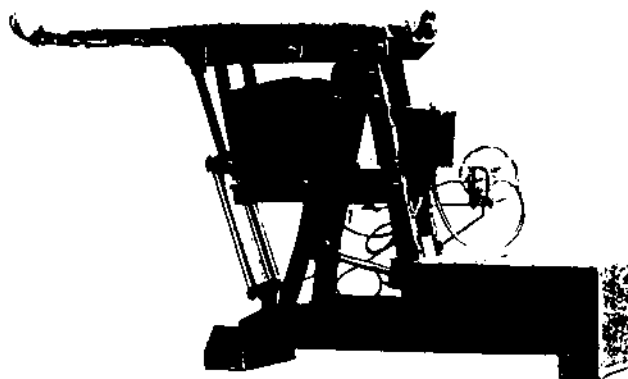


Fig. 2-1-3 Dummy cow



Fig. 2-1-4 Semen collection room

Left: dummy cow;

right: stanchion for tether bull for mounting

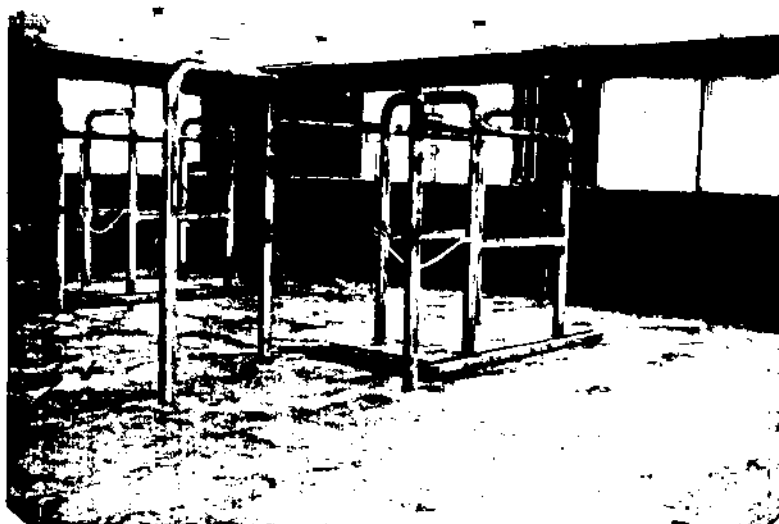


Fig. 2-1-5 cattle squeezer

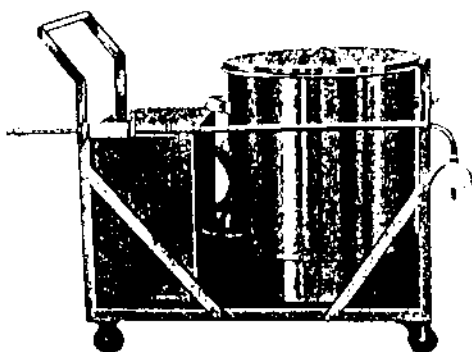


Fig. 2-1-6 Prepuce douche and mozzee

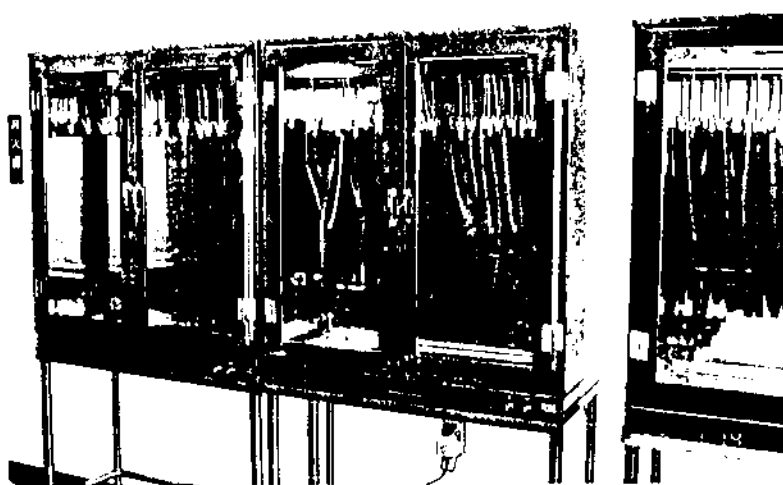


Fig. 2-1-7 Box for sterilizing and storing inner rubber liner



Fig. 2-1-8 Storage of artificial vagina with inner rubber liner attached) in incubator



Fig. 2-1-9 Semen collection using dummy cow



Fig. 2-1-10 Semen examination plate



Fig. 2-1-11 Slide warmer

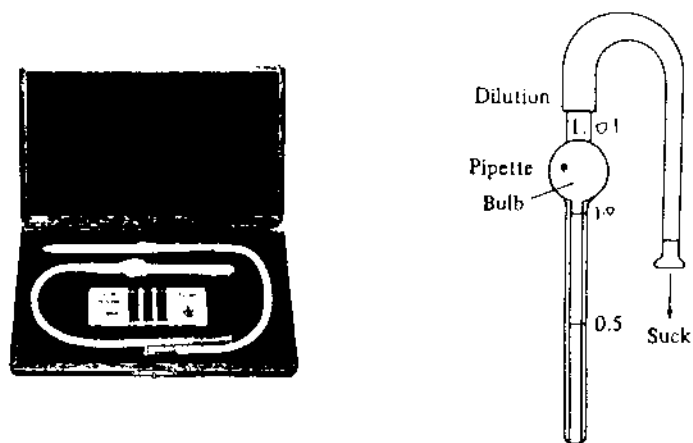


Fig. 2-1-12 Hemocytometer set and pipette

Hemocytometer

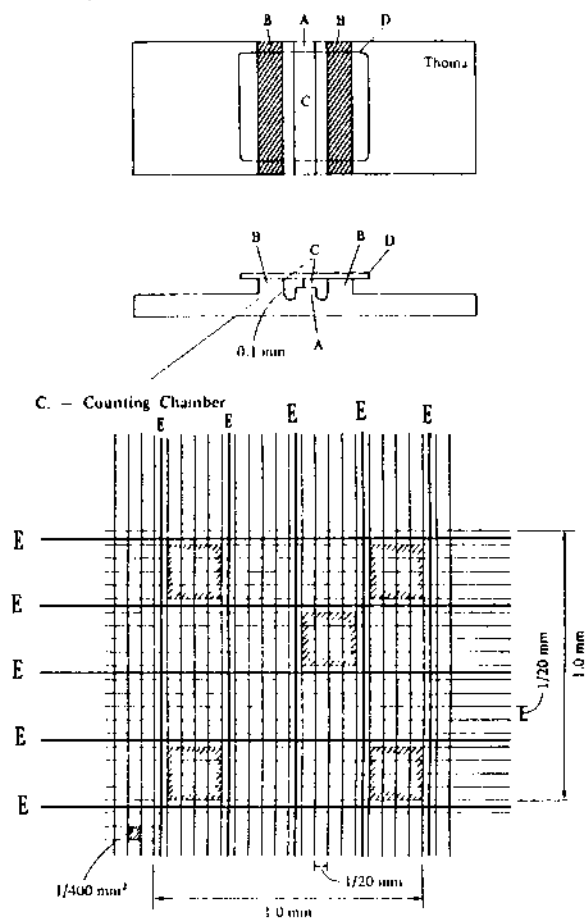


Fig. 2-1-13 Hemocytometer

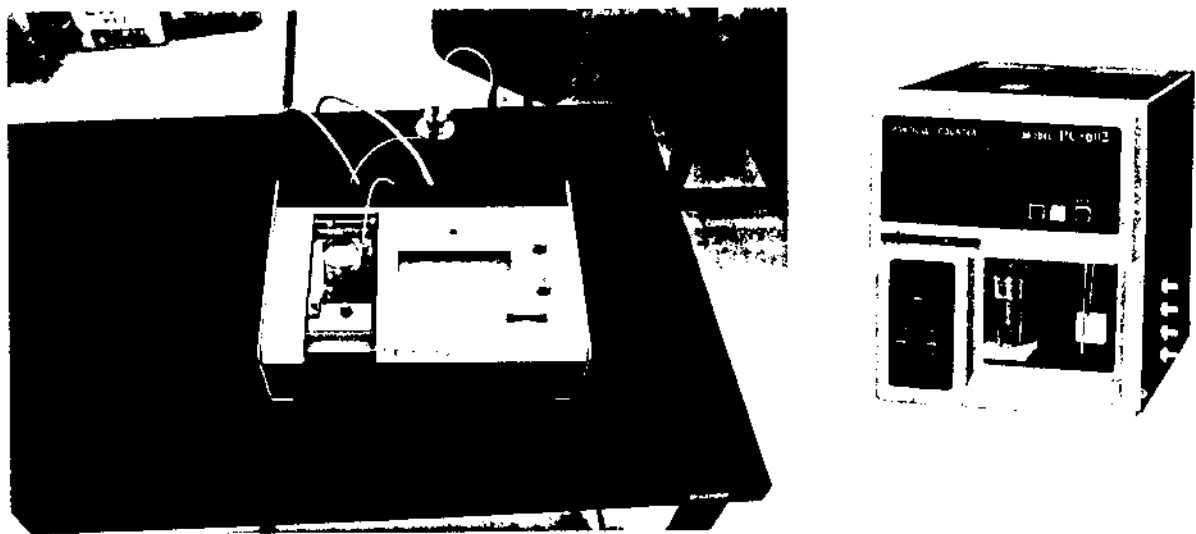


Fig. 2-1-14 Spectrophotometer Call counter

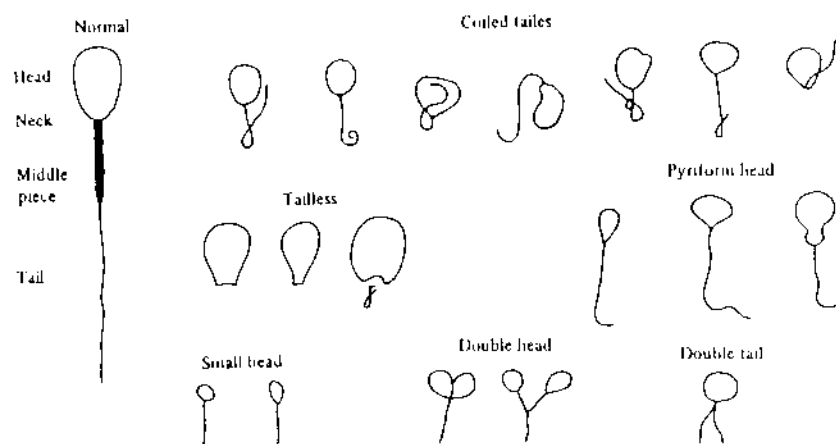


Fig. 2-1-15 Abnormal spermatozoa

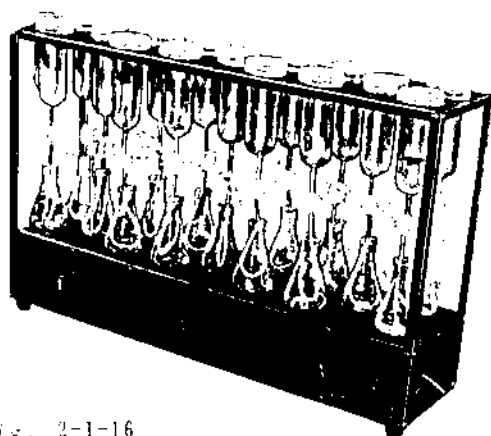


Fig. 2-1-16

Drip dilution apparatus for preparing the second dilution

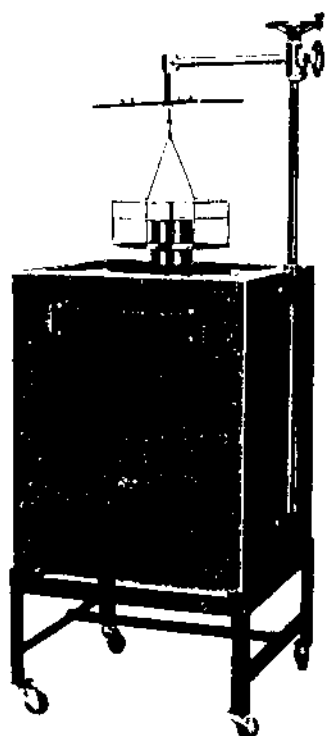


Fig. 2-1-17 Simple rapid freezer



Fig. 2-1-18 Left: automatic temperature recorder;
right: large freezer for horizontal placement

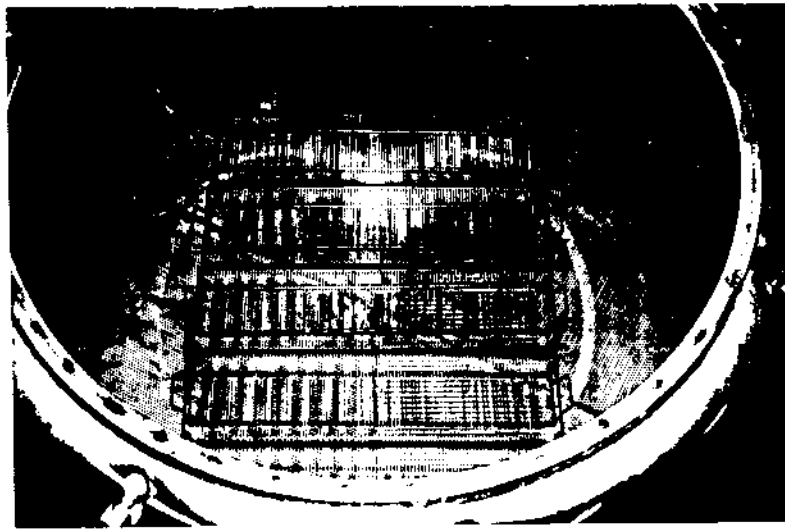


Fig. 2-1-19

Freezing horizontally-placed straws in large freezer

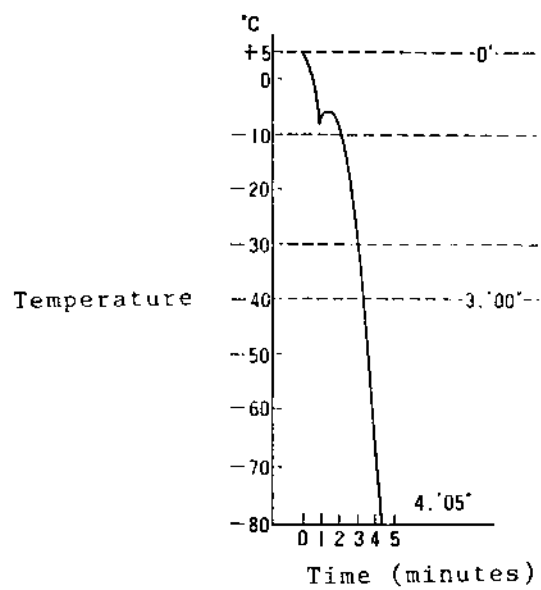


Fig. 2-1-20

Temperature curve during freezing (Nishikawa, 1973)

2 Straw Insemination Procedures

2-1 Thawing and injection of Frozen Semen

(1) Thawing

There are 2 methods of thawing: thawing in a yard and thawing in the station and the method of thawing differs depending on the situation.

Thawing in the a yard is carried out as follows; frozen semen is carried to the farmer's yard in a frozen semen container by the personnel who will conduct the insemination, and is allowed to thaw immediately before insemination.

Thawing in the station is carried out as follows; frozen semen is thawed in the substation by personnel who do not have a frozen semen container. Liquid semen is carried to the place of insemination in a vacuum bottle.

In the case of thawing in the yard, semen can be used for insemination immediately after thawing. Thawing is generally conducted at 30 - 40°C. Examine a cow in estrous to determine the state of estrous and determine if she is at the appropriate period for insemination. If insemination is decided upon, wipe and disinfect the external genitalia of the cow to prepare for insemination. Place the frozen straw in luke warm water (35 °C) in a plastic beaker of approximately 600cc immersing the straw (where the frozen semen is present) in water with the cotton stopper downward. When using a 0.5cc straw, frozen turbid semen is thawed and becomes semi-transparent within 10 - 15 seconds, and bubbles start to come from the cotten stopper at the bottom of the straw. Immediately after the completion of thawing, remove the straw from the water and place the straw in an injector. Then carry out insemination. It is considered that the viability and metabolic capability of spermatozoa

thawed at 30 - 40°C are better than those thawed at 4°C or 20°C, since rapid thawing is possible at 30 - 40°C. However, if semen is left to stand in lukewarm water after the completion of thawing, the viability of spermatozoa decreases. Therefore, pay attention to the timing of removal since it is important to remove the straw immediately after thawing (within 10 - 15 seconds). If lukewarm water is not available, water at 4 - 5°C or approximately 20°C can be used. In this case, revival of spermatozoa is somewhat poorer than in the case of thawing in lukewarm water.

When thawing is carried out at 4°C, the ratio of the number of surviving spermatozoa is 60% (3 hours after thawing) and 10% (6 hours after) compared to that counted immediately after thawing. However, when thawing is carried out at 38°C the surviving ratio 3 hours after thawing is 81% and at 6 hours is 60%. The surviving ratio when thawing is carried out at 20°C is between the values at the temperatures shown above. The average time needed for passing from -60°C - 0°C (the temperature range most injurious to frozen semen) is 61 seconds when thawing at 4°C; whereas it is only 7 seconds at 38 °C.

Thawing in the station is conducted in a similar way to that described above. Frozen semen is thawed in a plastic beaker containing water at 5 °C. Thawed semen is placed in a vacuum bottle containing water at 5°C and small pieces of ice before being used for insemination. Ensure that the temperature of the water in the vacuum bottle does not go below 4 - 5°C. Conduct insemination as early as possible after thawing and within 8 hours at the latest.

The viability of spermatozoa revived from frozen semen is low compared with that of fresh semen. The viability of spermatozoa introduced into the reproductive organ of a cow is low and the time in which the ability to conceive is retained is short for thawed semen. Therefore, it is necessary to choose an

appropriate period of insemination and to perform correct injection in order to obtain a high rate of conception. Injections must be made in the correct area, i.e. deep into the endocervix near the internal orifice of the uterus or the corpus uteri.

(2) Injection of semen

The technique of semen injection is an important factor in artificial insemination and this process has a marked influence on the conception rate. As a foundation for obtaining a higher conception rate, personnel who practice artificial insemination should also act as leaders in teaching the management of breeding animals. Cows that are scheduled for insemination should be in a physiologically normal conditions in which reproduction is controlled by sex hormones, should show appropriate signs of estrous and should produce normal ovarian follicles.

The following techniques are required for semen injection; appropriate handling of semen, sterilization of implements for injection, determination of the appropriate period for insemination, and practice of accurate and hygienic injection at the proper site.

(2-1) Amount of semen for injection

The usual standard for the amount of semen for injection in Japan is 0.5cc - 1.0cc (the number of spermatozoa injected is 25 to 50 million). At present, 0.5cc of frozen semen for injection is employed but in other countries 0.25cc of frozen straw semen is used.

(2-2) Site of injection

The site of injection is deep into the endocervix near the

internal orifice of the uterus. In the method of injection into the uterus, the site of injection is the uterine body.

It is necessary to take care not to cause fenestration with the injector in the uterus since the long diameter of the uterine body is only 4 - 6 cm. When cows are in estrus due to a pregnancy, there may be the danger of miscarriage if the fetal membrane is broken with an injector. Therefore, it is necessary when making an injection into the uterus, that the injector is placed at the internal orifice of the uterus from where only the foremost part of the tip of the injector can enter the uterus.

(2-3) Detection of estrous and optimum time of insemination

① Estrous detection

The primary factor in good reproduction is to clearly detect the estrous cycle. Detection of standing estrous

(a state in which the cow allows herself to be mounted) is the most certain method. The time of pasturage after milking in the morning or the duration of exercise in the paddock is the best time for observation. At least 4 - 5 cattle are needed to detect a cow in standing estrous .

Apart from standing, other symptoms also show that cows are in heat (Table 2-2-1).

There is congestion or swelling of the external genitalia. Transparent and sticky mucus is discharged and sometimes adheres to the tail and buttocks. In cows kept in a cow shed all the year round, the following symptoms are observed; the cow in estrus remains standing while other cows lie down, she approach her neighbors in an unnatural way, she nestles up to people who happen to pass by, she shows a decrease in

milk production, and she has a poor appetite. In cows exercising in a paddock, the following symptoms are observed; cows move around more actively than usual, they sniff at the external genitals of other cows, they nestle up to other cows and she may place her chin on the hip or the back of other cows, and she tries to mount other cattle. When one cow runs away from other cows advances (which is seen at the beginning of heat) then this is not a period of estrous. Such symptoms and behavior appears about 8 hours before the start of heat. The cow then stops running away from other cattle's advances, bellows fiercely, becomes restless and nervous, and sometimes raises and wags her tail. This is estrus and lasts for 12 - 18 hours. After estrus the cow again refuses to stand near other cattle, but tries to mount them, sniffs them, and discharges mucus from the external genitalia.

Cows in estrus lose hair on the skin of the coccyx or on the front of the ischium tuberculum, or suffer grazes when they allow themselves to be mounted by other cattle. Sometimes, a bloody discharge is secreted from the genitals about 2 days after the termination of heat.

In order to clearly detect these symptoms, it is important to make accurate records of everyday observations of cows and the estrus cycle after delivery.

From results of investigations, it is known that 60% of estrus activities are seen during the period from 6 o'clock in the evening to 6 o'clock the next morning and most of the remaining 40% are seen soon after feeding in the morning. Observations in the early morning are particularly important and if observations at night when daily work has been completed are also carried out, satisfactory records will be obtained.

② Optimum time of insemination

Injection of semen at the appropriate time is important in obtaining a high conception rate.

The optimum time of insemination is between the start of estrous (about 7 hours after a cow begins standing) to the end of estrous . The period before this is too early. Insemination should be performed within 4 hours of the end of estrus since any later and the rate of conception becomes low because the ovum ages (Fig. 2-2-1).

In practical artificial insemination, factors, such as the time since after the start of estrous (time at which estrous is detected) and data from the examination of the rectum used to understand the degree of development of the ovarian follicles, are taken into account and the optimum time for insemination is determined.

In the findings of the ovarian follicles in the examination of the rectum, most ovarian follicles in the middle stage of estrous are elastic and hard, the diameter of the follicle is approximately 1cm in many cases, and the wall of the follicle is of a certain thickness. In contrast, follicles at the end of estrous and near the time of ovulation have a diameter of about 1.5cm (the diameter of some follicles is about 2cm), the wall of the follicle is thin and soft (loses its hardness) and has the feeling of an uneven surface when examined with the hand. The period when the ovarian follicles are in such a condition is the optimum time for insemination.

When an examination of the rectum is not performed in insemination using the endocervical forceps method, the optimum time for insemination is judged from the data of observations such as the degree of congestion of the vaginal mucosa, from the degree of swelling and relaxation of the uterovagina, and from the volume and color of the mucus in the endocervix. Observation is carried out with the vagina opened using a colposcope or with the inside of the vagina irradiated with the light in a colposcope or a pencil-type flashlight.

When a cow is in a state appropriate for insemination, congestion in the uterovagina changes and this part relaxes, the amount of the mucus discharged from the endocervix gradually decreases, and the mucus which is colorless and transparent in the middle of estrous becomes semi-transparent and slightly grayish-white towards the end. This is the right time for insemination.

In practice, when standing estrous is observed in the morning, insemination is conducted in the evening of the same day, and when standing estrous is observed between the afternoon and evening, insemination is conducted the next morning (Table 2-2-2).

(2-4) Method of injection

① Method of injection deep into the endocervix

i . Rectovaginal method

When the hand is inserted into the rectum during insemination using the rectovaginal method or in a rectal examination, the fingernails should be cut short and a cut ends should be made less sharp by

polishing with a file. Otherwise, the rectal mucosa could be damaged.

When a plastic sheath tube straw injector is used, disinfect the semen straw tube and a straw cutter with alcohol-soaked cotton, and cut the sealed side of the straw at right angles near the upper level of the semen. Put the straw in a sterilized and disposable sheath tube (disposable), insert the body of an injector then fix the sheath tube to the body.

Injection is carried out using the following procedures: use a thin polyethylene glove for the rectal examination on the hand to be inserted into the rectum and apply a lubricant such as soap or vaseline to the surface of the glove. Insert the injector into the vagina which is opened with the thumb and forefinger of the other hand. The external genitalia should have previously be disinfected with alcohol-soaked cotton. At first, gently push the injector obliquely upward about 10cm. When the tip of the injector reaches the vestibule of the vagina, move it to the horizontal position and continue to push it slowly. When the tip of the injector almost reaches the external orifice of the uterus, hold the injector gently in this position. Slowly insert the gloved hand into the rectum. Gradually push the hand in time to the groans of the cow, and take hold of the uterocervix from the rectal side. Grasp the region near the external orifice of uterus in the hand and place the little finger at the side of or under the external orifice of the uterus. Next, lead the tip of the injector, which is located near the external orifice of the uterus into this orifice with the tip guided by the little finger.

Push the injector carefully until the tip reaches the centre of the endocervix. Move the hand forward along the uterocervix, put the forefinger on the internal orifice of the uterus, insert the injector deep into the endocervix near the internal orifice of the uterus while moving the uterocervix. Then inject semen. Keep the hand, in which the uterocervix is held, for approximately 30 seconds to 1 minute after the completion of injection in this position and gently remove the injector ensuring there is no backflow of semen (Fig. 2-2-2).

ii . Endocervical forceps method

Wipe the external genitalia, disinfect the vulva with alcohol-soaked cotton, and prepare semen and the injector in a similar way to the method for rectovaginal injection. Open the vulva with thumb and forefinger, insert a disinfected closed colposcope into the vulva obliquely upward about 10cm. Then, keeping the injector horizontal, insert it deep into the vagina and observe the position and the appearance of the external orifice of the uterus. Insert hook tip of the disinfected endocervical forceps into the external orifice of the uterus about 2cm, clasp the upper left side of the uterovagina with the forceps, pull it forward carefully and press it together with the colposcope with the thumb. Then, carefully insert the tip of the injector-deep into the endocervix and inject the semen. Leave the injector in this position for about 1 minute then gradually remove the injector from the vagina. Return the uterocervix to its original position with the forceps still in place, move the forceps from the external uterus to

outside the vagina, and pull out the colposcope while closed. Although congestion is often seen at the site which is clasped with the forceps, no special treatment is needed. However, it is necessary that implements should suitably disinfected and injection should be carried out under hygienic conditions. It is also important that semen for injection should be treated hygienically and it should contain a suitable amount of antibacterial agent (Fig. 2-2-3).

② Method of injection into uterus

Prepare semen and the injector in a similar way to the rectovaginal injection method described above. Insert the tip of the injector into a site near the external orifice of the uterus, insert the gloved hand into the rectum, and hold the uterocervix. Insert the injector into the endocervix, carefully push it in until the tip of the injector reaches deep into the endocervix. Put the forefinger of the hand holding the uterocervix, onto the internal orifice of the uterus to check the position of the internal orifice. Then, carefully push the injector. At first, there is slight resistance at the internal orifice but this resistance disappears when the tip of the injector enters the body of the uterus. Keep the tip of the injector in this position and inject the semen into the uterus. When the injection is completed, leave the hand holding the uterocervix in this position, remove the injector by gently pulling backward, let go of the uterocervix, pull the injector out of the vagina, and pull the hand from the rectum. Following this, remove the sheath tube of the injector, take the used straw out and discard it together with the cease tube after injection has been completed, wash and sterilize the injector, and store

the injector in its case.

Semen injection should be carried out carefully. Wash the external genitalia and remove soiling such as feces, wipe the area with a dry towel then disinfect with alcohol-soaked cotton, and inject the semen hygienically. Take care not to damage the reproductive organs by mishandling. Sometimes, the tip of an injector contacts the ring-shaped inner wall of the endocervix. If an injector is pushed forcibly, the mucosa may be damaged and bleeding will occur. So never push the injector forcibly. When there is the impression that the tip of the injector is stuck, pull the injector slightly backwards to remove the tip from the wall and try another direction, then slowly push the injector into the uterocervix. In order to obtain a satisfactory conception rate, it is very important to practice careful injection under hygienic conditions.

2-2 Instruments Needed for Injecting Semen

(1) Semen injector

Conventionally, an injector consisting of a straw semen injector and straw beak tube has been used widely, although recently a cease tube straw injector has been used.

There are 2 types in the conventional injector: an injector for rectovaginal injection (approximately 50cm long) and an injector for endocervical forceps injection (approximately 40cm long). In each type, there are 2 subtypes; i.e. for a 1.0cc straw and a 0.5cc straw. Injectors and beak tubes are kept in a sterilization containing case 70% alcohol for disinfection (JP) and is always carried in a state ready for use. At the upper part of the handle of the injector, there is a device for attaching a penlight to illuminate the inside of the vagina.

It is convenient to use a penlight when injections are carried out using the forceps method in a dark place such as a cattle shed.

A sheath tube straw injector is 45cm long and is of 2 types; an injector for a 0.5cc straw and an injector for a 0.25cc straw. Since the part of the injector which is inserted into the female reproductive organs is entirely covered with disposable sheath tube sterilized with ethylene oxide gas, insemination can be carried out hygienically and handling of an injector is simple (Fig. 2-2-4). In addition to these 2 types, there is a pipette-type injector. On the base part of a long and narrow 1cc glass or plastic pipette is a cap and semen is sucked up and then injected. In overseas countries, frozen semen in an ampul is used in many cases. Here, semen in the ampul, after thawing, is sucked into the plastic pipette injector before injection. The pipette injector is used widely as is the straw injector. A glass pipette injector is sterilized by dry heat or by boiling before use. When sterilization by boiling is carried out, suck up sterilized physiological saline or 5 - 6% sterilized glucose solution into the pipette before use in order to remove any remaining water in the pipette. After use, wash the pipette in water and sterilize it again before storage.

A plastic pipette injector is kept sterilized in the package. It is discarded when insemination is completed.

(2) Colposcope and light on the colposcope

A colposcope is used to open the vagina of a cow in the forceps method of injection deep into the endocervix. It is also used to examine the uterovagina. The shape of the colposcope is specific to each type of livestock and depends on the volume and the shape of the vagina. Even in the same species of livestock, there are colposcopes of different sizes and colposcopes for multiparae and nulliparae. Generally, a colposcope with a side opening is used. Sterilize the

colposcope with boiling water, leave it to cool to a temperature near that of the body, and disinfect the surface with alcohol-soaked cotton before use. After use, wash it with water and disinfect it with alcohol-soaked cotton before string. When an examination of the vagina is difficult because the location such as a cow shed is dark, attach a light to the colposcope in order to make the examination of the inside of the vagina or the uterovagina easier.

(3) Forceps for endocervical injection

The forceps for bovine endocervical injection has 3 hooks at the tip, and is, generally, more slender than endocervical forceps.

Endocervical forceps for bovine artificial insemination can be divided into halves for convenient storage. Store the part with hooks in the disinfection case in which alcohol for disinfection (JP) is present and always carry it in the disinfected state. Before use, take the part with hooks out of the disinfection case and combine it with the other part and then wipe and disinfect the whole forceps with alcohol-soaked cotton before use. After use, wash the whole instrument with water, place the part with hooks in the disinfection case, and disinfect the other part with alcohol-soaked cotton before storing.

(4) Fingertip disinfection

Alcohol-soaked cotton is carried in a portable metal container. The alcohol-soaked cotton is used for wiping and disinfecting the fingertips, straw semen tubes, straw cutter, colposcope and external genitalia. Always carry this portable container of alcohol-soaked cotton.

(5) Disinfection of instruments for injection

If treatment is not hygienic, contaminating bacteria may

proliferate in the reproductive organs of a cows and cause various diseases. Therefore, it is particularly important to disinfect the instruments, fingers of the person who conducts the insemination, and the external genitalia of the recipient cow. The method of disinfection differs depending on the instruments. It is necessary to carry out disinfection which does not harm the spermatozoa or reproductive organs. It is important to understand and conduct disinfection properly according to the type of instruments.

(5-1) Boiling disinfection

For disinfecting metal instruments, such as the semen injector, forceps for endocervical injection, and colposcope, boiling disinfection is employed.

Instruments are immersed in boiling water at 95 - 100°C for 15 - 30 minutes to sterilize them.

Since water remains in a semen injector when disinfected with boiling water, it is necessary to remove the remaining water by washing (purging) the inside of the injector with sterilized physiological saline or sterilized 5 - 6 % glucose solution (glucose solution for injection in an ampul can also be used) before sucking up the semen.

To cool instruments rapidly after boiling disinfection, pour the hot water out, with the sterilizer (with the cover on it) inclined. Wrap the disinfected injector and colposcope with sterilized gauze or other such materials and carry in its case to avoid bacterial contamination.

In autumn and winter, a colposcope becomes cold during transportation. It is not good to insert a cold colposcope into the vagina since it causes tension in the reproductive organs. Disinfect the colposcope by applying hot water and

check that the temperature of the colposcope is down to body temperature. Disinfect the surface of the colposcope with alcohol-soaked cotton before use.

(5-2) Alcohol disinfection

Personnel who conduct insemination should carry a portable disinfection container in which alcohol-soaked cotton for fingertip disinfection is contained. Alcohol-soaked cotton is used for disinfection of the fingertips after washing, the straw cutter, the surface of the straw before its upper end is cut, and the external genitalia of the cow after washing. Alcohol used for this disinfection is 70% alcohol for disinfection (JP). As alcohol is highly volatile, the alcohol content decreases with time. Since the disinfectant effect of cotton which was immersed in alcohol some time ago is very low, immerse the cotton in alcohol just before use.

(5-3) Disinfection with invert soap and cresol soap

These agents are used for the disinfection of the external genitalia before inserting the injector to introduce semen.

Since the external genitalia of a cow may be soiled with feces, wash this area well with water or warm water to remove the feces then clean the genitals with a 200-fold dilution of invert soap solution and 1% cresol soap solution. These disinfectants have a very bad effects on spermatozoa and must be handled very carefully.

References

1. Text for Short Class of Livestock Artificial Insemination

Revised edition, Japanese Association of Specialists in Livestock
Artificial Insemination

2. Advanced Livestock Artificial Insemination

Revised edition, Asakura-shobo

3. Text for Livestock Artificial Insemination

Comprehensive revision, Bun-eido

Table 2-2-1 Symptoms of cow estrus

Strong signs	1. Standing Allowing other cattle (cow or bull) to mount her, and remaining standing even after being mounted
Supporting signs	1. Restless movement, excitement 2. Congestion in the eyes 3. Bellowing 4. Poor appetite 5. Reduced production of milk 6. Approaches other cattle, sniffs at the genitals of others, and tries to mount them 7. Congestion and swelling in the external genitals, and discharges mucus

(Source: Text for Short Class of Livestock Artificial Insemination)

Table 2-2-2 Appropriate time for insemination from a practical viewpoint

(Source: Text for Short Class of Livestock Artificial Insemination)

Time of estrous detection	Appropriate time for mating	Delayed
Early in the morning (9 a.m. or earlier)	In the afternoon of the same day	Next day
In the morning (from 9 a.m. to noon)	In the evening of the same day or early next morning	10 a.m. or later on the next day
In the afternoon (12 a.m. or later)	Next morning	2 p.m. or later on the next day

Before estrous	Standing estrous	After estrous	Surviving ova
6 - 10 hours	18 hours	10 - 14 hours	6 - 10 hours
	End of standing estrous 	Ovulation 	
Too early	Appropriate	Optimum	Appropriate
			Too late

(Source: Text for Short Class of Livestock Artificial Insemination)

Fig. 2-2-1 State of estrous and appropriate time for insemination

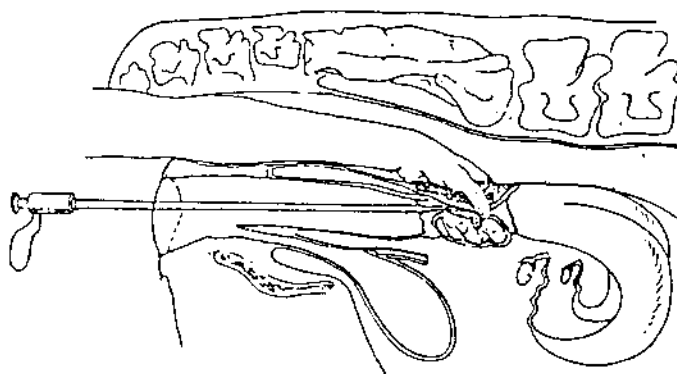


Fig. 2-2-2 Injection by the rectovaginal method

(Source: Text for Short Class of Livestock Artificial Insemination)

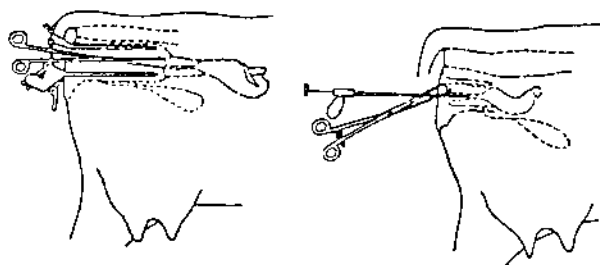


Fig. 2-2-3 Injection by the endocervical forceps method

(Source: Text for Short Class of Livestock Artificial Insemination)

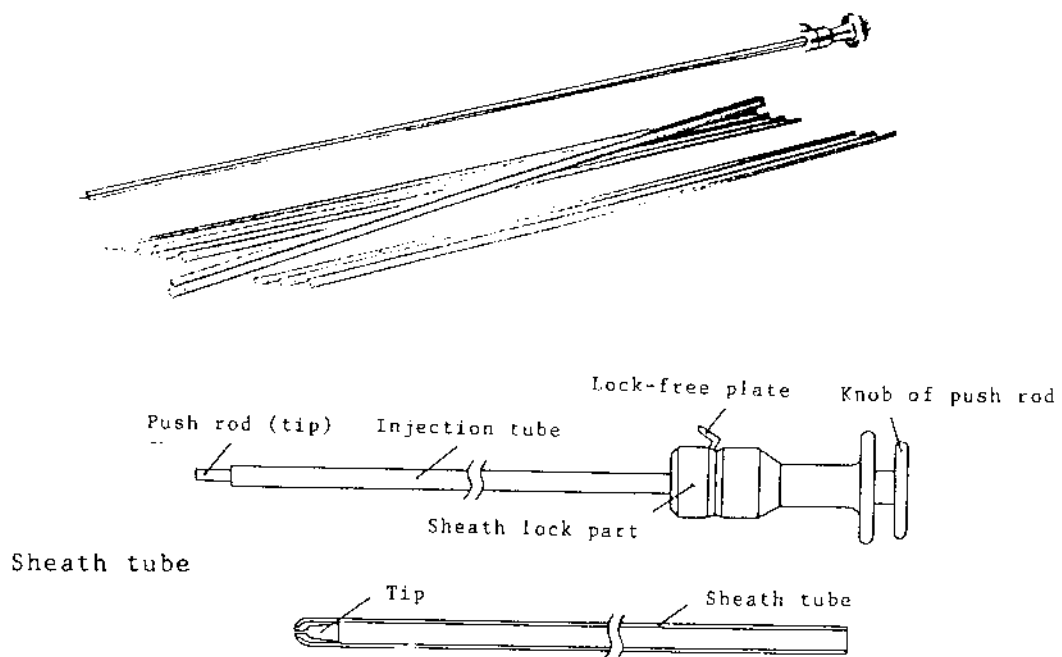
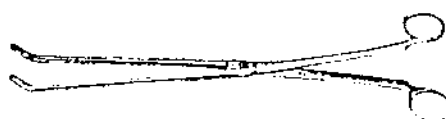


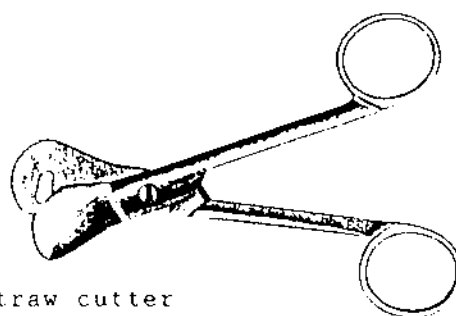
Fig. 2-2-4 Straw semen injector and its structure



Straw tweezers



Straw forceps



Straw cutter

Fig. 2-2-5 Instruments for handling frozen straw semen

3 Maintaining Insemination Records

Animal identification and a good system of record keeping are essential elements of a successful AI program. Cow identification systems allow animals to be treated as individuals. Records show the estrous cycle of each cow and provide a history of insemination service and calving information. Many recording systems are available for both dairy and beef herds. Selecting the methods is the dairyman's or cattleman's responsibility. Accurate and complete records are necessary for successful AI and efficient herd management. This chapter describes information that should be recorded, and gives several examples of how it can be done.

3-1 Dairy Herd Records

The first step to successful AI is a good animal identification system. Most dairymen find ear tags or neck chains work well and help them keep accurate records.

In this section, three types of dairy herd recording systems are shown. A barn breeding chart (barn chart) is a multiple cow record system, while an individual cow record system (ICR) keeps a card on file for each cow or heifer in the herd. There is the other example for heat prediction for better reproduction management.

(1) Barn chart

Generally, the barn chart is used with smaller size herds. Insemination information for up to 45 cows may be recorded on the chart with additional records on the insert (Fig. 2-3-1).

Besides space for breeding records, the barn chart also has a "Heat and Gestation Table" for handy reference. By locating insemination dates on the table, dairymen can easily determine dates for dry-off, calving and possible return to heat.

(2) Individual Cow Record System

The second system for records which a dairyman might choose is the Individual Cow Record System (ICR). As the name suggests, a record card is kept on file for each cow or heifer in the herd. More comprehensive than the barn chart, these file cards provide the dairyman with a written history of the cow's health, milk production from the time she enters the herd. One card covers four lactations (Fig. 2-3-2).

Additional cards may be added should a cow remain in a herd for more than four lactations.

Filed by cow or heifer identification number, cards for the entire herd can be kept in files or in a ring-type notebook.

Color-coded file signals may be attached to the monthly sequence at the top of each cow card. These signals identify the reproductive status of each cow as well as those cows with problems. Refer to the table for suggested use of colored file signals (Table 2-3-1).

Efficient herd management requires a daily check of the records. Therefore, all records should be located in the barn or dairy office and be easily accessible.

(3) Heat prediction chart

Heat prediction charts are particularly useful for heat detection in large herds and those with 'quiet' heat problems. The chart consists of columns 21 days long. A cow's estrus period is recorded beside the date it occurs, then her next possible heat is expected around the date directly beside the previous entry, as shown in Fig. 2-3-3.

For each cow, all heats are recorded and the previous heat crossed out. The cow number is circled at mating. Cow 65, for example,

was on heat on January 12th. She came on heat for a third time on February 23rd and was mated again.

3-2 Beef Herd Records

Animal identification is essential in beef herds. By combining proper identification and records, each female becomes an individual. The cattleman knows which cow is inseminated to which bull and whether or not she has been inseminated before. Plus, it's easier to identify cows in heat, and generally, get a tighter grip on overall herd management.

(1) Identification systems

Many methods such as hot iron branding, freeze branding, ear tags, tattoos, dyes and paints, brisket chains are available.

Brands serves as an excellent form of permanent identification. Calves ear tagged at birth should be branded at weaning time.

Although branding with hot irons is the traditional procedure, many cattlemen are finding that freeze branding also works well and is more humane. With this method, a very cold iron destroys pigment cells that produce dark hair. Where irons were applied, white hair then grows. A freeze brand causing permanent hair removal can be used on animals with light colored coats.

Ear tags are also widely used. A double-tagging system consisting of a large easy-to-read plastic tag in each ear gives extra identification security should one of the tags be lost.

Tattoos provide permanent identification. However, they are impossible to read without putting animals through a chute. For easier identification, use ear tags or brisket tags in addition to tattoos.

Dye and paint are often used to apply temporary identification

shortly before the breeding season. Although the brands only last 2 to 4 months, they can be very large and easy to read from a distance.

Brisket tags are used successfully in some parts of the world. Neck chains are not recommended for commercial herds because they are more costly, plus there's the danger of an animal choking or getting hung up on a fence or bush.

(2) Record forms

There are three types of records in this section which cattlemen may find helpful in managing beef herds, for example.

An individual beef cow record is the most detailed (Fig. 2-3-4). It provides space for recording insemination information for individual cows, along with calf weights, weaning age and remarks on pregnancy or cow health. Pedigree information may be recorded on the reverse side of each card.

"Shirt pocket" size record books are available for convenient use in the field. In a heat detection record book the cattleman may record dates of last calving, heat detection, heifer size, temperament and breeding information (Fig. 2-3-5).

A beef herd record book provides space for calf birth dates, sex, calving ease notes, birth weight, sire number and weaning weight (fig. 2-3-5).

These books are handy for field reference. However, all information should be transferred to permanent herd records such as the individual beef cow record cards.

3-3 What Should be Recorded?

Insemination information should be recorded immediately following the

procedure. Completing records later can result in lost information and inaccuracy.

Date of insemination -- month, day and year -- must be recorded at the time of breeding.

Cow identification can be taken from ear tags, neck chains, brands, tattoos, barn names or registration papers.

The bull code number consists of three parts. Using 29 H1234 as an example, the first number identifies the AI organization processing the bull's semen.

The capital letter or letters identify the breed of the bull. "H" stands for Holstein in this case. The four-digit number "1234" identifies the specific sire within the breed. Normally, there are several bulls of different breeds with the same four-digit code even in one institution, so breed letters must be written down.

The four-digit collection number or collection date printed on each straw must also be recorded. In the unlikely event there is a question regarding semen quality, the collection number will allow for a quality control check of the semen.

Immediately after removing the straw from the thaw water, double check bull identification. Act quickly to avoid exposing the straw to temperature extremes.

Table 2-3-1 Using Colored File Signals

GREEN

Identifies the month of calving. Allows dairymen to predict when cows should be returning to estrus (heat) after calving.

WHITE

Identifies the month of insemination. When the cow is inseminated, the green tag is removed and the white one attached to the appropriate month. If insemination is repeated, the signal is moved accordingly.

YELLOW

Identifies month cow is due to calve. For efficient herd management, cows are generally pregnancy checked 45 days following the last insemination. If the cow or heifer is pregnant, the white signal is removed and a yellow file signal is attached the month the cow is due to calve.

RED

Indicates reproductive complication. It may mean difficult calving, cows not coming into heat or having irregular heats, or cows continually returning to heat. The red signal is attached to the current month, and the other signal is left on to indicate the last event of the heat cycle.

BLUE

Indicates month heifer should be vaccinated for brucellosis.

[illegible]

Fig. 2-3-1 The Barn Breeding Chart.

Fig. 2-3-2 The Individual Cow Recording System.

Fig. 2-3-3 The Heat Prediction Chart.

Fig. 2-3-4 The Individual Beef Cow Record.

Heat Detection Record

[illegible]

Beef Herd Record Book

[illegible]

Calving Ease Codes: 1 = No assistance 2 = Some assistance 3 = Mechanical assistance 4 = Caesarean 5 = Abnormal presentation

Fig. 2-3-5 The Heat Detection Record Book and the Beef Herd Record Book.

4 Reproductive Disorders

Reproductive disorders in cattle may result from any abnormalities in the processes from sexual behavior to delivery of healthy calves, including estrus, ovulation, fertilization, movement of eggs in the oviduct, the growth of the embryo, implantation, the growth of the fetus, and delivery. Several factors, such as the environment (climate, nutrition and feed management, heredity, infectious agents, endocrine disorders and stress) are considered to affect the reproductive process. These factors affects reproduction, not always singly but in combination. Reproductive disorders should be considered as a wide concept including anestrus causing inability to mate, infertility such as failure to ovulate and conceive, and early embryonic mortality, fetal mortality, diseases before and after parturition, and deaths of newborn animals (Fig. 2-4-1).

Reproductive disorders in bulls are excluded from the scope of this chapter.

4-1 Etiologic Classification of Reproductive Disorders

Reproductive disorders are etiologically classified into four categories of hereditary and genetic disorders, infectious diseases, endocrine diseases and diseases having other pathologic etiologies.

They are, however, often classified according to the diseased organs. For example, into ovarian disorders and uterine disorders.

(1) Hereditary Diseases

Hereditary abnormalities are caused by such factors as recessive genes, mutations, abnormal chromosomes, and failure in development and differentiation stages. In particular, abnormal animals showing hereditary defects should be culled out as early as possible.

(1-1) Freemartin

In the case of twins or multiplets of either sex, females become sterile to an extent greater than 90%. The anastomoses of fetal membranes is considered to cause abnormal sexual differentiation but the mechanism is unknown. Recently, the potential involvement of H-Y antigens has been studied. Freemartins are diagnosed by confirming a mosaic composition of blood groups and chromosomal chimeras, and the following rapid differential method can also be used.

[Rapid differential method of freemartin determination]

A newborn female calf suspected of being a freemartin can be diagnosed by measuring the vaginal depth using a small test tube. The depth of the vagina in normal female calves is 12-15cm but the vagina of a freemartin is as short as 5-7cm. This examination should be conducted using a lubricated test tube about 1.0cm in diameter. A calf having a closed hymen may be misdiagnosed as a freemartin.

(1-2) Hypoplasia and Aplasia of Reproductive Organs

In the reproductive organs (ovary, oviduct, uterus, cervix and vagina), several degrees of hypoplasia and aplasia are observed. Notably, in the case of ovarian hypoplasia, it is necessary not to confuse it with delayed puberty or ovarian atrophy caused by extreme malnutrition in nulliparous heifers. Double external orifices of the cervix are sometimes found, but this does not always indicate sterility unless cervical stenosis is observed.

(2) Infectious Diseases

Major infectious diseases causing reproductive disorders are summarized in Table 2-4-1. Other infectious diseases may

influence reproductive performance if they affect the overall condition of the animals. It is necessary to survey local and endemic diseases.

(3) Endocrine Diseases

Most endocrine diseases causing reproductive disorders are secondary hormonal disorders induced by various factors such as nutrition, stress and lactation. Most ovary dysfunctions and fertilization failures, and early embryonic mortality are included in this category and details of each disease will be described later.

(4) Other Pathological Etiologies

There are causes other than those mentioned above:

(4-1) Injuries

Attention must be paid because there are other injuries occurring to genital organs in addition to accidental problems at the time of natural mating or delivery. Adhesion of the ovaries or oviducts may occur as an aftereffect of the manual removal of corpora lutea or the follicular cysts may rupture. Intrauterine perforation by an inseminator sometimes leads to uterine abscesses. One cannot remove retained placenta without injuring the uterine membranes or cotyledons even though this may be carried out carefully.

(4 2) Genital Organ Tumors

Tumors are rarely found in the ovaries and uterus. Differential diagnosis must be made between tumors and abscesses and the prognosis is not good.

4-2 Ovarian Diseases

Major ovarian diseases are summarized in Table 2-4-2.

(1) Ovarian Cysts

Roberts defined ovarian cysts as anovulatory follicles remaining in the uterus for more than 10 days and which are more than 2.5cm in diameter. This disorder is found especially in cows with high milk yield but occasionally in beef cattle. Kesler made a review of this disease.

Cow with ovarian cysts normally exhibit frequent or irregular estrus but some cows show no estrus. Frequent estrus is called nymphomania and cows with this disorder show signs of continuous estrus behavior, arelaxed sacrospinous ligament and raised tail root. However, most anestrus cows are not discovered until the rectal palpation is performed because they exhibit no special external signs.

There are two kinds of ovarian cysts; follicular cysts and luteal cysts. Follicular cysts comprise one or more cysts which have follicular walls. Corpus luteal cysts usually comprise one cyst having walls somewhat thicker than those of follicular cysts. This wall results from the proliferation of luteal tissues and it is considered that the inner wall of follicular cysts changes into this wall type after a period of time. Corpus luteal cysts must be differentiated from cystic corpus luteum. Cystic corpus luteum are recognized as completely normal if there is a normal estros cycle.

As shown by the time course assays of hormone levels in cows with ovarian cysts (Kesler et al.,1980), the signs of ovarian cysts are not static but change from time to time, showing no tendency common to all diseased cows. It may be that this disease is not present before diagnosis is carried out, i.e, the

time of onset cannot usually be determined.

As a direct cause of ovarian cysts, low secretion of LH from the pituitary gland is thought to be important but the detailed mechanism is unknown. The presence of hereditary factors, the relationship to milk yield, nutritional status and season, and the effect of stress are also considered important.

(2) Persistent corpus luteum

A persistent corpus luteum means that corpus luteum do not regress and an estrous cycle is not exhibited. Attention must be paid to the fact that abnormal intrauterine contents are involved in most cases. The contents include pyometra, maceration of the fetus, myxometra and mummification of the fetus. It is because luteolytic factors are not produced or do not function due to the presence of such abnormal contents that estrus does not return. A uterine examination and re-examination after an appropriate interval are necessary as a cow having silent estrus may often be misdiagnosed as having persistent corpus luteum.

(3) Ovarian Dysfunction

Feeding of poorly nutritious feed especially low-energy feed, induces delayed puberty in nulliparous heifers and delayed return of estrus after parturition. The cow develops anestrus, and the ovarian surfaces are flat without corpus luteum and noticeable follicles.

Nulliparous heifers whose genital organs show retarded growth due to malnutrition, are referred to as heifers with ovarian hypoplasia. Ovarian quiescence and ovarian atrophy are almost the same. In particular, cows with atrophied and hard ovaries and a small uterus are known as those with ovarian atrophy.

4-3 Uterine Diseases

(1) Endometritis

In addition to the infectious diseases shown in Table 2-4-1, there are other diseases caused by non-specific resident bacteria. As these bacteria infect the animals via the vagina, attention must be paid to the onset resulting from dystocia and delayed fetal membranes as well as to artificial introduction of bacteria into the uterus. In application of artificial insemination techniques, there is a high possibility that the use of contaminated semen or inseminators cause endometritis. For example, it is reasonable to think that inappropriate artificial insemination of a cow at the luteal phase forces vaginal bacteria into the uterus, causing endometritis because the uterus at the luteal phase is highly sensitive to such bacteria.

As the signs of endometritis vary widely, it is possible that mild signs are not discovered for a long time and persist without conception. Daily observations by farmers are recommended. Endometritis is sometimes diagnosed by an increase in leukocytes in a smear preparation from diagnostic uterine irrigation or mucus taken from estrus.

As treatment, intrauterine administration of a drug solution after uterine irrigation (omitted in mild cases) is performed.

(2) Pyometra

Infectious pyometra is caused by trichomoniasis but general cases occur following to pyoendometritis. It also occurs as a result of bacterial infection of an early dead fetus. It is misdiagnosed as conception because the uterine wall becomes thin and the uterus becomes distended and is fluctuant on palpation. Persistent corpus luteum are frequently seen.

In the case accompanied by persistent corpora lutea, PGF2 α or its analogs are administered. If this is insufficient, intrauterine administration of a drug solution is carried out in the same way as in endometritis.

4-4 Repeat Breeders

If mating has been performed at all stages of estrus for three or more successive cycles and conception has failed without known causes, the cow is known as a repeat breeder.

In the event that a more precise examination method is established in the future, more information will be gathered and it may be seen as a specific disease. Possible causes are an imbalance of hormones, a bad environment for the oviducts and uterus, early embryonic mortality, immunologic incompatibility, a time lag of insemination, and thermal stress.

When a cow is suffering from very mild sub-clinical endometritis, embryonic mortality may occur even 8 to 16 days after fertilization.

In this case, the estrus cycle repeats itself at its normal duration. If it returns to a long cycle of about 30 days, embryonic mortality at older ages is noted.

4-5 Diseases During Gestation and Before and After Parturition

The major diseases during gestation and before and after parturition are summarized in Table 2-4-3.

(1) Abortion

Abortion is where the fetus dies before it become viable outside the maternal body and is discharged. Abortion in cattle is said to occur if gestation is 260 days or less.

(1-1) Infectious Abortion

Major infectious abortion are listed in Table 2-4-1. In addition to these diseases, sporadic abortion may occur when cows are infected with serious infections such as parainfluenza type III, foot-and-mouth disease, rinderpest and piroplasmosis.

(1-2) Non-infectious Abortion

Non-infectious abortion are as follows;

- ① Toxicity: Due to chemicals, poisonous plants.
- ② Hormones: Administration of high doses of estrogens, glucocorticoids and PGF_{2α}. Deficiency of progesterones. Habitual abortion.
- ③ Nutrition: Starvation and malnutrition.
Deficiency of vitamin A and iodine.
- ④ Heredity: Embryonic mortality, malformation of fetus and abnormal fetal chromosomes.
- ⑤ Physical: Administration of a drug or artificial insemination into a pregnant uterus.
Stress (transportation, fever or surgery).
- ⑥ Others: Multiple fetuses, allergy.

(2) Mummification and Maceration of the Fetus

If a dead fetus is not aborted the placenta and fetal fluid are absorbed, and the uterus itself is contracted in a process known as mummification. Maceration of the fetus is, by contrast, a state in which the dead fetus is macerated without participation of putrefactive bacteria. Both are frequently

accompanied by persistent corpus luteum.

The treatment of first choice is to discharge the fetus by administering estrogens or PGF 2 α .

(3) Prolonged Gestation

A cow who exhibits no signs of parturition even after 300 days of gestation is suspected of having this disorder. Parturition begins with a signal from the fetus but in the case of prolonged gestation, parturition does not start because most fetuses have a malformed head or abnormalities of the pituitary-adrenal system. Most of the fetuses are stillborn or die soon after birth.

4-6 Diagnosis of Reproductive Disorders

At diagnosis of reproductive disorders, it is important to communicate with the owner as well as to understand the reproductive history of the sick animal as clearly as possible. At the same time, it is necessary to note the nutritional state of the cow, the feeding and management conditions, the presence of other chronic diseases of the udder, digestive organs, legs and hooves.

Recently, the the most up to date technology has been applied to the diagnosis of reproductive disorders. For example, a rapid assay method for sexual hormones, especially for progesterones, is possible and ultrasonic diagnostic apparatus has been introduced into the livestock industry, while the rectal palpation method is still a practical and effective technique used in the field.

Even where rectal palpation is the main diagnostic method, diagnosis should not be carried out only on the basis of the ovarian and uterine findings, but accessory reproductive organs such as the uterine cervix, the vagina and the external genitalia should be observed at the same time. This is necessary because the accessory

reproductive organs are under the direct control of ovarian hormones and, therefore, change according to ovarian changes. The overall diagnosis of these organs makes it possible to carry out accurate diagnosis without mistakes.

(1) Rectal Palpation

When rectal palpation is performed for the purpose of clinical reproductive examinations, the cervix, the uterus, the ovaries and the oviducts can be examined. As already mentioned, this examination depends on the examination of individuals. Therefore, it is important to improve the technique and keep records of the same standards.

(1-1) Remarks for performing rectal palpation and the examination method

Remarks concerning rectal palpation are as follows;

- ① Clip the nails well so as to minimize hemorrhage from the rectum. The trust of the owner will be lost with frequent and excessive hemorrhage.
- ② Regardless of the comments of the owner and the reproductive record, diagnosis of pregnancy must firstly be carried out which makes it possible to avoid incorrect treatment. Owners occasionally mistake individual cows for others and stray cows may have mated naturally in some areas.
- ③ In high multiparous cows, the uterus often hangs down into the abdominal cavity and rectal palpation is sometimes difficult. An easy examination can be achieved by holding and pulling the cervix backward and then inverting the uterine horns to reposition it in the pelvic cavity. When inverting the uterine horns, do

not hold the uterus itself because of the possibility of an existing pregnancy. Be sure to put the fingers around the uterus and hold the broad ligament. (Fig. 2-4-2)

- ④ The rectal membranes sometimes become tensioned due to air intrusion into the rectal lumen during examination, which makes it difficult to palpate. In such cases, forceful palpation must be avoided. Palpation makes it possible to stimulate evacuation by massaging the membranes or to relax the membranes by holding and pulling backward the folds of the membranes after inserting the hand forward. (Fig. 2-4-3).

(1-2) Remarks on the examination of each organ

① Ovary

Even if there are no abnormalities such as adhesion and swelling of the ovaries, care must be taken to check the presence of corpus luteum when examining of the ovaries. In the presence of clearly normal corpus luteum, there is at least no possibility of ovarian cysts or ovarian dysfunction. It is necessary, however, not to make misdiagnosis of ovarian dysfunction for ovaries with small follicles in estrus or ovaries after ovulation, without observing regressed corpus luteum in cows before and after estrus. In the examination of cows showing a normal estrous cycle, the approximate number of days after estrus can be estimated from the shape and softness of corpus luteum.

