Manual
for Diagnosis and Treatment
of Reproductive Disorders in Dairy Cattle

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Japan Livestock Technology Association
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Chapter 1  Reproductive physiology of dairy cattle

1. Structure of reproductive organs

Female reproductive organs are roughly divided into the ovary, oviduct, uterus, uterine cervix, vagina and vulva.

Their locations in the body are as shown in Figures 1-1 and 1-2.

![Reproductive organs of cows](image)

**Figure 1-1** Reproductive organs of cows

![Reproductive organs of cows (partial section of the dorsal part)](image)

**Figure 1-2** Reproductive organs of cows (partial section of the dorsal part)

1. Ovary
2. Proper ligament of ovary
3. Abdominal opening of uterine tube
4. Ovarian fimbria
5. Ampulla of uterine tube
6. Isthmus of uterine tube
7. Mesosalpinx
8. Uterine horn
9. Volum uterus
10. Caruncle
11. Broad ligament of the uterus
12. Interstitial ligament
13. Uterine cavity and isthmus
14. Uterine cervix
15. Internal uterine orifice
16. External uterine orifice
17. Cervical canal
18. Vaginal part of cervix
19. Vagina
20. Vaginal vestibule
21. Orifice of longitudinal duct of ophophoron
22. Greater vestibular gland
23. Outlet of greater vestibular gland
24. External urethral orifice
25. Pudendal lip
26. Clitoris

(1) Ovary

A cow has the left and right ovaries and follicular growth and maturation, ovulation, and luteal formation and regression occur in these ovaries of mature cows according to the estrous cycle.
(2) **Oviduct**

The oviduct following the ovary serves as a route through which an emitted ovum is transported and is the location where the ovum encounter sperm and fertilization takes place. The oviduct consists of the infundibulum with fimbriae, ampulla and isthmus (see Figure 1-3.). Fertilization occurs in the ampulla portion. A fertilized ovum descends within the oviduct and enters the uterus while undergoing cleavage. A fertilized ovum after the start of cleavage is generally referred to as an embryo.

A fertilized ovum that started cleavage in the oviduct develops through the 2, 4, 8, …cell stages into the morula stage. It further develops to form internal cavities (blastula cavities) in the gaps among cells where it stores protein-rich fluid. An embryo at this stage is referred to as a blastocyst.

An embryo enters the uterus when it is at the stage of morula or blastocyst, about 7 days after ovulation.

![Diagram of Oviduct and Cleavage Stages](image)

Figure 1-3  Descent of an ovum in the oviduct (cow)

(3) **Uterus**

An embryo that enters the uterus is implanted on the endometrium and continues to grow. It then gradually develops an external shape characteristic of adult cattle. The internal structures become similar to those of adult cattle to carry out functions. An embryo at this stage onward is referred to as a fetus. The pregnancy period is divided into the ⊗ ovum period, ⊠ embryonic period and ⊡ fetal period.
The endometrial surface is covered with mucous membranes that contain semispherically elevated structures called caruncles. After the fetal membrane is closely attached to the endometrium and attains implantation, only the portion of the fetal membrane that matches the caruncle develops and forms cotyledons. This is referred to as a cotyledonary placenta. A cow has about 80-120 caruncles in its uterus (see Figure 1-2 (p.1)).

4. Uterine cervix

The lumen of the uterine cervix is referred to as the cervical canal. Circular folds are present on the internal surface of the cervical canal of cattle and the lumen is rigidly closed. The exit of the cervical canal on the uterine side is referred to as the internal uterine orifice and the one on the vaginal side the external uterine orifice. The external uterine orifice protrudes into the vagina and this portion is referred to as the intravaginal part of the uterus.

5. Vulva

The pudendum refers to the end of the urogenital canal of female cattle and the exit to the outside of the body is referred to as the vulva.

2. Sexual maturity, estrous cycle, pregnancy, parturition, and return of estrus after parturition

1. Sexual maturity and breeding age

Sexually mature cows repeat estrus at certain intervals. In Holsteins, the ovary starts functioning at the age of 6-12 months (puberty) but cows are usually capable of pregnancy and calving at and after the age of 6-18 months. However, they are still developing at this age so they are usually subjected to mating after reaching 14-22 months old (breeding age) because their subsequent growth may be hindered. The time of puberty and sexual maturity varies significantly depending on individuals and is affected by various environmental factors as described in detail in Chapter 1 Section 4. The first ovulation after puberty does not show any estrous signs in most cases and the first estrous cycle is short.

2. Estrous cycle

1. Emergence of estrous cycle

In non-pregnant sexually mature cows, a cycle consisting of follicular growth, ovulation, and luteal formation and regression is repeated in the ovary and estrus emerges when the ovarian follicle is mature. These periodical changes are referred to as the estrous cycle. The estrous cycle is generally divided into the estrous phase and the luteal phase, the latter of which is further divided into the luteal formation stage (early luteal phase), functional luteal stage (luteal peak phase) and the luteal regression stage (end luteal phase).

2. Length of estrous cycle

In temperate zones including Japan, the length of the estrous cycle ranges from 18 to 25
days with that of nulliparous being 20 days and that of multiparous 21 days. As described in detail in Chapter 1 Section 4, this cycle is influenced by breed, season and age and extends to 22 days in old cows. In the Northern Hemisphere, the estrous cycle is longer in spring and autumn than in summer and winter.

3 Changes that occur in the reproductive organs during estrous cycle

i. Growth of ovarian follicles

A cow originally has several tens of thousand to a hundred thousand primordial ovarian follicles in its ovary. The process in which these primordial ovarian follicles mature is shown in Figures 1-4 and 1-5 (p.5). They develop through the primary and secondary ovarian follicles into vesicular ovarian follicles (graaflian follicles) and emit ova. However, not more than 200 primordial ovarian follicles mature and emit ova during a cow's lifetime and the remaining follicles degenerate in the ovary.

ii. Maturity of ovarian follicles

A mature ovarian follicle is 12-24 mm in diameter and is enveloped with an external theca layer, internal theca layer and granulosa layer. An oocyte is enveloped with granulosa cells to form a germ hill and projects into the follicular antrum (see Figure 1-5 (p.5)). The follicular antrum is filled with follicular fluid.

Figure 1-4 Schematic view of bovine ovary

1. Hilum of ovary 2. Blood vessel
3. Epithelium 4. Ovarian alboginea
5. Primordial ovarian follicle 6. Primary ovarian follicle
7. Secondary ovarian follicle 8. Growing mature ovarian follicle
11. Corpus luteum at functional luteal stage 12. Corpus luteum at luteolysis stage
15. Interstitial gland 16. Connective tissue
Development from the primordial ovarian follicle to the stage before the formation of follicular antrum occurs in cows without hypophysis. The final development from the formation of follicular antrum to ovulation depends on gonadotropins. This second-stage development is shorter than the first-stage development. Maturity of an oocyte (resumption of meiosis and maturity of cytoplasm) only occurs after an LH surge.

iii. Ovulation

Ovulation normally occurs after estrus and between 2 and 20 hours after estrus although it rarely occurs before the end of estrus. It most commonly occurs between 10 and 15 hours after estrus.

Changes that occur within the ovary before and after ovulation are as follows. Following ovulation surge, the pituitary gland releases a large quantity of luteinizing hormone (LH), which activates proteolytic enzymes such as collagenase contained in ovarian follicles and plasmin. Consequently, follicular walls become coarse-textured, permeability increases and the ovarian follicle expands rapidly. The pressure inside the ovarian follicle does not increase because of reduced tension in the follicular walls before ovulation but part of the ovarian follicle expand because of a reduction in blood supply. This expanded portion is referred to as a stigma. An ovum is discharged through this stigma. Ovulation is not completed instantly but takes some time (Figure 1-6 (p.6)). Follicular fluid first leaks through a tiny hole in the follicular wall (stigma) and then an oocyte enveloped by granulosa cells is discharged by the action of prostaglandin produced within the ovarian follicle, as suggested by a study.
iv. Formation and regression of the corpus luteum

Immediately after ovulation, the follicular antrum is filled with blood, some follicular fluid and leaked lymph fluid. Subsequently, luteal cells are formed by both surrounding granulosa and internal theca cells, the internal portion is enriched and the corpus luteum is formed (see Figure 1-7 (p.7)). The corpus luteum is completed within 7-8 days of ovulation with a longitudinal length of 20-25 mm (functional corpus luteum). It continues developing and reaches a maximum longitudinal length of 20 mm or more within 13-15 days of ovulation. After the functional corpus luteum stage, the corpus luteum starts rapid regression from the 17th to 18th day of the estrous cycle and shrinks and hardens. It also changes in color from orange during the functional corpus luteum stage to yellowish orange and finally to reddish brown (corpus luteum). The corpus luteum does not have the same function as the corpus luteum or excrete hormones. The pregnant corpus luteum remains on the ovarian surface for a long period after regression and is referred to as the corpus albicans.
Upper:
The early luteinization stage after ovulation. Multiplied granulosa cells are seen forming characteristic folds of various sizes centripetally and forming corpus luteum.

Lower:
Corpus luteum at functional luteal stage. The head of enriched spherical corpus luteum is protruding like a mushroom from a rupture on the ovarian follicle.

Figure 1.7  Formation of the corpus luteum
('In a simian ovary)

4 Estrous signs

Estrous signs in cows include a sharp look, increased sensitivity to sounds, reduced appetite, reduced rumination and loose stools. Estrous cows howl with a peculiar loud cry and wander about for bulls. They may mount on and be mounted by other cows raised together. They stand still and allow other cows to mount them when they are at estrous peaks. This behavior is referred to as a standing estrus and represents the estrous phase in a strict sense. The pudendum becomes congested and swollen and a large amount of mucus leaks from it. The cervical mucus in the estrus stage has increased electric conductivity and lowered pH values as shown in Figure 1-8 (p.8). Increased conductivity suggests an increase in salinity of the mucus and coincides with the fact that the endocervical smear of estrous cervical mucus reveals salt crystals (see Figure 1-9 (p.8)). The development of equipment for detecting estrus using conductivity as an indicator has been attempted.

The mounting and standing behavior of estrous cattle is most active between midnight and early morning and the duration of estrus as a textbook describes ranges from 10 to 20 hours, although in some cases estrus lasts for only a few hours. The duration of estrus is influenced by many environmental factors as described in detail in Chapter 1 Section 4.
Optimum time of insemination

Optimum time of insemination is described in detail in Chapter 4 Section 2-(6).

See Chapter 1 Section 4 for a detailed description of the effects on the optimum time of insemination of various environmental factors such as the shortening of the duration of estrus due to a recent tendency to high-lactation cows.

Identifying the start of estrus in cattle is important in determining the optimum time of insemination and achieving high conception rates. However, monitoring cattle continuously is difficult so various tools such as heat mount detectors, tail paint, chin balls and pedometers have been used and teasers (bulls for detecting estrus) that are treated in various ways have been experimentally introduced.

Bleeding after ovulation

Some cows leak blood-like mucus or blood from the pudendum 1-4 days after the end of estrus. Leaks occur 2 days after the end of estrus in most cases and the incidence and amount of blood leaked is higher in nulliparae than in multiparae. Bleeding occurs at uterine mucous membranes. This bleeding indicates the end of estrus and helps predict the following estrus.
Hormonal control of estrous cycle

Hormones that control the estrous cycle include those indicated in Table 1-1.

<table>
<thead>
<tr>
<th>Hormone-producing organs</th>
<th>Hormones</th>
<th>Chemical property</th>
<th>Molecular weight</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothalamus</td>
<td>Gonadotropin releasing hormone (GnRH, LHRH)</td>
<td>Peptide</td>
<td>1,182</td>
<td>Promotes release of FSH and LH.</td>
</tr>
<tr>
<td></td>
<td>Corticotropin releasing hormone (CRH)</td>
<td>Peptide</td>
<td>Approx. 4,900</td>
<td>Controls GnRH secretion. (Controls gonad function under stress).</td>
</tr>
<tr>
<td></td>
<td>Oxytocin (Stored in the posterior lobe of the pituitary gland. Also produced by the ovary)</td>
<td>Peptide</td>
<td>1,007</td>
<td>Promotes uterine contraction, calving, transportation of sperm and ova, and milk ejection. Most likely to have luteolytic action.</td>
</tr>
<tr>
<td></td>
<td>Melatonin (Secreted by the pineal body)</td>
<td>Peptide</td>
<td>232</td>
<td>Involved in the seasonal expression of reproductive activity of seasonal breeders.</td>
</tr>
<tr>
<td>Anterior lobe of the pituitary gland</td>
<td>Follicle stimulating hormone (FSH)</td>
<td>Glycoprotein (heterodimer of and subunits)</td>
<td>41,000</td>
<td>Promotes follicular growth, spermatogenesis and estrogen secretion.</td>
</tr>
<tr>
<td></td>
<td>Luteinizing hormone (LH)</td>
<td>Glycoprotein (heterodimer of and subunits)</td>
<td>26,000</td>
<td>Promotes ovulation and luteal function. Promotes secretion of progesterone, estrogen and androgen.</td>
</tr>
<tr>
<td></td>
<td>Progesterin (PRG)</td>
<td>Simple protein</td>
<td>22,000</td>
<td>Promotes lactation and in some animal species, luteal function and progesterone secretion. Enhances maternal behavior. Promotes tissue and bone growth.</td>
</tr>
<tr>
<td>Placenta</td>
<td>Human chorionic gonadotropin (hCG)</td>
<td>Glycoprotein</td>
<td>14,500</td>
<td>Shows LH action. Maintains pregnant corpus luteum in primates.</td>
</tr>
<tr>
<td></td>
<td>Pregnant man serum gonadotropin (hMG, etc)</td>
<td>Glycoprotein</td>
<td>53,000</td>
<td>Shows mainly FSH action. Promotes formation of the accessory corpus luteum in horses.</td>
</tr>
<tr>
<td></td>
<td>Placental lactogen (PL)</td>
<td>Glycoprotein</td>
<td>20,000 - 50,000</td>
<td>Controls nutritional supply from mother to fetus.</td>
</tr>
<tr>
<td></td>
<td>Protein B</td>
<td>Unknown</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Progesterone/corpus luteum</td>
<td>Steroid (C-21)</td>
<td>314</td>
<td>Promotes estrous behavior and helps reproductive tracts to prepare for implantation in conjunction with estrogen. Promotes secretion by the endometrium. Maintains pregnancy. Promotes growth of mammary glandular cells. Controls gonadotropin secretion.</td>
</tr>
<tr>
<td></td>
<td>Testosterone/testis</td>
<td>Steroid (C-19)</td>
<td>Approx. 290</td>
<td>Promotes growth of and maintains accessory reproductive glands. Promotes secondary sexual character, sexual behavior and spermatogenesis. Shows assimilation action.</td>
</tr>
<tr>
<td></td>
<td>Inhibin</td>
<td>Glycoprotein (heterodimer of and subunits)</td>
<td>14,000</td>
<td>Controls FSH release.</td>
</tr>
<tr>
<td></td>
<td>Activin</td>
<td>Simple protein (heterodimer of subunits)</td>
<td>Approx. 28,000</td>
<td>Promotes FSH release.</td>
</tr>
<tr>
<td></td>
<td>Oxytocin</td>
<td>Peptide</td>
<td>1,007</td>
<td>Interacts with prostaglandin secreted by the endometrium to promote luteolysis.</td>
</tr>
<tr>
<td></td>
<td>Relaxin</td>
<td>Peptide</td>
<td>Approx. 6,000</td>
<td>Involved in opening of the cervix canal, uterine contraction, maintenance of pregnancy and relaxation of pelvic symphysis.</td>
</tr>
<tr>
<td>Uterus</td>
<td>Prostaglandin F₂ (PGF₂)</td>
<td>Unsaturated fatty acid</td>
<td>Approx. 350</td>
<td>Causes uterine contraction and luteolysis.</td>
</tr>
</tbody>
</table>

(Prepared based on Lees, 1987, etc.)
GnRH: gonadotropin releasing hormone, FSH: follicle-stimulating hormone, LH: luteinizing hormone, PGF$_{2\alpha}$: prostaglandin F$_{2\alpha}$.

Figure 1-10  Changes in GnRH, FSH, LH, estradiol-17β, progesterone, PGF$_{2\alpha}$ levels in blood in the estrous cycle
The changes in the levels of these hormones in peripheral blood during the estrous cycle are illustrated in Figure 1-10 (p.10). Gonadotropic hormone releasing hormone (GnRH), produced and secreted by the hypothalamus and directly transported to the anterior lobe of the hypophysis through the hypophyseal portal vein, is involved in the secretion by the pituitary gland of follicle stimulating hormone (FSH) and luteinizing hormone (LH).

At the time of an LH surge by the pituitary gland during the estrous phase, large quantities of FSH are also released, forming the first FSH peak. Unlike LH, FSH levels in the blood increase after ovulation as well. This second FSH peak, which is not as high as the first one, is produced because ovarian follicles that serve as a matrix for producing inhibin are lost as a result of ovulation and the control of FSH secretion is lost.

Ovarian estrogen (E) is secreted mainly by ovarian follicles, progesterone (P₄) by the corpus luteum and testosterone (T) by the testes. These steroid hormones are biosynthesized from cholesterol through the pathway shown in Figure 1-11. These steroid hormones produced by the ovary work on the uterus and control its function. P₄ along with E works on the reproductive tract at the implantation stage to help prepare for implantation of embryos and plays an important role in maintaining the pregnant status. At the time of delivery, blood P₄ levels decrease and E levels increase in advance of delivery to form a birth canal and thus both hormones cooperate to facilitate normal delivery.

Prostaglandin F₂α (PGF₂α) is a major hormone secreted by the uterus and is secreted from the endometrium at the luteolytic stage to facilitate luteal regression. It also facilitates the shrinkage of the uterus at the time of delivery.

![Figure 1-11: Biosynthesis pathway of sexual steroid hormones (Niswender, 1974)]
(3) Pregnancy

Pregnancy refers to the condition of cows during the period in which an emitted ovum is fertilized and develops and until a fetus is delivered.

1. Gestation length

The gestation length of cattle is 278 days on average; that for male fetuses is 1-2 days longer than for female fetuses, that for primiparous and secondary fetuses of young cows is 1-2 days shorter than for fetuses of middle-aged cows and that for twins is 3-6 days shorter than for monotocious fetuses.

2. Pregnancy diagnosis

Identifying the pregnant status as early as possible after fertilization or mating is important in improving productivity and reproductive disorders. Changes in the mother’s body resulting from pregnancy and pregnancy signs showing the presence of a fetus are used in pregnancy diagnosis and the method used in pregnancy diagnosis should ensure early, accurate and simple diagnosis and must not involve harm to the mother’s body or fetus.

In practice, ultrasonography, non-return method, determination of milk or blood P₄ levels, palpation of the amniotic sac, fetal membrane slip, asymmetry of uterine horns, swelling of the pregnant horn and fluctuation, cervical mucus tests and palpation of the fetus and placental lobes are performed. See Chapter 4 for details.

3. Maintenance of the pregnancy

Important factors for the maintenance of pregnancy include the progestational proliferation of the endometrium, uterine expansion, reduction in the contraction of uterine muscle and uterine cervix closure. P₄ and E are involved in all these factors. P₄ levels in peripheral blood after conception are in the range of 4-8 ng/ml and the main source of P₄ production is the corpus luteum throughout pregnancy. However, the P₄ secretion from the corpus luteum decreases in late pregnancy and the placenta and adrenal glands make up for this decrease.

4. Location of the fetus in the uterus

The location of the fetal body relative to the mother’s body is expressed as “presentation” and “position” and the locational relationships of the fetal head and extremities are expressed as “fetal attitude.”

i. Presentation

If the long axes of the fetus and the mother are parallel to each other, the presentation is referred to as “longitudinal lie” and if these axes cross, it is referred to as “transverse lie” or “oblique lie.” A head presentation is when the head of the fetus faces the pelvic cavity; a breech presentation is when the breech of the fetus faces the pelvic cavity (also known as back or abdominal presentation). In cattle, about 90% of presentations are head presentations and 10% are breech presentations.
ii. Position

A position indicates which of the uterine surfaces the fetal spine faces: superior, inferior or lateral. In cattle, fetuses assume the lateral position in late pregnancy.

iii. Fetal habitus

Fetal habitus refers to the locational relationships of the fetal head/neck part and fore and hind legs. During pregnancy, the fetus assumes a ventrally rounded posture according to the uterine shape. At the time of delivery, the fetus extends its fore legs, head and hind legs to pass through the tight birth canal.

(4) Parturition

After the fetus develops sufficiently in the uterus to be able to live in the external environment, it is released from the mother’s body. This process is referred to as delivery. Parturition is induced by the expulsive power generated by the combination of the contraction of uterine muscle, which is accompanied by labor pain, and abdominal pressure (straining) resulting from the contraction of abdominal muscle.

1. Signs of Parturition

The pudendum is red due to congestion and soft because of swelling. The mucous plug that blocked the uterine cervix begins to soften from 2-3 days before parturition and is fluidized. It then flows into the inner part of the vagina and eventually leaks from the vulva in the form of mucus similar to starch syrup. The intravaginal part of the uterus swells to the size of a fist and the external uterine orifice expands to the extent such that it allows 2-3 fingers to be inserted. Because the pelvic ligament relaxes as the parturition approaches, both sides of the tail head are depressed so that the tail head looks elevated. This is particularly apparent 2-3 days before parturition.

The swelling of the udder becomes increasingly noticeable as the delivery time approaches and trial milking may show that the initial relatively clear honey-like secretion changes to an opaque colostrum.

As parturition approaches, pregnant cows show uneasiness, look at their abdomen frequently, wander about inside the cow shed restlessly, repeat lying and standing up and scratch the floor with their fore legs. They urinate more frequently and leak urine sporadically.

2. Parturition process

The birth canal is a route through which the fetus passes at the time of parturition and consists of the bony birth canal and soft birth canal. The bony birth canal is a pelvic cavity that is surrounded by the pelvis consisting of the hipbone, sacrum and coccygeal bone. The joints of these bones are fixed with ligaments but they relax by the action of E and relaxin as the delivery time approaches, resulting in an enlarged pelvis. The soft birth canal consists of the uterine cervix, vagina and vulva and is enlarged at the time delivery.

A virtual line along which the fetal vertical axis may pass the pelvic cavity is referred to
as the pelvic axis. The fetus must be drawn along this pelvic axis. The pelvic axis of cattle is undulated vertically Figure 1-12; the fetus must be drawn upward until the fetal head emerges completely, horizontally until the chest emerges and downward until the full body emerges.

Figure 1-12  Bovine pelvis (From Stoss, modified by S. Hoshi and Y. Yamauchi, 1982)

The delivery process is divided into the opening period (1st stage), expulsion period (2nd stage) and the afterbirth period (3rd stage). During the opening period, the birth canal is formed to prepare for delivery. In the expulsion period, the opening of the uterus is fully open and the fetus is delivered. The afterbirth period refers to a postpartum period until the afterbirth is discharged.

During the opening period, the fetal sac enters the cervical canal in advance of the fetus to dilate it. Opening labor pains last for a period of 3-6 hours, during which the fetus is more active and changes its position from lateral to superior positions.

The expulsion period starts with contraction or straining of the abdominal wall. Straining occurs 8-10 times every time the uterus contracts. The allantoic chorion ruptures since it is attached to the placenta and cannot move with the fetus. The amnion is relatively mobile so it is exposed outside the vulva and forms the foot sac. The rupture of the allantois is referred to as the first rupture of bag and that of the amnion as the second rupture of bag. Delivery is smooth once the fetal chest passes the vulva. The newborn's umbilical cord may be spontaneously broken at birth but it is not cut before the mother moves in some cases.

During the afterbirth period, contraction of the abdominal wall almost subsides but uterine contraction still continues. The contraction force is reduced but its frequency is increased. The fetal placenta is exfoliated and discharged as a result of afterbirth labor. It is normally discharged in 6-8 hours but may not be discharged in a fixed time. In these cases it is referred to as the retained placenta, which occurs frequently in cattle because of cotyledonary placenta.

Major hormones involved in parturition

Parturition is induced by hormonal changes in the fetal hypothalamo-pituitary-adrenal system, as demonstrated by a study of sheep. As the parturition time approaches, adrenocorticotropic hormone (ACTH) is probably secreted by the fetal pituitary gland and in response to this, a large quantity of cortisol is secreted by the fetal adrenal glands. This cortisol acts on the placenta to secrete a large quantity of estrogen (E) and to reduce the secretion of progesterone (P₄). Increased estrogen stimulates the production and secretion of prostaglandin F₂α (PGF₂α) by the placenta and also act on the maternal
uterus to produce and secrete more PGF<sub>2α</sub>. In addition, estrogen and the relaxin secreted by the ovary help relax the birth canal. As a result of reduced P<sub>4</sub> secretion and increased E secretion, the proportion of P<sub>4</sub> and E levels in the maternal blood changes, the uterine muscle is more sensitive to oxytocin (OT) and contraction starts. After the start of parturition, the fetus stimulates the cervical canal wall to dilate and also stimulates OT secretion by the nervus lobe of hypophysis. As a result, the fetus is given birth. These mechanisms demonstrated in sheep are considered to be common to cattle.

Changes in the levels of these hormones in peripheral plasma of cattle around parturition have been reported as shown in Figure 1-13. These hormonal level changes reflect the mechanisms of parturition induction described above.

![Graph showing hormone concentrations](image)

**Figure 1-13** Trends in hormone concentrations in the peripheral plasma of the cow around the time of parturition. (Day 0 at parturition)

4 Physiology of puerperium

After parturition, involved organs such as the uterus try to reverse the changes produced by the effects of pregnancy and parturition and restore to their original state. This process is referred to as puerperium and normally lasts for 3-4 weeks. Involved in the restoration of reproductive organs are oxytocin (OT) and prostaglandin (PG). The PG level rapidly increases at delivery, reaches its peak within 3 days of parturition and returns to the baseline level in about 15 days. Uterine contraction disappears in 4 days and the pregnant horn is reduced to half in 5 days in breadth and in 15 days in length. The cervical canal tightens to the extent that inserting a hand is difficult in a day and
narrowed to the extent that it only allows two fingers to be inserted in 4 days. Liquid
excretion, or lochia, is the most abundant between 2 and 3 days after parturition and
disappears within 14-18 days of parturition. Uterine restoration in terms of size
(uterine involution) is completed in 25 days or more but the normalization of the
intrauterine bacterial flora may take 40-45 days. See Chapter 1 Section 4 for more
details.

5. Return of estrus after parturition

The ovary is static immediately after deliver, however ovarian follicles start growing in
about a week at the earliest and the ovary is activated quickly as soon as ten days have
passed. The first ovulation takes place in about 2 weeks after parturition at the earliest
and about 20 days in most cases. Development of ovarian follicles is delayed in
nursing cows compared to milking cows and the first ovulation occurs earlier in cows
that take in more energy.

The mean number of days between parturition and the first estrus varies significantly
from report to report, however it is estimated to be 20-70 days and is greatly affected by
the age, parity, season and nutritional and lactation conditions. It is known to increase
in high-lactation cows as well as in cows that suffer abnormal parturition and diseases.

The estrus at the time of the first ovulation after parturition is dull in nature and does not
show estrous signs in many cases. However, the incidence of dull estrus is lower in
cows that have experienced ovulation more times. The corpus luteum that is formed
after the first postpartum ovulation generally grows poorly and is short-lived.

3. Latest progress in the studies of mechanisms of controlling reproductive function

1. Changes in hormone concept

Hormones have been conventionally considered to be chemical messengers that are
secreted by endocrine organs and transported to remote target organs to transmit
information. However, recent advances in research have revealed various substances
that are difficult to differentiate from hormones. Studies of the mode of action have
also demonstrated that a certain hormone not only exhibits an endocrine action but also
plays an important role in facilitating reproductive function by means of paracrine and
autocrine action and that neurotransmitters and immune-related substances that have not
been considered hormones are involved in reproductive function (see next section).
These substances include insulin-like growth factor (IGF), epidermal growth factor
(EGF), transforming growth factor (TGF), nerve growth factor (NGF), immune-related
cytokines such as interleukin 1 (IL-1) and neuropeptides such as dopamine, enkephalin
and substance-P. These substances are now included as hormones and referred to as
new hormones compared to conventional hormones, which are called classic hormones.

2. Hormonal mode of action

Recent advances in research have shown that hormones can act locally on cells adjacent
to the hormone-secreting cells or, in some cases on the hormone-secreting cells
themselves instead of being transported to remote locations. The mode of action of
hormones is shown in Figure 1-14 (p.17). Signal messengers with paracrine and
autocrine action that have not been considered hormones are now included as hormones.
(3) Feedback mechanisms

Reproductive function is controlled by hormones secreted by endocrine organs such as the hypothalamus, pituitary gland and gonad. An assumption is made that the mechanism depends on the hypothalamus-hypophyseal-gonadal axis and the hormones secreted by higher organs control the functions of lower organs. However, research shows that hormones secreted by lower organs may control the functions of higher organs and these mechanisms are referred to as feedback mechanisms. Feedback mechanisms include promotive (positive) and suppressive (negative) ones (Figure 1-15).
(4) Receptors

Hormones transmit signals by binding to the receptors of target cells. In the case of peptide or protein hormones such as GnRH, LH and FSH, if these hormones bind to receptors on cell membranes, second messengers are produced within cells and control cell function in place of the hormones.

The receptors of steroid hormones secreted by the gonad are located on cytoplasm or within nuclei. Steroid hormones are fat-soluble and have generally low molecular weight so they easily pass through cell membranes and bind to intracellular receptors to transmit signals. Unlike peptide hormones, steroid hormones do not need second messengers.

Reproductive functional disorders have been attributed to lack of hormones but the latest studies show that these disorders include some cases of hormone irresponsiveness resulting from abnormal receptors and abnormal action after the binding of hormones and receptors.

(5) Assay methods of hormones

The determination of hormones relied on bioassays in early studies. Sensitivity, specificity, accuracy and reproducibility have dramatically improved since immunoassay was developed. Radioimmunoassay is particularly sensitive. Due to various constraints incidental to the use of radioisotopes, however, enzyme immunoassay (EIA) that uses enzymes instead of isotopes as tracer has been developed. Free from constraints accompanying the use of isotopes, EIA can be employed by ordinary laboratories. Determination kits for progesterone (P₄) are available on the market. Kits operable in the field are also available for the determination of blood and milk P₄ levels and pregnancy diagnosis.

(6) Pulsatile secretion of hormones

Dramatically improved sensitivity of the determination methods of hormones significantly reduced the quantity of samples required for measurement. This allowed frequent determination of hormone levels at short intervals and hence the analysis of minute fluctuations in blood hormone levels. The results of these analyses show that hormones are secreted at certain intervals in a pulsatile manner instead of being secreted in certain amounts continuously (refer to Figure 1-10 (p.10)). As a result of pulsatile secretion of a hypothalamic hormone GnRH, FSH and LH are secreted by the pituitary gland in a pulsatile manner and then E₂ and P₄ by the ovary. The interval, frequency and amplitude of these pulses depend on the stage of estrous cycle and play important roles in the control of reproductive function.

(7) Ultrasonography

Dramatic advances in ultrasonographs allowed visualizing the organ and tissue structures without resorting to surgery (refer to Figure 1-16 (p.19)). Using this method, morphological changes in organs such as the development of the ovarian follicles and formation and regression of the corpus luteum can be visualized. Studies of morphological changes in the ovary using this method revealed that ovarian follicles are present even in the ovary of cows in the luteal phase and alternate between growth and regression regularly. This process is referred to as follicular waves.
Figure 1-16
View of an ovarian follicle and corpus luteum of a cow in the estrous cycle using ultrasonography
A: Dominant follicle, B-F: Other follicles, G: Corpus luteum
Material cow: Multiparous Holstein
Estrous cycle: at 6 days after ovulation (Day 6)
Equipment: Aloka SSD-610
Probe: Aloka UST-556T-7.5
(7.5 MHz, linear type)
(Laboratory of Theriogenology, Kitasato University)

(8) Follicular waves

Follicular growth and regression take place in bovine ovaries even in the luteal phase and follicular waves are generally repeated 2-3 times during the estrous cycle. This process is shown in Figure 1-17. Although more than one ovarian follicle starts growing simultaneously, the growth rate of only one of them increases rapidly when it reach a diameter of 4-5 mm and the remaining ovarian follicles stop growing and remain at the same size. The ovarian follicle that continues to grow is referred to as the dominant follicle. The dominant follicle continues to grow but in the luteal phase it begins to regress at a certain time point because of a lack of an LH surge and hence ovulation occurs due to high blood P₄ levels. If this first-wave dominant follicle starts regression, the second-wave follicles begin to grow and follow the same process. If the growth of the second-wave dominant follicle falls in the estrous phase, there is an LH surge and resultant ovulation take place. If the second-wave dominant follicle regresses rather than ovulates, then the third wave occurs and the third-wave dominant
follicle ovulates. With cattle, follicular waves take place 2-3 times during an estrous cycle in most cases and once or 4 times in rare cases.

(9) **Discovery of new hormones, inhibin and activin**

The follicular fluid of mature follicles of cows has been found to contain a relatively large proportion of inhibin that inhibits FSH secretion by the pituitary gland. This finding indicates that unlike LH secretion, FSH secretion is dually controlled by GnRH and inhibin. Inhibin is a glycoprotein hormone with a molecular weight of about 32,000 and also a heterogeneous dimer consisting of α and β subunits. Inhibin is produced mainly by the granulosa cells of the ovarian follicle in cows and the Sertoli's cells of the testes in bulls and is transported via the blood flow to the pituitary gland, where it inhibits FSH secretion by the pituitary gland. Studies of inhibin revealed the presence of activin having a physiological function opposite inhibin, a function to promote FSH secretion. Activin is produced by the same organs as inhibin. Activin is a homogeneous dimer consisting of the same β subunit as inhibin with a molecular weight of about 24,000 and plays an important role in cell differentiation as well. It is bound to follistatin in the blood so that it does not induce the pituitary gland to release FSH.

4. **Factors affecting reproductive function**

The reproductive process of dairy cattle includes sexual maturity, estrous cycle (follicular maturation, estrus, mating, ovulation and the formation and regression of corpus luteum), pregnancy, parturition and postpartum recovery of reproductive function. Factors affecting these reproductive functions include age, heredity, nutrition, lactation, season, summer heat stress and diseases.

(1) **Sexual maturity**

The period from birth to sexual maturity depends on the nutritional conditions and breed and is normally 7-18 months averaging 10 months. The body weight at the time of sexual maturity is 50% of that of adults in beef cattle and 35-45% in dairy cattle. The period required for sexual maturity depends on nutritional conditions to a greater extent than on breed.

① **Breeds**

The Zebu and Brahman require 6-12 months longer periods for sexual maturity than the European breeds.

② **Nutrition**

The time of sexual maturity is closely related to the body weight of cattle, which is related to nutrition. If a heifer is on a low-nutrition diet and showing poor growth, the ovarian follicles will not mature nor does estrus occur even after it reaches the standard sexual maturity time. In contrast, heifers that are in nutritionally good condition and growing well attain sexual maturity earlier than the standard.
Diseases

Diseases that adversely affect the growth delay sexual maturity.

(2) Factors affecting the length of estrous cycle

The length of the estrous cycle in the bovine is 20 days on average (normal range: 18-22 days) in nulliparae and 21 days (normal range: 18-24 days) in multiparae. The length is affected by days after calving and the occurrence of diseases in lactating cows.

(1) Parity

The length of the estrous cycle is one day shorter in heifers on average than in cows, as described above. Follicular waves occur mostly three times within an estrous cycle in lactating cows and two times in heifers. This explains the difference of one day in the length of estrous cycle between heifers and cows.

(2) The days after calving

The first postpartum estrous cycle is several days shorter than the subsequent cycles in many cases because the life span of the corpus luteum formed after the first ovulation is shorter than normal.

(3) Diseases

Mastitis may shorten the estrous cycle because of the early regression of the corpus luteum. A possible mechanism is that endotoxin produced by the causal bacteria enters the blood vessel and resulting in production of prostaglandin, which causes luteolysis.

(4) Ovarian dysfunction and embryonic death

In some cases of follicular cysts, estrus is repeated on short cycles. In the cases of persistent corpus luteum, on the contrary, the estrus cycle is extended.

The length of the estrous cycle is prolonged if embryonic death occurs after the 25th day of mating.

(3) Factors affecting estrous signs, duration and behavior

According to textbooks, the duration of estrus in cattle is 15 hours on average with a range of 2-30 hours. However, recent studies indicated that the duration of estrus has remarkably shortened in recent years (Table 1-2 (p.22)). Factors affecting the estrous duration include breeds, seasons, the presence/absence of sires, nutrition, milk yield, parity and the number of cows that exhibit estrus simultaneously. Factors that suppress estrous signs and behavior and shorten estrous duration are particularly important in reproductive management.

Endocrine mechanisms that express and suppress estrus are shown in Figure 1-18 (p.22).

As seen in the figure, progesterone (P<sub>4</sub>) that is secreted by the adrenal cortex in response
to nonspecific stress is thought to suppress estrus. Important causes of this nonspecific stress include nutritional disorders, high milk yield, summer heat and diseases.

The environment and the presence/absence of other estrous cows are also related to the intensity and duration of estrus.

Table 1-2 Major reports concerning bovine estrous duration

<table>
<thead>
<tr>
<th>Presenter</th>
<th>Estrous duration</th>
<th>Type of cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trimberger, 1948</td>
<td>17.8h</td>
<td>Multiparous dairy cattle</td>
</tr>
<tr>
<td>Hurnik et al., 1975</td>
<td>7.5 - 10.1h</td>
<td>Multiparous dairy cattle</td>
</tr>
<tr>
<td>Xu et al., 1998</td>
<td>8.6h</td>
<td>Multiparous dairy cattle (89 cows)</td>
</tr>
<tr>
<td>Dransfield et al., 1998</td>
<td>9.6h (SD6.9)</td>
<td>Multiparous dairy cattle (2661 cows)</td>
</tr>
<tr>
<td>Nebel et al., 2000</td>
<td>7.1h (SD5.4)</td>
<td>Multiparous dairy cattle (2055 cows)</td>
</tr>
<tr>
<td>Diskin, 2000</td>
<td>14.5h</td>
<td>Nulliparous dairy cattle</td>
</tr>
</tbody>
</table>

Figure 1-18 Mechanism of expression and suppression of estrous behavior in cattle
(Adapted from Allrich, 1994)

1 Breeds
The Zebu and Brahman have weaker estrous signs and 3-6 hours shorter duration of estrus than European breeds. Seasonal variation in the duration of estrus in Brahman cattle raised in the State of Florida, US, is presented in Table 1-3.

Table 1-3 Seasonal estrous duration of Brahmons raised in the State of Florida (Hansen, 1997)

<table>
<thead>
<tr>
<th>Season</th>
<th>Estrous duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>8.6 ± 2.3</td>
</tr>
<tr>
<td>Summer</td>
<td>5.5 ± 1.2</td>
</tr>
<tr>
<td>Autumn</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td>Winter</td>
<td>6.8 ± 1.6</td>
</tr>
</tbody>
</table>
Age

In cattle the estrous duration and cycle are shorter in younger animals than in adults. As seen in Table 1-4 that shows the relationship between the parity and estrous behavior in dairy cattle, the estrous behavior was more active in multiparous than in nulliparous and was more intense as the parity increased up to four. The proportion of cows that showed mounting behavior 12 hours after the discovery of estrus was only 17.7%, indicating that most of the cows studied had estrous duration shorter than 12 hours.

Genetic factors have been also found to be involved in the intensity of estrous behavior.

Bleeding after estrus was observed in 90% or more in nulliparous and 40-50% in multiparous.

Table 1-4  Effects of the parity on estrous behavior (the number of mountings) at the onset of estrus and 12 hours after in dairy cattle (Gwazdauskas et al., 1983)

<table>
<thead>
<tr>
<th>Time of observation</th>
<th>Estrous onset</th>
<th>12 hours after estrous onset</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard error</td>
</tr>
<tr>
<td>Nullipara</td>
<td>5.5</td>
<td>0.3</td>
</tr>
<tr>
<td>Para I</td>
<td>6.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Para II</td>
<td>7.0</td>
<td>0.4</td>
</tr>
<tr>
<td>Para III</td>
<td>6.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Para IV</td>
<td>7.9</td>
<td>0.5</td>
</tr>
<tr>
<td>≥ Para V</td>
<td>7.9</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Nutrition

Low energy intake during early lactation period may lead to weak estrus. Stress due to a lowered energy fulfillment rate is supposed to suppress the expression of estrus.

Lactation

There are not necessarily clear relationships between milk yield and the duration and intensity of estrus. One study reported that if cows with a relatively high milk yield are compared with cows with a low milk yield in the same herd, high-yielding cows tended to have weaker estrous signs.

Ambient temperature

A certain relationship was observed between the daily maximum temperature and estrous behavior. At a temperature up to 25°C, the number of standings per hour increased as the temperature rose. At a temperature of 30°C or higher, however, the number decreased (Figure 1-19 (p.24)). The estrous behavior of cattle is weak on hot days because they avoid exercise as much as possible to prevent their body temperature from rising. Adrenal P₄ (progesterone) secreted as a result of heat stress suppresses the estrus expression by E (estrogen).

Shortened estrous duration and weakened estrus during summer are conspicuous in a
European breed (*Bos taurus*) and mild in intensity in the Zebu (*Bos indicus*).

![Graph showing relationship between estrous behavior and maximum daily temperature](image)

\[ Y = 5.7597 + 2029 \times 1 - 0040 \times 1^2 \]

**Figure 1.19** Relationship between estrous behavior (number of times of mounting per hour) of cows and maximum daily temperature (Gwazdauskas et al., 1982)

6. **Management conditions**

The management condition also has a significant effect on the intensity and duration of estrus.

i. **Density of cattle**

Sufficient space is necessary for the mounting and standing. A lack of space may lead to inapparent estrous behavior.

ii. **Floor**

Cattle dislike being mounted on concrete floors. The duration of estrus tends to be longer and the signs stronger when they are on grassland and soil or the floor covered with straw (Table 1-5 (p.25)). Table 1-6 (p.25) shows the results of a study in Thailand that compared estrous behavior in Holsteins in the summer hot and relatively cool seasons. Observation in the first year revealed that the number of standing was significantly less in the summer hot season. However, cattle were raised in cowsheds with concrete floors in that season. In the second year, cattle had access to a stock yard with dust in the summer hot season as well as in the cool season and the result was that neither the number of standing nor the duration of estrus decreased significantly, indicating that the hard floor had a greater effect on estrous behavior than summer heat.
Table 1-5  Comparison of estrous duration and behavior of cattle raised on either concrete or soil floor

<table>
<thead>
<tr>
<th></th>
<th>Soil</th>
<th></th>
<th>Concrete</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard error</td>
<td>Mean</td>
<td>Standard error</td>
</tr>
<tr>
<td>Number of cases observed</td>
<td>69</td>
<td></td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Estrous duration</td>
<td>13.8</td>
<td>0.6</td>
<td>9.4</td>
<td>0.8</td>
</tr>
<tr>
<td>Number of mountings (in 30 min)</td>
<td>7.0</td>
<td>0.6</td>
<td>3.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of standings</td>
<td>6.3</td>
<td>0.5</td>
<td>2.9</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Britt et al., 1986

Table 1-6  Comparison of estrous duration and behavior of dairy cattle in a relatively cool or hot season in a subtropical climate (Rodtian et al., 1996)

<table>
<thead>
<tr>
<th>Division</th>
<th>1st year</th>
<th></th>
<th>2nd year</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Relatively cool season</td>
<td>Hot season</td>
<td>Relatively cool season</td>
<td>Hot season</td>
</tr>
<tr>
<td>Feeding form</td>
<td>Cowshed and stockyard</td>
<td>Only cowshed</td>
<td>Cowshed and stockyard</td>
<td>Only cowshed</td>
</tr>
<tr>
<td>Floor</td>
<td>Concrete and soil</td>
<td>Only concrete</td>
<td>Concrete and soil</td>
<td>Only concrete</td>
</tr>
<tr>
<td>Number of mountings</td>
<td>8.9 ± 1.5</td>
<td>2.8 ± 1.6</td>
<td>7.2 ± 1.6</td>
<td>6.7 ± 1.1</td>
</tr>
<tr>
<td>Number of mountings/standings</td>
<td>29.7 ± 3.5</td>
<td>11.4 ± 3.8</td>
<td>24.1 ± 3.6</td>
<td>26.5 ± 2.6</td>
</tr>
<tr>
<td>Estrous duration</td>
<td>11.3 ± 1.3</td>
<td>7.1 ± 1.4</td>
<td>12.4 ± 1.4</td>
<td>10.6 ± 1.0</td>
</tr>
</tbody>
</table>

7 The presence/absence of cows showing estrus simultaneously

Estrous behavior is more active as the number of cows showing estrus simultaneously increases (Table 1-7). The number of standing in cows in estrus is greater when the number of estrous cows is two than when the number is one and when the number is three than when the number is two. If a large proportion of cows in a small herd are pregnant, more than one cow will show estrus simultaneously less frequently resulting in fewer chances of discovering estrus.

Table 1-7  Relationship between the number of cattle in estrus and estrous behavior

<table>
<thead>
<tr>
<th>Number of cattle in estrus</th>
<th>Average number of mountings during estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.2</td>
</tr>
<tr>
<td>2</td>
<td>36.6</td>
</tr>
<tr>
<td>3</td>
<td>52.6</td>
</tr>
<tr>
<td>≥ 4</td>
<td>49.8</td>
</tr>
</tbody>
</table>

Hurnick et al., 1975
Diseases

Cattle with locomotor disorders have a smaller number of mounting and standing. Some of such cows may show the standing even when they are not in heat because they are not able to escape due to pain from being mounted.

Time of the day

Estrous behavior is most intensive between midnight and early morning because they are not disturbed by milking nor feeding. Effect of diurnal endocrinological rhythm is also suggested.

Factors affecting ovulation

Ovulation in dairy cattle is most likely to be suppressed during a few weeks after parturition. Ovarian follicles cannot ovulate before they mature and secrete E, which causes an LH surge, and LH receptors are present in follicular ovarian cells in sufficient quantities. An increase in the frequency of LH pulses causes ovarian follicles to mature and produce E.

Ovulation does not occur until the frequency of LH pulses increases and an LH surge occurs. In addition, ovulation does not occur unless there are sufficient quantities of LH receptors in ovarian follicular cells. The most important cause of suppression of the increase in LH pulses is energy deficiency. Suppression of the LH surge is caused by nonspecific stress including energy deficiency. Energy deficiency also suppresses the production of LH receptors.

Factors affecting fertilization, embryonic growth and implantation (Factors affecting conception rate)

Genetic factors

A well-known factor is chromosomal aberration. Fertilization by an ovum or a sperm with abnormal chromosomes may lead to chromosomal aberration at the time of cleavage and so forth and the fatal effect of this chromosomal aberration causes embryonic death and abortion.

Age and parity

The conception rate is lower in multiparous than in nulliparous and tends to decrease as the parity increases (Table 1-8.). One reason for this tendency is that with multiparous bacterial infection of the uterus is likely to occur at the time of calving resulting in a deteriorated intrauterine environment due to inflammation of the endometrium and so forth. Another reason is that multiparous are likely to have problems arising from stress from lactation and nutritional problems such as energy deficiency and excess proteins.

<table>
<thead>
<tr>
<th>Parity</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nullipara</td>
<td>54.2</td>
</tr>
<tr>
<td>Para I</td>
<td>44.2</td>
</tr>
<tr>
<td>Para II</td>
<td>41.3</td>
</tr>
<tr>
<td>Para III</td>
<td>40.1</td>
</tr>
<tr>
<td>Para IV</td>
<td>26.5</td>
</tr>
<tr>
<td>≥ Para V</td>
<td>17.5</td>
</tr>
</tbody>
</table>

Gwazduskas et al., 1986
The number of days postpartum and the number of times of insemination

The interval between parturition and the day of first insemination affects the conception rate. Too early insemination may result in low conception rates. After parturition, the uterine muscle contracts and discharges a large amount of lochia remaining in the uterus, which rapidly decreases in size. The uterus returns to its original size in 20-30 days after calving. However, regeneration of the endometrium takes 30-40 days and the cleanup of bacteria that invaded the uterus takes 40-45 days. In the case of dystocia and retained placenta, uterine involution takes more days.

Thus, too early insemination postpartum may result in the embryo entering the uterus before the endometrium returns to its normal state and intrauterine bacteria are eradicated and a high conception rate is unattainable.

Table 1-9 shows the results of a study in southern Ontario, Canada, that compared reproductive performance according to the days from calving to the first insemination in dairy cattle with a 2-month reproductive disease-free postpartum period. The conception rate was significantly low in cows inseminated within 39 days after calving. A high conception rate at the first insemination, a small number of times of insemination per conception and the highest first conception rate were shown by a group of cows inseminated within 60-79 days postpartum. Insemination performed later than this showed high conception rates but is uneconomical because of the extremely long open periods.

<table>
<thead>
<tr>
<th>Days to 1st insemination</th>
<th>Number of cows</th>
<th>Days open</th>
<th>Conception rate after 1st insemination</th>
<th>Number of inseminations per conception</th>
<th>Final conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 39</td>
<td>21</td>
<td>97.5</td>
<td>19.0</td>
<td>2.39</td>
<td>85.7</td>
</tr>
<tr>
<td>40 - 59</td>
<td>179</td>
<td>91.5</td>
<td>39.7</td>
<td>2.02</td>
<td>90.5</td>
</tr>
<tr>
<td>60 - 79</td>
<td>421</td>
<td>98.8</td>
<td>50.4</td>
<td>1.77</td>
<td>91.2</td>
</tr>
<tr>
<td>80 - 99</td>
<td>302</td>
<td>119.7</td>
<td>50.3</td>
<td>1.79</td>
<td>90.7</td>
</tr>
<tr>
<td>100 - 119</td>
<td>186</td>
<td>135.0</td>
<td>54.3</td>
<td>1.70</td>
<td>87.1</td>
</tr>
<tr>
<td>120 -</td>
<td>170</td>
<td>184.0</td>
<td>43.5</td>
<td>1.88</td>
<td>82.9</td>
</tr>
</tbody>
</table>

Nutrition

Nutritional conditions significantly affect the conception rate of cattle. Energy deficiency and excess protein intake during the period between drying off and 1-2 months after calving have significant effects.

Body condition scores (BCS) are widely used as an indicator of the degree of energy intake in dairy cattle. In an ideal state, BCS of dairy cattle is maintained at 3.5 through the dry period to parturition and at 2.5 or higher up to the lactation peak.
Significant loss of energy intake after calving may result in a significant reduction in BCS. Reduction in BCS is closely related to the conception rate. A study showed that if the BCS at the time of calving was 3.5 and the value 30 days after calving 3.0, the first insemination conception rate was 40%. As the BCS decreased from this by 0.5 points, the conception rate decreased by about 10% (Table 1-10). The mechanism by which postpartum energy deficiency lowers the conception rate is shown briefly in Figure 1-20. Postpartum energy deficiency results in decreased conception rates because it causes postpartum ovarian dysfunction and delays the first ovulation postpartum significantly, causes weak or silent estrus leading to a failure to perform insemination at an appropriate time and adversely affects the ova and follicular membrane cells of growing ovarian follicles. As a consequence, reduced fertility of the ova and reduced luteal function after ovulation follows.

Because dry matter intake decreases after parturition, large amounts of high-protein concentrate feed is often supplied to make up for the loss. Excess intake of proteins may increase ammonia and urea levels in the blood. Thus, the levels of urea nitrogen in the plasma and milk are used as indicators of protein intake. The conception rate is known to decrease as these urea nitrogen levels increase. A possible mechanism is that an increase in the urea nitrogen level in blood decreases the intrauterine pH and adversely affects the intrauterine environment resulting in embryonic mortality.

Table 1-10  Relationship between the degree of reduction in BCS and conception rate in postpartum dairy cattle (Butler, 2000)

<table>
<thead>
<tr>
<th>BCS at delivery</th>
<th>BCS on day 30 postpartum</th>
<th>Conception rate after 1st insemination</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5</td>
<td>3.0</td>
<td>40%</td>
</tr>
<tr>
<td>2.5</td>
<td>3.0</td>
<td>30%</td>
</tr>
<tr>
<td>2.0</td>
<td>2.0</td>
<td>20%</td>
</tr>
</tbody>
</table>

Figure 1-20  Postpartum energy deficiency and reduction in conception rate in cows

- Intensity of estrus

The more apparent the estrus, the easier the detection of estrus and the determination of the optimal time for insemination, and the higher the conception rate (Table 1-11 (p.29)).
Estrus is primarily the situation in which a female approves mating with a male so the act of standing, or acceptance of mounting by other cattle, is the most reliable estrous sign.

Table 1-11  Results of analysis of variance by the least squares method regarding the conception rate of artificial insemination (732 cases) over 3 years in dairy cattle on the Kansas State University Farm in the US (Stevenson, et al., 1983)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity of estrus</td>
<td>2</td>
<td>.85*</td>
</tr>
<tr>
<td>Time from estrus onset to insemination</td>
<td>3</td>
<td>.53*</td>
</tr>
<tr>
<td>Semen thawing method</td>
<td>2</td>
<td>.36</td>
</tr>
<tr>
<td>Ease for an inseminator to pass through the cervical canal</td>
<td>1</td>
<td>.17</td>
</tr>
<tr>
<td>Absence/presence of clear mucus at insemination</td>
<td>1</td>
<td>1.18*</td>
</tr>
<tr>
<td>Inseminator</td>
<td>2</td>
<td>.15</td>
</tr>
<tr>
<td><strong>Bull</strong></td>
<td>19</td>
<td>.17</td>
</tr>
<tr>
<td>Number of inseminations</td>
<td>3</td>
<td>.82*</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>4</td>
<td>.89**</td>
</tr>
<tr>
<td>Maximum temperature on the day of insemination (continuous measurement)</td>
<td>1</td>
<td>.79†</td>
</tr>
<tr>
<td>Maximum temperature on the day of insemination (4-time measurement)</td>
<td>1</td>
<td>.98*</td>
</tr>
<tr>
<td><strong>Error</strong></td>
<td>692</td>
<td>.22</td>
</tr>
</tbody>
</table>

†  P < 0.10
*  P < 0.05
** P < 0.01

Conception rates were compared between insemination after the detection of estrus by standing behavior and that after the detection of estrus by mounting behavior and other signs. The result showed that the conception rate was the highest with standing, the second highest with mounting and significantly low with other signs (Table 1-12 (p.30)). In addition, the conception rate was higher in cases where transparent mucus was observed at the time of artificial insemination than in cases where such mucus was not observed (Tables 1-12, 13 (p.30)).

Another study showed that the conception rate after artificial insemination in the cases of standing estrus and estrous signs other than standing estrus was 51.3% and 45.7%, respectively (Table 1-13 (p.30)).
### Table 1-12  Relationships between the intensity of estrus or the presence/absence of clear estrous mucus at insemination and the conception rate in dairy cattle (Stevenson, et al., 1983)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of cows</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intensity of estrus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standing</td>
<td>548</td>
<td>50%</td>
</tr>
<tr>
<td>Mounting</td>
<td>102</td>
<td>46</td>
</tr>
<tr>
<td>Others</td>
<td>82</td>
<td>34</td>
</tr>
<tr>
<td>Estrous mucus at insemination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>375</td>
<td>39</td>
</tr>
<tr>
<td>Present</td>
<td>357</td>
<td>48</td>
</tr>
</tbody>
</table>

### Table 1-13  Relationships between estrous signs at insemination and conception rate in dairy cattle (Heersche & Nebel, 1994)

<table>
<thead>
<tr>
<th>Estrous signs</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standing</td>
<td>51.3%</td>
</tr>
<tr>
<td>Other than standing</td>
<td>45.7</td>
</tr>
<tr>
<td>Roaring</td>
<td>50.0</td>
</tr>
<tr>
<td>Restlessness</td>
<td>49.6</td>
</tr>
<tr>
<td>Milk reduction</td>
<td>49.3</td>
</tr>
<tr>
<td>Mounting</td>
<td>49.2</td>
</tr>
<tr>
<td>Bristling up in the tail head part</td>
<td>48.8</td>
</tr>
<tr>
<td>Vulvar mucus</td>
<td>44.2</td>
</tr>
<tr>
<td>Complete reddening of the heat mount detector</td>
<td>43.2</td>
</tr>
<tr>
<td>Bloody mucus in the vulva</td>
<td>33.0</td>
</tr>
<tr>
<td>Partial reddening of the heat mount detector</td>
<td>30.2</td>
</tr>
</tbody>
</table>

### Intrauterine environment

An embryo moves from the oviduct into the uterus within 4-5 days of fertilization and is implanted in further 30-40 days. Meanwhile, the embryo absorbs necessary nutrients from the secretion (uterine milk) secreted by the uterine glands. Any abnormality in the properties of uterine milk may cause degeneration and death of embryos. The major cause of this abnormality is endometritis. Most cases of bovine endometritis are the chronic form of postpartum metritis. Thus, in cases that had been diagnosed as endometritis about one month after calving, the conception rate after artificial insemination performed 60 days after calving or later decreased significantly (Table 1-14.). The conception rate was also low in cases that had been diagnosed as endometritis 54 to 60 days postpartum (Table 1-15 p.31)).

Another important factor that affects the intrauterine environment is luteal function.

### Table 1-14  Effects of endometritis one month after calving on subsequent reproductive performance in dairy cattle (Nakao et al., 1992)

<table>
<thead>
<tr>
<th></th>
<th>High-lactation cow herd</th>
<th>Normal-lactation cow herd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Endometritis</td>
</tr>
<tr>
<td>Number of cows</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>Days to 1st-time AI</td>
<td>67 ± 19</td>
<td>73 ± 22</td>
</tr>
<tr>
<td>Conception rate of 1st-time AI</td>
<td>40.9</td>
<td>28.1</td>
</tr>
<tr>
<td>Final conception rate</td>
<td>86.4</td>
<td>90.6</td>
</tr>
<tr>
<td>Non-pregnant days</td>
<td>88 ± 33</td>
<td>105 ± 55</td>
</tr>
</tbody>
</table>
Table 1-15  Effects of endometritis 54-60 days after calving on the conception rate after 1st insemination in dairy cattle (Hamon et al., 2001)

<table>
<thead>
<tr>
<th>Uterine condition</th>
<th>Number of cows</th>
<th>Number of pregnant cows</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>55</td>
<td>38</td>
<td>69%</td>
</tr>
<tr>
<td>Mild endometritis</td>
<td>27</td>
<td>11</td>
<td>41%</td>
</tr>
<tr>
<td>Moderate endometritis</td>
<td>22</td>
<td>6</td>
<td>27%</td>
</tr>
<tr>
<td>Severe endometritis</td>
<td>11</td>
<td>3</td>
<td>27%</td>
</tr>
</tbody>
</table>

7 Luteal function

An embryo enters into the uterus within 4-5 days of fertilization and this is due to the action of P₄ secreted by the corpus luteum. The corpus luteum is formed within about 5 days after estrus and there is an increase in blood P₄ levels. The oviduct relaxes by the action of P₄ and facilitates the passage of embryos. P₄ induces the production of uterine milk required for the development of the embryo that enters the uterus. P₄ stimulates the growth of the uterine glands and facilitates them to produce uterine milk.

Thus, a delay in the formation of the corpus luteum and an insufficient secretion of P₄ from the corpus luteum may delay the descent of an embryo in the oviduct, reduce uterine milk produced by the uterine glands, inhibit the development of the embryo and, consequently decrease the conception rate.

8 Summer heat and seasons

The facts that the conception rate decreases in summer, that the conception rate and the survival rate of embryos decrease by experimental exposure of cows to summer heat and that the conception rate increases in cows that are managed such that their body temperature will not rise in summer clearly show that summer stress has adverse effects on conception.

Figure 1-21 (p.32) shows by-month first insemination conception rates in parturum dairy cattle on a farm in southern Florida, US. The conception rate during the summer heat period from June to September was significantly low. Figure 1-22 (p.32) shows the relationship between the maximum temperature of the day after artificial insemination and the conception rate. The highest conception rate was observed at a maximum temperature between 5°C and 20°C. A maximum temperature of 30°C or higher significantly decreased the conception rate.

High ambient temperatures cause a reduction in the conception rate because an increase in the body temperature adversely affects embryonic development. A body temperature of 30°C or higher is thought to decrease the conception rate. The body temperature of cattle increases at an ambient temperature of 27°C or higher and increases up to 40.5°C at an ambient temperature of 35°C. A reduction in the conception rate due to summer heat stress is more noticeable in high-lactation cows.

Embryos are also more susceptible to summer heat stress in their earlier stages and are less susceptible in their later developmental stages. For instance, in one study, summer heat stress was applied to superovulated cows at 1, 3, 5 or 7 days after insemination and the embryos were recovered at day 8 to study the survival rate and the
rate of development to blastocysts of the embryos. The survival and development rates of embryos were significantly low if summer heat stress was applied on the day after insemination but summer heat stress applied from 3 days after insemination onwards had no adverse effects on the survival or development rate (Figure 1-23 (p.33)).

Figure 1-21  By-month first insemination conception rate in postpartum Holsteins cows in a large-scale farm in southern Florida, US (Foote et al., Proc. 10th Int. Congr. Reprod. AI)

Figure 1-22  Relation between the maximum ambient temperature of the day after insemination and conception rate in dairy cattle (Gwazdauskas et al., 1986)
Artificial insemination has long been performed according to “morning-afternoon or afternoon-morning rule”. If the duration of estrus is shorter than the normal 12-20 hours, artificial insemination must be performed at an earlier time point.

Figure 1-24 (p.34) presents the theoretically optimum time of insemination in the case of estrous duration of 20 hours. Ovulation occurs in a mean time period of 12 hours after the end of estrus and the ovulated ovum retains the fertilization ability for 10 hours. Ova have the greatest fertilization ability for two hours after ovulation and the ability gradually decreases afterwards. Sperm deposited into the uterus require 5-6 hours to acquire fertilization ability, which is retained by the sperm for 24 hours after injection. The optimum time for insemination is when sperm that acquired and retain great fertilization ability can encounter an ovum that retains great fertilization ability in the ampulla of oviduct.

Thus, the optimum time for artificial insemination is a period from 24 hours before ovulation, or 8 hours after estrus to 4 hours before ovulation, or 8 hours after the end of estrus. Insemination earlier than the optimum time results in the sperm starting to age before they encounter the ovum since more than 24 hours lapse before ovulation. Insemination later than the optimum time results in the ovum passing two hours or a longer time and starting to age before a sufficient number of sperm that acquired fertilization ability reach the ampulla of oviduct. Consequently, the optimum time for insemination corresponds to the period between 8 to 28 hours after the onset of estrus. During this period, the highest conception rate is achieved with insemination performed at around the end of estrus.

According to one study conducted in the US regarding the relationship between the onset of estrus to insemination interval and the conception rate (Figure 1-25 (p.34)), the highest conception rate was achieved when insemination was performed 8-12 hours after estrus onset and the conception rate was significantly lower when insemination was done from 16 hours after estrus onset onwards. The data shows that insemination
at the recommended time of 20-28 hours after estrus onset is too late. This trend is related to the fact that the duration of estrus in dairy cattle has become significantly shorter than it was previously thought. The duration of estrus among the group of cows in the study ranges from 7 to 10 hours. The optimum time for insemination in cows with duration of estrus of 8 hours is shown in Figure 1-26 (p.35). In the case with estrous duration of 8 hours, ovulation occurs in 20 hours after estrus onset. This means that the optimum time for insemination corresponds to the period of 16 hours starting from the discovery of estrus onset. Insemination later than this results in the ovum starting degeneration before the sperm acquire fertilization ability. If a degenerating ovum encounters sperm that acquired fertilization ability and fertilization occurs, the conception rate is low due to the embryonic death. Figure 1-27 (p.35) indicates that as the estrus to insemination time increases, the degeneration rate of embryos at 7 days after insemination significantly increases.

Figure 1-24  Optimum time for insemination in the case of estrous duration of 20 hours

Figure 1-25  Relation between the interval from onset of standing estrus to insemination and the conception rate (2661 cows from 17 herds) (Nebel et al., 2000)
Other factors

Other factors that affect the conception rate after insemination include the methods of thawing semen, the locations where semen is deposited and variations in insemination skills among individual technicians.

The thawing of frozen semen is most commonly performed at 35°C for 30-40 seconds. A previously recommended method is to thaw frozen semen at 35°C for 12 seconds and immediately deposit it into the uterus. However, research shows that the conception rate is about 2% higher when semen is thawed in 30-40 seconds (Table 1-16 (p.36)). In thawing frozen semen, the entire semen in the straw should be quickly warmed to 35°C, the same temperature as the hot water used for thawing. Frozen semen thaws within 12 seconds. At this time point, however, the semen near the surface is warmed
to 35°C but the inner portion is not. If the straw semen is infused into the uterus as is, sperm in the inner portion may be affected by low temperature and sperm motility is weakened during the process of insemination. By extending the thawing time to 30-40 seconds, semen in the inner portion is warmed to 35°C and all the sperm recover normal motility.

Table 1-16 Relationship between frozen semen-thawing method and conception rate

<table>
<thead>
<tr>
<th>Year</th>
<th>Presenter</th>
<th>Straw</th>
<th>Thawing method</th>
<th>Number of inseminated cows</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1979</td>
<td>Almquist et al.</td>
<td>0.3 ml</td>
<td>32 - 35°C, 12 sec, 30 sec</td>
<td>10682, 10742</td>
<td>70.1, 72.0</td>
</tr>
<tr>
<td>1980</td>
<td>Senger</td>
<td></td>
<td>35°C, 12 sec, 30 sec</td>
<td>13543</td>
<td>70.1, 72.0</td>
</tr>
<tr>
<td>1981</td>
<td>Pace et al.</td>
<td>0.5 ml</td>
<td>37°C, Room temperature, Ice water</td>
<td>Approx. 100, 100, 100</td>
<td>69.6, 66.0, 65.0</td>
</tr>
<tr>
<td>1982</td>
<td>Almquist et al.</td>
<td>0.5 ml</td>
<td>35°C, 9 sec, 40 sec</td>
<td>18057</td>
<td>64.4, 66.3</td>
</tr>
</tbody>
</table>

Methods of thawing frozen semen include ice thawing, air thawing, 35°C thawing and 35°C thawing plus ice preservation (to protect thawed semen from undergoing cold shock at low ambient temperatures in winter). A study was conducted to compare the survival rate of sperm when thawed by these methods and incubated at 37°C for 3 hours and the rate of sperm with normal acrosomes. The result showed that these rates were the highest with the 35°C thawing method (Table 1-17.).

Table 1-17 Effects of thawing temperature on the sperm survival rates and normal-acrosome rates when 0.5-ml French-type straw semen is used

<table>
<thead>
<tr>
<th>Thawing method</th>
<th>Incubated at 37°C for 3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Survival rate (%)</td>
</tr>
<tr>
<td>5°C (ice water)</td>
<td>30.3</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>21.4</td>
</tr>
<tr>
<td>35°C (warm water)</td>
<td>51.4</td>
</tr>
<tr>
<td>35°C (warm water) followed by 5°C (ice water)</td>
<td>41.1</td>
</tr>
</tbody>
</table>

Nebel (1997)

The sites for semen deposition also have significant effects on the conception rate (Table 1-18 (p.37)). If semen is correctly deposited into the uterus, sperm enter into the bilateral uterine horns evenly and allow a conception rate almost equal to that attained by infusing half volume of the semen into each uterine horn (Table 1-19 (p.37)). However, if semen is deposited into the cervical canal because of difficulty in passing the insemination gun or pipet through the cervical canal, the conception rate is significantly reduced because the deposited semen flows backward and an insufficient number of sperm can reach the uterine horns. If the insemination gun or pipet is erroneously inserted into the uterine horn over the uterine body, the resultant conception rate is also reduced. If ovulation occurs in the ovary on the same side as the uterine
horn into which semen was erroneously injected, the conception rate is not lowered. In opposite cases, conception occurs quite rarely.

Table 1-18  Analysis of variance by the least squares method concerning the conception rates of artificial insemination (2600 cases) over 5 years on the Virginia State University Farm in the US (Gwazdaukas et. Al., 1986)

<table>
<thead>
<tr>
<th>Factors</th>
<th>Degrees of freedom</th>
<th>Mean squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parity</td>
<td>5</td>
<td>2.19**</td>
</tr>
<tr>
<td>Number of inseminations</td>
<td>4</td>
<td>.73*</td>
</tr>
<tr>
<td>Bull</td>
<td>74</td>
<td>.32*</td>
</tr>
<tr>
<td>Time of estrus onset</td>
<td>1</td>
<td>.09</td>
</tr>
<tr>
<td>Inssemnation time</td>
<td>1</td>
<td>.06</td>
</tr>
<tr>
<td>Time from estrus onset to insemination</td>
<td>1</td>
<td>.0004</td>
</tr>
<tr>
<td>Inssemnator</td>
<td>3</td>
<td>.08</td>
</tr>
<tr>
<td>Location of semen injection</td>
<td>1</td>
<td>5.28**</td>
</tr>
<tr>
<td>Feeding location</td>
<td>2</td>
<td>.01</td>
</tr>
<tr>
<td>Maximum temperature on the day of insemination</td>
<td>1</td>
<td>.11</td>
</tr>
<tr>
<td>(continuous measurement)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum temperature on the day of insemination</td>
<td>1</td>
<td>.16</td>
</tr>
<tr>
<td>(4-time measurement)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum temperature on the day following insemination</td>
<td>1</td>
<td>1.57**</td>
</tr>
<tr>
<td>(continuous measurement)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum temperature on the day following insemination</td>
<td>1</td>
<td>2.64**</td>
</tr>
<tr>
<td>(4-time measurement)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumulative temperature on the day of insemination</td>
<td>1</td>
<td>.27</td>
</tr>
<tr>
<td>Cumulative temperature on the day following insemination</td>
<td>1</td>
<td>.10</td>
</tr>
<tr>
<td>Parity x number of inseminations</td>
<td>20</td>
<td>.37*</td>
</tr>
<tr>
<td>Parity x inseminator</td>
<td>12</td>
<td>.40*</td>
</tr>
<tr>
<td>Error</td>
<td>2283</td>
<td>.23</td>
</tr>
</tbody>
</table>

a  P < 0.054
*  P < 0.05
** P < 0.01

Table 1-19  Comparison of conception rates between uterine body insemination and uterine horns insemination

<table>
<thead>
<tr>
<th>Insemination method</th>
<th>Number of inseminated cows</th>
<th>Conception rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Half dose into each of the uterine horns</td>
<td>114</td>
<td>67%</td>
</tr>
<tr>
<td>Uterine body</td>
<td>110</td>
<td>64%</td>
</tr>
</tbody>
</table>

(6) **Factors affecting development of the fetus and placenta**

Embryos start the implantation process when they are about 25 days old and eventually form placentomes. A placentome is a combined organ of the cotyledon and caruncle where capillaries from the dam and fetus are distributed side by side. At this location, nutrients and oxygen contained in the maternal blood are transferred into the fetus's
blood. Thus, development of the placenta where blood components are exchanged is closely related to development of the fetus. A bovine fetus develops at an accelerating rate as the pregnancy stage proceeds. The growth rate is particularly high from 180-200 days of pregnancy onwards. Although studying the growth of the placenta during pregnancy is not easy, the determination of estrone sulfate (E1S) levels in peripheral blood may help estimate the growth condition. Like the growth curve of the fetus, blood E1S levels rapidly increase from around 200 days of pregnancy onwards, indicating the rapid growth of the placenta during this period. Genetic and nutritional factors are associated with the growth of the fetus and placenta.