The lower surfaces of the ovaries are attached to the mesovarium. The mesovarium is held in position with the middle and ring fingers of the hand and palpated with the tips of the thumb and the fore finger. One end of

the mesovarium is held by the ovarian ligament and the other by the ovarian fimbria, which is part of the tubal fimbria. By checking both ends, the position of corpora lutea and ovaries can be determined. When palpating the underside of the ovaries, the ligament and the fimbria can be distinguished because the former is harder and sharply rises up. The exact changes in ovaries can be understood at re-examination of even 2 or 3 weeks later if the approximate positions of the corpus luteum and ovaries and described in the record of ovarian findings.

② Uterus

The size (expressed as finger widths), contractility and elasticity, thickness of the uterine wall, and internal touch are examined. It is important to carry out this examination at a fixed position a little deeper than the bifurcation of uterine horns. A location at the uterine body is unsuitable for examination because the size and thickness of the uterus varies considerably according to the individual calving numbers and nutritional state.

Contractility, elasticity, and the thickness of the uterine wall increase due to the actions of estrogens, these parameters become more marked during estros. Even in the luteal phase, however, it is abnormal if they are palpated extremely low.

Just before estrus, mucus that has been secreted in the uterus can often be felt.

(1-3) Recording the findings of rectal palpation

Records of findings of the rectal palpation should be kept

at a fixed standard. The form adopted by the Japanese Agricultural Mutual Aid Association is shown in Fig. 2-4-4. It is recommended that not only the findings for the uterus and ovaries but also those of the cervix, vagina and vulva are recorded. Excretion of mucus and pus from the external genitalia is also recorded at the same time.

The ovarian findings are illustrated. It should not be a simple sketch of the shape but a visual diagram showing the positions of the corpora lutea, follicles and induration.

Changes in the estrus cycle of normal cows are shown in Table 2-4-4 according to this recording method.

(2) Other diagnostic methods

- (a) Smear preparation of mucus from estrus and cervical mucus, and biopsy of uterine membranes
- (b) Assay of sexual hormones, especially of progesterones
- (c) Ultrasonic diagnostic apparatus

4-7 Control and Treatment of Reproductive Disorders

(1) Control of Reproductive Disorders

Except for hereditary problems, most reproductive disorders can be controlled.

(1-1) Improvement of Feed Supply

A deficiency of energy as well as of protein has a serious effect on reproduction. In cows, adequate feed meeting the milk yield is necessary and overfeeding is considered to induce ovarian cysts.

Phosphorus and magnesium deficiencies in feed cause weak estrus and ovarian dysfunction. Deficiencies of vitamins A and E are said to be the cause of irregular estrus cycles and anestrus.

(1-2) Improvement of Feed Management

Thorough improvement of the feed environment of cows, proper exercise, hoof cutting, washing of cows, and observations of estrus, the control of reproductive disorders or minimized losses can be achieved. Stress affects reproduction and, for example, by always keeping the rump area clean, changes of external reproductive organs and the presence of mucus can be easily detected.

(1-3) Maintenance of Reproductive Records

It is recommended that all records concerning reproduction (age, calving number, dates of final parturition, mating and estrus, and records of treatment) are maintained so they are always available. Dairy farmers should be aware of the reproductive cycle of an individual cow by keeping a reproduction calendar for 21 days to record the onset of estrus in the herd.

(2) Treatment of Reproductive Disorders

The basics for treatment of reproductive disorders are early diagnosis and early treatment. Diseases have serious effects on the productivity of cows and, if they are untreated, recovery will take a long time. One of the most important points is that the relationship between the uterus and ovaries should be taken into consideration. Persistent corpus luteum are accompanied by abnormalities of the uterus, and ovarian cysts left untreated for a long time result in a slackening of

the uterus. Luteolytic factors are produced in the endometrium.

Consequently, when treatment of reproductive disorders takes longer than expected, it is recommended that the uterus be concurrently treated even in ovarian diseases, and vice versa. Generally, the drugs listed in Table 2-4-4 are used in treatment and are typical drugs used in Japan. As they include drugs which are not available or are expensive in some regions, it will be necessary to make some modifications where necessary.

(References)

- 1) Jainudeen, M. R. and Hafez, E. S. E. 1987. Reproductive Failure in Females in 'Reproduction in Farm Animals' 5th ed. E. S. E. Hafez (ed.). Philadelphia, Lea & Febigar.
- 2) Kesler, D. J., and H. A. Garverick. 1982. Ovarian cysts in dairy cattle: a review. J. Anim. Sci. 55:508.
- 3) Kesler, D. J., et al. 1980. Reproductive hormone and ovarian changes in cows with ovarian cysts. J. Dairy Sci. 63: 166
- 4) Roberts, S. J., et al. Veterinary Obstetrics and Genital Disease 2nd ed. NY. Lithographed by Edwards Brothers, Inc., Ann Arbor, MI.
- 5) Guide for Clinicopathological Examination in Livestock Mutual Aid 1987. the Economic Bureau of the Ministry of Agriculture, Forestry and Fisheries (ed.). National Agricultural Mutual Aid Association.
- 6) Guide for Treatment of Special Diseases and Injuries in Livestock Mutual Aid. 1985. National Agricultural Mutual Aid Association.

Table 2-4-1 Infectious Diseases Causing Reproductive Disorders in Cattle

Name of Disease	Infectious Agents	Effect on Reproduction	Route of Infection	Diagnosis	Treatment
(Bacterial) Campylobacteriosis	Campylobacter fetus	Embryonic mortality Early abortion	Natural mating Contaminated semen	Agglutination of vaginal mucus Fluorescent antibody technique Bacterial isolation	Vaccination Artificial insemination
Leptospirosis	Leptospira pomona Lept. canicola icterohaemorrhagiae grippotyphosa hardjo	Late abortion (haemolytic anaemia)	Contamination of feed and drinking water with urine of infected animals	Agglutination reaction Bacterial isolation	Vaccination Cleaning of the environment Antibiotic treatment of carriers
Brucellosis	Brucella abortus	Abortion Weak neonates Retained fetal membranes Low performance in bulls	Contamination of feed and drinking water with aborted fetus	Bacterial isolation Serum agglutination Complement fixation	Vaccination Culling out of positive animals
Listeriosis	Listeria monocytogenes	Late abortion Retained fetal membranes Endometritis (Neurological signs)	Contaminated feed (silage) and the environment	Bacterial isolation Histopathological examination	Cleaning of the environment Antibiotic treatment
Tuberculosis	Mycobacterium bovis	Endometritis, Salpingitis Abortion Infertility	Contaminated feed and Environment Natural mating Contaminated semen	Tuberculin skin test Bacterial isolation	Culling out of positive animals
Infection of genital organs with non- specific bacteria	Various bacteria Corynebacterium, Pseudomonas, E. Coli, Micrococci, Salmonella, Enterococci etc.	Endometritis Long term anestrus Low reproductive performance	Contaminated environment	Clinical diagnosis	Cleaning of the environment Antibiotic treatment
(Fungal)	Aspergillus fumigatus absidia mucor	Middle to late abortion Necrosis of the placenta	Inhalation of spores from moldy feed	Fungal isolation	Cleaning of the environment Proper storage of feed

Table 2-4-1 (Continued)

Name of Disease	Infectious Agents	Effect on Reproduction	Route of Infection	Diagnosis	Treatment
(Protozoal) Trichomoniasis	Trichomonas fetus	Early abortion Pyometra Infertility	Natural mating	Detection of the fungus	No mating Artificial insemination Culling out of positive animals
Toxoplasmosis	Toxoplasma gondii	Late abortion Weak neonates, Stillbirth, Retained fetal membranes	Contaminated feed and drinking water (with oocytes)	Histopathological examination Serum reaction	Prevention of oral infection with oocytes
(Viral) Bovine viral diarrhea mucosal disease	BVD-MD virus	Early abortion Abnormal fetus; cerebellar agenesis (Respiratory diseases)	Contaminated environment Contaminated semen	Viral isolation Serum neutralization test	Vaccination
Infectious bovine rhinotracheitis; Infectious pustular vulvovaginitis	IBR-IPV virus	Middle to late abortion Transient infertility Vulvovaginitis	Contaminated environment Contaminated semen	Viral isolation Serum neutralization	Vaccination
Akabane disease	Akabane virus	Abortion, Stillbirth Abnormal fetus: arthrogryposis hydrocephaly	Carried by blood-sucking insects	Viral isolation, HI test	Vaccination, Extermination of blood-sucking insects
Bluetongue disease	Bluetongue virus	Late abortion Central nervous disorders	Probably contaminated semen	Viral isolation Serological examination	Vaccination(non-pregnant cows)
Epidemic bovine miscarriage	Chlamydia	Late abortion, Stillbirth, Retained fetal membranes Vesiculitis Abnormal fetus; (yellow liver, enlarged lymphoid tissues)	Natural mating Contaminated feed	Viral isolation Complement fixation	No mating Antibiotic treatment

Table 2-4-2 Classification of Major Ovarian Diseases

Name of Disease	Findings in Ovary and Uterus	External Signs	Cause	Measures and Treatment
Ovarian Dysfunction (Follicular hypoplasia; Ovarian hypoplasia, Ovarian quiescence, Ovarian atrophy)	- Absence of corpus luteum - Absence of growing follicles - Ovaries of both sides palpated flat and smooth - In cases of ovarian hypoplasia and atrophy, the uterus is small and shows no contraction and elasticity	Anestrus	- Lowered secretion of GnH from pituitary gland - Nutritional deficiency (energy, P) - Factors such as heredity, age, the environment and lactation - Occurs subsequent to exhaustive systemic diseases	- Improvement of feed amount - PMSC 750-1,500 IU - HCG 2,000-5,000 IU - GnRH 100-200 μg - Give priority to the treatment of the other disease if any.
Ovarian cyst Follicular cyst	- Absence of corpus luteum - Giant follicles not less than 2.5cm in diameter persist without ovulation - Relaxation and swelling of uterine wall - Sometimes accompanied by endometritis	- Two types; nymphomania and anestrus - In nymphomania, continuous estros signs, swollen external genitals and relaxed sacrospinous ligament are observed	- Low secretion of LH from pituitary gland - The effects of heredity, lactation, nutrition, seasons and stress are suggested but the detailed mechanism is not known	- HCG 3,000-6,000 IU - GnRH 100-200 μg - Concomitant endometritides should be treated - Into markedly relaxed uterus, 50ml hypertonic glucose solution (40-50%) is injected
Luteal cyst	- Presence of follicles with luteinized inner wall - Do not confuse this with cystic corpus luteum (luteal cyst has no ovulation grooves)	Anestrus	- Low LH secretion from pituitary gland - Considered as an old follicular cyst	- PGF2α 20-30 mg - PG analog (Estrumate) 500 μg
Ovulation failure (Delayed ovulation Anovulation)	- Diagnosed as delayed ovulation or anovulation by examination in estrous - Anovulation may affect follicular cysts or ovarian dysfunction - Subclinical endometritis may be concomitant	Prolonged estrus	Low FSH secretion is considered as well as low LH	(The following treatments are carried out at the time of artificial insemination) - GnRH 100-200 μg - HCG 2,000-3,000IU - PSH 10AU - Accompanying endometritis is to be treated
Persistent corpus luteum	- Functional corpus luteum persists without pregnancy (identified by the re-examination 14-21 days later) - Intrauterine foreign bodies (degenerated fetus, pus, mucus frequently occur)	Anestrus	The production of luteolytic factors is inhibited by the presence of intrauterine foreign bodies	- PGF2α 20-30 mg - PG analog (Estrumate) 500 μg

Table 2-4-3 Diseases during gestation and before and after parturition

Name of Disease	Cause	Signs	Treatment
[During gestation]			
Abortion	(See text)		
Mummification	Fetal death	Contraction and sclerosis of dead fetus	Removal of fetus and placenta
Maceration of fetus	Fetal death	Maceration of soft tissues in dead fetus	(same as above)
Prolonged gestation	Hereditary abnormality of fetus	Gestation over 300 days	Induced delivery
Edema of fetal membrane	Fetal deformity, placental dysfunction, incompatibility between fetus and maternal tissues	Abnormal increase of fetal fluid Feeble labor pains at parturition	Artificial abortion Cesareotomy (if necessary)
[Before and after parturition]			
Dystocia	Oversize and malformation of fetus Abnormal attitude posture and position of fetus, torsioned uterus, abnormal birth canal	Birth difficulty Successive occurrence of injuries to birth canal and uterine prolapse	Obstetrics, Lamination or cesarean section (if necessary)
Injuries to birth canal	Dystocia due to oversized fetus	Rupture of uterus, cervix and vagina, Rupture of perineum and vulva	Surgical suture
Retained fetal membranes	Inflammation of placenta due to infection Feeble postpartum labour pains	Postpartum retained fetal membranes	Do not attempt manual removal, Uterine irrigation after decidualation
Vaginal prolapse	Excessive relaxation of pelvic ligament (late gestation)		Repositioning and pressing, Surgical treatment
Uterine prolapse	Severe tenesmus at dystocia		(same as above)
Postpartum metabolic disturbance			
Milk fever and downer	Hypocalcemia	Paresis	
Grass tetany	Hyponagnesemia	Convulsion and tetany	(Omitted)
Ketosis	Hypoglycemia	Loss of body weight and milk yield	

Table 2-4-4 Changes in Ovaries and Accessory Reproductive Organs during Estrus Cycle (in Normal Cows)

	Ovary		Uterus				Others			Remarks	
	Left	Right	Size	Shape	Contractility and Elasticity	Hypertrophy	Inner palpation	Uterine cervical canal	Vagina		Vulva
Estrus stage			Finger width 1.5 1 2.5	Circle-semi-circle	$\frac{1}{2}$ +	$\frac{1}{2}$ +	—	Swelling Open	Congestion Glossy	Swelling Deep wrinkle	In normal cows, grown follicles and regressive corpus luteum can be palpated.
2 days after			"	"	$\frac{1}{2}$ +	$\frac{1}{2}$ +	—	Open	Presence of mucus Hemorrhage	Deep wrinkle	Bilateral ovaries are nearly the same size. Excitement of the uterus at estrus persist. Delayed relaxation of the uterus
4			1.5 1 2.0	"	+	+	—	Closes narrowly	Dry mucus	Reduction Small wrinkle	Small and fragile new corpus luteum is palpated in ovulated ovary.
6 1 7			"	"	+	+	—	Tight	"	Reduction, Small wrinkle	Fully grown corpus luteum is weak and easily ruptures by light palpation. The uterus is soft and smooth.
9 1 10			"	"	+	+	—	"	Slightly wet	"	Small follicles are growing in the ovaries which sometimes causes increased contractility in the uterus.
12 1 14			"	"	$\frac{1}{2}$ +	+	—	"	Dry	"	Pay attention not to misdiagnose a corpus luteum as a follicle because a corpus luteum, which is covered with ovarian epithelium, is smooth and is palpated like a follicle.
16 1 17			"	"	($\frac{1}{2}$) +	($\frac{1}{2}$) +	—	Slightly swelling	"	Deep wrinkle	Hardness of corpus luteum is marked. The projection is palpated with marked force. Uterine contractility and elasticity is greater.
19 1 20			1.5 1 2.5	"	$\frac{1}{2}$ +	$\frac{1}{2}$ +	+	Swelling Open	Congestion Slightly transparent mucus	Swelling Deep wrinkle	Corpus luteum regresses to 60-80% of size of the functional stage. The uterus is swollen and continually contracts.
21			Development of estrus signs								

(Note) ["] in the table indicates the same as the above column.

(Guide for Clinicopathological Examinations in Livestock Mutual Aid, 1987)

Table 2-4-5 Drugs Used for Reproductive Disorders

	Action	Target Disease and Dose	Side Effects
(Ovarian hormones)			
Estrogen	Increase in uterine bactericidal action, uterine contraction and congestion Relaxation of cervix	Retained fetal membranes and pyometra Termination of conception by administering after mating Artificial abortion by overdosing Mummification and maceration of fetus	Estradiol 2-5mg 4-8 10-20/3-4days 4-8 * Long-term overdosing induces ovarian cysts and ovarian atrophy
Progesterone	Progestational proliferation of endometrium Inhibition of myometrium Contraction of cervix Maintenance of gestation	Synchronization of estrus Prevention of habitual abortion Follicular cysts	Various synthetic progesterones Progesterone 100-200mg/ 7-10days 750-1,500mg
(Aimed at LH action)			
HCG	(the same as LH) Induction of ovulation Increase in function of corpus luteum	Follicular cysts Ovarian dysfunction Delayed ovulation	HCG 3,000-10,000IU 1,500-3,000 1,500-3,000 * Production of antihomone by frequent overdosing
GnRH	Promotion of LH secretion from the pituitary gland	(same as above)	Fertirollin acetate 100-200µg
(Aimed at FSH Action)			
hMG	(the same as FSH) Follicle stimulation	Ovarian dysfunction	hMG 750-1,500IU * Multiple fetuses Follicular cysts
FSH		Ovarian dysfunction Follicular cysts Delayed ovulation	p-FSH 10-20mg 20-40 10 * Multiple fetuses
(Aimed at regression of corpus luteum)			
PGF _{2a}	Regression of corpus luteum Induction of ovulation Contraction of uterus	Synchronization of estrus cycle Persistent corpora lutea and pyometra Luteal cysts Mummification and Maceration of fetus	PG 15-25mg 4-6 (i.c.)
PG analogues	(same as above)	(same as above)	Cloprosterol 500-750µg
(For intrauterine administration)			
Antibiotics	Cleaning of infected uterus	Endometritis	Penicillin 300,000U+ Streptomycin 0.3mg(i.u.) Ampicillin 500mg(i.u.)
Iodophor	Cleaning of infected uterus Stimulation of uterine membrane	Endometritis Feeble estrous	2% PVP Iodine 30-50ml(i.u.) Lugol's solution 30-50ml(i.u.)
Hypertonic glucose solution	Treatment of relaxed uterus	Auxiliary treatment for follicular cysts	40-50% Glucose 50ml(i.u.)

i. u.: Intrauterine administration

i. c.: Intrauterine-horn administration

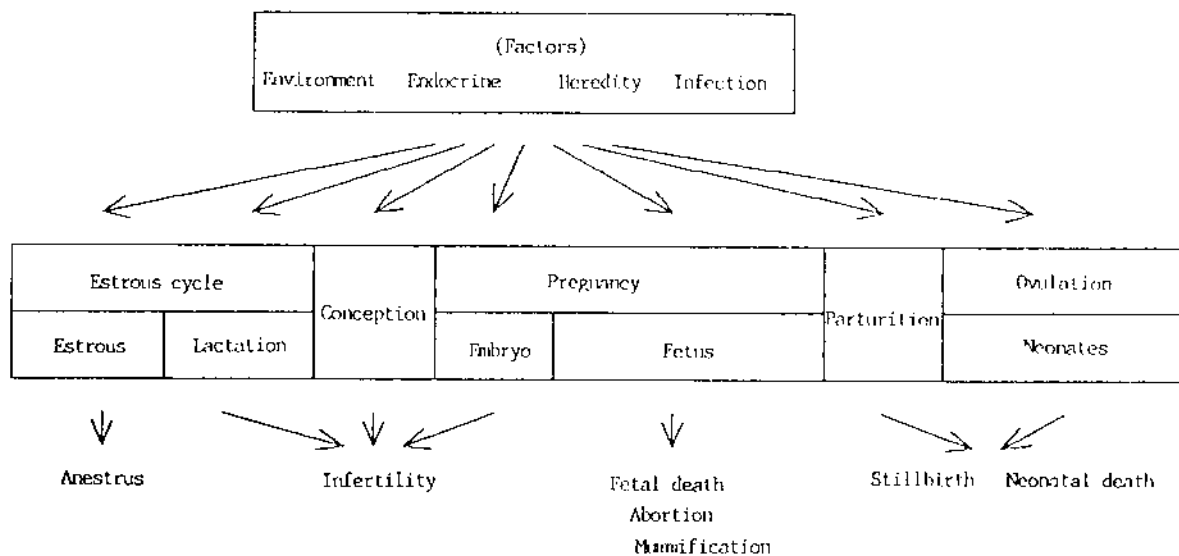
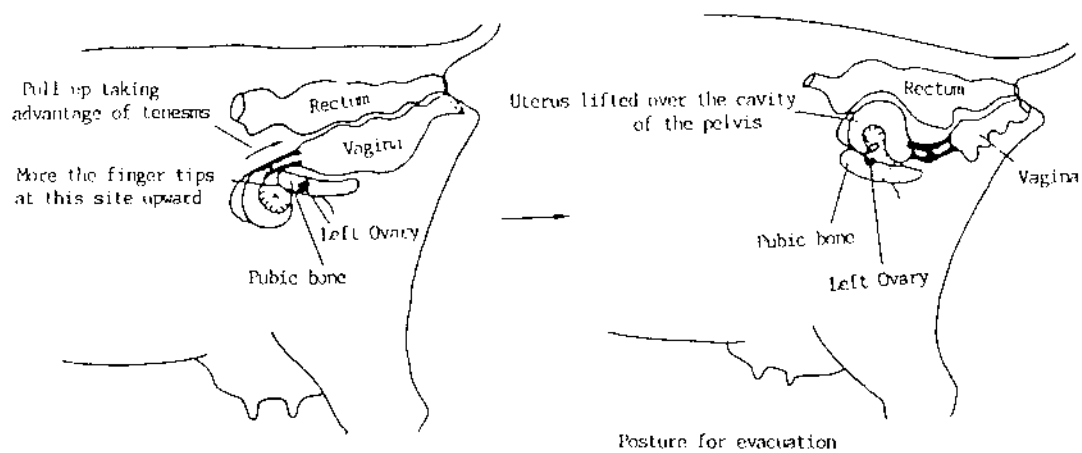


Figure 2-4-1 Reproductive Processes and Factors Having Adverse Effects on them

(E.S.E. HAFEZ, 1987)



Uterus lifted over the cavity of the pelvis (in cows)

Figure 2-4-2

(Guide for Clinicopathological Examinations in Livestock Mutual Aid, 1987)

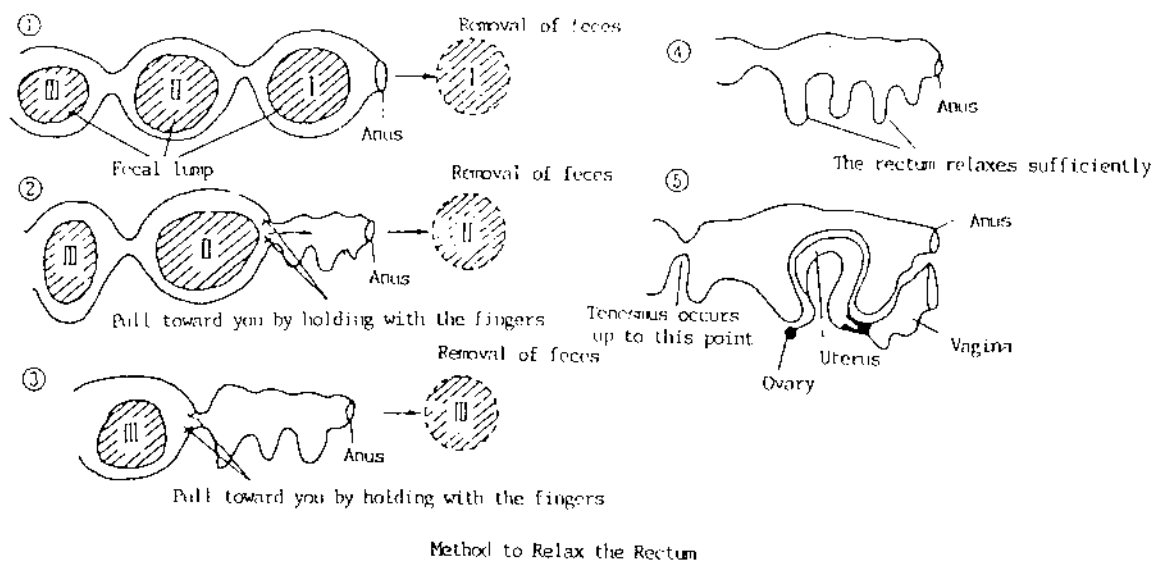


Figure 2-4-3
(Guide for Clinicopathological Examinations in Livestock Mutual Aid, 1987)

Findings	Diagram of ovary	Remarks	Findings	Diagram of ovary	Remarks	Treatment	Diagram of ovary
Follicles in estrous		Draw a part of the contour of an ovary	Regressive corpus luteum or site of induration		Paint this black	Crushing of follicles with fingers	
Ovulation site		Show as a wedge shape	Follicular cyst		Draw inside the contour of an ovary	Sucking of intraovarian fluid	
Corpus luteum		Draw slanted lines Increase the number of slanted lines according to the mass	Follicles other than follicles of estrous		Draw inside the contour as shown	Removal of corpus luteum	
Regressive corpus luteum		Fill with crossed slanted lines for a solid corpus luteum Paint this black	Luteal cyst		Draw inside the contour as shown	Intraovarian parenchymal injection	
			Cystic corpus luteum		Draw luminal fluid of corpus luteum	Intrafollicular injection	
						Artificial insemination	

* Draw as a cross section with the mesovarian margin of the ovary at the bottom and the direction of the ovarian ligament on the inside and along the longer axis of the ovary.

Figure 2-4-4 Illustration of ovarian findings

PART III FEEDING AND MANAGEMENT OF CATTLE

1. Feeding and Management of Dairy
 Cows
2. Feeding and Management of Beef
 Cattle

III Feeding and Management of Cattle

1. Feeding and Management of Dairy Cows

1-1 Feeding and Management of Heifer

It is important in feeding and management of breeding cattle to make the most of their genetic characteristics. Good milk production can be achieved by cows which have healthy bodies and a good intake of feed. It is important to rear breeding cattle correctly so that their stomachs are large and strong. Their skeleton should be strengthened as well as the legs. Most importantly, special care must be taken so that they develop a good udder.

(1) Feeding and management during suckling

(1-1) Management at birth

Immediately after a calf is delivered its respiration must be checked. If the calf is apparently dead due to swallowing amniotic liquid, vomiting and attempts at artificial respiration will often save its life. The umbilical cord should be disinfected with tincture of iodine in order to prevent inflammation and the calf must drink colostrum. Colostrum has higher nutritional value than normal milk (see Table 3-1-1) as well playing a role in immunizing the calf, triggering excretion of meconium, and controlling its physiological functions. The first feed on colostrum should be within the first hour of birth since the components of colostrum deteriorate as time passes and the absorption of immunoglobulin by the calf ceases after about 24 hours. Surplus colostrum may be preserved by freezing or fermenting, so it can be used for other calves born of diseased mother. Calves should be separated from their mothers as soon as possible to prevent disease and to

help in the general management of calves. Special care must be taken to prevent diarrhea, in particular, up to 3 weeks from birth and to prevent colds up to about 1 month after birth.

(1-2) Digestion in a calf

A dairy cow, being a ruminant, has four stomachs of which the rumen plays the main role in digestion in a calf immediately after birth. However, as the rumen is not yet mature the abomasum plays an important role since the abomasum of a calf is larger than the rumen. The volume of the abomasum (1 - 2l) accounts for 70 % of the stomachs, while that of rumen accounts for 30 %. The rumen will become larger than the abomasum about 1 month after birth. The development of the rumen is faster in cows with a shorter suckling period and in cows receiving pellet feed from an earlier age. The mucosa of the rumen develops due to stimulation of lower fatty acids, and the volume of the rumen develops due to the physical properties of roughage.

Liquid feed such as milk and milk substitute are digested by enzymes such as chymosin and pepsin while they are in the abomasum. Milk protein is better digested than other proteins and proteins, such as those in fish and soybean, are poorly digested in a calf. Of the saccharides, lactose and glucose are well digested, while sucrose and starch are poorly digested saccharides and cause diarrhea more often than other nutrients. In particular, high concentrations or excessive saccharide may cause this disorder.

(1-3) Suckling methods and precautions

Suckling methods include those using whole milk, fermented milk and milk substitute. From the viewpoint of diarrhea the use of milk and fermented colostrum have produced good

results. However, in terms of feed costs and labor saving, milk substitute is used. The suckling period can be long-term suckling (over 90 days) and short term suckling (40 - 50 days). Short-term suckling is widely practiced because it has beneficial effects on the development of calf rumen and can save on feed costs and labor.

Milk substitute mainly consists of de-fatted powdered milk, animal fats, vitamins and minerals. Milk substitute not containing de-fatted powdered milk often causes diarrhea and it should be diluted 6 - 8 times. Subsequent feed volume differs according to feeding methods, i.e. the constant volume method or the increasing volume method. Growth of calves is about the same for either method.

Another suckling method is one in which colostrum or whole milk is fed at the beginning and milk substitute is added later. The concentration of milk substitute in the mixed milk should be gradually increased until colostrum or whole milk are not included.

A third method is one in which whole milk is fed in the same way as milk substitute when given alone. In another method, fermented colostrum is slightly diluted then used. The feeding standards of the National Livestock Breeding Center are shown in Table 3-1-2.

Attention must be paid to the temperature and volume of milk, the time of feeding and the cleanliness of feeding equipment since calves have low physical strength and a small stomach. The temperature of milk must be 38 to 40 °C and the volume must be up to 10 % of the calf weight. Feeding should be at regular times during a day with regular intervals. Feeding equipment must be carefully disinfected especially when supplying whole milk or milk substitute since residual milk will putrefy. In addition to this, attention must be paid

to the preservation of whole milk or milk substitute in particular in hot regions since milk is apt to putrefy in such regions. A sudden change in the kind of feed, excessive concentration of milk substitute and a detrimental or sudden change in the feeding environment will have a bad influence on the health of calves and may cause diarrhea. Pathogens and microorganisms may also cause diarrhea in calves.

(2) Management from weaning to 12 months of age

(2-1) Feeding of formula feed and weaning

Two kinds of formula feed are fed to young calves and these are calf starter or synthetic milk, and formula feed for breeding cattle.

Calf starter mainly consists of high-quality vegetable protein, grain, vitamins and minerals. When using food leftover or when keeping calf starter for a long time special care must be taken since the feed easily undergoes changes.

Calf starter can be given in small volumes from one week of age and the volume should be increased with intake according to the free-feeding method. Intake has a close relationship with suckling and a small volume of milk consumed results in larger intake of calf starter. The feed volume of calf starter is less than 2 - 2.5kg and if more nutrient is necessary formula feed for breeding cattle can be supplemented.

Formula feed for breeding cattle is given with the calf starter from when suckling feed is reduced or from one month of age. Calf starter should be stopped between the age of 3 - 6 months and all the feed is formula feed for breeding

cattle from then on. This feed is given until 12 months of age. There is another feeding method in which formula feed is switched to concentrated cow feed and is given to calves at 6 months of age when the rumen of the calf is mature enough. We recommend the former method to stimulate the growth of calves.

The time for weaning is determined by the intake of calf starter. In short-term suckling, a calf can be weaned when the intake reaches 1 - 1.5kg per day. At this stage the calf should show sufficient growth for weaning. More importantly, the animal's weight increase should be 0.6 - 0.7kg per day at this stage. If the weight increase is lower than this, it is better to delay weaning since the appropriate time for weaning is also affected by intake and quality of hay and/or the formula feed for breeding cattle.

(2-2) Feeding of roughage and other management

Good quality hay should be fed as roughage until 6 months of age since the rumen has not matured sufficiently. The free feeding method is used at this stage in order to stimulate development of the rumen and succulent feed should be avoided until 6 months of age to help the growth of calves. Restricted grazing can be introduced from 7 to 12 months of age when free grazing can be introduced.

In addition to the management outlined above, dehorning and removal of supernumerary teats should be carried out. There are several dehorning methods such as cauterization, the potassium hydroxide method and the dehorner method. Of these methods, cauterization is practiced widely, and dehorning should be carried out before weaning. The removal of supernumerary teats should be carried out at 10 months of age since the teats may cause leakage of milk or mastitis.

(3) Management after 12 months of age

(3-1) General management

Roughage may be used as the main feed from 12 months of age when the rumen is almost as mature as that of a cow. Formula feed can be changed to concentrated feed for cows and the volume of formula feed must be reduced to 1 - 2kg. Feeding of succulent feed or free grazing may be introduced at this stage. Some concentrated feed should be fed to supplement the succulent feed or free grazing should be allowed since it is almost impossible to maintain normal growth of a calf without concentrated feed. Roughage of relatively bad quality can be used for a heifer at this stage and may well result in acceptable growth of the animal. The composition of formula feed for breeding cattle is shown in Table 3-1-3.

The ovaries of female calves gradually mature from 6 months of age due to the effect of gonadotrophic hormone secreted from the hypophysis. Reproductive organs such as the uterus, and vagina as well as the mammary glands mature at 8 - 10 months of age under the influence of estrogen and progesterone secreted from the ovary. At this time estrus can be observed and may be observed earlier in well nourished calves.

Mating may commence from 15 - 16 months of age or from when the animal reaches 350 - 400kg. Even if the animal shows poor growth, it should be mated at 18 months of age. Obesity has a bad effect on reproduction and growth of mammary glands.

(3-2) Growth

Dairy cattle mature at around 5 - 6 years of age. The time taken for each part of the body to achieve 90% maturity is short for the height and in belly girth, is medium for body length, chest girth and size of the rear half of the body (hip width for example), and it takes a long time for the weight to increase. In other words, in dairy cattle the body length increases first then width increases and weight is gained last.

Bone plays an important roles in growth. Bone comprises protein as the substrate with calcium and phosphorus deposited on this substrate and these substances form about 65% of bone. Essential nutrients for the growth of bone are vitamins A and D in addition to the substances stated above. A lack of protein may suppress the growth of bone and a lack of calcium, phosphorus or vitamins may result in fragile bone. If growth of some animals is poor compared to ordinary growth, feed management for other animals needs to be improved especially during the periods where poor growth was observed. Normal growth values for dairy cattle in Japan is shown in Table 3-1-4.

(3-3) Nourishment during raising

Growth of reproductive organs is closely related to nourishment during raising. The first estrus cycle and first ovulation are observed earlier in well-nourished animals than those that are poorly nourished. This is because the development of reproductive organs is more closely related to actual growth, and more specifically to its weight than age. In addition, estrus cycles are often long in well-nourished animals.

Milk production in a cow is related to nourishment during raising. Milk production is the highest in a cow receiving an intermediate level of nourishment (DG 0.8 - 0.7) during

raising and is medium in cows receiving poor nourishment (DG about 0.45) and is lowest in a poorly-nourished animal (about 0.9 as DG). Note that production shows a high negative correlation with nourishment from 7 - 10 months of age. Less parenchyma and more fat is observed in cows receiving good nourishment during raising since development of mammary gland cells was hindered by nourishment in the period in which cells, especially mammary duct cells, show marked development. A weak effect on the development of mammary glands is observed with good nourishment on or after the 13 the month.

1-2 Feeding Management of Milking Cows

The most important feeding period for milking cows is between 1 month prior to giving birth and about 2 months after. Milk production during lactation is largely determined by feed management along with genetic features of the cow. Special care must be taken to prevent diseases that often occur during this period such as milk fever, post-partum astasia and changes in the abomasum as well as dystocia due to obesity and a decrease in milk production along with breeding disorders due to insufficient nutrition.

(1) Structure of the udder and milk production

The udder is suspended by a central suspensory ligament which also divides the udder into two halves along the length of the body. Each half is divided again from left to right to form four quarters altogether. In each quarter there are mammary gland cells which produce milk and connective tissue which supports these cells. Lower milk production may result from an udder containing a large amount of connective tissue although the udder may have a similar shape to a normal udder. May mammary gland cells form mammary alveoli and the organ is connected to the mammary lobe, mammary lobule, mammary duct and mammary cistern.

Milk is produced in the epithelial cells of the mammary gland. These cells produce milk components by taking nutrients from the blood and about 500kg of blood is needed to produce 1kg of milk. The origins of the components of milk from the blood are shown in Table 3-1-5.

Various hormones are involved in milk production (Table 3-1-6). Milk production starts after birth due to decreased secretion of progesterone and increased hormones such as prolactin, insulin and glucocorticoids. Among these, prolactin or lactogenic hormone as it is sometimes known, plays an important role in controlling milk production. Growth hormone is involved in stimulating the development of mammary glands and in milk production as well as in the overall growth of the animal. The concentration of this hormone in blood is high in the early stage of lactation. Oxytocin is also important in lactation and is secreted by a suckling (milking) stimulus such as massaging the udder. This stimulates lactation by causing contraction of myoepithelial cells which surround the mammary gland cells.

Milking must take place within several minutes of excretion of oxytocin which commences 30 seconds after initial stimulation and lasts only several minutes, i.e. during oxytocin secretion. If not, a lot of milk will remain in the udder and the residual milk will decrease the subsequent milk production. Without milking, milk production may gradually decrease since the milk remaining in the mammary gland cells increases the pressure within the udder which consequently increases pressure on the blood vessels around the cells until milk production stops.

(2) Feed management

(2-1) Function of the rumen

The rumen plays an important role in digestion in a dairy cow as in other ruminants. The volume of the rumen is about 160 - 250 L but depends on the weight of the cow. Feed taken into this organ is digested or degraded by microorganisms such as bacteria and protozoa present in the rumen.

Of the nutrients, most carbohydrate is degraded and absorbed in the rumen as volatile fatty acids such as acetic, propionic and butyric acids. The composition of milk is affected by the volatile fatty acid components which differ according to the quality of feed. The percentage of milk fat increases with the content of acetic acid which comes from roughage. As concentrated feed increases so does propionic acid and the fat ratio decreases.

About 70% of protein is degraded to amino acids and ammonia in the rumen by the action of microorganisms, and most amino acids are absorbed in the small intestine as microbial proteins. Proteins which are not degraded in the rumen bypass this organ and are digested and absorbed in the small intestine.

Fats are degraded to fatty acids and glycerol by lipase secreted from microorganism in the rumen. Fatty acids are then degraded to propionic acid which is absorbed in the rumen along with glycerol. The volume of fats in feed must not exceed 5% of dry matter since digestion decreases with high fat consumption and it suppresses the function of microorganisms.

(2-2) Nutrient Requirement and Intake

High milk production in a cow during lactation can be achieved by increasing and prolonging peak production which has steeply increased after delivery and by maintaining peak

volume. However, the nutrient intake of a cow reaches only 80 - 90% of nutrient requirements during lactation, since the peak intake of dry matter is observed 70 - 90 days after delivery while the peak milk production is 40 - 60 day after delivery. The weight of a milk cow shows a marked decrease after giving birth due to the use of nourishment stored in the body for milk production while intake of nourishment is insufficient (Figure 3-1-1). This decrease is most marked in cows producing a high volume of milk.

Nutritious feed must be given to cows soon after giving birth to supplement nutrition. Concentrated feed can be given as a supplement along with the some volume of roughage. Roughage is essential in cow feed as it is in feeds for other ruminants. The volume of roughage should be more than 40% of dry matter or more than 17% in crude fiber. Concentrated feed alone may well cause problems such as indigestion.

The componets of concentrated feed used in early lactation (about 3 months after delivery) should be high in calories and high in protein (CP 16 - 20%, TDN 70 - 75%) and the calorific content and the amount of protein should be decreased with a decrease in lactation. The increase in concentrated feed per day must be less than 0.5 - 0.9kg and a sudden increase in feed must be avoided. The volume of concentrated feed can be 2/5 to 1/3 of the feed for a cow with high milk production and can be 1/3 to 1/4 of the feed for a cow with low milk production. Frequent feeding of concentrated feed in one day in small amounts at a time is good for maintaining homeostasis of the rumen. Recently, bypass protein, or heated soybean, and bypass fats, or protected fats, have been fed to cows with high milk production.

(2-3) Factors that affect dry matter intake

High milk production can be attained by a high intake of dry matter. Such intake is greatly affected by factors such as weight, milk production, frequency of feeding, number of days after delivery, feed volume of concentrated feed, water content of feed, and heat. Among these factors, weight, milk production and temperature are closely related to dry matter intake as shown by Milligan's formula;

$$DM = 0.0185 W + 0.305 (0.4 + 0.15 MF) MP \times T$$

DM: dry matter intake of a cow during lactation (kg)

W : weight (kg)

MF: milk fat percentage (%)

MP: milk production (kg)

T : correction factor for temperature

Here, the correction factors for temperature are:

under -15°C : 1.16	-15 to -5°C : 1.07
-5 to 5°C : 1.05	5 to 15°C : 1.03
10 to 25°C : 1.00	25 to 35°C : 0.90
at or over 35°C :	
0.65 (if temperature falls at night),	
0.90 (if temperature does not fall at night)	

(3) Milking management

Pay attention to the following during milking.

- (i) Do not frighten the cow or get her excited before milking.

Blood capillaries contract under the influence of adrenaline secreted from the adrenal cortex when a cow is frightened or excited. Such a stimulus causes

obstruction due to the contraction of myoepithelial cells which otherwise would be caused by oxytocin.

(ii) Massage the udder before milking.

Massaging the udder will stimulate lactation as it stimulates the hypophysis to secrete oxytocin which triggers the contraction of myoepithelial cells that surround the mammary gland cells.

(iii) Carry out preliminary milking before applying the milking machine to the udder.

Milk with a high bacteria content can be discarded by preliminary milking. In addition mastitis can be detected earlier.

(iv) Apply the milking machine within one minute of massaging the udder.

Milking must be completed within 5 - 6 minutes after massaging, since peak secretion of oxytocin occurs about 1 minute after massaging and secretion will gradually decrease until it stops after 5 - 6 minutes. If milking has not been completed within this time a lot of milk will remain in the udder.

(v) Remove the milking machine as soon as milking is finished.

Excessive milking will cause mastitis due to damage to the teat and mammary cistern of the cow. In contrast, early removal may well affect the next milking due to residual milk in the udder.

(vi) Carry out dipping.

Infections from various kinds of bacteria in the udder can be prevented by dipping the teats in disinfectant after milking.

(vii) Disinfect the milking machine.

Infections such as mastitis can be prevented by disinfection of teat cups after milking prior to application to the next cow.

(viii) Maintain the vacuum pressure in the pipe within -30 to -40 mmHg.

A change in vacuum pressure may cause the milking machine to fall off or mastitis.

(ix) Disinfect the milking machine and the pipe to prevent growth of bacteria.

(x) A cow udder, soon after giving birth, requires massaging with hot water to reduce edema in the udder.

(4) Breeding management

Breeding management is important to attain high breeding efficiency. The time between conception and delivery should be less than 85 days in order to achieve one delivery a year which is the ideal reproduction rate for cows in Japan. Artificial insemination can take place usually 60 days or more after delivery although first anestrus ovulation may be observed about 20 days after delivery and estrus may be seen more than 30 days after delivery.

Good nutrition of a cow before and after delivery is expressed in a body condition score of; 3.5 - 4 for a cow before

delivery, 2.5 - 3 for a cow at peak lactation soon after delivery and about 3.5 for a cow in the latter half of lactation. Obesity before delivery may cause dystocia at delivery and some disorders after birth, such as endometritis and follicular cysts, as well as disorders in reproductive function such as a delay in first estrus. Insufficient nutrient soon after delivery due to the lack of intake compared to nutrient requirements may well lead to miscarriage or the death of early embryos in cows.

(5) Dry up

A Dry up period is essential for mammary glandular cell to rest and grow and for the mother to rest the reproductive organs as well as improve the nutritional status of the whole body. The ideal length the Dry up of period is 45 - 60 days.

A shorter period, not more than 40 days, may not increase milk production for the next period of lactation due to insufficient rest and growth of the mammary glandular cells. A longer period of not less than 60 days may cause obesity and may not increase milk production for the next period of lactation because a long non-lactating period has no effect on stimulating the growth of mammary glandular cells.

There are two methods to stop lactation, i.e. a rapid method and a gradual method. In the rapid method, a cow is milked twice in 2 or 3 days after discontinuation of feeding concentrated and succulent feeds, thereafter milking is stopped immediately. However, mastitis may occur in cows showing high milk production if this method is used. In the gradual method, after discontinuation of feeding of concentrated and succulent feeds, a cow is milked once per day until milk production decrease to 5 - 10kg per milking, then milking is stopped.

In either of these methods, mastitis treatment for the non-

lactating period should be applied and the teats should be dipped in disinfectant after the last milking. Cessation of lactation occurs within 2 - 3 weeks after discontinuation of milking while the udder may enlarge for a time due to lactation within the udder although this milk is absorbed. A long period between delivery and cessation of lactation may cause bad nutrition in the cow and may affect her health as well as development of embryos.

Management of cows after lactation stops includes provision of feed which is mainly roughage, and exercise and exposure to sunshine. Additional nourishment for the growth of embryos should be provided from 2 - 3 months before delivery. Concentrated feed should be given little by little from 2 - 3 weeks before delivery in order to improve the condition of the rumen, more specifically the microorganism within the rumen, for the increase in nutrition requirement for milk production after delivery.

1-3 Feeding Standards

Nutrient requirements according to the Japanese Feeding Standards are shown in Tables 3-1-7, 3-1-8 and 3-1-9. Pay attention to the following when using this standard.

- (i) Correction of the values of nutrient requirement may be necessary according to the actual conditions such as the environment, strength of an individual cow and amount of exercise, since the values are calculated for average cows bred by standard management.
- (ii) The actual content of feed which is given to cows must be measured since different feed, in particular different roughage, has very different components.
- (iii) Nutrient requirements for breeding cattle should be used for

cows under 36 months of age since these cows show marked growth.

- (iv) The volume of dry matter should be 3.5 - 4% of the weight of a cow at peak lactation, 3 - 3.5% of body weight for a cow in the middle and latter period of lactation and 1.5 - 2% of body weight for a cow in the non-lactating period.
- (v) Actual feed volumes should be 110 - 120% of the nutrient requirements by estimating variations in factors such as the quality and individual differences in roughage.
- (vi) Roughage should not be less than 40% of the total dry matter and crude fiber should not be less than 17% of this.
- (vii) Feed volumes of various minerals and the balance between them must strictly follow the nutrient standard for calves and milking cows since the requirement, in particular that of calcium and phosphorus, is high in these stages. The ratio of calcium and phosphorus should be maintained at 2 : 1.
- (viii) The amount of dry matter should be changed according to the temperature correction factor for dry matter intake described above. Feed intake of cows with high milk production may decrease with temperature above 25°C and the decrease will result in a decrease in milk production and the quality of milk. In this case, the temperature in the cowshed should be lowered and the energy value of feed should be increased.

Table 3-1-1 Changes of major components in colostrum

(Folly, Otterby, 1978)

component	colostrum (milking after delivery)					normal milk
	1st time	2nd time	3rd time	4th time	5th to 6th times	
total solids %	23.9	17.9	14.1	13.9	13.6	12.9
milk fat %	6.7	5.4	3.9	4.4	4.3	4.0
non-fat solids %	16.7	12.2	9.8	9.4	9.5	8.8
protein %	14.0	8.4	5.1	4.2	4.1	3.1
immunoglobulin %	6.0	4.2	2.4	-	-	0.09
casein %	4.8	4.3	3.8	3.2	2.9	2.5
lactose %	2.7	3.9	4.4	4.6	4.7	5.0
ash content %	1.11	0.95	0.87	0.82	0.81	0.74
calcium %	0.26	0.15	0.15	0.15	0.16	0.13
magnesium %	0.04	0.01	0.01	0.01	0.01	0.01
vitamin A ug/ml	295	190	113	76	74	34

Table 3-1-2 Example of feeding of dairy calf

agen in months	0			1			2			3			4			5			6			7			ll	remarks
	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days	1st 10days	2nd 10days	3rd 10days		
weight	42	47	52	57	62	68	75	81	91	110	110	120	139	168	196	217										
whole milk calf starter	3 0.1	4 0.2	5 0.3	5 0.7	4 0.8	2 1.0	1.1	1.2	1.3	1.4	1.5	1.4	1.2	1.0	1.0										or milk substitute	
formula feed for breeding cattle							0.7	1.0	1.1	1.1	1.2	1.3	1.5	2.0	2.5	2.5									free grazing	
hay	0.1	0.2	0.3	0.5	0.7	0.8	0.9	1.0	1.2	1.2	1.2	1.5	2.0	2.5	3.0	5.0										

Table 3-1-3 Major nutrient content in breeding feed for cow (NRC 1989)

feed	crude protein (%)	total digestive nutrients (TDN) (%)	crude fiber (%)	calcium (%)	phosphorus (%)	vitamin A IU	vitamin D IU
calf starter	18	80	0	0.60	0.40	2,200	300
feed for 3-6 months of age	16	69	13	0.52	0.31	2,200	300
feed for 6-12 months of age	12	66	15	0.41	0.30	2,200	300
feed for 12 months of age or over	12	61	15	0.29	0.23	2,200	300

Table 3-1-4 Values for growth or milk cow

Table 3-14 Values for growth of milk cows																
1. Values for growth of Holstein		age in months	at birth	2	4	6	8	10	12	14	18	24	30	36	48	60
weight	average deviation	41.5 10.0	85.7 17.9	140.8 28.5	192.9 37.6	241.7 46.4	287.1 54.2	329.1 61.0	367.5 66.4	434.0 75.7	509.4 87.2	559.9 94.0	591.7 96.6	827.6 100.4	661.1 103.6	
height	average deviation	75.7 5.0	86.9 5.3	98.2 5.5	107.1 5.5	114.0 5.6	119.3 5.6	123.4 5.6	126.6 5.7	131.1 5.7	135.5 5.9	138.9 6.0	141.3 6.1	141.4 6.1	142.6 6.2	
rump length	average deviation	23.1 2.2	28.3 2.6	33.2 3.0	37.2 3.2	40.4 3.4	43.0 3.6	45.2 3.8	46.9 3.8	49.4 3.9	51.8 4.0	53.4 4.0	54.5 4.0	55.2 4.0	56.2 4.0	
girth	average deviation	17.3 2.4	22.5 2.9	27.9 3.4	32.5 3.7	36.3 4.0	39.5 4.2	42.1 4.4	44.4 4.5	47.9 4.5	51.6 4.5	54.2 4.5	56.1 4.5	58.0 4.5	59.6 4.5	
chest girth	average deviation	79.8 6.7	99.6 8.6	117.6 9.3	132.3 10.1	144.2 10.8	153.9 11.3	161.7 11.9	168.2 12.1	177.9 12.3	187.8 12.9	194.5 13.1	199.1 13.1	201.6 13.2	205.6 13.2	

Note 1. The values are according to the Holstein Cattle Association of Japan

2. Value within average ± deviation is normal growth.

2. Values for growth of Jersey

	age in months	at birth	2	4	6	8	10	12	14	16	18	24	30	36	48
weight	average deviation	27.1 4.9	55.2 8.4	98.2 14.1	138.8 18.8	176.3 21.2	209.0 22.8	241.8 23.7	274.7 32.9	333.6 30.4	414.2 31.9	421.1 45.1	436.3 35.9	463.4 84.0	486.2 48.7
height	average deviation	66.7 7.3	77.8 3.1	89.2 3.4	97.4 3.8	102.8 11.0	107.0 8.8	111.3 3.4	114.7 3.4	119.3 2.8	123.5 2.7	128.7 2.9	130.4 3.5	130.4 3.5	130.4 3.5
rump length	average deviation	20.7 1.6	24.9 1.7	29.7 1.8	33.4 1.7	36.4 4.0	39.0 1.7	40.9 1.6	42.8 1.8	45.8 1.6	49.2 2.2	50.0 1.9	50.9 2.4	52.0 2.2	52.0 2.2
girth	average deviation	14.2 1.0	18.5 1.6	23.9 1.6	28.1 1.8	32.0 1.6	34.4 1.7	37.0 1.8	39.3 2.0	43.2 1.7	47.1 1.7	49.6 2.0	50.7 1.9	52.7 2.3	53.0 2.7
chest girth	average deviation	68.6 4.0	86.5 4.7	105.1 5.5	118.4 6.4	130.4 4.9	137.6 5.8	145.0 6.5	151.5 6.7	163.4 5.9	175.5 6.3	178.0 8.4	179.4 6.5	184.8 7.6	186.8 7.0

Note 1. The values are actual measurements according to the National Livestock Breeding Center, Iwate Station.

2. The values were measured for cows born between Jan 1 1970 to Dec 31 1989.

3. The number of cases differs according to the age in months (149 maximum, 31 minimum). The height at 48 and 60 months of age are corrected.

Table 3-1-5 Components of milk produced from components of blood

Components of blood	Intermediate (synthesized in mammary gland cells)	Components of milk
acetic acid	→ fatty acid	→ milk fat
fatty acid	→	→ fat globules
glucose	→ glycerol	→
	→ lactic acid	→ lactose
amino acid	→ casein	→ calcium caseinate
calcium	→ calcium	→ protein
minerals	→ minerals	→ calcium
water	→ water	→ minerals
		→ water

} non-fat solid

Table 3-1-6 Function of mammary gland and effect of hormones

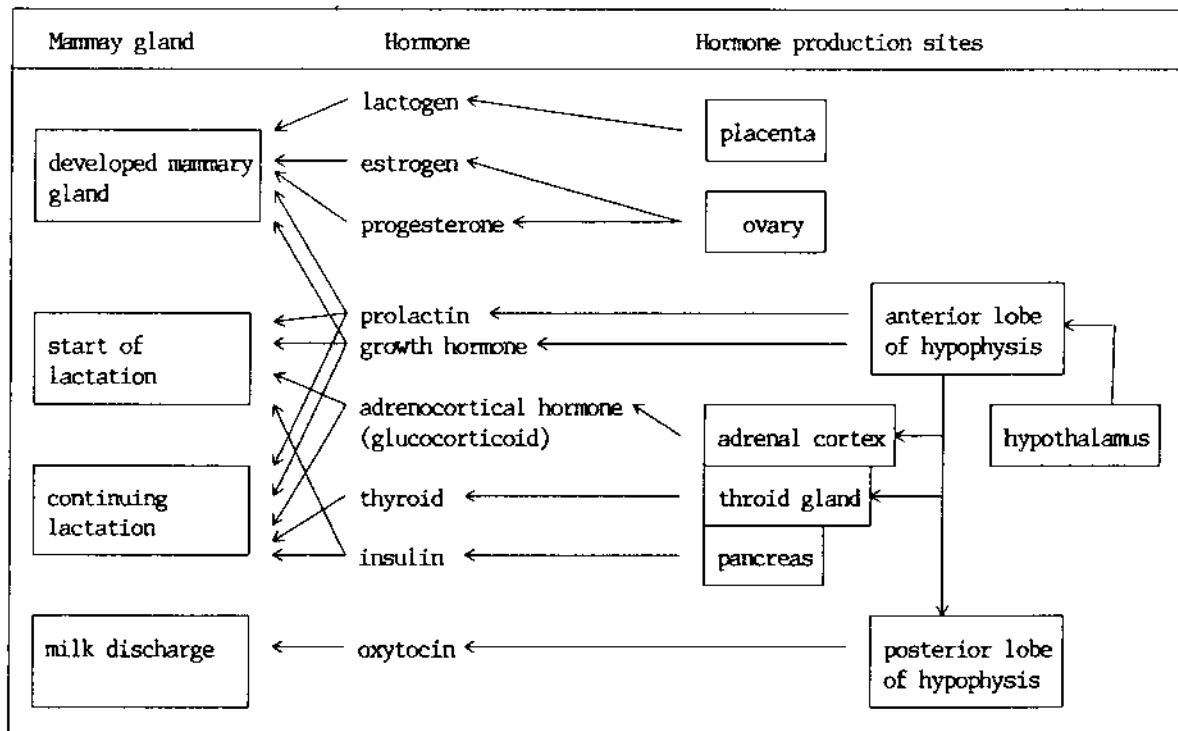


Table 3-1-7 Nutrient requirements for cow (Japan Feed Standard 1987)

weight	age	weight increase per day	dry matter	crude protein	digesti- ble crude protein	total digesti- ble nutrient	digesti- ble energy	calcium	phos- phorus	vitamin A	vitamin D
(kg)	(weeks)	(kg)	DM (kg)	CP (g)	DCP (g)	TDN (kg)	DE (Mcal)	Ca (g)	P (g)	(100 IU)	(IU)
45	1	0.3	0.7	116	99.5	0.79	3.50	5.7	3.9	3.0	270
50	3	0.4	1.2	162	130	1.03	4.53	9.4	5.1	3.3	300
		0.5	1.2	192	156	1.10	4.83	12	6.1	3.3	300
		0.6	1.2	221	182	1.16	5.13	14	7.2	3.3	300
75	8	0.6	2.2	321	221	1.66	7.31	19	7.6	5.0	450
		0.7	2.2	358	251	1.74	7.67	22	8.7	5.0	450
		0.8	2.2	395	281	1.82	8.02	25	9.7	5.0	450
100	13	0.6	2.9	379	244	2.04	8.98	19	7.9	6.6	600
		0.7	2.9	415	273	2.13	9.39	21	9.0	6.6	600
		0.8	2.9	452	302	2.22	9.79	24	10	6.6	600
150	24	0.6	4.1	466	276	2.72	12.0	20	8.7	9.9	900
		0.7	4.1	502	304	2.83	12.5	23	9.8	9.9	900
		0.8	4.1	537	333	2.94	13.0	26	11	9.9	900
200	34	0.6	5.2	546	3.6	3.33	14.7	22	10	13	1200
		0.7	5.2	581	333	3.46	15.3	25	11	13	1200
		0.8	5.2	616	361	3.59	15.8	28	12	13	1200
250	45	0.6	6.3	617	332	3.88	17.1	24	12	17	1500
		0.7	6.3	651	360	4.02	17.7	26	13	17	1500
		0.8	6.3	686	387	4.16	18.4	29	14	17	1500
300	55	0.6	7.2	681	356	4.37	19.3	26	14	20	1800
		0.7	7.2	715	383	4.53	20.0	29	14	20	1800
		0.8	7.2	749	411	4.69	20.7	32	15	20	1800
350	65	0.4	7.9	669	324	4.46	19.6	23	13	23	2100
		0.6	7.9	736	377	4.82	21.3	28	15	23	2100
		0.8	7.9	804	431	5.16	22.7	33	17	23	2100
400	77	0.3	8.3	663	309	4.54	20.0	21	15	26	2400
		0.5	8.6	751	369	5.03	22.2	26	17	26	2400
		0.7	8.6	818	423	5.40	23.8	30	18	26	2400
450	92	0.2	8.1	627	286	4.45	19.6	21	16	30	2700
		0.4	9.1	758	359	5.17	22.8	25	17	30	2700
		0.6	9.1	824	412	5.58	24.6	30	19	30	2700
500	110	0.1	7.9	669	300	4.32	19.0	20	16	33	3000
		0.3	9.6	840	386	5.25	23.1	25	18	33	3000
		0.5	9.6	907	439	5.70	25.1	29	20	33	3000
550	135	0.1	8.4	711	316	4.62	20.4	22	18	36	3300
		0.3	9.9	866	398	5.50	24.2	27	20	36	3300
		0.5	9.9	932	450	5.97	26.3	31	21	36	3300
600	173	0.1	8.9	751	333	4.92	21.7	24	19	40	3600
		0.2	9.8	840	377	5.41	23.9	26	20	40	3600
650	261	0.0	8.5	700	304	4.69	20.7	23	20	43	3900

Note: Calf is fed in short suckling period described in (1)-a-(b). Concentrated feed is given at 2.5kg per day until the weight reaches 450kg and is not given before it reaches 500kg. Assumed dry matter contents in milk substitute, calf starter and concentrated feed which is used after 13 weeks of age are 92%, 88%, 88% and the their assumed protein content (in dry matter) are 22%, 18% and 12%.

Table 3-1-8 Nutrient requirements for maintenance of a cow (Japan Feeding Standard 1987)

weight	dry matter	crude protein	digestible crude protein	total digestible nutrient	digestible energy	calcium	phosphorus	vitamin
(kg)	DM (kg)	CP (g)	CP (g)	TDN (kg)	DE (Mcal)	Ca (g)	P (g)	A (100IU)
350	5.0	365	219	3.02	13.3	12	9.2	23
400	5.5	404	242	3.34	14.7	14	12	26
450	6.0	441	265	3.65	16.1	16	14	30
500	6.5	478	287	3.95	17.4	18	15	33
550	7.0	513	308	4.24	18.7	20	17	36
600	7.5	548	329	4.53	20.0	21	19	40
650	8.0	581	349	4.81	21.2	23	20	43
700	8.5	615	369	5.09	22.4	25	22	46
750	9.0	647	388	5.36	23.6	27	23	50

Note: Use nutrient requirements for cows until first delivery. Nutrient requirements for a cow between first and second deliveries and for a cow between second and third deliveries must be 115% and 105% of the value stated in this table due to a weight increase.

Table 3-1-9 Supplementary nutrient requirement for a cow in the last stage of pregnancy and for a lactating cow (Japan Feeding Standard 1987)

Supplementary nutrient requirement for maintenance of a cow in the last stage of pregnancy (within 2 months of delivery)

crude protein	digestible crude protein	total digestible nutrient	digestible energy	calcium	phosphorus	vitamin
CP (g)	CP (g)	TDN (kg)	DE (Mcal)	Ca (g)	P (g)	A (100IU)
367	220	1.66	7.3	16	8	20

Note: Assumed increase in intake of dry matter in this period is about 5kg.