1. Retarded development of the fetoplacental system and weak calves resulting from inbreeding and so forth

Retarded growth of the fetus in humans is well documented. The delivered fetus is small and weak despite a normal pregnancy period. Causes include abnormal development of the placenta. The fetus is not supplied with sufficient nutrients from the dam due to inadequate development of the placenta and shows growth retardation. Abnormal development of the placenta is considered a genetic disorder.

With cattle as well, a small and weak calf may be born due to retarded intrauterine growth despite a normal pregnancy period. This occurs relatively frequently in Japanese Black cattle. Inbreeding is considered to be the principal cause of this; retarded growth of the fetus occurs when a certain maternal line is crossed with a bull of a certain line.

2. Effects of nutrition during the pregnant period on development of the fetoplacental system

Nutritional conditions of the mother during the pregnant period also have effects on development of the fetoplacental system. For instance, research shows that if energy intake by the dam during the intermediate to late pregnant period is low, the weights of the placenta and fetus are low and the incidence of weak calves and retention of the fetal membranes is high. If the intake of proteins during the intermediate to late pregnant period is inadequate, both the placenta and fetus are small in size and weak calf syndrome and retention of the fetal membranes are likely to occur. Too high or low BCS during the dry period of the dam cause weak newborns and retention of the fetal membranes more frequently.

3. Other factors affecting the body weight of newborn calves

The mean body weight of newborn calves depends on breeds (Table 1-20.). The body weight of newborns is directly correlated to the body weight of the dam and the sire. The newborn from a multipara is larger than that from a primipara. Male newborns are 1-4 kg heavier than female newborns.

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Newborn bodyweight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sindhi</td>
<td>24.4 kg</td>
</tr>
<tr>
<td>Jersey</td>
<td>25.0 kg</td>
</tr>
<tr>
<td>Aberdeen Angus</td>
<td>27.2 kg</td>
</tr>
<tr>
<td>Guernsey</td>
<td>30.9 kg</td>
</tr>
<tr>
<td>Hereford</td>
<td>32.7 kg</td>
</tr>
<tr>
<td>Shorthorn</td>
<td>32.7 kg</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>33.1 kg</td>
</tr>
<tr>
<td>Holstein</td>
<td>41.8 kg</td>
</tr>
<tr>
<td>Brown Swiss</td>
<td>45.9 kg</td>
</tr>
<tr>
<td>Charolais</td>
<td>47.7 kg</td>
</tr>
</tbody>
</table>
(7) Factors affecting the pregnancy period

(1) Breeds

The pregnancy period of cattle varies depending on the breed; 280 days on average for Holstein Friesian, 285 days for Japanese Black and 292 days for Brahman (Table 1-21). The pregnancy period depends on the breed of the fetus rather than on the breed of the dam. For instance, if an embryo from a Japanese Black cow is transferred to a Holstein cow, the pregnancy period is 285 days, the same period as the Japanese Black.

The pregnancy period also varies from individual to individual to a relatively great extent. Figure 1-28 shows the distribution of pregnancy periods among Holstein Friesian cattle. It indicates a great variation ranging from 260 to 310 days. The cause of these variation in the pregnancy period is not well understood.

<table>
<thead>
<tr>
<th>Bovine breeds</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aberdeen Angus</td>
<td>273-282</td>
</tr>
<tr>
<td>Ayrshire</td>
<td>277-279</td>
</tr>
<tr>
<td>Jersey</td>
<td>277-280</td>
</tr>
<tr>
<td>Holstein</td>
<td>278-282</td>
</tr>
<tr>
<td>Shorthorn</td>
<td>281-283</td>
</tr>
<tr>
<td>Guernsey</td>
<td>282-285</td>
</tr>
<tr>
<td>Hereford</td>
<td>283-286</td>
</tr>
<tr>
<td>Brown Swiss</td>
<td>288-291</td>
</tr>
<tr>
<td>Brahman</td>
<td>292</td>
</tr>
<tr>
<td>Japanese Black</td>
<td>285</td>
</tr>
</tbody>
</table>

(2) Sex of the fetus

A male fetus has been reported to have a 1-2 days longer pregnancy period than a female fetus. As seen in Figure 1-28, however, there are almost no differences in the mean pregnancy period between male and female fetuses.

(3) Number of fetuses

Twin pregnancy is known to have a 3-6 days shorter pregnancy period than single pregnancy. A study in Hokkaido reported that twin pregnancy had a mean pregnancy period of 275 days, which was 5.5 days shorter than that of single pregnancy. However, a substantial number of cattle with twin pregnancy showed a pregnancy period of 280 days or more.

(4) Genetic factors

Genetic factors affect the pregnancy period. For instance, the pregnancy period was several days longer depending on the maternal line and some cows mated with a certain bull showed a longer pregnancy period. Some cows with a congenital hypophysial defect showed a long pregnancy period.
Summer heat

The pregnancy period may be several days shorter during a summer heat season. This occurs because summer heat stress causes an increase in adrenocortical hormone levels in the blood of the dam and induces parturition.

Factors affecting the time required for parturition and the degree of calving ease

The calf delivery process in cattle starts with labor pains with long intervals. Then, the cervical canal dilates, part of the allantois enters the vagina through the cervical canal and the head part of the fetus enters the cervical canal. The period up to this stage is referred to as the first stage of labor, or the opening stage, and lasts for 3-6 hours. The entrance of the fetus into the birth canal stimulates the posterior lobe of hypophysis to secrete a large quantity of oxytocin, which causes strong contraction of the uterus and abdominal walls, and in the case of normal cephalic presentation, the fetal forefeet first pass the birth canal. Next, the head, chest, abdomen and hip pass the birth canal in this order and the fetal expulsion process is complete (the second stage of labor or the expulsion stage). The expulsion period lasts for 1-4 hours. Labor pains continue after delivery of a fetus and eventually the placenta is expelled in about 5 hours.

A number of factors affect the time required for parturition and the calving cattle.

1. Age and parity

Primiparae require 30 minutes to one hour longer for parturition than multiparae. The incidence of dystocia in primiparae is 4-5% compared to 1-2% in multiparae. In primiparae, it is more likely that the fetus size is large in relation to the pelvic size canal because the pelvis has not yet developed sufficiently so the bony birth canal is tight.

In old-aged cattle uterine inertia often causes dystocia. Uterine inertia often results in fetal malpresentation which may cause extended time for parturition and weak calf syndrome.

2. Breeds

The incidence of dystocia is highest in Holsteins Friesians among other dairy breeds and is relatively low in Jerseys. In Holsteins Friesians the fetus has a relatively large skeleton and is often delivered at an early age when pelvic bone is not well grown. As the consequence a relative fetal oversize occurs. This is the main reason why Holstein heifers have often been mated with beef breeds with low birth weight. Beef breeds are used because the calves are raised as beef cattle. In Jerseys the fetus has a small skeleton and passes through the birth canal easily.

3. Genetic factors

Bulls have a significant genetic effect on the fetal skeleton and weight. The incidence of dystocia depends on the bull. In European countries, calving ease is included in the evaluation parameters for Holstein bulls used for insemination.

Dystocia may also result from fetal deformity due to congenital anomalies. Schistosomus reflexus occurring in cattle relatively often is representative of these anomalies.
Nutrition

Over conditioned cattle during the dry period are more likely to have dystocia due to uterine inertia. Hypocalcemic cattle may also have uterine inertia.

Calving environment

The sources of stress such as noise and the approach of man may temporarily suppress labor and lead to dystocia.

Factors affecting newborn health

Newborn calves start spontaneous respiration immediately after birth, try to stand up, acquire immunocompetence through colostrum and continue to grow. Many factors are related to health of newborn calves.

Gestation period

The lungs of newborns must be sufficiently mature at the time of parturition, so that they can start spontaneous respiration. The lungs of the fetus are generally mature sufficiently to permit life outside the dam after 95% of the gestation period. Delivery earlier than this may result in a failure of spontaneous respiration.

Conditions at calving

The proportions of causes of deaths of the fetus and newborn are 25.8% for dystocia, 21.9% for weak calves and 7.1% for injury at birth. A total of about 55% of all causes are related to abnormalities at the time of parturition. Asphyxia neonatorum is the direct cause of neonatal deaths associated with abnormal delivery.

Of the two types of asphyxia neonatorum, one type occurs at the time of or immediately after birth and another type occurs several hours after birth. The former includes cases in which a long time has passed after labor onset and the fetus is already in an asphyxial state and other cases in which the fetus is exhausted after a long time of excess traction and falls into an asphyxial state immediately after birth. The latter type includes cases in which the fetus is delivered earlier than expected time before the fetal lungs are sufficiently matured. In these cases the fetus is normal at first but develops dyspnea gradually and falls into an asphyxial state in several hours.

If labor continues for a long time, the fetus falls in hypoxia because blood vessels distributed within the uterus contract, the placenta exfoliates partially and blood supply to the fetus decreases. A long-time traction of the fetus may lead to hypoxia.

Newborns that are delivered more than two weeks earlier than due date develop dyspnea due to pulmonary emphysema and atelectasis that occur upon inhaling air because surfactant is not present on alveolar membranes in sufficient quantities.
Feeding of colostrum after birth

Colostrum contains a large amount of immunoglobulin and newborns acquire immunity through the ingestion of colostrum. A newborn can absorb immunoglobulin from its small intestine during the period of several hours after birth. Thus, colostrum must be fed in sufficient quantities within 2-4 hours at the latest, and 15 minutes if possible, after birth. The appropriate quantity of colostrum to be fed is about 4 liters for a newborn with a body weight of 45 kg.

Delayed ingestion or supply of colostrum may lead to a failure in acquiring immunity and to occurrences of infectious diseases.

Factors affecting the postpartum recovery of ovarian function

Postpartum recovery of ovarian function and nutritional factors

Development of ovarian follicles, ovulation and luteinization are controlled by gonadotropins secreted from the anterior lobe of hypophysis. In the late pregnancy period, the secretion of gonadotropins by the hypophysis is suppressed by the negative feedback action of P₄ and E secreted by the pregnant corpus luteum and the placenta so follicular development and ovulation are suppressed. The negative feedback is lost after the expulsion of the placenta and the secretion of gonadotropins recur. The ability of the cow to secrete gonadotropins is recovered within 10-20 days after calving and postpartum ovarian cyclicity commences (Figure 1-29).

The pregnant corpus luteum of the ovary starts to regress rapidly several days before delivery and loses the ability to secrete P₄ after parturition. Thus, the ovary immediately after calving is in a quiescent state. Follicle-stimulating hormone (FSH), a gonadotropin that promotes growth of ovarian follicles, is secreted several days after delivery so ovarian follicles start to grow several days after parturition.

Figure 1-29 Postpartum recovery of the anterior pituitary functions and ovarian and uterine involution in cattle
After the dominant follicle matures and ovulates, the corpus luteum is formed, the second ovulation occurs after a relatively short luteal phase and a normal ovarian cycle is resumed (Figure 1-30). The first ovulation is not accompanied by estrus but estrus occurs from the second ovulation postpartum.

![Figure 1-30 Normal postpartum recovery of ovarian function in cows](image)

If ovarian function is recovered smoothly after calving, the conception rate after the first insemination is high. If the postpartum recovery of ovarian function is delayed, however, the first insemination conception rate is low. The reason why higher conception rates are attained when ovarian function is recovered more smoothly can be explained as below. The conception rate is higher in cases in which the blood P₄ level is higher during the cycle preceding the present estrous cycle. One reason may be that as the P₄ level in the luteal phase increases the intrauterine environment improves and the estrus after luteolysis is clearer so ovulation and luteinization take place smoothly. The P₄ level in the luteal phase is higher after the second ovulation than after the first ovulation and is higher after the third than second ovulation. For instance, if the first insemination takes place about 70 days after delivery, then it follows that cows with early postpartum recovery of ovarian function are inseminated after the third luteal phase in which the P₄ level is high. Consequently, they have greater chances of conception (Figure 1-31 (p.44)). On the other hand, cows with late recovery of ovarian function are inseminated after the first luteal phase with relatively low P₄ levels so high conception rates are not expected.

The dominant follicle in the first follicular wave must ovulate for the ovary to recover its normal function. The secretion of FSH occurs within several days after calving and in response to this the first follicular wave emerges. The frequency of LH pulses must increase and LH receptors must be present in theca cells in sufficient quantities for the dominant follicle in the first follicular wave to mature and ovulate. An increase in the frequency of LH pulses stimulates the dominant follicle to mature and produce estrogen and causes an LH surge, resulting in ovulation. Unless the frequency of LH pulses increases sufficiently, the dominant follicle degenerates halfway without maturing and results in postpartum ovarian quiescence (Figure 1-32 (p.44)). If the frequency increases to some extent but not to such an extent that the dominant follicle matures, then the dominant follicle remains without undergoing atresia and results in a follicular cyst because LH stimulation persists.
Postpartum energy deficiency may lead to a suppression in LH pulses and a failure of ovulation of the dominant follicle in the first follicular wave. Energy deficiency also suppresses the production of insulin-like growth factor 1 (IGF-1) and the production of LH receptors in ovarian follicles and causes ovulation disorders.

Nutrition during the period from the dry period to postpartum lactation peak

Over condition of cows due to excess intake of nutrients during the dry period, periparturient diseases and energy deficiency during an early lactation period resulting from a lack of dry matter intake delay the recovery of postpartum ovarian function. BCS is used as an indicator of the energy intake level in cattle. An extremely lean condition is expressed as 1.0 and an extremely fat condition as 5.0, and BCS values for
conditions between these two are determined to the nearest 0.25.

The BCS of an ideal nutritional status is in the range of 3.00-3.75 for the time of parturition, 2.50-2.75 for the lactation peak, 3.00-3.50 for the period 150-200 days after calving and 3.00-3.75 for the dry period (Figure 1-33). Particularly important are BCS values in the dry period. Excessively over conditioned dry cow with a BCS value of 4.0 or higher are highly susceptible to dystocia, retention of fetal membranes, metritis and ovarian diseases. Since these cows have dry matter intake after parturition and suffer from nutritional deficiency, nutrients contained in their body tissues are mobilized. As a result, cows show weight loss and significantly low BCS during a postpartum period.

![Graph showing changes in body condition scores in dairy cattle under normal nutritional conditions.](image)

**Figure 1-33** Changes in body condition scores in dairy cattle under normal nutritional conditions (Wallace, 1996)

In cattle with a BCS value having decreased by 1.0 point or more within 5 weeks postpartum, the first postpartum ovulation occurred 13 days later, time to the first insemination was 11 days longer and the first insemination conception rate was 19% lower, compared to cows with a decrease in BCS values of less than 1.0.

The mechanism that energy deficiency within one month after calving adversely affects the conception rate 60-80 days postpartum is as follows. A primordial ovarian follicle requires 60-80 days after the start of development to mature and ovulate. Thus, an ovarian follicle that matures and ovulates 60-90 days after delivery must have started development immediately after calving when the energy intake level was the lowest (Figure 1-34 (p.46)). Energy deficiency causes a lack of IGF-1 that is necessary for initial growth of the ovarian follicle and leads to a reduction in quality of the dominant ovulatory follicle and luteal function.
Figure 1.34  Theoretical development process of the first to sixth ovulation follicles in postpartum cows. The shaded portion shows energy balance (Mcal/day). (Britt, 1994)

3) Age and parity

Postpartum recovery of ovarian function occurs later in primiparous cows than in multiparous cows. A suggested reason is that the cow is still growing at the time of the first calving in life so it requires nutrition for its growth as well as for maintenance and milk production. Thus, a primipara is likely to suffer from severer nutritional deficiency during an early gestation period.

4) Conditions at calving

In cows with dystocia the postpartum recovery of ovarian function tends to be delayed. One reason is that stress from dystocia may suppress the secretion of gonadotropins by the hypophysis. Another reason is that a high incidence of reproductive diseases and metabolic disorders resulting from dystocia may lead to a low dry matter intake and resultant energy deficiency.

5) Postpartum reproductive diseases

Diseases that are likely to occur after dystocia and retention of the placenta, such as metritis, lochiotremata and chronic endometritis, are often accompanied by the extension of the luteal phase after the first postpartum ovulation and result in delay in the recovery of ovarian function.

6) Postpartum diseases

Diseases that are likely to occur in postpartum cows, such as milk fever, ketosis, abomasal displacement, mastitis and hoof diseases, cause a reduction in dry matter intake and energy deficiency and result in delay in the recovery of ovarian function due to ovarian quiescence (Figure 1.35 (p.47)).
(11) **Factors affecting postpartum uterine involution**

The postpartum uterus completes the involution after the extrusion of lochia by uterine contraction and the restoration of endometrial tissues.

1. **Decrease in the uterine size and volume of lochia**

   The bovine uterine weight rapidly decreases from 9 kg after delivery to 1 kg in 20-30 days and 750 g in 50 days. The width of the uterine cervix and uterine horn returns to their normal size in 30-40 days (Figure 1-29 (p.42)). The postpartum uterus contains large quantities of fetal fluid, sloughed surface of the caruncles and blood resulting from the rupture of umbilical vessels and separation of fetal membranes. The lochia amounts to 1,000-1,600 ml within 48 hours of delivery and decreases to 40-100 ml in 10-20 days. Lochia is reddish brown at first but gradually turns transparent. It is no longer seen by 24 days after calving.

2. **Collapse and repair of endometrial tissues**

   A caruncle is 70 mm long, 35 mm wide and 25 mm high on the day after parturition but it returns to its normal size by 40-60 days after calving and is 4-8 mm across and 4-6 mm high. Endometrial tissues show necrosis and collapse 5 days postpartum and the entire necrotic maternal placenta falls within 19 days of postpartum leaving rough surfaces (Figure 1-36 (p.48)). The caruncle is covered with mucosal epithelia at 30 days after delivery onwards and uterine involution is complete within 40-60 days of delivery.
Intrauterine bacterial contamination after parturition

The pressure inside the postpartum uterine is lower than the atmospheric pressure and the cervical canal is relaxed and dilated so the bacteria in the birth canal and around the vulva may be aspirated with air into the uterus and cause microbial contamination. The bacterial contamination rate in the uterus 3-15 days after calving is 93%. However, bacterial infection is rarely established within the uterus excepting the cases of dystocia and retention of the fetal membranes and intrauterine bacteria decrease with uterine involution and estrous expression and are almost eliminated within 50 days of delivery (Figure 1-29 (p.42)).

Factors affecting postpartum uterine involution are as follows:

i. Conditions at calving

Secondary uterine inertia often occurs after dystocia and twin birth and uterine involution is delayed due to a lack of uterine contraction and a delay in lochial disappearance.

ii. Retention of fetal membranes

Retention of the fetal membranes may result in intrauterine infection and an extended time to uterine involution.

iii. Periparturient diseases

Hypocalcemia may lead to primary uterine inertia and delay uterine involution.

iv. Parity

Uterine involution generally tends to occur earlier in primiparae than in multiparae.
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Chapter 2  Definition and types of reproductive disorders

1. Definition of reproductive disorders

A reproductive disorder is defined as a status in which the reproductive function of a female or male animal is temporarily or persistently suspended or disturbed and abnormal calves are produced. Causal factors include inappropriate feeding environments and methods, genetic defects, nutritional disorders, systemic diseases, reproductive anomalies and diseases, abnormal secretion of various hormones and inappropriate breeding management.

Of reproductive disorders, one type that leads to a failure of conception due to the anomalies and diseases of the reproductive organs is sometimes referred to as sterility and another type that leads to conception but results in an embryonic or fetal death and subsequent absorption and abortion is referred to as infertility. The type of sterility that is caused by the absence of gametes or defective reproductive organs and allows no chance of conception is referred to as absolute sterility. The type of sterility in which conception is temporarily impeded as a result of a failure in detecting reproductive diseases and estrous signs and performing insemination in a timely manner is referred to as relative sterility.

2. Manifestations of reproductive disorders and reproductive diseases

(1) A case in which an animal with anomalies of the ovary or uterus develops abnormal estrous conditions such as anestrus and fails to mate even if it has attained 12 months old or 40 days have passed since the last delivery.

Freemartin

In bovine unlike-sexed twin and multiple pregnancy, about 92% of female fetuses fail to have normal sexual differentiation and are likely to have anomalies of the reproductive organs that lead to absolute sterility. This sterile cow is referred to as a freemartin.

The cause has not yet been clarified, however, one suggested reason is that the blood vessels of female fetal membranes are anastomosed with the blood vessels of male fetal membranes in the early embryonal phase to produce an exchange of blood so that the sex-determining region Y (SRY) deriving from the male, which masculinizes reproductive organs, masculinizes the ovarian primordium of the undifferentiated female fetus and induces the secretion of androgen.

Findings in clinical diagnosis include the length of the vagina being 1/3 the normal length or less, hypertrophy of the clitoris and rough and long pubic hair. In a vaginal examination of calves, a test tube 2 cm in diameter and 20 cm long can be inserted 12-18 cm into the vagina in normal calves but only 8-10 cm into the vaginal vestibule in freemartins since they lack a vagina. By rectal palpation, a hard cylinder/ cone-shaped object is normally felt 5-10 cm in front of the vaginal vestibule but the cervical canal, uterus and ovary are not palpable.

Detailed examinations include examinations of chimeras of XX/XY by chromosome tests of cultured leukocytes and chimeras of blood types. By detecting Y chromosome-specific repetitive sequences by PCR (polymerase chain reaction), accurate diagnosis can be made in a short period using an extremely small amount of blood.

There is no cure and conception is not possible.
Ovarian subfunction

A state in which nulliparae and multiparae, within 12 months of birth and 40 days after delivery, respectively, develop no ovarian follicles or ovarian follicles develop to some extent but repeat atresia and regression without ovulating, resulting in continued anestrus. This disorder is divided into ovarian hypoplasia, ovarian quiescence and ovarian atrophy.

i. Ovarian hypoplasia
   Both the right and left ovaries grow insufficiently and are extremely small, flat and inelastic. Growth of the uterus is inadequate.

ii. Ovarian quiescence
   The shape of the ovary is normal, however, the ovarian follicles do not grow or grow to some extent but repeat atresia and regression without ovulating. The uterus is small and inelastic in some cases and is normal in shape in some cases. In multiparous cow, postpartum uterine involution is delayed in many cases.

iii. Ovarian atrophy
   Both the right and left ovaries are dwarfed, indurated and flat with smooth or wrinkled surfaces and they neither form ovarian follicles or corpus nor ovulate. The uterus is small and inelastic.

The direct cause of this disorder is a reduction in the ability of the anterior lobe of the hypophysis to secrete gonadotropins (GTH), i.e. follicle-stimulating hormone (FSH) and luteinizing hormone (LH), and this condition is related to a reduction in gonadotropin-releasing hormone (GnRH) secretion due to the suppressed function of the hypothalamus. The predisposing causes include inappropriate feeding and management such as, in particular, qualitative and quantitative deficiencies of feed and an inferior feeding environment, and in nulliparous cows, parasitic diseases of the alimentary canal during the raising stage and in multiparous cows, systemic wasting diseases during the perinatal period. Genetic factors are also involved in ovarian hypoplasia in nulliparous cow.

Diagnosis should be based on rectal palpation and if the first diagnosis fails, an additional examination should be performed 7-14 days afterwards. Diagnosis should be made by confirming that nothing has changed compared to the findings in the previous examination of the right and left ovaries.

If poor health is caused by inappropriate feeding and inferior feeding environments, stockkeepers should be instructed to improve these predisposing factors to eliminate causes. If systemic diseases are causing the disorder, these diseases should be treated and normal nutritional and health conditions restored. Hormone preparations should be administered after or in parallel with these procedures. Complicating uterine diseases should be treated.

Efficacy of the treatment with hormone formulations depends on the stage of the follicular wave at the time of administration. Thus, if rectal palpation reveals a growing ovarian follicle 10-15 mm in diameter in the ovary, 1,500-3,000 international units (IU) of human chorionic gonadotropin (hCG) or 100 µg fertirelin acetate or buserelin acetate at a dosage of 10-20 µg as buserelin, both gonadotropin-releasing
hormone analogues (GnRH analogues), should be administered once by intramuscular injection for the purpose of inducing follicular maturation and ovulation and forming corpus luteum afterwards. The interval between the first and second ovulation after hormone therapy is usually in the range of 8-15 days, which is shorter than normal. Therefore, stockkeepers should be instructed to observe estrous signs 8, 20 as well as 30 days after treatment.

If no growing ovarian follicle is present in the ovary, intramuscular or subcutaneous injection of 500-1,000 IU of pregnant mare serum gonadotropin (PMSG) or simultaneous injection of 500-1,000 IU each of PMSG and hCG should be administered once for the purpose of inducing follicular growth, maturation and ovulation and forming corpus luteum. In addition, intravenous or intramuscular injection of 200-400 Rab.U of bovine anterior pituitary gonadotropin (APG) or intravenous injection of 20-40 A.U. of swine anterior pituitary follicle-stimulating hormone (APFSH) should be administered once. Mating with a cow with estrus that appears several days after PMSG administration may lead to a multiple pregnancy due to superovulation.

In addition, for the purpose of normalizing GTH secretion from the anterior lobe of the hypophysis in “cow” with ovarian quiescence, controlled internal drug release device (CIDR) and progesterone releasing intravaginal device (PRID: capsule containing estradiol-17β), which are indwelling types of luteal hormone sustained-release preparation, were inserted into the vagina and maintained in the same position for 12 days with good results.

A normal estrous cycle after treatment indicates that the treatment was successful. In spite of treatment with hormone preparations, some ovarian follicles fail to ovulate and fall into atresia and regression and some ovaries fall into quiescence even if the corpus luteum is formed after ovulation. Thus, if rectal palpation for luteal formation 7-14 days after treatment reveals the absence of efficacy, the type and dosage schedule of hormone preparations should be reconsidered. If repeated treatment of ovarian hypoplasia in nulliparous cow by this method has no effect on the ovary, then the prognosis is diagnosed as poor.

3 Ovarian cyst

Ovarian cyst refers to a condition in which an ovarian follicle grows to more than 2.5 cm in diameter without ovulating and is divided into follicular cyst and luteal cyst. The frequency of follicular cyst is higher than that of luteal cyst.

i. Follicular cyst

An ovarian follicle grows to more than 2.5 cm in diameter without ovulating, persists for a long time and then regresses. This process is repeated.

Because this disorder occurs in obesity due to oversupply of concentrate feed and deficiency of exercise and in poor nutrition due to qualitative and quantitative deficiency of feed, stress from inappropriate feed types and feeding management as well as the oversupply of feed containing estrogen-like materials and genetic factors such as a predisposition to follicular cyst are known to be involved in the disorder.
The direct cause of the disorder is abnormal secretion of GTH due to reduced function of the anterior lobe of the hypophysis, or transient mass release of LH that induces ovulation, that leads to a failure of LH surge. This indicates that it may be caused by an abnormality in the LH releasing mechanism resulting from decreased sensitivity to a rise in estradiol concentrations in the hypothalamus anterior lobe of the hypophysis system. Another mechanism is that progesterone secreted by the adrenal gland acts on the higher-level organ hypothalamus to suppress the release of GnRH.

Symptoms include nymphomania in which estrous signs appear constantly or frequently and sexuality increases or a sign in which estrous signs appear irregularly, but estrous signs are absent in many cases. In the case of a long-term process, the both sides of the tail head are depressed and the tail head is elevated due to loosened Lig. Sacrotuberale latum.

The disorder is diagnosed if one or more large-size ovarian follicles 2.5 cm or more in diameter are present in one or bilateral ovaries by rectal palpation (Photograph 2-1 (p.81)), these follicles increase in size or remain at the same size at the time of reexamination 7-14 days later and the corpus luteum is absent. Ovaries with growing ovarian follicles are swollen and contractible and those with degenerating ovarian follicles are relaxed. The disorder is complicated by endometritis in many cases.

Care must be taken because there is an ovarian condition similar to this disorder in which a luteal cyst, cystic corpus luteum, a large-size ovarian follicle and corpus luteum (a large-size ovarian follicle and a normal growing ovarian follicle in the estrus) are coexistent as seen in Table 2-1 (p.55) and Photograph 2-2 — 2-6 (p.81). If differentiating between these conditions is difficult, reexamination should be performed 7-14 days later. Diagnosis of follicular cyst is made if ultrasonography reveals that no luteal layers are present near the follicular fluid of the ovary or a large-size ovarian follicle and if progesterone concentrations are 5 ng/ml or lower in whole milk and 1 ng/ml or lower in plasma and skimmed milk.

In some cases within 40 days postpartum in which an ovarian follicle that grew to 2.5 cm in diameter or larger persists for some time and undergoes regression to atresia while another ovarian follicle that grew anew ovulates, the disorder may be healed spontaneously but reexamination should be performed 7-14 days later and the disease treated as needed.

If the cause of this disorder is related to feeding and management, and especially inappropriate feeding, stockkeepers should be instructed to improve such conditions. In addition, 100-200 μg of firtirelin acetate or buserelin acetate at a dose of 10-20 μg as buserelin, both GnRH analogues, should be administered once by intramuscular injection for the purpose of inducing the anterior lobe of hypophysis to cause endogenous LH surge. 5,000-10,000 IU of hCG should also be administered once by subcutaneous or intramuscular injection to supplement GTH that has LH action. If these treatments fail, intravenous or intramuscular injection of 200-400 Rab.U bovine APG or intravenous injection of 20-40 A.U. swine APFSH or subcutaneous or intramuscular injection of 40-60 A.U. swine APFSH should be administered once. Administration of these hormone preparations when
a new ovarian follicle grows in the follicular wave of an ovarian follicle coexisting with the cystic follicle induces ovulation and subsequent luteinization so the efficacy of the hormone therapy can be enhanced. Care must be taken when using hCG, bovine APG and swine APFSH preparations because antihormones are produced in the body and reduce therapeutic effect if they are used repeatedly.

Table 2-1 Differences in secretion of steroidal hormones in ovarian cysts and similar forms (by palpation)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Schematic section views of the ovary (left and right)</th>
<th>Endocrinological characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cyst</td>
<td></td>
<td>a: high levels of OE₂ and BE₂, granulosa layer present or growing</td>
</tr>
<tr>
<td>Follicular cyst (FC)</td>
<td></td>
<td>b: low levels of OE₂ and BE₂, granulosa layer degenerated or lost</td>
</tr>
<tr>
<td>Luteal cyst (LC)</td>
<td></td>
<td>c: intermediate type of a and b</td>
</tr>
<tr>
<td>Cystic corpus luteum (CCL)</td>
<td></td>
<td>OE₂ low being expressed in pg/ml and OP high being expressed in μg/ml</td>
</tr>
<tr>
<td>Coexistence of large-size ovarian follicles and corpus luteum (FC₁)</td>
<td></td>
<td>BP at the level of 1-3 ng/ml (luteal layer present in cystic cavity)</td>
</tr>
<tr>
<td>Estrus (ES)</td>
<td></td>
<td>Similar to follicular cyst (FC)</td>
</tr>
</tbody>
</table>

(Cited from Hoshino)

Notes 1: OE₂, OP, BE₂ and BP represent estradiol and progesterone levels in cystic follicular cavity fluid and peripheral blood, respectively.
2: Steroidal hormone levels were determined by radioimmunoassay.

For the purpose of normalizing the release of GTH, especially LH, from the anterior lobe of the hypophysis, PRID was inserted into the vagina and retained at the same position for 12 days with good results.

If rectal palpation performed 7-14 days after treatment with the aforementioned hormone preparations reveals luteinization in the ovary, then the treatment is diagnosed as effective. The responses of the ovary to treatment include one in which an ovarian follicle less than 2.0 cm in diameter coexisting with the cystic ovarian follicle ovulates and forms the corpus luteum, one in which the cystic ovarian follicle ruptures and the corpus luteum is formed afterwards and one in which the cystic ovarian follicle degenerates to atretic corpus luteum or undergoes retrogression to atresia. The response in which the formed corpus luteum regresses within 25 days of administration, estrus emerges as ovarian follicles develop and normal corpus luteum is formed after ovulation is diagnosed as healed.
Stockkeepers should be informed beforehand that there are some cases in which estrus appears around 10 days after administration and an estrous cycle with duration of about 10 days is repeated. At the time of the first estrus around 20 days after treatment, findings suggest as if the disorder is not yet healed because the liquid content of the cystic ovarian follicle that was present in the ovary at the time of treatment remains as is without being absorbed. However, if clear estrus appears and new ovarian follicles 1.5-2.0 cm in diameter are present in the ovary, insemination should be performed without hesitation.

If treatment fails or the disorder recurs, the type of hormone preparation and dosage schedule should be reconsidered for additional treatment. If the disorder recurs in spite of various treatments mentioned above, the case should be judged as having no chance of healing.

ii. Luteal cyst

A state in which ovarian follicles grow to 2.5 cm or larger but do not ovulate, part of the entire formed cystic follicle walls luteinizes and the cystic follicle forms an internal cavity that retains fluid. Once formed, a luteal cyst continues to exist for a long time and suppresses the growth of normal ovarian follicles so an anestrous state persists.

The disorder is often complicated by follicular cysts so its diagnosis should follow that of follicular cysts.

Differentiation from follicular cysts or cystic corpus luteum formed after ovulation and from normal corpus luteum is often difficult if rectal palpation reveals thin luteal layers on part or the entire cystic walls (Photograph 2-2&3 (p.81)) and thick luteal layers, respectively. The diagnosis of this disorder is made if ultrasonography reveals the presence of luteal layers around the cavity containing fluid or the determination of progesterone levels in milk or plasma reveals that the level in whole milk and that in plasma and skimmed milk are more than 5 ng/ml and 1 ng/ml, respectively, and reexamination 14-20 days later reveals no changes.

The disorder is likely to be confused with cystic corpus luteum, however, differentiation is possible because of the fact that the cystic corpus luteum is formed after ovulation so that it has processus (Photograph 2-4&5 (p.81)) and maintains a normal estrous cycle. Care must be taken, however, because rectal palpation may fail to detect the processus of a cystic corpus luteum with the major axis of at least 3.0 cm.

As a treatment for luteal cyst, either one of prostaglandin (PG) F₂₅₆ preparations, i.e. 12-15 mg of dinoprost or tromethamine dinoprost at a dose of 15-25 mg as dinoprost, or one of PGF₂₅₆ analogues, i.e. 500 μg of cloprostenol or 5 mg of etiproston tromethamine, should be administered once by intramuscular injection. Alternatively, 1 mg of fenprostalene should be administered by subcutaneous injection to facilitate the regression of the luteal layers of the cystic walls and the growth of ovarian follicles.

If ovarian follicles develop within 10 days of treatment, followed by the occurrence of estrus and ovulation, and a normal corpus luteum is subsequently formed, the
case is diagnosed as healed. Care must be taken when making a diagnosis because cavity fluid sometimes remains after the disappearance of the luteal layers of a luteal cyst appearing as if it shifted into a follicular cyst.