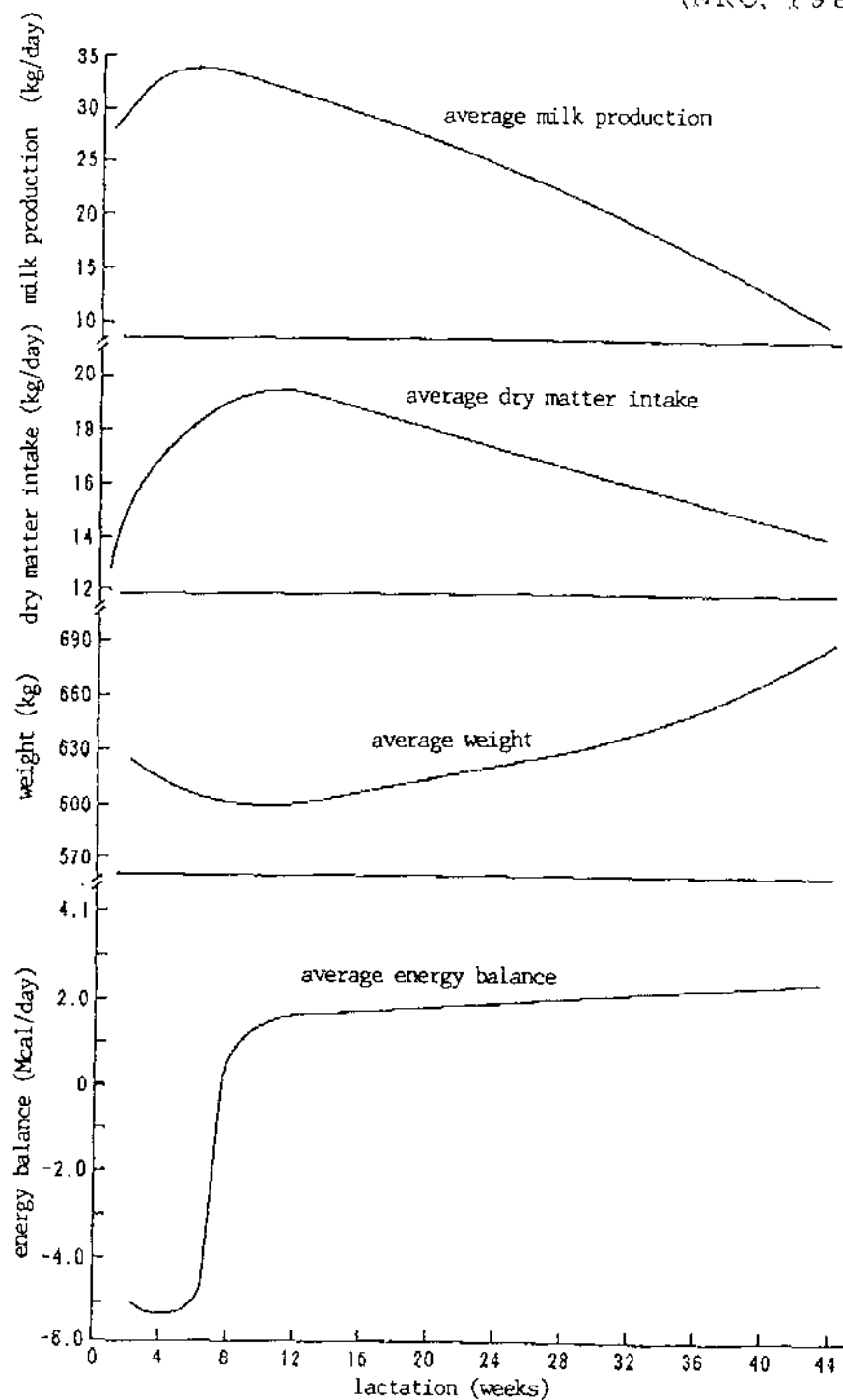
Supplementary nutrient requirement for maintenance of a cow during lactation

milk fat ratio	crude protein	digestible crude protein	total digestible nutrient	digestible energy	calcium	phosphorus
(%)	CP (g)	CP (g)	TDN (kg)	DE (Mcal)	Ca (g)	P (g)
2.8	64	41	0.270	1.19	2.6	1.6
3.0	65	43	0.280	1.23	2.6	1.6
3.5	69	45	0.305	1.35	2.7	1.7
4.0	74	48	0.330	1.46	2.8	1.8
4.5	78	50	0.355	1.57	2.9	1.9
5.0	82	53	0.380	1.68	3.0	2.0
5.5	86	56	0.405	1.79	3.1	2.1
6.0	90	58	0.430	1.90	3.2	2.2

Note: For a cow which produces not less than 20kg of milk, add 5% of the total nutrient requirement, which is the sum of the value in Table 3-1-8 and the volume in this table, to the total requirement for every 10kg increment in milk production.

Figure 3-1-1 Change in milk production, dry matter intake, weight of lactating cow.

(NRC, 1989)



2. Feeding and Management of Beef Cattle

2-1 Breed

It is estimated that there are more than 800 breeds of cattle throughout the world. The major breeds around the world which could be improved for beef production are shown in Table 3-2-1. In addition to cattle, animals such as water buffalo and some kinds of partially domesticated animals are used for meat production as well as drafting and milk production in some areas. Examples of the kinds of cow are Gaul, Gayal, Banteng, Balinese, Maduresea, Grati and Kouprey. Buffalos are found in humid areas.

In Southeast Asia, most cattle bred for beef are a dual-purpose breed which is also used for drafting. In this dual purpose breed, many animals are hybrids between local breeds and European breeds, the major breed of which is zebu and its varieties (Figure 3-2-1). The home of the zebu is India and there are many varieties of this breed in the world. This breed has a hump, high heat tolerance and high resistance to cattle tick fever, and is suitable for breeding in tropical and subtropical areas.

It is possible to produce hybrids between this breed and European breeds, and the hybrids are widely used for beef cattle in many countries. This breed is bred for beef production anywhere in Southeast Asia except in the countries where consumption of beef is prohibited by religion. Examples of the major varieties of zebu bred in this area are Nellore and Brahman.

2-2 Feed Management of Breeding Cattle

(1) Basic Considerations

(1-1) Individual Identification

Effective individual identification is an important factor in beef cattle management since such cattle are often bred in herds. The identification is also essential in distinguishing cattle of one's own herd from outside cattle. Individual markings should be applied to the

cattle immediately after they are born or newly introduced into the herd.

Here are some identification methods used today:

- Ear marking and a neck tag,
- Tattooing the ear,
- Branding with a hot iron on the horn, the hoof or the skin,

(branding methods also involve application of liquid nitrogen or dry ice which marks the skin by freezing, but this method is costly in tropical areas) — Application of a muzzle pattern and a diagram of hair color marking

- Recording the position of the whorl,
- Writing a number on the hair with decolorant (hydrogen peroxide), spray paint or hair dye.
- Clipping,
- Cutting the ear,

Some marks can be identified only from close up, and others can only be seen when the cattle stands in a specific posture. Some marks need to be reapplied after a short time, and others are costly due to the use of special equipment. Of these methods, ear marking is widely used because of its advantages such as ease, clarity of identification, and durability.

(See " (3) Management of Calves b. Individual Identification" for the methods of applying ear marks and tattooing.)

(1-2) Composition of Herd

Feeding can be made easier and herd management can be simplified by gathering cattle having similarities into groups and breeding each group if many cattle are together. Some such groups include (i) mating groups (cow for A.I. or pasture breeding), (ii) delivery group, (iii) pregnant group, (iv) raising group, (v) weaning group, (vi) fattening group. The structure differs according to the size, management methods and the land use of the farm. Herd management can be more effective by placing groups which require frequent observations and gathering, such as mating cow groups, in a paddock near to the maintenance facility such as the farmyard.

(1-3) Observations

The purpose of observations of the herd is to oversee individual conditions such as their health, estrus and state of delivery in order that appropriate treatment may be applied and that the number of animals in the herd can be counted to try to prevent straying and theft. A hard of cattle in or around a barn can easily be watched over, but in the grazing season, some cattle out in the field may not be watched over very carefully since the whole herd is widely scattered and it may take time for the observer to move from one herd to another. In this case, the frequency of visits to each group must be planned according to the requirements of each group.

Individual health such as wounds, the state of nutrition and walking style should be observed in order to detect diseases. A body condition score can be used to check the nutrition of parents (Table 3-2-2, Figure 3-2-2). The state of estrus can be understood by factors such as the behavior of a cow and the condition of her vulva. An estrus detector such as an estrus mount detector and chin ball can be used in addition. The condition of delivery should be observed carefully for primipara, which often causes dystocia, and cows who have experienced problems in previous deliveries. Prevention of loss of calves by assisting in delivery or attempting artificial respiration may be necessary. Prevention of straying and theft can be accomplished by periodically matching actual cattle numbers with the cattle numbers in the herd record.

(1-4) Management of the Bodies of Cattle

Management of the bodies of cattle consists of periodic checks such as weight measurement and inoculations and non-periodic checks such as copulation, hoof cutting, castration and treatment of diseases. The latter activities are carried out according to the situation of each animal. The specific items in the periodic checks are determined by the structure of the herd and should be carried out for each group in the herd according to the program set out for the year. Non-periodic checks can be carried out effectively by improving the method of observation and gathering since these checks often involve individuals only.

(1-5) Inspection, Repair and Maintenance of Facilities and Equipment

Equipment and facilities such as fences, collecting fences, water facilities and feeding facilities must be inspected and maintained before commencement of grazing or housing. The barn and other facilities must be disinfected before use.

(1-6) Organization of Records

Preparing records is the best way to improve management skills. Problems on the farm can be detected and a solution can be found by organizing and analyzing daily records. Items included in such records are, for example, delivery and growth of calves, cattle diseases, feeding and other farm activities. These items may be recorded in relation to each other and checklists may be prepared for some items which must be observed daily to ensure that observation works.

(2) Practical Feed Management

(2-1) Improvement of Feed System

Management of livestock can be improved in Southeast Asia in order to develop agriculture and food production. The following are goals for improvement of management;

- Effective use and management of pastures, natural grasslands and wasteland,
- Attention to be paid to forage crops in terms of economic crop production,
- Use of local crops which can replace imported feed,
- Improvement of digestivity of feed such as straw, crushed cane stalks (residue of cane sugar) and stems of bananas, in addition to making an evaluation of nourishment in feed. The improvement of digestivity can be accomplished by applying better physical or chemical treatment when storing and processing feed.

Some of these objectives may be omitted when they are applied to specific areas in Southeast Asia where the source of feed widely differs from place to place.

The major source of feed for livestock used by farmers includes

residues from reaped rice and wheat fields where cows are freely kept, and weeds or wild grass near roads, river banks or under plantation crops such as coconuts, where the animals are kept freely or are tethered. Weeds or wild grass are used as green forage every morning and evening by some farmers. The volume of such feed is very changable according to the weather. In the dry season, insufficient feed production will cause a decrease in animal reproduction due to a serious lack of nutrient. However, concentrated feed is mainly made from agricultural byproducts partly because commercial feed is costly.

(2-2) Feed Supply and Management

① Important Functions for Breeding Cows

Cows are evaluated by their efficiency and reliability in producing good calves and some of the desirable functions of cows include;

- Strong estrus, little dystocia and tolerance to repeated reproduction,
- High milk yield for nursing
- Good health, in particular strong legs and hooves, and
- Good intake of roughage and high feed efficiency.

It is essential in the management of cows to provide sufficient nutrient and a clean environment to maintain exercise and the health of animals.

② Basic Feed Supply

The basic feed supply provides sufficient feed to heifers and cows to maintain their health and to deliver calves as economically as possible.

Values for feed such as nutrient requirement, necessary volume of dry matter and the nutrient value of feed should be calculated according to a feed standard for the kind of feed, weight of individual cattle and stage of reproduction. The Japanese Feeding Standards are shown in Tables 3-2-3, 3-2-4, 3-2-4, 3-2-5, 3-2-6 as examples. Ideally, the feeding standard must reflect the area where it is applied, for example, the climate.

The Japanese Feeding Standards in the tables show the required volume

and a suitable balance of various nutrients necessary in the maintenance, growth and reproduction of beef cattle and for the production of beef. Generally, the nutrient requirement do not change for different breeds of cattle with the same weight on the same milk yield per day. However, if the tables are used in an area of different environment conditions, the values should be arranged according to the features of the breed such as size of the body and milk yield as well as temperature of the environment and management conditions.

The feed for a cow should satisfy the values shown in the standard tables, i.e. the values for dry matter (DM), total digestive nutrients (TDN), and digestive crude protein (TDN). Actual feed volume is determined by adding the volumes corresponding to the safety factors for each condition to the volumes in the standard table, since the table shows the minimum nutrient requirements. The safety factors for roughage are shown in Table 3-2-7. These safety factors are for changes in components and digestibility of roughage and the percentage change in feed intake. As shown in this table, feed intake is not equal to the volume of feed given to the animal.

③ Types of Feed

Feed sources in tropical area vary according to regions, and different feed sources have different feed values. It is essential in feed management to obtain feeds of the highest quality at the lowest cost from the local area and to use them to satisfy the nutrient requirements.

Here are some feed sources for livestock besides roughage fed in grazing in reaped fields, rice fields for example, or in natural grassland.

- Grass for fodder and hay, such as elephant grass, napier grass, or guinea grass,
- Trees such as *Leucaena leucocephala*, *Gliricida maculata*, *Sesbania grandiflora* (Tables 3-2-8, 3-2-9).
- Agricultural byproducts such as copula refuse, rice bran, poultry manure, cassava chips, stalks or stems of tubers, crushed cane stalks and leaves and stems of bananas. These byproducts are highly utilized.

④ Feed Volume and Supply Methods

In the housing method, roughage such as hay and silage is mainly fed ad libitum, and concentrated feeds such as rice bran and soybean waste are used according to the stage of reproduction of the parent cattle.

Here is an easy way to determine the feed volume;

- Collect data on the cattle group such as the average weight of cattle, average weight gain per day per head, and stage of reproduction.
- Based on this data, calculate the required nutrients and dry matter per head according to the Standard Feeding Tables.
- Determine percentage IDN and DCP and water content for each obtainable feed through feed analysis or from the table of feed composition.
- Determine the kinds of feed and calculate the volumes of TDN, DCP and dry matter per head for each feed which comprises the total feed actually given. Make sure the volume will satisfy the feed requirement.
- Calculate dry matter in total feed and check if dry matter approximates to the dry matter intake per head.

⑤ Feed Supply during Gestation, Parturition and Nursing

Concentrated feed is not necessary for cows until 2 months before delivery if high-quality roughage is given in free feeding, but it should be supplied as a supplement if the quality of roughage is low.

Extra nutrients must be given for the cow within 2 months of delivery and after delivery for marked growth of the fetus in the former period and for lactation in the latter period. For extra nutrients, some values shown in the standard feeding table for cows should be added to the values of nutrient requirements for cow maintenance. Examples of calculating the volume are shown in Tables 3-2-10 and 3-2-10.

Continuous insufficient nutrition during this period impairs the growth of suckling calves as well as a delay the recovery of reproductive function and a return to estrus in mothers and a decreases the conception rate after delivery.

⑥ Nutrient Requirement in Hot Climate

Environmental factors may influence the management of beef cattle to varying degrees according to the condition of cattle such as breed, age and accommodation of the cattle and general herd management. However, temperatures ranging between 15 to 25°C will not influence feed intake and feed efficiency. Beef cattle, for example, may well show a slow weight increase in an area where they suffer stress from high temperatures. When the environmental temperature is in excess of their tolerance limit, a decrease in feed intake will be observed as well as an increase in respiratory rate and rise in body temperature. The decrease in feed intake will slow down their weight gain.

Under such temperature conditions, high energy feed of high feed efficiency should be used, and the TDN value should be 10 - 20% higher than that in the feeding standard required for maintenance of a cow. In addition to changing feed quality, preparing a cool environment such as constructing a standard area, showering and fanning the cattle will improve feed efficiency.

(2-3) Estrus

① Signs and Detection of Estrus

Estrus indicates a time when cow libido increases. More specifically, cows approach bulls and will partake in copulation. Some of the signs include restless movement, frequent bellowing, swelling of the vulva and discharge of mucus. Cows in estrus will mount or let themselves be mounted by other cows in the herd. Some of the suckling male calves in the same herd will follow the cows about.

The best way to detect if a cow is in estrus is frequent and periodical observation of females. However, the chin ball and estrus mount detector methods may help busy farm managers who, for example, have to take care of large herds. In the chin ball method, a device containing dye is hung under the chin of bulls whose seminal ducts have been ligated, and the loins and rumps of cows in estrus are colored when the bulls mount the cows (Figure 3-2-3). In the estrus mount detector method, bags

containing dye are attached to the loins of cows, and the skin of cows in estrus is colored due to breakage of the bags when they are mounted (Figure 3-2-4).

② Estrus Cycle and Return of Estrus

Estrus repeats itself every 21 days on average and the cycle is between 18 days minimum and 25 days maximum in cows with normal ovaries, except during gestation or if they are suffering disorders such as excessive malnutrition. Careful observation should be carried out periodically for cows, in particular, 20th day after the last estrus. Note that cows with good nutrition may show a short estrus cycle.

The first estrus cycle after delivery occurs about 40 - 70th day after birth in many cows but it may occur earlier than 20th day or as late as 6 - 7th month after delivery. The duration between delivery and the first estrus cycle differs according to factors such as the strength of stimulation by sucking, recovery of the uterus and nutrition of the cow.

(Feed management for cows in the last stage of gestation and after delivery was discussed in (b) Feed Supply Management, and (v) Feed Supply in Gestation, Parturition and Nursing). Careful observations should be carried out in cows after delivery, too, to detect the return of estrus. Insemination should be postponed in a very early return of estrus since the uterus may not be fully recovered. Conception within 80 days after delivery can achieve one delivery per year. Development of the ovaries and recovery of the uterus of animals who have shown no clear estrus for 3 months after delivery can be assumed to show delayed recovery.

(2-4) Insemination

Sire progeny should show high meat production. Artificial insemination is recommended among other insemination methods since the semen collected from sires can be used over wide area. Pasture breeding can be used in mating cows who were unsuccessful in artificial insemination in order to improve the conception rate of the herd. Sires of breeds whose calves are of small birth weight may be used to mate heifers in order to prevent accidents at delivery.

The duration of estrus in a cow is 21 hours on average, ranging from 16 to 26 hours. Short and weak estrus may indicate an imbalance of hormones and long estrus may obstruct ovulation. Ovulation takes place about 28th hour after the commencement of estrus or 6th to 10th hour after the end of estrus.

The ovum is fertilized in the ampulla of the oviduct and the fertilization ability of spermatozoa lasts about 40 hours while that of the ovum is as short as 6 - 10 hours. Due to this short duration, the key to insemination is to find the period of ovulation in cows. Suitable periods for insemination are shown in Figure 3-2-5. Insemination should be undertaken in the afternoon if estrus is observed in early the morning, in the evening or early next morning if it is observed in the morning, and next morning if it is found in the afternoon. This is because conception is most successful during the medium to the latter stage of estrus which is 9 to 24 hours after the commencement of estrus. The person managing insemination of cows must receive a precise report on cows in estrus from the manager of the herd, such as the time estrus was observed and the state of estrus. A suitable time should be decided by observation of outward signs and an internal examination since the duration of estrus and the time of ovulation differ in individuals.

(2-5) Pregnancy

① Signs and diagnosis of pregnancy

Estrus ceases due to gestation since the corpora lutea of pregnancy are formed in the ovary in gestation and restricts the growth of ovarian follicles which stimulate estrus.

Some methods to correctly confirm gestation are rectal palpation on fetal membrane by experienced personnel (Figures 3-2-6 and 3-2-7) and an observation of corpora lutea of pregnancy, both of which can be undertaken about 40 days after insemination. Gestation is predicted by the cessation of estrus but this is not accurate since the possibility of wrong diagnosis is 15% if the cessation of estrus 60 days after insemination is presumed to indicate gestation.

Some of the outward signs of gestation are an increase in appetite,

good nutrition, a more docile manner and relaxed motion. The udder and vulva swell 5 months after the beginning of gestation.

② Gestation period

Gestation period for a cow is about 280 days on average, and it differs slightly according to factors such as breed, age, and calving number of the cow as well as sex, size and nutrition of the fetus within the cow. For example, the gestation period of young cows may be short, and that of cows with a high calving number may be long. A male fetus may result in a longer gestation period than a female fetus, and a fetus with good growth, or a large one, may result in a short gestation period in the mother. There are many causes of a long gestation period as stated above, but prolonged gestation, which is a gestational disorder, may be caused by genetic factors.

The expected date of delivery can be calculated by the insemination date and the length of gestation. Changes in condition of a cow must be carefully observed every day especially within one week before the expected date of delivery, since she might deliver before that date.

(2-6) Delivery

① Signs and Process of Delivery

When delivery approaches, some signs can be observed, for example, movement of the cow is slow, the vulva expands and becomes loose, both sides of the root of the tail become indented the tail near the rump rise upwards and the udder is enlarged. One or two days before delivery, these signs are much more obvious and other signs can be observed; eg, thick string-like mucus is discharged from the vulva and milk shows a yellow color. When delivery is very close, the cow will lose her appetite and will walk about in a restless and uneasy manner.

Normal delivery is as follows.

Opening period: Delivery begins with labor pains. The first few labor pains are weak and have long intervals but these intervals get shorter as time passes. The birth canal is open by the fetal sac at this

time, and the fetus is pushed into the canal changing its position in a stated sequence.

Expulsion period: The fetus will enter the canal with its head forward and with its fore legs stretched out in normal cases. As time passes, the fetal sac bursts and the amniotic fluid discharges from the sac (the first rupture of the sac). This fluid will lubricate the canal and this makes delivery of the fetus easier. The fore legs of the fetus can be observed through the translucent amnion after it ruptures. Amniotic fluid then flows from the vulva by breaking of the amnion (the second rupture of the sac). The fetus will be expelled smoothly after the head and the shoulders appear. It takes about 2 hours, or 3 to 4 hours in some cases, from the commencement of the labor pains to expulsion of the calf.

Postpartum period: Delivery is completed by expulsion of the afterbirth after labor pains occurring after delivery following expulsion of the calf. The afterbirth should be disposed of so as to keep the site clean and safe, though the cow might eat it. If not, the afterbirth rots at the site or stray dogs may come close to the site for the afterbirth.

② Dystocia

Some of the factors which cause dystocia are abnormal gestation period, insufficient size of cow and fetus, genetic, abnormalities of individual sires and abnormal position of fetus, i.e. buttocks first. Watching the movement of cows is essential in detecting dystocia for the first 3 - 4 hours in the course of delivery. The newborn calf may be pulled out from the birth canal using a rope if time passes without expulsion of the calf although its fore legs and head have emerged normally. The rope should be tied at the fetlocks of the fore legs, and the calf must be pulled just at the time when a labor pain is observed. Other cases of dystocia must be treated by veterinarians.

(2-7) Preparation and Aftercare for Delivery

① Preparation

In the housing method, mothers should be brought into the calving pen

when signs of delivery are observed and clean bedding should be laid in the pen. Some of the requirements for delivery are buckets, disinfectant, cloth, cotton, tincture of iodine, scissors, a rope, lubricant (cooking oil), and some light. These should be checked and the necessary personnel such as a veterinarian and experienced persons involved in caring for and helping delivery of the cow should be asked to stand by since delivery may be at night.

② Management of Newborn

If the calf appears dead due to swallowing amniotic fluid, make the calf vomit out the liquid or try artificial respiration. Swing the calf upside down by its hind legs to make the calf vomit the liquid. Push the breast in artificial respiration.

The muzzle, mouth and body of the calf should be wiped with soft bedding or cloth if the mother, such as a heifer and nervous cow, does not lick the body of the calf and dry it as a normal mother would do.

The umbilical cord breaks naturally. It should be disinfected with tincture of iodine to prevent inflammation.

Make sure that colostrum, or milk expressed within 3 - 5 days of delivery is fed to the calf since it has an important effect on the calf such as immunizing the calf and triggering the excretion of meconium. Feed colostrum artificially if the mother, who might be tired due to the first delivery or dystocia, refuses to feed it or if the calf is too tired to suck by itself.

③ Aftercare for the Mother Cow

It is essential for a mother cow to rest quietly after delivery. The afterbirth will be expelled 2 - 3 hours after delivery. No expulsion of afterbirth 8 - 12 hours after delivery indicates retention of the placenta.

In this case, the afterbirth must be removed artificially as soon as possible by a veterinarian in order to prevent bleeding disorders which may be caused by such disorders such as endometritis due to retention.

The teats of the cow showing high lactation may swell due to sucking.

In this case, feed volume for the cow may be decreased for a while or

the remaining milk may be removed.

2-3 Management of Calf

(1) Feeding

The abomasum which plays an important role in digesting milk is more mature than other stomachs in a calf in the suckling period. Lactation by the mother cow peaks at 2 - 3 weeks after delivery and decreases from then on. Solids must be fed in addition milk from 2 months of age for the calf since the nutrients in milk alone is insufficient for the growth of the calf in that age when the rumen is mature enough to digest solids.

The rumen develops as a result of taking solids, high-quality roughage for example.

The calf should be kept and fed separately from the mother if she is a heifer or produces little milk, or in a season when grass feed is insufficient. Calves kept separately from the cow can be weaned easily with little stress, and growth after weaning will be better than calves kept otherwise.

In feeding a calf which has been separated from the cow, high-quality hay is mainly given and concentrated feed may be used as a supplement. Basically, the feed volume per day is about the volume a calf can eat. Concentrated feed for the calf contains approximately 13% of the DCP and 70% of the TDN volumes, and its volume is about 0.1 - 1.0% of the weight of the calf (Table 3-2-12).

In housing, a place for the calf for separation may be an enclosure in the barn, of 40 cm width and about 100 cm height, into which a calf can go. Bedding should be layed on the floor of the enclosure to keep it dry. In grazing, an enclosure into which a calf can go should be built near the area where the mother cows gather, a watering trough, for example, and concentrated feed should be fed 1.0 - 1.5 kg per day per calf before weaning.

(2) Individual Identification

Individual markings should be applied to every individual to

distinguish one from another. In particular, in grazing the mark should be applied to a calf immediately after it is delivered so that matching of the calf and the mothers is easier afterwards.

Ear marking is widely used due to its advantages such as economy, the ease of marking, the accuracy in identification in the herd and durability. Tattooing of the ear which is permanent should be carried out along with ear marking since the ear marking may fade while grazing. These two marks may be put on ears on each side, i.e. ear marking on the left ear and tattooing on the right ear.

There are various kinds of ear markings. The kind shown in Figure 3-2-8 has various colors on the plate. The individual number of the calf as well as that of the parent and the date of birth can be written on the plate. Such information including the colors are useful in the management of cattle.

The procedure for tattooing is as follows (Figure 3-2-9);

- 1) Lay the calf down on a flat place and hold it down.
- 2) Clean an area of the ear where there are no blood vessels and cartilage.
- 3) Spread the dye for tattooing over the area.
- 4) Set a number on the tattooing tool and grip the chosen area of the ear in the tool to impress the number.
- 5) Rub the number with fingers or a brush.

(3) Castration

The main purpose of castration is make the animal more docile, to improve the quality of beef, and to prevent natural mating within the herd. Bulls should be castrated at 2 - 3 months of age since the stress caused by this treatment is low and recovery of the affected part is fast in calves in the early stage of suckling. There are two methods for castration; bloodless castration and bloody castration. The spermatic cord crushing method is one of the conventional methods (Figure 3-2-10).

This method is widely used since stress in the calf due to treatment is low and treatment itself is easy and can be completed in a short time. The procedure is as follows;

- 1) Place the calf in a treatment stall and tether it in the

standing position.

- 2) Pull the right testis down. Press the spermatic cord which is 3 - 4 cm above the testis and position it on the right side of scrotum.
- 3) Grip the cord lightly in the crushing tool by holding the blade of the slip preventer downward. Make sure the cord is gripped in the blade.
- 4) Squeeze the handle quickly and hold it firmly for 20 - 30 seconds.
- 5) Palpate the cord with the fingers and make sure that it is cut, then loosen the tool.

Do the same for the left spermatic cord.

- 6) Apply tincture of iodine to the affected part.

Make sure one of the two cords on different sides is cut each time, and a different part is gripped in order to crush a different cord. Do not injure the fingers or hands of helpers. The scrotum swells 4 - 5 days after treatment, and atrophies about 1 month after treatment.

The bloody method is unhygienic especially in tropical areas since insects, flies for example, remain around the treated part where there is blood.

(4) Dehorning

Dehorning is desirable when breeding many cattle in a herd. Some advances of dehorning are prevention of injury due to the movement of cattle, in particular, when feeding in a group, and accidents involving animal managers. Dehorning may be performed during the suckling period when the horns are small. Consequently, there is little pain for the animal and treatment is easy.

There are several tools for dehorning according to each method; a saw, hot pipe or iron, a rubber ring which is put applied to the base of a horn, and a dehorner. There are several types of the dehorner according to the size of the horn (Figure 3-2-11).

Dehorning may be avoided in very humid seasons. If not, special care must be taken for hygiene of the wound caused by this treatment, since the wound slowly heals in a season where suppuration is possible and where

insects such as flies, remain on this part.

(5) Weaning

Weaning should be completed ideally at 6 months of age, or practically at 5 - 7 months of age of the calf, from when milk production by the mother decreases. Roughage fed in the course of weaning promotes the development of stomachs and growth of the calf. A delay of weaning will affect reproduction of the mother since she requires extra nutrients for preparation for the next delivery.

The calf and the mother should be kept apart, even during grazing, so that they cannot see each other. It is also desirable to keep them in different barns or paddocks. They may cry and be restless for 2 - 3 days after separation. Fences and doors of the barn or the paddock must be solidly built since some of separated animals will try to escape in order to meet other animals. The calves should be kept from the herd of mothers for about 3 months.

Roughage alone must be given on the first day of weaning and 1 - 2 kg of concentrated feed must be added from the second day. High-quality roughage must be fed to supply sufficient nutrient to the calf. The composition of concentrated feed is explained in "a. Feeding". Note that sufficient should be given. Weaning will be completed in 7 - 10 days. Calves must then be brought out for grazing or must be given silage if they have been housed. When the feed intake of a calf increases, the volume of concentrated feed must be controlled since calves are apt to develop obesity.

Measure the weight and withers height of the calf on the day of weaning so that the data can be used for selection and weeding out of the calves from mothers.

At the time of weaning, the mother cow should have stopped lactating.

Hay alone must be given at 5 - 6 kg per day for the cow without succulent feed. Usual feed should be fed one week after this.

2-4 Management of Breeding Cattle

(1) Selection of renewal out Cattle for Reproduction

Cows may be used for reproduction until 10 years of age or 8 deliveries if the cow is of good health. Basically, 1/8 of the cows for reproduction should be weeded out for a year while heifers replace them.

The following cows may be weeded out:

- Those who easily fall ill.
- Those with low fertility.
- Those of wild character.
- Those who are physically deficient.
- Those producing calves showing poor weight gain.

Heifers replacing cows can be selected in the period between 12 months of age and the first insemination since both the advantages and disadvantages of heifers can be seen in this period. Good cows for reproduction are rugged, show high reproduction, good nursing ability and a docile character. With this goal, calves having the following conditions may be selected for replacement:

Those who are healthy.

Those who show good growth, or have a large frame.

Those who have strong legs and hooves, and long legs.

Those who have a strong, straight backbone and strong loin muscles.

Those who have a wide and deep chest.

Those who have a big mouth and wide jaw.

Those who have developed ribs, and a good volume.

Those who are not obese.

Those who are not nervous.

(2) Feeding and Management

(2-1) General Feed Management of Breeding Cattle

After weaning, the calves should be reared in three groups; female calves for reproduction, female calves to be weeded out and male calves. The calves of the first group should be raised so that they become hardy and productive cows, using enough well-balanced nutrient and exercise.

Few diseases or accidents occur in the rearing period, or period between weaning and first delivery, thus the management in this period is

easier. There is no need to prepare a barn for the cattle in this period if there are simple shelters or copses and watering troughs. Grazing is better than housing in terms of exercise for calves. Supplementary feed is not needed for the rearing of cattle if the volume of grass in the pasture is sufficient and water and salts are provided.

In housing, the position of facilities such as cow shed, a manger, a watering trough and a mineral salt trough should be kept apart so that the movements of the calves increased.

Ensure that nutrient for heifers is sufficient since the heifers are still growing and need extra nutrient for growth and for the fetus in particular in the late stage of gestation. Heifers should be given sufficient exercise in the paddock since insufficient exercise will cause obesity which stimulates dystocia.

Heifers, after delivery or during lactation, need nutrient for their own growth as well as for lactation. Ensure that they can take sufficient nutrients for growth. Extra feed may be prepared in a manger so as to keep cows from eating all the feed. A separate facility should be built to relax the burden of lactation for the mothers as well as to stimulate the growth of newborn calves.

(2-2) Feed Management of Breeding Cattle

Some of the basic concepts of feed management for breeding cattle are described above. The volume of feed can be determined from the Feeding Standard Tables which shows the nutrient requirements for the maintenance of breeding cattle as well as the extra nutrient required in some particular stages of heifer growth. Make sure the determined volume is given to them to satisfy their requirements (Table 3-2-13).

(3) First Estrus and Insemination

First estrus of heifers can be observed at 10 - 12 months of age. This differs according to the breed, the growth and the nutrition of a heifer.

The time of first insemination can be determined by growth and age of the heifer. In the case of Angus and Hereford, insemination can be

carried out when the age, the withers height, and the weight of heifer reach about 14 - 16 months, 110 cm and 300 kg or more, respectively, if it has good nutrition and has shown a good condition during growth.

Insemination of young heifers may affect the growth of the animal in particular in the nursing period since nursing requires the most labor effort from the animal through the process of reproduction which includes estrus, insemination, gestation, delivery and nursing. Some experiments on insemination of young heifers were carried out to increase the calving number of a cow, but insemination in this period was not related to the increase in productivity in breeding a herd.

2-5 Management of Sire

(1) Management as Pasture Bull

In the grazing season, sires are often used as pasture bulls, while housed, they are used for artificial insemination as well as for mating with cows kept in the same paddock in a herd.

Here are some notes on pasture breeding;

- Ensure that semen and the sire are normal before commencing pasture breeding.

- The number of cows in a herd should be about 40 - 50, of which heifers should comprise half this number.

The service period should be 2 - 3 months. It is not desirable to keep the sire in the herd for whole year since the sire may be exhausted after such long service, and its strength and conception rate decreases.

A paddock for grazing should be as small as possible and rational grazing should be practiced.

- Periodically observe the sire to detect mating and disease in the animal.
- Take the sire from the paddock soon after the service period is over since the animal will be exhausted or, more specifically, decrease its weight by 100 - 150 kg in service due to the increase in motion.

(2) Management in the Rest Period

After service, the sire should be kept in a pasture with plenty of grass for grazing, or be fed high-quality roughage when housed. Concentrated feed should be fed according to the requirement determined by the weight of the animal so that obesity in the sire, including a breeding sire, can be avoided in the period until the next service. The volume of feed can be determined according to the Feeding Standard. A standard table and an example of feeding are shown in Table 3-2-14 and 3-2-15.

Sufficient roughage should be fed even after the weight of the animal has recovered, while a deficiency of micronutrients should also be avoided. Exercise is important for the sire in the rest period when the movement is restricted while housed. The recommended frequency of exercise is 2 - 3 times per week and a minimum of 1 hour each time. Free exercise in a large paddock may help strengthen the feet and hooves of the animal.

It is essential for the sire to exercise for semen production and collection. The recommended frequency of collecting semen is twice per week, the same frequency as exercise. The feeding method is the same as the rest period and obesity should be avoided.

Prospective sires should be tamed while in the breeding period by frequent contact with herdsman during exercise so that they can be caught easily when needed.

2-6 Management of Fattening Cattle

(1) Fattening Method

The term "fattening" means to produce high-quality beef from the accumulation of fat in the meat by providing feeds with a high nutrient content. In Southeast Asia, fattening is rarely practiced since the feed is poor and most of the cattle have a dual purpose as a draft breed. In Japan, there are many kinds of fattening methods and the management of fattening cattle differs according to the method. The management method cannot remain fixed since it must be changed according to factors such as

the characteristics of newly introduced cattle, conditions of feeding and standards which the beef market demands.

The fattening methods widely practiced in Japan are listed in Table 3-2-16 and some principles of management are explained here.

(2) Management when Introducing Cattle

Newly introduced cattle need about 2 weeks to acclimate to a new environment. They should be given a rest with water and high-quality hay fed in the free feeding method.

In housing, the composition of feed for the introduced cattle should be changed gradually to reach the composition used at the beginning of the fattening of the herd, since the kind of feed which has been given to the new cattle is often unknown. For this reason, the feed volume of concentrated feed should be about 1 kg and that of silage should be 2 - 3 kg for new cattle in the first few days. The feed volume can be gradually increased when the condition of the body of the cattle and the shape of their faces indicates adaptation of the cattle, and when left over decrease.

When grazing, an on and off system of grazing is recommended for cattle introduced for the first time. Some of the necessary treatment for cattle should be completed in this period, such as ear marking, castration, general examinations, application of vermifuges and vaccination for diseases such as diseases of respiratory system.

When fattening cows in pens, the recommended herd for fattening calves is composed of about 10 cattle of the same sex, age, size and weight. Changes in the composition of the herd or transfer of the herd in the course of fattening should be avoided since these changes will delay the growth of cattle.

(3) Management in Fattening Period

(3-1) Measuring Weight and Feed Intake

The weight of each cattle should be measured at introduction of the cattle or at the beginning of fattening and should be measured and

recorded every month until transfer. If a decrease or small increase of body weight from the last month is observed, examine if diseases are present, infestation of ectoparasites, stable fly (*Stomoxys calcitrans*), a marked change of environmental temperature and other environmental changes in the barn, all of which might be indicated by an abnormal change in body weight.

Feed intake of the whole herd should be measured. The volume of concentrated feed and the approximate volume of roughage must be measured before feeding and recorded.

(3-2) Daily Observation and Management

Observations of fattening cattle must be made every day in order to detect and treat abnormal cattle as early as possible. Some of the key points in observation are feed intake, respiration, manner of walking, swelling of the belly, movements of a cow or bull and its vitality, conditions of feces and urea, and conditions of the muzzle, eyes, head and ears.

Hoof cutting should be practiced at introduction of cattle and again at 5 - 6 months before transfer. The frequency and time of hoof cutting differs according to the length of the fattening period. Excessively long hooves decrease feed intake of a cow or bull as and cause accidents such as fractures of the legs.

Drinking water should be examined every day and the watering trough should be cleaned. Bedding should be added at 2 - 3 kg per cattle per day in a pilling up method, or whole bedding, as it gets dirty, should be exchanged with new bedding about twice a month.

(4) Environmental Stress

Environment factors which affect the production of beef in the course of fattening are divided into three categories; heat factors, physicochemical factors and biological factors. Examples of heat factors are environmental temperature, humidity and sunlight while physicochemical factors include light, water, the quality of feed, number of fattening cattle and the structure of a barn. Biological factors comprise pathogenic microorganism, parasites and pest insect all of which are involved in diseases. These factors are related to each other and affect production. However, among these factors the effect of heat, the environmental temperature, in particular, has often been discussed in recent years.

The relationship between environmental temperature and feed intake is shown in Table 3-2-17. Removing stress in cattle caused by a humid climate may be the most important factor for effective fattening management in Southeast Asia.

2-7 Major Facilities and Tools

(1) Facilities

(1-1) Cow shed

There are many different styles of barn according to simplicity; a shelter, as the simplest example, to a complete low barn. The style of barn on a farm should be decided by carefully considering factors such as feeding method, labor, cost and location. Grazing in a free barn is widely practiced in the management of rearing cattle (Figures 3-2-12 and 3-2-13). A free barn basically consists of a feeding place, resting place paddock in between the two sites, and creeper and calving pen are built in the barn if necessary.

(1-2) Corral

A corral is a place enclosed by a fence to drive in a herd of cattle which are not accustomed to individual management (Figure 3-2-14). This facility is useful in the management of a herd to divide it and to catch a cow or bull for treatment which includes measuring its size, checking its identification, installing identification marks, medical treatment such as applying vermifuges, vaccination, general health examinations and treatment of disease, and insemination.

The site of the corral should be a place which is useful to drive the herd as well as an area which is well drained. A place near a barn or paddock, or a place between two barns may be an economical site to build a corral. This facility must be functional in treating cattle, and be strongly built since the density of the herd driven into the facility will be high. A drive-in entrance and a shoot should be prepared at the corners of the corral in a pasture.

(1-3) Shelter woods and Shelter

A sheltering copse is essential for pasture in tropical area, where the climate is extremely hot, to keep cattle from suffering from heat stress. A mineral salt trough may be installed in this area since most of the cattle will come into the area from strong sunlight (Figure 3-2-15).

(2) Equipment for General Management

(2-1) Treatment Stall

A simple treatment stall can be helpful for management of cattle to tether a cow or bull in order to apply treatment such as insemination, castration, hoof cutting, medical treatment and nursing a calf with its mother who has rejected nursing (Figure 3-2-16). The stall is usually installed in a corner of the paddock or gathering place but a portable one is useful in treating cattle in a pasture or if the cattle cannot be carried.

(2-2) Halter

A halter is a rope or leather band fastened round a head of a cow or bull to control the animal and it can be used when the animal is moved to a treatment stall or is tied in one location for some time, or when a sire is given exercise or training. A rope halter is very useful and advantageous since it is inexpensive and very adjustable to the size of a cow or bull as well as to the needs for specific uses. It can serve as another useful tool, besides a muzzle ring, in Southeast Asia where cattle and water buffalo are often used and trained as draft animals. A simple knot for a halter is shown in Figure 3-2-17 as well as other useful knots which can be used in the farm are shown in Figure 3-2-18, 3-2-19, 3-2-20, 3-2-21.

Table 3-2-1
A comparison of abilities of beef cattle

BREED	Reproductive efficiency	Calf mortality	Birth weight	Weaning weight	Yearling weight	Age at puberty	Average mature weight: bulls	Average mature weight: cows	Crossbreeding use as sire	Crossbreeding use as dam	Feed efficiency	Carassability	Carass quality	Rank
Angus	4.86	4.14	4.43	2.86	2.57	4.71	2.86	3.00	3.14	4.71	3.14	2.71	5.00	1st
Barzona	3.20	3.60	3.80	3.20	3.40	2.80	3.60	3.40	3.40	3.00	3.00	3.00	2.60	37st*
Beefalo	1.80	3.20	3.00	2.80	2.80	2.20	3.60	3.00	2.40	2.60	2.80	3.20	2.20	44th
Beef Friesian	3.43	2.86	2.86	4.43	4.14	3.43	4.00	3.71	3.14	3.29	3.29	3.57	2.43	15th
Beefmaster	3.40	3.60	3.60	3.80	3.80	3.00	3.80	3.60	3.20	3.00	3.40	3.00	2.60	21st
Blonde d'Aquitaine	2.50	2.33	2.50	4.00	4.33	2.17	3.67	3.33	4.33	2.17	4.17	4.33	2.17	31st*
Braford	3.33	4.00	4.00	3.17	3.50	3.00	3.67	3.67	2.67	3.17	3.00	3.33	2.33	26th
Brahman	2.14	3.43	3.86	3.00	3.00	2.43	3.43	3.43	3.29	3.29	3.00	3.43	2.43	41st
Brangus	3.33	4.17	4.17	3.67	3.67	3.00	3.67	3.67	3.50	4.17	3.00	3.17	3.50	4th
Brown Swiss	2.14	2.57	2.86	4.29	4.14	2.57	4.14	3.86	3.00	3.57	3.14	4.00	2.57	25th
Charbray	2.67	2.50	2.33	2.83	3.17	2.00	3.33	2.83	2.83	2.33	3.00	3.00	2.00	45th
Charolais	2.86	2.00	2.57	4.57	4.86	2.14	4.57	4.00	4.71	1.86	3.57	4.86	1.86	16th
Chianina	3.00	2.29	3.00	4.29	4.57	2.71	4.71	4.14	3.86	1.57	3.43	4.29	1.71	22nd*
Devon	3.43	3.43	3.43	3.43	3.57	3.43	3.14	3.00	2.86	3.14	3.29	3.00	3.14	27th
Gasconne	3.67	3.67	3.67	3.33	3.33	3.33	2.67	3.00	3.00	2.67	3.00	3.33	2.67	35th
Galloway	4.00	3.67	3.88	2.83	2.83	3.67	3.00	3.17	2.33	3.00	3.00	3.33	3.50	29th
Gelbvieh	4.33	3.00	2.50	4.00	4.17	2.67	4.00	3.50	3.67	3.83	3.67	4.33	2.83	6th
Hays Converter	3.71	3.14	3.14	4.00	3.86	3.29	3.57	3.43	3.14	4.14	3.57	3.57	2.86	10th
Hereford	4.50	3.83	4.00	2.50	3.17	3.50	3.33	3.33	3.33	3.67	3.67	3.50	3.33	9th
Holstein	3.43	3.14	2.86	4.43	4.29	3.29	4.14	3.86	2.86	3.57	3.29	3.71	2.29	13th
Jersey	4.86	4.43	4.29	2.57	1.28	4.71	1.28	2.43	1.43	4.00	2.57	2.57	4.00	40th
Limousin	3.00	3.00	3.29	3.57	3.71	2.86	3.57	3.43	3.86	2.86	3.86	4.71	2.29	18th
Lincoln Red	3.50	3.33	3.33	3.67	3.83	3.67	3.83	3.33	3.50	3.50	3.67	3.17	3.00	11th
Longhorn	2.50	4.25	3.75	2.00	2.25	2.00	2.50	2.25	1.50	2.00	2.00	2.75	1.50	47th
Luing	3.67	3.67	3.67	3.33	2.67	3.00	3.33	3.33	2.33	3.00	3.00	3.33	3.33	34th*
Maine-Anjou	3.29	2.43	2.29	4.57	4.71	2.71	3.00	4.14	4.57	3.57	3.86	4.00	2.57	8th
Marchigiana	2.57	2.57	2.71	4.14	4.29	2.57	4.29	3.43	3.86	3.57	3.43	4.43	1.71	22nd*
Meuse-Rhine-Issel	3.00	3.00	2.50	3.75	3.25	2.50	4.00	3.75	3.50	3.50	3.25	3.50	2.75	28th
Milking Shorthorn	3.43	3.57	3.43	3.57	3.43	3.29	3.43	3.14	2.86	3.71	3.29	3.14	3.14	24th
Murray Grey	3.86	3.71	4.14	3.00	3.14	4.14	3.00	3.14	2.29	3.43	3.29	3.14	4.14	17th
Normande	3.40	3.20	3.20	3.80	4.00	3.20	4.00	4.00	3.40	3.60	3.20	3.40	2.80	12th
Norwegian Red	3.40	3.40	3.60	3.60	3.60	3.40	3.80	3.60	2.80	3.80	3.20	3.40	3.00	14th
Pinzgauer	3.60	4.00	3.60	3.60	3.60	3.80	3.80	3.80	3.60	3.80	3.40	3.80	2.80	3rd
Polled Hereford	4.50	4.00	4.00	3.00	3.17	3.67	3.33	3.33	3.50	3.83	3.33	3.17	3.33	7th
Red Angus	4.86	4.29	4.57	2.86	2.86	4.71	2.86	3.00	3.14	4.57	3.00	2.71	3.14	5th
Red Poll	3.83	4.00	4.00	3.33	3.17	3.67	2.83	2.67	2.83	4.17	2.83	3.33	3.17	20th
Romagnola	2.80	2.40	3.60	3.40	3.80	2.60	4.00	3.80	3.60	2.00	3.00	4.00	2.20	37th*
Salers	2.75	3.00	3.25	3.50	3.50	2.75	3.25	3.00	3.00	2.75	3.25	3.50	2.50	42nd
Santa Gertrudis	2.14	3.17	3.17	3.57	3.57	2.86	3.86	3.86	3.43	3.14	3.14	3.43	2.71	30th
Scotch Highland	3.00	3.67	3.50	2.17	2.33	3.17	2.17	2.33	1.83	1.67	2.67	3.00	3.00	46th
Shorthorn	3.57	2.86	3.86	3.00	2.86	3.71	3.14	3.29	2.86	3.43	3.14	2.29	3.29	36th
Simmental	3.29	2.43	2.57	4.71	4.71	3.43	4.00	3.71	4.57	4.00	3.86	4.00	2.43	2nd
South Devon	3.50	3.17	3.17	3.67	3.67	3.17	3.50	3.67	3.33	3.17	3.50	3.33	3.00	19th
Sussex	3.40	3.40	3.60	3.20	3.20	3.40	3.00	2.80	3.00	3.00	3.00	3.20	3.00	37th*
Tarentaise	3.60	3.80	4.00	3.20	2.60	3.40	3.00	2.60	2.40	3.60	3.00	3.40	3.20	33rd
Welsh Black	3.50	3.25	3.75	3.25	3.00	3.25	3.25	2.75	2.50	3.00	3.00	3.25	3.25	39th
Zebu	2.17	3.33	3.67	2.50	2.67	2.50	3.17	3.00	3.17	3.00	2.50	3.33	2.17	43rd

* Denotes Tie.

These 47 breeds were compared as to their potential value to a commercial cow-calf man with 250 grade English breed beef cows. This cow-calf man is willing to crossbreed and use artificial insemination to upgrade his own herd. The breeds were scored by each of our seven beef experts as follows: 5.00—Excellent; 4.00—Good; 3.00—Average; 2.00—Unsatisfactory, and 1.00—Poor.

Table 3-2-2

(1) Standards for body condition score (Ballows et al. 1878)

body condition score	1	2	3	4	5	6	7	8	9	10
bodycondition class	thin				moderate			good		
backbone & ribs	They are obviously protected by no muscle and fat, or muscle is scarce. The tip is sharp, but slightly rounded in cattle with score 4				Light pressure from the hand can reveal the backbone and ribs. Some meat or fat layer can be felt.			Very firm pressure from the hand can distinguish the backbone. The ribs are well covered with soft meat and fat.		
withers	It has a sharp peak. The frame is obvious. Each vertebra is very sharp and obvious. Cattle with score 4 have a slight amount of meat.				It is rounded and smooth. Firm pressure from the hand can feel each projection in cattle with score 7.			The shape of the frame is not obvious. Accumulation of subcutaneous fat is obvious.		
rump	Hip bone & ischium sharply project with slight meat. The rump is markedly indented.				Hip bone & ischium are rounded, and the rump seems flat. Cattle with higher score have some fat.			It is round and accumulation of fat is obvious. The are a between the two hip bones is completely flat.		
head of tail	Both sides of the tail head are indented and the bones at this part are sharp.				Cattle with higher score has a rounded tail head and some accumulation of fat.			It is rounded and accumulation of fat is obvious. Fat loss is observed.		

(Suzuki)

Table 3-2-3 Nutrient requirement for the maintenance of a cow

weight (kg)	1日当 たり乾 物量 DM(kg)	粗蛋白質 CP(kg)	可消化粗 蛋白質 DCP(kg)	可消化養 分総量 TDN(kg)	可消化エ ネルギー DE(Mcal)	カルシ ウム Ca(g)	リン P(g)	vitamin A (1,000IU)
350	5.0	0.38	0.20	2.5	11.2	10	12	23
400	5.5	0.42	0.22	2.8	12.4	12	13	26
450	6.0	0.45	0.24	3.1	13.5	14	15	30
500	6.5	0.49	0.26	3.3	14.6	15	16	33
550	7.0	0.53	0.28	3.6	15.7	17	18	36
600	7.5	0.56	0.30	3.8	16.8	18	20	40

Table 3-2-4

Extra nutrient for maintenance of a cow in the last 2 months of gestation

粗蛋白質 C P (kg)	可消化 粗蛋白質 D C P (kg)	可消化 養分総量 T D N (kg)	可消化 エネルギー D E (Mcal)	カルシウム C a (g)	リン P (g)
0.12	0.09	0.9	4.0	14	5

Note: Extra DM per day for maintenance of a cow can be 1.5 kg in the last 2 months of gestation.

Table 3-2-5 Extra nutrient for maintenance of a cow in lactation (per 1 kg of milk production)

粗蛋白質 C P (kg)	可消化 粗蛋白質 D C P (kg)	可消化 養分総量 T D N (kg)	可消化 エネルギー D E (Mcal)	カルシウム C a (g)	リン P (g)
0.08	0.06	0.4	1.7	2.5	1.1

Note: Extra DM per day during milk production for maintenance of a cow can be 0.5 kg during lactation.

Table 3-2-6 Average grazing intake of a grazing cow per day (dry matter)

cow	weight (kg)	TDN of (dry matter %)							
		60		65		70		75	
		grass	units	grass	units	grass	units	grass	units
breeding cow	150		4.5 106		4.8 111		5.0 116		5.2 122
	200		6.2 116		6.5 122		6.8 128		7.1 133
	250		7.1 112		7.4 118		7.8 124		8.2 130
	300		8.1 112		8.5 118		9.0 124		9.4 131
	350		8.9 110		9.4 116		9.9 122		10.4 129
	400		9.7 108		10.3 115		10.9 122		11.5 128
	450		10.5 107		11.1 114		11.8 121		12.5 128
cow	500		11.0 104		11.7 111		12.5 118		13.2 125

Table 3-2-7

Example of safety factors

Safety factors for feed volume	
(i) Change of roughage components	
a. Feed or reaping time which is not described in the Japanese Feeding Standard Table: Add 0 - 5% to similar feed or reaping time.	
b. Components of silage or hay which are not described in the Table: Add 5% to the material components.	
(ii) Change in digestion	
Cow in the early stage of lactation: Add 5%	
Fattening cattle: Add 3 - 8% (DG should also be considered)	
(iii) Change of feed intake (the value should be adjusted according to the actual intake)	
a. Hay: 5 - 10%	
b. Straw: 10 - 20%	
c. Succulent feed: 0 - 5%	

Table 3-2-8

The Nutritive Values of Some Common Tree and Shrub Browse Species.

SPECIES	DRY MATTER BASIS			GROSS ENERGY (Mcal/kg)	TDN (%)	ME (Mcal/kg)
	DRY MATTER (%)	CRUDE PROTEIN (%)	CRUDE FIBRE (%)			
<i>Acacia</i> spp.	91.3	22.2	10.4	-	56.2	2.03
<i>Artocarpus heterophyllus</i>	36.6	14.0	22.1	-	73.5	2.66
<i>Artocarpus integer</i>	35.3	11.5	37.1	2.57	55.8	2.02
<i>Fragaria fragrans</i>	35.3	9.6	22.4	-	72.8	2.63
<i>Lantana aculeata</i>	28.0	14.2	35.8	2.73	62.3	2.25
<i>Leucaena leucocephala</i>	13.3	27.8	10.9	3.37	78.5	2.83
var Hawaiian						
<i>Manihot esculenta</i>	23.1	19.5	16.9	2.64	81.1	2.93
<i>Melastoma malabathricum</i>	33.5	10.8	24.9	2.52	70.2	2.54
<i>Sesbania grandiflora</i>	86.6	22.6	18.4	-	70.1	2.53

Source: Devendra, C. (1979), Malaysian Feedstuffs, MAHDI.

Note: All samples analysed green leaves except for the *Acacia* and *Sesbania* which are in the form of meal and dried leaves, respectively. TDN and ME are calculated for cattle.

Table 3-2-9

Chemical Composition and Nutritive Value of Available Tree and Shrub Forage in Bangladesh.
(% on DM Basis)

BOTANICAL NAME	CRUDE PROTEIN	ETHER EXTRACT	CRUDE FIBRE	NITROGEN FREE EXTRACT	TOTAL ASH	CALCIUM	PHOSPHORUS	DCP	TDN
<i>Cajanus cajan</i>	22.06	1.73	9.50	62.71	4.00	0.12	0.49	14.5	75
<i>Musa sapientum</i>	10.03	5.53	20.95	51.33	12.16	1.26	0.27	-	-
<i>Ficus bengalensis</i>	10.55	2.98	22.54	50.14	13.79	1.51	1.75	2.1	43
<i>Bambusa tulda</i>	15.05	2.21	23.88	47.02	11.81	0.45	0.13	9.8	47
<i>Odina woodier</i>	13.25	6.29	7.97	61.87	10.62	1.44	0.26	-	-
<i>Artocarpus hetero-</i> <i>phyllus</i>	12.24	3.16	19.46	52.06	12.28	1.67	0.17	-	-
<i>Erythrina indica</i>	27.94	7.93	13.18	39.19	11.13	3.74	0.34	-	-
<i>Mangifera indica</i>	12.96	2.58	18.01	53.59	12.86	1.72	0.26	11.3	61

Source: Bangladesh Livestock Research Institute, 1990.

Table 3-2-10

Example 1 (Grazing)

-
- (i) Kind of herd: Cows in the last stage of gestation
Average weight: 550 kg
- (ii) Dry matter per day 11.0 kg
-

	DCP	TDN
Requirement for maintenance	0.28 kg	3.6 kg
Requirement for the last stage of gestation	0.09 kg	0.9 kg
Total	0.37 kg	4.5 kg
(added an extra 50%)	0.56 kg	6.8 kg

(iii) Pasturage

(orchard grass; before heading time to heading time)

DCP 1.5%, TDN 12.1%, Water content 80.5%

(iv) Intake 50 kg

DCP: $50 \text{ kg} \times 0.015 = 0.75 \text{ kg}$

TDN: $50 \text{ kg} \times 0.121 = 6.05 \text{ kg}$

(v) Water content 80.5% (=dry matter 19.5%)

Dry matter intake: $50 \text{ kg} \times 0.195 = 9.75 \text{ kg}$

Check: The calculated dry matter intake is sufficient in comparison with assumed dry matter intake, i.e., 11.0 kg.

Table 3-2-11
Example 2 (Housing)

- (i) Kind of herd: Lactating cows
Average weight: 550 kg
(ii) Dry matter per day $7.0 \text{ kg} + 8 \times 0.5 \text{ kg} = 11 \text{ kg}$

	DCP	TDN
Requirement for maintenance	0.28 kg	3.6 kg
Requirement for lactation (assumed lactation is 8 kg)	0.48 kg	3.2 kg
Total	0.76 kg	5.8 kg

- (iii) Corn silage
DCP 1.0%, TDN 12.2%, water content 81.0%
Hay
DCP 6.0%, TDN 50.3% water content 16.3%
Formula feed
DCP 8.0%, TDN 70.0%, water content 13.0%

- (iv) Corn silage 10 kg fed
DCP $10 \text{ kg} \times 0.01 = 0.1 \text{ kg}$
TDN $10 \text{ kg} \times 0.122 = 1.22 \text{ kg}$
Hay 10 kg fed
DCP $10 \text{ kg} \times 0.06 = 0.6 \text{ kg}$
TDN $10 \text{ kg} \times 0.503 = 5.03 \text{ kg}$
Formula feed 1 kg fed
DCP $1 \text{ kg} \times 0.08 = 0.08 \text{ kg}$
TDN $1 \text{ kg} \times 0.70 = 0.7 \text{ kg}$
Total
DCP $0.1 + 0.6 + 0.08 = 0.78 \text{ kg}$
TDN $1.22 + 5.03 + 0.7 = 6.95 \text{ kg}$

- (v) Water content
Corn silage 81.0% (= dry matter 19.0%)
Formula feed 16.3% (= dry matter 83.7%)
Formular feed 13.0% (= dry matter 87.0%)
dry matter intake:
 $10 \text{ kg} \times 0.19 + 10 \text{ kg} \times 0.837 + 1 \text{ kg} \times 0.87$
 $= 11.14 \text{ kg}$

Check: The calculated dry matter intake, i.e. 11.14 kg, is approximately the same with assumed dry matter intake, i.e. 11.0 kg.

Table 3-2-12

Example of feeding

		(kg)					
age in months		2	3	4	5	6	7
hay		0.5	0.5	0.5	1.0	1.0	1.5
concentrated	bull	0.7	0.9	1.2	1.4	1.7	2.0
feed	heifer calf	0.6	0.8	1.0	1.2	1.4	1.6

Table 3-2-13

Nutrient requirement for breeding heifer

weight	(daily gain)		粗蛋白質	可消化粗蛋白質	可消化養分総量	可消化エネルギー	カルシウム	リン	vitamin A
(kg)	DG(kg)	DM(kg)	CP(kg)	DCP(kg)	TDN(kg)	DE(Mcal)	Ca(g)	P(g)	(1,000IU)
175	0.4	4.0	0.41	0.24	2.3	10.2	15	9	12
	0.6	4.3	0.48	0.29	2.7	11.7	20	11	12
	0.8	4.5	0.55	0.34	3.0	13.1	25	12	12
	1.0	4.7	0.61	0.39	3.2	14.3	29	13	12
	1.2	4.8	0.67	0.44	3.5	15.3	33	15	12
200	0.4	4.4	0.43	0.24	2.6	11.3	16	10	13
	0.6	4.7	0.50	0.30	2.9	13.0	20	11	13
	0.8	5.0	0.57	0.34	3.3	14.5	24	12	13
	1.0	5.2	0.63	0.39	3.6	15.8	28	14	13
	1.2	5.3	0.68	0.44	3.8	16.9	32	15	13
250	0.2	4.6	0.40	0.20	2.5	11.1	12	10	17
	0.4	5.2	0.47	0.25	3.0	13.3	16	11	17
	0.6	5.6	0.54	0.30	3.5	15.3	20	12	17
	0.8	5.9	0.60	0.34	3.9	17.1	23	13	17
300	0.2	5.3	0.44	0.22	2.9	12.8	13	11	20
	0.4	5.9	0.51	0.26	3.5	15.3	17	12	20
	0.6	6.4	0.58	0.31	4.0	17.6	20	13	20
	0.8	6.8	0.63	0.34	4.4	19.6	23	14	20
350	0.2	5.9	0.48	0.23	3.2	14.3	14	13	23
	0.4	6.6	0.55	0.27	3.9	17.2	17	14	23
	0.6	7.2	0.61	0.31	4.5	19.7	20	15	23
400	0.2	6.6	0.51	0.24	3.6	15.8	16	14	26
	0.4	7.3	0.58	0.28	4.3	19.0	18	15	26
	0.6	8.0	0.64	0.31	4.9	21.8	20	16	26
450	0.2	7.2	0.55	0.25	3.9	17.3	17	16	30
	0.4	8.0	0.62	0.29	4.7	20.7	19	16	30
	0.6	8.7	0.67	0.32	5.4	23.8	20	17	30

Table 3-2-14 Nutrient requirement for sire

weight (kg)	1日当 たり増 体量 (kg)	1日当 たり肥 均量 (kg)	粗蛋白質 CP(kg)	可消化粗 蛋白質 DCP(kg)	可消化養 分総量 TDN(kg)	可消化エ ネルギー DE(Mcal)	カルシ ウム Ca(g)	リン P(g)	vitamin A (1,000IU)
250	1.0 1.2 1.4	5.5 6.2 6.9	0.68 0.78 0.87	0.43 0.49 0.55	4.0 4.6 5.2	17.6 20.1 22.9	32 37 41	16 18 19	17 17 17
300	1.0 1.2 1.4	6.3 7.1 7.9	0.71 0.81 0.90	0.43 0.49 0.55	4.6 5.2 6.0	20.2 23.1 26.3	32 36 40	17 18 20	20 20 20
350	1.0 1.2 1.4	7.1 8.0 8.9	0.74 0.83 0.93	0.43 0.48 0.54	5.1 5.9 6.7	22.7 25.9 29.5	31 35 39	18 19 21	23 23 23
400	1.0 1.2 1.4	7.9 8.8 9.9	0.77 0.86 0.96	0.43 0.48 0.53	5.1 6.5 7.4	25.0 28.6 32.6	31 34 38	18 20 21	26 26 26
450	1.0 1.2	8.6 9.6	0.80 0.89	0.43 0.48	6.2 7.1	27.4 31.3	31 34	20 21	30 30
500	0.8 1.0	8.3 9.3	0.74 0.82	0.38 0.43	5.9 6.7	26.0 29.6	28 31	21 21	33 33
550	0.6 0.8	8.1 9.0	0.68 0.76	0.34 0.39	5.6 6.3	24.5 27.9	26 28	21 22	36 36
600	0.6 0.8	8.6 9.6	0.71 0.78	0.35 0.39	5.9 6.8	26.2 29.8	26 28	22 23	40 40
650	0.4 0.6	8.4 9.2	0.67 0.73	0.32 0.35	5.6 6.3	24.7 27.8	25 27	23 24	43 43
700	0.4 0.6	8.8 9.7	0.69 0.76	0.33 0.36	5.9 6.7	26.1 29.4	26 28	24 25	46 46
750	0.2 0.4	8.6 9.3	0.66 0.71	0.30 0.33	5.6 6.2	24.8 27.5	25 27	25 26	50 50
800	0.2	9.1	0.68	0.31	5.9	26.1	27	27	53

Table 3-2-15 Example of feeding according to the Japanese Feeding Standards

sire	weight	DG	nutrient for maintenance		examples of feeding						TDN	sufficiency of TDN
			DM	TDN	concentrated feed	bran	hay	straw	silage			
breeding sire	K ₂	K ₂	K ₂	K ₂		K ₂	K ₂	K ₂	K ₂	K ₂	K ₂	%
	500	0.8	8.3	5.9	①	4	1	8	-	-	7.5	127
	600	0.6	8.6	5.9	②	4	1	6	-	5	7.4	125
	700	0.4	8.8	5.9	③	4	1	4	-	8	7.0	119
	800	0.2	9.1	5.9								
sire	800		8.5	5.5	①	4	-	5	-	10	7.3	111
	900		9.3	6.0	②	3	2	5	3	-	7.6	109
	1000		10.1	6.4	③	2	4	5	4	-	7.9	123

Table 3-2-16 Major methods for production of beef cattle

cattle	age at the beginning	weight at the beginning kg	fattening months	DG kg	final weight kg
beef cattle (bull)	ideal fattening 7~8 months	220~250	17~18	0.5~1.0(0.8)	600 or more
	young fattening 6~8 months	180~240	10~12	0.6~1.0(0.8)	540 or more
beef cattle (cow)	ideal fattening 3~5 years	370~420	10~12	0.4~0.8(0.6)	600 or more
	ordinary fattening 5~6 years	370~420	5~6	0.7~1.1(0.9)	540 or more
dairy cattle (bull)	young fattening 6~7 months	200~250	12~13	1.0~1.2(1.1)	600 or more
	early young fattening 1 week	45~50	18~20	1.0~1.2(1.1)	600 or more

Note: There are some other fattening methods such as old beef bull
fattening, young beef heifer fattening and young beef bull calf
fattening.

Table 3-2-17

Correlation between environmental temperature and feed intake of the cattle

Environmental Temperature	Change of feed intake (ratio to nutrient requirement in NRC Feed Standard (%))
35°C or above	High humidity, high temperature at night or shaded from sun: marked decrease in the intake Cattle with full feeding: 10 - 35% decrease Cattle with restricted feeding for maintaining the same weight: 5 - 20% decrease Shade, cooling, low fiber feed: relaxation of decrease
25 - 35°C	Intake: 3 - 10% decrease
15 - 25°C	Intake: the stated value in the Feeding Standard
5 - 15°C	Intake: 2 - 5% increase
-5 - 5°C	Intake: 3 - 8% increase
-15 - -5°C	Intake: 5 - 10% increase
-15°C or below	Intake: 8 - 25% increase Excessively low temperature (-25°C or below), wind, snow, storm: temporary decrease Roughage alone cannot achieve an increase in intake.

(NRC, 1981)

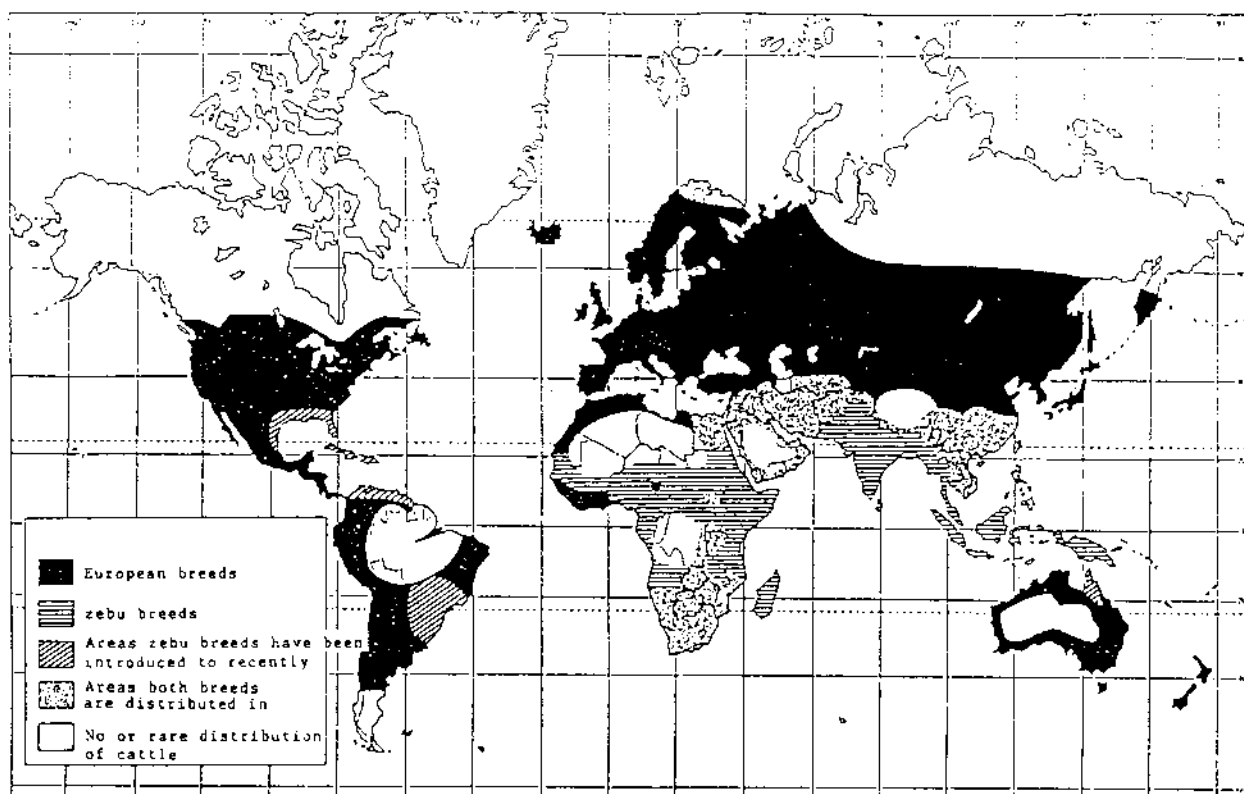


Figure 3-2-1 Distribution of cattle breeds

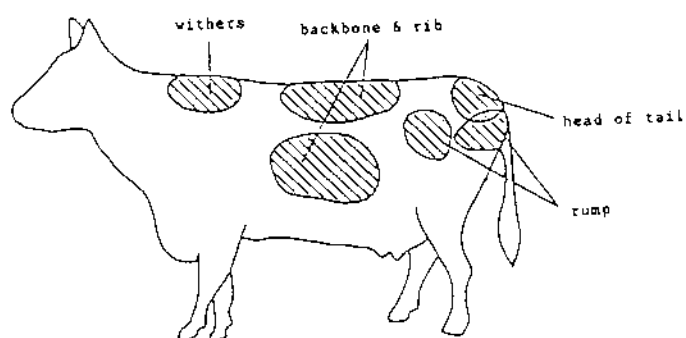
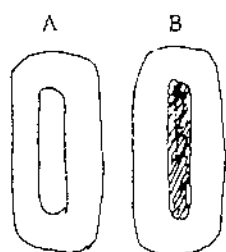


Figure 3-2-2
Parts to be observed for body condition score



A: condition before estrous (no color)
 B: condition in estrous (red)

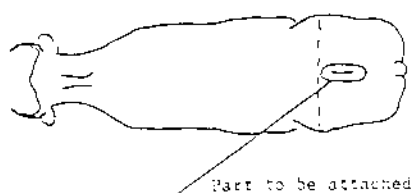
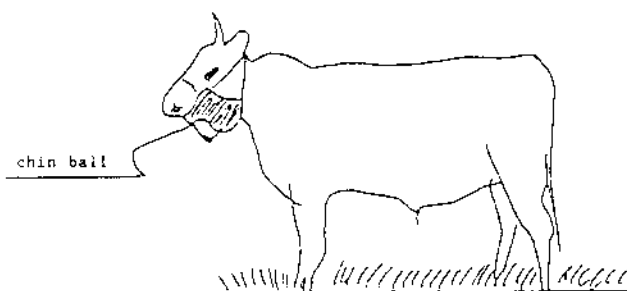
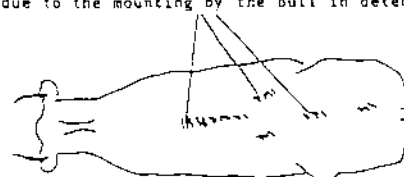


Figure 3-2-4 Estrous mount detector method



bull for estrous detector with chin ball

parts which are dyed due to the mounting by the bull in detection



cow in estrous

Figure 3-2-3 Chin ball method

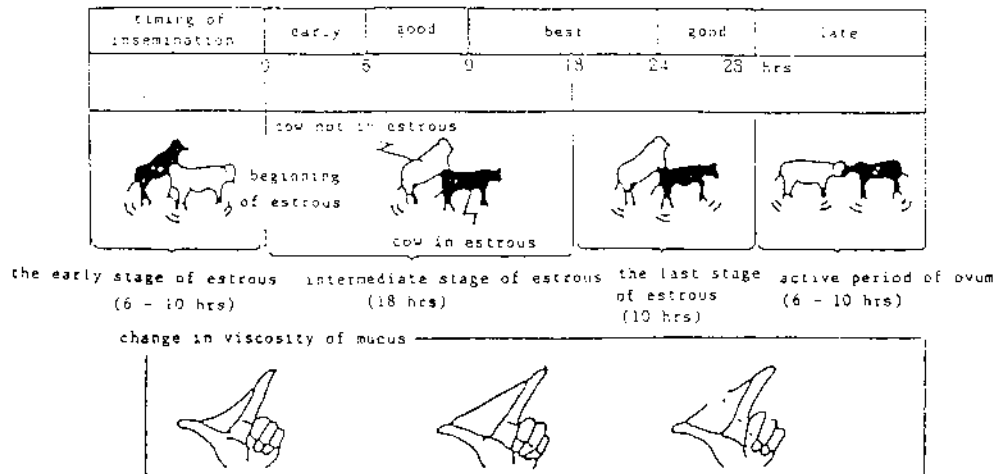


Figure 3-2-5 Signs of estrous and suitable period for insemination

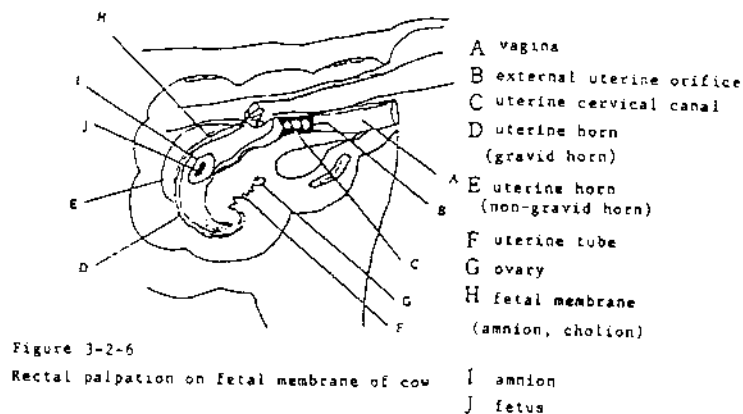


Figure 3-2-6

Rectal palpation on fetal membrane of cow

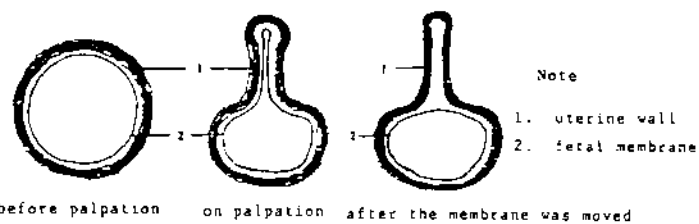


Figure 3-2-7

Diagram of cross-section of gravid horn of cow
(explanation of palpation of fetal membrane)

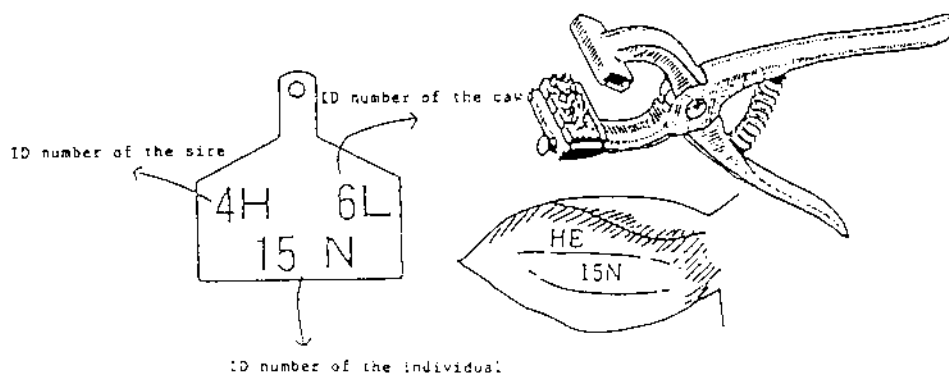


Figure 3-2-8 Ear marking

Figure 3-2-9 Tattooing tool and part for tattooing

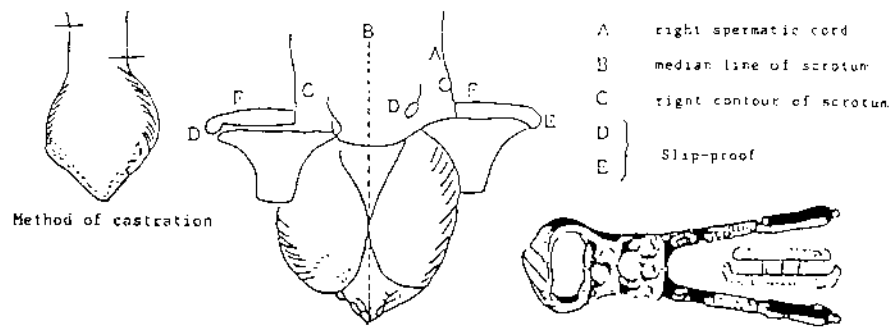


Figure 3-2-10 Method and tool for castration

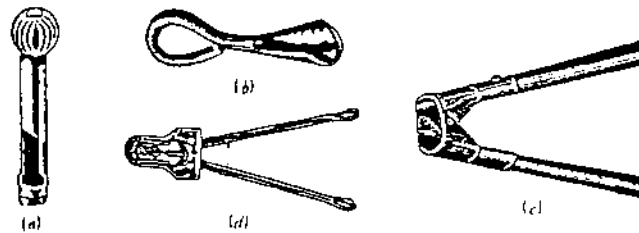


Figure 3-2-11 Dehorning tools for various ages of calf: (a) tube for dehorning, for 1 - 3 months of age; (b) spoon for dehorning, for 3 - 5 months of age; (c) Barnes's dehorner, for 5 - 12 months of age; (d) Leavitt's clippers, for 12 - 24 months of age (O.M. Franklin Serum Co., Denver, Colorado)

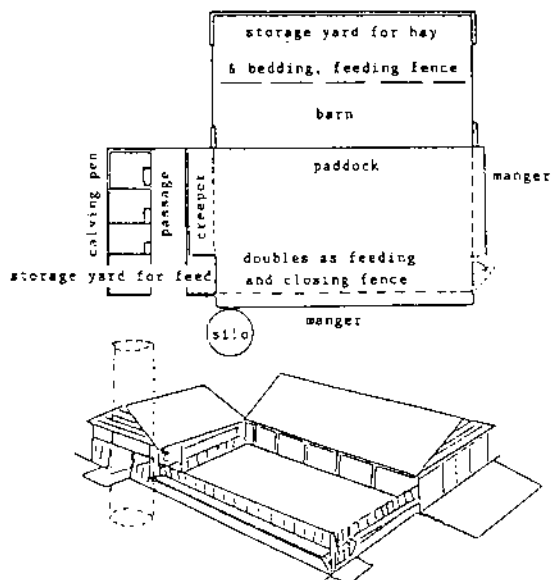


Figure 3-2-12 Example of free barn for 50 cattle

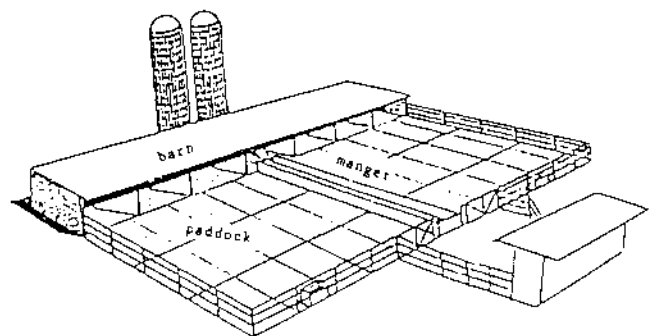


Figure 3-2-13 Example of free barn for 50 - 80 cattle

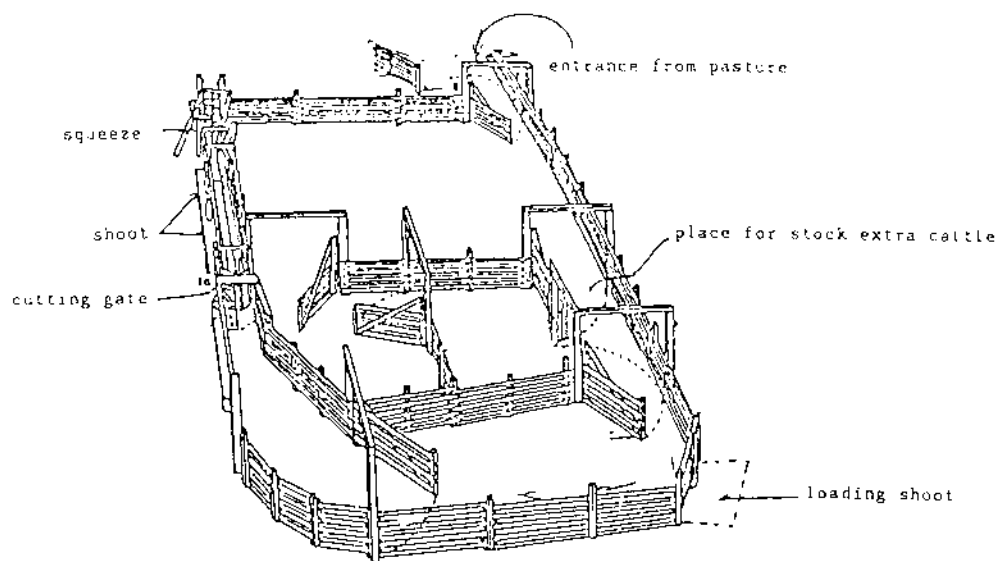


Figure 3-2-14 Example of corral

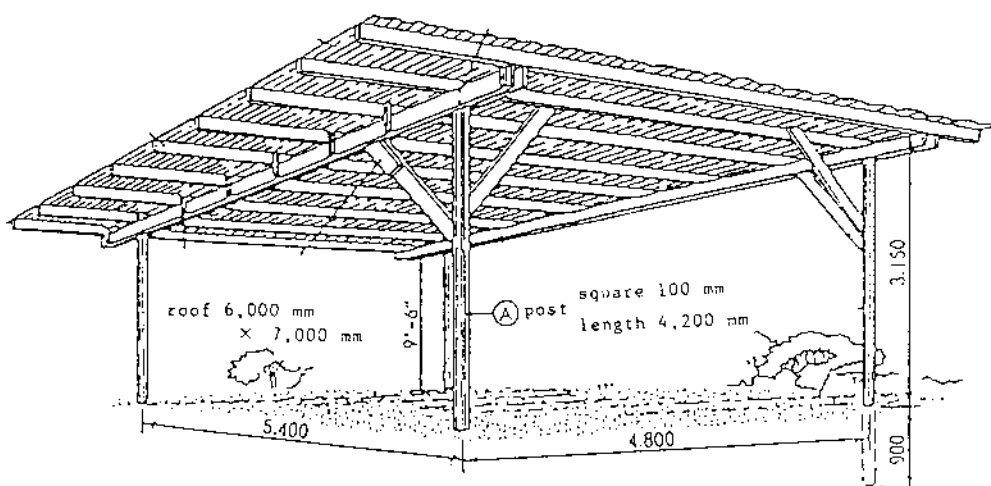


Figure 3-2-15 Example of shelter

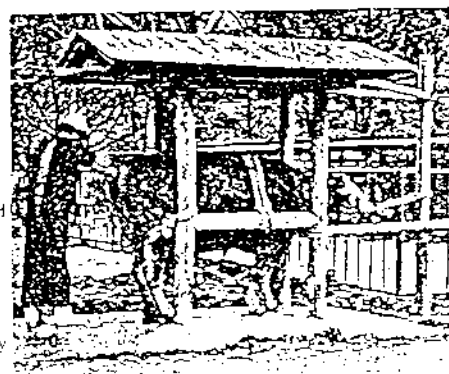
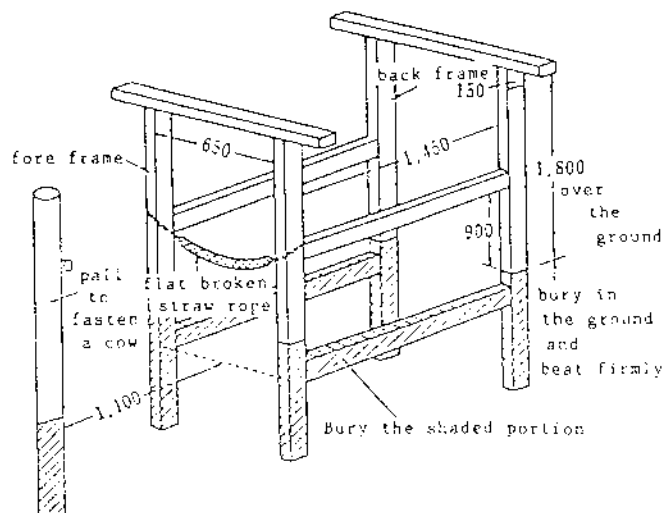
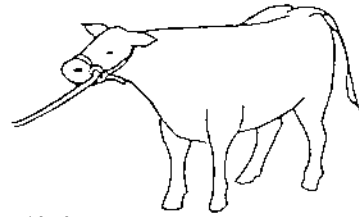
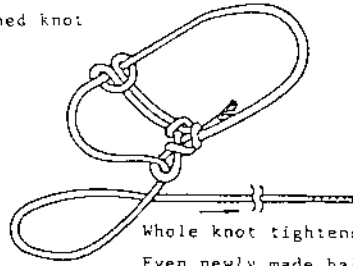


Figure 3-2-16 Treatment stall

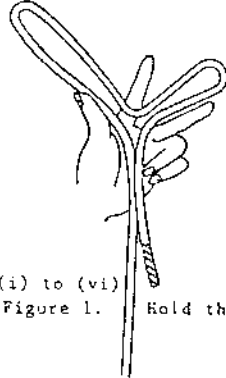
Finished knot



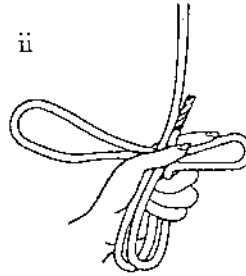
Whole knot tightens as it is pulled.
Even newly made halter tightens in this method.

Procedure

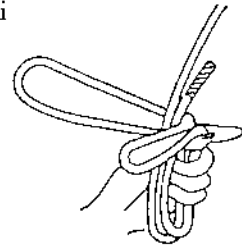
i



ii

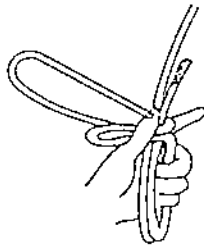


iii

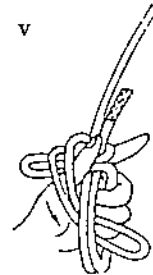


procedure through (i) to (vi)
is the same as in Figure 1. Hold the short end.

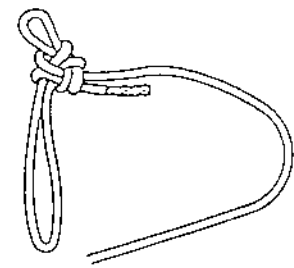
iv



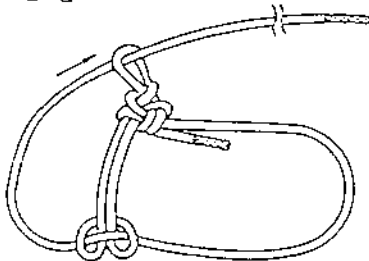
v



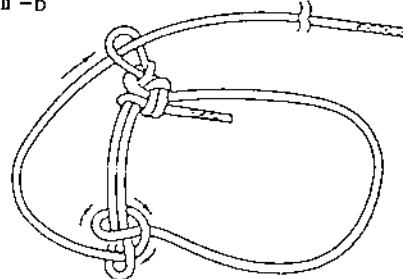
vi



vii-a



vii-b



This part of the knot is different from (vii)-a. The part will n
tighten but will not loosen neither.

Figure 3-2-17 Method to make halter

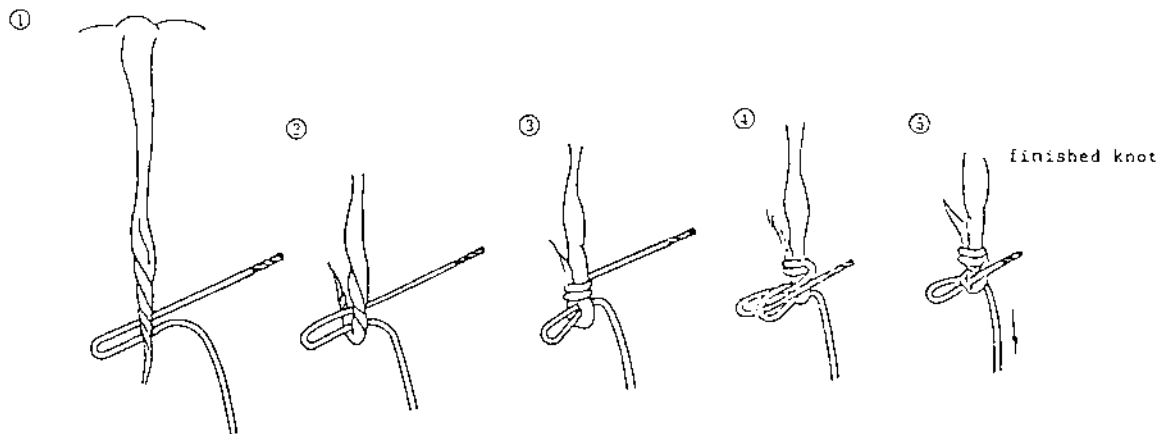


Figure 3-2-18 Method to fasten a switch

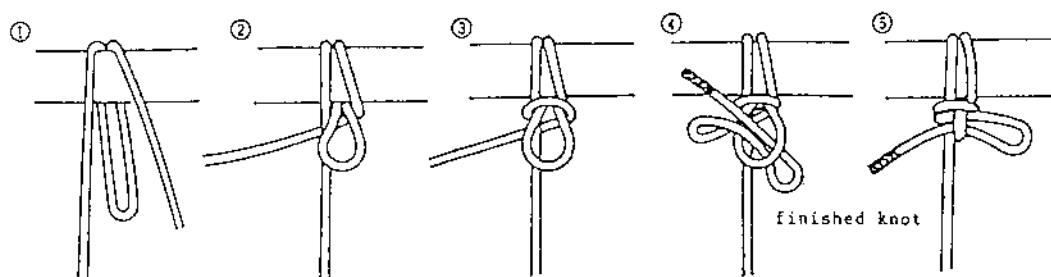


Figure 3-9 Knot to fasten on post or fence (a)

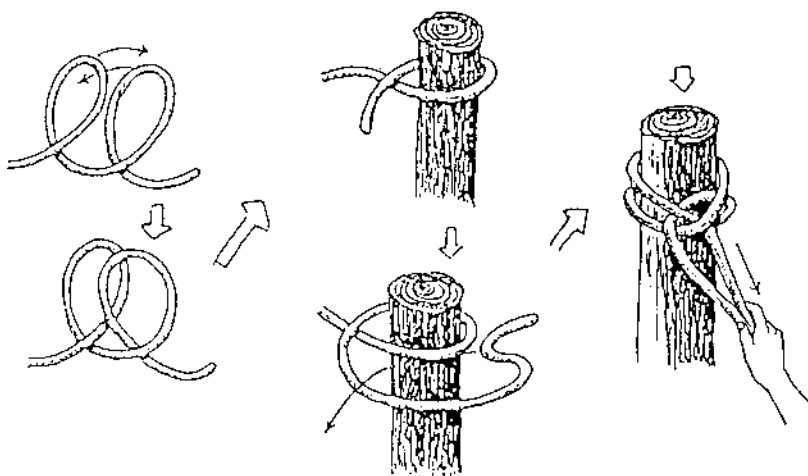


Figure 3-2-20 Knot to fasten on post or fence (b)

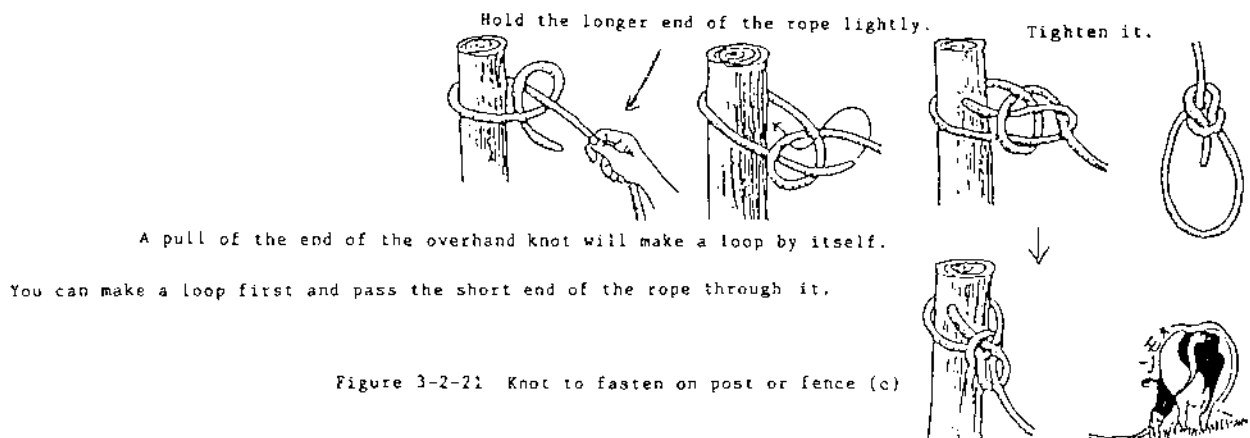


Figure 3-2-21 Knot to fasten on post or fence (c)

References

- Encyclopedia of Breeding, Yokendo, 1989.
- World Cattle, Yokendo.
- Encyclopedia of Agricultural Technology. Beef Cattle, Nosan Gyosan Bunka Kyokai.
- Guidebook for Promotion Beef Cattle Production, Nikuyougryu Seisan Shinkou Suishin Kaigi.
- Basic Knowledge of Grazing of Beef Cattle and Applications, Zenkoku Nikuyougryu Kyokai.
- Guidebook for Feeding Beef Cattle "Foreign Breeds", Zenkoku Nikuyougryu Kyokai.
- Guidebook for Feeding Beef Cattle (Reproduction), Zenkoku Nikuyougryu Kyokai
- Text Book for A.I. Technology, Nihon Kachiku Jinkou Juseishi Kyokai.
- Livestock in Southeast Asia, Nihon University Agriculture and Veterinary Medicine Department International Area Study Institute.
- A.L. Neuman, Beef Cattle.
- Study of Dairy Farming "Knots as Tool", Rakunou Jijo Sha, 1989.
- Guidebook for Raising Angus and Hereford, Hokkaido Nougryu Kaihatsu Kosha.
- Study of Dairy Farming, Jan 1978, July and August 1983, Yokendo.
- Consultation on Dairy Farming, December 1991.
- Dairy Farming Technology, October 1991.
- Dairy Farming in the World, October 1989.
- Anima. December 1984, Heibonsha Ltd., Publishers.
- Angus Journal, October 1975.
- Asian Livestock, October and November 1991.

PART IV BREEDING OF DAIRY CATTLE

1. Genetics and Breeding in Dairy Cattle
2. Progeny testing of Dairy Bulls in Japan
3. Type Evaluation of Dairy Cows
4. Outline of Dairy Cattle Registry System in Japan

IV Breeding of Dairy Cattle

1. Genetics and Breeding in Dairy Cattle

1-1 Introduction to Genetics and Cattle Breeding

The principles of inheritance in animals (including livestock) are studied in the field of animal (livestock) genetics, and these principles are applied to the field of livestock breeding for the improvement of livestock. In practice, however, animal genetics and livestock breeding are interchangeable terms because these two fields are closely linked. Research into and application of animal genetics are classified into Mendelian genetics, population genetics, and quantitative genetics.

Mendelian genetics was founded by G. Mendel in 1865, before being rediscovered in 1900. In 1906, W. Batson first reported Mendelian inheritance in chickens. Following that, in 1906 W. Johanson created the concept of gene, genotype and phenotype. Mendelian genetics does not have direct importance in the genetic improvement of livestock, but underlies the other two fields of genetics. In addition, genes related to Mendelian inheritance may be used as marker genes for individual identification and registration.

Population genetics is the study of Mendelian genetics in populations of animals and plants. It was founded by G.H.Hardy and W. Weinberg in 1908 and greatly contributed to knowledge of genetic changes in qualitative characters in populations. It

offered information on genetic changes within a population that are caused by selection of favorable genes or inferior gene frequencies. Such information forms the foundation of the study of evolution.

The field of quantitative genetics covers characters controlled by polygenes and the environment (for example, milk yield, growth rate, reproduction). Quantitative genetics is thus difficult to define, but it is the most important branch for the genetic improvement of livestock. This is because genetic gain in quantitative characters produces the highest yield in economic terms. The founders of this field were R.A. Fisher and S. Wright who combined genetics with the statistical concepts of F. Galton, and K. Pearson. In the field of livestock breeding before the rediscovery of Mendel's law, R. Bakewell, who was himself a breeder, successfully took advantage of accurate family records to improve the abilities and uniformity of his livestock. At this turning point, a registration system was framed for horses (1871) and cattle (1822), and after that the system was applied to many other livestock (e. g. to Holstein in 1874) in various countries.

J. L. Lush, an American scientist, made a great contribution to the systematization of livestock breeding, and also developed a fundamental theory of progeny testing. One of his graduate student, C. R. Henderson developed a highly accurate method for estimating breeding values by using a best linear unbiased prediction (BLUP). Presently, simultaneous male-female comparison in animal models is popular as an evaluation method for breeding values of livestock.

In the 1960s, many economic characters of livestock and poultry were genetically improved, which contributed considerably to human well-being. All these improvements were based on accurate records of target characters and individual identification. A remarkable examples in this process were the utilization of records of dairy herd performance tests. At the beginning, the

purpose of dairy herd performance tests was to contribute to operation of dairy farming through information on management and reproduction. The data accumulated are now used, though, for the evaluation of genetic values for bulls, through the development of statistical genetics and computers. On the other hand, selection by breeding value with a high degree of accuracy, along with the spread of artificial insemination as a reproduction technique, has made a great contribution to the genetic improvement of dairy cattle.

Thanks to recent developments in molecular genetics, it has become possible to artificially change the sequences on the double helical structure of DNA (genetic material) for the purpose of producing transgenic (gene transfer) animals. Under these circumstances, a field of study has appeared where livestock carrying useful gene groups are attempted to be reproduced by this technique with the aim of utilizing them for livestock breeding. There is much hope for future developments in this field.

1-2 Qualitative Characters and Inheritance

With regard to the genetic expression of qualitative characters such as hair color, horns and blood group, gene actions are strong and the number of genes related to the characters is small. The environment has only a small influence on expression, and the characteristics show a discontinuous distribution. On the other hand, with regard to quantitative characters such as milk yield, growth, reproduction and type, the number of genes related to the characters is large, indicating genetic control by so-called polygenes. Accordingly, the environment has a large influence on expression, and the characters show a continuous distribution. The genetic bases of all these genetic events are described below.

(1) Genes and chromosomes

Genes carry genetic information, are transmitted from cell to

cell and from generation to generation, perform replication, and cause mutation. They are substances common to all living things, and their genetic information is expressed systematically through decoding and transcription.

Genes are found on a chemical substance called DNA (deoxyribonucleic acid), which forms two stands linked to each other at carbohydrates through two base pairs, namely, adenine and thymine, and guanine and cytosine.

Figure 4-1-1 shows the double helical structure of DNA and how replication proceeds. Complete genetic expression is not the function of one nucleotide sequence, but of several units which control genetic expression through units of structural genes by regulation, promotion and operation. Most DNA is found in chromosomes in cell nuclei, but some is also found in mitochondria of cytoplasm.

Gene effects can be classified from a practical point of view into 6 types, as follows:

- i . Dominant or recessive effects. With respect to hair color, for example, black (B) is dominant over red (b).
- ii . Heterosis or overdominant effects. This effect is of practical importance when the first generation (F_1) shows greater vitality than its parents.
- iii . Co-dominant effects. Each allele of heterozygotes has its own phenotype. A typical example is hair color of Shorthorn cattle and blood groups.
- iv . Epistatic effects. This effect relates to interactions between genes at different loci.
- v . Pleiotropic effects. One specific gene manifests itself

in multiple phenotypic expressions.

vi. Additive effects. Most characters of economic importance are controlled by polygenes, a group of minor genes.

All these gene effects combine with each other and the effects of the environment to produce different phenotypes. i - iii are interallelic effects, while iv - vi are non-interallelic effects.

Chromosomes are classified into autosomes and the sex chromosomes (which determine sex). The number of chromosomes in cattle is 60, meaning $2n$ is 30. Accordingly, there are 29 pairs of autosomes (AA) and one pair of sex chromosomes (XX or XY). Males and females are expressed genetically as AA-XY and AA-XX, respectively, as for all mammals.

Chromosomes divide in mitosis (of somatic cells) or meiosis of sex cells (Fig. 4-1-2). When sex cells divide in the process of formation of sperm and ovum, diploid cells become haploid cells, but they become diploid again at fertilization (Fig. 4-1-3). Naturally, diseases caused by chromosomal aberrations can occasionally occur.

(2) Genes related to lethal condition and disease

Even in Holstein, 9 inferior recessive genes are regarded as important targets of culling in recent populations produced by artificial insemination (AI) (Table 4-1-1). Measures to counter disease caused by such genes require an enormous cost, but some genes, for example, the BoLa gene related to mastitis, can be used as marker genes. Another example is the B gene of κ -Casein, which can be used to increase the productive efficiency of cheese.

1-3 Genetic Resources for Dairy Cattle

The efficiency of converting feed nutrients of dairy cattle is, in general, higher than that of any other livestock species. However, it is cannot always be expected that there will be a breed suitable to specific dairy farming operations in particular conditions in a particular country. In addition, there should be fairly large heritable variations in the same breed. There are also large genetic differences in the efficiency of milk production between lines selected with the aim of attaining the specified goal of improvement. Table 4-1-2 shows an international comparison of Holstein-Friesian Cattle which began in the 1970s and was reported in 1983 in Poland. It is worth noting that meat productivity was a character for selection in European Friesian, which showed a lower value for lean meat productivity than North American Holstein (Table 4-1-3).

Following that, European contries undertook drastic Holsteinization from Friesian of the European type to Holstein of the North American type (Table 4-1-4). The U. K., which had held the most negative opinion toward Holsteinization, finally in 1987 undertook Holsteinization through multiple ovulation and embryo transfer (the MOET-Nucleus breeding method), and sampled the genetic resources retained by the U. S. In 1991, the first AI-bull produced by this method was supplied to AI centers.

In sampling species for the purpose of utilizing the genetic resources of dairy cattle, it is important that newly introduced cattle be suited to the new environmental conditions of dairy farming operations. Holstein, for example, requires special conditions such as a mild to cool climate and abundant nutrition resources, and may not be able to adapt to extremely different conditions. When there is a specific cattle adapted to a certain local region, it is necessary to plan cross-breeding or other selective breeding after comparative tests.

Reproduction techniques such as AI and ET were developed and many diseases have been overcome with the progress of breeding science. This situation has made it possible to internationally compare genetic abilities in dairy cattle with high accuracy for a given environment. Noticeably, differences at the genetic level in a starting line cause large differences in investment efficiency even when breeding high-performance cattle that is the most suited to a particular environment.

Such international comparisons suggest that frozen semen or fertilized ova should be introduced as genetic resources, rather than adults. Adaptation to the environment during rearing is important, but the MOET breeding system, which includes the introduction of fertilized ova, may possibly produce its best results when the cytoplasm is related to genetic abilities.

1-4 Breeding Goals in Dairy Cattle

The expected products of dairy cattle include milk, and as a by-product, lean meat. There are small differences in the economic importance of milk yield between areas supplying milk for drinking and those supplying raw milk for cheese, butter, powdered milk, and so on. Overall, however, there are no marked differences between these. In general, recent dairy farming has shown a marked tendency to place greater importance on the content of protein (P) than fat (F) in milk. At present the important P:F ratios are from 1.0 : 1.0 to 6.0 : 1.0, that is from 1.2 : 1.0 in Canada to 3 : 1 in the U. S and 6 : 1 in the Netherland. The most important selection character in dairy cattle is milk yield. However, some countries such as Canada and the U. S. use several type characters in their selection indices, and put such comprehensive ability indices to practical use.

(1) Aspects of dairy cattle breeding

The economic ability of dairy cattle is greatly affected by

the environment. It is therefore necessary to continuously accumulate highly accurate, high cost performance records over fixed time spans.

The following factors directly interfere with improvement of dairy cattle.

- i . Expensive mature cows
- ii . Long period of growth required for cows to reach milk-producing age
- iii . Low breeding efficiency of cows
- iv . Milk production performance dependent on cows alone
- v . Many different records obtained from cows
- vi . Overlapping generations
- vii . Reproduction dependent on AI (40,000/year/bull)
- viii . Dairy farmers permitted to decide semen selection
- ix . Some dairy farmers joining dairy herd performance tests and some not
- x . No distinctions between breeding and commercial populations
- xi . Genetic levels and economic efficiencies compared internationally

Since the pressure of international economic interaction is becoming stronger and stronger, the goal of improving the genetic resources of dairy cattle is necessary in order to obtain a high level which can cope with the age of internationalization.

(2) characters in breeding program

Most characters of economic importance are controlled by polygenes, which have additive gene effects. It is estimated that a group of 100 to 200 genes are involved in milk yield. Most of these genes are on chromosomes, but some are in the mitochondria. However, almost all of these genes are difficult to identify.

Furthermore, the environmental or rearing conditions mask the genetic potential of cows, which affects the genetic evaluation of the important characters in dairy cattle (Fig. 4-1-4). Therefore it is necessary to systematize dairy herd performance tests to fully understand the environmental factors shown in the figure and to evaluate the genetic value of characters.

Selection is necessary for genetic improvement of economically importance in dairy cattle. Such selection requires genetic ranking of bulls and cows based on accurate values, for genetic evaluation. A bull and a cow at the top of such a ranking are intentionally mated to produce young bulls which will be examined in progeny tests.

Multiple characters are frequently employed in breeding programs, but too many characters (n) may possibly cause a reduction in selection efficiency by a factor of $1/\sqrt{n}$. Efficiency is also affected by heritability and genetic correlations (positive or negative, and variance). Accordingly, the following characters are incorporated into breeding programs: i. characters with a large economic contribution; ii. characters which can be accurately measured and genetically evaluated; iii. characters which show heritable variation possibly leading to effective selection responses in breeding (Table 4-1-5). The selection of multiple characters requires useful information on genetic correlations between the most important character and other characters (Table 4-1-6). When a positive genetic correlation is observed, selection of a target characters can be expected to cause positive correlations in genetic improvement with other characters. On the other hand, when negative correlations are observed, its selection may cause genetic deterioration in other characters.

(3) Selection of productive characters

Characters relevant to milk production are actually the most important characters in breeding programs for dairy herds, and have the following features:

- i . The characters are important from an economical point of view.
- ii . The characters can be measured accurately in dairy herd performance tests by dairy farmers. Genetic evaluation is possible in both bulls and cows.
- iii . The characters show genetic variances which can be expected to lead to genetic improvements.

Appropriate genetic gain cannot be expected without accurate ranking according to genetic evaluation of bulls and cows on the basis of dairy herd performance tests in which dairy farmers have participated. In advanced nations in the area of dairy farming, selection indices for important characters, that is, the milk components (fat and protein), are used to good effect in the improvement programs conducted by AI centers or dairy farmers.

(4) Selection of nonproductive characters

Efficient reproduction is important for management of dairy farming. Characters are monitored in relation to reproduction by dairy herd performance tests. Such characters, however, show very low heritability, and it is difficult for them to be included in ordinary genetic improvement programs. However, there is self-selection pressure. In other words, normal, efficient reproduction is maintained if there is adequate nutrition, health care, disease prevention, estrus detection, and appropriate artificial insemination. In this

process, however, care should be paid to calving difficulty in crossbreeding and young cow.

Characters of type are taken into consideration by registration agencies or AI centers. Many such characters have no definite relation to economic values. A certain character of type is chosen as a character for selection only when it is thought to contribute to the genetic improvement of milk yield, even if it is important in a registered population of cattle or a dairy herd for individual sales. Characters of type are measurable and genetic, though they are slightly but negatively correlated to milk yield. Recently, a linear score system has been introduced, and the evaluation values for AI-bulls obtained by this system are now used for corrective mating of dairy herds.

Feeding and milking efficiency are characters of economic importance. Fortunately, there is a positive correlation between these characters and milk yield, leading to self-selecting pressure. Resistance to mastitis shows low heritability and selection efficiency. This character is in serious question from an economic point of view, but it can possibly be controlled by proper management and treatment.

Longevity is very important, but it shows low heritability. This character, however, is positively correlated to milk yield, and may be slowly improved genetically.

Originally, meat production performance was not a character for selection in Holstein, though Holstein shows high lean meat production performance and excellent growth rates.

1-5 Dairy Herd Performance Tests

It is difficult to manage dairy herds efficiently if there are no accurate records of dairy herds. Continuously and accurately

recorded data on dairy herds can be effectively used as information for evaluating dairy herds and for individual management. Locally or nationally accumulated data on dairy herds is also used as information for evaluating bulls and cow in order to make genetic rankings. The ranking obtained by this means should be utilized as the basic data for genetic improvements.

Records of reproduction, data of calving, diseases, reasons for culling, and so on, are used as information for the health, reproduction and management of dairy herds. On the other hand, family records of parents and of other related cattle are important data on which genetic evaluation values for bulls and cows are based. Figure 4-1-5 shows clearly the annual changes in milk yield based on data from dairy herd performance tests performed in Hokkaido.

1-6 Selection Responses in Dairy Cattle

(1) Factors related to genetic improvement

The selection response in terms of genetic gain (ΔG) is:

$$\Delta G/L = \frac{r_{GG} \times i \times \sigma_A}{L}$$

r_{GG} = Accuracy of evaluation

i = Selection intensity

σ_A = Genetic standard deviation

L = Generation interval

The accuracy of evaluation is the correlation between predicted breeding values and true breeding values. The selection intensity is the selection differential expressed as units of standard deviation. The genetic standard deviation is a measure of the variation of breeding values for characters considered. The generation interval is the

average number of years between generations. There are large differences between bulls and cows in their contribution to genetic gain. Such differences originate mainly from differences in accuracy of evaluation and differences in selection intensity. The generation intervals in ordinary progeny tests are about 7 years for bulls and about 4.5 years for cows. The contribution of bulls to genetic improvement is 75 - 95 % when progeny tests are performed.

(2) Pathways of genetic improvement

Genetic gain in a given population is the sum of four pathways: i. a dam having a young cow (dc); ii. a sire having a young cow (sc); iii. a dam having a young bull (db); and iv. a sire having a young bull (sb).

$$\Delta G/L \text{ year} = \frac{\Delta G_{sb} + \Delta G_{db} + \Delta G_{sc} + \Delta G_{dc}}{L_{sb} + L_{db} + L_{sc} + L_{dc}}$$

- i . Dam having young cow (contributes to approximately 2% of genetic gain)

This path shows moderate selection accuracy and low selection intensity. The generation interval is about 4.5 years.

- ii . Sire having young cow (contributes to approximately 26% of genetic gain)

This path shows high selection accuracy and moderate selection intensity. The generation interval is about 6 years.

- iii . Dam having young bull (contributes to approximately 32% of genetic gain)

This path shows relatively high selection accuracy and high selection intensity if an elite cow list is used. The generation interval is about 5.5 years.

- iv . Sire having young bull (contributes to approximately 36% of genetic gain)

This path shows very high selection accuracy and high selection intensity. The generation interval is about 7 years.

Accordingly, the total proportion genetic gain in AI-bulls that is due to these pathways is 92% (36% + 32% + 26%). It can thus be seen that on the basis of dairy herd performance tests, AI bulls contribute greatly to genetic improvement in dairy cattle.

1-7 Evaluation of Bulls and Cows

A genetic evaluation system based on dairy herd performance tests is indispensable for supplying AI-bulls and renewing cows to achieve effective genetic gain in dairy cattle. Analyses of data from dairy herd performance tests show that phenotype (P) can possibly be divided into additive effect (G_a), dominance-epistatic effect (G_{nt}), permanent effect (E_{perm}), adjustable environmental effect (E_{adj}), herd-year effect (E_{hy}), temporary environmental effect (E_{temp}) and error effect (e).

Elite bulls and cows should be selected on the basis of highly accurate breeding value rankings, and should be at the top genetic level internationally. Data from these bulls and cows makes it possible for AI stations to systematically produce young bulls for progeny testing. Under these conditions, it becomes possible to effectively place strong selection pressure on the target characters.

(1) Evaluation of bulls (Progeny Testing)

There are many aspects to the evaluation of bulls, and these aspects are in turn affected by many factors. For example, the heritability of characters is directly linked to the reliable evaluation values, which requires adjusted mating plans where an appropriate number of daughters are intentionally arranged for each bull. If concerned with milk yield, 60 or more daughters are generally required. In practice, about 400 doses of frozen semen per young bull are used for progeny testing.

Progeny testing (evaluation of bulls) for genetic improvement of dairy cattle shows the highest efficiency under the following conditions:

- i . Low heritability
- ii . Sex-limited characters
- iii . Characters requiring measurement after slaughter
- iv . Characters which do not cause long generation intervals
- v . Characters which cause low breeding efficiency of cows
- vi . Supplied for AI after selection according to genetic values

The methods of evaluating bulls have changed as follows. At first, dam-daughter comparisons were employed. Then, contemporary comparisons were attempted and modified. In 1970, best linear unbiased prediction (BLUP) was used for evaluation in northeastern America, and following that, the maternal grand sire (MGS) model was developed by including the maternal pedigree in BLUP. Recently, simultaneous male-female evaluation has spread which is another form of BLUP and named as animal model which includes all individual relationships (Table 4-1-7). This method has been made possible in large part by the development of quantitative genetics and super computers.

The genetic evaluation values of ordinary AI bulls are made public twice a year in the form of sire summaries of 8 characters for milk production performance, 20 characters for type and additional information on calving difficulty, temperament and milkability, as shown in Figure 4-1-7. Rankings of each character according to these genetic values are used to good effect in the selection of elite bulls or bulls which are suited to the goal of improving dairy farms. Ranking with a high accuracy is important because differences in genetic values between the respective bulls are reflected in the distribution of daughter cows (Figure 4-1-8).

(2) Evaluation of cows

The genetic values of cows are expressed as cow indices. Elite cow lists formed on the basis of cow indices are basic information used to find embryo donors for embryo transfer (ET) and bull dams for young bull production. Figure 4-1-9 shows a herd improvement information sheet used by dairy farmers in Hokkaido. Thanks to such sheets, distinction can be made between genetic and environmental effects. In addition, changes in producing ability or characters in a herd that is intended to be genetically improved can be understood from such a sheet, because the producing ability is the result of both genetic and permanent environmental effects.

1-8 Dairy Cattle Improvement Strategy

(1) Strategies for genetic improvement

(1-1) General strategy

In order to genetically improve livestock and poultry, in general, foreign animal resources are introduced when it

is not possible to provide native resources suitable to the anticipated environment. On the other hand, when a suitable native breed exists, an improvement strategy may be started by comparing the breed with an introduced breed or a crossbred. Figure 4-1-10 shows a general improvement strategy. In this method, however, some introduced breeds may not adapt to an extreme environment if there is a large interaction between genetic and environment. In any case, various comparative tests need to be performed. When a specific breed or line is considered to be the optimum one, native breeds are renewed by the new breed or grading is performed. If heterosis is important, high combining ability is used in the breeding system. On the other hand, a population of synthetic variety is created, and genetic improvement is attempted by selection within the population. If there is a native population at this point in time, it is appropriate to introduce frozen semen and fertilized ova as genetic resources with consideration for disease prevention. When cytoplasm is involved in the expression of genetic ability, the best way to sample genetic resources is by introduction of fertilized ova.

(1-2) Strategies for dairy cattle

North American Holstein, which has adapted to regions with abundant nutritional resources and a mild to cool climate, was introduced into nations with advanced dairy farming, and is now bred for characteristics appropriate to the particular nation. In most of these nations, various organizations supporting the genetic improvement of dairy cattle are cooperating with each other to systematize dairy herd performance tests, genetic evaluation of bulls and cows, artificial insemination and researches on breeding (Fig. 4-1-11). Computers are involved in every aspect, and breeders' associations take part in this

system in some nations. Basically, the system consists of four large groups, and genetic improvement of dairy cattle never proceeds without any one of these groups. The introduction of genetic resources from a target population may be considered as basically the same situation.

Opportunities for selection of dairy cattle are classified into three steps, as shown in Figure 4-1-12. In the first step, the strongest selection is possible because of the high accuracy and percentage of selection on the side of the bulls. In the second step after progeny testing, accuracy of selection is high, but there is a difference in selection percentage between countries, as shown in Table 4-1-8. As seen in this table, selection pressure is 1/20 in some countries but 1/10 in most countries. In the U.S., progeny tests are conducted on a total of 1470 bulls a year, resulting in a top group of AI-bulls put under fairly high pressure. In the third step, there is also some selection pressure in mating on dairy farms. In practice, however, such pressures are not so high because the farms tend to avoid inbreeding and they select multiple characters including type as targets of selection. Usually, the AI bull for an individual cow is determined with consideration for the limited supply of semen from top-ranked AI-bulls.

(2) Inbreeding

Inbreeding is mating between individuals which are related to each other. AI and ET for the breeding of dairy cattle have spread. In populations of dairy cattle showing great genetic improvement, elite bulls and cows are selected and brought together. In addition, selection pressure after progeny testing contributes greatly to genetic improvement.

International comparisons have become easier in Holstein, and young bulls of elite cattle in target populations are supplied as AI-bulls in various countries, and this genetic structure creates many opportunities for inbreeding. Inbreeding reduces milk yield, milk fat and milk protein according to the degree of inbreeding. On the other hand, inbreeding increases the mortality of calves, and lowers the efficiency of breeding (Table 4-1-9). For this reason intentional inbreeding is not recommended. In order to prevent inbreeding on dairy farms, it is necessary for AI centers to intentionally create several pedigree groups. In particular, this point should be considered in the case of creation a MOET-Nucleus breeding population when sampling from a target population.

(3) Application of embryo transfer

Embryo transfer (ET) involves large expenses because of technical fees and the cost of medicine. Accordingly, the most efficient way to utilize this method is to produce young candidate bulls for AI by combining elite bulls and cows. This method is more effective in populations with accurate genetic rankings, high individual prices and an international market. ET can lead to the following general possibilities:

- i . Production of young bulls for AI
- ii . Production of cows for replacement
- iii . Cow progeny testing
- iv . Increasing the number of top cows in dairy herds
- v . Effective utilization of expensive semen
- vi . Export of embryos or granting assistance to developing countries
- vii . Collection of embryos from old cows or cows with low

reproductive ability

- viii . Breed changes
- ix . Production of registered cattle from non-registered cows
- x . Creation of uniformity in dairy herds
- x i . Production of desired cows and bulls
- x ii . Recessive gene tests
- x iii . Production of cattle for genetic and experimental researches
- x iv . Performance of progeny testing on small numbers of cows
- x v . Realization of a commercial base using this method

(4) Selection within dairy herds

The goals of improvement in dairy cattle should be different for different dairy farms since the goals should depend on the economic conditions of their particular dairy herds. Selection pressure on type in dairy herds with the sale of individuals as the main purpose is not the same as that in dairy herds with the production of milk as the main purpose. In a similar manner, there should be differences in the importance of selection with respect to the constituents of milk between areas producing drinking milk and those producing raw milk cheese.