4 Silent heat

A clinical condition in which in spite of the normal ovarian cycle, i.e. periodic follicular growth, ovulation and luteinization, estrus does not occur normally with regression of the corpus luteum and follicular growth. Because of unclear estrous signs, the condition disturbs mating and causes a reproductive disorder but conception by insemination is possible by identifying the optimum time for insemination from information about internal estrous signs and the follicular growth status obtained from vaginal and rectal inspections.

Possible causes include abnormal secretion of GTH, quantitative imbalance between estrogen and progesterone, the high threshold level of sex hormones for nervous excitation involved in the expression of estrous behavior and psychological factors, although the direct cause is not well understood. Silent heat frequently occurs in cows with high milk yield, fat cows, cows raised in cowsheds under unfavorable feeding management conditions, low-class cows in a herd and cows with algetic diseases of the hoof.

If rectal palpation reveals that internal estrous signs are clear with mature ovarian follicles present in the ovary but they ovulate without external estrous signs and if the corpus luteum is present in the ovary, a reexamination 7-14 days later reveals changes in the location and shape of the corpus luteum and external estrous signs are not present during this period, then the case is diagnosed as silent heat. In making a diagnosis, the fact that many cases of the first and second postpartum ovulation in the ovarian cycle are quiet ovulation and that there are many cases in which careless stockkeepers overlook external estrous signs should be noted and care must be taken as to the differentiation between a retained corpus luteum and pregnancy.

To treat a case in which rectal palpation reveals the corpus luteum that was formed after silent heat, either one of PGF₂₀ preparations, i.e. 12-15 mg of dinoprost or tromethamine dinoprost at a dose of 15-25 mg as dinoprost, or one of the PGF₂₀ analogues, i.e. 500 µg of cloprostenol or 5 mg of etiprostol tromethamine, should be administered once by intramuscular injection. Alternatively, 1 mg of fenprostalene should be administered by subcutaneous injection. Studies showed that the administration of a GnRH analogue, fertyrelin acetate, at a dose of 100 µg 54 hours after treatment with the aforementioned drugs followed by insemination 18-24 hours later generally provided satisfactory conception rates. Intramuscular injection of 2-5 mg of estradiol benzoate, a follicular hormone preparation, in the cases with a regressive corpus luteum and growing ovarian follicles induced estrous signs. An infusion of 30-50 ml of povidone iodine solution (containing 20 mg of povidone iodine or 2 mg of effective iodine per milliliter) into the uterus in the luteal phase induced clear estrous signs 6-11 days later.

In addition, to normalize endocrine abnormalities by adjusting blood progesterone levels, CIDR was inserted into the vagina and retained in the same position for 7 days with good results.
Insemination should be performed if estrus occurs after treatment. In some cows with poor nutritional and health conditions or algetic diseases of the hoof, quiet ovulation occurs after treatment, development of ovarian follicles that occurs simultaneously with luteolysis is quite late or ovarian follicles undergo regression to atresia without ovulation and shift to ovarian quiescence. Thus, causal factors must be improved before treatment.

3. Retained corpus luteum (persistent corpus luteum)

A clinical condition in which the functioning corpus luteum continues to exist for a longer period than normal in the estrous cycle in the absence of pregnancy. Progesterone secreted by the corpus luteum suppresses the growth of ovarian follicles resulting in an extended period of anestrus.

There are two possible mechanisms for the disorder. One mechanism is that the presence of foreign materials such as a mummified fetus, pus and mucus in the uterus or abnormalities in the endometrium such as chronic inflammation inhibit the production and/or release of luteolytic factors by the uterus. Another is abnormal secretion of GTH from the anterior lobe of the hypophysis and this mechanism may be involved in the disorder of the type occasionally seen in high-lactation cows that is not accompanied by uterine abnormalities.

Diagnosis should be made by confirming that rectal palpation reveals the corpus luteum in the ovary and a reexamination 10-14 days later reveals no changes in the location or shape of the corpus luteum. Diagnosis of the disorder is also made if the progesterone level does not decrease to lower than 5 ng/ml and 1 ng/ml in whole milk and plasma and skimmed milk, respectively, in the examination of progesterone levels in milk or blood that is performed continuously at 3 to 4-day intervals for more than 25 days.

Care must be taken because there may be foreign matter in the uterus. If the presence of mucous matter in the uterus is suspected, ultrasonography should be performed and as required, a sample of the content of the uterus should be taken via the cervical canal for the study of its properties. This must be distinguished from the early stage of pregnancy.

As a treatment for the disorder, either one of PGF₂₀ preparations, i.e. 12-15 mg of dinoprost or tromethamine dinoprost at a dose of 15-25 mg as dinoprost, or one of PGF₂₀ analogues, i.e. 500 μg of cloprostenol or 5 mg of etiprostanon tromethamine, should be administered once by intramuscular injection to facilitate luteolysis and growth of ovarian follicles. Alternatively, 1 mg of fenprostalene should be administered by subcutaneous injection. The corpus luteum was enucleated with fingers through the rectal walls in an old-fashioned treatment for the disorder, however, this method must not be employed because ovarian function may be impaired due to bleeding, and occasionally lethal bleeding, from the ovary and adhesions near the ovary. Foreign matter in the uterus must be removed.

If luteolysis occurs within 10 days of treatment with the above-mentioned drugs and ovarian follicles develop and ovulate followed by the formation of a new corpus luteum and a normal estrous cycle, then the case is diagnosed as healed. Treatment must be made if the corpus luteum that was present at the time of treatment is still present 10
days later. Care must be taken because a shift to ovarian quiescence and an ovarian cyst may occur after treatment or a fluid such as mucus may be retained within the uterus and result in recurrence of the disease.

Pyometra

A condition in which pus or purulent exudate collects in the uterine cavity. This occurs because purulent exudate resulting from purulent endometritis is not expelled into the vagina because the cervical canal is closed.

The disease often follows delayed recovery of the uterus due to dystocia and retained placenta in the perinatal period and it sometimes results from infection by bacteria such as Actinomyces pyogenes and protozoa such as Trichomonas fetus that enter the uterus at the time of insemination of a pregnant cow showing estrus.

Purulent exudate that collected in the uterine cavity suppresses the production of luteolytic factors by the uterus and causes retained corpus luteum resulting in an extended anestrous period. A vaginal examination often reveals dry vaginal mucous membranes and a closed external uterine orifice presenting findings similar to the pregnancy period. Rectal palpation reveals that the uterus is expanded, subsiding into the abdominal cavity, presenting fluctuation and has no contractility with thin uterine walls. The uterus feels similar to the one in the second to third month of pregnancy in many cases so differentiation from pregnancy is necessary. If a differential diagnosis fails, reexamination must be performed a few weeks later. Changes in the uterus according to the number of days after mating are observed in the case of pregnancy but not in the case of pyometra. Systemic symptoms and changes in blood properties are absent if a large amount of pus accumulates in the uterus. Ultrasonography reveals the pus in the spherically enlarged uterine cavity as a hypechoic image but it does not show the presence of the fetus or conceptus in that area.

As a treatment for the disorder, either one of PGF₂α preparations, i.e. 12-15 mg of dinoprostan or tromethamine dinoprostan at a dose of 15-25 mg as dinoprostan, or one of PGF₂α analogues, i.e. 500 µg of cloprostenol or 5 mg of etiprostol tromethamine, should be administered once by intramuscular injection to facilitate luteolysis and the growth of ovarian follicles. Alternatively, 1 mg of fenprostalene should be administered by subcutaneous injection. Luteolysis occurs in 3-5 days after treatment, an ovarian follicle develops to produce estrogen, the cervical canal relaxes and dilates, uterine contraction is enhanced and the purulent exudate in the uterus is expelled. Treatment procedures for endometritis should be followed as required if the purulent exudate is still present after treatment.

The judgement of recovery should be made in the same way as endometritis. The possibility of recovering and conception is greater if the diagnosis is made and treatment is given earlier. If the discovery and treatment of the disease is late, the endometrium is destroyed, the uterine walls become fibrous and a complete recovery and conception may be difficult to attain.
Mucometra and hydrometra

A condition in which various amounts (30 milliliters to several liters) of fluid is retained in the uterus. These fluids include various types of liquid ranging from watery and viscous mucus to semifluid mucous masses containing denatured tissue fragments.

The disorder is considered to be unrelated to microbial infection. Histopathological findings show cystic degeneration of the endometrium and atrophy of the uterine walls although their causes are unknown. The disorder accompanies follicular cysts and occurs in individual animals with anomalies of the uterus, cervical canal and vagina and persistent, rigid and imperforate hymen.

Rectal palpation reveals that both of the uterine horns are enlarged and thick, uterine walls are thin and have fluctuation and liquid matter is present in the uterine cavity. A viscous property of the liquid indicates mucometra and a watery property hydrometra. In some nulliparous cows with persistent hymen, the vagina as well as uterus is expanded.

Ultrasonography reveals a near circular or irregular-shape echo-free mass (liquid matter) in the uterus that includes a low-level echo (mucus) or minute high-level echoes (cells, tissue fragments).

The disorder is accompanied by a retained corpus luteum in many cases and causes the host to remain in an anestrous state so care must be taken not to make a misdiagnosis by confusing with pregnancy or pyometra.

Persistent hymen must be removed, if present, for treatment. In case the corpus luteum is present in the ovary, either one of PGF₂α preparations, i.e. 12-15 mg of dinoprost or 20-33 mg of tromethamine dinoprost (15-25 mg as dinoprost), or one of PGF₂α analogues, i.e. 500 μg of cloprostenol or 5 mg of etiprostol tromethamine, should be administered once by intramuscular injection. Alternatively, 1 mg of fenprostalene should be administered by subcutaneous injection. Follicular cysts must be treated if they are complicating the disorder.

The disorder is diagnosed as healed if the liquid matter retained in the uterus has disappeared within 40 days of treatment. In general, the disorder often recurs after apparently successful treatment and there are few cases in which treatment results in complete recovery and conception. In cases in which persistent hymen was removed for the treatment of the disorder, care must be taken because scars formed after treatment may lead to vaginal stenosis and resultant dystocia.

A case in which estrus and mating does not lead to conception due to the disorders of the vagina, cervical canal, uterus, oviduct and ovary.

Vaginitis

Inflammation of the vagina.

The disorder results from infection by bacteria or vaginal irrigation with irritating disinfectant and high-temperature wash, associated with dystocia, vaginal prolapse, coitus and insemination. It also occurs as a result of retention of the placenta,
endometritis and cervicitis in many cases. In the case of bacterial infection, major
causal bacteria include indigenous bacteria in the vulva and vestibule of the vagina such
as Staphylococcus spp., Streptococcus spp., E. coli and Actinomyces pyogenes. Vaginitis inhibits conception if the disorder accompanies endometritis and cervicitis but it heals spontaneously if it is mild in severity and without complications.

In many of the cows with the disorder, pyoid or cloudy mucus discharges from the
vulva irregularly and the vaginal mucous membrane is congested, swollen and has
pyoid mucus on the vaginal walls. In the case of severe vaginitis, stimulation from a
vaginal examination and so forth causes pain. In the case of complications by
cervicitis and endometritis, discharge of pyoid exudate from the external uterine orifice
and congestion of ectocervical mucous membranes are present.

For treatment, vaginal irrigation with less irritant physiological saline or a zwitterionic
or cationic detergent should be performed and drugs such as sulfanilamides and
antibiotics applied to or infused into the vagina.

The disease requires some time to heal but the prognosis is generally good. Diseases
that are secondary to endometritis and cervicitis can be healed by treating these
diseases.

2 Urovagina

A condition in which part or most part of the urine flows backward into the vaginal
floor and is retained temporarily or persistently.

The disorder occurs because the broad ligament of the uterus and supporting tissues
near the vagina that support the uterus and vagina relax due to aging, weakness,
malnutrition and perinatal injuries and the inner part of the vagina sinks due to a loss of
strength. The disease is accompanied by impaired ovarian function such as follicular
cysts and ovarian subfunction in most cases and occurs rarely in animals with a normal
estrous cycle. Thus, its close relationship to the secretion of sex steroid hormones is
suggested.

In cows with the disease, the vulva faces upward because the vagina sinks forward so
the urine is retained in the inner part of the vagina submerging the exterior uterine
orifice. The vaginal mucous membrane is reddened and emits a strong urinary odor
and the cervical mucus is suspended from the vulva like a thread since it is mixed with
urine and rendered watery. Cervicitis or endometritis follows if the disease lasts long.

Treatment must be tailored for the individual causes of the disease because urine
collects in the vaginal floor soon after it is removed. Thus, marasmus and malnutrition
must be improved by supplementing with nutrients and providing sufficient exercise
and care. Appropriate treatments must be given to cows with inflammation of the
uterus, cervical canal and vagina.

For cows with mild urovagina in which urine is retained in the vaginal floor temporarily,
the urine should be removed and the vagina irrigated with physiological saline or 5%
glucose solution before infusing semen into the inner part of the cervical canal or the
internal uterine orifice area. Surgery has been tried to restore the vagina if the vagina
sinks to a serious extent, however, the disease recurs in many cases.
There are chances of conception in mild cases or cases of temporary retention of urine. In cases in which the disease results from marasmus and malnutrition, the improvement of constitution is needed and takes a significantly long period. There are no chances of recovery in cases of a depressed back due to aging and emaciation.

3 Cervicitis

Inflammation of the cervical canal.

It often accompanies endometritis and results from bacterial infection at the time of abortion, dystocia and retained placenta. It also results from injuries, and especially sticking injuries, to the cervical walls due to inappropriate handling of equipment for insemination and the diagnosis/treatment of uterine diseases.

The ectocervical part is congested and swollen and the third plicae circularis of the cervical canal is congested and, because it is swollen, turned over, exposed outside the external uterine orifice showing complicated morphology with its mucous membrane turned red or dark purple. A pyoid exudate discharges from the external uterine orifice. In some cases of old cervicitis, the external uterine orifice is maintained wide open and allows access to the uterus even during the luteal phase.

Care must be taken because cervicitis is present independently quite rarely and is complicated by endometritis and vaginitis in most cases.

For treatment, the ectocervical part must be washed with warm water containing less irritant disinfectant or physiological saline and povidone iodine solution or an antibacterial agent applied to or infused into the cervical canal. The disease may heal spontaneously after repeated estrous cycles so care should be taken to maintain normal ovarian function while at the same time treating complicating endometritis and vaginitis.

The disease is diagnosed as healed if the congestion of the ectocervical region disappears and normal cervical mucus is present. If the disease is accompanied by endometritis, however, diagnosis of it being successfully treated is also necessary. The prognosis is generally satisfactory.

3 Endometritis

Endometritis refers to inflammation of the endometrium and is the most frequently occurring disease of all uterine diseases, being one of the major causes of conception failures. The disease inhibits sperm from ascending by reducing their motility, inhibits the growth of embryos and, if implantation is attained, causes early fetal death and abortion.

The disease is divided into either the infectious type that is caused by bacterial, viruses, fungi and protozoans or the noninfectious type. In most cases of the infectious type, causal bacteria are Staphylococcus spp., Streptococcus spp., E. coli, Actinomyces pyogenes, Pseudomonas aeruginosa and other noninfectious indigenous bacteria that are present in the vulva, vestibule of the vagina, cow's body and cow sheds. Mixed infection with two or more bacterial species often occurs. Infectious bacteria include Campylobacter fetus and Brucella abortus. Bacterial endometritis is mainly caused by
spontaneous or artificial infection of the uterus with bacteria through the vagina that results from coitus, insemination, embryo transfer and examinations and equipment for the diagnosis and treatment of uterine diseases or from procedures for perinatal disorders such as dystocia and retained placenta. The mechanism of bacterial infection of the uterus is related to the nutritional and health conditions of cows and is closely related to sex steroidal hormones. Thus, estrogen acts by protecting the uterus against bacteria and progesterone acts by suppressing this protective action and making the environment suitable for bacterial growth. The uterus in the early luteal phase is known to be particularly susceptible to bacterial infection and endometritis. The causes of endometritis of the noninfectious type include artificial factors such as the infusion of an irritate chemical solution into the uterus and uterine irrigation with high-temperature physiological saline.

Endometritis is divided into the acute and chronic types according to clinical findings and the type that is associated with discharging of abnormal secretion is referred to as secretive endometritis and the type that is not latent endometritis. Secretive endometritis is further divided into catarrhal endometritis in which hyaline mucus or hyaline mucus containing grayish-white streaks discharges from the external uterine orifice and purulent endometritis associated with discharging of purulent mucus.

Diagnosis is based mainly on a vaginal examination and, as required, on diagnostic uterine irrigation and an endometrial biopsy. By microscopically observing Giemsa-stained specimens of centrifugal precipitates of the cervical mucus and washings from uterine irrigation, determination of the white blood cell count and a bacteriological examination are performed. The result of a bacteriological examination will help determine the sensitivity of pathogens for subsequent treatment with antibacterial agents. Findings in a histological study of the endometrium help determine the prognosis. Care must be taken when making a diagnosis because the disease is often complicated by ovarian diseases such as ovarian cysts and ovarian quiescence.

As a basic treatment, the inside of the uterus is washed with sterilized physiological saline (40-42 °C, a total of 2-4 liters) to remove its content and an intrauterine infusion for endometritis is administered. In cases of mild endometritis that are not associated with a large amount of abnormal secretion, omitting uterine irrigation from the treatment procedure mentioned will not reduce the efficacy. In the absence of high effectiveness, however, uterine irrigation must be performed instead of uselessly repeating medications.

Intrauterine infusions include a combination of ampicillin 500 mg (potency), benzylpenicillin procaine 400,000 units and dihydrostreptomycin sulfate 400 mg (potency) and povidone iodine solution (20 mg of povidone iodine, or 2 mg of effective iodine, per milliliter). If kanamycin or another drug is used according to the result of a drug susceptibility test, these drugs in the amount of about 10% of the dose for systemic treatment should be dissolved in distilled water for infusion before use. The quantity of infusion should be 30-50 ml, a sufficient amount to cover the entire surface of the endometrium. If antibacterial agents are used for treatment, the relevant standards for use must be followed considering the possibility of these agents remaining in the body and appearing in the milk.

Stockkeepers should be informed beforehand that the intrauterine infusion of povidone
iodine solution 2-7 days after ovulation, i.e. in the early luteal phase, causes estrus to occur 4-11 days after infusion and shortens the estrous cycle to 8-15 days while infusion 15-17 days after ovulation, i.e. in the late luteal phase, prolongs the estrous cycle by 2-4 days. Infusion in the early luteal phase shortens the estrous cycle because temporary inflammation of the endometrium occurs after infusion and luteolytic factors produced during the recovery process are transported through vessels to the ovary where they directly act on the corpus luteum. Infusion in the late luteal phase prolongs the estrous cycle because inflammation of the endometrium occurs and delays the production of luteolytic factors.

To promote the self-cleaning action of the uterus associated with the appearance of estrus, tromethamine dinoprost, a PGF₂α preparation, at a dose of 15-25 mg as dinoprost was administered once intramuscularly in the luteal phase with satisfactory results. However, uterine irrigation and intrauterine administration should be performed as required. Ovarian diseases such as ovarian cysts must be treated if they are present.

The disease is diagnosed as healed if an examination performed in the estrous period or 7-14 days after treatment according to the diagnostic method mentioned above reveals a loss of abnormal findings. The prognosis is generally satisfactory, however it is poor in cases in which the disease persists and the uterus is extremely loosened and suspended and the uterine wall is extremely thinned or thickened.

Myometritis and perimetritis

These refer to inflammation of the myometrium and perimetrium, respectively, and myometritis is often accompanied by endometritis or perimetritis.

Some cases of these diseases result from severe endometritis, injuries of the uterus and cervical canal at the time of dystocia, injuries of the uterus at the time of the correction of presentation, embryotomy and cesarean section and rough excision of retained placenta. Some cases result from injuries such as perforations in the uterus due to medical instruments at the time insemination, embryo transfer and the diagnosis and treatment of uterine diseases. Perimetritis sometimes results from peritonitis.

In the case of myometritis, rectal palpation reveals thickening and induration of the entire or localized part of the uterine wall and, in some cases, abscesses. Abscesses range from 2 cm to the size of a child's head and some sink into the abdominal cavity because of their weight. In the case of perimetritis, on the other hand, connective tissue fibers grow on the surface of the perimetrium (serous membrane) in the form of strings in some cases, both the uterine horns adhere to each other in some cases and the uterine horn adheres to the oviduct, ovary, broad ligament of the uterus, peritoneum, bladder or rumen in some cases. In acute cases, the disease shows the same symptoms as peritonitis and an examination of the uterus by rectal palpation causes pain.

For treatment, the disease is treated according to the same method as endometritis in case the disease is secondary to endometritis and no abscess is present. In the case of early-stage myometritis that was initially caused by perforations in the uterus, an antibacterial agent is infused into the uterus or injected focally. If any abscesses are present, however, the disease is unlikely to be healed.

The disease does not impede conception as long as adhesions and induration do not
affect uterine function. However, if the adhesion is extensive and severe and an abscess persists after treatment, there is little chance of recovery and conception or, if conception is attained, abortion may occur.

6 Salpingitis

Salpingitis refers to inflammation of the oviduct. The disease causes infertility because inflammatory changes occur in the oviduct, an exudate collects within the duct, mucous membranes are thickened, the duct is closed and the movement of sperm and the ovum and thus fertilization are inhibited.

If the cause of the disease is bacterial infection, it results from endometritis or pyometra and from the ascent of the causal bacteria of retained placenta or puerperal metritis. If the cause of the disease is artificial, it results from inflammation associated with a perforation in the uterus produced at the time of uterine irrigation, insemination or embryo transfer and from repeated manual rupture of cystic follicles at the time of rectal palpation, rough excision of the corpus luteum and violent palpation of the oviduct.

The diagnosis of the disease is based on the examination of the oviduct by rectal palpation and requires years of experience and careful observation. In cases of mild salpingitis, differentiation from the normal oviduct is difficult. In acute cases, the swollen oviduct can be felt since an exudate is retained in the lumen. In chronic cases, induration of the oviduct resulting from the growth of connective tissues is felt. If the atresia of the oviduct is suspected, tubal insufflation is performed. The percentage of antemortem detection of salpingitis by clinical examinations is low. The disease is detected as a result of autopsies in most cases.

If the disease is mild, the treatment method for endometritis is followed. If the atretic oviduct occurs unilaterally, insemination after confirming the growth of an ovarian follicle in the ovary on the same side as the normal oviduct may lead to conception. If the atretic oviduct occurs bilaterally, embryo transfer may be considered after confirming the normal functions of the ovary and uterus since there is no chance of fertilization.

7 Hydrosalpinx and pyosalpinx

Hydrosalpinx refers to a condition in which adhesion of the mucous membranes of oviduct and atresia of the lumen occur and a transparent and watery secretion is present in the atretic lumen. If the secretion is purulent, the condition is referred to as pyosalpinx.

Both diseases are secondary to salpingitis in many cases. Hydrosalpinx is sometimes present in the cases of congenital anomalies of the reproductive organs due to a recessive gene.

The diagnosis of the disease is based on the examination of the oviduct by rectal palpation. With hydrosalpinx, the oviduct enlarges to 1.2 cm or more in diameter. If there is a large amount of secretion, the oviduct wall is thinned and has fluctuation so that the condition is sometimes misdiagnosed as an ovarian cyst. With pyosalpinx, the fimbria of oviduct adheres to the ovary or tissues surrounding the ovary in many cases and the oviduct expands to the size of the little finger or larger due to retention of
secretion. However, distinguishing between these diseases by rectal palpation is not possible. Ultrasonograms of the secretion in the oviduct may help differentiate these diseases.

There is no cure for the diseases.

8 Ovulation failure

Ovulation failure refers to an abnormality in the ovulation process and includes delayed ovulation and anovulation.

i. Delayed ovulation
A condition in which there is a long period between estrus onset and ovulation although ovarian follicles grow in the ovary, estrus emerges and ovulation eventually occurs.

ii. Anovulation
A condition in which ovarian follicles undergo regression to atresia, atretic corpus luteum or cysts without ovulating although ovarian follicles grow and estrous signs appear.

This disorder is directly caused by abnormal LH secretion from the anterior lobe of the hypophysis, or the delay, lack or loss of LH surge, but may also be related to abnormal secretion of FSH and estradiol.

The diagnosis of ovulation failure involves repeating rectal palpation over the estrous period to confirm the presence/absence of ovulation by major ovarian follicles in the ovary. The diagnosis of delayed ovulation is made if ovulation occurs 36 hours after estrus onset followed by luteinization since ovulation normally occurs 32 hours after estrus onset in cows. Because estrus is detected by using the swelling and congestion of the vulva and the discharge of mucus from it as indicators, insemination is often repeated from several days before estrus onset and a misdiagnosis of the disease is made. The diagnosis of anovulation is made if ovulation does not occur after the presence of estrous signs and follicular growth, as indicated by rectal palpation, and findings in an examination of the ovary performed 7-10 days after the loss of estrous signs reveal follicular atresia, regression or cysts and a lack of luteinization.

If growing ovarian follicles are present, treatment includes administering 100-200 µg of fertirelin acetate or 10-20 µg of buserelin, both GnRH analogues, once intramuscularly to stimulate the anterior lobe of the hypophysis to induce endogenous LH surge as a means of remedying abnormal LH secretion. In addition, to supply LH, an intramuscular or subcutaneous injection of 1,500-3,000 IU of hCG or an intravenous or intramuscular injection of 200-400 Rab. U of bovine APG or an intravenous injection of 20-40 A.U. of swine APFSH is administered once.

Insemination is performed 9-24 hours after administration of hormone preparations depending on the status of estrus before and after the hormone administration. If insemination results in a failure and estrus returns, the hormone preparations mentioned above are administered within a short period from estrus onset and insemination is repeated.
Administration of the aforementioned hormone preparations before estrus onset results in ovulation 30-36 hours after the administration. Thus, occurrence of ovulation 2 days after administration indicates that the treatment was effective. If ovulation occurs within 24 hours of administration, the ovulation was triggered by an LH surge that was induced by endogenous GnRH and not by exogenous hormones. In the case of absence of ovulation after treatment, rectal palpation is performed 7-14 days after treatment to see if additional treatment is necessary because a shift to ovarian quiescence or ovarian cysts may have occurred.

3 Luteal hypoplasia

Luteal hypoplasia refers to a condition in which luteal tissues are formed insufficiently after ovulation. It is divided into hypoplastic corpus luteum and cystic corpus luteum depending on the shape of the corpus luteum.

i. Hypoplastic corpus luteum

The diagnosis of the disease is made if the corpus luteum grows insufficiently after ovulation, secretes progesterone insufficiently and undergoes regression too early with the estrous cycle shortened to about 10 days. A corpus luteum like this is often formed after the first ovulation following birth or delivery and after treatment of cows with ovarian quiescence with hCG or GnRH analogues. After the treatment of follicular cysts or in the cases of urovagina and endometritis, an estrous cycle of about 10 days is sometimes repeated due to insufficient luteinization after ovulation.

Because of poor luteinization, possible causes include reduced ability of the anterior lobe of the hypophysis to secrete LH and a lack of LH receptors in the ovarian follicles that ovulated and the corpus luteum that was formed later. Because the disease occurs in cases of urovagina and mild endometritis, luteolytic factors that are produced during the recovery phase of transient inflammation associated with endometritis in the early luteal phase may be one of the causes.

For treatment, 1,500-3,000 IU of hCG is administered intramuscularly or subcutaneously or 100-200 μg of furtirelin acetate or 10-20 μg of buserelin, both GnRH analogues, is administered intramuscularly during the period from onset of the following estrus to ovulation. Administration of the aforementioned hormone preparations to cases with existing hypoplastic corpus luteum may not prolong the estrous cycle in many cases. If present, urovagina and endometritis must be treated concurrently.

The disease is diagnosed as healed if a normal corpus luteum is formed after treatment.

ii. Cystic corpus luteum

For several days in the early luteal phase, a cavity retaining fluid is often present in the central part of the bovine corpus luteum but the cavity gradually becomes occupied by luteal tissues as the corpus luteum grows until finally it is lost. Cystic corpus luteum refers to a condition in the functional luteal stage in which the cavity within the corpus luteum is so large that the surrounding luteal layer is thin, and the cavity retains fluid.
Diagnosis of the disease is made if by rectal palpation at 2-3 days after ovulation, i.e. in the early luteal phase, the corpus luteum is found to be larger than normal and a sense of strain or fluctuation is felt because of fluid retention and if these conditions persist until 7-14 days after ovulation. Palpation presents findings quite similar to those of ovarian cysts in many cases. Processus of the corpus luteum protruding from the ovarian surface like a mushroom are present (Photograph 2-4&5 (p.81)) because the disease develops after ovulation but rectal palpation may fail to detect them. The length of the estrous cycle is within the normal range.

An ultrasonographic examination of the corpus luteum reveals inside a circular or irregular-shaped echo-free image 1.0 cm or more in diameter. The thickness of the luteal layer ranges from extremely thin to thick depending on the case. The range of progesterone levels in blood varies from normal to low.

Views differ as to whether fluid is retained in the luteal cavity because of decreased ability to form the corpus luteum due to abnormal function of the anterior lobe of the hypophysis to secrete LH or if luteinization is insufficient because of fluid retention after ovulation.

Treatment is unnecessary if the luteal layer of the cystic corpus luteum is thick because such corpus luteum retains normal function. If the cavity inside the corpus luteum is exceptionally large and the surrounding luteal layer extremely thin, a solid corpus luteum can be formed in 3-4 days by eliminating the fluid inside the luteal cavity by pressing with fingers from over the rectal wall or by aspirating it with an ovarian syringe. Eliminating the fluid inside the luteal cavity by the seventh day from insemination is effective in achieving conception if the fluid was formed after insemination.

The disease is healed if a normal corpus luteum is formed after treatment.

**Ovaritis**

Ovaritis refers to inflammation of the stroma of the ovary and adhesion of the ovary to the surrounding tissue often occurs. Although ovarian follicles grow and estrus occurs in the early stage of the disease, these ovarian follicles cannot achieve ovulation and undergo atresia and regression or, in some cases, degenerate to cysts. In the case of chronic ovaritis, connective tissue grows on the stroma of the ovary and inhibits growth of ovarian follicles, resulting in anestrus due to a loss of ovarian function. Abscesses are rarely formed.

Artificial causes include violent palpation of the ovary in rectal palpation, rupture of ovarian cysts and cystic corpus luteum by pressing with fingers and removal of the corpus luteum. The disease also results from bacterial endometritis and salpingitis and from peritonitis associated with injuries due to metal foreign matter and tuberculosis.

Diagnosis in the early stage of the disease is relatively easy because rectal palpation reveals slightly to severely swollen ovaries and mild pain is present. In chronic cases, adhesion of the ovary to the surrounding tissue impedes palpation but diagnosis is possible because it is shrunk and inelastic.
No appropriate treatments are available. The disease may heal spontaneously if the degree of inflammation and adhesion to surrounding tissue is mild. The prognosis is poor if the disease occurs bilaterally and severely, but if the disease occurs unilaterally and estrus and growing ovarian follicles are present, insemination may lead to conception.

If stockkeepers choose conception, delivery and milking in spite of poor prognosis, embryo transfer may be tried after confirming the normal estrous cycle and ovarian function.

(3) **A condition in which no abnormality is present in the reproductive organs but no conception occurs even after three times of mating**

1. **Repeat breeder**

   Repeat breeder refers to an infertile condition of unknown cause in which in spite of the normal estrous cycle and normal results of examinations of the ovary and accessory reproductive organs, no conception occurs after three times of mating at each estrus.

   The causes of the disease vary but fertilization failure and early embryonic death are considered to be the main causes. The causes of fertilization failure include congenital or acquired anomalies or infectious diseases of accessory reproductive organs such as the oviduct and uterus as well as reduced fertility of the ovum and sperm due to poor properties of semen and untimely insemination. The causes of early embryonic death include mild bacterial infection of the uterus and abnormalities in the environment of the oviduct and uterus due to quantitative imbalance between estrogen and progesterone involved in movement, growth and implantation of the embryo. Involvement of chromosomal aberration is also probable.

   Because of a lack of definitive diagnostic criteria, infertile cows are indiscriminately diagnosed as the disease in some cases. Examinations performed from different angles may reveal that some of these cases are caused by other disorders such as ovulation failure, atretic oviduct and latent endometritis.

   Treatment includes improving the feeding environment and breeding management, which are thought to be predisposing factors. If early embryonic death is considered to be the cause, underlying factors are assumed and the following diagnostic treatment procedures are performed.

   If mild bacterial infection of the uterus is suspected, uterine irrigation is performed and a drug containing an antibacterial agent is infused into the uterus in the luteal phase before insemination or within 1-2 days of insemination, according to the treatment method for endometritis. To promote luteinization, 1,500-3,000 IU of hCG is administered intramuscularly or subcutaneously or 100-200 µg of fertirelin acetate or 10-20 µg of busrelin, both GnRH analogues, is administered intramuscularly at the time of insemination or within 3-7 days of ovulation. If implantation failure of the embryo due to reduced luteal function is suspected, 200-500 mg of long-acting progesterone is administered intramuscularly 4-5 days and 10-12 days after insemination.

   Healing of the disease is diagnosed if conception occurs after the improvement of the
feeding environment and breeding management or treatment. If no conception occurs in spite of the treatments mentioned above, there is no chance of recovery.

(4) Abnormalities during the pregnancy and perinatal periods

1. Fetal death

   i. Fetal mummification

   A condition in which a fetus that died in the mid-pregnancy period, fetal fluid, fetal membrane and placenta shrink and harden and turn chocolate color because body fluids from them are absorbed aseptically and remain in the uterus for a long period.

   Causes of fetal death vary. Malnutrition, summer heat, infection with bovine viral diarrhea mucosal disease (BVD-MD) virus and *Neospora caninum*, torsion of umbilical cord and a recessive gene on the autosome are known to cause the disease. Estrus is absent because the production of luteolytic factors by the endometrium is suppressed and the corpus luteum is retained.

   A vaginal examination reveals pregnancy findings, rectal palpation reveals a failure of growth of the fetus and uterus matching the fetal age, fetal fluid is quite little or lost and the uterus lacks fluctuation. The uterine wall is thin and rough, the placenta is not palpable and the fetus feels like a hard body. Vibrations characteristic to the mid-pregnancy period are not felt on the uterine artery and the persistent corpus luteum is present in the ovary. These conditions occur one or more months after fetal death when the fetus has undergone sufficient mummification. In practice, fetuses in various stages of mummification are found.

   For treatment, either one of PGF₂α preparations, i.e. 12-15 mg of dinoprost or tromethamine dinoprost at a dose of 15-25 mg as dinoprost, or 500 µg of cloprostenol, a PGF₂α analogue, is administered once by intramuscular injection to facilitate regression of the retained corpus luteum and growth of ovarian follicles and promote contraction of the uterus and dilation of the cervical canal to expel the mummified fetus. Alternatively, 1 mg of fenprostalene is administered subcutaneously. If the expulsion is delayed due to insufficient dilation of the cervical canal and contraction of the uterus, 10-20 mg of estriol or 5-8 mg of estradiol benzoate is administered once intramuscularly as a supplementary method.

   The mummified fetus is usually expelled in 2-4 days after injection. If the mummified fetus is retained in the vagina with a colicky symptom, the fetus is removed with a hand. If these procedures fail, a cesarean section is performed.