A future course of breeding depends on the AI-bull selected for mating. Accordingly, farm manager should always select an appropriate bull with consideration for future improvement. Although mating shows no effects on the production of milk for about three years, after that it affects earnings from the dairy herds for about 15 years.

Characters that can be improved are divided into the

following three groups (Table 4-1-10) since the importances and times of selection can be classified:

- i . Target characters for selection with the aim of improvement
- ii . culling criteria for maintaining dairy herds
- iii . Mating in order to avoid extreme characteristics

Table 4-1-11 shows the expected selection gain with various degrees of importance placed on the genetic transmitting ability (ETA) of milk yield and of type. The results are expressed as genetic correlation of -0.10. When it is intended that milk yield be improved to its maximum without changing type, the importance of milk yield may be increased 5- to 10-fold. This is similar to selection related to milk components. Accordingly, the selection of milk fat and protein is a strategy for the genetic improvement of milk yield without decreasing the component percentages (Table 4-1-12).

Noticeably, a marked tendency to place high selection pressure on milk protein is seen in almost all nations with advanced dairy farming. On the other hand, in Holstein, selection pressure on type is observed in relation to large-sized dairy herds and management systems. It is a problem, though, that dairy farmers with milk yield as their goal continue to conduct strong selection on type scores. They have to consider that AI centers usually keep up the strong selection pressures placed on type during selection of elite bulls and after progeny testing.

1-9 MOET-Nucleus Breeding Method

Genetic gains in dairy cattle and beef cattle are smaller than in chickens and pigs. This is because monotocous of cattle leads to relatively weak selection and a long generation interval. If many calves are produced from one cow over a certain period of time, selection according to records of half-sibs and full-sibs becomes possible. In the case of dairy cattle, large-scale dairy herd performance tests conducted in developed countries require a certain degree of socioeconomic structure. In addition, such tests cannot be conducted without the development of education, communication, transportation, and so on. There are some doubts about the effectiveness as a breeding strategy of such large-scale dairy herd performance test systems being planned in developing countries, as they may disregard the above conditions.

With Holstein, the target population is in North America, and the international sampling of genetic resources is possible. In this case, the international market for elite bulls and cows has become extremely large. Recently, the MOET-Nucleus breeding method has been employed by an increasing number of countries that are advanced in the field of dairy farming, such as the U.K., Netherland, Denmark, Germany, France, and Canada.

In MOET, multiple ovulation is induced by hormonal treatment, fertilized ova are collected, and embryo transfer is performed on recipient cows. The Nucleus breeding method is now used for breeding chickens and pigs. In this system, the breeding and producing populations are different because many offspring are obtained from just one individual. At first, a breeding population is created using an elite individual, and the genetic level is then raised by highly accurate, strong selection within the population. The resulting breeding population is multiplied, and used as livestock for production in the fields.

Breeding efficiency is a 'bottle-neck' in genetic improvement of dairy cattle and beef cattle. If this difficulty could be solved by MOET, the rate of improvement in cattle would become the same

as that in chickens or pigs. In addition, the generation interval in cattle would be shortened. Since technical fees and the cost of medicine required for MOET are enormous, practical application of the breeding population is important and leads to a large investment effect. In the case of Holstein, there is a large population that has been based on dairy herd performance tests in North America. This population has achieved large genetic gains and genetic levels recognized in the international market. It is possible to select elite bulls and elite cows from this population, and to effectively create MOET-Nucleus breeding populations. Such MOET populations would possibly maintain high genetic levels not directly affected by the genetic levels of native populations. Accordingly, fertilized ova and semen from populations controlled in this way may have genetic levels which can respond to the international market. In other words, this method may contribute not only domestically to genetic improvement of cattle, but also internationally to economic effect.

Genetic improvement requires a long period of time and the costs are large. Accordingly, the goal of improvement and the conditions of evaluation or testing should be planned with due consideration for the expected production conditions and the necessity of marketing. On the other hand, in developing countries, using breeding plans which are just copies of those in developed countries should be avoided.

As described in "General Strategy", at first genetic resources and a breeding method with a high productive efficiency are selected by evaluation of breeding stocks crossbreds with the aim of making the most of the genetic resources. For this purpose, the first step is to make performance tests on breeding stocks and crossbreds. Continuous introduction of semen and fertilized ova is recommended if imported stocks show as high a productive efficiency as is expected and no problems in interactions between genetic-environment, and diseases. The possibility of selection of target stocks under the process of development of such

breeding systems may be extended by the international breeding values.

Even if the goal of improvement in a developing country is similar to that in a developed country, there may be extremely large differences in environment. In such cases, interaction between genetic and environment must not be neglected, and this requires an improvement plan particular to that developing country. When a breeding plan is dependent on the introduction of livestock, following a breeding structure which is practiced in the target population and supported by various organizations should be avoided. Naturally, if a developing country has a specific market, production conditions and livestock, a breeding plan of its own should be made.

The limit of genetic gain per year caused by ordinary reproduction and progeny testing is about 1.5% for milk field, while those by the MOET method and MOET-Nucleus breeding method are about 2.3% and 3.5%, respectively. The MOET method has been, or will be developed into the following methods:

- i . MOET in dairy herd performance tests
- ii . MOET-Nucleus breeding method for mature cows
- iii . MOET-Nucleus breeding method for young cows
- iv . Cloning MOET-Nucleus breeding method (in the future)

Technically speaking, i - iii can be practically utilized at present.

Table 4-1-13 (Smith, 1988) shows the advantages of the MOET-Nucleus breeding method. This method is more practicable than breeding plans based on dairy herd performance tests, even if a society's infrastructure is not sufficient in terms of communication, roads, education or distribution. Capital, talent, technology and resources can be concentrated on. In addition, this method makes it easier to introduce new technologies, and has

led to the new concept of center systems which include other livestock species.

Table 4-1-14 (Smith, 1988) shows the disadvantages of the MOET-Nucleus breeding method. In response to these disadvantages, it is now proposed that improvement targets be specified and that multiple MOET-Nucleus breeding populations be created as safety measures against disease. On the other hand, training of technical experts is also required in order to spread this method since dairy cattle from a MOET-Nucleus breeding population are expected to display high ability on commercial dairy farms.

This method requires the systematization and application of science, and the initiative and participation of private citizens. The development and practical application of this method requires long-term investment for 10 to 20 years, indicating that some sort of preparation of sufficient working capital is necessary.

Figure 4-1-13 shows an example of the MOET-Nucleus breeding method for dairy cattle in the Netherlands. Netherland has attracted attention because its breeding program is performed under a high selection pressure of 1/20 according to dairy herd performance tests and progeny tests as well as because the MOET-Nucleus breeding method has been employed. This Delta Nucleus breeding program is a completely open system which characteristically includes Nucleus breeding dairy herds. In this system, the world's leading genetic resources suitable to Netherland's improvement goals are continuously introduced in the form of semen and fertilized ova. In addition, in this system, young cows are transferred to three test fields, and each year performance tests are conducted to compare the new young cows and domestic young cows.

1-10 Summary

Dairy cattle efficiently convert non-edible resources into milk

which contains high quality edible substances such as milk protein, milk fat, minerals and lactose. With respect to genetic improvement, it has been confirmed that not only characters of milk and meat production, but also many other characters not associated with production, show considerable genetic variation and that these characters are changed by artificial selection. Genetic gain in these characters has made an incalculably great contribution value to dairy farming operations.

Investment in feed, rearing management or health in certain regions has a large effect in improving overall on-site milk yield of dairy cattle. This investment effect, however, is only a temporary environmental effect. In contrast, genetic improvement is a permanent effect, and this fact needs to be recognized. In addition, genetic improvement of dairy cattle has a strong connection to the establishment of dairy farming as an industry which can respond to the recent moves towards internationalization.

Table 4-1-1 Lethal Recessive Genes in Holstein

Genetic disease	Symbol for gene	Symptoms
Achondroplasia (Bulldog)	BD	Bulldog-like head and forehead, hernia
Atrichia (Hairless)	HL	Little body hair or down
Protonged gestation	PG	Long-term gestation, weight of fetus: 50-70 kg, no signs of labor
Imperfect skin	IS	Developmental disorder of the skin of lower limbs, eyes and nose of fetus
Syndactylism (Mule-foot)	MF	Short hoofs and insufficiency of one or two limbs
Dwarf	DF	Small size, stumpy limbs, potbelly (possible to diagnose at age of 12 months)
Porphyria (Pink tooth)	PT	Photosensitivity, mahogany, teeth or urine
DUMPS	DP	Deficiency of uridine phosphate synthase, death of embryo
MAC-1 deficiency	BLAD	Deficiency of bovine leukocyte adherence factor
Hemophilia	Bh	Deficiency of blood coagulation factor XI

Table 4-1-2 International Comparison of Milk Yield in Holstein
(Least Square Method)

Country	Milk yield		Milk fat percentage	
	Field(kg)	High nutrition	Field(%)	High nutrition
U. S.	298	432	-0.08	-0.28
Israel	210	252	-0.06	-0.14
Canada	171	255	-0.04	-0.03
New Zealand	144	26	0.11	0.17
Sweden	24	61	-0.01	0.00
U. K.	13	95	0.00	0.01
Denmark	-148	-264	0.02	0.02
West Germany	-152	-37	-0.02	0.04
Netherlands	-154	-131	0.06	0.17
Poland	-406	-573	0.02	0.09

Jasiorowski (1983)

Table 4-1-3 International Comparison of Daily Gain and Feed
Conversion Ratio of Fattening in Male
Crossbred by Holstein bulls.

Country	Number of animal	Daily gain of lean meat	Country	Number of animal	Feed conversion ratio (oats kg/lean meat kg)
U. S.	27	417.9(g)	U. S.	27	12.76
Israel	27	414.0	Israel	27	13.13
Sweden	28	409.5	West Germany	28	13.25
Poland	29	409.2	Poland	29	13.27
Canada	28	405.4	U. K.	28	13.39
Netherlands	26	405.3	Canada	28	13.39
U. K.	28	403.2	Sweden	28	13.48
West Germany	28	397.6	Netherlands	26	13.57
Denmark	29	383.3	Denmark	29	14.14
New Zealand	27	375.5	New Zealand	30	14.73

Leklewski (1982)

Table 4-1-4 Percentage of North American Holstein Genes in Young
candidate bulls in European Friesian

Country	1980	1988
Denmark	53%	100%
France	80	100
West Germany	72	100
Netherlands	24	98
U. K.	26	55

Table 4-1-5 Heritability of Characters in Dairy Cattle

Character	Heritability	Character	Heritability
Milk yield	.15- .40	Mastitis	.00- .20
Milk fat, milk protein		Feed efficiency	.30- .55
Lactose	.20- .40	Temperament	.05- .20
Maximum daily yield	.10- .30	Miking rate	.20- .50
Persistency of lactation	.02- .10	Feeding speed	.10- .20
Milk components ratio	.40- .70	Days open	.01- .10
Live weight gain/day	.20- .40	Days dry	.15- .35
Mature weight	.20- .70	Calving interval	.00- .10
Chest girth	.30- .60	First conception rate	.00- .10
Withers height	.20- .60	Age at Calving (Months)	.15- .70
Udder depth	.25- .60	Calving difficulty	.03- .15
Hip width	.25- .30		
Type score	.10- .35	Longevity	.00- .10
Ketosis	.00- .35	Stayability	.02- .05

Table 4-1-6 Genetic Correlation Coefficient between Milk Yield and Characters in Dairy Cattle

Character	Genetic correlation coefficient	Character	Genetic correlation coefficient
Milk yield		Mastitis	-.30- .40
Milk fat, milk protein		Feed efficiency	.85- .95
Lactose	.70- .95	Temperament	
Maximum daily yield	.80- .90	Miking rate	.00- .15
Persistency of lactation	.40- .60	Feeding speed	
Milk components ratio	-.20- -.60	Days open	.40- .70
Live weight gain/day	-.05	Days dry	-.20- -.40
Mature weight	.30- .50	Calving interval	.40- .70
Chest girth	.00- .40	First conception rate	.35- .60
Withers height	.30- .60	Age at calving (Months)	.05- .27
Udder depth	-.10- .15	Calving difficulty	.05- .05
Hip width	-.10- .80		
Type score	-.25- .40	Longevity	.40- .65
Ketosis		Stayability	.35- .65

Table 4-i-7 Sire Evaluation with Respect to Milk Yield Characters in Various Countries

Name of country	Characters	Parity and gestation period (days)	Method	Conditions required for publication		
				Number of daughters	Number of herds	Repeatability
Argentina	M, F	First, 305	SELUP	20	5	—
Australia	M, F, F%, P, P%.	All, 305	A. M.	20*	5	—
Austria	M, F, F%, P, P%. Net.	1-3, 305	A. M.	20-40	—	—
Belgium	M, F, F%, P, P%. Net.	First, 305	SELUP	50	—	—
Canada	M, F, F%, P, P%.	All, 305	A. M.	—	5	55
Czechoslovakia	M, F, F%.	First, 305	SELUP	30+	—	—
Denmark	M, F, P. Net.	First, 305	IHMC	—	—	60
West Germany	M, F, F%, P, P%. Net.	1-3, 6, 305	S & M BLUP	20	5	—
Finland	P, FCM. Net.	First, 305	SELUP-MGS	20	—	—
France	M, F + P, F% + P%	All, 305	A. M.	—	—	70
East Germany	M, F, F%, P, P%.	All, 305	MCC	40*	—	—
U. K.	M, F, F%, P, P%.	First, 305	SELUP-MGS	5*	—	—
Hungary	M, F, F%. Net.	All, 305	SELUP	—	—	60
Ireland	M, F, F%, P, P%, F + P.	First, 305	SELUP-MGS	20*	—	57
Israel	M, F, F%, FCM.	1-3, 365	BLUP-MGS	—	—	60
Italy	M, F%, P%.	First, 305	A. M.	20	5	—
Netherland	M, F, F%, P, P%. Net.	First, 305	A. M.	—	—	55
New Zealand	M, F. Net.	All, 305	MCC	20	6	60
Norway	FFCM. Net.	First, 305	A. M.	100	—	—
Poland	M, F, M%.	First, 305	BLUP	10	—	—
South Africa	M, F, F%, P, P%.	1-2, 300	BLUP	—	—	40
Spain	M, F, F%.	All, 305	Anim. M.	20	3	—
Sweden	M, F, F%, P, P%, FCM. Net.	First, 305	A. M.	15*	—	—
Swiss	M, F%, P%.	First, 305	SELUP	40*	—	—
U. S.	M, F, F%, P, P%, PTAS	1-5, 305	A. M.	10	—	30
Northeastern, U. S.	M, F, P, Stayability, ETAS.	First, 305	A. M.	20**	—	—
Yugoslavia	M, F, F%.	First, 305	CC	20	—	—
Japan	M, F%, P%, SNF%, Value.	First, 305	SELUP-MGS (A.M.)	15	5	—
Hokkaido (cows)	M, F, F%, SNF, SNF%, ETAS.	All, 305	A. M.	—	—	—

Net, PD \$, ETA \$, ¥ Value, ETA ¥ = Economic effect, First = Record of first lactation,

All = Record of all lactation, SELUP = Sire model, SELUP-MGS = MGS model (S expresses a single trait model),

MELUP-MGS = MGS model for multiple traits model, Anim. M. = A. M. = Animal model,

IHMC = Improved contemporary comparison, MCC = Modified contemporary comparison, CC = Contemporary comparison

* = Effective number of daughter cows, (A. M.) = Amended in 1992

Table 4-1-8 Number of Progeny Tests and Selection Pressure
in Young Holstein Bulls in Various Countries (1990)

Name of country	Number of Young Bulls Per Year	Selection pressure
U. S.	1470	1/10
Canada	341	1/10
France	650	1/20
West Germany	500	1/10
Netherland	350	1/20
Italy	260	1/10
Denmark	300	1/10
U. K.	100	1/10

Table 4-1-9 Degeneration with Inbreeding (Young, C. W. '69)

		Coefficient of inbreeding		
		6.25 %	12.5 %	25.0 %
Milk yield	(kg)	-136	-273	-545
Milk fat	(kg)	-4.0	-8.2	-16.4
Milk fat percentage	(%)	+0.02	+0.04	+0.12
Birth weight	(kg)	-0.68	-1.36	-2.73
Weight at the age of 1 year	(kg)	-4.55	-11.40	-27.30
Weight at the age of 2 year	(kg)	-9.10	-18.20	-27.30
Mature weight	(kg)	?	?	?
Withers height of 1 year of age	(cm)	-0.60	-1.2	-2.4
Withers height of 2 year of age	(cm)	-0.4	-0.8	-2.4
Withers height as adult	(cm)	0	0	0
Chest girth at 1 year of age	(cm)	-1.0	-2.0	-4.0
Chest girth at 2 year of age	(cm)	-1.2	-2.4	-4.8
Chest girth as adult	(cm)	-0.8	-1.6	-3.2
Mortality *	(%)	112	125	150

Note: * As a percentage of when the coefficient of inbreeding is 0.

Table 4-1-10 Method of Selecting traits in Dairy Herd Improvement
Strategies on Dairy Farms

Classification	Traits
Traits selected	Milk yield, milk fat, Solids-not-fat, milk protein, economic effect
Target traits in selection	Percentage of milk components (fat, protein, solids-non-fat, total solid), reproductive ability, temperament, type (linear test)
Extreme traits are avoided in mating	Hind limb, udder support, mastitis, calving difficulty

Table 4-1-11 Expected Gain per Year with Different Economic Weights
on Milk Yield and Type (Freeman, '84)

Relative weights		Expected gain/year	
ETA of milk yield	ETA of type	ETA of milk yield	ETA of type
1	Limited	106	0
1	0	106	-0.02
1	1	73	0.18
2	1	95	0.10
3	1	101	0.06
5	1	104	0.02
10	1	106	0
20	1	107	-0.01

$$\sigma_{ETA M} = 274, \quad \sigma_{ETA M} = 0.7, \quad \gamma_c = -0.1$$

Table 4-1-12 Expected Gain of Traits by Selection of Single Character
(Mitsumoto)

	Character for selection				
	Milk yield	Milk fat	Protein	Milk fat percentage	Protein percentage
Milk yield	+++++	++++	+++	--	---
Milk fat	++++	+++++	+++	+	-
Protein	++++	++++	+++++	-	-
Milk fat percentage	--	++	-	+++++	+++
Protein percentage	---	-	-	+++	+++++
Milk fat + Protein	++++	+++++	+++++	0	-

Note: + means genetic increase, -- means genetic decrease.

Table 4-1-13 Comparison Between MOET-Nucleus Breeding Method and Dairy Herd Performance Test System (Smith, C.)
(Advantages of the MOET breeding program)

1. Possibility of creation of genetically elite populations
2. Increased improvement rate
3. Reduced investment cost
4. Early realization of economic effect (shortening of generation interval)
5. Possibility of effective selection of target for improvement
6. Possibility of control over test conditions
7. Possibility of difficult and precise measurements which are impossible in dairy herd performance tests
8. Centralization of technological talents
9. Possible utilization of advanced biotechnology
10. Easy development and utilization of new technology
11. Feasible establishment of centers for improvement of different species or lines
12. Feasible establishment of centers for study and applied examination
13. Feasible establishment of research study centers for technical experts
14. Independence of socioeconomic foundations

Table 4-1-14 Disadvantages of MOET Nucleus Breeding Method
(Smith, C)

-
1. Vulnerability of genetic resources caused by simplification of breeding unit
 2. Risk of disease and loss
 3. Selection under environmental conditions different from those of ordinary dairy farmers
 4. Insufficient measures to counter the interaction between genetic and environment
 5. Restrictions by administration and management agencies
 6. Cattle show-like use rather than practical genetic improvement
 7. Insufficient cooperation in industrial field and dairy farms
 8. Necessity of capital for establishment and management
 9. Necessity of capital investment under long-term contract
-

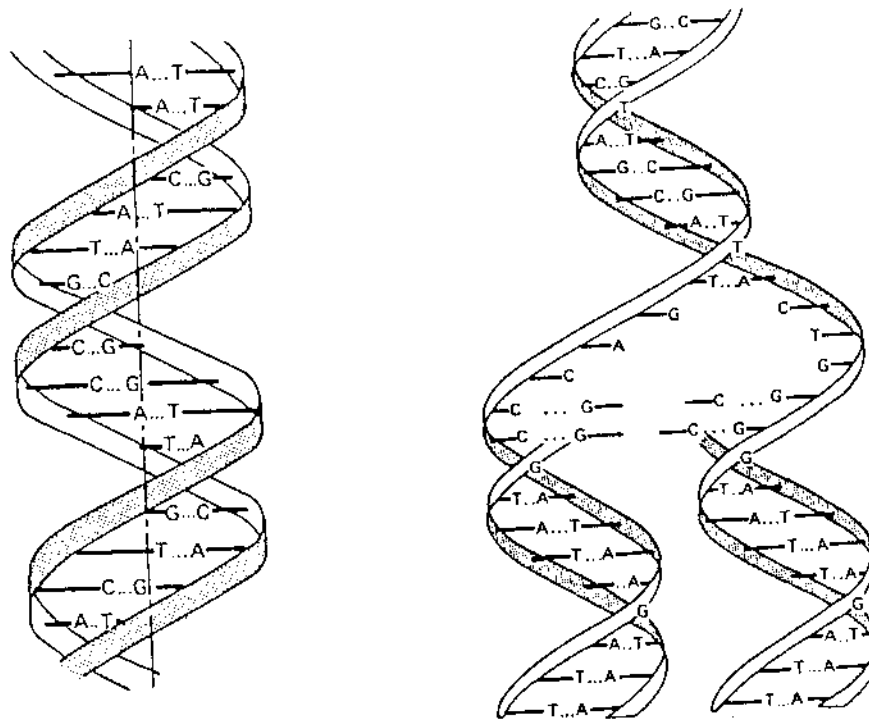


Figure 4-1-1 Structure and Replication of DNA

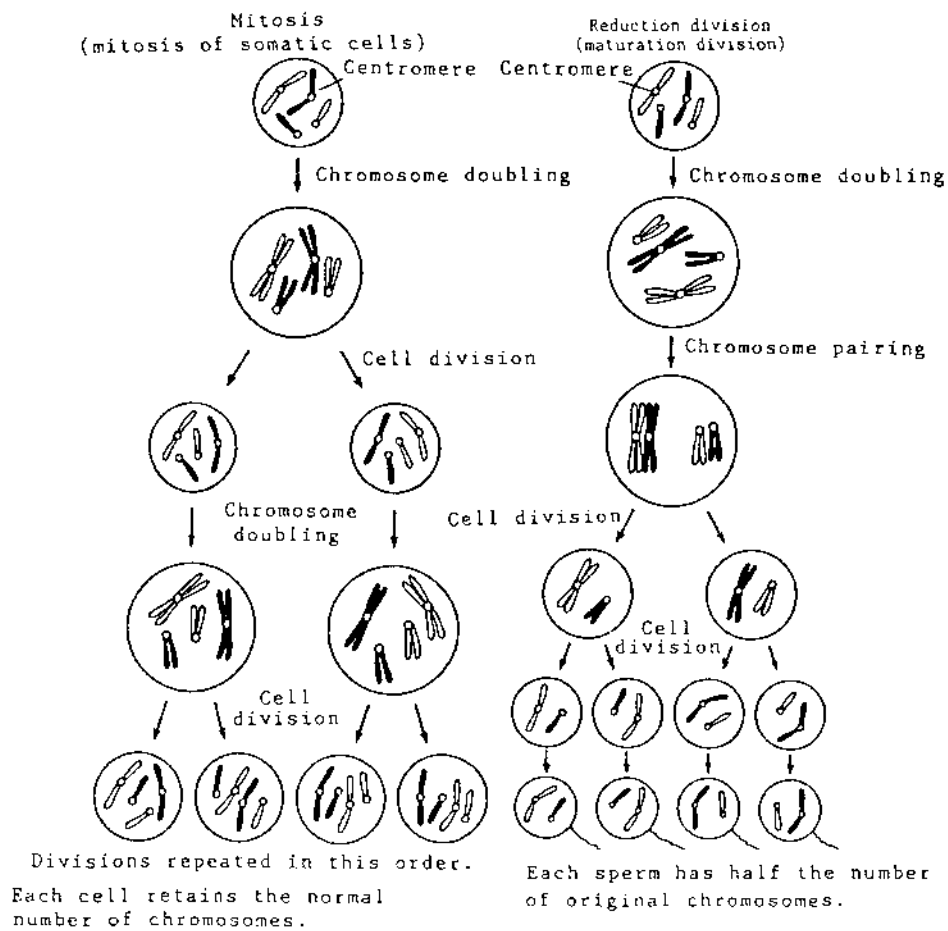


Figure 4-1-2 Comparison of Mitosis and Meiosis

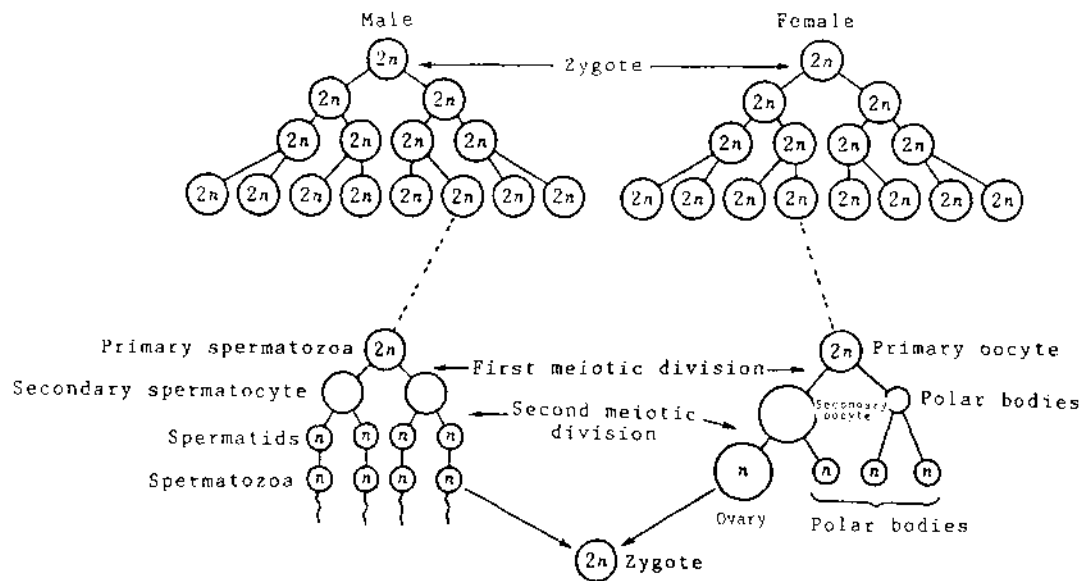


Figure 4-1-3 Cycle of Diploid and Haploid in Animals

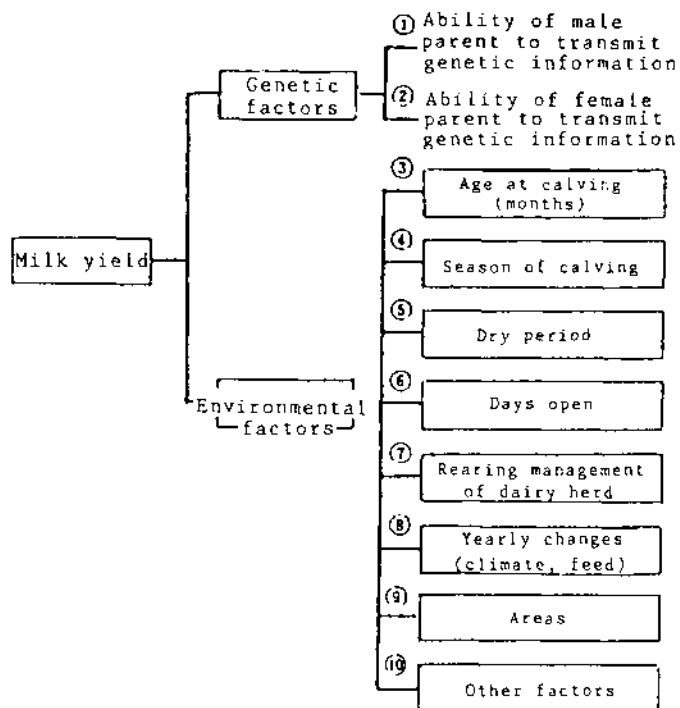


Figure 4-1-4 Various Factors Related to Milk Yield

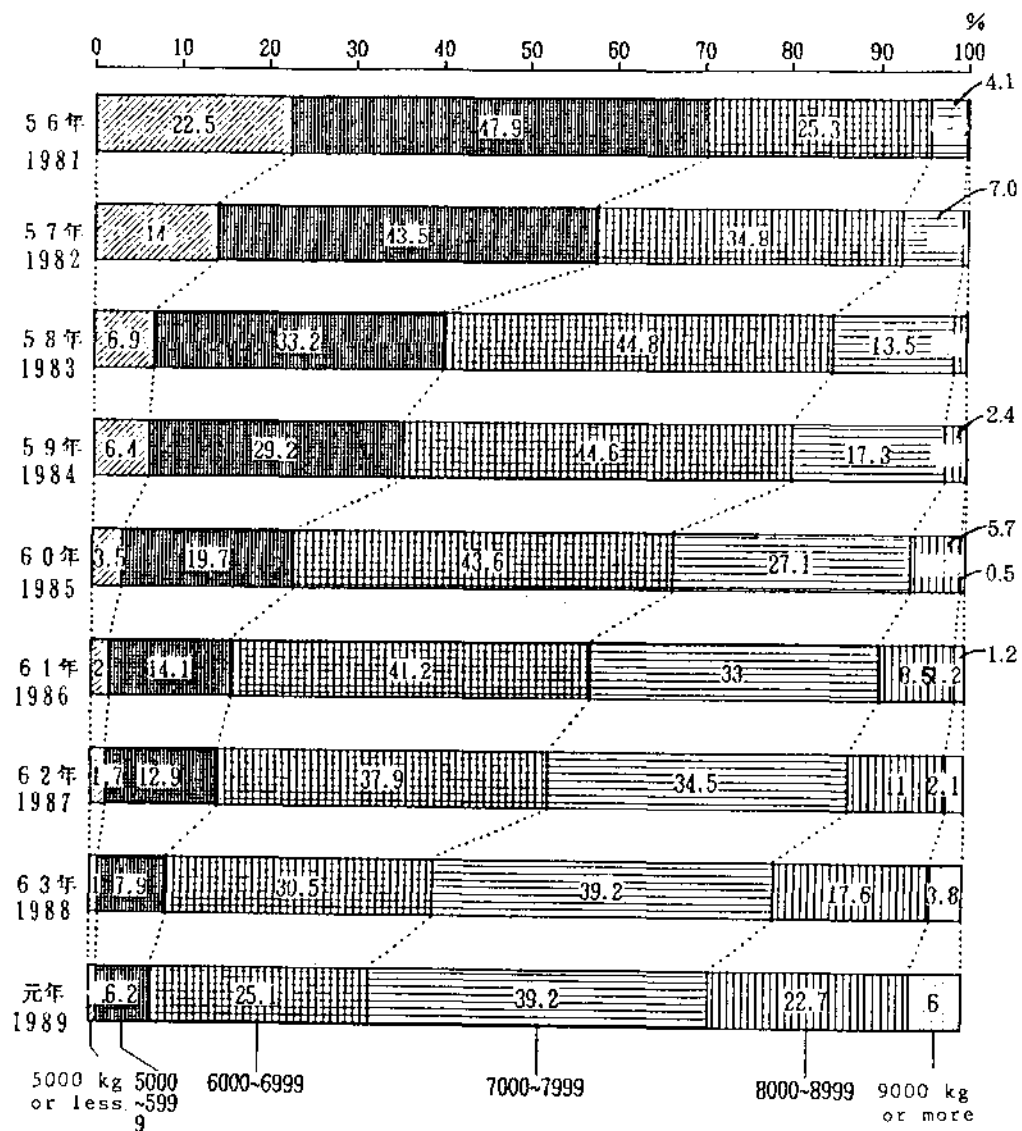
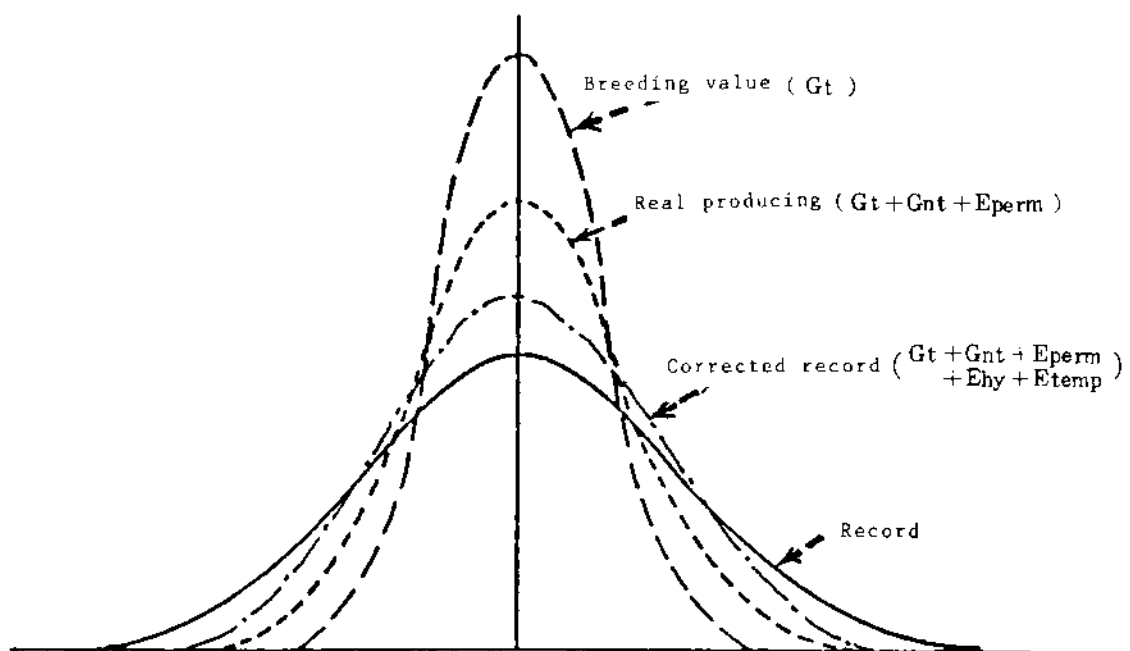


Figure 4-1-5 Annual Changes in Milk Yield Expressed as Percentage of Farming Households



$$P = G_t + G_{nt} + E_{perm} + E_{adj} + E_{hy} + E_{temp} + e$$

Figure 4-1-6 Factors Affecting Variation, and Differences in Variation Magnitude (Mitsumoto)

デイクシーリー ネットド ボーイ イーデー

P548

48547 S61.06.10

Economic effect + ¥78738

BV	M	+1557 _{kg} ± 304 (ETA	+779 _{kg} ± 152)	Repeatability	60 %
				18 herds	18 bulls
F		+81 _{kg} ± 11 (	+41 _{kg} ± 5)	Record in progress	100 %
F%		+0.44% ± .14 (	+0.22% ± .07)		
SNF		+132 _{kg} ± 26 (	+66 _{kg} ± 13)	17 herds	17 bulls
SNF%		+0.19% ± .10 (	+0.10% ± .05)	Record in progress	100 %
P		+53 _{kg} ± 26 (	+26 _{kg} ± 13)	17 herds	17 bulls
P%		+0.22% ± .10 (	+0.11% ± .05)	Record in progress	100 %

Milking rate	kg/min				
Percentage of milk yield from fore and hindudders	kg (				
DC	kg (				

Owner or management, Kachikukairyo-jigyodan
(Livestock Improvement Corporation)

Sire 1806201A ハイッテイアファームズ ネットド ボーイ
Maternal grandsire 1650414A エスタブリッシュデー パリアント

Character	Degree 2	1	0	1	2	Degree: STA
Milk yield	low					2.86
Milk fat percentage	low					1.71
Solids-not-fat percentage	low					1.06
Protein percentage	low					1.65
Withers height	low					0.99
Strength	Weak					0.12
Body depth	Shallow					1.39
Sharpness	Lacking					1.57
Rump angle	High pins					0.57
Rump length	Short					0.62
Rump width	Narrow					0.29
Side view of hind leg	Posty					1.11
Hock angle	Small					1.42
Attachment of fore udder	Weak					0.15
Height of rear udder	low					0.03
Width of rear udder	Narrow					0.88
Udder support	Weak					0.96
Udder depth	low					1.44
Test placement	Internal side					2.11
General appearance	low					0.99
Dairyness	low					1.13
Body	low					0.22
Mammary	low					0.91
Final score	low					0.22

29 herds 32 bulls

Figure 4-1-7 Examples of Results of Evaluation of Daily Bulls
(September 1991)

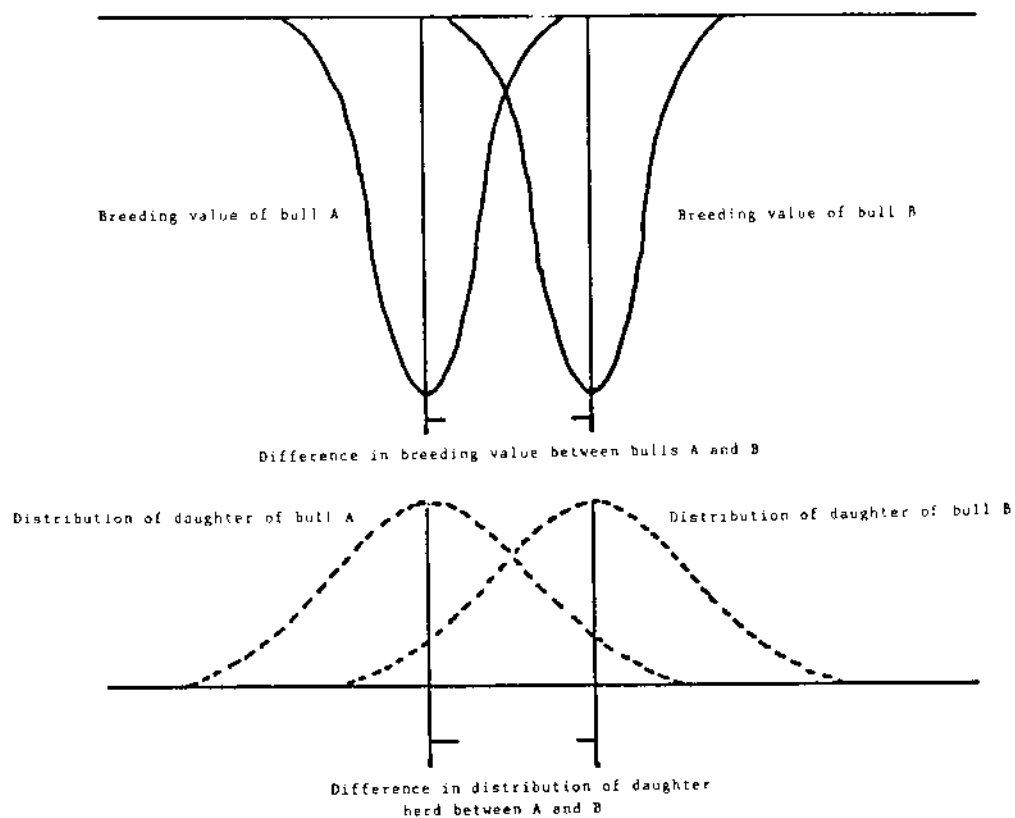


Figure 4-1-8 Differences in Breeding Values Between Bulls, and Expected Distribution of Daughters

→

Note: M = milk yield, F = milk fat, PT = milk fat percentage, SMP = solids-not-fat, SNF = solids-not-fat percentage.

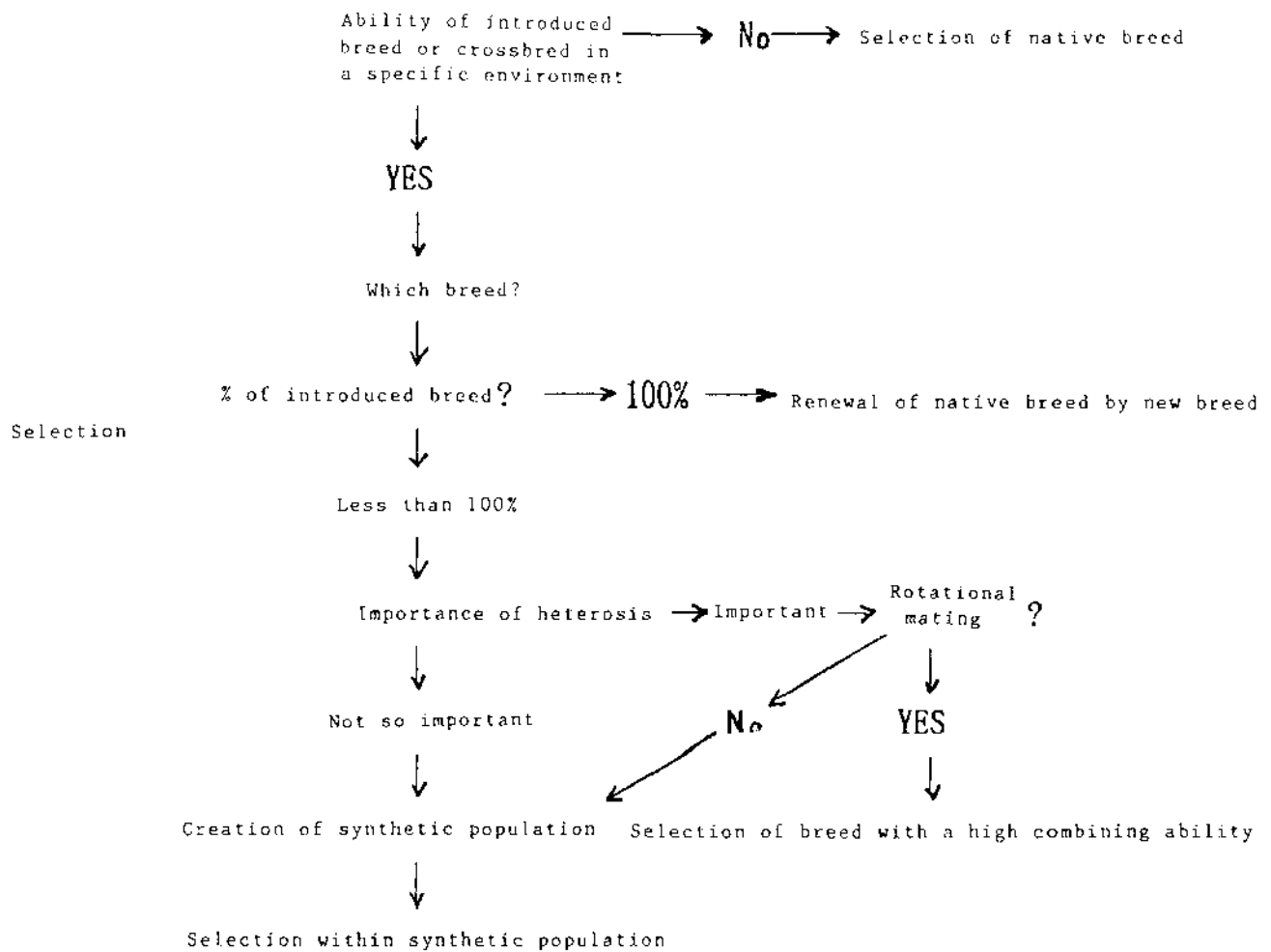


Figure 4-1-10 General Strategy for Genetic Improvement of Livestock and Poultry

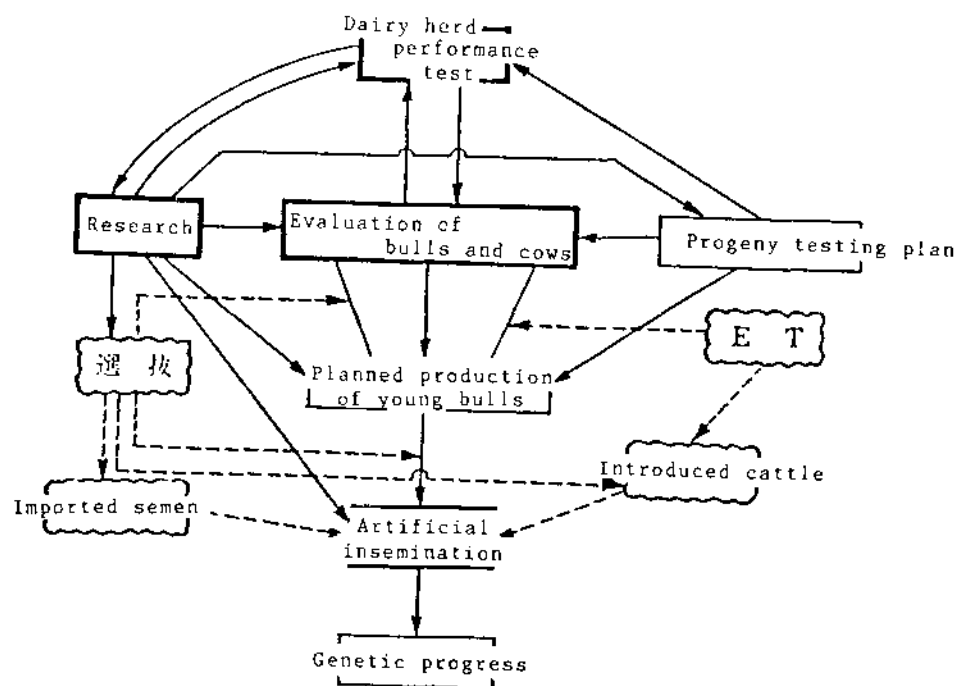
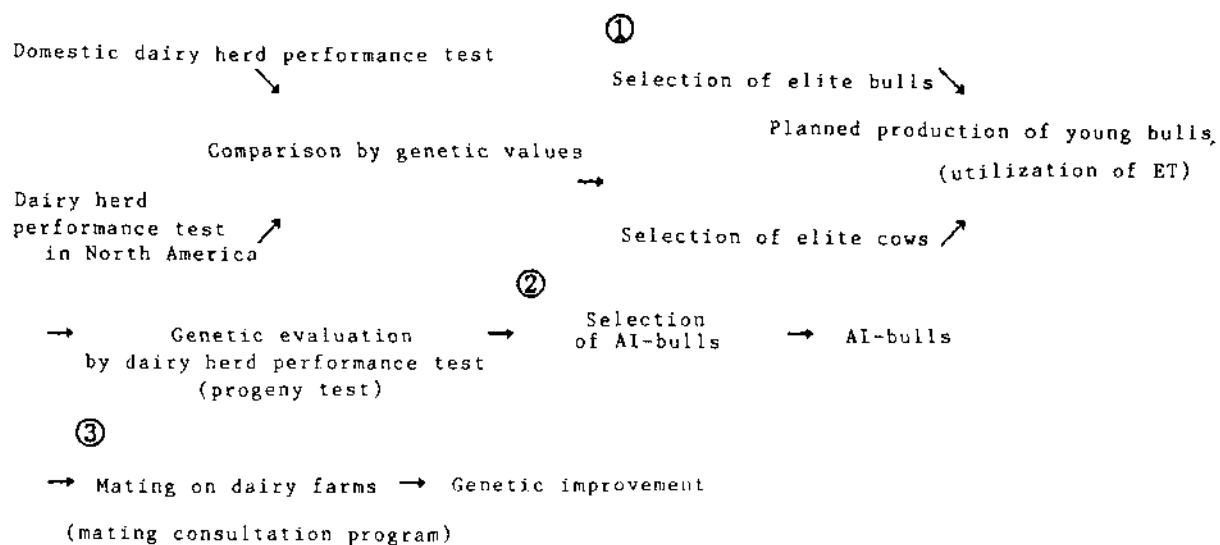


Figure 4-1-11 System Contributing to Genetic Progress (Mitsumoto)

Figure 4-1-12 Opportunities for Selection in Dairy Cattle (Mitsumoto)



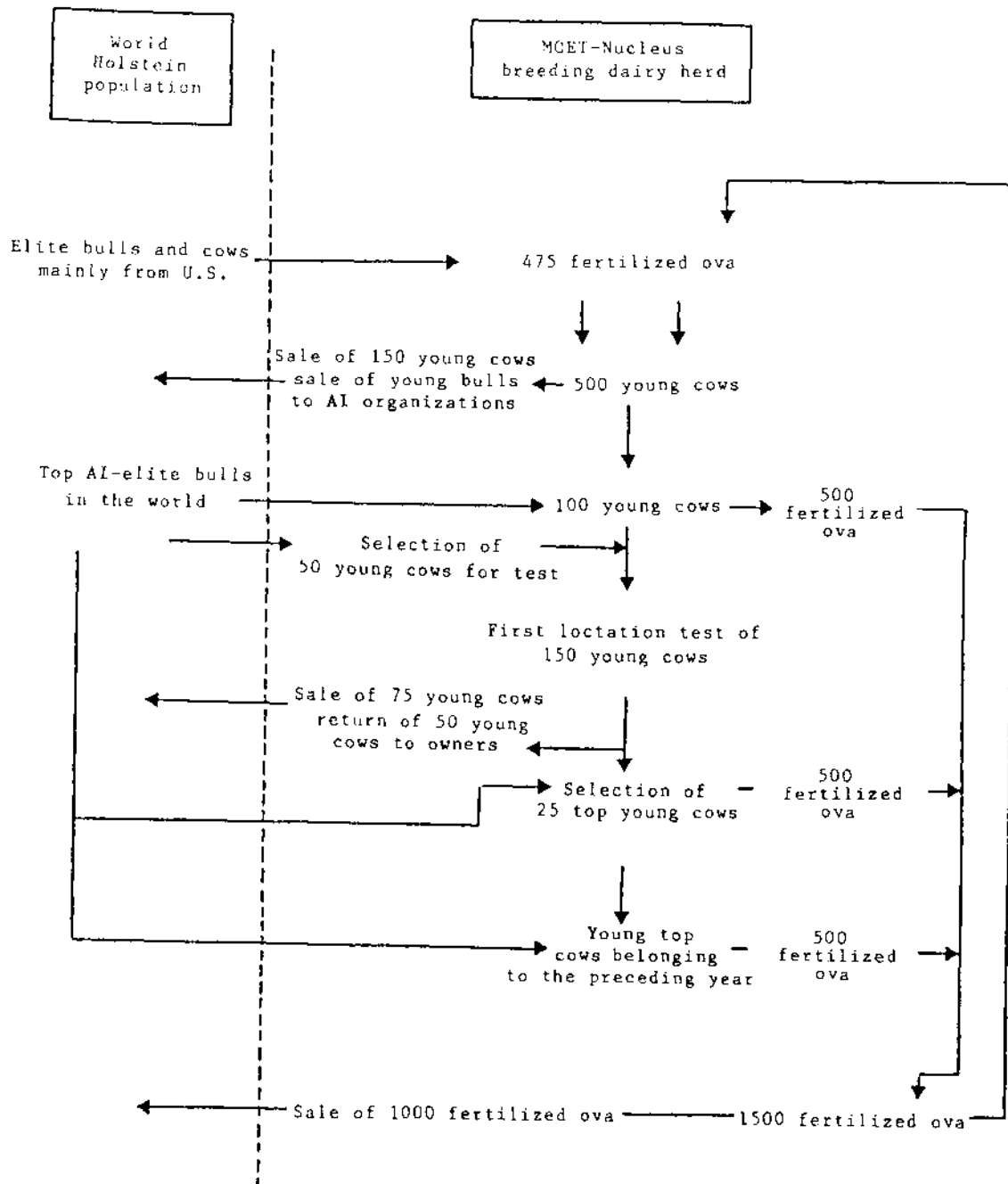


Figure 4-1-13 Delta MOET-Nucleus Breeding Population Cycle in the
Netherland
(Mitsumoto)

2. Progeny Testing of Dairy Bulls in Japan

2-1 Introduction (Dairy Farming in Japan)

As of February 1, 1991, a total of 60 thousand farmers keep 2.1 million dairy cows in Japan. Those over two years of age are 1.4 million, and the cows in milk 1.1 million. The average herd size is 34.6, including all age classes, and 23.6 with only those over two years. Ninety-nine percent of the dairy cattle in this country are Holsteins with a tiny minority of Jersey cattle. Annual milk production per cow (national milk production/total number of cows in milk) is about 6,400 kgs.

Almost all of the dairy cows and heifers are bred artificially with frozen semen. The estimated total number of units of dairy bull semen annually used is 2.4 million. The leading semen suppliers of dairy bull semen are the Livestock Improvement Association of Japan (LIAJ) and Hokkaido Livestock Improvement Association (HLIA), taking up altogether about two thirds of the national sales.

Importation of livestock semen from overseas began in 1984. Annual import of dairy bull semen has been gradually increasing from about 20,000 units in 1984 to 143,000 in 1990. All the imported semen has been from USA and Canada.

The official milk recording program came into existence in 1974. It has shown a rather rapid expansion since then. In 1991, about 42 percent of the cows are enrolled in the milk recording program (Table 4-2-1).

2-2 Reorganization of Dairy Bull Progeny Testing Programs

Up until 1988, proven sires came from two official dairy bull progeny testing programs. One was carried out by the National Livestock Breeding Stations (NLBS) for the young bulls produced in these governmental establishments. The other was undertaken by the Livestock

Improvement Association of Japan (LIAJ) under the sponsorship of the National Government. Such young bulls as produced by the private breeders and farmers through contract mating and purchased by the Government were to be progeny-tested in this latter program. The NLBS program was initiated in 1969 and the LIAJ program in 1971. These two programs were separate ones, but the method of progeny testing and data analysis were much the same for both.

The system of progeny testing dairy bulls in these programs was very different from that in other countries where this kind of work was run to the extent that all the daughters of the test bulls were milk recorded in special stations. Part of the barns of two National Livestock Breeding Stations and many prefectural government institutions were utilized for this purpose. This station system was the only choice available at the time when these programs were started, because no nationwide milk recording program existed yet in Japan.

By the end of 1988, about 180 Holstein proven sires were selected and stationed for service in the four bull centers and one depot of the LIAJ located in different regions of the country. The LIAJ bull centers kept 51 proven Holstein sires active in 1988. Almost all of the Holstein bull semen produced for sale by the LIAJ centers was from the proven sires.

Meanwhile, with progress of the progeny testing programs for dairy bulls, the following limitations and problems came to be felt increasingly:

- ① While there is growing interest among farmers in the use of proven sires, the stations' total capacity for accommodating the daughters of the test bulls is limited, barring further expansion of the program.
- ② Only the Government-owned bulls can be progeny-tested and selected as official proven sires. In some regions, efforts were made by private breeders to prove sires within such regions on the basis of

the data available from the milk recording program which developed rapidly. The dairy farmers as users of the semen are claiming for one unified system of sire evaluation, instead of the "official" and "private", or "local", proof figures which are not convertible mutually.

The Government decided in 1984 on remodeling and expansion of the progeny testing programs such that:

- ① The NLBS program and the LIAJ program be reorganized into one;
- ② The new program include the progeny testing of private-owned young bulls meeting certain requirements, upon request of the owners of bulls, as well as the Government-owned young bulls; and
- ③ Sire evaluation by a unified method, based on the milk recording data as well as station data, be made not only for the bulls progeny-tested under the new system, which requires "waiting", but also for any other bulls which can be evaluated.

It was expected that more young bulls would be progeny-tested in the new program than in the old ones so that a larger number of selected proven sires might be available for use and/or higher genetic quality of proven sires might be secured than ever through intensified selection.

The batch of the young bulls test-mated in 1983 was the last one involved in the old programs, being proved in 1988. The test bulls mated in 1984, the first group in the new program, had their first evaluation in May 1989, along with a large number of sires used in the past and outside this program. We have now the sixth sire evaluation (November 1991). Two evaluations a year has become a routine practice.

Fig.4-2-1 summarizes the differences between the old and new programs. Hereafter, description will be focused on the new program.

2-3 Test Bulls

The young bulls to be progeny-tested (referred to as "test bulls") may be classified by ownership of the bull as follows:

- (1) Those owned by the National Government (Government-owned bulls), which are further classified into:
 - a. The bulls purchased by the Government from among the bull calves produced by planned mating under the contract between the Government and farmers, and
 - b. The bulls produced by the National Livestock Breeding Stations (NLBS); and
- (2) Those owned by private organizations or individuals (private bulls).

2-4 Production of Test Bulls Through Planned Mating

- (1) Selection of bull sires and bull dams

Selection of individual bull sires and establishment of the general selection criteria for bull dams are made every year by the committee of the Livestock Improvement Association of Japan (LIAJ), consisting of university professors and researchers in animal breeding, dairy cattle specialists and representatives of dairy organizations, and so forth.

The number of the bull sires selected for the planned mating each year is around 30. Those selected include both domestic and foreign bulls, the latter being used in the form of imported semen. A large portion of the foreign sires are US ones and the remaining are Canadians.

The general criteria for cows to be selected as bull dams, decided by the committee for the 1991 season follow:

For production (ME, 2X, 305 d. basis):

CBV¥(yen)	Not less than 77,000, or
[CBVF(k)	Not less than +36, and
[CBVSNF(k)	Not less than +75, or
[CBVP(k)	Not less than +20.

For conformation:

Final score (pts.)	Not below 82 (after 1st calving), or Not below 85 (after 2nd calving or on), and
Mammary system (%)	Not below 82 (after 1st calving), or Not below 85 or 83* (after 2nd calving or on).

* This 83% is applicable only when the animal is outstanding in the proof for production.

The both requirements for production and conformation should be satisfied.

(2) Contract

When the owner of a bull dam thus selected agrees to use the semen of the bull sire specified by the Government on his cow concerned, a contract will be concluded to the effect that, if such mating results in a bull calf which is apparently normal, the Government purchases it at a predetermined price. The semen for the planned mating is provided to the herd owner free of charge. If the calf born is female, it belongs to the herd owner.

(3) Collective raising of the bull calves and preliminary selection

The bull calves produced by the planned matings and bought by the Government are all moved into one of the Livestock Breeding Stations for collective raising. A preliminary selection is made on them at around 12 months of age mainly on the basis of the growth, conformation and semen conditions. Half of them will be culled. The other half saved are transferred to the different bull centers of LIAJ for semen collection and "waiting".

2-5 Requirements to be Met by Private Bulls

For a private bull to be accepted as test bull, it must satisfy the criteria regarding its sire and dam. These criteria are set out each year by a committee of LIAJ, composed of learned persons and representatives of organizations relative to dairy cattle breeding. Applicable are the criteria for the year in which the action for acquisition of the bull (mating, purchase, etc.) is taken.

The criteria for 1991 follow:

(1) Sire

- i As for a domestic sire, any one of the conditions of i -1 and the condition of i -2 below should, in principle, be satisfied.

i -1 Milk production

i -1-1

Proof*	
"Economic effect"	$\geq +53,000$ yen
Repeatability	$\geq 65\%$

i -1-2

Proof*	
Fat yield	BVF $\geq +50\text{kg}$
SNF yield	BVSNF $\geq +99\text{kg}$
or Protein yield	BVP $\geq +32\text{kg}$
Repeatability	$\geq 65\%$

i -1-3

Proof*		
Fat yield	BVF	≥ +50kg
Fat %	BVF%	+
SNF %	BVSNF%	+
Protein %	BVP%	+
Repeatability		≥ 65%

i -2 Conformation

Proof*	
STA (final score)	+
STA (mammary system)	+

* Dairy Sire Evaluations (1991-I).

ii As for a foreign sire, the conditions below should, in principle, be satisfied.

USA*			CANADA**	
Fat yield	PTAF	≥ +54 lb	BCAF	≥ +13
Protein yield	PTAP	≥ +44 lb	BCAP	≥ +13
	Rel.	≥ 65%	R	≥ 65%
Conformation	PTAT	+	FC	+
	UDDER COMP.	+	UDDER	+

* Holstein Association, Sire Summaries, 1991 Vol. 1.

** Agriculture CANADA, Dairy Sire Appraisal (1991, I).

A sire of any other country than USA and Canada is required to have proofs considered comparable enough to the above.

(2) Dam

i) As for a domestic dam, it should have undergone the type classification of the Holstein Breeders' Association of Japan and milk recording with the results satisfying, in principle, either of the conditions presented in i -1, and also the condition of i -2 below.

i -1 Milk production

i -1-1

Proof*	
CBV¥	≥ 77,000 yen

i -1-2

Proof*		
Fat yield	CBVF	≥ +36kg
SNF yield	CBVSNF	≥ +75kg
or Protein yield	CBVP	≥ +20kg

* Cow Evaluations for Selection of Dams to Produce Domestic Test Bulls (June, 1991).

i -2 Conformation

Result of type classification		
Final score	Post-2nd calving or later	≥ 85 pts.
	Post-1st calving	≥ 82 pts.
Score %, mammary system	Post-2nd calving or later	≥ 85%
	(83% if production proof is outstanding.)	
	Post-1st calving	≥ 82%

- ii As for a foreign dam (including a donor cow), the conditions below should, in principle, be satisfied.

	USA *	CANADA**
Fat yield	CIF ≥ +42 lb	CIF ≥ +9
Protein yield	CIP ≥ +34 lb	CIP ≥ +8
Final score	VG or higher	VG or higher
Mammary system	VG or higher	VG or higher

* USDA-DHIA, Elite Cow Index (1991.1)

** Agriculture CANADA, Cow Indices (1991.1)

A dam of any other country than USA and Canada is required to have proofs and classification results considered comparable enough to the above.

- iii As for an imported dam, either of the conditions presented in i or in ii above should be satisfied.

2-6 Test Bulls Selected in 1991

The number of young bulls selected as test bulls in 1991 is given in the table below, as classified by ownership and sources.

Ownership	Sources		Total
	Domestic	Imported	
Government:			
NLBS	5	0	5
Planned mating	30	0	30
Private	3	140	143
Total	38	140	178

The private bulls come largely from the two leading AI organizations, i.e., the Livestock Improvement Association of Japan (LIAJ) and the Hokkaido Livestock Improvement Association (HLIA). Many of the domestic test bulls have been produced with imported semen. Most of the imported young bulls are from the United States, the remaining small part being from Canada.

2-7 Test Mating

The semen of the test bulls is distributed to many different herds nationwide for test mating.

(1) General planning for test mating

The Livestock Improvement Association of Japan (LIAJ) is responsible for making a general test mating plan each year. For 1991, the number of necessary test matings was figured out as follows:

No. of test bulls	No. of milk recorded daughters needed		No. of necessary matings
	Per test bull	Total	
178	36	6,408	51,264

For the above calculation of the number of necessary matings, the effective heritability value for milk yield is assumed to be 0.31 after removal of herd-year effect and other systematic (fixed) effects. It is also assumed that 8 test matings are necessary finally to obtain one lactation record of daughter usable for sire evaluation.

(2) Distribution of semen for test mating

The test bulls' semen is distributed nationwide for production of sample daughters to herds participating in the milk-recording program. All of the forty-seven prefectures cooperate for the test mating.

Allotment of test matings to different prefectures is based partly on the number of milk-recorded herds and partly on the total number of cows kept in those herds in each prefecture.

The owners of the test bulls are requested to provide about 800 units of semen from each bull. The part of such semen for use in all the prefectures except in Hokkaido region is sent firstly to the stock point in the Maebashi Bull Center of the LIAJ, and the part to be used in Hokkaido to the stock point in the main AI center of the HLIA. In turn, according to the semen distribution plan set out by the LIAJ, as classified in two ways by test bull and prefecture, the Maebashi Center sends the semen of different bulls to the semen handling organization in each prefecture except for Hokkaido, where the main AI center sends semen to subcenters in the region.

The LIAJ has concluded a contract for implementation of test mating with an appropriate organization in each prefecture that represents the milk-recording associations at prefectural level.

2-8 Milk-Recording of Daughters

The daughters of the test bulls are expected normally to be milk-recorded in the herds of their own birth. The herd owners are requested to make best efforts to keep them at least until they complete their first lactation.

In the milk-recording program, 305-day milk yield is based normally on 10 recording day measurements. As for milk composition such as fat %, protein %, and SNF %, measurement is made once a month with the sample taken on the recording day.

For some years following reorganization of the progeny testing program, the daughters of the test bulls were milk-recorded in both farmers' herds and special stations in use under the old system. Use of the special stations, however, is to be dropped for the daughters of 1990 and following groups of test bulls. This complete change to field system was decided after a few year experience of sire evaluation with field data. After 1993, no special stations will be used, although the daughters of 1989 and earlier test bulls are still to be milk-recorded therein.

2-9 Sire Evaluation

The traits that sire evaluation, whether a formal genetic evaluation or else, concerns include milk production traits, management characters, beef production characters, and conformation characters.

(1) Milk production characters

(1 1) Traits included

Milk yield, butterfat % and yield, SNF % and yield, and protein % and yield.

(1-2) Sources of data

① Data from the milk recording program accumulated to a recent date. Only the data satisfying the following conditions are used:

- ①-1 The record of a Holstein or grade cow registered in herdbook;
- ①-2 The officially approved first lactation record, with calving between 22 and 36 months of age;
- ①-3 Two milkings a day; and
- ①-4 The record of a complete lactation of 240 days or longer, or the incomplete lactation record based on at least five supervised recording day measurements.

② Data from the station testing, accumulated in the old and new progeny testing programs up to a recent date. Only the records meeting the following conditions are used:

- ②-1 The complete 240-day record based on everyday measurement of milk production; and
- ②-2 At least five measurements of milk composition at appropriate intervals during the test period.

(1-3) Cutting or extension of record

A record of lactation longer than 305 days is cut on the 305th day.

A record of lactation terminating between the 240th and 305th days, inclusive, is used as it is.

A record of lactation in progress is used after extension to the 305 day basis if five or more supervised recordings in field testing or five or more measurements of milk components in station testing have been made.

All the 240-day records from station testing are also extended.

Extension of record is made for milk yield, fat yield, SNF yield and protein yield, using the following formula:

$$Y = Y_1 + (B_0 + B_1X)(305 - t),$$

where Y: Estimated 305-day yield.

Y_1 : Amount produced from the calving day to the preceding day of the latest supervised recording.

x : Measurement on the last supervised recording day.

t : Number of days from calving to the last supervised recording.

B_0, B_1 : Coefficients in correspondence to region, calving month, and the number of days from calving. These coefficients have been determined by application of the fifth power regression curves to the data from the milk recording program.

(1-4) Total number (rounded) of records used in November 1991 sire evaluation:

952,000 for milk yield and fat % and yield,

786,000 for SNF % and yield, and

389,000 for protein % and yield.

(1-5) Method of sire evaluation

The best linear unbiased prediction (BLUP) is employed for sire evaluation.

① Statistical model

MGS model is used:

$$Y_{ijklmnop} = HY_i + G_j + S_{jk} + \frac{1}{2}G'_l + \frac{1}{2}S'_{lm} + A_n + M_o + e_{ijklmnop}$$

where

$Y_{ijklmnop}$: Observed value

HY_i : Effect of the i^{th} herd-year(fixed)

G_j : Effect of the j^{th} sire group(fixed)

S_{jk} : Effect of the k^{th} sire in the j^{th} sire group(random)

G'_l : Effect of the l^{th} maternal grandsire group(fixed)

S'_{lm} : Effect of the m^{th} maternal grandsire in the l^{th} maternal grandsire group (random)

A_n : Effect of the n^{th} calving age(fixed)

M_o : Effect of the o^{th} region-calving month (fixed)

$e_{ijklmnop}$: Effect particular to each observation

② Numerator relationship matrix with sires and maternal grandsires is used.

(1-6) Expression of genetic merit

The genetic merit of each evaluated bull is expressed in BV (breeding value):

$$\hat{BV}_{i,k} = 2(\hat{G}_i + \hat{S}_{i,k})$$

The genetic base for the two 1989 evaluations was defined as the average BV of the bulls born in 1978. For evaluations thereafter, the genetic base is to be shifted by one year in the spring

evaluation every year.

(2) Management characters

(2-1) Traits included

Milking rate, fore-rear udder ratio of milk secretion, calving ease, temperament, and milking ease.

(2-2) Sources of data

For management traits, data come from different sources. Information about calving ease comes from the mates of test bulls and/or their daughters. Hearing from herd owners who keep the test bulls' daughters is done for temperament and milking ease. The station testing of daughters can provide data on milking rate and other milkability traits. In general, however, the system of data collection on these management characters has not been established well enough to allow a formal genetic evaluation of sires. Efforts are being made, for the time being, to analyze data in one way or another and furnish farmers with information just indicative of sire's transmitting ability.

(2-3) Method of analysis

The Harvey's least squares analysis is applied to the station data to estimate sire effect for milking rate and fore-rear ratio with the following model:

$$Y_{ijk} = M + H_i + S_j + e_{ijk}$$

where

Y_{ijk} : Observed value
 M : Population mean
 H_i : Effect of the i^{th} station

S_j : Effect of the j^{th} sire
 e_{ijk} : Effect particular to each observation

As for calving ease, temperament, and milk ease, records are classified into a number of classes, i.e., "easy, no assistance", "slight assistance", "two- to three-men assistance", "difficult, several-men assistance", and "very difficult, surgery needed or death of cow" for calving ease, "gentle", "nervous", and "rough" for temperament, and "fast", "medium", and "slow" for milking ease. Then, the "standardized relative frequency (SRF)" is computed for each of the traits of each sire as follows:

$$SRF = (P_i - \bar{P}) / \sqrt{\bar{P}(1 - \bar{P})N_i}$$

where

P_i : Relative frequency of the i^{th} sire's records classified into the specified class(es): "easy, no assistance" and "slight assistance" for calving ease, "gentle" for temperament, and "fast" for milking ease.
 $\bar{P}$: Average P over all the sires
 N_i : Number of i^{th} sire's records

(3) Beef production characters

For beef production, only daily weight gain from 7 to 17 months of age is measured on a certain number of sons from each Government-owned test bull.

Analysis of data is made using the method of least squares with about the same model as for the milking rate and fore-rear ratio mentioned above.

(4) Conformation characters

(4-1) Traits included

Scores for general appearance, dairy characters, body capacity, mammary system, as well as final score, and also fifteen linear characters.

(4-2) Sources of data

The records of the type classification plan designed specifically for a part of the daughters of test bulls, as well as the records of herd classification service conducted as a routine by the Holstein Breeders' Association of Japan are the main sources of data. Only the records satisfying the following conditions are eligible for use:

- ① The record of a Holstein or Holstein grade registered in herdbook,
- ② The record of a first calver which calved not earlier than 22 months nor later than 36 months of age, and
- ③ The record of classification made within 10 months after calving.

(4-3) Method of sire evaluation

The BLUP with the following model and the numerator relationship matrix is applied for sire evaluation:

$$Y_{ijklmnopq} = CHY_{ij} + G_k + S_{kl} + G'_m + S'_{mn} + A_o + L_p + e_{ijklmnopq}$$

Where

- $Y_{ijklmnopq}$: Observed value
- CHY_{ij} : Effect of the j 'th herd-year in the classification on by the i 'th classifier (fixed).
- G_k : Effect of the k 'th sire group (fixed)
- S_{kl} : Effect of the l 'th sire in the k 'th sire group (random)
- G'_m : Effect of the m 'th maternal grandsire group (fixed)

s'_{mn} :	Effect of the n^{th} maternal grandsire in the m^{th} maternal grandsire group (random)
A_o :	Effect of the o^{th} month of age at the time of classification (fixed)
L_r :	Effect of the p^{th} stage of lactation (fixed)
$e_{ijklmnopq}$:	Effect particular to each observed value (random)

2-10 Publication of Sire Evaluations

The computation work for sire evaluation in the new program is done regularly (twice a year) at the Computer Center of the Livestock Improvement Association of Japan (LIAJ) for publication in the "Dairy Sire Evaluations" by LIAJ under the supervision of the Livestock Bureau, Ministry of Agriculture, Forestry and Fisheries.

As regards milk yield, butterfat yield and %, SNF yield and %, protein yield and %, and conformation characters, sire evaluation is published only if the bull has at least 15 daughters, distributed over 5 or more different herds, with records (usable for sire evaluation) of the trait concerned.

The "Dairy Sire Evaluations" consists of four sections according to the categories of bulls such that:

- Section I - Bulls having undergone the whole process of progeny testing, including "waiting", as described earlier.
- Section II - Bulls (under 15 years of age) once evaluated in the old program of progeny testing and re-evaluated in the new program.
- Section III - Other bulls (under 15 years of age).
- Section IV - Bulls over 15 years of age.

Information items for each bull follow:

(1) Identification

Name, registration number, date of birth, final type score, and names of sire and maternal grandsire.

(2) Milk production characters

- ① BV (breeding value) estimate and ETA (estimated transmitting ability). The latter, being simply half of the former, is given just for convenience to those who have been familiar to PD. All the figures are on the first lactation (assumed to begin at 26 months of age) basis.
- ② CR (confidence range) for the BV estimate and ETA.
- ③ Numbers of daughters and herds.
- ④ Percent of records in progress. Percent of lactation records in progress used for sire evaluation by extension to 305 days.
- ⑤ Economic effect. Economic merit of using the bull, expressed in terms of increase or decrease of income expected from milk production of his average daughter. To calculate, the following formula is used:

$$\text{Economic effect} = \frac{1}{2}BV_M \times A + \frac{1}{2}BV_F \times B + \frac{1}{2}BV_{SNF} \times C,$$

where

- A: Average raw milk price – (2-year class average fat % x 1/0.1 x extra payment per 0.1 fat % + 2-year class average SNF % x 1/0.1 x extra payment per 0.1 SNF %),
- B: Price per kg fat, and
- C: Price per kg SNF.

(3) Management characters

① Rate of milk flow and fore-rear udder ratio of milk secretion.

The rate of milk flow is presented in terms of the least squares sire effect, while the fore-rear ratio in terms of the least squares sire mean.

② Calving ease, temperament and milking ease. A graphical presentation is given on the basis of the standardized relative frequency (SRF) calculated for each of these traits.

(4) Beef production character

DG (daily gain in live weight). The least squares sire effect and sire mean are presented for daily gain of weight.

(5) Owner of the bull and availability of semen.

(6) Graphical presentation of STAs (standardized transmitting ability) for different traits.

Different traits have different genetic variations, and it is rather difficult only with BV or ETA value to see at once the relative position of the bull in the bull population at large. For supplementation in this respect, STA is calculated for each important trait in the following manner:

$$\text{STA} = \frac{\text{ETA for the bull} - \text{Average ETA for all bulls evaluated}}{\text{Standard deviation of ETA for all bulls evaluated}}$$

The STAs are computed for 4 milk characters, 15 linear conformation traits as well as 5 scored type traits, including the final score, and arranged on a compact diagram, where the relative position of the bull regarding the trait concerned is indicated by the length and direction (to the right or left of the middle point) of a horizontal bar.

2-11 Scheduled Changes in Evaluation Procedures and Organization to do
Evaluation Job

For milk production and conformation traits, introduction of BLUP with the animal model in place of the MGS model now in use is scheduled from the 1992 autumn run to enable simultaneous evaluation of bulls and cows. At the same time, job of sire evaluation for these traits will be taken over by the Animal Breeding Center of the Ministry of Agriculture, Forestry and Fisheries from the Livestock Improvement Association of Japan.

Table 4-2-1 Milk Recording Program

Present status of milk recording program
(as of 31 March 1991)

No. of prefectures involved	47
No. of milk recording associations	349
No. of milk-recorded herds	17,287
No. of cows enrolled	543,176
No. of all cows (Feb. 1)	1,285,000
Percentage of cows enrolled	42.3%

Yearly average for 305-day production

Year	Cow No.	Milk kg	Fat %	Fat kg
1975	6,271	5,826	3.6	208
1980	98,266	6,339	3.7	232
1981	121,832	6,330	3.7	232
1982	149,782	6,372	3.7	233
1983	178,187	6,704	3.6	244
1984	193,560	6,821	3.6	247
1985	210,840	7,008	3.65	256
1986	206,203	7,171	3.65	262
1987	211,786	7,346	3.66	269
1988	233,183	7,507	3.67	276
1989	245,625	7,693	3.69	284

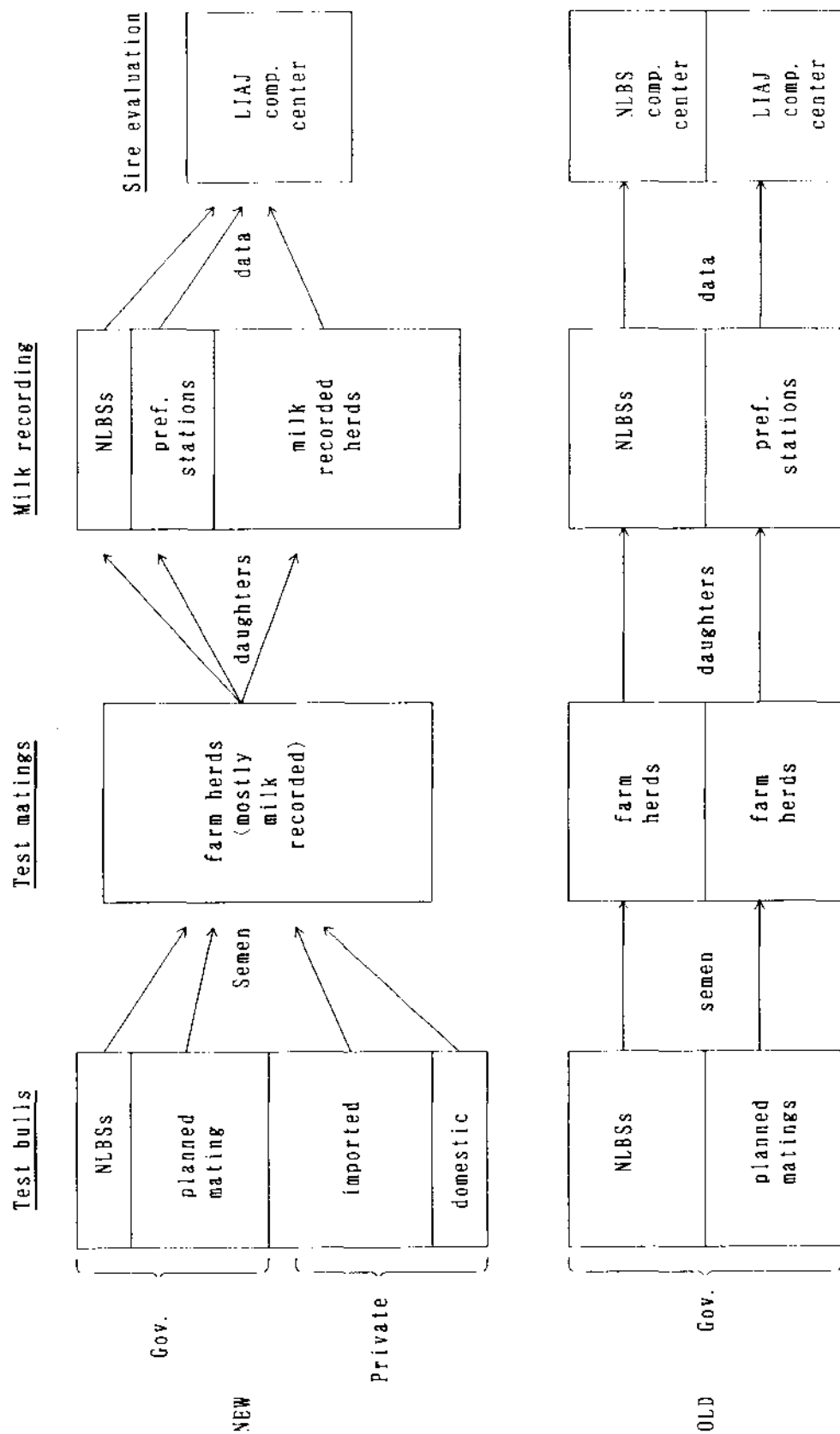


Fig 4-2-1 Procedures of Progeny Testing of Dairy Bulls

3. Type Evaluation of Dairy Cows

3-1 Significance of Type Evaluation

Milk production is the overall requirement for dairy cows and it is best if accompanied by well-balanced milk constituents. This potential, however, cannot be judged by the maximum daily milk yield during peak lactation or by records made over a short-term such as one lactation period. This is because the final judgement of the value of cows is formed on the basis of milk yield per day of life including the rearing period.

It is very important for improvement of milk yield and milk composition that dairy cows are in good health and have a strong constitution suitable for the intake of the necessary amounts of energy. Strength and durability are thus required for dairy cows to maintain their health and to retain milk production.

Type traits relating to reproduction efficiency is also an important factor which has a direct influence on profitability. In addition, great importance should be placed on mammary system and legs and feet among the type traits in relation to productivity and profitability, as described later.

Productivity, strength and reproduction required in dairy cows have all been improved by improving cattle over many years, resulting in a type conformation showing such functions as seen in Figure 4-3-1.

In this manner, type traits clearly accompany functions such as productivity and strength, indicating that the significance of type evaluation is to observe these functions at an early stage. An outline of the profitability due to the type traits of dairy cows can be estimated by type evaluation, and can lead to efficient selection or culling in combination with records of pedigree and milk production testing. Methods for evaluating cows types are

guidelines for dairy farmers and specialists when they find a promising cow or decide to cull poorer animals in the early stages. If there are no records of milk production testing records, in particular, the records of type evaluation are useful in the primary selection.

3-2 The Role of Type Evaluation

(1) Use as a Means of Selection or Culling

The term "improvement" means selection and breeding of individuals with superior breeding value (genetic potential). Genes, however, cannot be seen by external appearance. Genetic potential is therefore estimated through pedigree, milk production tests and the evaluation of type traits. Consequently, type evaluation can be used effectively as a means of selection and culling out.

(2) Securing Replacement Cows

Selection and culling out are the head or tail of improvement methods: suitable animals are picked out by the former and unsuitable animals are removed by the latter. For example, target cows for culling out are cows which show a weak attachment of the udder, cows which have weak legs and loins indicating a short economic lifespan, and cows which suffer from diseases such as reproductive disorders. Cows selected according to such criteria are used as replacement cows.

In heifers without records of milk production tests, type evaluation has the role of gauging genetic inheritance of characteristics with the aim of finding promising animals or those which may help in further improvement.

(3) Foundation Underlying Milking Ability

Type includes important factors regardless of the correlation between type and production. This is because type conformation is closely related to strength and longevity, in particular, mammary system and hind legs milk producing ability play an important role underlying an animal's milk producing ability.

- (4) Mutually dependent relationship between production testing and type evaluation

A cow whose udder hangs down due to genetic defects indicates a low value as a replacement even if she shows excellent results in milk production tests. However, a cow which shows problems in lactation such as a low milking rate is not a suitable replacement cow even if she has a favorable conformation.

Type characteristics or some other traits which cannot be evaluated in milk production are evaluated by type evaluation. On the contrary, traits which cannot be evaluated by way of type evaluation are evaluated by milk production tests. These two evaluations are thus inter-related.

3-3 Conformation and Economy

Any characteristics selected in an improvement plan for cattle should accompany improved economy. Where type traits are targeted for selection, the following conditions should be considered: (i) the target character is of economic value; (ii) the character is genetic and shows selection effect; (iii) the character can be accurately measured and evaluated.

- (1) Are the type traits having economic value ?

- (1-1) Relations to lifetime production

Table 4-3-1 shows the relationship between type (final scores) and the lifetime production (records of 3 or more

lactation periods). As can be seen from the table, accumulated milk yields during the respective periods increase with an increase in final score in type evaluation. In other words, the type characteristics of an animal are closely related to lifetime production.

(1-2) Relationship between udder traits and productivity

Table 4-3-2 shows the relationship between herdlife or lifetime milk yield and the characteristics of the mammary system which are most important type traits. Cows showing a long herdlife and a high lifetime milk yield have the following highly evaluated features: "strong ligaments in the udder"; "high and wide attachment"; and "favorable size and position of teats".

(2) Traits to be inherited

Characteristics examined in type evaluation should be genetic if they are used as criteria in selection.

Table 4-3-3 shows the heritability of the main characteristics of dairy cows. The values for type traits are no lower than those for lactation and reproduction. Heritability is rather high for the body size and mammary system, both of which are particularly important for uniformity of a dairy herd. This leads to economy and increased labor productivity (labor saving in management due to mechanization).

3-4 Dairy Cows with Functional Character

What are the requirements for good dairy cows? The main requirements are listed in Table 4-3-4. From this table, we can see that good dairy cows are those which are healthy, work efficiently and show a high productivity and profitability.

"Functionality" in addition to economy is also required of dairy cows. Functional dairy cows are healthy, have a long duration of milk production, show high feed efficiency, show good reproductivity, are resistant to diseases such as mastitis and are easy to raise. In other words, functional dairy cows are cows which show a high lifetime milk yield due to efficient milk production and good economics.

What kind of characteristics lead to functional cows? Functional cows, in general, are defined as those which "have high milk production and characteristics suitable for management of a cow shed, live long, and pass high milk producing potential to their offspring".

The main functional characteristics of mammary system, rump, legs and feet are described below.

(1) Measurement and Size of a Cow's Body

The body size and balance among body parts along with the formation of the body are calculated by body measurements and are used as a supplementary means of individual evaluation. In addition, the results of body measurements are used in dairy herd management as information regarding the progress of cattle improvement and the state of growth.

In Japan, a moderate or appropriate withers height (137 cm - 150 cm) is considered to be favorable. A moderate body width accompanied by strength is also required in order to take in a large amount of feed and produce a large amount of milk. In addition, the following characteristics are advantageous; the overall outline of the body is clean-cut and angular, and the space between the ribs is wide, indicating a sufficient spacing.

Body measurements of cows are performed in the following positions: cows are kept standing on firm ground in which the

hooves do not sink; the head is kept slightly higher than the top line; the head, neck and top line are kept straight; the fore legs are kept straight; and the region from the hock to the pastern of the hind leg is kept perpendicular. Figure 4-3-2 shows the measurements while Table 4-3-5 shows standard growth values for Holstein cows in Japan.

(2) Mammary System

Mammary systems (Figure 4-3-3) are the most important areas among those related to functional characteristics.

- i . Suspension of udder: Strong suspension and a good balance of udder are favorable. Good cows are considered those who show a higher position of the udder floor than the hock. Cows showing an udder floor lower than the hock are considered to have a short lifespan.
- ii . Rear udder: High and wide attachment and the same width from the top to the bottom are good.
- iii . Fore udder: The fore udder needs to be of an appropriate length, and to attach strongly and smoothly to a point somewhat in front of a perpendicular line from the anterior end of the hip bone.
- iv . Quality of udder: The udder is required to be soft and flexible and contract immediately after milking.
- v . Teats: Teats must be thin cylinders about 5 - 7 cm long and hang down from the center of each quarter of the udder, and the space between the fore teats must not be excessively large.

(3) Rump

The rump houses the reproductive organs and the udder. The rump needs to be wide and moderately long, while the position of the pin bone (the upper edge of tuber ischii) needs to be slightly lower than that of the hip bone. The structure of the rump is also important due to its relationship to the animal's posture.

- i . Rump width: Importance is attached to the thurl width in particular. This is because the region measured as the thurl width is the narrowest part of the birth canal.
- ii . Rump length: The reproductive organs are housed inside the pelvis if cows are not pregnant, requiring a suitable rump length of at least 55 cm.
- iii . Rump angle: It is desirable for the position of the pin bone to be slightly lower than that of the hip bone. If so, lochia can be easily eliminated, and a urovagina is prevented, leading to improvement in reproduction results.

(4) Legs and Feet

- i . Legs and feet: The legs and feet supporting the body weight (Figure 4-3-5) are an important part along with the mammary system, because defects in the legs and feet have a marked effect on production and efficiency.
- ii . Legs: It is desirable that the legs have suitable thickness, well-developed joints, muscles and tendons as well as dry and firm flat bones. The following legs are undesirable: legs too straight; sickle-shaped legs; legs too close or too apart at the hocks; in rear leg position, too far back or too far forward; and crampy condition in legs.
- iii . Feet: Thick feet of suitable size, a good shape and excellent quality are desirable. It is also important that the toe is closed and the sole is flat.

3-5 Structure of Cow Body (Skeletal Structure)

Criteria for functionality are based on scale of points in principle. In order to understand these standards, it is important to understand the structure of each part of a cow's body. This is briefly explained below.

(1) Names of Each Part of a Cow's Body

The following terms are general names which depict several areas.

- i . Back line: From withers to tail head (chine + loin + rump).
- ii . Back junction: Linking the back (thoracic vertebrae) and loin (lumber vertebrae). This part is usually smooth, but is prominent in some cows. A markedly prominent junction leads to lack of strength.
- iii . Hypochondrium: A term used as a common name for the area of the 13th rib (the last rib).
- iv . Crops: The posterior end of the shoulder to which the shoulder blade adheres. This site is examined to see whether adhesion of the shoulder and the extent of ribs are good or bad.
- v . Flank: There are upper and lower flanks. The extent to which the ribs are opened and the state of barrel are examined using the upper flanks, which are evaluated for body capacity. Also, the thinness and distinctness of the flank are examined using the lower flanks, which are evaluated for the dairy character.
- vi . Buttocks: The periphery of the pin bone from the back view.

(2) Names of Bones: Division and Features of Skeleton

A total of 200 bones form a cow skeleton. The skeletal features are described below.

- i . Classification of skeleton: The skeleton is broadly divided into the following 3 classes: head bones (frontal bones + jaw bone); abdominal bones (vertebrae + ribs + sternum); and leg bones (fore leg bones + hind leg bones).
- ii . Processes of development and growth: Development and growth of the tissues and organs are not the same. The nerves, skeleton, muscles and fat develop in this order according to their physiological importance in development. The highest growth rate in the growth layer of the epiphysis is observed one year after birth and then between one to two years. As shown in Figure 4-3-8, ossification occurs, in general, from the lower part to the upper part and from the fore part to the hind part. This ossification is thought to be completed after 6 years of age. Accordingly, exercise in young cows is important in order to strengthen their legs and feet, and emphasis is placed on methods of rearing young cows in consideration of this growth period. In fact, growth in width continues after growth in height has ceased.
- iii . Protection of important organs: The most important organs in the body are protected by bones. The brain, spinal cord, and heart and lung are protected by the frontal bone, vertebrae and ribs, respectively. However, the mammary organs related to milking are not protected by bones, indicating that they have to be protected.
- iv . Fore leg bones: Most bones are connected through joints. The adhesion between leg bones and the abdomen, however, are supported only by muscles. Defects in a cow shed or a

lack of exercise may cause cows to show weak adhesion of the soulders. Fatigue during grazing may become serious in cows showing extremely weak adhesion of the shoulders.

- v . Strength of the loin: Few cows have a weak back, but many cows have weak loins. This is considered to be due to the supporting structure formed by the ribs and sternum, while there is no supporting structure in the loin, which supports heavy organs. The strength of the loin, is considered to be related to the position of the thurl. In other words, it is desirable that the thurl lies between the hip bone and the pin bone, forming the fulcrum of the rump. In addition, the more backward the thurl lies, the more forward the pelvis leans, leading to a weak loin.

3-6 Scale of Points on Holstein Cows

A functional type conformation is necessary for cows to exhibit and maintain sufficient milk production. Accordingly, it is important to breed cows having improved type traits and marked physical ability. It is necessary for us to develop methods to select a healthy and hardworking cow. In this section, an outline of type evaluation is described according to "Scale of Points for Holstein Cows" of The Holstein Cattle Association of Japan (Table 4-3-6).

Scale of Points for Holstein Cows is a yardstick type evaluation based on the ideal type in measuring the type traits of dairy cows, and is also used as a practical index when dairy farmers buy, sell, select or cull dairy cows. The contents described in Scale of Points have been improved by revisions to meet the needs of the times.

(1) Ideal type

An ideal type for Holstein cows is shown in Figure 4-3-9. The established standards for the ideal type of Holstein cows in

Japan are as follows: angular shape; high feed efficiency; 6 years of age; 4 - 5 offspring; and a milk yield of 8,000 kg or more for 305 days. The standards at 5 months after delivery are shown below. Cows in the field are evaluated with this ideal type in mind.

- i . The size is expressed by body weight and withers height, which are set at 670 kg (670 kg - 780 kg) and 143 cm (137 cm - 150 cm) respectively.
- ii . The posterior abdomen full of capacity. The rump length and hip width show the same value, and the ratio to the withers height are set at 0.410 - 0.415. For the width of the posterior abdomen, importance is attached to the thurl width.
- iii . The udder is long and wide, and shows strong attachment. The fore udder is attached to the somewhat forward point of a perpendicular line from the anterior point of the hip bone. The rear udder shows high and wide attachment, and has substantial capacity. In addition, the suspensory ligament is strong, and the positions of the bottom surface of the udder are almost horizontal and higher than the point of the hock.
- iv . The milk yield ratio of the fore udder to the rear udder is set at 48 : 52.
- v . The teats are rather thin and suitable for machine milking. Length of about 5 cm. They are suspended from the center of each quarter of the udder. The space between the fore teats should be less than 25 cm.

(2) Fundamentals of type evaluation

The bases of decisions relating to good or bad cows are as follows: 1. whether a cow has a favorable size and

characteristics of the breed; 2. whether a cow is an efficient converting feed to milk; 3. whether a cow has a good mammary system; 4. whether a cow has sufficient durability required for long herd life; and 5. whether it has a gentle temperament. These items are judged according to the Scale of Points. It is difficult, however, to understand the Scale of Points without a trained eye. Supplements for the contents of judgement standards are thus shown for reference in Table 4-3-7.

(3) Improvement in judging techniques

Holsteins are very delicate cattle and their type traits are affected by the environment and rearing management in a similar manner to their milk producing ability.

Even the same cows show different conformation and vitality according to the time of year, namely, the best time for lactation or the non-lactating period. It is thus important to observe many cows under various conditions. It is also necessary to take notice of the following matters when evaluating cows.

- i . The following items are documented; age, parity, dates of delivery and mating and time of milking.
- ii . Fussy judgement of details from the beginning leads to misjudgement of the general nature and makeup. Accordingly, the outline of strong and weak points in cows are grasped by observing the whole body from the side, the rear, the front views at a distance of 4 - 5m. The details are then observed at close range, and palpation is performed if necessary.
- iii . Palpation is performed to observe the following items: the state of the skin and hair of the neck and near the last rib; the state of the ribs; the quality of the udder and

teats; and the state of subcutaneous tissue on the lower flank.

- iv . The location for type evaluation must be well lit, flat and moderately spacious. Cows look small in an extremely spacious environment while they look large in a small place or under a low ceiling.

3-7 Type Evaluation by Linear Type Classification System

Type evaluation is performed not solely with the aim of evaluating individuals and increase the value. At the same time details of type traits are collected and analyzed for the purpose of grasping the genetic effects of characters in sires and using the results as criteria for their selection. In practice, data are obtained in more detail by numerically expressing features in each part of the body in addition to general appearance, dairy character, body capacity, and mammary system. Such data on genetic ability of sires in relation to type traits are used as information for improvement of type traits. In linear type classification, this information is used for improvement of individuals as well as dairy herds.

In linear type classification, the biological features of type traits are expressed on a continuous scale of features (1 - 50). Measurement is made on both features related to potential profitability and functionally important characters. The characters evaluated are classified into primary traits (15 items) and secondary traits (9 items). The primary traits are of economic value and are satisfactory factors to genetically select for progeny tests of bulls. The economic and genetic importance have not yet been clarified for secondary traits, but the items are employed for the purpose of collecting as much information as possible. Totalization and analysis of results of linear type classification make it possible to practically understand and evaluate the genetic features of sires.

Figure 4-3-10 shows the characteristics, terms and evaluation standards (with respect to primary traits) employed in linear type classification.

3-8 Type Evaluation and Dairy Cattle Breeding

It is necessary for the stability of dairy farming management to have good quality cows showing excellent milk producing ability, successive reproduction, good durability and high feed efficiency. It is, thus, basically important to make efficient use of scientific information for improvement such as the results of type evaluation and progeny tests on the basis of pedigree background. In addition, the improvement of dairy cows should be performed with respect to both type traits and lactation characteristics on a parallel basis without giving overemphasis to any side, with the aim of selecting sires having beneficial economic features.

References

1. Reviewing type conformation of dairy cattle.
Consider the Habitus of Dairy Cattle,
1991, The Holstein Cattle Association of Japan.
2. The A B C of judging techniques of Holstein Cattle.
1973, The Holstein Cattle Association of Japan
3. Dairy Farming
1984, Dairy Japan Co., Ltd.
4. Manual of Dairy Herd Classification Techniques.
Hokkaido Holstein Association
5. Sire summary on the progeny test for Dairy Bulls in 1991 - II
Livestock Improvement Corporation

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Table 4-3-1 Relationship between Total Milk Yield and Final Scores in Cow as Registry of Production Record.

Final score of type evaluation		Less than 80 points	80 points or more and less than 85 points	85 points or more	Total or average	Average score
Registered with respect to 3 test records from the first-lactation production to third-lactation production.	Number of cows (percentage)	6,062 head (16.3%)	24,867 head (66.8%)	6,278 head (16.9%)	37,201 head (100%)	82.0 points General appearance 81.6 Dairy character 82.7 Body capacity 82.8 Mammary system 81.8
	Mean total milk yield (per one lact.)	20,126 kg (6,709)	21,710 kg (7,237)	23,204 kg (7,734)	21,704 kg (7,235)	
Registered with respect to 5 test records from the first-lactation production to 5th-lactation production	Number of cows (percentage)	1,296 head (10.7%)	2,889 head (65.1%)	2,924 head (24.1%)	12,109 head (100%)	82.6 points General appearance 82.3 Dairy character 83.5 Body capacity 83.4 Mammary system 81.7
	Mean total milk yield (per one lact.)	35,544 kg (7,109)	37,556 kg (7,511)	39,957 kg (7,991)	37,921 kg (7,584)	
Registered with respect to 7 test records from the first-lactation production to 7th-lactation production	Number of cows (percentage)	221 head (6.7%)	2,134 head (64.3%)	962 head (29.0%)	3,317 head (100%)	83.1 points General appearance 82.8 Dairy character 84.1 Body capacity 83.8 Mammary system 82.0
	Mean total milk yield (per one lact.)	50,755 kg (7,251)	53,265 kg (7,609)	55,591 kg (7,941)	53,773 kg (7,681)	

(The Holstein Cattle Association of Japan)

- Note: 1) The data were derived from records of milk production tests registered and confirmed as of August 8, 1990.
2) Records of 3 milking frequencies were excluded, and results for 305 days or those corrected in terms of 305 days were used as records of milk production.
3) Best score was used as final score.

Table 4-3-2 Results of Type Evaluation at First Lact., Longevity and Continuous Calving Ability

Type characteristics	Herdlife (days)	Lifetime milk yield (kg)
Strong suspensory ligament of the udder	+ 57	+ 315
Rear udder attached highly and widely	+ 42	+1,048
Teats of ideal size and position	+ 32	+ 567
Weakened suspensory ligament of udder	- 58	-1,303
Rear udders loosely attached	- 60	- 942
Rear udder too large	- 26	- 942
Crampy legs	- 96	-1,508

Table 4-3-3 Heritability of Major Traits of Dairy Cows

Traits	Heritability
Lactation characteristics	
Milk yield	0.2 - 0.4
Butterfat percent	0.5 - 0.6
Non-fat solids percent	0.4 - 0.6
Protein percent	0.4 - 0.7
Total solids	0.4 - 0.6
Milking rate	0.5 - 0.6
Reproduction characteristics	
Frequency of mating required for conception	0.1 or lower
Calving interval	0.1 or lower
Type traits	
Height	0.6 - 0.7
Chest girth	0.3 - 0.5
Body weight	0.3 - 0.5
Final score	About 0.3
Length of teat	0.9 or higher
Thickness of teat	About 0.4
Space between teats	About 0.5

Table 4-3-4 Requirements for Good Dairy Cows

1. High milk production

Good cows should produce a lot of milk with a well-balanced milk composition, and show not only a high milk yield during one lactation period but also good production over a lifetime.

2. Good udder

Good cows should have an udder and teats suitable for machine milking, show good milking rate, and be resistant to mastitis.

3. High reproduction ability

Good cows should have normal reproductive functions and require few efforts to conceive, resulting in successive calvings.

4. Sufficient strength

Good cows should have a strong appetite, eat their feed, have characteristics resulting in efficient conversion of ingested feed into milk, and have strong digestive organs as well as a strong back, loins, legs and feet which support the digestive organs.

5. Gentle disposition

Good cows should be gentle and easy to raise.

Table 4-3-5 Standard Growth Values for Holstein Cows

Months	Body weight		Withers height		Hip width		Rump length	
	Mean value	Normal growth range	Mean value	Normal growth range	Mean value	Normal growth range	Mean value	Normal growth range
At birth	41.5kg	31.5- 51.5kg	75.7cm	70.7- 80.7cm	17.3cm	14.9-19.7 cm	23.1cm	20.9-25.3 cm
1	62.5	49.2- 75.8	81.3	76.3- 86.3	19.9	17.3-22.5	25.8	23.4-28.2
2	85.7	67.8-103.6	86.9	81.6- 92.2	22.5	19.6-25.4	28.3	25.7-30.9
3	113.7	90.2-137.2	92.9	87.5- 98.3	25.3	22.1-28.5	30.9	28.1-33.7
4	140.8	112.3-169.3	98.2	92.7-103.7	27.9	24.5-31.3	33.2	30.2-36.2
5	167.3	134.1-200.5	103.0	97.5-108.5	30.3	26.7-33.9	35.3	32.2-38.4
6	192.9	155.3-230.5	107.1	101.6-112.6	32.5	28.8-36.2	37.2	34.0-40.4
8	241.7	195.3-288.1	114.0	108.4-119.6	36.3	32.3-40.3	40.4	37.0-43.8
10	287.1	232.9-341.3	119.3	113.7-124.9	39.5	35.3-43.7	43.0	39.4-46.6
12	329.1	268.1-390.1	123.4	117.8-129.0	42.1	37.7-46.5	45.2	41.4-49.0
14	367.5	301.1-433.9	126.6	120.9-132.3	44.4	39.9-48.9	46.9	43.1-50.7
16	402.5	331.0-474.0	129.1	123.4-134.8	46.3	41.8-50.8	48.3	44.4-52.2
18	343.0	358.3-509.7	131.1	125.4-136.8	47.9	43.4-52.4	49.4	45.5-53.3
24	609.4	422.2-596.6	135.5	129.6-141.4	51.6	47.1-56.1	51.8	47.8-55.8
30	559.9	365.9-653.9	138.9	132.9-144.9	54.2	49.7-58.7	53.4	49.4-57.4
36	591.7	495.1-688.3	141.3	135.2-147.4	56.1	51.6-60.6	54.5	50.5-58.5
48	627.6	527.2-728.0	141.4	135.3-147.5	58.0	53.5-62.5	55.2	51.2-59.2
60	661.1	557.5-764.7	142.6	136.4-148.8	59.6	55.1-64.1	56.2	52.2-60.2

(The Holstein Cattle Association of Japan)

Table 4-3-6 Scale of Points for Holstein Cows

Parts for evaluation and its description	Score
1. General Appearance Attractive style and carriage with a harmonious blendings of all parts, revealing vigorousness, refinement, femininity, with gentle temperament and light and sound walk.	30
(1) Breed characteristics Coat color; distinct black and white note: [animals with solid black, solid white, intermingled with black and white are ineligible for Herd Book Registry] Size; a standard mature cow in milk should weigh about 670 kg. Head; moderate in length, clean-cut, broad muzzle with large, open nostrils, clear and strong jaws, large bright eyes with thin eyelids and gentle impression, forehead between eyes is broad and moderately dished, bridge of nose straight, ears medium size, desirable form, good quality and alertly carried. Horns; medium in length, pointing forward and moderately curved inward, horn base with good quality, rather fine, with dense structure.	(8)
(2) Shoulder, Chine and Loin Shoulder; moderately length, set desirably against body, connecting smoothly chest and withers, tightly connected middle part of body. Chine; strong, long and appearing straight with vertebrae well defined. Loin; broad, long, strong, and nearly level.	(5)
(3) Rump long, wide and voluminous, top-line nearly level from loin through tail head. Hips; wide, nearly level with chine and loin being not coarse but moderately defined. Thuris; wide apart. Pin Bones; wide apart and slightly lower than hips, well defined, rump level and wide. Tail head; slightly above and well shapely set between pin bones Tail; long and tapering end with well balanced and thick switch.	(8)
(4) Legs and Feet Length being well proportioned with the depth of body, wide apart, squarely set, clean-cut and strong. Fore Legs; straight Hind Legs; nearly perpendicular from pastern top up to hock, wide apart with rear view and nearly straight, tube bone being flat and tight, tendons well defined, pasterns being moderately long, strong, elastic, hocks clearly defined and well shaped. Feet; desirable shape and quality with deep heel and level sole.	(9)
2. Dairy Character Giving an impression of vigorousness, angularity with a stretch as a whole, with moderate fleshiness corresponding to stage of lactation, free from coarseness, with indication of a high conversion efficiency of food into milk.	20
(1) Neck; rather longer and leaner, blending smoothly into shoulders and chest, clear-cut throat dewlap. Withers; well defined and moderately wedge-shaped appearance combined upper edge of shoulder blades with dorsal processes of the vertebrae slightly mounting on it. Ribs; wide apart among bones, rib bone broad, flat and long. Flanks; deeply arched and well defined. Thighs; outside surface being flat and moderately rounded out and its rear view being wide apart, slightly curving inward well cut-up between thighs, providing sufficient room for the udder and its attachment.	(13)
(2) Skin and Hair stretchy, elastic skin and fine and glossy hair bristles.	(7)

3. Body Capacity Having body capacity corresponding to size, robust constitution, full of vigorousness, appearing ample digestive capacity.	20
(1) Fore Ribs, Chest full crops, with well sprung deep and long fore ribs, blending into the shoulders, wide chest floor between front legs and fullness at the crops and the point of elbow.	(8)
(2) Barrel strongly supported and deep, with ribs highly and widely sprung, depth and width of barrel tending to increase toward rear.	(12)
4. Mammary System A well developed, strongly attached, capacious udder of fine texture, indicating heavy production and a long period of usefulness.	30
(1) Udder	(27)
(1) Texture soft, pliable, elastic, and well collapsed after milking.	[8]
(2) Capacity and Shape long, wide and of moderate depth extending well forward, strongly attached, reasonably level floor. rear attachment, high and wide. quarters evenly balanced and symmetrical.	[14]
(3) Teats uniform in convenient length and diameter for milking, cylindrical in shape, appropriately apart and plumb down.	[5]
(2) Mammary Veins long, thick, prominent, tortuous and branching and enters numerous large wells. veins on udder being numerous, reticulated and clearly defined.	(3)
Total	100

Table 4-3-7 Supplements for Scale of Points for Holstein Cows

General appearance (30 points)

A rough evaluation of a cow is judged from her appearance.

Attractive style: This does not mean a delicate and frail figure but a gentle and beautiful figure indicating a strong constitution.

Harmonious blendings: This indicates balance between the anterior, middle and posterior abdomen (head, neck, abdomen and rump), between the abdomen and the legs, or between the abdomen and mammary organs. A cow is not considered to be well-balanced if the head is large, the neck is short, or the legs are too long.

Refinement: Refinement is seen in the head and neck.

Light and sound walk: This means a gait with great composure. In other words does not sway on the hocks, walk with dragging toes or take a outward posture.

Dairy character (20 points)

These indicate the physiological function of converting feed to milk and features expressing this function.

Vigorousness, angularity with a stretch as a whole: Good quality and strength are judged generally from the vividness, angular shape and stretchability.

Moderate fleshiness corresponding to stage: This means the body condition corresponding to stage of lactation.

Body capacity (20 points)

Body capacity includes the fore ribs, chest and barrel.

Corresponding to size: This means that the cow has sufficient body length and chest girth (chest depth and chest width) in proportion to the withers height. The following cows are not desirable: those having a large volume but lacking general balance; and those having too small or too large a volume.

Mammary system (30 points)

These are most important for dairy cows, and determine their value.

Strongly attached: Good or bad attachment has a marked influence upon reproduction and lifetime milk yield.

Heavy production and a long period of usefulness: The overall value of dairy cows is determined by their lifetime milk yield. Strong system organs are thus necessary

Size: Body weight is the basis. Withers height and chest girth in proportion to the body weight are as follows.

Sex \ Item	Body weight		Withers height	
	Base	Range	Base	Range
Female	670 kg	570 - 780kg		137 cm - 150 cm

i. Cows within this range do not receive a cut in points, or may have a small cut in points if they do. Cows outside the range are evaluated according to the degree of deviation.

ii. Wide ranges for these items are favorable because such ranges may lead to the production of cows suitable for various environments.

Neck: This area directly reflects the whole body. It expresses characteristics of the breed, balance quality and strength.

Length (of shoulder): This indicates the length of the shoulder blade.

Hip bone: It is necessary that the top-line is virtually horizontal, but the rump itself does not need to be flat. A slightly lower rump showing a wide thurl and pin bone is important for reproduction.

Buttock: These indicate the periphery of point of pin bone (rear view).

Balance between length (of legs and hooves) and body depth: Standards for balance in chest depth and leg length are 55 % and 45 % of withers height, respectively.

Fore and hind legs: Cows need to have firm muscles and tendons, and strong legs (related to firmness of the body and quality).

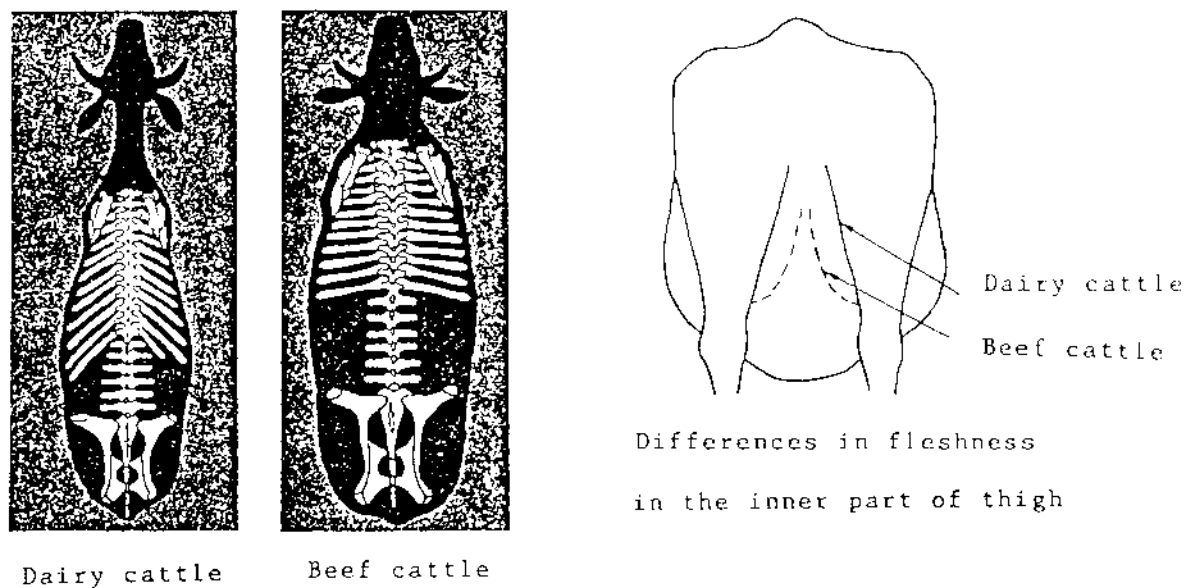
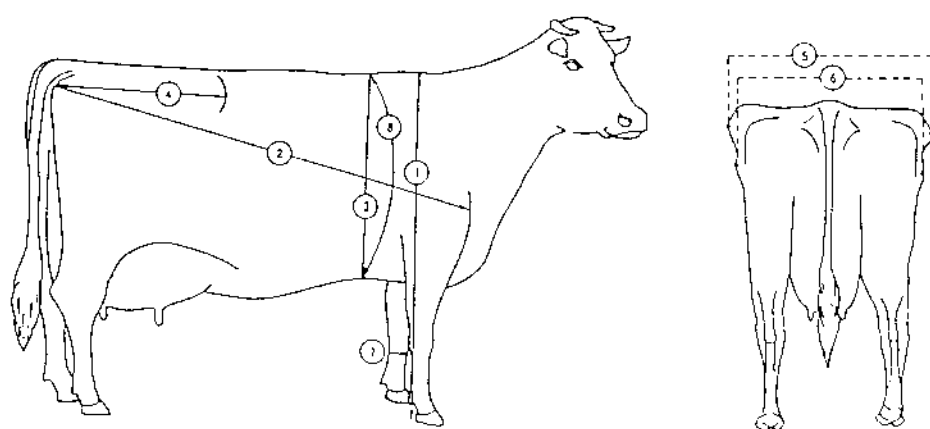


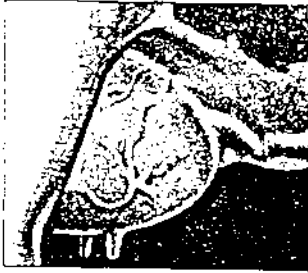
Figure 4-3-1 Differences in Characteristics of Dairy Cattle and Beef Cattle



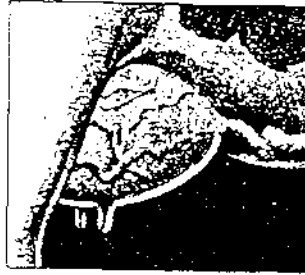
1. Withers height: From withers to ground.
2. Body length: Line from shoulder to buttocks (to pin bone).
3. Chest depth: Distance between withers to lower part of chest.
4. Rump length: Line from foremost point of hip bone to pin bone.
5. Hip width: Greatest width between right and left outermost points of hip bone.
6. Thurl width: Greatest width between right and left thurl hip joints.
7. Cannon girth: Girth of thinnest part of fore shank.
8. Chest girth: Girth of abdomen passing the 8th rib at the crops

Figure 4-3-2 Body Measurements of Cows

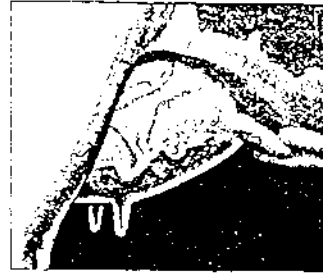
Attachment of fore udder



Extremely weak
attachment of fore
udder



Moderate attachment
of fore udder



Smooth and strong
attachment of fore udder

Width of rear udder



Rear udder with
narrow posterior part

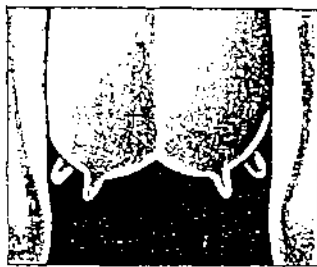


Rear udder
showing broadness

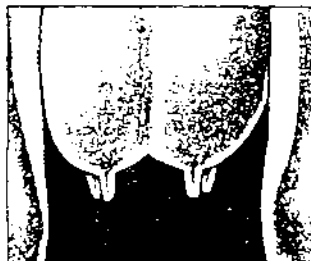


Rear udder having
intermediate broadness

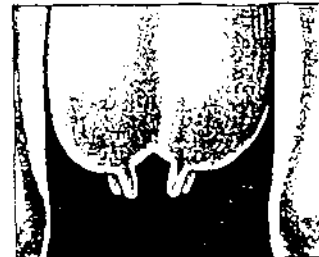
Rear view of teat placement



Teats part right and left
and point outward extremely



Teats hang down properly
from the center of udder



Right and left teats are
extremely close to each other
and attached to the inside of
the udder

Figure 4-3-3 Attachment and Shape of Udder



Pin bone is clearly higher than the hip bone.



Pin bone is slightly lower than the hip bone.



Pin bone is markedly lower than the hip bone.