   The disease is diagnosed as healed if estrus occurs after the mummified fetus has been expelled.

   ii. Fetal maceration

   Fetal maceration is one of the postmortem changes that occur when the fetus dies in the uterus but abortion does not occur and is characterized by a condition in which the fetus dissolves without the action of bacteria leaving in the uterus a thick,
viscous cream-like fluid and the fetal skeleton. The cervical canal is closed during the early stage but relaxes gradually until bacteria enter the uterus and decompose the fetus. In many cases the cause of fetal death is unidentified.

Estrus is absent. By a vaginal examination, the exterior uterine orifice is slightly open and a dirty, bad smelling fluid discharges from it. In cases in which a long time has passed since fetal death, this fluid may contain fetal hair, hoof or bone fragments. Rectal palpation reveals the hard fetal skeleton in the lowered pregnant horn. The persistent corpus luteum is often present in the ovary. Normally, no apparent systemic symptom is present.

Treatment are the same as fetal mummification if the dead fetus is in the uterus and the corpus luteum is retained in the ovary. If the decomposition of soft tissue advances and bones are separated, administration of PGF₂α preparations will result in some of the bone fragments being retained in the uterus in many cases. If the macerated fetus is removed, treatment methods for endometritis are followed. However, removing all the bone fragments is difficult whether they are removed through the cervical canal or through an incision in the abdominal wall.

Although conception is possible if the macerated fetus is discovered in the early stage and eliminated, chronic endometritis persists due to remaining bone fragments. Prognosis is poor if the degree of uterine relaxation and thickening is significant.

2 Abortion

Abortion refers to a condition in which the fetus is delivered live or dead before reaching the stage of viability and in which the delivered fetus is generally of a macroscopical size. A case in which a viable fetus is delivered before the completion of gestation period is referred to as premature birth and a case in which a nonviable fetus is delivered during this period or a fetus dies immediately before or during the delivery process is stillbirth.

Abortion is divided into infectious abortion that is caused by infection with pathogens such as bacteria, viruses, protozoans and fungi and noninfectious sporadic abortion depending on the cause. Infectious diseases of the reproductive organs that cause infectious still abortion in cows, pathogens, clinical conditions, diagnosis and countermeasures are summarized in Tables 2-2 (p.72) and 2-3 (p.72). Sporadic abortion is caused by falling of the fetus in the uterus, bruising in the abdomen, low nutrition and poisoning, serious systemic diseases, a shortage of progesterone secretion, increased secretion of adrenocortical hormones due to stress, inappropriate handling of the uterus during gestation period and genetic factors such as fetal chromosomal aberration.
### Table 2-2  Abortion in cows due to viruses and protozoans
(modified from Gibbons, 1972)

<table>
<thead>
<tr>
<th>Name of diseases</th>
<th>Pathogen</th>
<th>Clinical conditions</th>
<th>Diagnosis</th>
<th>Countermeasures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akabane disease</td>
<td>Akabane virus</td>
<td>Abortion, premature birth, stillbirth, malformation (hydrocephalus, articulation curvature of calves)</td>
<td>Viral isolation Serologic reaction Fluorescent antibody technique</td>
<td>Vaccination</td>
</tr>
<tr>
<td>Chuanzai disease</td>
<td>Chuanzai virus</td>
<td>Abortion, premature birth, stillbirth, malformation (hydrocephalus, cerebellar hypoplasia of calves)</td>
<td>Viral isolation Serologic reaction Fluorescent antibody technique</td>
<td>Vaccination</td>
</tr>
<tr>
<td>Aino virus infection</td>
<td>Aino virus</td>
<td>Abortion, premature birth, stillbirth, malformation (cerebellar hypoplasia, articulation curvature and wryneck of calves)</td>
<td>Viral isolation Serologic reaction Fluorescent antibody technique</td>
<td>Vaccination</td>
</tr>
<tr>
<td>Infectious bovine rhinotracheitis (IBR)</td>
<td>Bovine herpes-virus 1</td>
<td>Abortion, respiratory symptoms, postural reflexes, balanoposthitis</td>
<td>Viral isolation Serologic reaction Fluorescent antibody technique ELISA</td>
<td>Vaccination Isolation Discontinuation of mating</td>
</tr>
<tr>
<td>Bovine viral diarrhea mucosal disease (BVD-MD)</td>
<td>BVD-MD virus</td>
<td>Enteritis, diarrhea (primary), abortion, mummification, malformation (cerebellar hypoplasia)</td>
<td>Viral isolation Serologic reaction</td>
<td>Vaccination Isolation</td>
</tr>
<tr>
<td>Rift valley fever</td>
<td>Rift valley fever virus</td>
<td>Abortion, hepatic necrosis</td>
<td>Viral isolation Serologic reaction</td>
<td>Rudication of external parasites Restriction of movement</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td><em>Trichomonas foetus</em></td>
<td>Sterility, pyometra, abortion (1-4 months of pregnancy)</td>
<td>Detection of <em>Trichomonas</em> sp. by culture of preputial content</td>
<td>Insemination Treatment of infected cows</td>
</tr>
<tr>
<td>Neosporosis</td>
<td><em>Neospora caninum</em></td>
<td>Abortion, stillbirth, mummification, non-purulent encephalomyelitis, non-purulent myositis</td>
<td>Histopathological study Immunohistochemical examination of interstitial protozoa</td>
<td>Prevention of approach of dogs</td>
</tr>
</tbody>
</table>

### Table 2-3  Abortion in cows due to infection with bacteria, Chlamydia spp. and fungi
(modified from Frank and O’Berry, 1966)

<table>
<thead>
<tr>
<th>Name of diseases</th>
<th>Pathogen</th>
<th>Clinical conditions</th>
<th>Diagnosis</th>
<th>Countermeasures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis</td>
<td><em>Brucella abortus</em></td>
<td>Abortion (6-9 months of pregnancy) Conception failure Orchiitis Epizootic dermatitis</td>
<td>Serological reaction Bacterial isolation Clinical conditions</td>
<td>Vaccination* Examination and slaughter Isolation, disinfection</td>
</tr>
<tr>
<td>Campylobacteriosis</td>
<td><em>Campylobacter fetus</em></td>
<td>Temporary sterility Abortion (4-7 months of pregnancy)</td>
<td>Agglutination test of vaginal mucus Bacterial isolation</td>
<td>Artificial insemination Treatment of infected cows</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td><em>Leptospira pomona</em></td>
<td>Hemolytic anemia Abortion (late gestation period) Agalactia</td>
<td>Serological reaction Bacterial isolation</td>
<td>Vaccination* Treatment (Antibiotics)</td>
</tr>
<tr>
<td>Leptospirosis</td>
<td>Others</td>
<td>Abortion (late gestation period)</td>
<td>Serological reaction Bacterial isolation</td>
<td>Vaccination* Treatment (Antibiotics)</td>
</tr>
<tr>
<td>Listeriosis</td>
<td><em>Listeria monocytogenes</em></td>
<td>Encephalitis Abortion</td>
<td>Bacterial isolation Clinical condition Histopathological study</td>
<td>Isolation, disinfection</td>
</tr>
<tr>
<td>Chlamydiosis</td>
<td><em>Chlamydia psittaci</em></td>
<td>Abortion (late gestation period) Hepatic lesions of the uterus Orchiitis Seminal vesiculitis</td>
<td>Isolation of pathogen Serological reaction Fluorescent antibody technique</td>
<td>Isolation Elimination of stress (prevention)</td>
</tr>
<tr>
<td>Mycotic abortion</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Abortion</td>
<td>Fungal isolation Lesions of aborted fetus and placenta</td>
<td>Avoid using moldy feed</td>
</tr>
</tbody>
</table>

Note  * Not practiced in Japan.
Eliminating causes and predisposing factors is the basis of treatment. In the case of infectious abortion, hygienic management is important and vaccination is employed. To prevent habitual abortion in which abortion occurs at the same stage each time in spite of a lack of infection or exogenous sensitization in particular, 50-200 mg of progesterone is administered several times intramuscularly at 2 to 3-day intervals starting from 2 to 3 weeks before the abortion-prone period or 300-600 mg of its depot preparation (long-acting preparation) is administered 3 or 4 times.

Occurrence of abortion of 5% or higher is a serious matter requiring diversified examinations of causes for the establishment of a proper treatment method.

3 Prolonged gestation

Prolonged gestation refers to a condition in which the gestation period far exceeds the normal range without signs of delivery or the start of delivery. Gestation with a period of 300 days or longer is generally recognized as being the disease. The fetus is excessively large or malformed in most cases.

The cause of the disease on the part of the dam is thought to be the failure of a series of endocrine mechanisms. These mechanisms include increased secretion of fetal hypophysis-derived adrenocorticotropic hormone that triggers delivery, increased secretion of fetus-derived adrenocortical hormone, an increased estrogen level in the dam's blood, production of PGF2α in the placenta, increased contraction of the uterus due to a drop in progesterone secretion and an increase in oxytocin receptors in the uterus and the start of labor. Causes on the part of the fetus include a recessive gene on the autosome that is supposed to be a predisposing factor of the abnormality in the hormone secretion mechanism mentioned above. Manifestations of the disease occurring from this cause include a giant fetus, facial malformations, cerebral deficiency, hypophys deficiency, severe adrenal hypoplasia or deficiency as well as stunning and malformation of the fetus. An example of nonhereditary congenital malformation is an outbreak of prolonged gestation associated with fetal malformation due to alkaloidal intoxication resulting from ingestion of Veratrum stamineum by pregnant cows.

Diagnosis is made if prepartum prodromal signs are absent when the due date of calving has passed, such as mammary tension, relaxation of the sacroiliac and sacrotuberous ligaments, shift to a depressed state, significant loosening of the vulva and discharge of cloudy, viscous mucus.

For treatment, either one of PGF2α preparations, i.e. 12-15 mg of dinoprost or tromethamine dinoprostone at a dose of 15-25 mg as dinoprost, or 500 μg of cloprostenol, a PGF2α analogue, is administered once by intramuscular injection. Alternatively, 1 mg of fenprostalene is administered subcutaneously. If parturient signs do not appear within 72 hours of injection, injection is retried at the same dose as above or 10-20 mg of dexamethasone is administered once intramuscularly. In addition, 10-20 mg of estriol or 5-8 mg of estradiol benzoate may be administered concurrently. If diagnosis was delayed and the fetus grew too large, delivery through the birth canal is significantly difficult in many cases if delivery is induced. In these cases, cesarean operation should be performed from the beginning. Dexamethasone is known to temporarily reduce immunocompetence of the body so the presence of infection should be confirmed beforehand and useless repeated administration avoided.
The disease is healed if the fetus has been delivered. In the case of induced delivery by means of medication, the fetus may die because labor pains are so mild that delivery takes a long time. Thus, continuous observation is necessary after a procedure to induce delivery has been performed. Care must be taken because fetal malformation, and especially malformation of the head, may lead to dystocia. Retained placenta occurs more frequently in the case of induced delivery than in spontaneous delivery.

① Uterine torsion

A condition in which the pregnant uterus is twisted around its long axis.

Because the great curvature of the bovine uterine horn is not supported by the broad ligament of the uterus, a pregnant uterus that has sunk into the abdominal cavity in the form of a sac is easily moved by the action of the stomach and intestines. As the gestation period advances, factors such as the growth of the fetus, fetal membrane and placenta and an increase in fetal fluid cause imbalance in weigh and size between the pregnant and non-pregnant horns. An increased estrogen level in the late gestation period also relaxes supportive tissue such as the broad ligament of the uterus. In these circumstances, torsion of the pregnant uterus is likely to occur as a result of spontaneous straining associated with the lying and standing behavior of the dam, fetal movement and labor.

The disease that occurs in the mid-gestation period (6-7 months of gestation) occurs in the uterine body in front of the interior uterine orifice. Symptoms include sudden restless behavior, decreased appetite and tympanites. Rectal palpation reveals a strained uterus with reduced fluctuation, folds that show the direction of torsion and strained bilateral broad ligaments of uterus and uterine arteries. The disease that occurs during the period from the opening to expulsion periods is without uterine torsion because the fetus has moved to the uterine body and is likely to occur in the uterine cervix that has become elastic, relaxed and open. Labor pains persist for a long time but the fetal sac and fetus are not seen from the vulva. By palpation with a hand in the vagina, a number of spiral folds are felt formed on the vaginal wall running from the posterior to anterior portions. The direction of torsion is identified from the clear folds formed on the upper vaginal wall or from the fact that the pudendal lip on the opposite side of torsion (left pudendal lip in the case of right torsion) is swollen.

For treatment, the most appropriate method of reducing and delivering a fetus for the case in question must be determined by taking into account fetal size and the clinical conditions of the dam. If the torsion is slight (around 90°), the fetus is drawn according to the methods for dystocia. The fetal head (the hip in the case of posterior presentation) is guided from the uterine cervix to the vaginal birth canal. If the fetal hoof and head are palpable, repositioning is performed in a standing posture. If the uterine body is twisted, the uterine cervix is twisted without dilating sufficiently, the fetus is impalpable, the dam is unable to assume a standing posture due to weakness or reposition is unfeasible in a standing position, reposition is performed by letting the dam lie on the same side as torsion with the fore and hind legs bound separately and turning the cow quickly. If these methods are found to be ineffective, a cesarean section is performed. A cesarean section is applicable only when the uterus is not necrotized.

The prognosis of the dam and calf is satisfactory if the torsion was detected in the early stage and remedied but poor if the torsion was severe and lasted long.
Dropsy of fetal membrane

This condition, in which a large amount of fetal fluid is retained in the fetal membrane cavity divides into hydramnios and hydrallantois. These occur independently but are sometimes combined. Hydrallantois is thought to occur more frequently than hydramnios.

i. Hydramnios

This disease is associated with a genetically or congenitally abnormal fetus. Because the swallowing of amniotic fluid is impeded in an abnormal fetus, an increasing amount of amniotic fluid collects in the amniotic cavity resulting in an expanded amniotic cavity. Thus, expansion of the abdomen occurs in the late gestation period and gradually becomes noteworthy. Dystocia often occurs due to expansion of the uterus, uterine inertia and fetal malformation and the fetus is abnormal and dies without exception.

In mild cases, observation is continued while waiting for delivery. In severe cases, abortion is induced by administering PGF₂α or a PGF₂α analogue or dexamethasone according to the treatment method for prolonged gestation.

The prognosis of the reproductive ability of the dam is satisfactory.

ii. Hydrallantois

This disease is usually associated with uterine diseases. The placenta is significantly swollen with edematous and necrotic changes. The cause of the disease is thought to be abnormalities in the allantoic chorion function including the permeability of vessels that exudate and absorb body fluids.

The disease may be unnoticeable until delivery if it is mild. In sever cases, symptoms suddenly appear during a period of 5-20 days after the mid-gestation period with significant expansion of the abdomen that may suggest twins or triplets. The dam shows reduced appetite, arrest of rumination, constipation as well as fibrillated pulsation and rapid breathing. Rectal palpation reveals an unusually expanded uterus but the fetus is impalpable. The abdomen continues to expand as the disease progresses and the dam will be weakened and eventually unable to stand. Dystocia is likely to occur due to malpresentation and uterine inertia and the fetus dies within the uterus or immediately after birth.

For treatment, abortion is induced immediately as in hydramnios. Severe cases may require cesarean section. Because of severe dehydration, the dam needs a large amount of transfusion.

The prognosis of the reproductive ability of the dam is poor if it escapes death.

Dystocia

Dystocia refers to a condition during the delivery process in which the first stage (opening period) or the second stage (expulsion period) is so prolonged that delivery is difficult or impossible without assistance.
Dystocia occurs from causes derived from the fetus more frequently than from those derived from the dam. Causes derived from the fetus include faulty fetal disposition, fetal gigantism and fetal malformation and those derived from the dam faulty uterine disposition and abnormalities in the birth canal and labor.

Diagnosis should include inquiring of stockkeepers about the number of times of delivery, time that has passed since delivery, the presence/absence of discharge of fetal fluid and actions that have been taken to relieve dystocia. If a long time has passed since delivery onset, the general condition of the dam should be examined carefully. Disinfected fingers are inserted slowly through the birth canal to check for injuries, the degree of dilation of the uterine cervix, status of the fetal sac and fetal presentation and position. To check whether the fetus is alive or not, palpation of the fetus is performed from over the fetal membrane if rupture of the bag has not yet occurred or directly if the rupture has occurred at the time of examination.

The procedure includes performing reposition and assisting in delivery if the cause is faulty fetal disposition. When infeasible and when the cause is fetal gigantism and malformation, cesarean section is performed. When the fetus is dead, the fetus is delivered by means of fetotomy.

If the cause is uterine hernia of the dam, the fetus is delivered quickly by cesarean section and so forth. If the cause is an abnormality in the birth canal, the procedure involves moistening the birth canal with mucilage using disinfected fingers, relaxing and dilating the insufficiently dilated portion by massage and trying to deliver gradually. When infeasible and when the cause is severe stricture and obstruction, the fetus is delivered by incision of the insufficiently dilated, constricted or obstructed part or by cesarean section. If the cause is uterine inertia, after confirming that the birth canal is secure, labor is promoted by administering oxytocin, or stimulating the dorsal part of the transitional zone between the vestibule of vagina and vagina, or administering 20-150 units of oxytocin, an uterotonic, intravenously, intramuscularly or subcutaneously or 1.7-12.0 mg/kg of sparteine sulfate subcutaneously or intramuscularly.

The prognosis is satisfactory if the fetus is delivered with assistance or by fetotomy without injuring the birth canal. Sequelae include puerperal metritis, puerperal fever and peritonitis and severe cases of these conditions result in poor prognosis. If the birth canal is severely injured and healed, stricture of the cervical canal and vagina may lead to recurrence of dystocia.

Vaginal prolapse

This refers to a condition in which part or the entire vaginal wall protrudes from the vulva.

It tends to occur during the mid- to late gestation period and sometimes after delivery. The possible mechanism is that increased abdominal pressure presses the uterus and vagina backward and high amounts of estrogen produced by the placenta relaxes the supportive tissue of the vagina and vulvar sphincter. If the birth canal and vulva is injured or relaxed due to dystocia, the disease is likely to occur frequently during the following gestation. A long period of raising on an inclined floor with the front part higher and the rear part lower is a major cause of the disease that occurs in the mid- to
late gestation periods. In infertile cows, the disease often occurs during the estrous phase but infrequently during the luteal phase. Involvement of estrogen in the pathogenicity of the disease is also suggested by the fact that it often accompanies follicular cysts.

During the last stage of pregnancy, a reddened vaginal membrane is often exposed from the vulva in a hemispherical form when in a prone position but is reduced in a standing position. In severe cases, the entire vaginal wall along with the ectocervical portion protrudes to produce a mass the size of a human head, which will not be reduced if in a standing position. As time advances after the prolapse, the vaginal membrane degenerates to necrosis in summer and dries to necrosis in winter due to insufficient blood supply, contamination with feces, mechanical stimulation and drying. In some cases, the back part of the prolapsed vaginal wall serves as a hernial sac, into which the bladder and fatty tissue enter. Cases in which the bladder enters the reversed vaginal sac are prone to dysuria.

In mild cases, the disease heals spontaneously after delivery with satisfactory prognosis but tends to recur in the following gestation. Treatment of severe cases of the disease involves disinfecting the vulva and surrounding parts with cationic or zwitterionic detergent, washing the prolapsed vagina with physiological saline or mildly irritating disinfectant and reducing in a low-front and high-back standing position. A vulvar truss is installed after reduction. In the case of poor results with straining induced, vulvar suture is performed, or opening of the pudendum is restricted by passing a tape underneath the skin surrounding the vulva and both ends are ligated, or abdominal fixation or these methods are combined. When present, follicular cysts are also treated.

If the entire vaginal wall prolapses, spontaneous healing is unlikely and reduction may not improve conception failure.

§ Uterine prolapse

Uterine prolapse refers to a postpartum condition in which part or the entire uterus is reversed and prolapses from the cervical canal to the outside of the vulva.

The disease is often caused by dystocia due to fetal gigantism, postpartum persistent labor pain, retained placenta and relaxation of the uterus and birth canal. Raising pregnant cows on a floor with a steep high-front and low-back slope is known to be a major predisposition to the disease.

In some cases, the uterus is reversed and prolapses outside the vulva in the form of a dark red sac, which has a number of placentas lined in parallel on its surface. Part or most of these placentas have fetal placentas attached to them or all of them have lost fetal placentas. The prolapsed uterus is contaminated with blood and feces, has congestion and edemas due to a circulation failure, and these conditions worsen as time passes resulting in necrosis. The disease is sometimes associated with secondary laceration, perforations or partial defects of uterine tissue. Straining occurs repeatedly during the period shortly after prolapse but gradually weakens and disappears in the long run. Appetite decreases with the passage of time and pulse and respiration rates increase, causing dysstasia and, in some cases, collapse.
Stockkeepers should be instructed to keep the prolapsed uterus clean while covering it with a moistened cloth or a plastic sheet to protect it from drying. Reduction should be started immediately if only a short time has passed since prolapse, the uterus has no or few injuries and the congestion and edema are mild. If a long time has passed and the uterus has injuries and advanced congestion and edema with clear systemic symptoms, reduction is performed carefully in combination with symptomatic treatments by use of cardiotonics and transfusion. When performing reduction, the cow is made to assume a low-front and high-back standing posture, when feasible, and in the case of strong straining, extradural anesthesia of the coccygeal vertebrae is performed beforehand.

Reduction involves irrigating the surface of the prolapsed uterus with moderately warm sterile physiological saline or a mildly irritating disinfectant followed by wiping. If the fetal membrane placenta is attached, it is detached with fingers but should not be detached forcibly. The prolapsed uterus is placed on a disinfected broad and strong rubber cloth, which is held by two assistants, and is held slightly higher than the vulva. The operator reduces the prolapsed uterus gradually from the cervical canal by taking advantage of the pauses of straining. Meanwhile, the uterus is sometimes irrigated with sterile saline or Ringer's solution to keep it clean and warm and prevent it from drying. After reduction, the uterus is redressed with fingers inserted deep inward. After redressment, 1-2 g of aureomycin is infused into the uterus to prevent infection of the uterus. In addition, 30-50 units of oxytocin is administered intravenously, subcutaneously or intramuscularly as an uterotonic. To prevent uterine prolapse from recurring, the cow with a vulvar truss installed is tethered on a floor with a low-front and high-back slope and, as required, vulvar suture is performed. In cases in which the injuries and contamination of the prolapsed uterus are serious, an antibacterial agent is administered locally and systemically for 3-5 days and symptomatic treatments are continued because puerperal metritis is likely to follow.

If injuries of the prolapsed uterus are absent or mild and systemic symptoms are absent, the disease does not affect the following conception in many cases. However, because adhesion and incuration may remain on the reproductive organs, stockkeepers should be guided to receive examinations of the reproductive organs 20-30 days after delivery.

9) Retained placenta

The placenta is usually expelled 3-8 hours after delivery of the fetus. In some cases, the placenta is not expelled within more than 12 hours of delivery of the fetus. This condition is referred to as retained placenta.

The causes of this condition are varied and have not yet been clarified. In some cases it occurs because detachment of the fetal placenta from maternal placenta is difficult because of placental inflammation, in some cases it occurs because the detached placenta is not expelled due to early contraction of the cervical canal and uterine inertia. Generally speaking, it preferentially occurs in cows with high milk yield, cows that are obese during the dry up period and cows that take insufficient exercise. It frequently occurs in cows that have undergone abortion, premature birth, stillbirth, dystocia, twin and triplet delivery and induced delivery. The disease is sometimes caused by lack of selenium and vitamin E.

Diagnosis includes confirming that part or the entire fetal placenta is retained in the
uterus and part of the fetal membrane is suspended from the vulva when 12 hours have passed since delivery of the fetus. The detached placenta may be simply retained in the vaginal floor so the fetal membrane suspending from the vulva should be pulled lightly to determine whether the placenta is detached or not. General conditions should also be checked.

Although there is a controversy, no treatment procedures are performed for retained placenta, in principle, if there are no systemic symptoms such as fever and anorexia. If the retained placenta is left untreated while waiting for spontaneous extrusion and the fetal membrane suspended from the vulva is likely to cause odors and contamination of cowsheds, the fetal membrane is cut at the vulva while pulling the membrane lightly. Part of the placenta remaining in the uterus softens and liquefies spontaneously as time passes, discharges as exudate-like lochia and is expelled within 7-10 days of delivery. If the placenta liquefies and remains in the uterus without being expelled, a PGF₂α preparation or a PGF₂α analogue is administered. An antibacterial agent for endometritis is infused into the uterus after expulsion. If systemic symptoms are present, an antibacterial agent is administered into the uterus and systemically.

The conventional manual removal of the placenta is no longer employed because it causes injuries of the uterus, if however great care is taken, and bacteria that infect the injuries cause puerperal metritis and peritonitis associated with systemic symptoms. The method is also likely to cause persistent diffuse adhesion and induration of the reproductive apparatus resulting in reproductive inability. The method must not be used in the presence of systemic symptoms because the condition may worsen rapidly.

If the fetal placenta and lochia are removed without problems and the uterus recovers satisfactorily, the disease has no effects on the following conception. In the case of poor recovery, intrauterine administration or uterine irrigation is performed within one month of delivery according to the treatment method for endometritis. If the disease is accompanied or followed by puerperal wound infection, various diseases occur one after another resulting in poor prognosis.

16 Puerperal metritis

Puerperal metritis refers to inflammation of the myometrium or perimetrium. Myometritis is often accompanied by endometritis or perimetritis.

It is caused by bacterial infection of the injuries of the uterine wall due to rough procedures for repositioning of the fetus, cesarean section and fetotomy at the time of dystocia and for retained placenta. Perimetritis also follows peritonitis. The disease that occurs from delivery to the puerperal period shows a serious infectious systemic symptom with the inflammation spreading from the endometrium to perimetrium and to the surrounding region. Causal bacteria include *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Actinomyces pyogenes* and *Clostridium* spp.

The disease is characterized by bad smelling reddish brown discharge from the uterus and is identifiable mainly by rectal palpation. In the case of myometritis, thickening, induration and, in some cases, abscesses of the entire or localized part of the uterine wall are palpable. Abscesses reach from 2 cm to the size of a child's head in diameter and may sink into the abdominal cavity because of their weight. In the case of
perimetritis, fibrous connective tissue grows on the perimetrium in the form of strings in milder cases. In severer cases, both the uterine horns adhere to each other and the uterine horns adhere to the oviduct, ovaries, broad ligament of the uterus, peritoneum, bladder or rumen. In addition to rectal palpation, hematology to identify the characteristics of the disease and bacteriological examination of the discharge at the external uterine orifice are performed as appropriate.

In cases in which systemic symptoms such as fever and anorexia are present, systemic and local administration of antibacterial agents is performed to the full. Where a large amount of lochia is retained in the uterus and systemic symptoms will not disappear, a PGF$_{2\alpha}$ preparation or a PGF$_{2\alpha}$ analogue is administered to expel the retained matter. If the treatment fails, a soft rubber tube is used to siphon the retained matter.

In cases in which abscesses are absent and adhesion and induration are of a severity that does not affect the uterine function, the disease will not impede conception. If adhesion is extensive and strong and abscesses remain after treatment, the disease results in sterility.
Photograph 2-1  Follicular cyst (FC)
A cystic follicle is present in each ovary.

Photograph 2-2  Luteal cyst (LC)
Atretic corpus luteum derived from a cystic follicle is present in the right ovary.

Photograph 2-3  Luteal cyst (LC)
Part of the cystic wall that luteinized is present in the lower part of the right ovary and a cystic follicle undergoing regression to atresia is present in the upper part.

Photograph 2-4  Cystic corpus luteum (CCL)
Corpus luteum with a thin luteal layer that has processus and retains fluid at the center is seen in the left ovary and an ovarian follicle is seen in the right ovary.

Photograph 2-5  Cystic corpus luteum (CCL)
Corpus luteum with a thick luteal layer that has processus and retains fluid at the center is seen in the right ovary and an ovarian follicle is seen in the left ovary.

Photograph 2-6  Coexistence of a gigantic ovarian follicle and corpus luteum (FCL)
A gigantic ovarian follicle undergoing regression to atresia and normal corpus luteum with processus are seen in the left ovary.
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Chapter 3  Diagnostic method for reproductive disorders

Diagnostic methods for reproductive disorders in cows include external examination, vaginal examination, rectal palpation, ultrasonic examination as well as the determination of steroidal hormone levels in blood, bacteriological examination of the uterine content and endometrial biopsies. Of these, external examination, vaginal examination and rectal palpation are the most frequently used techniques when performing clinical examinations of a number of cows in the field.

This chapter describes rectal palpation, which is the most commonly used in a clinical setting, and ultrasonic examination.

1. Rectal palpation

Rectal palpation is the most widely used in making diagnoses of reproductive disorders in cows and pregnancy diagnosis. It is a basic technique that will remain a main reproductive examination if a simple method of determining blood steroidal hormone levels in the field and ultrasonic examination are introduced. However, since rectal palpation is a sensitive examination that relies on the sensation of the fingers, subjective views are likely to affect the result and differences in experience and sensation among individuals may lead to errors in the results. To eliminate this problem as much as possible and to standardize technical levels and improve accuracy, the following points must be noted in performing rectal palpation. First, the findings of the uterus, cervix, vagina and vulva are likely to be regarded low compared to a finding of the ovary showing dynamic changes but these findings must be examined comprehensively. Thus, in this chapter, external examination, simple vaginal examination and internal examination are included in rectal palpation. Next, findings should be recorded in a unified format so that they can be shared among specialists.

Rectal palpation is described in the following sections according to the Guidelines for Clinical Pathological Study (compiled by the Economic Affairs Bureau, Ministry of Agriculture, Forestry and Fisheries) used for a livestock mutual aid program.

1) Methods of rectal palpation

Prior to rectal palpation, sufficient information is obtained from stockkeepers, including the reproduction history of the sick cow, dates and conditions of delivery, history of perinatal period diseases, observations of estrus and insemination and treatment history.

Next, external examination is performed to check nutritional condition, hair, abnormalities in the hooves and feces as well as the presence/absence of spilled mucus on the floor or the body, shape of the labium and condition of labium mucosa. External examination must precede internal examination because findings in external examination change to some degree after internal examination.

The obstructive tail is fixed to the back or upper part of the body while performing vaginal examination. The labium is washed and disinfected and a vaginal speculum is inserted into the vagina slowly. The vaginal speculum is easily inserted by inserting it slightly upward for the first 10 cm and then downward. The vaginal speculum is inserted up to the external uterine orifice and opened sufficiently. By lighting the inside with a flashlight, the shape of the vagina, the presence/absence of congestion and swelling of the vaginal mucosa, the shape and dilation status of the external uterine
orifice, and the quantity and properties of spilled fluids, if any, are quickly examined. When performing bacteriological and cytological examination of mucous samples taken from the external uterine orifice and vagina, a sterile cotton swab may be used with a sheath tube for insemination instead of a sampler. The vaginal speculum is removed slowly after vaginal examination has been completed.

When inserting a hand, the arm must be kept in parallel to the rectum to avoid generating a sense of incongruity. The feces are removed from the rectum. Simply pulling the rectal wall will not be sufficient to relax the rectal wall so the rectal wall must be pushed with the hand inserted to the inner part of the rectum. This operation is repeated several times to relax the rectal wall sufficiently. The rod-shaped uterine cervix under the rectum is held and drawn near. With the hand moved forward, the uterine horns are held between the thumb and middle finger at the point where the horns separate, the forefinger is placed under the ventral ligamentum interconucale (Photograph 3-1 (p.100)) and the entire uterus is lifted into the pelvic cavity (Photograph 3-2 (p.100)) so that the uterus is turned over. The forefinger must be placed under the ventral and not dorsal ligamentum interconucale because the dorsal ligamentum interconucale is so thin that it might be torn (Photograph 3-3 (p.100)). This method allows detailed palpation of the uterine horns and oviduct because only the hand is left in the rectum at the time of palpation so that rectal tenesmus is reduced. If holding the uterine horns between the thumb and middle finger is not possible, the hand is placed under the uterine horn on the same side as the hand, the hand is lightly held to lift, and turned over so that the end of the horn comes to the fore. The opposite horn, which is slightly lifted at this time point, is lifted similarly and turned over. The uterus is lifted onto the pelvis and turned over. Detailed palpation is also possible with this method. Inability to lift the uterus by these methods indicates the possibility of adhesion of the uterus and the broad ligament of the uterus.

Palpation of the uterus involves applying light pressure to the horn while holding it between the thumb and other fingers and sliding the thumb across the horn. Palpation is performed on both of the horns to see if there is any difference. The ovaries are also lifted into the pelvic cavity along with the uterus. Palpation of the ovaries is performed by holding and fixing the proper ligament of the ovary between the middle and ring fingers (Photograph 3-4 (p.100)) and pressing lightly with the thumb, forefinger and middle finger. The intensity of the pressure should be such that when a spoon held with the thumb and forefinger is shaken lightly it does not move. By doing this, the depth in the ovary (size) and hardness (softness, hardness) of the corpus luteum or fluctuation of ovarian follicles are palpable. If the pressure is too low, distinguishing the corpus luteum from ovarian follicles may be difficult resulting in a misdiagnosis. However, the pressure should not be so high as to eliminate the corpus luteum or rupture the ovarian follicle.

(2) Recording rectal palpation findings

① Properties of spilled mucus

An observation is made to see if mucus or blood is attached to the pubic hair, tail, hind legs or floor.

The property of spilled mucus is classed as watery, sticky or starchy. Spilled mucus in
each class is examined for color, degree of opacity and the presence/absence of purulent or bloody matter. Blood or pus may be present instead of mucus. If purulent matter is present, vaginoscopy is performed with a vaginal speculum to examine if vaginitis, cervicitis or endometritis is present.

② Shapes of labium

The shapes of labium is classified into four grades as shown in Figure 3-1. The shapes of labium is affected by estrogen ($E_2$) and progesterone ($P_4$) labium swell and relax, if estrogen is dominant, and tight, if progesterone is dominant. Thus, labium is ① loose and ② swollen during the estrous phase and is loose in the case of follicular cysts. It is ③ contracted and ④ tight during the luteal phase.

![Diagram of labium shapes](image)

(Note) 
①: Deep wrinkles
②: Small wrinkles

Figure 3-1 Shape of the labium: ① loose, ② swollen, ③ contracted and ④ tight. Labium swell and relax, if estrogen is dominant, and contract and tighten, if progesterone is dominant.

③ Findings in labium mucous membrane

The labium is opened slightly with fingers and the labium mucous membrane is observed to see whether there is congestion or not and whether the mucosal surface is wet or dry.

The labium mucous membrane is congested and moist when $E_2$ is dominant and is free from congestion and dry when $P_4$ is dominant.

Examination by a vaginoscope should be performed as required because mucus, blood or pus can be detected in cows without signs of spilled mucus if the labium is opened.

④ Findings in the vagina

In normal conditions the vaginal mucous membrane shows findings similar to those of the labium. If the vaginal mucous membrane has inflammation, however, it reddens and swells more than the labium and occasionally has abscesses. In granular vaginitis, numerous millet-like tubercles 1-2 mm in diameter are present on the vaginal mucous
membrane. Morphological abnormalities such as stricture and trabeculation are rarely present in the vagina. Vaginal examination may reveal a double external os of the cervix.

3. Findings in the uterine cervix

The diameter of the external uterine orifice is examined from the rectum by palpation with the thumb and recorded to the nearest 0.1 cm. The size is generally 3-4 cm (intermediate) and may be expressed as larger (large) or smaller (small). The large size is often observed in the case of delayed uterine involution or cervicitis and the small size in the case of contraction due to hormonal abnormalities. The diametral size of the cervix is expressed in terms of finger width as compared to the forefinger. It can also be expressed in terms of centimeters. The length is expressed to the nearest 0.1 cm by comparing with the finger. By pressing the cervix from above and below at the point indicated by △ in Figure 3-2, the hardness of the cervix is classed as very hard, hard, or soft and the status of the cervical canal as open, contracted or tight. If examining the status of the cervical canal is difficult in this way, a rod for dilating the cervical canal is inserted. If the rod passes through the internal uterine orifice smoothly, the condition is expressed as open. The cervix is thick and the cervical canal dilates if $E_2$ is dominant. The cervix is thin and the cervical canal closes if $P_4$ is dominant.

Fig. 3-2. Location of the cervix for palpation: The size of the cervix is measured at the point marked by the symbol ◊ and the dilation status is measured at the point marked by the symbol △. The size of the external uterine orifice is measured with the thumb.

Y. Makihara et al. proposed a method for observing the hardness of the cervix using plicae circularis as an indicator. This method involves holding the cervix with the palm as if to enclose it and palpating the plicae circularis by pressing and rubbing the cervix with the thumb. In this method, hardness is evaluated on a four-grade scoring system as shown in Figure 3-3 (p.87). As seen in Table 3-1 (p.87), progesterone level increases as the score increases from S1 to S4. Table 3-2 (p.87) indicates the possibility of applying this method in early pregnancy diagnosis.
Figure 3-3  Schematic view of cervical scores: Soft, smooth and has no internal induration by pressure palpation (S1), smooth but has internal induration by pressure palpation (S2), plicae circularis is palpable by pressure palpation (S3) and plicae circularis is palpable by light palpation (S4).

Table 3-1  Mean blood level of progesterone by cervical scores

<table>
<thead>
<tr>
<th>Score</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>8</td>
<td>10</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Mean blood level of progesterone (ng/ml)</td>
<td>2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.7</td>
<td>6.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a-b, a-c: P < 0.05

1999 Y. MAKIHARA et al.

Table 3-2  Conception performance by cervical scores 17-33 days after insemination

<table>
<thead>
<tr>
<th>Score</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows inseminated</td>
<td>6</td>
<td>61</td>
<td>47</td>
<td>6</td>
</tr>
<tr>
<td>Number of cows with conception</td>
<td>2</td>
<td>31</td>
<td>38</td>
<td>6</td>
</tr>
<tr>
<td>Conception rate (%)</td>
<td>33.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>100</td>
</tr>
</tbody>
</table>

a-b: P < 0.01

1999 Y. MAKIHARA et al.

5. Findings in the uterus

The uterine horns are examined at the part between ⋆ and ▲ as shown in Figure 3-4 (p.88) for the size, shape, contraction and elasticity, thickness and internal condition and findings in the examination are recorded.

The size of the uterus is recorded in terms of finger width, by comparing with the forefinger, or to the nearest 0.1 cm. The uterus during the estrous phase is generally swollen and is 1.0-1.5 finger widths in normal nulliparous cows and 1.5-2.5 finger widths in multiparous cows. The size during the luteal phase is smaller than that during the estrous phase and is around 1.0 finger width and 1.5-2.0 finger widths, respectively. The uterine horns involute until both of them are of the same size within 30-40 days of delivery. In some cows with dystocia, retention of fetal membrane and postpartum diseases, however, the involution process tends to be slow and the right and left horns have different sizes.
Figure 3-4 Location of the uterus for palpation: The part from ★ to ▲ of the uterine horn is examined. Both of the right and left horns are examined.

The shape of the uterus is recorded according to the sectional shape of the uterine horns as shown in Figure 3-5. During the estrous phase, when the uterine cavity expands and the endometrium thickens, the shape is round or semiround in normal nulliparous cows and semiround in multiparous cows. During the luteal phase, the shape is semiround and semiround or oval, respectively. A flat status is abnormal and is present in the case of ovarian quiescence and prolonged follicular cyst.

Figure 3-5 Shape of the uterus: A flat shape at any time indicates an abnormal finding.

The contraction and elasticity of the uterus is particularly strong during the estrous phase when secretion of estradiol (E₂) increases and secretion of P₄ decreases.

+++ Rectal palpation directly causes strong and continuous contraction of the uterus, which feels like a round sausage.

++ Rectal palpation causes contraction but strong contraction does not continue.

- Rectal palpation causes mild contraction and can identify the outline of the uterus.

± Stimulation as strong as that caused by palpation does not induce contraction. Strong massage causes contraction of the uterus but it loosens as soon as stimulation is removed.

- Strong massage does not cause contraction.
The rating for normal cows during the proestrus is from +++ to ++ and the contraction will gradually become intermittent during the postestrus. The rating is normally + during the luteal phase.

Contraction findings and elasticity findings are almost the same.

The thickness of the uterus represents the thickness of the uterine wall (the thickness from the perimetrium to endometrium) and is recorded according to Figure 3-6. The rating of ++ is normal during the estrous phase and the rating of + during the luring the luteal phase. The rating of +++ during the luteal phase suggests acute uteritis or luteal hypofunction. The rating of − is abnormal whether during the estrous or luteal phase.

![Figure 3-6](image)

Figure 3-6  Thickness of the uterus: +++: the uterus looks like an edema and thickens and swells during the estrous phase when progesterone levels decrease and estrogen levels increase. −: A normal state of the luteal phase of a cow receiving a sufficient effect of progesterone. −: A condition during the estrous and luteal phases in which the uterus is weak and thin is abnormal. This condition occurs in the cases of prolonged endometritis and follicular cysts. However, care must be taken because the pregnant horn 30-45 days after insemination is also thin.

The internal conditions of the uterus are recorded according to Figure 3-7. Internal conditions include fluctuating or sticky retained fluids (mucus, pus, etc.), a dead fetus, fetal membrane and fetal fluid. The rating of normal cows during the proestrus is + due to the retention of watery secretion but the rating is − during other periods.

![Figure 3-7](image)

Figure 3-7  Internal conditions of the uterus: The part from ★ to ▲ of Figure 3-4 (p.88) is examined. Otherwise the retained matter is not palpable.
7) Findings in the ovaries

The ovaries are examined mainly for major follicles, the corpus luteum, indurated parts and other important findings and the results are visualized by means of, for instance, illustration. The illustration does not need to be realistic but may be simplified as long as it is understandable.

As a general rule, the shape of the ovary is expressed as a section plane on the long axis of the ovary by placing ovaries such that either the mesovarium edge is at the bottom and the proper ligament of ovary is inside or the mesovarium edge is vertical and the proper ligament of ovary is at the bottom. Findings in the examination of follicles, the corpus luteum and the indurated parts of ovaries induration are recorded according to Figure 3-8 (p.91).

8) Record sheet for rectal palpation

The record sheet we use in rectal palpation is like the one in Figure 3-9 (p.92). All information from stockkeepers and findings in the ovaries must be entered. The sheet is devised to avoid entering omissions by adopting a system in which the appropriate answer is circled for items from the properties of spilled fluid to uterine findings. By analyzing rectal palpation findings comprehensively, estimating time in the estrous cycle is possible. Thus, the expected date of the next estrus can be indicated. By trying to follow this process, more sophisticated rectal palpation will be possible.

3) Pregnancy diagnosis by rectal palpation

Pregnancy diagnosis is the most important technique of reproductive examination and requires the most careful attention of technicians. Early and accurate pregnancy diagnosis provides an effective means of performing breeding management for stockkeepers.

A cow undergoing conception shows the process as indicated in Table 3-3 (p.93) and diagnosis of pregnancy by rectal palpation is possible from 30 days after insemination. Diagnosis is quite easy from 35 days after insemination if the uterus is turned over and the uterine horn can be palpated up to its end.

The pregnant horn swells and the uterine wall at the swelling point thins in the days from 30 days after insemination. Fluctuation due to fetal fluid, the fetal membrane and the vasa that develop into the fetal membrane can be palpated at the swelling point.

Pregnancy diagnosis involves holding the swelling part of the horn with the thumb and forefinger, applying low pressure two or three times to examine whether fluctuation is present, closing the fingers lightly and sliding them across the horn and confirming the fetal membrane and thread-like vasa that develop into the fetal membrane.

The size of the pregnant horn is 2.0-2.5 finger widths 30 days after insemination, 2.5-3.0 finger widths 35 days and 3.5 finger widths 40 days after insemination. The non-pregnant horn also swells around 40 days after insemination. The labium and cervical canal contract and soft and large corpus luteum is present in the ovary on the same side as the pregnant horn.
Figure 3-8  Recording of ovarian findings
Cattle number | Date of examination | Stockowner | Abbreviated name | Date of birth
---|---|---|---|---
Parity | Last parturition: | Parturient condition: | Retained placenta: Absent Present | Disease history:
Number of times of insemination | Last insemination: | Current milk yield: kg |

Information from the stockkeeper

<table>
<thead>
<tr>
<th>Spilled mucus</th>
<th>Left</th>
<th>Ovary</th>
<th>Right</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>Clear</td>
<td>Cloudy</td>
<td>Purulent</td>
</tr>
<tr>
<td>Watery</td>
<td>Sticky</td>
<td>Starchy</td>
<td></td>
</tr>
<tr>
<td>Labium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Loose</td>
<td>Swollen</td>
<td>Contracted</td>
</tr>
<tr>
<td>Labium and vaginal mucosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congested</td>
<td>Present</td>
<td>Absent</td>
<td>Wet</td>
</tr>
<tr>
<td>Uterine cervix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Cervical canal</td>
<td>Open</td>
<td>Contracted</td>
<td>Tight</td>
</tr>
<tr>
<td>Uterus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Size</td>
<td>1.0</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>BCS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormalities in hooves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td>Round</td>
<td>Semiround</td>
<td>Oval</td>
</tr>
<tr>
<td>Contraction and elasticity</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Thickness</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Internal condition</td>
<td>+++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Expected date of estrus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3.9 A record sheet sample for rectal palpation
Table 3-3  Changes in the findings in the ovaries, uterus and so forth from insemination to the 35th day of pregnancy

<table>
<thead>
<tr>
<th>Ovary (insemination)</th>
<th>After 10 days</th>
<th>After 18 days</th>
<th>After 21 days</th>
<th>After 26 days</th>
<th>After 35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>1.5 to 2.0</td>
<td>1.0 to 2.0</td>
<td>1.0 to 2.0</td>
<td>1.0 to 2.0</td>
<td>Pregnant horn 1.5 to 2.0</td>
</tr>
<tr>
<td>Contraction</td>
<td>Round</td>
<td>Round to semiround</td>
<td>Round to semiround</td>
<td>Round to semiround</td>
<td>Semiround</td>
</tr>
<tr>
<td>Elasticity</td>
<td>-- to +++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Thickness</td>
<td>++ to +++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Internal condition</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td>Fetal fluid (fluctuation)</td>
</tr>
<tr>
<td>Cervical canal</td>
<td>Open</td>
<td>Contracted</td>
<td>Contracted</td>
<td>Contracted</td>
<td>Contracted</td>
</tr>
<tr>
<td>Vaginal mucosa</td>
<td>Wet</td>
<td>Dry</td>
<td>Dry</td>
<td>Dry</td>
<td>Dry</td>
</tr>
<tr>
<td>Labium</td>
<td>Swollen</td>
<td>Contracted</td>
<td>Contracted</td>
<td>Contracted</td>
<td>Contracted</td>
</tr>
</tbody>
</table>

If rectal palpation 30-40 days after insemination reveals that the corpus luteum is present in both of the ovaries, both the uterine horns swell to the same extent and the fetal membrane and vasa that develop into the fetal membrane are palpable, twin pregnancy is indicated.

If swelling of the uterus is 2-3 days delayed or the corpus luteum is small and hard or the labium and cervix are loosened, an hCG or P₄ preparation should be administered and reexamination performed because embryonic death or abortion is likely. The diseases likely to be mistaken for pregnancy findings include pyometra and mucometra. Differential diagnosis for distinguishing these diseases is shown in Table 3-4.

Table 3-4  Differences in uterine findings from rectal palpation among the cases of pregnancy, pyometra and mucometra

<table>
<thead>
<tr>
<th></th>
<th>Pregnancy</th>
<th>Pyometra</th>
<th>Mucometra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of the horn</td>
<td>Asymmetric except in the case of twin pregnancy</td>
<td>Symmetric in many cases</td>
<td>Symmetric or asymmetric</td>
</tr>
<tr>
<td>Contraction</td>
<td>Observable until the 3rd month of pregnancy</td>
<td>Weak</td>
<td>Absent</td>
</tr>
<tr>
<td>Thickness</td>
<td>Present in locations other than the swollen part, which is thinned.</td>
<td>Present or absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Flat. Rough at 70 days of gestation onwards because of growth of caruncles.</td>
<td>Coarse textured</td>
<td>Flat</td>
</tr>
<tr>
<td>Fetal membrane and vasa that develop into fetal membrane</td>
<td>At 30 days of gestation onwards</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>Median uterine artery</td>
<td>Develops on the pregnant horn side and shows clear pulsation at 3 months of gestation onwards</td>
<td>Does not develop and is of the same size on both sides</td>
<td>Does not develop and is of the same size on both sides</td>
</tr>
</tbody>
</table>
An accurate diagnosis is made 30-45 days after insemination by the presence of the fetal membrane and vasa that develop into the fetal membrane. All technicians must master this skill.

4) Estimating blood levels of progesterone from rectal palpation findings

Performing the determination of blood P₄ levels by the Enzyme immuno assay (EIA) method is easier and less expensive than with the conventional Radio immuno assay (RIA) method but determining them on a regular basis is not practical.

However, if rectal palpation is performed as described above and the results are examined comprehensively, the estimation of blood P₄ levels may be possible. The following is an example of this.

H.Koumoto et al. created an equation for estimating blood P levels from rectal palpation findings using the multiple regression analysis of the method of quantitative theory I group and examined estimation accuracy and reproducibility in the field. They recognized the usefulness of the equation in the clinical setting. Table 3-5 (p.95) shows the items, findings and categorical values (values on the left are based on findings including ovarian findings and those on the right are based on findings other than ovarian findings) of rectal palpation used in estimating blood P₄ levels. Categorical values corresponding to the rectal palpation findings obtained are totaled and constants are added to them to make logarithmic P₄ levels, which are converted to antilogarithms (P = 10⁷) to estimate blood P₄ levels. Findings in the examination of the ovaries and accessory genital organs were substituted in the estimation equation to examine the correlation between the observed values and estimates of blood P₄ levels. The correlation coefficient (r) was 0.9481 (see Figure 3-10 (p.96)). Findings in the rectal palpation of 98 new cows were also substituted in the estimation equation to examine the correlation coefficient between the observed values and estimates. The correlation coefficient (r) was 0.9607 (see Figure 3-11 (p.96)). The correlation coefficient between the observed values and estimates of blood P₄ levels was analyzed using only findings in the examination of accessory genital organs except ovarian findings. The correlation coefficient (r) was 0.8242. Findings in the rectal palpation of 98 new cows were also substituted in the estimation equation to examine the correlation coefficient between the observed values and estimates. The correlation coefficient (r) was 0.8951. These results show that findings in the accessory genital organs well reflect blood P₄ levels.

In rectal palpation, examining findings in the accessory genital organs is as important as those in the ovaries.
Table 3-5  An equation for estimating blood progesterone levels from rectal palpation findings; the left categorical values are used when estimating from the findings in the ovaries and accessory genital organs and the right categorical values when estimating from the findings only in the accessory genital organs.

<table>
<thead>
<tr>
<th>Items</th>
<th>Findings</th>
<th>Categorical values</th>
<th>Estimation from findings in the ovary and accessory genital organs</th>
<th>Estimation from findings in the accessory genital organs only</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>j</td>
<td></td>
<td>X1ij</td>
<td>X2ij</td>
</tr>
<tr>
<td>Uterus</td>
<td>Size</td>
<td>1</td>
<td>≤ 1.0</td>
<td>0.088</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1.5</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>2.0</td>
<td>-0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>2.5 &lt;</td>
<td>-0.079</td>
</tr>
<tr>
<td>Shape</td>
<td></td>
<td>1</td>
<td>Round/semicircular</td>
<td>-0.027</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Oval</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Flat</td>
<td>0.038</td>
</tr>
<tr>
<td>Contraction/elasticity</td>
<td></td>
<td>1</td>
<td>+ + + + to + + +</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>+</td>
<td>-0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>±</td>
<td>0.023</td>
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<td></td>
<td></td>
<td>4</td>
<td>-</td>
<td>-0.054</td>
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<tr>
<td>Thickness</td>
<td></td>
<td>1</td>
<td>+++ to ++</td>
<td>0.055</td>
</tr>
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<td></td>
<td></td>
<td>2</td>
<td>+</td>
<td>0.091</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>-</td>
<td>-0.056</td>
</tr>
<tr>
<td>Internal condition</td>
<td></td>
<td>1</td>
<td>+ +</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>+</td>
<td>-0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>Cervix</td>
<td>Size</td>
<td>1</td>
<td>≤ 1.5</td>
<td>-0.002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>2.0</td>
<td>-0.005</td>
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<tr>
<td></td>
<td></td>
<td>3</td>
<td>2.5 &lt;</td>
<td>0.017</td>
</tr>
<tr>
<td>External uterine orifice</td>
<td>Size</td>
<td>1</td>
<td>Large</td>
<td>-0.079</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Intermediate</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Small</td>
<td>0.031</td>
</tr>
<tr>
<td>Vagina</td>
<td>Congested</td>
<td>1</td>
<td>Present</td>
<td>-0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Absent</td>
<td>0.007</td>
</tr>
<tr>
<td>Dry</td>
<td></td>
<td>1</td>
<td>Wet</td>
<td>-0.005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Dry</td>
<td>0.007</td>
</tr>
<tr>
<td>Vagina labium</td>
<td>Shape</td>
<td>1</td>
<td>Loose</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Swollen</td>
<td>-0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Contracted</td>
<td>-0.018</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>Tight</td>
<td>0.036</td>
</tr>
<tr>
<td>Spilled mucus</td>
<td></td>
<td>1</td>
<td>Present</td>
<td>-0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Absent</td>
<td>0.006</td>
</tr>
<tr>
<td>Ovary</td>
<td>Shape</td>
<td>1</td>
<td>Follicular cyst</td>
<td>-0.578</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>Luteal cyst</td>
<td>-0.149</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>Cystic corpus luteum</td>
<td>0.373</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>Estrus</td>
<td>-0.990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>Luteal phase 3-5</td>
<td>-0.169</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>Luteal phase 6-8</td>
<td>0.162</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7</td>
<td>Luteal phase 9-11</td>
<td>0.401</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8</td>
<td>Luteal phase 12-15</td>
<td>0.571</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9</td>
<td>Luteal phase 16-18</td>
<td>0.536</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td>1</td>
<td>Nullipara</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>l</td>
<td>-0.014</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>3 or more</td>
<td>-0.010</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td></td>
<td>0.365</td>
</tr>
</tbody>
</table>

\[ P = 10^Y, \quad Y = \sum_{i=1}^{11} X_{1ij} + \text{constant} \quad \text{or} \quad Y = \sum_{i=1}^{12} X_{2ij} + \text{constant} \]

where, \( j = 1-2 \) when \( i = 8, 9, \text{ or } 11 \), \( j = 1-3 \) when \( i = 2, 4, 5, 6, 7 \) or \( 13, j = 1-4 \) when \( i = 1, 3 \) or \( 10, j = 1-9 \) when \( i = 12 \).

The values of \( i \) number 12 are missing in \( X2 \) and replaced by the values of \( i \) number 13.

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Figure 3-10  Correlation between blood progesterone levels determined by RIA method and those estimated from the findings in the ovaries and accessory genital organs.

Figure 3-11  Accuracy of the estimation of blood progesterone levels from findings in the ovaries and accessory genital organs: Correlation between blood progesterone levels determined by RIA method and those estimated from the findings in the ovaries and accessory genital organs was confirmed in 98 new cows.
2. Ultrasonic examination

Like rectal palpation, ultrasonic examination is advantageous in that it can be performed on the same subject without invading living tissues. Unlike rectal palpation findings, the images generated are objective, which is the greatest advantage of ultrasonic examination. The method is especially useful when the ovaries and uterus cannot be examined by rectal palpation because of obesity. However, the difference in the hardness of the corpus luteum in the ovary and elasticity of the uterus, which can be felt by rectal palpation, are difficult to evaluate by this method. It also takes longer to obtain images for use in diagnosis than for rectal palpation so examining a number of subjects on the same occasion is difficult.

The advantages and disadvantages mentioned above must be considered when performing ultrasonic examination as used in breeding examination.

(1) Ultrasonograph

Mobility is an important factor in performing ultrasonic examination of cows in the clinical setting. The equipment must be compact so a portable type of ultrasonograph is often used. A knapsack type of ultrasonograph has been developed recently and its mobility is improved.

Many types of probes are available varying depending on the frequency, scanning system and shape. Ultrasonic examination as used in breeding examination is performed via the rectum so a small-size probe of the linear or convex type with a frequency of 5 MHz or 7.5 MHz is suitable.

(2) Examination methods

A cow to be examined is retained in a stanchion stall with its tail fixed to the body. The feces in the rectum are removed and the rectal wall is sufficiently relaxed. A probe is inserted into the rectum and pressed on the rectal wall to visualize the cervix, uterus and ovaries. The reproductive organs are kept as is during examination. When the cross section of the uterine horn is examined, however, the uterus is turned over as in rectal palpation to obtain satisfactory images. When visualizing the ovaries, rectal palpation is performed beforehand to obtain ovarian findings. Based on these findings, the probe is operated along the long axis of the ovary such that the image of the corpus luteum and follicles are maximized.

(3) Findings in ultrasonic examination

Reading ultrasonographic images requires skills like other diagnostic imaging methods. One must be familiar with the images of normal reproductive organs before attempting to examine whether there are abnormalities in images.

① Findings in the normal estrous cycle

i. Uterine cervix

The vertical section image of the cervix is rectangular and the plicae circularis is echogenic (on the left of Photograph 3-5 a and b (p.101)). The lower part of the cervix is sometimes undulated. This indicates a condition in which the cervix is
contracted by the effect of $P_2$. The cervix is thicker during the estrous phase compared to the luteal phase and the lower part appears linear. An echo-free line resulting from retention of mucus is present in the cervix of the estrous phase. The circular muscle layer appears as a slightly echogenic circle in a cross sectional image of the cervix (on the right of Photograph 3-5 a and b (p.101)). The echo-free central part of the cervical canal is often visualized during the estrous phase.

ii. Uterus

Because the uterine body is short, ultrasonic examination of the uterus focuses on the uterine horns. Vertical sectional imaging of the horn shows a long-curved image. However, the shape varies depending on the location of the uterus so comparing images directly is difficult. On the other hand, cross sectional imaging shows a round or elliptic image with a slightly echogenic elliptical layer (circular muscle layer) at the center of the uterine wall (Photograph 3-5 c and d (p.101)). Thus, comparing individual images is easier with cross sections than vertical sections. During the estrous phase, the uterine cavity often appears as an echo-free expanded body due to the retention of watery secretion.

iii. Ovary

Ultrasonic images of the ovary distinguish follicles, corpus luteum and stroma of ovary. Follicles appear as echo-free circular images because of the presence of follicular fluid but those immediately prior to ovulation may appear as elliptic images. Follicles 2-3 mm in diameter or larger can be identified although it depends on the equipment (frequency of the probe) used. The corpus luteum appears as a slightly echogenic homogeneous image. Cystic corpus luteum is often viewed even during the normal estrous cycle. An ultrasonic image shows an echo-free portion within slightly echogenic corpus luteum. The stroma of the ovary is more echogenic than the corpus luteum. Its outline may not be well defined because of the surrounding tissue (Photograph 3-5 e and f (p.101)).

2 Abnormal findings

i. Uterine cervix

Ultrasonography rarely reveals malformations in the cervix but usually discloses, if any, acquired cervical cysts secondary to injuries and tears. This refers to a retention cyst of the cervical mucosa and appears as an echo-free circle 1-5 cm in diameter. Cervical abscesses resulting from injuries and tears are echogenic. Thus, ultrasonography distinguishes between cysts and abscesses of the cervix.

ii. Uterus

Abnormalities in the uterus can be identified by ultrasonography only when there is abnormal retained matter in the uterine cavity such as when pyometra or mucometra is present. With pyometra, the uterine cavity is dilated and heterogeneously echogenic. The uterine wall either is or is not thickened. The endometrial surface may be irregular and ill defined. With mucometra, the uterine cavity is dilated as in pyometra but is echo-free. The uterine wall is thin and the endometrial surface is well defined (Photograph 3-6 (p.101)).
iii. Ovary

Follicular cysts are most easily identified by ultrasonography. Ovarian cysts are classified into follicular cysts and luteal cysts. Distinguishing a luteal cyst from a cystic corpus luteum is difficult but distinguishing a follicular cyst is possible with ultrasonography.

A follicular cyst appears as an echo-free circle within an echogenic theca folliculi. With multiple follicular cysts, the central part is deformed as seen in Photograph 3-7 (p.102). Follicles are separated by echogenic septa.

In contrast, the surrounding part of a luteal cyst and cystic corpus luteum appears as a slightly echogenic layer because of the presence of a layer of luteal cells. The inside is echo-free because of the retention of fluid (Photograph 3-8 (p.102)). A luteal cyst is circular like a follicle because of the lack of ovulation but a cystic corpus luteum is often elliptic or vase-shaped because of ovulation.

(3) Pregnancy diagnosis by ultrasonic imaging

Many researchers reported that, in pregnant cows, vertical sections of the uterine horn obtained by ultrasonography revealed echo-free thread-like images within the cavity of the pregnant horn from 15 days after insemination and these images increased to 2-3 mm by 20 days after insemination (Photograph 3-9 (p.102)) and continued to grow until an embryo was seen. They also indicated, however, that diagnosing pregnancy from these echo-free images in the uterine cavity 25 days after insemination or earlier is difficult because of the difficulty in distinguishing watery secretion at the time of estrus from the pathologic fluid retained in the uterus. Because pregnancy diagnosis requires accuracy, as described in the section of rectal palpation, ultrasonography for pregnancy diagnosis should be performed 25 days after insemination or later. Photograph 3-10 (p.102) shows a cross section of the pregnant horn 28 days after insemination. The uterine cavity is about 2 cm in diameter and contains an embryo (E). At the time of examination, but not in the still picture, the beating of the heart can be observed on a real-time basis. Improvements in diagnostic ultrasonographic techniques and equipment are expected to enable early and accurate diagnosis.

However, confirming the continuance of pregnancy is necessary after early pregnancy diagnosis.
Photograph 3-1
Method of lifting the uterus
The forefinger is placed under the ventral ligamentum interconucale, which is at the point where the horns separate, and the entire uterus is lifted to this side. The arrow indicates the ventral ligamentum interconucale.

Photograph 3-2  Palpation of the uterus
The lifted uterus is held with the thumb and other fingers as if to enclose it and is palpated.

Photograph 3-3
Points to note when lifting the uterus
Ligamentum interconucale are divided into dorsal and ventral ligamentum interconucale. The dorsal ligamentum interconucale is thin and weak so it may rupture if the uterus is lifted by this portion. Thus, the finger must be placed under the ventral ligamentum interconucale. The arrow indicates the ventral ligamentum interconucale and the dotted arrow the dorsal ligamentum interconucale.

Photograph 3-4  Palpation of the ovary
The proper ligament of the ovary is held with the middle and ring fingers and palpated with the thumb, forefinger and middle finger.
Photograph 3-5  Ultrasonograms of the genital organs in the estrous and luteal phases (7.5 MHz)

The cervix is thicker and the circular muscle layer is larger during the estrous phase (a) than the luteal phase (b). The arrow in (a) indicates the mucus retained in the cervical canal. The cross section of the uterine horn reveals an expanded circular muscle layer in the estrous phase (c) compared to the luteal phase (d) as in the cervix. This indicates thickening of the endometrium. Within the ovaries (e and f), the follicle (F) is echo-free and the corpus luteum (CL) is slightly echogenic. A small amount of echo-free fluid is retained in the cavity of CL in the right picture.

Pyometra

Photograph 3-6  Ultrasonograms of pyometra and mucometra (7.5 MHz)

In the case of pyometra, the uterine lumen is echogenic and its boundary is ill defined. The uterine wall is not thinned in this case. In the case of mucometra, the uterine lumen is mostly echo-free and minute echogenic particles are suspended in it. The uterine wall is thinned. In any case, care must be taken not to confuse it with pregnancy. The space between the arrows indicates the thickness of the uterine wall.
Photograph 3-7  Ultrasonogram of a follicular cyst (7.5 MHz, removed ovary)

Multiple follicles are present in the right and left ovaries. The theca folliculi and the septum between follicles is echogenic. As seen in the picture, follicles appear as a semiround or sectorial shape if the ovary consists of many structures.

Photograph 3-8  Ultrasonogram (5 MHz, removed ovary) of a luteal cyst

The cavity is large and echo-free, similar to a follicular cyst. It is surrounded by a slightly echogenic luteal zone, which distinguishes it from a follicular cyst.

Photograph 3-9  Ultrasonogram of the horn on the 20th day of pregnancy (7.5 MHz)

Echo-free images 2-3 mm in diameter are present in the lumen of the right horn that are related to the retention of fetal fluid. There are no changes in the lumen of the left horn. Diagnosing pregnancy from only these findings is difficult. The arrow indicates fetal fluid in the uterine cavity.

Photograph 3-10  Ultrasonogram of the horn on the 28th day of pregnancy (7.5 MHz)

The uterine cavity, which is echo-free, is dilated due to the retention of fetal fluid. An embryo (E) is present. By this stage, minute changes in the image accompanying heartbeats are confirmable during the ultrasonographic process. Thus, confirming pregnancy is possible at this stage.
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Chapter 4  Guidelines for the control of reproductive disorders

As long as there is a chance of insemination, female livestock generally repeat pregnancy/delivery according to their species-specific reproductive characteristics unless there are abnormalities in the ovarian function and accessory reproductive organs.

A condition in which reproduction is temporarily or persistently prevented and suspended from any cause is generically referred to as a reproductive disorder.

Causes include feeding and management factors such as the environment of cow sheds and feed used, managerial factors such as the herd size and milking volume, personal factors of individual stockkeepers such as operation ability, management ability and observation ability, obstetric factors such as sterility, abortion, premature and still birth and dystocia and insemination factors such as the identification of estrous signs, management of semen and artificial insemination method. Thus, causes should be identified according to individual stockkeepers to enable them to remove or improve the causes of reproductive disorders and achieve stable breeding performance.

This chapter provides field guidelines in managing reproductive disorders of dairy cattle for stockkeepers, inseminators and veterinarians.

1.  Technical guidance for stockkeepers

To maintain high lactation and continuous calving and achieve high productivity and stable management, one delivery per year should be pursued. To achieve one delivery per year, insemination must be performed at 60-90 days after calving.

To that end, identifying estrus after calving is significant in stock farming and improving conception rates. As dairy cattle have been improved, there has been a dramatic improvement in milk performance. These herds do not necessarily show clear estrous signs during the period from 60 to 90 days after calving because this period corresponds to the lactation peak. Thus, detecting estrus is difficult.

Stockkeepers must pay the utmost attention to identify estrus at this stage since it is the basis of stock farming.

The following are major points in detecting estrus.

(1)  Detection of estrus

Sexual behavior of female livestock in the estrous stage is shown in Table 4-1 (p.106). There are differences among species. Here, a detailed description of sexual behavior in cattle is presented.

A cow herd continuously fed in cow sheds tends to keep standing when normal cows are lying, approach adjacent cows in an unnatural manner, come close to a person in front of them, have decreased (or increased) milk yield and have decreased appetite and considerably shorter rumination periods. The vulva is swollen and the pudendal mucosa is congested. Sticky mucus is excreted from the vulva in the proestrus stage and the mucus becomes fluid as estrus advances. It is watery at the estrous peak and sperm receptivity is the highest at this stage.

- 105 -
Table 4-1  Sexual behavior (estrus signs) in female livestock
            (partially revised from Hafez, 1987)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Goat</th>
<th>Hog</th>
<th>Horse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smelling</td>
<td>Smelling the body and genital region of male animals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urination</td>
<td>Frequent urination during teasing by males. Pollakiuria is not an estrous sign in sheep and hogs but is characteristic of the condition accepting males in horses.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crying</td>
<td>Normal but frequent cries</td>
<td>Cries characteristic of estrus</td>
<td></td>
<td></td>
<td>?</td>
</tr>
<tr>
<td>Exercise</td>
<td>Motility is generally increased. Smelling the genital region of males, females face the opposite direction to males and make a round movement.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mount other females</td>
<td>Wag the tail. Do not mount</td>
<td>Mount other females</td>
<td>Smell head to head and make a false quarrel</td>
<td>Do not mount</td>
</tr>
<tr>
<td>Posture</td>
<td>Stand still while males court</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Turn the head backward and lift and bend the tail</td>
<td>Prick up the ears. Stand still if pressed on the hip by a person</td>
<td>Open and close the pudendal lips to expose the clitoris (tightening). Open the hind legs, bend the hip and lift the tail</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response</td>
<td>Bend the back and lift the tail high</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>after mating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Estrus is a state in which a cow stands still and allows other cattle to mount it.

The acts of pursuing and mounting other cattle usually last several days around estrus.

Many cows in their late pregnancy period pursue and mount other estrous cows frequently. Thus, these acts serve as signs for identifying estrus but are not decisive.

A detailed observation report of an estrous cow's behavior is presented in Table 4-2. The result shows the significantly activated behavior of an estrous cow.

Table 4-2  Behavior in bovine estrous cycle
            (Miyake 1966)

<table>
<thead>
<tr>
<th>Division</th>
<th>Estrous cow</th>
<th>Non-estrous cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cows</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>No. of times of mounting/hr.</td>
<td>2 - 14 (9.6)</td>
<td>0 - 2 (0.4)</td>
</tr>
<tr>
<td>No. of times of being mounted/hr.</td>
<td>17 - 44 (29.6)</td>
<td>0 - 1 (0.2)</td>
</tr>
<tr>
<td>Number of steps/hr.</td>
<td>862 - 1821 (1279.4)</td>
<td>186 - 658 (31.3)</td>
</tr>
</tbody>
</table>

Note: values in the parentheses represent the means

The sexual behavior of an estrous cow is known to be more active in the morning than in the afternoon. A report shows that estrus mostly begins during the period from midnight to 6 AM and less frequently in the afternoon.

The acts of standing still and allowing other cows to mount are important clues for detecting estrus when they are having exercise in the paddock or when they are raised in free stalls.
In tethered cows under drylot feeding, the presence of estrous mucus either attached to the tail or pubic hair or on the stall or feces in early morning often indicates. The results of a survey of the means used to detect estrus in 1,513 inseminated Holsteins in Chiba are shown in Table 4-3.

<table>
<thead>
<tr>
<th>Measured</th>
<th>Total</th>
<th>Conceived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual number</td>
<td>1,513</td>
<td>945</td>
</tr>
<tr>
<td>Total number</td>
<td>4,227</td>
<td>1,635</td>
</tr>
<tr>
<td>Mucus</td>
<td>35.8%</td>
<td>34.5%</td>
</tr>
<tr>
<td>Vulva</td>
<td>21.9%</td>
<td>21.7%</td>
</tr>
<tr>
<td>Mounting</td>
<td>16.3%</td>
<td>17.2%</td>
</tr>
<tr>
<td>Behavior</td>
<td>13.9%</td>
<td>14.4%</td>
</tr>
<tr>
<td>Roar</td>
<td>9.0%</td>
<td>9.4%</td>
</tr>
<tr>
<td>Others</td>
<td>2.4%</td>
<td>3.1%</td>
</tr>
</tbody>
</table>

Chiba Prefectural Mutual Insurance Federation of Agricultural Cooperatives (1977)

Estrus was detected by the presence of mucus in 35.8% of the cases, by swelling of the vulva in 21.9% of the cases and by mounting behavior in 16.3%.