Figure 4-3-4 Shape of the Rump



Straight hocks



Hocks of a intermediate angle



Hocks extremely sickled and sloped

Figure 4-3-5 Rear view of hind legs

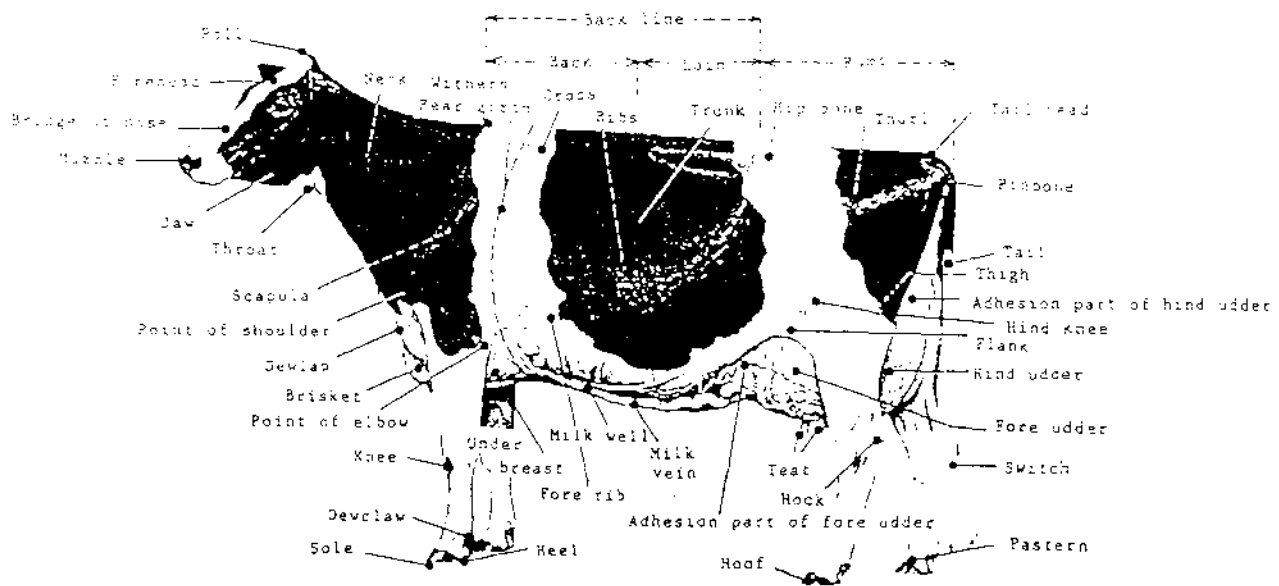


Figure 4-3-6 Parts of a Holstein Cow

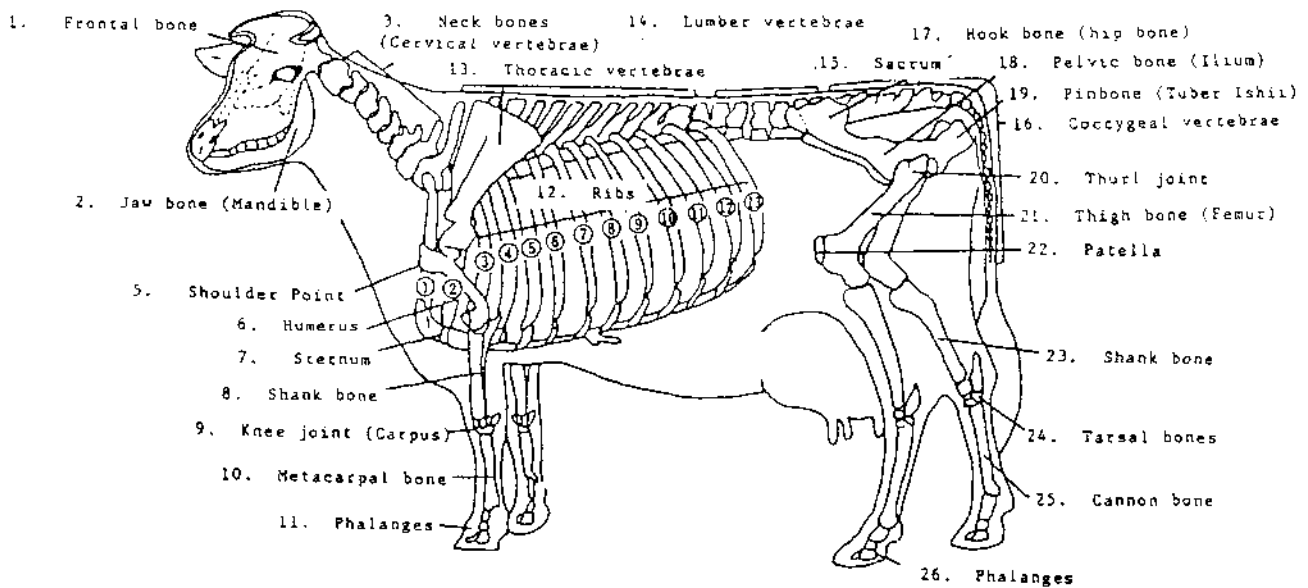


Figure 4-3-7 Skeleton of a dairy cow

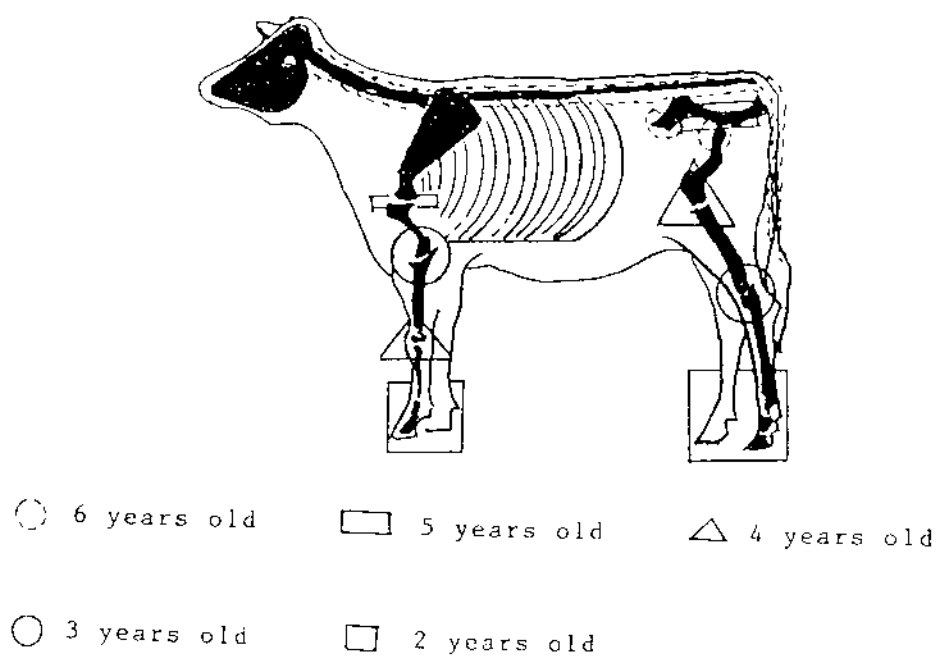
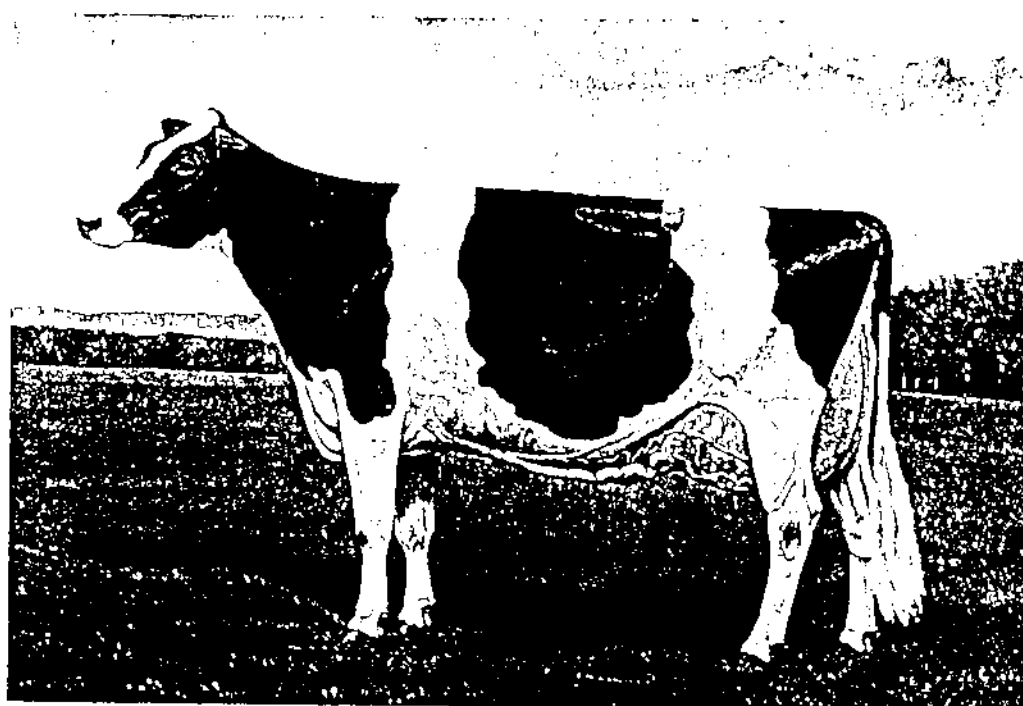


Figure 4-3-8 Skeletal Growth Layer of Epiphysis According to Age



(The Holstein Cattle Association of Japan)

Figure 4-3-9 Ideal type of Holstein Cow

○Stature

Evaluated by withers height
1 cm — 2

- 45 Extremely taller
- 35 Taller
- 25 Intermediate
- 15 Short
- 5 Extremely short

○Strength



1-5



25



45-50

- 45 Extremely strong and wide
- 35 Strong
- 25 Intermediate
- 15 Narrow and frail
- 5 Extremely narrow and frail

○Body depth



1-5



25



45-50

- 45 Extremely deep
- 35 Deep
- 25 Intermediate
- 15 Shallow
- 5 Extremely shallow

○Angularity



1-5



25



45-50

- 45 Extremely angular and prominent flat bone
- 35 Somewhat angular with clear flat bones
- 25 Intermediate
- 15 Lacks angularity to some extent
- 5 Thick and coarse

○Rump angle



1-5



25



45-50

- 45 Extremely lower pins than hooks
- 35 Somewhat lower pins
- 25 Slightly low pins
- 15 Somewhat higher
- 5 Extremely higher pins

○ Rump length



1-5



25



45-50

- 45 Extremely long
- 35 Long
- 25 Intermediate
- 15 Short
- 5 Extremely short

○ Rump width



1-5



25



45-50

- 45 Extremely wide
- 35 Wide
- 25 Intermediate
- 15 Narrow
- 5 Extremely narrow

○ Rear legs (side view)



1-5



25



45-50

- 45 Extremely sickled
- 35 Somewhat sickled
- 25 Intermediate set in hock
- 15 Posty leg to some extent
- 5 Posty and straight

○ Foot angle



1-5



25



45-50

- 45 Extremely steep angle
- 35 Somewhat steep angle
- 25 Intermediate
- 15 Low angle
- 5 Extremely low angle

○ Fore udder attachment



1-5



25



45-50

- 45 Extremely strong
- 35 Strong
- 25 Intermediate
- 15 Loose
- 5 Extremely loose

○ Rear udder height



1-5



25



45-50

- 45 Extremely high
- 35 High
- 25 Intermediate
- 15 Low
- 5 Extremely low

○ Rear udder width



1-5



25



45-50

- 45 Extremely wide
- 35 Wide
- 25 Intermediate
- 15 Narrow
- 5 Extremely narrow

○ Udder support



1-5



25



45-50

- 45 Extremely strong
- 35 Strong
- 25 Intermediate
- 15 Weakened
- 5 Extremely weakened

○ Udder depth



1-5



25



45-50

- 45 Extreme height of udder floor above hock
- 35 Udder floor above hocks
- 25 Somewhat higher udder floor than the hock
- 15 Somewhat low udder floor
- 5 Extremely low udder floor

○ Placement of teats (rear view)



1-5



25



45-50

- 45 Extremely close, having base of teats on inside quarter
- 35 Placed on somewhat inside quarter
- 25 Centrally placed on quarter
- 15 Placed on somewhat outside quarter
- 5 Extremely wide placed on outside of quarter

Figure 4-3-10 The illustration of linear type traits

4. Outline of Dairy Cattle Registry System in Japan

4-1 Introduction

(1) Definition and purposes of registration

Webster's New International Dictionary defines 'register' as 'to record formally and exactly, as for future use or service'. The word is commonly used in other situations, however, such as in 'cash register', 'registration of professional baseball player' and 'registered trademark'. It has a variety of meanings from expressing merely the formal concept of recording to making distinctions, or ensuring certain benefits or rights by recording a matter in a prescribed way.

The purposes of registration of livestock is to record, arrange and officially certify data for individual animals, including pedigree, milk production, type conformation, breeding records and the identity of the owner and so on, so that the data can be used effectively in the improvement of livestock. Consequently, the purpose of Breed Associations is to assist livestock breeders by storing and dispersing information needed for breeding and improvement, and to extensively promote public interest.

The basic method of dairy cattle and Herd Book Registry (H.B.Registry) has hardly changed during its century-long history. With the rapid development of dairy farming, however, the role of registraion in improving dairy cattle has changed remarkably. The burden on Breed Associations has grown heavier in recent years, in particular due to the development of Artificial Insemination, advanced data processing technology such as computers, increase in herd size and the sophistication of progeny testing and the like. At the same time, the public characteristics of the Breed Association has been enhanced tremendously.

Although Breed Associations have been, so to speak, holders and guardians of records of pure-bred cattle from the days when natural breeding and propagation were prevalent, they are now also expected

to play an additional role in supplying dairy farmers and related organizations with the information needed for improving dairy cattle. In this age when careful selection rather than propagation of cattle is prevalent and also male breeding stock are selected on a national basis as well as a more public basis.

(2) Origin of registration and modern registration

It is believed that modern livestock bloodline records were first used on a farm village in England in the 18th century, and that Breed Associations were established in Europe around the middle of the 19th century. It is believed that Robert Bakewell (1725-1795), an English farmer, established the foundations of modern livestock improvement. A century before Mendel's laws were re-discovered, Bakewell firmly believed that the characters of parents were transmitted to their offspring. Using this belief he succeeded in fixing excellent livestock characters by inbreeding. As breeding was performed in only certain districts in those days, even personal breeding records prepared by individual breeders could be used as information for mating since breeders knew the bulls used by fellow breeders and the reliability of records of dairy cattle purchased from these breeders.

However, with the increased demand for farm products, and the increased number of dairy cattle, cattle generations and breeders that occurred during the Industrial Revolution, it became difficult to trace the bloodline of fellow breeders' cattle using these records. The first registration body was established in the middle of the 19th century to prevent crossbred or mediocre cattle from being exported under the pretense of being pure-bred, as well as to offer the required information described above. The special importance of this export measure was proven by the fact that most dairy cattle Breed Associations were established at about the time when demand for overseas exports began to increase greatly.

The books in which Breed Associations record bloodlines are called

"herd books". This term is used for all livestock species throughout the world. The world's first herd book, for a thoroughbred horse, was published in 1791 in England. Subsequently, herd books for Shorthorn Cattle were published in England in 1822.

The following Breed Associations can be listed as earlier publishers of herd books on dairy cattle registration.

- The Ayrshire Breeders' Association of America (1863)
- The American Jersey Cattle Club (1868)
- The Association of breeders of Thoroughbred Holstein Cattle (1871)
- The Netherlands Herdbook Association (1874)

In Japan, the first herd book of Holstein cattle was published in 1913.

It has long been believed that the role of Breed Associations is to simply record progenitors, that is, the bloodlines of individual animals. This was adequate in the days when natural breeding was predominant, but it is not adequate today.

Useful information for improving dairy cattle has increased both in quantity and in quality as a result of the rapid development of breeding techniques such as Artificial Insemination and Embryo Transfer, advanced breeding theories such as estimations of breeding values, and data processing techniques by computers or statistics. Under these circumstances, it has become possible for Breed Associations to offer this useful information.

In addition to the conventional fundamental roles of operation of H.B.Registry and the like, it is now necessary for Breed Associations to supply dairy farmers wanting more productive cattle with the wide-ranging information needed for selection and breeding. Moreover, they are expected to feed information linked with bloodline records back to fellow organizations. For this purpose, they need to establish closer relationships with the organizations

that control Artificial Insemination, milk production testing or progeny testing, from which scores for genetic evaluation of male breeding stock and cow indices of female animals, among other information, are obtained, in return for offering various information required for the production and selection of young sires.

Thus, the feedback of information is the most important role of today's Breed Associations. It is strongly desirable that the information for improvement be systematized, made more sophisticated and supplied without delay.

(3) Relationship between improvement and registration of dairy cattle

Dairy cattle breeding may be defined as the selection of the parents of the next generation. The central principle is that offspring will be more like their parents than unrelated individuals and that if one selects desirable animals as parents, more desirable offspring will result than with no selection. However, in case of livestock (with conventional sexual reproduction), the offspring receive only a random half of the alleles in chromosomes of each parent, and therefore, the results are not completely predictable, especially with traits of low heritability. Moreover, genes may be partly recombined within each chromosome during germ cell formation, and quantitative characters may be controlled by the environment, which makes the above estimation more difficult.

For these reasons, the modern Breed Association is in a position of supplying the dairy cattle breeders with data or information needed to breed as accurately as possible, by preparing such data as animal identification, type and production information and others connected with pedigrees (genealogical chart). To select cattle for breeding as accurately as possible, it is essential that dairy farmers and relevant institutions be supplied with information concerning important characters that have been confirmed for given individuals. As shown in Fig. 4-4-1, such information can be obtained from four basic information sources. Pedigrees, milking

ability and external appearance have been recognized as the three main requirements for selecting individual cattle for breeding since early times. It is important to note that pedigrees are an information source, while milking ability and external appearance are characters.

The relationship between the registration and improvement of dairy cattle is summarized in Fig. 4-4-2.

(4) Kinds of registration and their importance

(4-1) Kinds of registration

The foundation of registration is the H.B. Registry. Pedigree of H.B. registry are records of blood relationships with progenitors (parents, grandparents and the like). Animal identification is a prerequisite for assuring accurate records. Therefore, H.B. registry contain markings for individual identification (color markings in the case of Holstein), in addition to data such as date of birth, bloodline (parents and other progenitors), breeder and owner and the like. Also, when cattle are traded, transfer certifying are necessary.

By transfer certifying, when the owner of Herd Book registered cattle changed by sales and purchases, the date of change (the date of transfer) are recorded on the herd book and registry certificate so that the whereabouts of Herd Book registered cattle is clear. In short, it provides additional information of H.B. Registry.

For all dairy cattle, milk yield in a given period (10 months or one year) and milk fat content are important characteristics. Therefore these values are officially measured (hereinafter referred to as "production testing") and the results are registered in the H.B. Registry. This type of registration based on production records is called Registry of Production

Record or Advanced Registry.

Furthermore, dairy cattle are evaluated externally in terms of functionality, durability, growth and other features. The records obtained in such a way are also commonly registered in linking with pedigree. This is called Registry of Type Evaluation.

Most of the registration systems that have been introduced around the world can be classified as one of the three main types mentioned above. In addition, most Breed Association in the world utilizes recognition programs in which cattle with excellent registration records are designated and recommended. These systems supply additional information by designating and announcing the identities of excellent cattle with markedly superior records of milking ability or type traits, or have good offspring records. In this way, the systems assist the spread and promotion of registration. In a broader sense, these systems can be called a kind of registration.

(4-2) Importance of registration

① Registration concerning ancestral lineage

i . H.B. Registry is the foundation of registration. Cattle are individually confirmed by H.B. Registry. Consequently, production testing and type evaluation (hereinafter referred to as "type classification") can be conducted only on cattle who are recorded in the herd book of a Breed Association.

ii . H.B. Registry is useful in ranking cattle for trade purposes because a certificate of H.B. Registry always contains details about color markings, the producer (breeder) and date of birth, by which the production records and type classification records of progenitors

of the cattle concerned can also be confirmed. It is especially helpful in estimating the potential of a heifer since her milk producing ability cannot be estimated reasonably without information about the progenitors, until she produces offspring and thereby displays her milk producing ability.

iii . H.B.Registry can be said to be a duty of dairy cattle breeders. As the dairy cattle improvement systems have been developed and made more sophisticated recently, more jointly and more collective information utilization have become necessary to make the most of them. Under these circumstances, it is necessary to gather and analyze records of as many cows as possible to obtain so-called proven sires and to determine the genetic potential of each cow. The ultimate goal of registration is to produce valuable cattle with high utility worth (breeding values). This approach leads to preparing high exchange values. This added values can compensate for registration fees. These expenses are usually offset by introducing registered cattle and using the semen of progeny tested proven sires. It naturally seems unfair when farmers do not strictly perform registration and only use what they produces. Some countries stop the supply of semen from progeny tested proven sires so dairy farmers who do not carry out registration strictly.

iv . A certificate of H.B. Registry is useful in avoiding inbreeding. Mating between near relatives such as between parents and offspring, between siblings, between grandparents and grandchildren, between uncles and nieces, may decrease potential, fertility, general vitality, body dimensions or other aspects of physique.

This phenomenon is called inbreeding depression. Furthermore, inbreeding increases the incidence of

defective genetic characters. In particular, unplanned and involuntary inbreeding, which only has demerits, is highly risky. Such risks can be avoided if there are certificates of H.B. Registry.

- v . In these days when advanced Artificial Insemination is available it is necessary to carefully select dams for producing young sires by using the largest possible gene resources and ensuring pedigree identified sample daughters for progeny tests which are evenly distributed in various environments. In addition, it may be necessary to prepare many cow herds with clear bloodline relationships in order to effectively apply a recently developed statistical method called the BLUP method (animal model).

With the development of embryo manipulation techniques such as Embryo Transfer, in vitro fertilization and nuclear transfer, it has become necessary for stricter selection of cattle offering embryos, ova and nuclei. Equally, the selection of sires for obtaining embryos has become more important. Thus the importance of H.B. Registry is still increasing. The rapid development of genetic engineering may allow the dream of being able to apply techniques such as gene transfer to dairy cattle to be realized. At present, however, there are practical difficulties in performing artificial gene recombination. Moreover, even if excellent individuals are produced by gene manipulation, it would be impossible to rapidly increase the numbers of such cattle. There is therefore no alternative but to carry out breeding by controlling natural gene recombination as deliberately as possible on the basis of pedigree information.

② Registration concerning milk production records

- i . Production testing is an indispensable means of realizing rational management of seeding and selecting individual cattle for breeding which have inherited excellent milking potential. Many of the dairy cattle Breed Associations in the world no longer undertake production testing, this having been taken over, in general, by other bodies, but instead they accept production testing records and register them, provided specific conditions are met. Why is it necessary for an application to be filed at a Breed Association for registration of an official milk production record? In order to utilize the tested record for breeding and improvement, it is necessary to store the record permanently in connection with the H.B. registry of cows which have been individually identified. If the records are not registered with a Breed Association in the form of a Milk Production Registry, it would be difficult to link the records with their ancestor's records, and the records may become extinct after the death or trade of that generation.

On the other hand, since a registered record is contained in and verified by certificates of H.B. Registry and extended pedigrees of progeny, as mentioned later, it can be used to evaluate and select cattle both for trade or private breeding.

- ii . Milk Production Registry provides data for genetic evaluation (e.g., cow indices) combined with pedigree information of each individual or a whole herd, and can be used in sales publicity.
- iii . Registered production records are collected and stored at Breed Association together with other data from

dairy farmers. These records are used in evaluating breeding stock of males and females and also in preparing certificates of extended pedigrees.

③ Registration concerning type classification records

Type is defined as the shape and structure of a dairy cattle's body inasmuch as it effects milk producing ability. To improve type, the majority of the more than 30 Holstein cattle Breed Associations around the world operate type classification programs by specialist classifiers. But why is improvement in type important in improving dairy cattle, and why are type classification systems necessary for type improvement?

- i . Sound type conformation are indispensable if milk producing ability is to be fully displayed. Of course, the most important character for dairy cattle is the capacity to produce milk. In order to fully display such an important ability, it is also necessary to simultaneously improve type traits, for example, udder attachment and legs and feet, which both enhance milk production potential, and characters helpful in handling the cows, such as disposition and milking speed. Type is to production what structure is to function. These characters can be improved by selection because they are inherited (heritability: 0.2 - 0.4), though they are affected by the environment. Although some genes governing these characters may determine both type and production characters (for example, growth hormone simultaneously maintains milk production and body growth), it is believed that in many cases these characters are governed by different genes on the same chromosome, or by different chromosomes. This is the reason why both characters need to be improved by selection.

The greater the improvement in the potential to produce milk, the more important improvement in type traits becomes. Moreover, the importance is increasing more and more as feeding management is mechanized and herd management is developed and becomes larger-scale. This is the same process as took place when the rice plant was improved: the more improved the rice plant, the more important stalk strength became. Under these circumstances, recent type classification methods are expected to become more objective and sophisticated.

- ii . Improvement in type traits is important for improving the life span of cattle and creating trouble-free herds.

It is important to lengthen the productive life of each cow and maintain a highly productive herd that has fewer troubles in order to increase milk yield per day of life and to reduce the production cost of milk. A longer life generally reduces the depreciation costs of cattle, increases the proportion of cows in peak productive age of milk secretion, and increases the average potential of a herd, though these advantages may be affected by beef prices and genetic trends. The proportion of cows in milk also increases. In addition, the rate and accuracy of selection increases because more calves can be obtained. From a global point of view, this can contribute to a reduction in feed resources. In this way, the lengthened life span of cattle brought about by improvements in type traits is also helpful in reducing milk production costs, the most important priority today.

- iii . Type classification is indispensable for effective type trait improvement. Originally, the goal of type classification was to obtain the most objective evaluation records with the least possible

inconsistency in order to improve type traits. To achieve this, type classification was carried out by the least possible number of qualified or authorized. These sort of type classification systems were established in the days of natural mating, when the dairy cattle populations to be evaluated were still small in size. However, in recent circumstances, where information relating to improvement is extraordinarily increased in the sense of diversified and collective use, type classification methods have needed to become more sophisticated. To meet this need, the descriptive type classification method was devised in the 1960s in countries with developed dairy farming. Following that, the linear type classification method, which is now spreading throughout the world, was created in the 1980s. In this method, linear (quantitative) evaluation of economically important characters, each of which is deemed to be a single trait biologically, is performed in addition to conventional relative evaluations based on the true-type method (a scoring method with a maximum score of 100), in order to analyze type trait characteristics at a single character level, for performing so-called trait selection and corrective mating. The results are fed back to dairy farmers after ranking relative to the herd, the district population and the national population.

In concert with this, proven sires selected by progeny testing are also evaluated and analyzed in detail in terms of inherited type traits through linear type classification of daughter cows. In this way, abundant information about type traits of the bulls are supplied.

- iv. To effectively improve type traits, it is not enough to perform type classification on an individual animal basis only. All cattle constituting a herd, or at least

all cattle used for breeding in a herd, must be evaluated. Moreover, overall information of the herd is needed to improve individual animals. For these reasons, dairy herd type classification is carried out on all cows in a herd.

Dairy herd type classification provides valuable information about each individual cow, revealing their ranking in the herd and the merits and demerits of their type traits relative to the whole herd. Another important aspect is that dairy farmers can know the ranking of their herds in the district or the national cattle population in a country, through participating in the nationwide classification.

Dairy herd type classification is also helpful in gathering type classification records without bias caused by selection. These records can be used to produce male breeding stock with excellent inherited traits. These merits are finally fed back to each dairy farmer. Today's dairy cattle improvement should be carried out under the concept of herd improvement and corrective mating, with the object of improving general potentiality combining type and production. To be more specific, breeding should be carried out with cows which are rationally selected according to not only milking potential but other important characters such as type traits, and with bulls which can enhance the merit of and correct the faults of the cow that will be mated, taking this into consideration to avoid inbreeding.

4-2 Registration systems for Holstein Cattle

(1) Holstein cattle in Japan

Today, most (99.8%) of the dairy cattle in Japan are pure-bred Holstein, or highly upgraded Holstein cattle which clearly display Holstein characteristics. The remainder consist of approximately 6,700 head of Jersey cattle and an extremely small number of other breeds.

There are several reasons why Holstein cattle account for this vast majority. In short, Holstein cattle have been recognized in Japan as a highly milk-productive breed; it is believed that a certain volume of milk can be obtained with a smaller number of Holstein cattle than with other breeds. Japan's dairy farming began in the suburbs of cities when each farmer maintained only one or two cows. This situation continued until dairy farming developed fully after World War II. In addition, at that time milk demand in Japan was mainly for drinking. Consequently, greater importance was placed on quantity rather than quality of milk. Although dairy farmers and traders were encouraged to breed Ayrshire and Jersey cattle in addition to Holstein cattle, Holstein gradually gained popularity for the above reasons and other breeds with a few exceptions, disappeared.

Most of the ancestors of Holstein cattle in Japan were imported from America and Holland. Herd Book registered Holstein cattle were first imported in 1889, with 20 coming from American and 10 coming from Holland. Although some vestiges of information about earlier imports than from these two countries can be found, the details are no longer known. It was from 1907 onwards that relatively larger numbers of cattle were imported. Subsequently, Holstein cattle were imported from Canada for the first time in 1916.

Some cattle have been imported from England and New Zealand in recent years, but the greater part of them have been imported from

America and Canada.

Holstein cattle in Japan can be classified into American or Canadian type, both of which have been improved for milk producing ability, but not into European type, which were improved with importance being placed on beef productivity, since Holstein cattle in Japan were improved with the underlying idea that beef productivity is an indirect result of improvement rather than a direct goal. It is well known, however, that all over the world, including in European countries, Holstein are rapidly approaching the American or Canadian type.

The level of milk producing ability of Holstein cattle in Japan can be estimated from a milk production registry (the, Registry of Production Record), overall records of DHI testing, relating raw milk production to numbers of cows in milk, as shown in Tables 4-4-1, 4-4-2 and 4-4-3.

The numbers of imported registered cattle and H.B. registry cattle produced from imported semen are in recent years shown by country and year in Tables 4-4-4 and 4-4-5.

(2) Organization of the Association

The Holstein Cattle Association of Japan (hereinafter referred to as the Association) is a public corporation with a membership system organized under Article 34 of the Civil Law and authorized by the Ministry of Agriculture, Forestry and Fisheries on August 1, 1948. It operates registration of Holstein cattle throughout the country under the approval of the Central Government.

The membership consists of regular members: the owners or lessees who breed Holstein and High Grade Holstein cattle, honorary members and assistant members (individuals or group members). The object of the Association is to assist cattle improvement and promote the mutual profitability of the members by carrying out registration and

other related matters.

The organization of the Association is shown in the upper part of Fig. 4-4-3.

Annual Conventions are Classified into Regular and Extraordinary ones. Regular Annual Conventions are held in June, and Extraordinary Conventions are held when necessary. In Annual Conventions, decisions are made on various subjects including annual plan of operation, budgets, settlement of accounts, revision of the Constitution of the Association, and the election or release of officers.

The Meetings of Delegates, consisting of 60 Delegates (for a three-year term) is elected from among the membership, and is a decision-making organ which agrees on which subjects should be dealt with in the Annual Convention, since it is impossible for all 30,000 or so members in Japan to attend meetings.

The Board of Directors Meeting consisting of 19 directors (three-year term) is elected at the Annual Convention from among the membership, and is an overall executive organ which conducts the affairs of the Association. Three or four Board of Directors Meetings are held each year to deliberate on subjects relating to the alteration of registration provisions, and the service and salaries of the staff in addition to matters for discussion at the Annual Convention.

A Board of Auditors, consisting of one to three auditors (three-year term) elected by the membership at Annual Convention inspects the assets of the Association, the settlement of accounts and business performance. Auditors attend the Board of Directors Meetings and Annual Convention and express their opinions there.

To assist on the farm or local basis registration activity, a number of Authorized Agents are designated in each prefecture. The

Authorized Agents are asked to promote and guide registration, arrange applications for registration, and guide or supervise production testing. More than 4,000 Authorized Agents from among technical employees of prefectural offices, institutes of the prefectural farm livestock experimental, agricultural cooperatives, public offices of cities, towns and villages and dairy farming organizations, are entrusted with the actual duties of the Authorized Agents.

The Advisory Council is an advisory body to which the president entrusts deliberations relating to the registration and improvement of Holstein and High Grade Holstein cattle. The president entrusts the duties of the Advisory Council to people nominated from the staffs of the Ministry of Agriculture, Forestry and Fisheries, prefectural offices, prefectural institutes animal industry, universities and from among dairy farmers.

Authorized organizations, established in each prefecture, are entrusted with registration affairs so that registration procedures are carried out smoothly at the prefecture level and throughout the country.

(3) Office structure of the Association

The organization of the Executive Office of the Association is shown in the lower part of Fig. 4-4-3.

Although the Association is a national organization, registration matters such as H.B. registry and H.B. registry are carried out by only two offices: The Tokyo Headquarters Office, which is responsible for Honshu, Shikoku and Kyushu, and the Hokkaido Branch Office in Sapporo, which is responsible for Hokkaido. The reason for this division is that the geographical and historical background of dairy farming in Hokkaido differs a great deal from that in Honshu, Shikoku and Kyushu. Dairy farming in Hokkaido has been greatly improved because of its longer history, larger herd size and

higher proportion of full-time dairy farmers. These circumstances led to requests for the establishment of a separate Hokkaido Branch Office, and this was opened in 1949, a year following the establishment of the main office.

Though registration is independently carried out by two offices on a self-supporting basis, registration matters including H.B. Registry and type classification are carried out using the same standards throughout the country.

For these reasons, the organization of the executive office utilizes the Headquarters Office and Hokkaido Branch Office. They have almost the same organization, except that the Managing Director and Executive Secretary are stationed at the Headquarters Office, and a Branch Office Manager is stationed at the Hokkaido Branch Office. For your convenience, the number of staff and their responsibilities are outlined below for each section of the Headquarters Office.

a. General Affairs Section: 4 people

Management of personnel, documents, property, meetings and matters involving membership; welfare of staff; other matters not covered by other divisions or sections.

b. Accounting Section: 3 people

Budgets and accounts, receipts and expenses, receiving applications for registration.

c. H.B. Registry Section: 7 people

H.B. Registry; transfer of Herd Book registered cattle; renewal, re-issue and correction of certificates of H.B. Registry; and other general matters related to H.B. Registry.

d. Type Production Registry: 3 people

Registry of Production Record; Registry of Type Evaluation; Advanced Registry (Bulls); general matters related to registration of High Grade Holstein cattle; extended pedigrees;

and recognition and award of superior cattle.

e. Computer Processing Section: 13 people

Computer operations; preparation of certificates of registration; preparation and storage of original registration records; preparation of Herd Book.

f. Research Section: 2 people

Research into dairy cattle improvement; arrangement and storage of printed materials; seminars and Advisory Council.

g. Registry Promotion Section: 1 person

Planning and performing registration promotion; publishing of Association Bulletin and other books; general matters related to dissemination and publicity of registration.

h. Computerize Planning Section: 2 people

Planning and development of computer service.

i. Classification Department: 4 people

Task of type classification; and general matters related to type classification.

(4) Characteristics of Holstein Cattle registration system in Japan

Originally, Holstein Cattle registration system in Japan followed the examples of Western systems. Inevitably, however, a unique Japanese system has been developed because of the special circumstances of dairy farming in this country.

(4-1) Open registration system

The registration system of the Association consists of separate systems for purebred Holstein and High Grade Holstein cattle. Both systems include registration concerning ancestral lineage, type and the like.

The objective of the High Grade Holstein cattle registration system is to detect and register cows born to unregistered cattle, provided it is possible they can be a foundation for improvement. In this system, the daughter of High Grade Registry B can be entered into purebred H.B. Registry.

In this way, these two registration systems are connected through a narrow bridge. The Association's registration system can be called an open system inasmuch as the High Grade Holstein cattle can be transferred to H.B. Registry after the cattle have been bred with pure-bred Holstein bulls over successive generations. This open registration system is similar to the Holstein cattle registration systems in Canada and England.

Today, there are ever some animals that happened to lose their ancestral records by chance in spite of having nearly the same genetic constituents as purebreds in Japan. So, from the point of view of ensuring the genetic resource, the Association have operated a High Grade Holstein cattle registration system and an open registration system.

A typical example of a closed registration system is the Holstein cattle registration system in America. The system has been devised so that an application for H.B. Registry is accepted only if the cattle concerned were born from pure-bred parents which can be traced back to progenitor cattle imported from Holland. In America, however, Breed Associations dealing with dairy cattle breeds other than Holstein operate an open registration system. Worldwide, open systems largely predominate.

Generally speaking, in countries where one breed dominates the dairy industry, the open registration system offers a unique opportunity to select the best individuals from the whole population regardless of parentage, and draw them into the more

intensely developed recorded and observed part of the population. In the case of developing countries, however, they may need to maintain a relatively "closed" status to protect the income of registered breeders until the breeding of dairy cattle in the country concerned is at an advanced level.

(4-2) Selective registration system

There are two methods of H.B. Registry. In one method, all cattle born to registered animals are registered unless disqualified due to defective characters, coat color and color markings or other features, but afterwards may be selected by some means (for example, Registry of Production record). In the other method, H.B. Registry is accepted only when the cattle concerned or his parent cattle are recognized following investigations as having type and production record above a specific level. The latter method is intended to include a certain degree of selection at the stage of H.B. Registry. This is called selective registration.

In 1962, the Association adopted a selective registration system for bulls. In this system, an application for H.B. Registry is accepted only for bulls whose sire was registered as Advanced Registry or selected as a proven sire, and his dam met the definite requirements in terms of type and production records. Selective registration systems tend to be uncommon around the world, but selective registration systems for bulls is used in some countries, with certain requirements being attached to dams only. The Holstein-Friesian Association of Australia, for example, operates a system similar to Japan's so far as requirements of dams are concerned. Canada also had a system like those in Japan and Australia, but in Canada it was discontinued in 1991 for the reason that the criteria for registration became unrealistically low to meet the needs of the time, an investigation of overall extended pedigrees was required in evaluating bulls, and simpler procedures were

required for registration.

(4-3) Restrictions regarding coat color and color markings

The black-and-white spotted characteristic of Holstein cattle can be used for animal identification since different individuals have different patterns even in identical twins. Thus Holstein cattle Breed Associations around the world have long used the spots when certifying individuals. The character is genetically dominant to red-and-white spots and brown line coat color, though it is genetically recessive compared with uniform black. For this reason, the Breed Associations have established regulations on these black and color markings, recognizing the value of safeguarding the purity of breed and the breed trademark. Cattle have been registered only when they met the regulations.

From the end of the 1960s, however, there was a marked worldwide trend towards the opinion that regulations on coat color and color markings should maximize profits for dairy farmers by effectively improving type and production traits of cattle using as many genetic resources as possible. So from around 1970, the regulations on coat color and color markings were relaxed by steps in advanced dairy-farming countries such as the U.S.A., Canada and England. As a result, registration became possible even for conventionally unaccepted cattle with nonstandard black-and-white spots (off-color spots) or red-and-white spots.

In general, the regulations were eased the most as they apply to H.B. registry of cows, H.B. registry of bulls and registration of High Grade Holstein cows.

In the same vein, Japan's regulations on coat color and color markings were relaxed in 1978 for applications for H.B. registry of cows and for registration of High Grade Holstein cattle, and in 1991 for applications for H.B. registry of bulls.

As a result, it became possible to register conventionally unaccepted cows with off-color or red-and-white spots. In the case of application for H.B. registry of bulls and for the registration of High Grade Holstein cows, however, only red-and-white cattle were made acceptable. The regulations of coat color and color markings are summarized in Table 4-4-6 by sex and kind of registry.

As shown in Fig. 4-4-4, off-color is defined in different ways in different countries. The figure shows that Japanese regulations on coat color and color markings were rather loose in the past, though they have been revised to an international level today.

(4-4) Registration of milk production

In Japan, the first milk production testing was carried out in 1911 by the Dutch Cattle Association of Japan, established in 1911. At the same time, it began to register cattle which reached a specific total milking yield and milk fat content. Thereafter, this Association and its successor were leaders in the field of milk production testing in Japan. The Central Government and Local Governments sometimes also played an important role in supporting milk production testing by offering economic aid.

The milk production testing project was promoted largely by dairy farmers who recognized the necessity of production testing but its development was hindered by its cost and its lack of labor. In particular, it sharply declined during World War II and the subsequent period of confusion.

Although priority was placed on 7-day and 30-day testing over 305-day inspections at that time, the former two were discontinued in 1953. Although inspection of milk fat percentage was included in earlier projects, inspection of

other milk constituents were not conducted until recently. For example, inspection of solids-not-fat percentage was introduced as an option in DHI testing (mentioned later) in 1974, and inspection of the protein percentage in milk was adopted in the same way in 1987. Since 1974, substantial development in milk production testing has been brought about in this country.

As for the official milk production testing program, it has been operated by the Milk Recording Societies established under the sponsorship of central and local governments since 1974, though being undertaken by the Livestock Improvement Association, Inc. as far as data processing is concerned. We call it Dairy Herd Improvement Test which contains the supervision plan and the owner-sampler testing plan. This Association has accepted that the supervision plan is official, and has added some regulations to it. At the same time we have been running production testing program which is called Testing Method A and Testing Method B (applied to High Grade Holstein) on an individual animal basis since 1956, but little testing has been carried out in this category recently because it has been replaced by the abovesaid plan on a herd basis.

(4-5) Registration of type conformation

A type evaluation system was started in Japan in 1911 with the establishment of the Dutch Cattle Association of Japan: the remotest ancestor of this organization.

Since that time, type evaluation has been a significant part of the progressive breeders' breeding program in Japan. During the period, several changes in the program have occurred, but since its beginning the basic evaluation system has remained relatively unchanged from its conception in 1911 until recently.

Generally in Japan, dairy farming operation size had been limited to such an extent that the usual herd size was only one

or two head by the time when substantial development was brought after World War II . As a result, a type evaluation system has been developed in the pattern characteristic of this country. Namely, up to recently, type evaluation has been operated on an individual animal basis. We switched to herd basis type classification rather too late in 1985.

With reference to appraisal yardsticks, in the early times either Dutch style score cards (scale of points) or American style score cards similar to American form were used for a long period.

The present cow and bull score cards were adopted in 1965. Some amendment of score cards in 1978 and 1985 relates chiefly to wording revision. The four major score card areas and its relative emphasis in the case of cows are shown in Table 4-4-7.

With regard to appraisal techniques, in 1976 we introduced the Descriptive Type Classification System which was designed to describe 14 specific functional type traits.

Further, we have adopted a Linear Type Classification System on April 1, 1986 after a two-year field trial. In this method, a typeappraiser (Classifier) evaluates each trait character on a scale ranging from 1 to 50 points, on the basis of biological values (small or large body; narrow or wide chest, etc.) .

As of December 1991, 14 Official Classifiers are engaged in type evaluation. The classifier rates each animal in four major areas, and from these ratings he arrives at a final score. This is an evaluation method on the basis of 100 points, which is given to True Type. At the same time, line type classification is also carried out.

As shown in Table 4-4-8, linear type traits consist of 15 primary traits and 9 secondary one. Each of these traits is

linearly evaluated on a scale of 1 to 50 points.

(4-6) Regulations regarding undesirable genetic abnormalities

The following three points are important in controlling undesirable genetic abnormalities.

- All defective calves should be reported to Breed Associations or other related organizations, upon being detected.
- The so-called "carriers" of congenital defects which carry undesirable recessive genes as heterozygote should be labelled as that.
- This information about congenital genetic defects should be made known to those who make decisions on mating and breeding.

When a defective character is found, it is also important to identify the pedigree (particularly, the sire) of the animal concerned. In addition, if the defective character of deformity is of an unknown type, it is necessary to establish an accurate diagnosis, whether it is genetic in origin or not. Then once defective characters have been determined, these and any characters which are possibly defective should be recorded under the pedigree records of the Association, as an initial measure.

It is effective and economical for a body such as Breed Association to manage records of defective characters.

The Association has published "Procedures regarding investigation on the undesirable genetic factors", in which the following ten undesirable genetic factors are designated for reporting to the Association in a prescribed manner:

- Prolonged Gestation
- Hairless (hypotrichosis congenita)
- Achondroplasia ("Bull-Dog", A, Band C type)
- Congenital blindness
- Mule-Foot (syndactylism)

- Dwarfism
- Taillessness
- Cleft palate (Palatoschisis)
- Genetic Muscle Contracture
- Teat abnormalities

(4-7) Application of blood typing test to registration

With the development and spread of new techniques such as Artificial Insemination and Embryo Transfer, the importance of confirmation of the parental relationship by blood typing test is increasing. As blood typing test are essential for ensuring the accuracy of H.B. Registry, they have become an essential part of registration procedures, playing an important role in improving dairy cattle in many countries.

In this Association, blood typing test service was introduced in 1964, after trial practice of a few years. It is operated under "The Rules and Procedures Regarding Blood Typing Test" which was adopted in 1964 and has been subject to some revision. Present programs are summarized in the following.

① Kinds of blood typing test

- i . Percentage test of bulls for H.B. registry application.
- ii . Blood type certifying of a single animal.
- iii . Parentage test in general.
- iv . Freemartinism determination.
- v . Monozygosity determination.

② Blood typing test is applied when:

- i . Determination of sire and/or dam is needed in the case of registration and progeny test, etc. (since 1964)
- ii . When freemartinism or monozygosity determination is

needed (since 1964)

- iii . Identification of individual animals is needed (since 1964)
- iv . Bulls are registered in the Herd Book (since 1971)
- v . A.I. semen is exported (since 1973)
- vi . There is a Donor dam and its offspring in Embryo Transfer (since 1978)
- vii . Spot checking in female animals applied for H.B. registry (since 1979)

③ Mandatory blood typing test in Bulls.

With the advent of frozen semen in 1970s, all bulls used artificially are required to be blood typed. Thus, we have stipulated that each bull applying to the Association for H. B. registry must be blood typed before the animal can be registered, at the expense of the applicants concerned, since 1971. Further we have decided that any bull calf submitted to H.B. registry must be blood typed along with his living unblood-typed dam since 1978.

④ Random checking

Another regular use of blood typing is the random sampling of female calves for parentage checking. This program calls for blood typing one of every 1,000 animals submitted to the H.B. registry, provided that most expense is borne by the Association in testing the calves selected and their dams.

⑤ Organization of blood typing

At the present time, blood typing of cattle is done in the Blood Typing Test Laboratory of Livestock Improvement Association, Inc. under the contract with this Association.

This arrangement was established in 1978. Prior to that

time, the blood typing was done by the blood type laboratory belonging to this Association. However, because of various circumstances, our laboratory was transferred to the abovesaid organization.

⑥ Number of blood type test

With reference to annual statistics, blood type testing is as shown in Table 4-4-9.

(4-8) Artificial Insemination system and registration in Japan

In Japan, commercial Artificial Insemination services to dairy farmers began in 1950, with the enactment of the "Livestock Improvement and Proliferation Law". Today, this law prescribes regulations concerning male breeding stock, natural breeding, Artificial Insemination, Embryo Transfer, licensing of Artificial Inseminators, livestock registration matters, and the like. Also, in the enforcement regulations of the law, provisions concerning the forms of certificates for Artificial Insemination and Embryo Transfer are incorporated.

An application for H.B. Registry of cattle produced by Artificial Insemination can be filed with a specific certificate for Artificial Insemination of livestock, provided that the cattle was produced from semen which was collected, labelled, and distributed in accordance with the provisions of the law. For High Grade Holstein cattle, application for Foundation Registry can be filed in a similar way. Nowadays, most (more than 99%) Herd Book-registered cattle are produced by Artificial Insemination.

In Japan, a cattle owner is permitted to inseminate his own cow even if he is not qualified as an artificial inseminator of livestock. Cattle produced by this so-called private insemination also are accepted for registration. In this case, however, obtaining a witness who is a qualified inseminator and

recording this in the margin of the certificate of Artificial Insemination is encouraged to ensure accuracy.

Few inseminators in Japan are employees of AI Centers: many belong to bodies such as agricultural cooperatives, while others are self-employed veterinarians or inseminators. In Japan, a certificate of Artificial Insemination is issued at the request of owners of livestock, unlike in the American and Canadian systems, in which certificates are issued in triplicate and distributed to the parties concerned immediately after insemination.

(4-9) Registration procedures of offspring resulting from Embryo Transfer

We recorded a male animal born August 6, 1964, as the first registered Holstein calf born as a result of Embryo Transfer. Although no regulations in this area were laid down at that time, this case was handled as a special case because no problems were found in the determination of parentage by blood type testing. Following a pioneering case of this, a few Embryo Transfers were begun around 1977 in Japan. Thus, as regulations necessary for the registration of calves born as a result of Embryo Transfer, we adopted "Regulations Governing Embryo Transfer and the Registration of Resulting Calves" in 1978.

In Japan, Embryo Transfer has recently been spread and promoted by Central Governmental aid, and it has been developed through projects actively performed by technologically advanced companies in order to improve dairy and beef cattle and increase the population size of beef cattle. As a result, Japan's technical level is approaching that of other countries with advanced dairy farming.

An outline of the Association's regulations of Embryo Transfer

is as follows:

- ① In the case of collecting embryos with the intention of applying resulting animals for H.B. Registry, the Donor Cow should be bred to only one bull at any one heat period or at two or three consecutive heat periods, as a general rule.
- ② When two or more of embryos are transplanted at one time per Recipient Cow with the intention of applying the resulting animals for H.B. Registry, they must be a group of embryos resulted from common parents.
- ③ When embryos are collected or transplanted, the person involved must submit a collection report or transplantation report, respectively, (within 100 days of transplanting) to the Association. In order to streamline the procedure, however, these will be discontinued in 1992.
- ④ An application for H.B. Registry must be filed by the recorded owner of the calf at the time of birth, accompanying the certificate of transplantation to which the certificate of embryo recovery was affixed. In this case, the owner of the donor on the date when the embryo is recorded as the breeder of the resulting animal, and is collected the applicant (recorded owner of the calf at the time of birth) is recorded as the owner (at the time of registration) of the resulting animal.
- ⑤ The donor dam must undergo blood typing immediately after the recovery of embryo. Also, the produced calf must undergo blood typing for parentage verification when applying for H.B. Registry. Whenever the Association deems it necessary, the recipient cow shall undergo blood typing.
- ⑥ When filing an application for H.B. Registry of a calf produced by an imported embryo or an imported recipient dam,

certificates related to recovery, transplantation, blood type and the like, issued by the relevant foreign Breed Association must be submitted with the application.

- ⑦ The resulting animal, once Herd Book registered, shall carry the suffix ET as a part of its name. Also, a resulting animal from split embryo shall carry the suffix ETS.
- ⑧ When filing an application for H.B. Registry of a calf produced by Embryo Transfer, an administrative fee (3,090 yen) must be paid to the Association in addition to a regular application fee.
- ⑨ When exporting an embryo or recipient dam, one or other of the certificates shown in Figs. 4-4-5 and 4-4-6 may be requested by the applicant (a handling fee of 3,090 yen will be charged).

The number of registered cattle produced by Embryo Transfer in each year since 1965 is shown in Table 4-4-10.

(5) Registration systems

As described previously, the Association operates two registration systems: a purebred Holstein cattle registration system and a High Grade Holstein Cattle registration system.

(5-1) Registration system of purebred Holstein Cattle

The requirements and procedures of the purebred Holstein cattle registration system for the main registration kind is outlined below. Fig. 4-4-7 shows the registration kind in this system and their relationships.

① Herd Book Registry

(i) Application procedures

An application for H.B. Registry can only be filed by the owner of the dam when the offspring has been born. This owner is called the "breeder". Application for H.B. Registry and a certificate of Artificial Insemination or natural breeding is to be submitted to the nearest Authorized Agent with the necessary registration fee and then the application is placed at the investigation of the Authorized Agent. The Branch Office or Authorized Organization involved (hereinafter referred to as the Branch Office) gather applications from Authorized Agents and send them to the Association. To summarize, an application has the following course: applicant → Authorized Agent → Branch Office → the Association.

(ii) Essential points in filing in an application form

The application form distributed from the Association is shown in Fig. 4-4-8. The following facts need to be entered in the appropriate blank spaces: the date of application, the sex, the type of coat color (red-and-white or off color), the registration numbers of the sire and dam, the name and address of the applicant (affixed with the applicant's seal), the code number and name of the Authorized Agent (affixed with the Authorized Agent's seal) and diagrams of the color markings. Clearly draw all color markings without any omissions since the diagrams will be used to determine whether the color markings are off color or not, as well as for animal identification. Boundary lines between the black and white parts are all that is necessary. Do not shade. Do not enter unnecessary data, an example of which is shown in Fig. 4-4-9, since the color markings drawing will be directly copied onto the back

of the certificate of registration using a photocopier.

Photos may be attached in place of the color marking drawings. The photos (one print of each of the right and left sides) should be taken in such a way that the color markings of the forehead and switch can be clearly seen. Attach each photo (11 × 7.6 cm) where the diagram would be.

(iii) Requirements of registration

The basic requirements of registration are shown in Fig. 4-4-7. In the case of H.B. Registry, the following additional requirements are also required:

- i . In the case of same sex twins, applications should be filed at the same time.
- ii . In the case of a female twin born with a male, the female will not be registered until it has been proved a breeder. If it has been found out that the animal is not a freemartin by means of the blood typing test, she may be registered before she freshens.
- iii . H.B. Registry is not permitted for cattle bearing hereditary defects which can significantly impair physiological functions, such as congenital blindness, mule foot and the like.
- iv . Unless the animal resulted from the natural breeding or Artificial Insemination of a licensed bull which passed the Breeding Stock Inspection, it should not be registered except in special cases the Association approves (such as breeding for scientific research, mating of an owner's bull with

a cow owned by the same owner).

v . An animal resulting from a female animal which has not yet reached the age of twelve months may be refused registration in the Herd Book, also an animal resulting from a dam whose gestation period is less than 265 days and more than 295 days may be refused registration in the Herd Book after a definite investigation.

vi . H.B. Registry application of imported cattle are acceptable only when the animal has been registered by a recognized foreign Herd Book Association.

As of February 1992, the following eight bodies have been recognized.

- The Holstein Friesian Association of America
- The Holstein Association of Canada
- The Cattle Registration Association of Friesland (FRS)
- The Cattle Registration Association of Netherlands(WRS)
- The Holstein Friesian Society of Great Britain and Ireland
- The New Zealand Friesian Association
- The Holstein-Friesian Association of Australia
- Verband Deutcher Schwarzbuntzuchter e.V. (VDS)

(iv) Processing of applications

A flowchart of the processing by which applications for registration are handled, from receipt to delivery, is shown in Fig. 4-4-10.

(v) Delivery of a certificate of H.B. Registry

A certificate of H.B. Registry is printed using a Chinese character printer and computer. A sample is shown in Fig. 4-4-11. Once a certificate has been drawn up it is sent as follows: the Association → Branch Office → Authorized Agent → applicant.

(vi) Registration fee for H.B. Registry differ according to the conditions of the cattle concerned, such as the sex, age in months and birthplace (domestic or overseas). The main charges are shown below.

i . Cows

Application filed within 6 months of birth:	3,910 yen
Application filed more than 6 months after birth:	7,210 yen
Cow produced from imported semen:	4,330 yen
Imported cow:	10,400 yen

ii . Bulls

Application filed within a year of birth:	25,750 yen
Application filed more than a year after birth:	52,530 yen
Bull produced from imported semen:	52,530 yen
Imported bull:	79,310 yen

The above charges are figured as of the date of receipt of applications received at the Headquarters Office of the Association.

② Several procedures after Herd Book Registry

After H.B. Registry procedure has completed, is accepted,

when necessary, the following procedures may be required: certification of transfer, cancellation or correction of H.B. Registry, or reissue or renewal of a certificate of H.B. Registry. These procedures are outlined below.

Certification of transfer: When Herd Book registered cattle are sold from the breeder to another person, the breeder files an application for certification of transfer by submitting the prescribed application form along with the certificate of H.B. Registry (charge: 2,580 yen).

Cancellation and correction of H.B. Registry: If a serious error (concerning parent animal or the date of birth, for example) is found after registration, the H.B. Registry must be cancelled. If a relatively minor error such as an omission of spots on the color marking drawing or an incorrect address is found, the registration must be corrected. After an H.B. Registry is cancelled, the animal can be registered again under a new H.B. Registry number and name, but another application form must be filed on the basis of the correct facts.

If a certificate of H.B. Registry is lost, torn or soiled, the necessary steps for reissue or renewal of the certificate are taken.

③ Registry of Production Record

Production testing is an indispensable step in a Registry of Production Record.

(i) Registry of Production Record on the basis of production testing independently carried out by the association

The Association operates an A-production testing method and a B-production testing method. The former is

applied to purebred Holstein cattle, and the latter to High Grade Holstein cattle, as a general rule.

In these testing methods, total milk yield volume is calculated from each milking of the cow during the testing period. The fat content of the milk is calculated from the composition of the milk and the total milk yield witnessed by the supervisor.

Both a 305-day testing and a one-year testing are used in the A- and B-testing methods. In both types of testing, the supervision has to be witnessed a specific number of times during a testing period: five times in the 305 day A-testing and six times in the one-year A-testing; three times in the 305-day B-testing; and four times in the one-year B-testing.

An application for Registry of Production Record is to be filed before Production testing. After completion of testing, the production record is to be submitted to the Association. An application for Registry Production Record is accepted when the fat index of the record is .80 or more. The fat index is calculated by assigning 100 as the standard value, as shown in Table 4-4-11.

(ii) Registry of Production Record on the basis Dairy Herd Improvement testing plan (DHI)

A Dairy Herd Improvement testing plan (DHI), which is carried out by separate bodies, is also used for Registry of Production Record. This system can be applied to both purebred Holstein and High Grade Holstein cattle. DHI testing is a monthly supervising method in which, as a general rule, the supervisor performs supervising once a month. Milk yield observed

on the day of supervising is used to estimate the total milk yield of that particular month, and of the 305 days or year. Even if supervising within a given month was not possible because of unavoidable circumstances, supervising at least eight times in a 305-day test, at least in a nine times in a 365-day test, are required respectively.

An application for Registry of Production Record must be filed before or during production testing. In extraordinary circumstances, it may be filed later, but it must be filed within six months of completion of testing. After the production testing, the Association receives the production records periodically from the computing center of the Livestock Improvement Association of Japan. The Association can then accept an application of Registry of Production Record. The Association will then promptly deliver a certificate of Registry of Production Record to the owner.

④ Registry of Type Evaluation

(i) Type classification

Type classification is an indispensable step in Registry of Type Evaluation. An application for Registry of Type Evaluation must be submitted to the Association via the Branch Office at the latest by the day two months before the type classification in the area concerned. Type Classification is conducted by Classifier of the Association according to the Scale of Points for Holstein Cattle, on the following animals:

- i . All females which have calved at least once in the dairy herd of the applicant (dairy herd classification), excluding:

- Cows 6 years of age or older
- Cows not used for breeding
- Cows raised for research purposes in national or prefectural institutes
- Cows which the Classifier regards as not-in-condition, and the like.

ii . In the previous rule, animals which missed the opportunity to undergo type classification because of special circumstances the Association approved, such as purchase, parturition and other reasons, may be type evaluated on an individual animal basis.

iii . Bulls older than 18 months.

Cattle cannot be examined again within nine months of the most recent classification.

(ii) Feedback of information on type classification

After completion of type classification, the Classifier submits a type evaluation report (shown in Fig. 4-4-12) to the Association, where the type classification records are brought together and analyzed in the Registry Department. The Dairy Herd Type Assessment (result of herd classification) shown in Fig. 4-4-13 is then sent to the applicant along with a Certificate of Type Evaluation Record. This is valuable information to an applicant for improving the type traits of their cattle.

(iii) Registry fee

The registry fee for Registry of Type Evaluation according to the type of appraisal (herd basis or individual basis appraisal) are as follows:

Herd basis appraisal: Charge for herd (20,600 yen a herd) plus charges for individual animals (4,640 yen a animal)

Individual basis appraisal: 8,650 yen

⑤ Recognition Program

Under the provisions of "Recognition Program of Merit cow and Elite cow", the Association runs a recognition project for recognizing Merit cows and Elite cows for payment (3,090 yen a cow).

To be recognized, the prescribed application form must be submitted to the Association. The criteria for recognition are shown in Fig. 4-4-7. The criteria for recognition of an Elite cow corresponds to the lowest criteria for a dam of H. B. Registry of male animals.

⑥ Award Program

Under the provisions of "Award Program of superior registered animals", the Association runs an award program for selecting and recommending superior registered animals as mentioned below.

- Gold Medal Dam (GMD)
- Longtime Producer (LP)
- Superior Production Sire (PS)
- Superior Type Sire (TS)
- Gold Medal Sire (GMS)

The criteria for selection are shown in Fig. 4-4-7. An application is not necessary since all cattle meeting the criteria are automatically selected.

(5-2) Registration of system of High Grade Holstein Cattle

As mentioned before, a certain number of cattle in Japan remain unregistered because owners of their progenitors, through certain circumstances, missed the opportunity for H.B. Registry.

Also, some unregistered cattle have been successively bred from excellent Holstein bulls over many generations. Among these cattle, individuals displaying coat color and other characteristics of Holstein cattle are called High Grade Holstein cattle, provided that they were born to Herd Book registered sires.

The purpose of the High Grade Holstein cattle registration system is to increase the gene resources used for improving Holstein cattle breeds as much as possible, by detecting and registering Holstein-line cows.

The main kinds of registration are as follows. Their mutual relationships are shown in Fig. 4-4-14 with their criteria for registration.

- Foundation Registry Symbol: K
- Preliminary Foundation Registry
 Symbol: 1K
- High Grade Registry A Symbol: 1H
- High Grade Registry B Symbol: 2H
- Registry of Type Evaluation
 Symbol: GT
- Registry of Production Record
 Symbol: GP

① Foundation Registry and Preliminary Foundation Registry

Foundation Registry and Preliminary Foundation Registry cattle may be called "Herd Book Registry" in High Grade

Holstein cattle. There are two types of Foundation Registry. Like the Preliminary Foundation Registry mentioned in the next paragraph, there are a Foundation Registry which is applied to the first generation to be picked up and registered, and the other type of Foundation Registry which is applied to the animal that resulted from a Herd Book registered sire and a High Grade Holstein dam (Foundation Registry, Preliminary Foundation Registry, High Grade Registry A, High Grade Registry B).

To be registered as a Foundation Registry which is applied to the first generation to be picked up and registered, the animal must meet the following three conditions:

- The animal must be regarded as the basis of improvement
- The date of birth must be confirmed
- The dam must have the coat color and other characteristics of Holstein cattle

These two types of registration are treated in almost the same way as H.B. Registry. The task of these registrations are usually performed by the Branch Office in each prefecture, including certifying of transfer, cancellation, correction and renewal of registration.

② High Grade Registry A

Foundation Registry animals which met the type or production criteria shown in Fig. 4-4-14 are registered as High Grade Registry A. Registration certificates of High Grade Registry A and High Grade Registry B (described below) show the color markings of individual animal since the certificates are issued in exchange for certificates of Foundation Registry. Thus these types of registration also serve the same function as Foundation Registry which is used for animal identification.

As a general rule, the task of these registrations is carried out by the Association's Headquarters Office or the Hokkaido Branch Office.

③ High Grade Registry B

Foundation Registry animals which met the type or production criteria shown in Fig. 4-4-14 are registered as High Grade Registry B. Registry of Type Evaluation (GT) and Registry of Production Record (GP) are conducted by the Association's Headquarters Office in the same manner as the purebred Holstein cattle registration system.

(5-3) Miscellaneous

① Extended pedigrees

The Association issues an extended pedigree certificate covering three to five generations on payment of a fee at the time of application, as a part of the feedback of registration information.

The amount of information available on a certificate of H.B. Registry certificate is limited by the size of the certificate, and because H.B. Registry is conducted when the animal are still very young. Therefore, extended pedigrees are issued to supplement certificates of H.B. Registry.

Depending on the quantity of officially certified records, the following data relating to the animal and its ancestors are included in an extended pedigree:

- Information on bulls

Final score of type appraisal (including the age in months

at the times of appraisal), type scores (%) for the major score card areas (general appearance, dairy character, body capacity), number of Advanced Registry, records of award program records of daughter cows (including the number of records and average values) and production records of daughter cows (number of records and average values).

- Information on cows

Final score of type appraisal (including the age in months at the times of appraisal), type scores (%) for the four major score card areas (general appearance, dairy character, body capacity, mammary system), records of recognition and award program, and production records (milk yield and fat percentage of each record and the like).

Extended pedigrees are still used less in Japan than in other countries with advanced dairy farming such as the U.S.A. and Canada. Thus it is necessary to further promote their use as valuable information for improvement.

② Export Certificate of livestock and semen

On application, the Association issues a certificate for export of Holstein cattle (or High Grade Holstein cattle) and of semen for payment (shown in Fig. 4-4-15 and 4-4-16) for the convenience of importing countries.

③ Registration activity statistics in recent years

Recently, registration has been decreasing owing to the difficult circumstances in dairy farming.

Table 4-4-12 shows the changes in the number of members, main registrations, dairy cattle, dairy farms and other factors, while Table 4-4-13 shows the results of registration by fiscal year.

4-3 Registration Systems of Jersey Cattle

(1) Jersey cattle in Japan

As described in Section 4-2, dairy cattle other than Holstein and High Grade Holstein are very few in Japan. Only 6,781 head of Jersey cattle were in Japan as of February 1, 1990, mainly in Okayama, Kumamoto, Akita and Gunma Prefectures. The recent raising situation of Jersey cattle is shown in Table 4-4-14.

Jersey cattle (two bulls and three cows) were first imported into Japan in 1877. Although many Jersey cattle were subsequently imported from the U.S.A., the breed did not become firmly rooted in Japan, and instead Holstein cattle became popular.

Registration of Jersey cattle was initiated by the Japanese Imperial Jersey Cattle Association, the first Japanese registration body, which was established in 1908. Bodies such as the Central Association of Animal Industry of Japan were the successors to this Association and have been performing registration on a small scale.