Excreted estrous mucus is shown in Photograph 4-1 (p.135). A swollen vulva is shown in Photograph 4-2 (p.135).

Postpartum recovery of reproductive function takes several dozen days. The ovary immediately after calving is in a stationary state and ovarian follicles start to grow within a week. The ovaries start to accelerate their growth 10 days after calving.

The first postpartum ovulation takes place about two weeks after calving at the earliest and mostly 20 days after calving in dairy cattle undergoing normal calving. This number of days depends on age, parity, season and nutritional conditions and is strongly affected by milk yield; it increases especially in high-lactating cows. However, some reports show that the first ovulation occurs about 15 days after calving in high-lactating cows if they are well fed.

Thus, observation of the estrus should be started as early as possible after calving (about 20 days after calving).

The conditions of a cow including weak estrus and metaestrual bleeding must be observed carefully and recorded in preparation for the next estrus.

As described above, failure in detecting estrus has a significant influence on dairy farming.

Thus, stockkeepers should make sure to detect estrous signs, however weak estrus.

Table 4-4 (p.108) shows the rates of detecting estrus by method. There is no significant difference between twice-daily, observation at fixed time and other methods.

Observation of the estrus should be performed at least twice daily in the morning and evening to increase the rate of detecting estrus and hence conception rates.
Cows should be subjected to once- to twice-monthly regular reproductive examinations by a veterinarian and all cows should be examined on the 30th day after calving onwards for the presence of estrus and uterine involution to improve reproductive performance of the herd as a whole.

Table 4-4 Rate of detecting estrus in cows by method

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3-times daily teasing</td>
<td>93.1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mounting by other cows</td>
<td>81.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>24-hour-day monitoring</td>
<td>100</td>
<td>97 - 100</td>
<td>89</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3-times daily, fixed-time monitoring</td>
<td>91</td>
<td>81 - 91</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Twice daily, fixed-time monitoring</td>
<td>90</td>
<td>81 - 91</td>
<td>72</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Observation during routine work</td>
<td>-</td>
<td>56</td>
<td>56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chin-ball method</td>
<td>98</td>
<td>98 - 100</td>
<td>80 - 87</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Heat mount detector method</td>
<td>-</td>
<td>-</td>
<td>98</td>
<td>96 - 98</td>
<td>-</td>
</tr>
</tbody>
</table>

(Sugic, 1980)

(2) Breeding register

To improve reproductive performance, updating and maintenance of the breeding register is essential. Basically, informations about all of the cattle in herd, for example the name of the cow, date of birth, date of last calving, presence/absence of metaestrual bleeding, date of the last estrus, date of the last insemination, name of the bull, estimated date of the next estrus and the presence/absence of pregnancy diagnosis, should be noted.

Recording the breeding history of individual cattle in the breeding register, leads to improvement of the stockkeeper's awareness and increases in the rate of detecting estrus and conception rates.

Tables 4-5 and 4-6 (p.109) show sample breeding registers. Alternatively, a white board may be installed in a cow shed.

<table>
<thead>
<tr>
<th>Insemination card</th>
<th>Station</th>
<th>Register No.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Registered Name</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Address</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>City</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>District</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Town</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Village</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date of birth:</th>
<th>BWB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of times</td>
<td>Date of insemination</td>
</tr>
<tr>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>-</td>
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<tr>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Producer's name</td>
<td>Registered No.</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------</td>
</tr>
</tbody>
</table>

Table 4-6 Breeding record sheet (for herds)
Management during the dry period

Management during the dry period has a significant influence on the postparturient occurrence of disease, reproductive performance and milk yield after calving.

Nutritional conditions during the periparturient period, especially 2-3 months before calving, are known to affect postpartum recovery of ovarian function and conception rates.

Ideal body condition scores (BCS) in individual lactation periods are shown in Figure 4-1. BCS adjustment must be performed from the middle to late lactation periods based on observation results. Obesity or emaciation during the dry period is the major cause of high incidence of diseases after calving. The BCS of primipara and bipara cows must be adjusted carefully.

![Figure 4-1 Ideal body condition](image)

To adjust the BCS of cows, especially obese cows, during the dry period, feed supply must be restricted considerably. However, management like this is known to cause unstable rumen conditions, decrease the blood levels of total cholesterol, albumin and betalipoprotein and favor perinatal diseases.

The influence of a postpartum reduction in BCS on reproductive performance is presented in Table 4-7. The result shows the close relationship between the prepartum nutritive condition and postpartum reproductive performance.

<table>
<thead>
<tr>
<th></th>
<th>Decrease in body condition scores after calving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>Days to primary ovulation</td>
<td>27</td>
</tr>
<tr>
<td>Days to return of estrus</td>
<td>48</td>
</tr>
<tr>
<td>Days to primary insemination</td>
<td>68</td>
</tr>
<tr>
<td>Conception rates of primary insemination (%)</td>
<td>65</td>
</tr>
</tbody>
</table>

Butler and Smith, March 1989 Journal of Dairy Science
The dry off period allows the organs exhausted by lactation, such as the liver, rumen and mammary glands, to rest sufficiently to recover from fatigue and prepare for the next calving.

The dry period is also necessary for the production of colostrum. If the dry period is absent or too short, there will be a great reduction in milk yield.

During the early dry period (from the onset of dry off to 2-3 weeks before calving), high-lignin roughage such as hay is given ad libitum to fully restore rumen function and form a sufficient mat within the rumen, which allows a high volume of dry matter intake immediately after calving onwards.

The most important point in feeding during the dry period is to minimize the fluctuation in rumen microorganism. Stabilization of the rumen microorganism after a change of feed is considered to take 3 weeks. Thus, an adequate amount of concentrate must be supplied as an energy source for microorganisms during the dry period.

In general, 1 kg of concentrate should be supplied daily although the quantity may vary depending on the quality of roughage and the nutritive condition of the cow.

Assuming that crude protein (CP) content is 11-12% on a dried basis, total digestible nutrients (TDN) is around 56% and neutral detergent fiber (NDF) is around 50%, Ca, P and salts must be limited within the required quantity. The following are the points to note in feeding and management during the late dry period (the period of 2-3 weeks before calving).

During this period, the rumen conditions must be achieved to meet the feed, especially formula feed, which is increased as milk yield rapidly increases.

Concentrate should be increased gradually starting from 2-3 weeks before the expected calving date and be supplied at the rate of 3-4 kg per day one week before calving.

Feeding with Ca should be avoided to prevent milk fever, leguminous hay and silage should be controlled because they have high contents of CP and Ca, and instead of it high-quality graminaceous hay should be fed. Grazing cattle should be placed on restricted grazing, if possible, and be fed with hay and concentrate.

Feed must not be changed for one week before and after calving.

Increasing vitamin preparation doses is also important in preventing postpartum diseases.

Raising dry and milking cows together in the same cow shed should be avoided as far as possible. If infeasible, at least the tethering place should be separated.

Feed for dry cows is totally different from that for milking cows so oversupply of concentrate must be prevented and feed for milking cows must never be used.
2. Technical guidance for inseminators

(1) Handling of insemination apparatus

① Equipment used for insemination

Insemination equipment
Vaginal speculum and illuminating lamp for vaginal speculum
Uterocervical forceps
Scissors for straw tube
Alcohol cotton case
Poly beaker and a thermometer

i. Insemination equipment

There are many types of insemination equipment with various structures and shapes according to the differences among animal species in the vaginal and cervical structures and the quantity of semen inseminated.

Insemination equipment should allow easy injection of semen in the amount required by individual animals and easy, hygienic handling and management and must be harmless to sperm and the female reproductive organs.

Insemination equipment consisting of a new-type inseminator for straw semen tubes and a straw catheter is widely used for cattle. Inseminators for straw semen tubes include a long type about 50 cm long for the recto-vaginal method and a short type about 40 cm long for the cervical forceps method. Both have two types adapted for 1.0 ml and 0.5 ml straws. The type for 0.5 ml straws is widely used. The type for 0.25 ml straws is not used commonly in Japan.

Photograph 4-3 (p.135) shows insemination instruments. The uppermost shows insemination equipment, the left instrument in the second section indicates a case for a straw catheter and the right one a straw catheter, the one in the third section shows a case for insemination equipment and the lowermost one shows a pair of scissors of a straw tube. Photograph 4-4 (p.135) also shows insemination instruments. The upper instrument is a vaginal speculum and the lower one is a pair of uterocervical forceps. Photograph 4-5 (p.135) shows a new type inseminator for straw semen tubes using a sheath tube. They are from the above-described insemination equipment, 0.5 ml straw, sheath tube and a plastic bag for equipment cover.

Insemination equipment and a catheter are carried in a case containing 70% alcohol for disinfection so they are maintained free of germs.

A mount for installing a penlight can be attached to the upper part of the grip of some insemination equipment and this type is useful for insemination by the cervical forceps method in a dark place such as a cow shed.

The process for using an inseminator for straw semen tube is as follows: cut the sealed part of the straw semen tube. Then insert the tube into a catheter. Draw out the core rod of the inseminator. After that, attach the catheter to the tip of the
inseminator. Insert the catheter into the inner part of the cervical canal and inject semen.

In the case of a new type inseminator for straw semen tube, cut the straw semen tube at the sealed part nearer to the semen surface, and then insert the tube into a sheath tube. Don’t insert the whole part of the straw into the sheath tube, but leave 1-2 cm from the cotton stopper exposed. Next, draw out the core rod of the insemination equipment and insert the exposed 1-2 cm part of the straw tube into the tip of the insemination equipment to connect the insemination equipment and sheath tube.

The sheath tube is then moved to the side of the insemination equipment and attached to the sheath locking part and the semen is injected. The straw tube is discarded after use.

An insemination pipette is used as insemination equipment for cattle in a limited number of cases. An insemination pipette consists of a 1.0 ml glass or plastic pipette and is attached with a cap at the base when used for aspirating and injecting semen. In abroad, frozen semen in glass ampoules is commonly used. The semen is thawed and aspirated and injected with a plastic insemination pipette. Insemination pipettes are used as widely as straw semen tube inseminators. A glass insemination pipette is dry-heated or thermally sterilized before use. In the case of thermal sterilization, sterilized physiological saline or 5-6% glucose solution is passed through the insemination pipette immediately before use to remove remaining water. After use, the used pipette is washed, sterilized and kept clean. A pre-sterilized plastic insemination pipette is packed and discarded after use.

ii. Vaginal speculum and illuminating lamp for vaginal speculum

Vaginal specula are used for injecting semen into the inner part of the cervical canal by the cervical forceps method as well as for examining the vagina and the portio vaginalis uteri. Their sizes vary depending on whether the cow is multiparous or nulliparous.

Commonly used vaginal specula are of the side opening type. A vaginal speculum is sterilized with boiling water and then its surface is disinfected with an alcohol soaked cotton swab before use. After use, the vaginal speculum is washed with water, disinfected with an alcohol soaked cotton swab and kept clean.

When examining in a dark place such as a cow shed and when examining the inside of the vagina is difficult, an illuminating lamp is attached to the speculum to light the inside for easier observation of the vagina and the portio vaginalis uteri.

iii. Uterocervical forceps

Uterocervical forceps are used for injecting semen by the cervical forceps method for cattle. The tip of uterocervical forceps used for insemination (cervical forceps method) in cows has a threefold hook and they are thinner than those generally used.
Some uterocervical forceps for insemination are of the joint type so they can be separated into two, front and back, parts. The front half with the hook is put in a case containing alcohol for disinfection and carried. The front half is removed from the case before use and connected to the back half. The entire body is disinfected with an alcohol soaked cotton swab and kept from contaminants. After use, the entire body is washed with water and the front half is kept in the case and the back half disinfected with an alcohol soaked cotton swab and kept in the case.

iv. Alcohol cotton case

A portable metal container for keeping alcohol soaked cotton swabs for disinfection. It is always carried to be used for disinfecting fingertips, straw semen tubes, scissors of straw tube, vaginal speculum and the vulva at the time of insemination.

(2) Disinfection method for insemination instruments

Disinfection methods are divided into physical, chemical and biological (antibacterial) methods.

A simple, rapid, economical and reliable disinfection method is preferred in performing artificial insemination. Disinfection methods vary depending on the equipment used. The method used must not be harmful to sperm or the reproductive organs. Thus, a disinfection method that is suitable for the equipment used must be selected and correctly understood.

Bacterial infection must be prevented throughout the insemination process, from collecting semen to insemination.

The following are disinfection methods.

(1) Physical disinfection

Thermal sterilization is the most reliable and commonly used method of all the physical methods.

i. Thermal sterilization

This method involves submerging equipment in boiling water at 95-100°C for 10-15 minutes. A Schimmelbusch disinfecter is usually used but a pot large enough to submerge equipment may be used instead. Equipment must be fully submerged in boiling water during thermal disinfection. A boiling sterilizer is closed during disinfection to prevent water loss due to evaporation, shorten the time to boiling, ensure disinfection and prevent contamination with foreign matter after disinfection. Adding a small amount of alkali (sodium carbonate) to the boiling water increases and ensures the effectiveness of disinfection.

Thermal disinfection is used for glassware, ceramics and metal and rubber apparatus. The disinfection time mentioned above refers to the time after boiling. Glassware and ceramics must be placed in the disinfecter before the water is heated to avoid rupture.
The vaginal speculum, insemination equipment and other glassware and rubber stoppers are usually disinfected by this method.

Rubber goods tend to be damaged by prolonged thermal disinfection so they should be placed in the disinfectant after boiling and heated for not more than 10-15 minutes.

The water remaining in the disinfected insemination equipment must be removed before use by passing aspiration sterilized physiological saline or 5-6% glucose solution through it.

To cool the disinfected equipment rapidly, the disinfectant is tilted with the lid on to discard hot water. The disinfected insemination equipment and vaginal speculum are kept in the case, wrapped with sterilized gauze, to protect from contaminants.

2. Chemical disinfection

This method uses chemicals including carbolic acid, phenols such as hexachlorophene and chlorhexidine, formalin, sapoated cresol solution, invert soap solution such as benzalkonium chloride and benzethonium chloride, 70% alcohol and quick lime.

In addition, disinfection is performed with gases such as ethylene oxide combined with carbonic acid gas and formaldehyde.

Carbolic acid, formalin, sapoated cresol solution, invert soap solution and ethanol (ethyl alcohol) are used at the concentrations of 5%, 2%, 2-3%, 1-2% and 70%, respectively.

Chemical disinfection is based on chemical reaction so the concentration of a chemical is in direct proportion to its effectiveness. However, excessively high concentrations may lead to decreased effectiveness as in alcohol. High concentrations are harmful to the body and equipment as well as uneconomical so chemicals must be used at specified concentrations.

Chemicals should be used at a temperature of 40-50°C as needed because they are more effective at elevated temperatures.

i. Disinfection with alcohol

Ethyl alcohol is used at the concentration of 70%. The inseminator for straw semen tubes and catheter for cattle are carried in a case for disinfection. These metal cases are filled with sufficient quantities of 70% alcohol.

The used inseminator and catheter are washed with water, shaken to remove water and kept in dedicated cases. The alcohol in the case is changed once a week to maintain disinfectant effectiveness.

With the cervical forceps method, the forceps are carried in a case containing alcohol so this alcohol must be changed once a week.

The inseminator for straw semen tubes, catheter and forceps should be disinfected
with boiling water as needed and kept in the case after being allowed to cool and dry.

Veterinarians and inseminators must carry an alcohol cotton case containing alcohol soaked cotton swabs whenever on duty.

Alcohol soaked cotton swabs are used to disinfect fingertips, scissors of straw tubes, the outside of the straw before cutting the tip, the tip of the forceps, the vulva after washing, the inside and outside of the vaginal speculum and so forth.

ii. Disinfection with invert soap and saponated cresol solution

These are used to clean and disinfect the vulva when inserting a vaginal speculum or an inseminator for the identification of the optimum time for insemination, insemination and pregnancy diagnosis.

The vulva of female livestock is unclean due to dirt. It is first washed with water or warm water to remove dirt and then cleaned and disinfected with invert soap solution diluted 200-fold with lukewarm water and 1% saponated cresol solution.

When the vaginal speculum is repeatedly disinfected with 1% invert soap solution and 2% saponated cresol solution before use, these disinfectants must be washed off the speculum with sterile water immediately before use.

These disinfectants are irritating to the vaginal mucous membrane and are quite harmful to sperm.

(3) Storage and handling of frozen semen

Collected semen is frozen and kept in canisters in an LN2 refrigerator filled with liquid nitrogen at -196°C. The structure of the refrigerator and inside temperature distribution are shown in Figure 4-2 (p.117).

Frozen semen is kept in an LN2 refrigerator filled with liquid nitrogen during transportation. Refrigerators with a capacity of 20-30 liters are used for transportation from centers to sub-centers and those with a capacity of 17 liters are used by inseminators. Refrigerators with a capacity of 30 and 10 liters are shown in Photograph 4-6 (p.136).

The quantity of the liquid nitrogen in the refrigerator must be checked regularly. If the quantity decreases to one third, liquid nitrogen must be supplemented to maintain the preservation temperature of -196°C.

When lifting the canister to take out frozen semen, the upper part of the canister must be about 10 cm lower than the opening of the refrigerator and the required number of straws must be drawn within 10 seconds. Frozen semen must be preserved at -130°C or lower. When taking out straws, special care must be taken not to allow the semen temperature to rise to -130°C or higher. Transfers of frozen semen from one canister to another to create space must take no more than 3-4 seconds for 1 ml straws and no more than 2-3 seconds for 0.25-0.5 ml straws.
Dewdrops may be present around the opening of the refrigerator due to a difference in temperature between the inside and outside of the refrigerator. If frost is present, however, there may be some problem with the refrigerator.

![Diagram of LN2 refrigerator with temperature labels and notes](image)

Figure 4-2 Structure and temperature distribution of an LN2 refrigerator

(4) **Thawing of frozen semen**

The method of thawing frozen semen varies depending on the site and is divided into on-site thawing and in-center thawing.

On-site thawing is when an inseminator brings frozen semen in an LN2 refrigerator to the farm and thaws it on the spot. In-center thawing is when frozen semen is thawed in the center or sub-center and then carried to the insemination site, kept in a thermos bottle.

In on-site thawing, frozen semen is thawed at 30-40°C because the thawed semen is directly used for insemination. After deciding insemination by observing estrous signs of estrous cattle and determining the optimum time for insemination, the vulva is cleaned and disinfected. A straw semen tube is placed in a poly beaker (capacity of about 600 ml) containing 35°C lukewarm water with its cotton stopper facing downward such that the semen part is submerged in the water, thereby observing thawed semen. With a 0.5 ml straw, if semen turns from opaque to translucent within 10-15 seconds and small air bubbles are produced from the cotton stopper, the straw is instantly removed from the water. The straw is placed in an inseminator and used for insemination according to given procedures. When thawing frozen semen, it must pass the zone of deleterious thawing temperatures ranging from -15°C to -30°C as quickly as possible. Thus, thawing at 30-40°C is thought to be favorable for the viability and metabolic activity of sperm after thawing. However, the straw semen tube must be removed from the water within 30-40 seconds, and immediately after it has been thawed. Otherwise, the viability of sperm is reduced.
Frozen semen may be thawed at 5°C on the spot, as required.

In in-center thawing, a straw semen tube is placed in a poly beaker containing ice water at 5°C to thaw following the same procedures mentioned above. The thawed semen is transported in a thermos bottle containing water at 5°C and small pieces of ice.

Care must be taken to ensure that the water temperature is not less than 4-5°C because of ice during transportation from the center. The time from thawing at the center to insemination should be minimized. The viability of sperm decreases over time so the time should be less than 8 hours.

The viability of sperm in thawed semen is lower than in fresh semen so the viability of sperm injected into the female reproductive organs is lower and the time of maintaining fertile ability is shorter than in fresh sperm. Thus, insemination must be performed at the optimum time for insemination and semen must be injected into the correct part of the uterus, i.e. the part covering from the inner part of the cervical canal to the uterine body, to achieve high conception rates. Correct handling procedures and techniques must be followed when transferring semen to prevent it from deteriorating during transportation and storage.

Bovine frozen semen is available in the form of pellet semen in addition to straw semen. Pellet semen is preserved in polyethylene bottles, which are placed in a refrigerator containing liquid nitrogen at -196°C.

Semen is thawed by placing and warming a glass ampoule containing 0.6-0.8 ml of thawing fluid in water at 35°C, opening the ampoule and placing one or two pellets in the ampoule. After thawing, an empty straw semen tube or an insemination pipette is used to aspirate and inject the semen.

(5) Insemination

Insemination technique is an important factor that affects the conception rate.

Inseminators must also provide guidance in feeding and management to help improve conception rates.

Insemination must be performed by taking care as to correct handling of semen, disinfection of equipment, determination of the optimum time for insemination and accurate and hygienic insemination.

1) Insemination equipment, insemination volume and the number of sperm

Inseminators for straw semen tube are usually used for insemination in cattle. Recently, insemination pipettes are being used as well. A 0.5 ml straw semen tube is more commonly used than a 0.25 ml straw semen tube.

For frozen semen, the goal of the number of sperm with a viability score of 3+ or higher after thawing is more than 20 million per straw. However, the required number of sperm depends on the location of insemination. If the location is the external uterine orifice, a larger number of sperm will be required because of loss. However, semen is now injected into the inner part of the cervical canal or the uterus in most cases. Thus,
a conception rate within the normal range is attainable when the number of sperm with a viability score of 3+ is 10 million per straw if a bull with normal fertile ability is used and semen is treated properly.

2) Location of insemination

Semen is injected into the inner part of the cervical canal near the internal uterine orifice with the endocervical insemination method and into the uterine body with the intrauterine insemination method. Care must be taken when injecting semen into the uterus not to perforate the uterine wall and damage the endometrium because the uterine body is only 2-4 cm long. Thus, only a small portion of the tip of an inseminator should be inserted into the uterine body beyond the internal uterine orifice.

3) Insemination methods

Insemination methods employed in Japan include the following. The rectovaginal method is shown in Figure 4-3 and the cervical forceps method in Figure 4-4.

![Figure 4-3 Injection of semen by rectovaginal method](image)

![Figure 4-4 Injection of semen by cervical forceps method](image)
i. Endocervical insemination

   (i) Rectovaginal method

Before proceeding with the rectovaginal method and rectal palpation, fingernails must be close cut and filed with a nail file to avoid damaging the rectal mucous membrane.

In on-site thawing using frozen semen, after straw semen is thawed the straw is immediately removed from the water, the scissors for the straw semen tube and the straw are disinfected with alcohol soaked cotton swabs and the straw is cut laterally at the sealed part near the semen surface. Next, a straw catheter is removed from the case and shaken well to remove remaining alcohol and the straw is inserted into the catheter. The straw is inserted into the catheter while pressing the straw tip strongly on the inside of the catheter tip, the core rod of the inseminator for straw semen tube is drawn and the catheter is connected to the inseminator.

In in-center thawing of frozen semen, semen thawed at the sub-center is transported in a thermos bottle maintained at 4-5°C, taken out of the thermos bottle immediately before use, the scissors and the straw tip are disinfected with alcohol cotton, the straw is cut laterally and fitted into the inseminator as in above.

Liquid semen in straw semen tubes is handled similarly except for the thawing process. If a glass or plastic insemination pipette is used, it is used with a rubber cap to aspirate a given volume of semen from the straw semen tube.

The following describe the procedure of insemination by the rectovaginal method. After cleaning the vulva and wearing a polyethylene glove for rectal palpation on the dominant hand, the glove is coated with mucilage such as soap and water and petrolatum and an inseminator is inserted into the vagina with the gloved hand while holding the vulva open with the thumb and forefinger of the opposite hand. The inseminator is inserted slightly upward for the first 10 cm and then horizontally after the tip of the inseminator has passed the vestibule of the vagina. When the inseminator has reached near the external uterine orifice, the inseminator is held as if to grasp lightly. Next, the gloved hand is gradually inserted into the rectum to grasp the cervical canal from over the rectal wall. To grasp the cervical canal, the cervical part near the external uterine orifice is first held with the hand and the little finger is placed beside or below the external uterine orifice. Next, the inseminator is inserted into the external uterine orifice by guiding the tip of the inseminator along the little finger. The inseminator is inserted carefully and when it has reached the central part of the cervical canal the hand is moved forward along the cervical canal and the forefinger is placed as if to touch the internal uterine orifice. The inseminator is inserted into the inner part of the cervical canal near the internal uterine orifice while carefully moving the cervical canal vertically and slightly forward and then semen is injected. The cervical canal with the inseminator is held for 0.5-1.0 minute after insemination and the inseminator is withdrawn slowly taking care that semen does not flow backward.
(ii) Cervical forceps method

The vulva is cleaned and disinfected with an alcohol soaked cotton swab. Then, semen and an inseminator are prepared in the same way as in the rectovaginal method. Uterocervical forceps are assembled, disinfected with an alcohol soaked cotton swab all over and the hook at the tip is made in contact with a fresh alcohol soaked cotton swab to prevent contamination. Next, the vulva is opened with the thumb and forefinger, a thermally disinfected vaginal speculum, without opening, is inserted about 10 cm slightly upward and then horizontally into the inner part of the vagina. The vaginal speculum is opened to open the vagina and observe the location and shape of the external uterine orifice.

The alcohol cotton is removed from the forceps, the tip of the forceps with one hook is inserted into the external uterine orifice about 2 cm to get hold of the upper left part of the portio vaginalis uteri, which is carefully drawn near and fixed with the thumb together with the vaginal speculum. Then, the inseminator is carefully inserted into the inner part of the cervical canal to inject semen. After insemination, the inseminator is maintained in the same position for about a minute and then withdrawn slowly. The cervical canal held with the forceps is replaced to its original position, the forceps are removed from the external uterine orifice and withdrawn from the vagina and the vaginal speculum is closed and withdrawn.

ii. Intrauterine insemination

Semen and an inseminator are prepared in the same way as in the rectovaginal method. The inseminator is inserted to near the external uterine orifice, the dominant hand with a polyethylene glove on is inserted into the rectum, the cervical canal is held with the hand, the inseminator is inserted carefully up to the inner part of the cervical canal and the internal uterine orifice is located with the forefinger of the hand that holds the cervical canal. The inseminator is further inserted, a sense of resistance is generated but disappears within a short period and the tip of the inseminator appears in the uterine body. The inseminator is held at this position and semen is injected into the uterus.

After insemination, the inseminator is withdrawn slowly from the cervical canal while holding the cervical canal with the opposite hand, the hand is removed from the cervical canal and the inseminator and the hand are withdrawn from the vagina and the rectum, respectively.

The catheter is removed from the inseminator, the straw is removed from the catheter and, after confirming its emptiness, discarded and the catheter and inseminator are washed and placed in the cases.

Special care must be taken that insemination is performed hygienically and the reproductive organs are not damaged by careless operations. Circular folds are present within the cervical canal and may obstruct the passing of the inseminator. Forcing the inseminator to pass through the canal may damage mucous membranes and cause bleeding and inflammation, resulting in sterility. As shown in Figure 4-5 (p.122), the cervical canal must be held horizontally with the hand inserted into
the rectum. If it is lifted, insertion will be difficult. If the inseminator is blocked, it is drawn a little to relieve its tip from the folds and is inserted in another direction. Thus, the inseminator is inserted slowly by carefully moving it vertically.

![Diagram showing how to grip the cervical canal](image)

**Figure 4-5** Good/wrong method for cervical canal grip at insemination

(6) **Optimum time for insemination**

To achieve satisfactory conception rates with artificial insemination, the optimum time for insemination must be selected so that fertile sperm can encounter a fertile ovum in the ampulla of the oviduct, the fertilization site. Ejaculated or injected sperm reach the ampulla of the oviduct within quite a short time, some within several minutes. However, a great number of sperm are required for fertilization to occur so about two hours are known to be necessary for a sufficient number of sperm to reach that point. The capacitation time necessary for sperm injected into the female reproductive organs is about three hours and the sperm retain the fertilizing ability for about 24 hours. In cattle, the time from estrus onset to ovulation is about 30 hours and ovulation generally occurs 10-15 hours after the end of estrus. The emitted ovum is said to retain fertilizing ability for 18-20 hours but actually it retains fertilizing ability for several hours within the oviduct. Thus, the ideal time for the encounter between sperm and an ovum appears to be between several hours before and after ovulation.

However, as shown in the relationship between the insemination time and conception rates in Figure 4-6 (p.123), high conception rates were obtained when insemination was performed during the period from 18 hours after estrus onset to 6 hours after the end of estrus and the conception rate was low when insemination was performed 6 hours after ovulation onwards. This suggests that the capacitation time required for sperm in the female reproductive organ is considerably long. Thus, the beginning or end of a standing estrus must be accurately identified to perform insemination at the optimum time.

In practice, the optimum time for insemination is determined by taking into account the time from estrus onset (detection of estrus) and the results of rectal palpation such as findings in follicular growth and luteal regression.
During mid-estrus, findings in rectal palpation show that ovarian follicles are hard with elasticity and the follicular wall is thick to some extent. Ovarian follicles are generally 1.0 cm in diameter. Toward the end of estrus and ovulation, some ovarian follicles become 1.5 cm (occasionally 2.0 cm depending on individuals) with the corpus luteum extremely hard after regression. The follicular wall becomes thin and rectal palpation reveals the loss of hardness and the presence of fluctuation. The optimum time for insemination is when ovarian follicles are in this status and the corpus luteum has fully regressed. When the cervical forceps method is used without rectal palpation, the vagina is opened with a vaginal speculum and, as required, an illuminating lamp for vaginal speculum or a penlight is used to light the inside to observe the quantity, color and properties of the cervical mucus for the determination of the optimum time for insemination. During the optimum time for insemination, the portio vaginalis uteri is less congested and loosen. The cervical mucus, which was colorless and transparent during mid-estrus, decreases gradually in quantity and becomes slightly grayish white and semitransparent in late estrus. Insemination is performed when these signs become apparent.

As a general rule, if monitoring of the estrus is conducted daily at certain times of the day, cows that have been found to show estrous signs in the morning are inseminated in the afternoon and those that have been found to show estrous signs in the afternoon are inseminated in the morning the following day.

3. Technical guidance for veterinarians

1. Early pregnancy diagnosis

Early identification of whether or not insemination resulted in conception helps to determine the course of action. In the case of conception, for instance, preventing abortion and making calving plans. In the case of a failure, estimating the date of the next estrus and ensuring insemination. In the case of reproductive disorders, proper diagnosis and early treatment helps early recovery from these disorders.

Pregnancy diagnosis refers to a technique involving identifying the changes in the mother's body resulting from conception and the signs of pregnancy accompanying the
generation of the fetus. A practical pregnancy diagnostic method must ensure early diagnosis and a rate of correct diagnosis of more than 85%. However, a rate of correct diagnosis of 100% is desirable as far as veterinarians are concerned. The method must also be simple, harmless to the mother and fetus and less costly.

A few methods of early pregnancy diagnosis employed in the field are described below.

1. Rectal palpation (fetal membrane slip)

This method has been widely used in the field and allows pregnancy diagnosis about 30 days after insemination onwards, though it requires advanced skills during the period from 30 to 33 days after insemination.

Field pregnancy diagnosis is usually made at 35-40 days after insemination. The method involves turning over the uterus, palpating the tips of the uterine horns and confirming clear fluctuation (fetal fluid) and dilation of the pregnant horn and the presence of the fetal membrane and vessels contained in the fetal membrane, as shown in Figure 4-7.

![Figure 4-7 Palpation regions in pregnancy diagnosis](image)

The fetal membrane develops in the uterine body and non-pregnant horn as well after the 40th day of pregnancy so the fetal membrane and vessels can be palpated on the uterine body and non-pregnant horn without turning over the uterus. Rectal palpation reveals the placenta in the pregnant horn on the 60th day of pregnancy onwards, the fetus in the fetal fluid on the 80th day of pregnancy onwards and the uterine artery and characteristic vibration in the pregnant horn on the 90th day of pregnancy onwards. However, findings of the uterine artery and vibration are not decisive indicators of pregnancy because they also occur in sterile cows with high parities.
Pregnancy must be confirmed by the finding of the fetal membrane referring also to findings in the accessory reproductive organs.

Early pregnancy diagnosis allows early detection of abnormalities. It allows the detection of cows with embryonic death and unstable pregnant conditions such as the lack of development of the uterus and vessels matching the days after insemination. It allows the detection of cows that have growing ovarian follicles and contraction and thickness of the uterus so they are at risk of abortion and need treatment with hormones such as hCG.

The following summarize points to note when making pregnancy diagnosis by rectal palpation.

i. Ovary
   (i) The number of pregnant corpus luteum, unilateral or bilateral?
   (ii) The presence/absence and quantity, if any, of fluid in the cavity of a pregnant corpus luteum
   (iii) The presence/absence, number and size of coexistent ovarian follicles

ii. Uterus
   (i) Whether thickening of the uterine wall is similar to that in estrus.
   (ii) Whether contraction is similar to that in estrus.
   (iii) Agreement between the size of the uterine horn and days of pregnancy
         The size of the pregnant horn resulting from development of the fetus, fetal fluid and appendages of the fetus (based on the middle finger)
         30 days of pregnancy: 2.0-2.5 finger widths
         35 days of pregnancy: 2.5-3.0 finger widths
         40 days of pregnancy: 3.5 finger widths
   (iv) Confirmation of the presence or absence of expansion of the non-pregnant horn (after the 35th day of pregnancy)

iii. Appendages of the fetus
   (i) Whether it is monotocous or prolificacy. If it is prolificacy, is it bilateral or unilateral pregnancy?
   (ii) Are the fetal fluid, fetal membrane and vessels in the fetal membrane readily palpable?
   (iii) Is the entrance of the appendages of the fetus into the non-pregnant horn confirmable (after the 35th day of pregnancy)?
(iv) Agreement between the vessels in the fetal membrane and days of pregnancy

- 30 days of pregnancy: silk thread like (size of vessels)
- 35 days of pregnancy: cotton thread like (size of vessels)
- 40 days of pregnancy: kite string like (size of vessels)

iv. Others

(i) The cervical canal is contracted.

(ii) The external pudendal lips are contracted. The external pudendal lips of a pregnant cow are shown in Photograph 4-7 (p.136).

v. Handling of a case of pregnancy with abnormal findings

(i) Treatment

If the contraction of the pregnant horn is strong, treatment with hormones such as hCG is necessary to improve luteal function and attain a stable pregnant status.

(ii) Reexamination

If abnormal findings are present, a reexamination is performed on the 60th to 90th day of pregnancy when implantation is completed.

Pregnancy diagnosis by rectal palpation leads to misdiagnosis in the case of pyometra and mummified fetus but differential diagnosis is not very difficult if meticulous care is taken when checking the points mentioned above. Photograph 4-8 (p.136) shows pyometra.

② Cervical mucus examination

Bovine cervical mucus is colorless, clear and fluid and increases in quantity during estrus but decreases in quantity and becomes thick and sticky during the luteal phase. In pregnancy, the change to a sticky property is great and cervical mucus becomes like jelly or toasted rice cake. Pregnancy diagnosis makes use of this characteristic.