After World War II, about 13,000 head of Jersey cattle were imported from Australia, New Zealand and the U.S.A. during the eight years from 1953 to 1960 as a part of a national project to improve and increase dairy cattle suitable for the promotion of dairy farming in mountainous areas. The Jersey Cattle Association of Japan was established in 1956 when the breeding of Jersey cattle was being greatly promoted. This is a membership organization consisting of Jersey cattle breeders, but it is not incorporated. Its staff consists of staff members of the Holstein Cattle Association of Japan, and its registration affairs are entrusted to the Association.

The Jersey breed, native to Jersey Island in the English channel, is an older breed than Holstein. It is famous for its highly fatty milk, though generally its milk yield is less than that of Holstein.

It became widely distributed throughout the world because of the following merits:

- Early maturity. In general, the first deliveries are at about two years of age.
- Ease of calving. A broad pelvis allows safe crossbreeding with beef cattle such as Charollais and Limousin.
- Docility. Quiet and easy to handle in large numbers due to mild temperament.
- Longevity. There are many instances of cows reaching into their teens.
- Adaptability. They can cope equally well with either hot or cold climates.

Their tolerance to heat is far greater than that of other European breeds — they are much sought after by Middle East countries and are being used extensively through A.I. in Third World countries to improve local breeds.

The basic color of the modern Jersey breed is brownish ranging from light to dark brown. Some have uniform coat color while others have so called brown-and-white spots (broken colors). Its characteristics, which vary somewhat between countries, are usually as follows: body weight, 420 kg; milk production, 4,000 kg; milk fat content, 5.2%; and milk protein content, 3.8%. The body weight and milk yield of Jersey cattle are about 30% less than those of Holstein cattle. The number of Jersey cattle imported into Japan in recent years is shown by year and exporting country in Table 4-4-15. The average milk yield of Jersey cattle in Japan seen in Dairy Herd Improvement Testing Plan (DHI) are shown by year in Table 4-4-16.

(2) Registration systems

The kinds of registry and their relationship in purebred and high grade Jersey cattle are shown in Figs. 4-4-17 and 4-4-18 respectively.

As can be seen in Fig. 4-4-18, the daughter of Supplementary Registry B animal in the High Grade Jersey registry system can be entered H.B. Registry in the Purebred Jersey Registry system. Therefore, it is an open system, like the registration system for Holstein cattle. The H.B. Registry system for Jersey bulls is also similar to that for Holstein cattle, that is, it is a selective system since criteria are specified for type and production of the parents of the bull concerned. Other matters, including type classification, production testing, recognition award program and application of blood typing test to registration are operated in the same way as in the registration system for Holstein cattle.

There are important differences, though, in the method of animal identification. As Jersey cattle do not have the same type of spots as Holstein cattle, tattoos are used to identify individuals for H.B. Registry and Foundation Registry.

Purebred Jersey or High Grade Jersey cattle are tattooed on both ears as shown in Fig. 4-4-19. The right ear is tattooed with a symbol expressing the prefecture and the last two digits of the year in which the tattooing is performed, while the left ear is tattooed with the year's serial number for each prefecture. Application for H.B. Registry or Foundation Registry is made after tattooing the life-long individual markings.

Recent registrations are shown by year in Table 4-4-17.

4-4 Concluding Remarks

- (1) Progress in biotechnology and dairy cattle registration

The need for more intense and accurate selection of excellent animals has been increased further by recent advances in embryo or cell manipulating techniques such as Embryo Transfer, in vitro fertilization (IVF), and nuclear transfer (commonly referred to as cloning).

Originally, registration was a means for increasing the accuracy of selection. Therefore, with the development of breeding techniques and selection methods, it is expected that more detailed information concerning the greatest possible number of characters should be supplied through registration. For example, registration may be required to supply information on characters related to dairy cattle management, such as fertility, disposition, feeding efficiency, disease resistance, and milkability (milking rate), as well as data related to milk production and type traits.

In order to obtain more sophisticated information, registration may be needed so that true protein contents can be used in place of conventional milk protein contents for more convenient dairy products manufacturing, and to supply bloodline information combined with the κ -casein genotype or marker genes as genetic engineering advances.

(2) Introducing new methods into registration

(2-1) DNA fingerprinting

As breeding processes have become remarkably complicated with the development of new techniques such as Artificial Insemination and Embryo Transfer, the importance of blood typing has increased more and more. It was fortunate that techniques for blood typing were developed and put into practical use in cattle at about the same time as registration procedures were becoming more complicated with the development of breeding techniques.

It is predicted that determining parentage relationships will become more and more complicated if nuclear and gene transfer are put into practical use. Fortunately, a new method called DNA fingerprinting has recently been practically applied in some countries. This method is extremely accurate (99.9% resolution rate in humans) because it analyzes genes themselves, unlike existing blood typing tests, in which gene products are utilized in antigen-antibody reactions. The current problem is in the high cost of this method.

For the time being, the DNA fingerpringing method will be used to assist the usual method of blood typing when highly complicated parentage problems arise.

(2-2) Electronic identification

Identification of individual animal is the first requirement for good herd management and breed improvement. Without positive identification of all animals in a herd, it is next to impossible to make genetic progress in the various important traits. The present methods of identifying Holstein cattle in most countries are as follows:

- Color markings (by color markings diagrams or photos)
- Freeze brand (branding marking numbers by breaking hair-root cells with dry ice or liquid nitrogen)
- Tattoos
- Ear tag

Instead of these time-consuming methods, recently a new technique called Electronic identification has been devised in some country. It is assured that it will spread gradually in future.

This method will be used by the Breed Association to confirm the

identity of individual animals for H. Book Registry and type classification, and will be applied to individual identification for feeding, Artificial Insemination, veterinary examination and treatment. This method provides not only completely accurate, automatic, and immediate individual confirmation, but also automatically inputs data into a computer.

An electronic transponder (ID chip) which stores the code for individual confirmation consists of about 10 figures and the code of breed name and country is implanted under the skin of the ear or the tail head of each animal. The stored data can be read by hand-held portable scanners.

One of the problems with this method is its high cost (\$5 to \$6 for a transponder and \$2,000 for a scanner). In addition, the method must be guaranteed to be safe in terms of beef consumption given that a unit made of metal is buried under the skin.

It is said that even in countries where the method has already been put into practical use, individual confirmation with the visual identification will still be used concurrently for some time to come.

(3) Registry of dairy cattle in future

- ① Feedback of information is the primary task of dairy cattle registration bodies. Their role is becoming important as more and more areas are affected by advanced improvement systems, and biotechnology has been steadily developing in recent years. They need to establish closer cooperation with bodies undertaking production testing inspection and progeny testing, Artificial Insemination bodies and related bodies so that dairy farmers and related organisations can be rapidly supplied with more detailed, versatile registration information available for effective improvement of dairy cattle.

- ② While preparation of databases and on-line processing of registration information is making progress, the Breed Associations need to rationalize systems of registration affairs and offer better services to members (dairy farmers) in line with new trends, including the advance of statistical processing techniques such as the BLUP method (animal model) and the practical development of new registration methods which are going to be introduced, as mentioned before.
- ③ Under circumstances in which the international distribution of genetic resources is increasing, the World Holstein Friesian Organisation will take a leading role in ongoing development of and research into an internationally standardized numbering system of H.B. Registry. International coordination of type classification methods is also under way. In advance of this, arrangements have begun for preparing an international standard of genetic evaluation of male breeding stock with INTERBULL as a pioneer. Although it may be difficult to prepare international standards of Holstein cattle registration in the near future, we can expect steady progress to occur as a result of these activities.

Table : 4-4-1 : Yearly Statistics the averages on production records of Registry of Production Records.

	1986	1987	1988	1989
number of records	31,358	35,308	40,314	41,692
milk production (kg)	9,004.5	9,188.6	9,446.6	9,588.9
butterfat production (kg)	334.6	341.5	352.4	357.7
butterfat percentage(%)	3.73	3.73	3.75	3.75

note; fiscal year are based on the date of registration.

Table : 4-4-2 : Averages of production records made in DHI production testing program (supervised testing)

	1986	1987	1988	1989	1990
number of records	206,203	211,786	233,183	242,754	261,670
milk production (kg)	7,171	7,346	7,507	7,705	7,798
butterfat production(kg)	262	269	276	284	288
butterfat percentage (%)	3.65	3.66	3.67	3.69	3.69
protein percentage(%)	—	3.02	3.06	3.09	3.09
SNF percentage (%)	8.62	8.62	8.63	8.64	8.62

Table : 4-4-3 : Average milk production per cow during milk secretion.
(these cows belongs to purebred Holstein or Grade Holstein)

	1985	1986	1987	1988	1989	1990
average per cow	5,640	5,676	5,869	6,130	6,380	6,384

unit: kg

source:Agricultural ministry

Table: 4 - 4 - 4 : Annual statistics concerning H.B.Registry Cattle re-registered after import.
(classified by fiscal year, countries and sex)

country \ sex \ fiscal year		1986	1987	1988	1989	1990
U.S.A.	male	45	66	65	106	147
	female	15	131	429	727	534
Canada	male	8	5	16	15	19
	female	14	80	105	110	161
others	male	0	0	0	0	0
	female	0	0	27	0	0
total	male	53	71	81	121	166
	female	29	211	561	837	695

note; fiscal year are based on the date of registration.

Table: 4 - 4 - 5 : Annual statistics concerning H.B.Registry Cattle resulted from imported semen.
(classified by fiscal year, country and sex)

country \ sex \ fiscal year		1986	1987	1988	1989	1990
U.S.A.	male	0	5	3	3	2
	female	729	1,215	2,815	3,537	5,633
Canada	male	0	4	2	4	2
	female	233	719	1,305	2,588	4,744
others	male	0	0	0	0	0
	female	0	0	0	0	1
total	male	0	9	5	7	4
	female	962	1,934	4,120	6,125	10,378

Table; 4—4—6 : Requirements qualifying for Registration application
from the viewpoint of coat color and color markings.

pattern of coat color and color markings	male animals (H. B. Registry)	female animals	
		H. B. Registry	Foundation Reg.
uniform white color: A	No	No	No
uniform black color: B	No	No	No
uniform red color : C	No	No	No
tri-color mixed (red, black, white) : D	No	No	No
red spots in B & W : E	No	Yes(Oc)	No
standard B & W : F	Yes	Yes	
Red and white : G	Yes(Red)	Yes(Red)	
B & W with black belly :H	No	Yes(Oc)	No
B & W with black switch of 50 % or more :I	No	Yes(Oc)	No
B & W with black spot on a leg or legs which encircled the hoof. :J	No	Yes(Oc)	No
Combination of above H, I, J :K	No	Yes(Oc)	No

note: Red and White animals, when H. B. Registered, shall carry the suffix
"Red" as part of its name. and so-called "Oc" female animals, when
Registered, shall carry the suffix "Oc" as part of its name.

Table: 4 — 4 — 7 : The Four Major Score Card Areas and its relative emphasis in each area.

Name of the Four Major Score Card Areas	Relative Emphasis	
	Female	Male
General Appearance	3 0 (%)	4 5 (%)
Dairy Character	2 0	3 5
Body Capacity	2 0	2 0
Mammary System	3 0	—

Table: 4 — 4 — 8 : Line Up of Linear Descriptive Type Traits.

Primary Traits	Secondary Traits
Stature	Shoulders
Strength	Chine and Loin
Body Depth	Rear Leg Position
Dairy Form	Rear Legs(rear view)
Rump Angle	Crampy
Rump Length	Pasterns
Rump Width	Toes .
Rear Legs (side view)	Udder Balance
Foot Angle	Teat Length
Fore Udder Attachment	
Rear Udder Hight	
Rear Udder Width	
Udder Cleft	
Udder Depth	
Teat Placement(rear view)	

Table 4-4-9: Annual Statistics on the Blood Typing Test

kinds of test year	parentage test of bulls for H.B. registry application.	blood type certifying of a single animal.	parentage test in general.	freemartinism determination.	monozygosity determination.	remarks
1964	0	19 (0)	13(0)	9(0)	0	<u>Adopted Rules and Procedures Regarding Blood Typing Test in 1964.</u>
1965	0	10 (0)	8(0)	3(0)	0	
1966	0	42 (0)	17(0)	13(0)	0	
1967	0	6 (0)	17(0)	22(0)	0	
1968	0	24 (0)	31(0)	31(0)	0	
1969	0	34 (0)	39(0)	40(0)	0	
1970	0	25 (0)	56(0)	45(0)	0	
1971	199	2 (0)	29(0)	33(0)	0	H.B. registry applying male animals were placed to blood typing test before registration since 1971.
1972	361	14 (0)	33(0)	35(0)	0	
1973	444	13 (0)	47(0)	52(0)	0	
1974	445	0 (0)	57(0)	43(0)	0	
1975	368	2 (0)	42(0)	37(0)	0	
1976	419	0	60(0)	27(0)	0	
1977	275	0	104(0)	26(0)	0	
1978	404	4 (0)	433(0)	95(0)	1 (0)	H.B. registry applying male animals have been blood typed along with his living unblood-typed dam since 1978.
1979	407	8 (0)	384(0)	88(0)	19 (0)	
1980	405	32 (0)	403(0)	118(0)	0	
1981	329(214)	46 (32)	412(279)	90(55)	4 (0)	Ruled E.T. regulations in 1978.
1982	276(198)	56 (34)	472(257)	87(46)	0	
1983	293(207)	114 (84)	468(298)	82(44)	0	Introduced a random sampling program of blood typing test in 1979.
1984	214(161)	137 (78)	451(249)	73(42)	0	
1985	253(197)	225(115)	500(313)	105(58)	0	
1986	277(229)	350(177)	687(431)	106(58)	0	Blood typing fees were increased approximately by 100% in 1988.
1987	285(230)	515(231)	907(556)	102(48)	0	
1988	245(187)	653(305)	835(433)	106(67)	2 (0)	
1989	233(169)	686(238)	979(469)	87(45)	2 (0)	
1990	292(218)	782(317)	1,126(514)	106(45)	0	
1991						
1992						
1993						

note: bracketed figures denotes the Hokkaido Branch Office's share.

Table : 4-4-11 : Standard butterfat production for calculating
" Fat Index"

Length of record	Ten months division		Yearly division	
milking frequency	2 X	3 X	2 X	3 X
Age when calving				
Junior Two-year-old	114.8	137.7	135.0	162.0
Senior Two-year-old	121.9	146.7	144.0	172.8
Junior Three-year-old	129.6	155.7	152.6	183.2
Senior Three-year-old	135.5	162.9	159.8	191.3
Junior Four-year-old	141.8	170.6	166.9	200.7
Senior Four-year-old	146.3	175.5	171.9	206.6
Full aged	148.9	179.1	175.5	210.6

(unit:kg)

Table:4 -4 -12:

Annual trends in numbers of Association Members, Registered Animals, Dairy Cattle and Dairy Farmers.

Year	Association Members		H.B.Registry		Advanced Registry		Production Registry	Type Registry	Dairy Cattle		Dairy Farmers
	Regular Members	Associate Members	Male	Female	Male	Female			Population		
									Number	Herd Size	
1948			544	2,624	0	283	0	4	170,850	---	---
1949	9,387	882	1,121	7,391	35	773	5	12	201,788	1.5	134,800
1950	10,791	594	765	9,048	17	993	17	23	203,825	1.5	133,024
1951	9,500	457	741	11,753	22	1,183	11	24	225,820	1.5	150,520
1952	14,160	626	570	14,638	29	1,963	64	25	275,590	1.5	183,149
1953	19,947	558	536	16,821	29	1,905	66	34	323,360	1.6	207,000
1954	25,014	10,365	631	21,249	44	2,258	87	72	356,470	1.6	225,000
1955	38,812	19,221	558	24,591	47	3,565	158	78	421,110	1.7	253,850
1956	41,316	17,941	512	28,211	61	3,188	71	54	497,410	1.7	283,190
1957	51,133	20,252	605	33,776	59	3,587	66	77	586,820	1.7	336,500
1958	41,692	33,142	578	37,723	67	3,940	102	125	654,340	1.8	364,800
1959	53,908	36,148	556	46,323	70	4,499	113	160	751,090	1.9	388,420
1960	65,341	26,065	627	52,629	84	4,081	146	194	823,500	2.0	410,420
1961	72,642	21,709	541	54,831	54	4,956	96	153	884,940	2.1	413,000
1962	79,818	23,739	653	81,470	104	8,918	199	394	1,001,690	2.4	415,710
1963	78,684	18,524	551	84,920	137	10,291	209	460	1,145,370	2.7	417,640
1964	87,066	13,429	535	100,977	122	10,960	219	626	1,238,300	3.1	402,470
1965	86,091	11,574	559	106,536	137	10,739	254	834	1,288,950	3.4	381,600
1966	107,049	11,473	703	130,052	110	11,321	312	1,125	1,309,970	3.6	360,730
1967	95,500	11,243	630	127,000	124	11,600	390	1,489	1,376,100	4.0	346,900
1968	99,998	10,665	600	158,000	134	13,000	385	1,936	1,489,000	4.4	336,680
1969	102,137	9,039	559	175,000	122	16,027	532	2,568	1,663,360	5.1	324,440
1970	98,356	4,317	621	168,600	135	20,573	831	2,885	1,804,000	5.9	307,600
1971	90,656	1,934	351	171,100	160	23,600	1,302	2,851	1,856,000	6.6	279,300
1972	64,702	1,326	348	164,900	108	18,400	1,374	3,345	1,819,000	7.5	242,900
1973	65,807	965	391	154,568	71	12,770	1,311	3,312	1,777,000	8.4	212,000
1974	54,729	2,043	400	150,832	115	12,130	1,340	3,800	1,752,000	9.9	178,600
1975	49,982	434	360	159,600	56	12,500	1,447	4,777	1,787,000	11.2	160,100
1976	50,321	412	370	178,400	98	16,000	3,030	9,481	1,811,100	12.3	147,100
1977	50,748	Discontin.	450	187,000	47	18,000	8,920	12,825	1,888,000	13.8	136,500
1978	50,247		380	205,000	64	20,800	15,400	17,212	1,979,000	15.3	129,400
1979	48,346		380	214,895	66	19,999	19,050	24,099	2,067,000	16.8	123,300
1980	47,478		360	219,000	33	24,000	27,750	25,928	2,091,000	18.1	115,400
1981	44,986		360	211,996	52	20,000	32,650	24,944	2,104,000	19.9	105,800
1982	42,567		280	213,000	59	16,600	33,700	29,601	2,103,000	21.3	98,900
1983	40,741		275	207,000	33	14,406	37,700	29,460	2,098,000	22.7	92,600
1984	37,624		235	204,000	23	8,694	42,593	51,156	2,110,000	24.1	87,400
1985	36,720		232	205,000	17	619	36,906	44,934	2,111,000	25.6	82,400
1986	33,676		260	205,000	61	96	49,890	42,333	2,103,000	26.8	78,500
1987	32,909		267	204,000	23	23	50,710	36,891	2,049,000	27.5	74,500
1988	36,168		252	211,000		2	56,163	41,310	2,017,000	28.6	70,600
1989	37,493		244	217,000	34		65,124	41,878	2,031,000	30.4	66,700
1990	33,863		279	224,000	97		74,127	39,419	2,067,000	34.6	59,800
1991											

Table:4-4-13: Yearly Statistics of Association Activities.

kinds of registry etc. year	1970	1975	1980	1985	1986	1987	1988	1989	1990
Regular Members	98,402	49,982	47,478	36,720	33,676	32,909	36,168	37,493	33,863
Associate Members	4,317	434	discont.						
Assistance Members									
individual	-----	-----	-----	142	260	203	153	168	307
organizational	-----	-----	-----	13	71	53	42	50	54
Authorized Agents(technicians)	5,385	5,168	4,886	4,542	4,096	4,204	4,125	4,031	4,078
Staff	198	158	151	121	119	113	111	103	109
Herd Book Registry									
male	622	360	360	232	260	267	252	244	279
female	169,687	159,600	219,000	205,000	205,000	204,000	211,000	217,000	224,000
Advanced Registry									
male	136	56	33	17	61	23	0	34	52
female	20,573	12,500	24,000	619	96	23	2	0	0
Registry of Type Evaluation									
male	-----	-----	-----	22	40	20	15	13	12
female	-----	-----	-----	44,912	42,293	36,871	41,295	41,865	41,011
Registry of Production Record	-----	-----	-----	36,906	49,890	50,710	56,163	65,124	77,777
Superior Production Sire	-----	-----	-----	0	12	0	0	0	0
Superior Type Sire	-----	-----	-----	0	4	0	0	0	0
Gold Medal Sire	-----	-----	-----	0	0	0	0	0	0
Elite Cow	-----	-----	-----	29	23	7	2	6	3
Merit Cow	-----	-----	-----	22	16	3	0	0	0
Gold Medal Dam	-----	-----	-----	0	15	0	0	0	0
Longtime Producer									
gold prize	-----	-----	-----	332	501	540	577	641	770
silver prize	-----	-----	-----	1,203	1,827	1,899	2,284	2,651	3,258
copper prize	-----	-----	-----	4,359	5,843	6,047	7,811	9,494	10,381
Animal Ownership Transfers	68,671	47,534	58,233	44,007	42,592	40,677	41,274	48,256	44,201
Renewal	40	23	55	43	20	20	15	45	32
Reissue	2,003	1,451	1,495	1,305	1,223	1,086	1,200	1,500	1,124
Correction	1,490	923	1,711	1,014	1,032	830	959	918	942
Preliminary Foundation Reg.	-----	-----	-----	-----	-----	-----	-----	-----	-----
Foundation Registry	47,643	46,010	50,443	42,483	39,597	39,374	47,892	45,273	45,094
High Grade Registry A	2,423	944	3,420	568	505	168	146	54	73
High Grade Registry B	1,431	459	2,160	2,340	1,900	1,432	1,554	1,257	1,179
Animal Ownership Transfers	674	153	264	219	189	126	136	121	85
Renewal	4	0	1	0	0	0	0	0	2
Reissue	23	3	12	11	10	1	5	3	1
Correction	34	6	12	3	2	1	4	1	3
Extended Pedigrees	659								
3-generations		47	128	112	158	85	26	5	14
4-generations		20	32	25	35	12	10	16	32
5-generations		81	123	21	27	16	2	9	9
Bulls		0	0	0	0	0	2	0	0

Table: 4-4-14 : Raising Situation of Jersey Cattle

item	fiscal year	1960	1970	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
population size	Male	75	42	9	10	9	10	4	7	7	6	20	9	11
	female	21,250	17,979	5,089	4,697	4,301	4,345	4,006	4,016	3,858	3,998	4,138	5,877	6,781
Dairy Farmers having Jersey		—	—	617	515	523	420	501	483	465	419	416	513	709
Herd size per dairy farmer		—	—	8.2	9.1	8.2	10.3	8.0	8.3	8.3	9.5	9.9	11.5	9.6

Table: 4-4-15 : Importation trends of Jersey Cattle in recent years

fiscal year		1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
U.S.A.	male	1	—	—	—	—	—	1	—	—	—	—	—	1	—
	female	—	5	—	—	—	—	—	16	36	331	171	168	64	—
Canada	male	—	—	—	—	—	—	—	—	—	—	—	—	—	1
	female	—	—	—	4	—	—	—	—	48	140	429	60	33	30
New Zealand	male	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	female	45	—	—	—	—	—	—	—	50	—	140	90	40	—
Australia	male	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	female	—	—	—	—	—	—	—	—	—	—	146	—	—	—
Total	male	1	—	—	—	—	—	1	—	—	—	—	—	1	1
	female	45	5	—	4	—	—	—	16	134	471	886	318	137	30

Table : 4-4-16 : Milk Producing Ability of Jersey Cattle in Japan (Statistics on DHI tested cattle)

fiscal year	1979	980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
number of records	506	500	528	477	504	451	398	360	418	538	744	954
milk production (kg)	3,892	3,918	3,730	3,839	3,988	4,121	4,443	4,649	4,836	4,664	4,807	4,802
fat percentage (%)	4.9	4.9	5.0	4.9	4.9	4.9	4.8	4.8	4.8	5.0	5.1	5.1
protein percentage(%)	—	—	—	—	—	—	—	—	—	3.73	3.75	3.71
SNF percentage (%)	—	—	—	—	—	—	9.15	9.18	9.20	9.31	9.32	9.29

Table : 4-4-17 : Annual Statistics on number of registered animals of Purebred and High Grade Jersey Cattle.

fiscal year	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990
H.B.Registry (female)	265	320	227	385	270	230	340	216	244	257	256	432	981	1,517	881
Guaranteed H.B.Registry	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—
Advanced Registry(female)	—	—	—	—	—	—	—	—	7	52	42	51	41	280	144
H.B.Registry(male)	2	4	1	3	0	1	3	3	2	4	0	5	5	4	1
Advanced Registry (male)	—	—	—	—	—	—	—	—	3	0	—	—	2	—	—
Supplementary Registry A	—	—	—	—	—	—	—	—	0	5	6	1	5	6	20

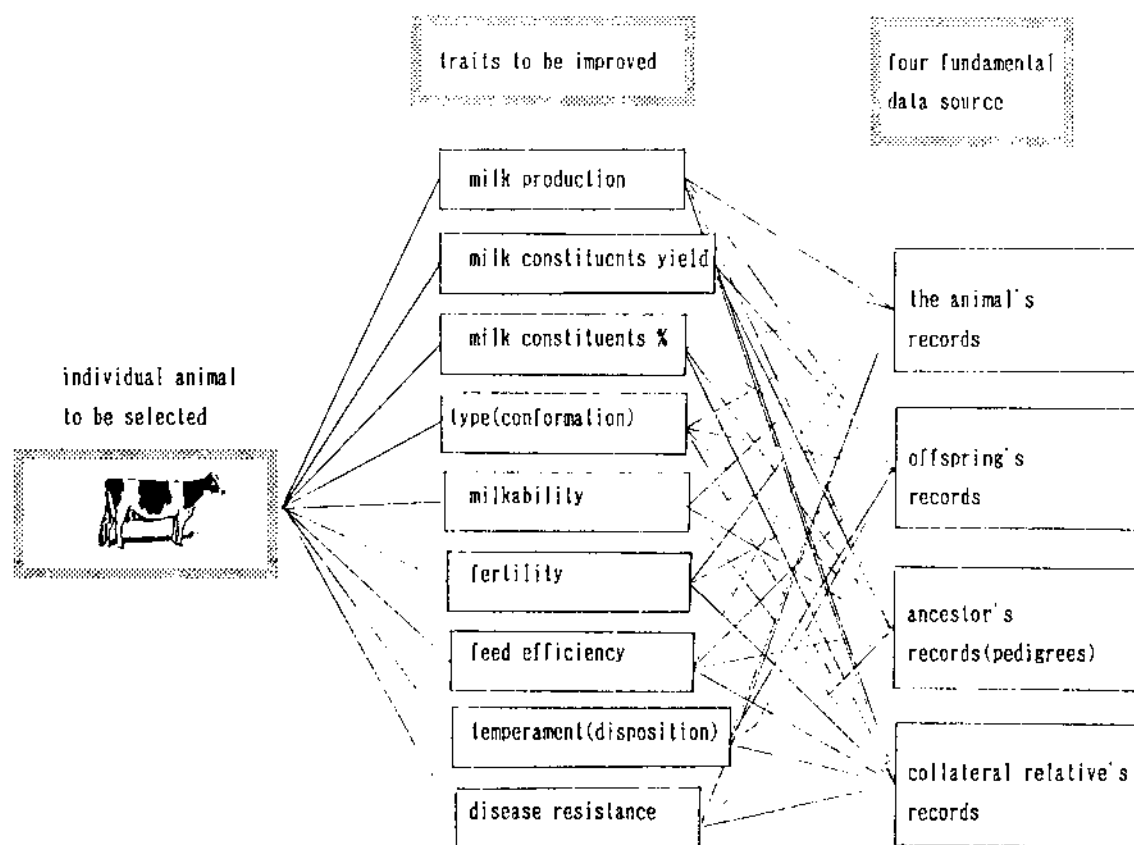


Fig. : 4-4-1 : Relation of traits to be improved and four fundamental data source

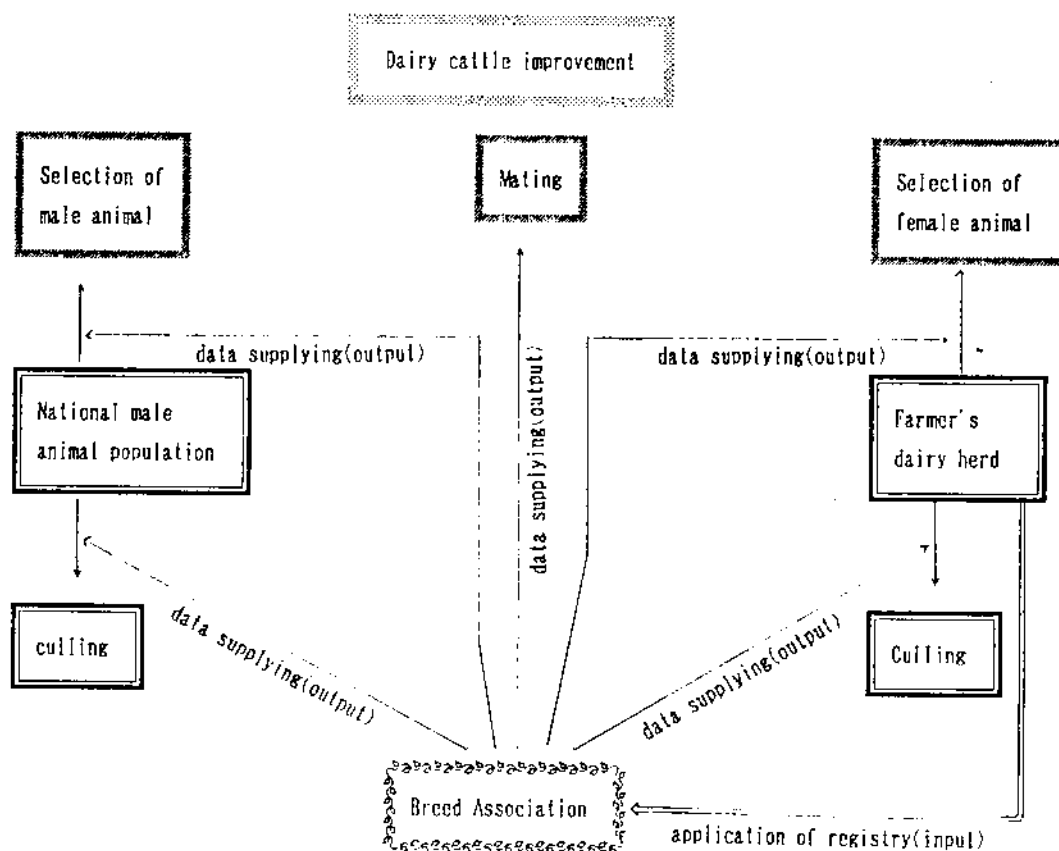


Fig. : 4-4-2 : The relation of the Breed Association to Breed improvement

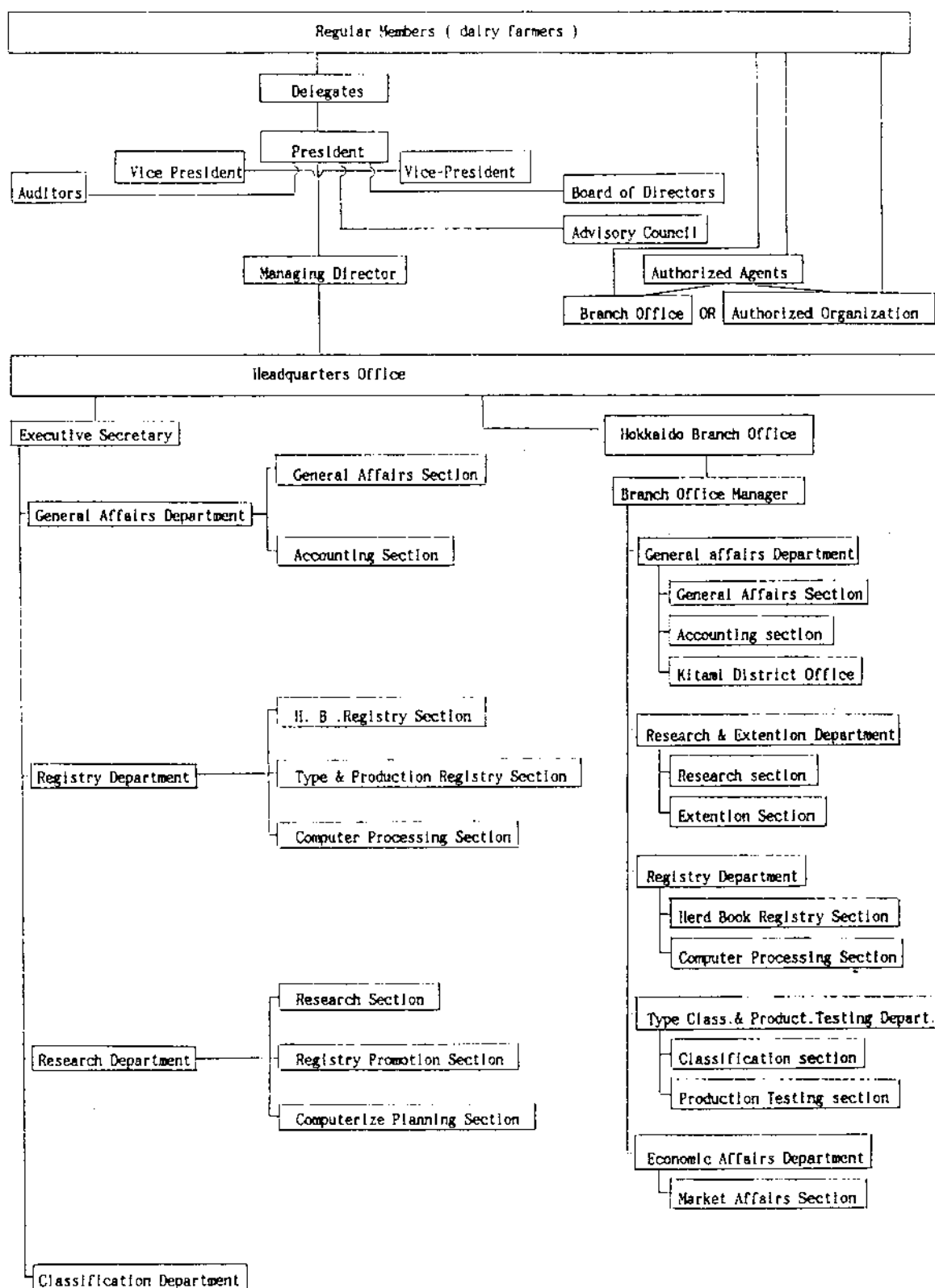


Fig. : 4 - 4 - 3 : NCAJ Organizational Structure
and Office Operational Structure

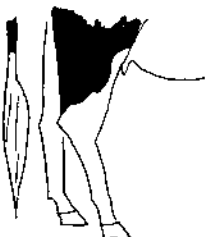
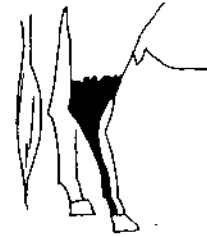
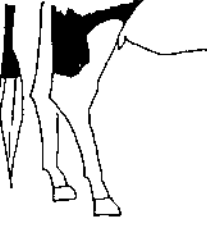
Example of color markings	U.S.A. and Canada	Japan	Remarks
	Standard Black and White	Standard Black and White	This is the typical standard black and white for Holstein Cattle
	Standard Black and White	Standard Black and White	In this case, the Switch is white in its 100% or more, also the black spot touching the hoof does not extend up to the hock, this color pattern is classified as standard black and white even in U.S.A. or Canada.
	non-standard Black and White (off-color)	Standard Black and White	In this case, the black spot touching the hoof extends up to the hock, so this is named as an off-color in U.S.A. and Canada, however, if the black spot does not encircle the hoof, it is classified as standard black and white in Japan.
	non standard Black and White (off-color)	Standard Black and White	In this instance, black spot on the tail spreads over the switch even to any extent, this is classified as a off-color in U.S.A. and Canada, however, if the switch is white 50% or more, it is classified as standard black and white in Japan.
	non standard Black and White (off-color)	non standard Black and White (off-color)	

Fig. : 4-4-4 : Difference between U.S.A. + Canada and Japan as for definition of off-color.

CERTIFICATE OF IDENTIFICATION OF EMBRYO FOR EXPORT

The Holstein Cattle Association of Japan
4-38-13, Hon-Cho, Nakano-Ku, Tokyo

This certificate is issued by The Holstein Cattle Association of Japan to identify the
fertilized ova or embryo undermentioned.

Donor dam: Name _____ Reg. No. _____

Service sire: Name _____ Reg. No. _____

Date of service: _____

Date of ova recovery _____ . Date of freezing _____

Intermediary host _____

Test-tube-labeled _____

or frozen and sealed in container labeled _____

Blood type of donor dam

Blood type of service sire

The above information is certified to be correct on the basis of the information provided
as produced under The Requirements of Regulations Governing Embryo Transfers and The
Registration of Resulting Calves of this Association, but does not constitute a guarantee
thereof.

Signature _____

Managing Director

F i g . : Official Form of Certificate of Identification of Embryo for Export.

CERTIFICATE OF IDENTIFICATION OF RECIPIENT DAM FOR EXPORT

The Holstein Cattle Association of Japan
4-38-13, Hon-Cho, Nakano-Ku, Tokyo

This certificate is issued by The Holstein Cattle Association of Japan to
identify the recipient dam of embryo transfer undermentioned.

Donor dam: Name _____ Reg. No. _____

Service sire: Name _____ Reg. No. _____

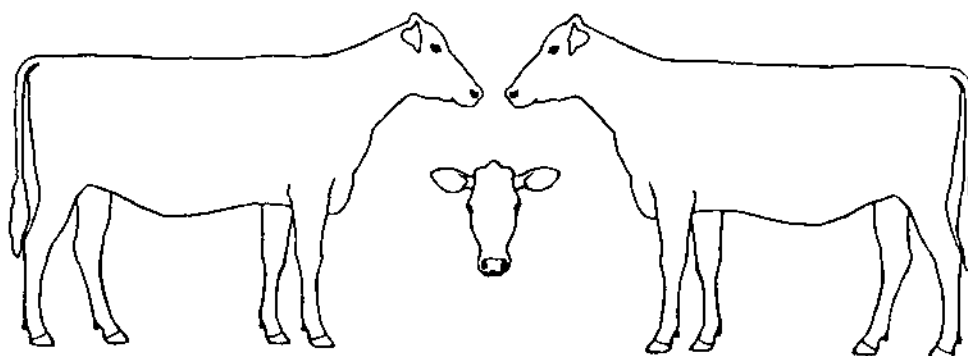
Date of service: _____

Date of ova recovery _____, Date of freezing _____

Recipient dam: Name _____ Reg. No. _____

Ear tag or tattoo: L _____ R _____

Color diagram of recipient dam



Date of ovum transfer _____

Blood type of donor dam

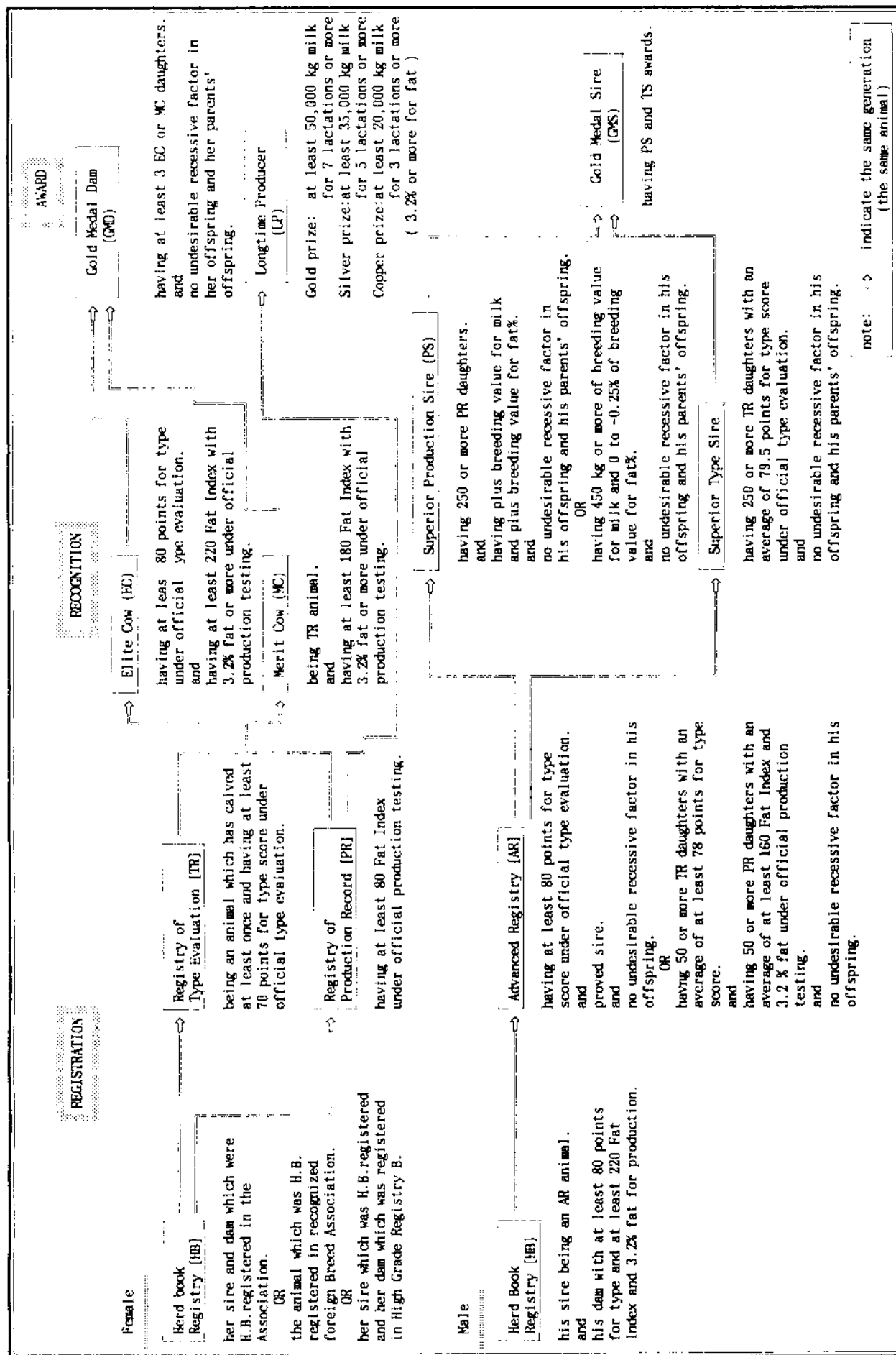
Blood type of service sire

The above information is certified to be correct on the basis of the information provided as
produced under the Requirements of Regulations Governing Embryo Transfers and The Registration
of Resulting Calves of this Association, but does not constitute a guarantee thereof.

Signature _____

Managing Director

F i g . : 4 - 4 - 6 ; Certificate of Identification of Recipient Dam for Export



To the President of the Holstein Cattle Association of Japan

[illegible]

NOTE.

1. The column of NC should not be entered 2. Enclosure by a thick line should be entered without fail. 3. Color markings diagrams should be entered clearly.

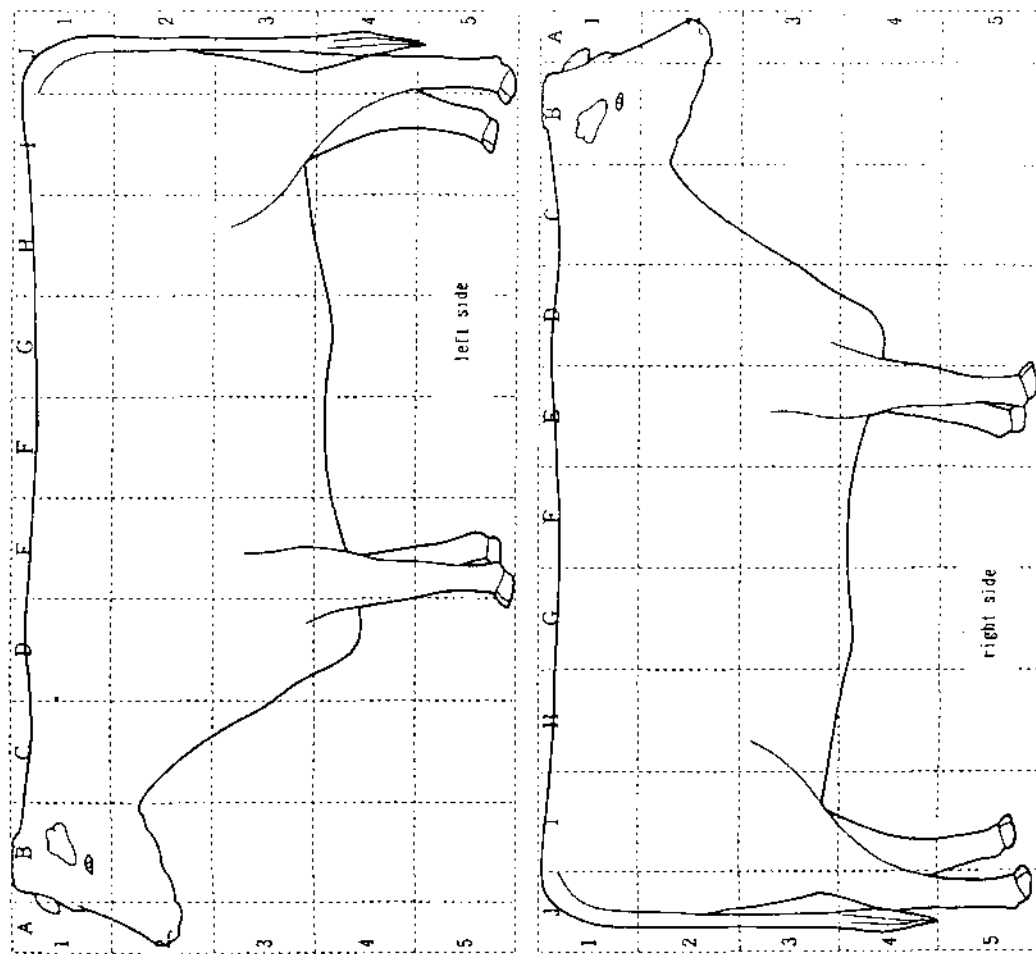
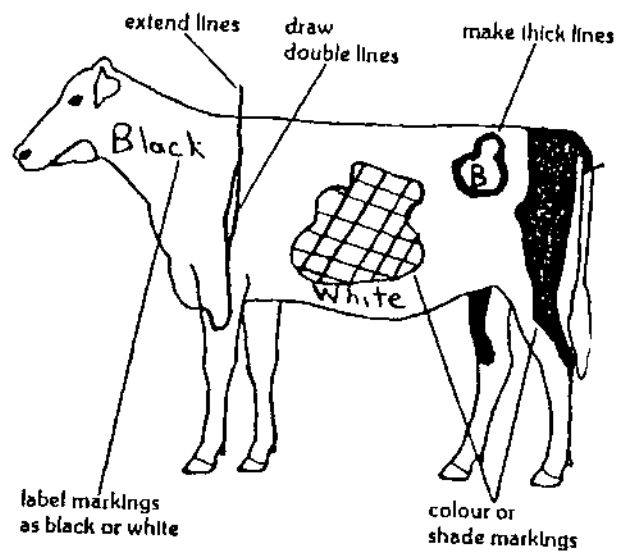
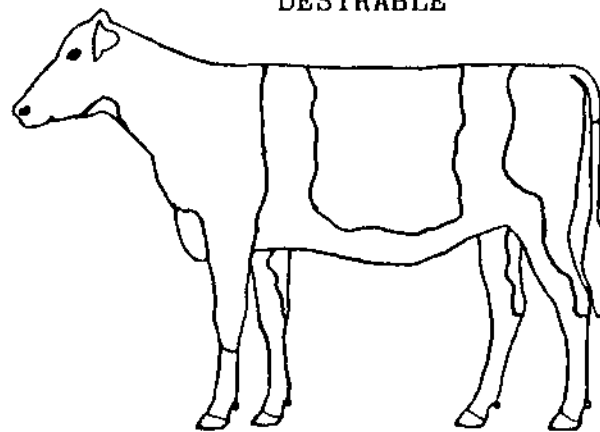


Fig. 4--4--8: Application form of Herd Book Registry

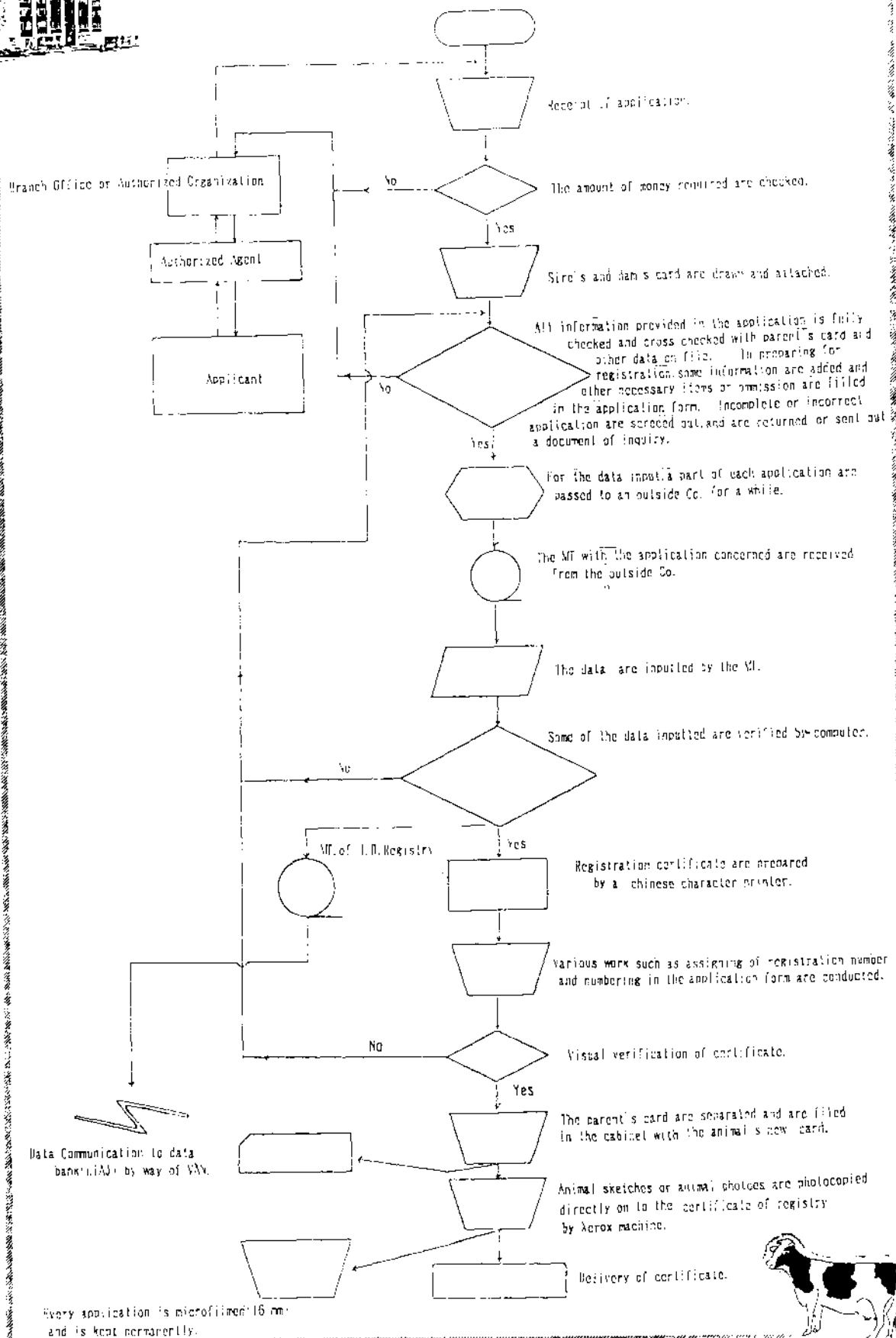
DO NOT



DESIRABLE



F i g. 4-4-9 : Example of color markings drawings



F : 3 . 4 - 4 - 1 0 : The processing procedure of Herd Book Registry application
from an application receipt to a certificate delivery

A specimen of H. B. Registry Certificate
(the reverse side: color markings diagram)

Certificate of H. B. Registry

血統登録証明書

Female

ホルスタイン種 雌牛

Name of the animal

H. B. Registry No. of the animal

NO. 5590377

イーステル アストロ グレース

MemberShip's code

父

ブライトン アストロ

母

イーステル アストロ

Owner's address

55-100-038-23

Owner's name

前田 孝志

Date of transfer

平01 11 08

Yende's address and name

特西郡富士宮市根原232 中島 邦彦

Birth date

平成 03年06月15日生

Sire's name and its H. B. Registry No.

Paternal grand sire

Paternal grand dam

Dam's name and its H. B. Registry No.

Maternal grand sire

Maternal grand dam

Date of registration

平成 03年08月31日

Membership's code

5402106717

The Association's name and seal

日本ホルスタイン登録協会

Fig. 4-4-11: The specimen of Herd Book Registry certificate

[illegible]

Fig. 4-4-12: Type Evaluation Report Form

THE HOLSTEIN CATTLE ASSOCIATION OF JAPAN NO.
38-13, HON-CHO 4-CHOME, NAKANO-KU
TOKYO, JAPAN

CERTIFICATE OF EXPORT

REGISTRATION NO. DATE REGISTERED

NAME

SEX BORN

SIRE NO. A. R. NO.

DAM NO. A. R. NO.

BRED BY

OWNED BY

EXPORTED BY

EXPORTED TO

RECORD OF ADVANCED REGISTRY (IF THIS ANIMAL IS REGISTERED IN A. R.)

(A) FEMALE (IF MALE, SEE (B) ON BACKSIDE OF THIS CERTIFICATE)

A. R. NO. DATE REGISTERED

(1) TYPE CLASSIFICATION

OFFICIAL SCORE POINTS DATE CLASSIFIED

(2) PRODUCTION

AGE (YR. MO.)	LENGTH OF RECORD (DAYS)	MILKING (X)	MILK (kg.)	FAT (kg.)	FAT %	PRODUCTION INDEX (%)

THIS CERTIFICATE IS ISSUED IN FULL RELIANCE UPON THE TRUTH OF THE STATEMENTS CONTAINED IN THE APPLICATION AND/OR THE REPORTS FILED THEREFORE, BUT IN NO EVENT IS IT TO BE DEEMED A GUARANTEE BY THE ASSOCIATION OF THE BREEDING OF THE ANIMAL AND/OR THESE REPORTS AND SHOULD SAID APPLICATION AND/OR REPORTS BE PROVED UNTRUE, THIS CERTIFICATE AND REGISTRY OF THE ANIMAL WILL BE CANCELLED

DATE

EXECUTIVE SECRETARY
FOR THE HOLSTEIN CATTLE ASSOCIATION OF JAPAN

Fig. 4-4-15: The form of certificate of export of livestock

CERTIFICATE OF SEMEN FOR EXPORT

BULL FROM WHICH SEMEN COLLECTED

NAME. _____

H. B. Reg. No. _____

NAME AND ADDRESS OF THE A. I. UNIT

NAME. _____

ADDRESS. _____

BATCH NUMBER AND DATE OF COLLECTION

BATCH NUMBER

DATE OF COLLECTION

NO. _____

NO. _____

NO. _____

NO. _____

DATE OF FREEZING. _____

NUMBER OF AMPOULES FROZEN. _____

NUMBER OF AMPOULES SHIPPED. _____

DATE OF SHIPMENT. _____

NAME AND ADDRESS OF CONSIGNEE

I HEREBY CERTIFY THAT THE ABOVE NAMED BULL WAS IDENTIFIED WITH HIS CERTIFICATE OF REGISTRY WHEN THE ABOVE SEMEN WAS COLLECTED, THAT EACH AMPOULE IS CORRECTLY LABELED AND THAT THE OTHER ITEMS ARE CORRECT IN EVERY PARTICULAR.

THE HOLSTEIN CATTLE ASSOCIATION OF JAPAN

BY _____

TITLE _____

DATE _____

F i g.: 4-4-16 ; Certificate of Semen for Export

Registry System of Purebred Jersey Cattle

registration

Recognition

Female

H. B. Registry

Sire must be H. B. Registered animal, and

Dam must be H. B. Registered animal or must be registered animal as a Supplementary Registry B.

Guaranteed H. B. Registry

All of the following requirements must be met.

- (1) Type classification score: appraised at 18 months or later, 74 points or more.
- (2) Milk production record: 117kg or more in butterfat production and 4.5% fat % or more in official milk records of 305-day 2X M.E. basis.

Advanced Registry

All of the following requirements must be met.

- ① In the case of daughter of Advanced Registry Animal or Guaranteed H. B. Registry Animal.
 - (1) type classification score: appraised at 24 months or later, 76 points or more.
 - (2) Milk production record: 142kg or more in butterfat production and 4.5 fat % or more in official milk records of 305 day, 2X M.E. basis.
 - (3) Undesirable recessives: not to be detected in the sample offspring.
- ② In the case other than the former.
 - (1) Type classification score: appraised at 24 months or later, 77 points or more.
 - (2) Milk Production record: 184kg or more in butterfat production and 4.5 fat % or more in official milk records of 305 day, 2X M.E. basis.
 - (3) Advanced Registry offspring: 2 h
 - (4) Undesirable recessives: not to in the sample offsprings.

Honorable Merit Cow

All of the following requirements must be met.

- (1) Advanced Registry offspring: 3 head or more.
- (2) Calving four times within 1400 days, or have three successive lactation records each of which reaches the standard A-1 of butterfat.
- (3) Have no progeny and sibs with undesirable recessives.

Male

H. B. Registry

The following requirements must be met.

its sire and dam are to be Advanced Registered.

or

its sire and dam are to be recognized as an animal with the same qualification as Advanced Registry.

Advanced Registry

All of the following requirements must be met.

- (1) Type classification score: 80 points or more at the age of 16 months or later.
- (2) Advanced Registry offspring: 10 head or more get from different dam respectively.
- (3) Undesirable recessives: not to be detected in the sample offspring of 25 head or more.

Honorable Merit Bull

All of the following requirements must be met.

- (1) Advanced Registry offspring: 30 head or more.
- (2) The average of type score of daughters: 76.5 points or more.
- (3) The average of type score of sons: 82 points or more.
- (4) The average of butterfat production index of daughters must be at least 130.
- (5) Yapp's Bull index must be at least 280 kg fat on a 305 day, 3X M.E. basis by survey of 5 pairs or more.
- (6) Have no progeny and sibs with undesirable recessives.

(revised in Aug. 11, 1989)
[to be applied to female animals only]

First Generation

When all of the following requirements have been met, the foundation stock is registered as a Foundation Registry.

- (1) The sire is an animal registered as H.B. Registry.
(2) The dam is an animal registered as Foundation Registry.

10

In case of the dam is an un registered animal, the animal itself is to be prospective as a foundation stock, also, its dam must have coat color and characteristics peculiar to Jersey breed.

Foundation Registry

All of the following requirements must be met.

- (1) The classification score:
appraised at 18 months or later, 74 points or more.
- (2) Milk production records:
117kg or more in butterfat production and
4.5% or more in official milk records of
305-day, 2X M.F. basis.

Supplementary Registry A (SPA)

Foundation Registry

(eligible to apply for SRB)

(In the case of failure.)

When her dam was registered as SRA, she can be registered as SRB.

Supplementary Registry B (SRB)

Foundation Registry

(eligible apply for H.B. Registry)

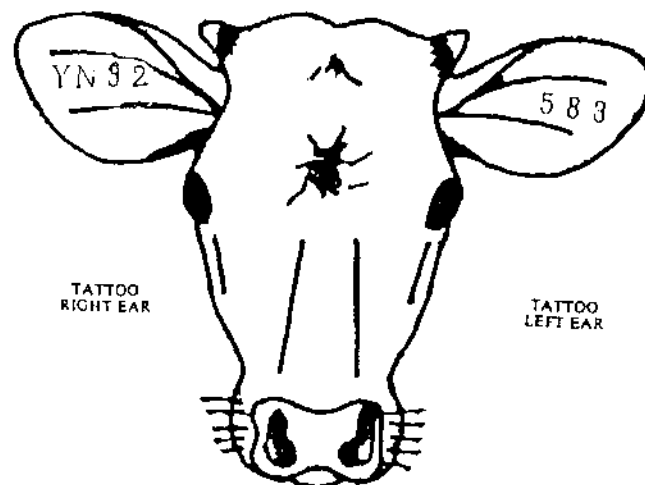
Enter into

(2nd Generation)

When her dam was registered as SSB, she can be entered into H. B. Registry.

(daughter: direct course)

note: (1) $\rightarrow$ indicates the next generation.
(2) $\leftrightarrow$ indicates the same generation.



F i g . 4 - 4 - 1 9 : tatooing letters and its position