A small amount (the head of a match) of mucus sample collected with cervical mucus sampling alligator forceps is placed on a slide glass and another slide glass is overlaid and rubbed together a few times while holding them between the thumb and forefinger. In pregnancy, the mucus attached to the slide glass looks as if frizzy fragments of thread are intertwined. In non-pregnancy, the mucus is not fragmented but looks like egg white or water and is attached to the glass in a threadlike or diffuse manner, sometimes accompanied by white spots and air bubbles. Frizzy fragments start to appear on the 20th day of pregnancy at the earliest and on the 35th day onwards in all cases. Pregnancy diagnosis by a cervical mucus examination may lead to a misdiagnosis if estrogen (hereafter E₂) has a temporary effect on the cervical canal in spite of conception and if the fetus undergoes embryonic death and remains in the uterus. The rate of correct diagnosis using this method is about 95% after the 35th day of insemination.
Progestrone method (enzyme immunoassay: EIA)

If pregnancy is established, blood progesterone (hereafter P₄) levels are maintained at the high levels of the functional luteal stage. Thus, by determining P₄ levels on the expected date of estrus, around 21 days after insemination, non-pregnancy (low P₄ levels) can be diagnosed with a probability of 100% and pregnancy (high P₄ levels) with a probability of 80-90% or more.

In cattle, P₄ levels in milk are 2-5 times higher than those in blood plasma so they can be determined to make pregnancy diagnosis with the same accuracy as blood levels.

P₄ levels in the plasma or milk are determined at 20-24 days after insemination. P₄ levels in plasma in pregnant and non-pregnant cows at 22-24 days after insemination are shown in Figure 4-8 and those in skim milk at 21-24 days after insemination are shown in Figure 4-9 (p.128).

Plasma cases with P₄ levels of 3 or more ng/ml are supposed to be pregnant and cases with P₄ levels of less than 3 ng/ml non-pregnant. Skim milk cases with P₄ levels of 1 or more ng/ml are supposed to be pregnant and cases with P₄ levels of less than 1 ng/ml non-pregnant.

Whole milk cases with P₄ levels of 10 or more ng/ml and less than 5 ng/ml are usually considered to be pregnant and non-pregnant, respectively, and cases with P₄ levels of 5-10 ng/ml are reexamined.

![Figure 4-8 Blood progesterone levels 22-24 days after insemination in pregnant and non-pregnant cases (Tanaka et al., 1988)]
Pregnancy diagnosis by rectal palpation as well as cervical mucus examination and the milk progesterone method has been described as above. The latter two methods are supplementary to rectal palpation so if accurate pregnancy diagnosis is pursued, findings in rectal palpation such as dilation of the uterus and presence of the fetal membrane and accompanying vessels should be primarily used to confirm the diagnosis.

(2) An example of a comprehensive diagnostic method for the reproductive organs mainly consisting of rectal palpation and treatment

Diagnosis of ovarian function based solely on ovarian findings in rectal palpation is often inaccurate and may lead to misdiagnosis.

The possibility of misdiagnosis can be remarkably reduced by examining ovarian function through an overall analysis of findings in palpation of the uterus and cervical canal and observation of the vagina, pudendal lips and mucus as well as ovarian findings.
A comprehensive diagnostic method will be described according to Table 4-8. In either case, if the first examination fails to lead to a decision, a second examination should be performed after 7-10 days.

Table 4-8  Judgement of ovarian findings from findings in the uterus, cervical canal, vagina and vulva (overall diagnosis)

<table>
<thead>
<tr>
<th>Uterine findings</th>
<th>Others</th>
<th>Judgement</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shape</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contraction/elasticity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membrane mucus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic lips</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hard, thick, open</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Plenty</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Loose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>? is an ovarian</td>
<td>Follicular cyst</td>
</tr>
<tr>
<td></td>
<td></td>
<td>follicle</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.9</td>
<td>2.5</td>
<td>Flat</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>? is the corpus</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>luteum</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>2.0</td>
<td>Round</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>? is an abnormal</td>
<td>Corpus luteum</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ovarian follicle</td>
<td>Abnormal ovarian</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>follicle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>2.0</td>
<td>Oval</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>? is the regression</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>of corpus luteum</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(left)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>? is an estrous</td>
<td>Estrus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>follicle (right)</td>
<td></td>
</tr>
</tbody>
</table>

① Palpation of the ovary does not help determine whether there is an ovarian follicle or corpus luteum.

Is the large body in the right ovary in column 1 of Table 4-8 really an ovarian follicle?
The uterus is large, flat, not contracted and the uterine wall is initially thick with clear internal feeling. The cervical canal is thick and its lumen is open. The vulva is significantly loose and leaks a large quantity of opaque mucus. These findings are the effect of \( E_2 \). Thus, the large body in the right ovary is an ovarian follicle. Because the follicle is more than 2.5 cm in diameter and other corpus luteum is impalpable, a diagnosis of a follicular cyst is made. A diagnosis to differentiate from the cystic corpus luteum and a luteal cyst can be made by closely examining the findings above. To treat follicular cysts, hCG and GnRH preparations are administered in most cases.

Special attention must be paid to whether continued estrus is present or not.

Is the body in the right ovary in column 2 of Table 4-8 (p.129) an ovarian follicle or corpus luteum?

The uterus is soft without intense contraction, elasticity or thickness. The cervical canal is thin, its lumen is closed and the vagina is dry. The pudendal lips are contracted. These findings are the effect of \( P_4 \). Thus, this body is the corpus luteum and judged normal because of a lack of internal feeling.

What are the bodies in the right and left ovaries in column 5 of Table 4-8? (p.129)

Because the accessory reproductive organs show estrous findings, either one must be diagnosed as an estrous follicle. The left body with the size of an ovarian follicle lacks fluctuation by palpation and is hard so it is judged as the regressed corpus luteum.

\( \square \) Examining luteal function and the effect of ovarian follicles

In column 3 of Table 4-8 (p.129), the corpus luteum and an ovarian follicle are detected in the left ovary. Are they functioning normally?

The uterus is thick with internal feeling and the cervical canal is somewhat thick and open. The quantity of vaginal mucus is large for the luteal phase and the vulva is loose. These findings indicate the effect of \( E_3 \). Thus, the ovarian follicle is abnormal and the corpus luteum is not functioning normally.

For treatment, to improve luteal function and induce ovulation and luteinization of the abnormal follicle coexistent with the corpus luteum, hCG preparations and luteal hormone are administered as required.

Findings in the accessory reproductive organs shown in column 4 of Table 4-8 (p.129) indicate the absence of effect of the ovarian follicle in the left ovary and normal luteal function.

(3) Administration of hCG preparations after insemination

This section describes hCG treatment after insemination among other hormone treatments for reproductive disorders. K. Hoshino et al. examined cows with abnormalities suspected from vulvar findings after insemination in the luteal phase and administered an oil or aqueous hCG preparation or swine anterior pituitary gonadotropin to improve luteal function. As a result, the conception increased in all treatment groups compared to the control without significant differences among treatment groups.
In another study, rectal palpation was performed during the period from 3 to 10 days after insemination and cows were divided into control groups with good and poor luteinization and a treatment group with poor luteinization receiving 3,000 IU of hCG. The results are shown in Table 4-9.

Table 4-9  Conception rate according to luteinization condition 3-10 days after artificial insemination

<table>
<thead>
<tr>
<th>Luteinization condition</th>
<th>Untreated Group</th>
<th>HCG-treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of conceived cows/number of cows examined (conception rate %)</td>
<td>Number of conceived cows/number of cows examined (conception rate %)</td>
</tr>
<tr>
<td>Good</td>
<td>65/102 (63.7)</td>
<td>-</td>
</tr>
<tr>
<td>Poor</td>
<td>35/167 (21.0)</td>
<td>199/438 (45.4)</td>
</tr>
</tbody>
</table>

(Note) Intergroup differences are significant with a level of significance of 1%.

(Yasumichi Saito, 1991)

The conception rate in the control group with good and poor luteinization and treatment group was 63.7%, 21.0% and 45.4%, respectively.

Comparison of blood P₄ levels between the conceived and non-conceived groups showed that blood P₄ levels were significantly higher in the conceived group on the 10th day of insemination onwards and the trend appeared on the 6th day.

The conception rates of 438 cows receiving hCG are shown in Table 4-10 according to the days from insemination to treatment. The conception rate of cows treated 3 days after insemination was 26.1%, which was similar to that in the control group, but the rate increased to 54.3% and 59.3% in cows treated 4 and 5 days after insemination, respectively. The conception rate decreased gradually in cows treated 6 days after insemination onwards although significant effects were present.

Table 4-10  Conception rate by the days from insemination to treatment with hCG

<table>
<thead>
<tr>
<th>Days after insemination</th>
<th>Number of conceived cows/number of treated cows</th>
<th>Conception rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>6/23</td>
<td>26.1</td>
</tr>
<tr>
<td>4</td>
<td>19/35</td>
<td>54.3</td>
</tr>
<tr>
<td>5</td>
<td>32/54</td>
<td>59.3</td>
</tr>
<tr>
<td>6</td>
<td>25/53</td>
<td>47.2</td>
</tr>
<tr>
<td>7</td>
<td>20/45</td>
<td>44.4</td>
</tr>
<tr>
<td>8</td>
<td>23/51</td>
<td>45.1</td>
</tr>
<tr>
<td>9</td>
<td>35/78</td>
<td>44.9</td>
</tr>
<tr>
<td>10</td>
<td>39/99</td>
<td>39.4</td>
</tr>
</tbody>
</table>

(Yasumichi Saito, 1991)

These results indicate that hCG treatment after insemination, and especially 5 days after insemination, is an effective treatment for reproductive disorders.
Response to uterine diseases

The uterus, one of the accessory reproductive organs, is under the direct control of ovarian hormones $E_2$ and $P_4$ and changes with their blood levels. Clinical manifestations of high $E_2$ and low $P_4$ levels in blood during estrus include congestion, swelling and increased contraction of the entire uterus. Manifestations of high $P_4$ and low $E_2$ levels in blood during the luteal phase include decreased congestion and contraction and elasticity of the uterus. Other accessory reproductive organs such as the oviduct, cervical canal and vulva also change similarly as a result of the effect of ovarian hormones. Thus, these changes appear as a result of the effect of ovarian hormones during individual stages of the sexual cycle but are prevented by abnormalities in ovarian function and $E_2$ and $P_4$ secretion. Not only is the uterus affected by ovarian hormones but it also produces a luteolytic factor (prostaglandin $F_2\alpha$) through the endometrium, promotes luteal regression and decreases $P_4$ secretion so it is strongly related to hormonal secretion in the sexual cycle. Thus, degeneration and loss of the endometrium due to endometritis may lead to a disturbance in hormonal secretion and abnormal ovarian function.

In many medical examinations of sterility in cattle, too much biased attention is paid to ovarian findings in rectal palpation compared to the uterus.

The functions of the ovary and uterus are closely related so this fact must be remembered when performing medical examinations.

Among other uterine diseases, postpartum uterine involution is often problematic.

Postpartum uterine involution is an important factor for the following conception and the delay in uterine involution affects the conception rate and is a major factor of reproductive disorders.

Rhythmic contraction movement of the uterus continues for 4 days after calving, the uterus undergoes involution through contraction and congestion, discharge of lochia stops and internal feeling in the uterine horn disappear within 2 weeks of calving and the uterus is restored to its normal state in 30 to 40 days. The size of the uterus is 2.5 finger widths at about 30 days after calving in dairy cattle and this is used as a guide to normal uterine involution. Sometimes, however, the uterus is more than 2.5 finger widths in size and retained matter is present in the uterine horn even after the 40th day of calving. One of the major known causes of the delay in uterine involution is perinatal diseases such as dystocia, prepartum and postpartum paraplegia, retention of the placenta, lochometra, puerperal infections and ketosis. At the time of dystocia, for instance, the best measures must be taken when giving assistance and obstetric treatment. Damage to the birth canal and cervical canal caused by careless interventions leads to metritis, cervicitis and vaginitis and to microbism resulting in endometritis and becomes a major cause of sterility due to delayed uterine involution. This is also true for manual removal of the placenta. Endometritis is the most common uterine disease. It is treated principally by uterine irrigation and subsequent intrauterine administration.

However, uterine irrigation is not widely employed in the field because it is time consuming and only intrauterine administration is performed.
Uterine irrigation using a balloon catheter is described below. First, the cow is retained, feces are removed from the rectum as far as possible, the anus and perineal region are cleaned, disinfected and wiped with gauze and a vaginal speculum is inserted. The tip of a balloon catheter with a core is inserted into the cervical canal as deep as possible, the vaginal speculum is removed and the catheter is guided into the uterine cavity by the rectovaginal method. The balloon in the uterine body is charged with 10–20 ml of air and the core is removed. The core should be moistened with physiological saline before being inserted into the catheter so that it can be readily removed. A silicone tube connected to a bottle containing 2–4 liters of physiological saline at 40–42°C is then connected to the balloon catheter.

A quantity of 100–200 ml is injected at a time and injection is stopped when the uterus becomes strained. The uterus is lightly massaged all over to discharge the washing. This washing operation is repeated, the washing is discharged thoroughly and an effective drug is injected. When performing uterine irrigation, the quantities of injected and discharged solution and the opacity of the discharged solution should be checked and cytodiagnosis and bacterial examination should be performed as required.

Figures 4-10 (p.134) and 4-11 (p.134) show the conception rates in cows with endometritis that underwent intrauterine administration with or without uterine irrigation. Study cows were 44 Holstein dairy cattle raised in Chiba Prefecture that discharged polluted mucus during estrus and more than 40 days had passed since their last calving. Of these, 22 cows underwent intrauterine administration and other 22 cows uterine irrigation followed by intrauterine administration.

The drug used was a mixture of penicillin and streptomycin. The conception rate within 60 days of treatment in cows that underwent only intrauterine administration and cows that underwent both uterine irrigation and intrauterine administration was 27.3% and 40.9%, respectively. Figures 4-10 (p.134) and 4-11 (p.134) show the conception rates in cows within 60 days of treatment according to the number of days from calving to treatment and parity. ● indicates conceived and ○ indicates non-conceived. In tripara or younger cows, there was no significant difference in the conception rate between the groups receiving only intrauterine administration or uterine irrigation plus intrauterine administration. In quadrupara or higher-parity cows, the conception rate in the group receiving only intrauterine administration was 14.3% compared to 44.4% in the group receiving uterine irrigation and intrauterine administration. This indicates that a combination of uterine irrigation and intrauterine administration is an extremely effective treatment for uterine diseases.

If treatment was given within 90 days of calving, there was no significant difference between the groups. If treatment was given on the 91st day of calving onwards, the conception rate in the group receiving only intrauterine administration was 23.1% compared to 43.8% in the group receiving uterine irrigation and intrauterine administration.

This also indicates the far higher effectiveness of uterine irrigation followed by intrauterine administration than the single use of intrauterine administration. When treatment was given on the 151st day onwards, in particular, all 8 cows in the group receiving only intrauterine administration failed to achieve conception while 3 of 4 cows in the group receiving intrauterine administration after uterine irrigation achieved
conception. Given these results, if a regular medical examination of reproductive diseases at 30 to 60 days after calving reveals endometritis and if intrauterine administration proves ineffective after 10-20 days by uterine findings (internal feeling, roughness, etc.) in reexamination, then uterine irrigation should be added. Uterine irrigation is an extremely effective treatment particularly for high-parity cows with a long history of uterine diseases.

<table>
<thead>
<tr>
<th>Days after calving</th>
<th>~90</th>
<th>91~150</th>
<th>&gt;151~</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadripara ≤</td>
<td>⬜️⬜️</td>
<td>⬜️⬜️⬜️</td>
<td>⬜️⬜️</td>
<td>7/17(41.2)</td>
</tr>
<tr>
<td>Tripara ≥</td>
<td>⬜️⬜️⬜️</td>
<td>⬜️⬜️⬜️</td>
<td>⬜️⬜️</td>
<td>5/15(33.3)</td>
</tr>
</tbody>
</table>

Conception rate (%)

- 3/9(33.3)
- 0/8(0)
- 6/22(27.3)
- 3/13(23.1)

○: Conceived
■: Non-conceived

Figure 4-10 Conception performance (Group receiving intrauterine administration)

<table>
<thead>
<tr>
<th>Days after calving</th>
<th>~90</th>
<th>91~150</th>
<th>&gt;151~</th>
<th>Conception rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadripara ≤</td>
<td>⬜️⬜️</td>
<td>⬜️⬜️⬜️</td>
<td>⬜️⬜️</td>
<td>4/9(44.4)</td>
</tr>
<tr>
<td>Tripara ≥</td>
<td>⬜️⬜️⬜️</td>
<td>⬜️⬜️⬜️</td>
<td>⬜️⬜️</td>
<td>5/13(38.5)</td>
</tr>
</tbody>
</table>

Conception rate (%)

- 4/12(33.3)
- 3/4(75.0)
- 2/8(33.3)
- 7/16(43.8)

Between a - b : p<0.05

○: Conceived
■: Non-conceived

Figure 4-11 Conception performance
(Grupo receiving intrauterine administration after uterine irrigation)
Photograph 4-1  Estrous mucus  Photograph 4-2  Swelling of the vulva

Photograph 4-3  Inseminator for straw semen tube  Photograph 4-4  Insemination instruments for cattle

Photograph 4-5  New type inseminator for straw semen tubes
Photograph 4-6  LN2 refrigerator (30l and 10l)

Photograph 4-7  The vulva of a pregnant cow

Photograph 4-8  Pyometra
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Chapter 5  Regional status and countermeasures

1. Reproductivity and productivity of breeds used in tropical dairy farming

(1) *Bos indicus* and *Bos taurus*

Domesticated cattle are classified into two major species, i.e. *Bos indicus* (Zebu) and *B. taurus*. These species are thought to derive from a wild species called “taurochs.” They both have 30 pairs of chromosomes but have different Y-chromosomes. They can be crossed with each other and the young, whether male or female, have normal fertility.

*Bos indicus* is distributed mainly in tropical and subtropical regions and *B. taurus*, also called a European species in temperate regions. Intermediate species are present in borderline regions between them. A few breeds of *B. taurus* are present that originated in tropical regions, such as N'Dama in Western Africa. This breed is famous for its trypanotolerance. Indigenous cattle in Latin America called “Criollo” were brought from Europe by the Spanish after the conquest.

Indigenous cattle in Southeastern Asia clearly have genes derived from both species as well as a wild species Banteng and species of the genus Bibos such as Balinese cattle.

The formation of these breeds is complicated in many cases. Table 5-1 shows a comparison of tropical and temperate dairy cattle species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Tropical breeds</th>
<th>Temperate breeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>Generally late maturing</td>
<td>Relatively early maturing</td>
</tr>
<tr>
<td>Heat resistance</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Disease resistance</td>
<td>Highly resistant to ticks, tick-borne disease, trypanosomosis, gastrointestinal parasites.</td>
<td>Sensitive to the diseases listed at left.</td>
</tr>
<tr>
<td>Breeds and breeding</td>
<td>• A variety of breeds including nondescript breeds. Confined to local regions.</td>
<td>• Mainly pure-line breeding. Relatively small number of breeds.</td>
</tr>
<tr>
<td></td>
<td>• Mainly natural selection. Crossbreeding with temperate breeds have been attempted.</td>
<td>• Artificial and intensive breeding.</td>
</tr>
<tr>
<td></td>
<td>• Dual-purpose or multipurpose.</td>
<td>• Milk production only.</td>
</tr>
<tr>
<td>Utilization of low-quality roughage</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Production system</td>
<td>Low input - low output</td>
<td>High input - high output</td>
</tr>
<tr>
<td>Feeding system</td>
<td>Small - scale farmers</td>
<td>Large herd size</td>
</tr>
</tbody>
</table>

(2) General characteristics of Zebu cattle

Most native tropical cattle belong to the Zebu species except for a few breeds. The external characteristic that distinguishes Zebu from the European species is the “hump” on the shoulder or at the back of the neck. The term “humped cattle” is often used to
mean Zebu cattle. The hump is composed of muscle, connective tissue and fat and varies in shape and size depending on the breed, sex and age. Its function is not well understood though the idea that it is a reservoir of fat in case of famine has now been rejected.

Other physical characteristics common to many Zebu cattle include a small body width, sloping rump and rather long legs. The skin is thin and loosely attached to the body and the dewlap is well developed. The body size varies widely and the mean body weight of mature female cattle ranges from 200 to 400 kg depending on the breed.

Zebu cattle are well adapted to tropical environments for the reasons below.

1. Heat resistance
   The high heat resistance of Zebu is due to low heat generation and good heat diffusibility. Efficient sweat glands are densely distributed and help release heat through evaporation. Short, smooth hair helps facilitate convection in the atmosphere. The small body size also helps improve heat resistance with the high surface area ratio to body volume.

2. Resistance to external parasites
   Zebu cattle are resistant to ticks and tick-borne diseases. For instance, Zebu cattle have the ability to resist attack by ticks by moving the skin. Research (Utech et al., 1978) shows that tick larvae develop into adults on Zebu in much lower proportions than on European species. Zebu cattle are considered to have some levels of resistance to many other diseases peculiar to tropical regions. To what extent this disease resistance is hereditary and whether they acquire immunity through exposure to these pathogens from early days has not been clarified.

3. Low nutritional requirements
   Zebu cattle generally have low nutritional requirements. The reasons for this include their small body size, low metabolic rates and, possibly, high digestive efficiency in spite of low supply of feed.

3. Major native tropical dairy breeds (See Photograph 5-1 (p.143-144).)

   [Note: Some photographs in Photographs 5-1 (p.143-144) and 5-2 (p.151) are unclear but they are included only for reference.]

1. Damascus
   Damascus is native to the oasis Ghuta in Damascus and distributed in Syria, Turkey, Iraq, Cyprus and Egypt. It is used for crossbreeding with other breeds. Its body size is medium, the trunk is thin, legs are long and thin and the hair is pale brown or reddish dark brown. It has a long head and short horns. The hump is lacking and the dewlap is well developed especially in males. The udder is medium-sized and the teats are thin and long. This breed is one of the finest dairy breeds in Western Asia. Israeli Holstein was created through repeated crossing of this breed with male Holstein. It has a milk yield of 1,500-3,000 kg, a lactation length of 200-300 days and a milk fat content of 4-5%.
2 Gir

Gir is native to Gujarat district in India and present in Maharashtra Province and Rajasthan Province as well. The physical characteristics of this breed include the broadly projecting long forehead and the forward hanging ears. The hair is white with scattered dark red and dark brown spots all over. Some are red all over in rare cases and some are dappled with yellowish red to near black colors. It has a milk yield of 1,200-1,800 kg per lactation. The age at first calving is 45-54 months and the calving interval is 515-600 days. In Brazil, Gir was crossed with Kankrej to produce the breed Indobrazil. Gir cattle are exported to the States of Texas, Florida and Louisiana in the US.

3 Hariana

Hariana is native to various parts of Hariana Province in India and is a major dual-purpose breed in northern India. The male is a strongly constituted draught breed and has been used widely in improving the draught ability of cattle in the Ganges Plain. Its physical characteristics include the long and thin face, flat forehead and projection of the area between the horns. The horns are small and the muzzle is usually black. The eyes are large and projecting. The udder stretches forward and milk veins are well developed. The teats are well developed, balanced and medium-sized. Total milk yield per lactation of a superior cow reaches 1,500 kg. The age at first calving ranges between 40-60 months and the calving interval between 480-630 days, depending on feeding and management practices.

4 Kankrej

Kankrej is native to the Gujarat district in India and is the heaviest breed of all the breeds in India. It is characterized by the broad chest, straight back and well-developed hump and the dewlap is developed to a medium degree. Total milk yield reaches 1,400 kg under favorable management conditions. The age at first calving is 45-47 months and the calving interval 486-510 days. The bulls have a superior draught capacity. This breed is exported to many countries. In Brazil, it is called Guzerat and excellent cow herds are present. It is crossed with beef breeds in some South American countries and the southern states of the US.

5 Ongole

Ongole is native to the Guntur district in India. It is large and muscular breed suitable for hard labor. Total milk yield is generally 600-1,000 kg on average but reaches 1,500 kg depending on the individual. The age at first calving is 38-45 months and the calving interval 470-530 days. It is exported to Sri Lanka, Fiji, Indochina, Indonesia, Malaysia and the US. This breed was used to develop the breed Santa Gertrudis in the state of Texas.

6 Rathni

Rathi is native to the central Thar Desert. It is a mixture of Sahiwal, Red Sindhi, Tharparkar, Dhamni and so forth but is the most strongly related to Sahiwal. Its body size is medium and the hair is short. The udder is well developed and milk veins protrude. Female cattle are gentle by nature and suitable for milking. Total milk yield per lactation is 1,325-2,093 kg and the calving interval 445-617 days.
Red Sindhi

Red Sindhi is native to Karachi and Hyderabad in Pakistan. It has a small body size but has good lactation capacity. The hair is red and the dewlap and forehead sometimes have white spots. Milk yield per lactation is 1,250-1,800 kg, age at first calving 39-50 months and calving interval 425-540 days. This breed is also exported to many countries including Sri Lanka, the Philippines, US, Malaysia, Iraq, Myanmar and Indochina. Female Red Sindhi has been crossed with Brown Swiss and Jersey to develop Karan Swiss and Jersind, respectively.

Sahiwal

Sahiwal is native to the Montgomery (now Sahiwal) district in Pakistan and is the most excellent breed in the Indian subcontinent. Although it resembles Red Sindhi, it is larger-sized and the skin is looser. The body is long and fleshy. The hair is reddish grayish brown or pale red, sometimes with white spots. The color of the muzzle and eyelashes is pale. A number of cow herds are maintained in India. Milk yield per lactation is 1,400-2,500 kg, age at first calving 37-48 months and calving interval 430-580 days. It is exported to Sri Lanka, Kenya, the West Indies and Latin American countries. A new breed Jamaica Hope was developed by crossing Sahiwal with Jersey.

Tharparkar

Tharparkar is native to the Tharparkar district of Sindh Province, Pakistan, and individuals especially superior in milk production are present in Rajasthan Province of India. It is a medium-sized and compact breed. Male Tharparkar has a good draught capacity. Milk yield per lactation is 1,800-2,600 kg, age at first calving 38-42 months and calving interval 430-460 days.

Sudanese

Sudanese is a generic name for Kenana and Butana. The body size of these breeds is similar to Red Sindhi and Sahiwal but the hair color varies from breed to breed: the hair of Kenana is bluish gray in most cases and the hair of Butana is generally red but mixed colors are sometimes present. The ears are long and each half hangs down. The hump is on the cervico-thoracic or thoracic part. The dewlap is large and outstanding. This breed is dual purpose for milk and draught, and suitable for arid regions. Kenana is considered to have superior milk producing ability to Butana.

Iraqi

Iraqi is native to southern Iraq and is a medium-sized, short-legged breed similar to Red Sindhi. The hair color is gold to bright reddish brown. The hump and dewlap are outstanding but not large. Average milk yield per lactation is 600 kg and calving interval 400 days. This breed is the target of breed improvement using temperate dairy breeds.
Photograph 5-1  Native tropical dairy breeds

1. Damascus
2. Gir
3. Hariana
4. Kankrej
5. Ongole
6. Rathi
7. Red Sindhi
8. Sahiwal
(9) Tharparkar

(10) Butana

(13-1) East African Zebu

(13-2) Indigenous Thai breed

(13-3) Indigenous Indian breed

(13-4) Indigenous Sri Lanka breed

(13-5) Indigenous Philippine breed
Lebanese

Lebanese is distributed in Lebanon and Syria. It has short strong legs, is short-bodied and medium-sized. The hair color is yellow to reddish brown or reddish brown with the tip black colored. Thickness of the skin is medium and the hair is short. The horns are of medium length and white. The dewlap is well developed. Milk yield per lactation is 1,000-2,500 kg.

Nondescript type

Most tropical cattle are of a nondescript type. These cattle are often called by their respective local names but do not possess characteristics common to respective breeds. They grow slowly, are late maturing and have small milk yield. The average milk yield is 340 kg (123-996 kg) and the lactation length 124-394 days (Bhat and Mukandam, 1979). Indigenous cattle in India, Thailand, the Philippines and Sri Lanka shown in Photograph 5-1 (13-1-5) (p.144) are examples of these cattle. They are well adapted to local environments and some have excellent reproductivity.

Reproductivity and productivity of native tropical dairy breeds

Milk producing ability is not well developed in many Zebu cattle. Their milk yield is often not sufficient to feed their own young. Female cattle generally don't let down milk in the absence of sucking stimulation by the calf and this makes the adaptation of Zebu cattle to modern dairy farming difficult. An attempt to milk cows by hand or milking machine in the absence of calf may completely stop lactation. Zebu cattle are both physiologically and sexually late in maturing and their estrous signs are weaker than those of the European species. Milk fat content and non-fat solids are higher than those of most European dairy breeds.

Sahiwal and Red Sindhi are the most widely used dairy breeds of all the Zebu breeds. Both these breeds are native to present Pakistan and quite similar to each other in appearance. Data from the Indian National Dairy Research Institute show that the abilities of these breeds are also similar. The body weight of mature female cattle is 300-350 kg, the average milk yield per lactation about 2,000 kg and milk fat content 5%. Milk yield exceeded 4,000 kg in some individual cows of both breeds. These breeds are widely used to improve the abilities of local breeds in many countries including southeastern Asia. In India, a cooperative project has been established to preserve and improve Sahiwal. A program for progeny tests by artificial insemination has been implemented in 8 institutes using 750 breeding females. In Kenya, pedigree Sahiwal is maintained and used to upgrade unimproved breeds and cross with the European species.

Tharparkar is the third most important breed native to Pakistan. It has a slightly larger body size than the above two breeds but has similar milk yield.

Many native Indian breeds have been improved to be dual-purpose breeds for milk and draught. Besides milk production and draught, Kankrej and Gir which are native to western India and Ongole which is native to southern India have great potential for meat production so they may be called multi-purpose breeds. These are exported to Latin American countries where they play an important role in milk and meat production.
Taneja (1999) reported on the reproductivity and productivity of native tropical dairy breeds as shown in Table 5-2. These data were based on information obtained largely from organized dairy farms because village-level data were not available. Data were summarized according to breeds and do not reflect the source of information, feeding and management conditions or time.

Table 5-2: Lactation performance in native tropical dairy breeds (V. K. Taneja, 1999)

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Body weight at maturity (kg)</th>
<th>Age at first calving (months)</th>
<th>Milk yield per lactation (kg)</th>
<th>Lactation length (days)</th>
<th>Calving interval (days)</th>
<th>Milk fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahiwal</td>
<td>301 - 544</td>
<td>37.4 - 48.8</td>
<td>972 - 2,523</td>
<td>184 - 354</td>
<td>405 - 571</td>
<td>4.3 - 5.2</td>
</tr>
<tr>
<td>Red Sindhi</td>
<td>317 - 454</td>
<td>39.0 - 50.9</td>
<td>835 - 1,869</td>
<td>231 - 345</td>
<td>435 - 562</td>
<td>4.5 - 5.2</td>
</tr>
<tr>
<td>Rathi</td>
<td>295 - 386</td>
<td>40.0 - 52.0</td>
<td>1,325 - 2,129</td>
<td>306 - 331</td>
<td>486 - 617</td>
<td>3.7</td>
</tr>
<tr>
<td>Kankrej</td>
<td>430 - 650</td>
<td>45.0 - 47.0</td>
<td>576 - 1,850</td>
<td>351 - 351</td>
<td>486 - 510</td>
<td>-</td>
</tr>
<tr>
<td>Gir</td>
<td>319 - 568</td>
<td>43.3 - 61.5</td>
<td>1,126 - 1,859</td>
<td>230 - 394</td>
<td>426 - 541</td>
<td>4.5 - 4.6</td>
</tr>
<tr>
<td>Ongole</td>
<td>363 - 591</td>
<td>36.0 - 54.0</td>
<td>613 - 1,590</td>
<td>217 - 279</td>
<td>485 - 637</td>
<td>5.1</td>
</tr>
<tr>
<td>Hariana</td>
<td>287 - 499</td>
<td>41.0 - 60.0</td>
<td>656 - 1,783</td>
<td>209 - 315</td>
<td>434 - 631</td>
<td>4.3 - 5.3</td>
</tr>
<tr>
<td>Tharparkar</td>
<td>293 - 544</td>
<td>37.5 - 53.0</td>
<td>911 - 2,449</td>
<td>240 - 326</td>
<td>399 - 474</td>
<td>5.0 - 5.2</td>
</tr>
<tr>
<td>Iraqi</td>
<td>272 - 363</td>
<td>33.0 - 45.0</td>
<td>609 - 1,035</td>
<td>193 - 277</td>
<td>391 - 454</td>
<td>5.0 - 5.2</td>
</tr>
<tr>
<td>Damascus</td>
<td>136 - 318</td>
<td>-</td>
<td>1,500 - 3,000</td>
<td>190 - 300</td>
<td>-</td>
<td>4.0 - 5.0</td>
</tr>
<tr>
<td>Libanese</td>
<td>230 - 350</td>
<td>-</td>
<td>1,000 - 2,500</td>
<td>-</td>
<td>-</td>
<td>4.0 - 5.0</td>
</tr>
</tbody>
</table>

(Africa)

| Creole   | 343 - 500                    | 30.0 - 31.0                  | 500 - 3,481                   | 180 - 300               | 365 - 420               | 4.6 - 5.1   |
| Boran    | 259 - 680                    | 35.0 - 52.0                  | 454 - 1,814                   | 139 - 313               | 334 - 420               | 4.1 - 6.8   |
| Sudanese | 250 - 500                    | 24.0 - 54.0                  | 454 - 2,723                   | 168 - 339               | 365 - 730               | 4.7 - 5.5   |

1. Age and body weight at first calving

Indigenous dairy breeds are generally late maturing and the possibility of improving sexual maturation is not well known. In a study at the Indian Veterinary Institute, the age at first calving was 30-33 months in 1.9% of Hariana cattle studied and 39 months in 21% (Dadlani et al., 1969). The age at first calving was 35-45 months in about 75% of Tharparkar cattle studied and less than 30 months in 12% (Dutt et al., 1974). Similarly, the age at first calving was less than 34 months in 19%, 35-44 months in 67.4% and more than 44 months in 13.5% in Sahiwal (Wahid, 1976). The male Sahiwal is late maturing and can be subjected to mating at the age of 30-36 months onwards in the field. However, semen can be collected at the age of 2 years onwards on farms with satisfactory feeding and management. The period during which a male can be subjected to breeding ranges from 4 to 8 years in the field and, on farms under good management, from 8 to 10 years.

Body weight at first calving ranges from 120 to 680 kg in tropical breeds. The large variation is due to differences in feeding and management systems, genetic capacity and age at first calving. Most Asian dairy breeds reach body weight at maturation in 30-35 months. The growth curve until maturation is almost linear and average daily weight gain is 200-332 grams.
2 Calving interval

Average calving interval ranges from 334 to 730 days depending on the breed. Sahiwal, Tharparkar and Red Sindhi have a shorter range (15 months) but Hariana, Gir and Rathi have a longer interval. African breeds generally have a shorter calving interval compared to Asian breeds. Calving intervals are reported to have low heritability and to be able to be improved by nutritional improvements and early mating.

3 Milk yield

There is a great variance in the milk yield of tropical breeds. Sahiwal, Tharparkar, Red Sindhi, Gir, Rathi, Kankrej, Damascus, Sudanese, White Fulani and Boran show satisfactory levels of milk yield (1,000-3,000 kg). However, most other breeds are multi-purpose breeds that have two or three purposes (milk and meat production and draught) and exist under unfavorable conditions such as insufficient feed, severe weather and diseases. The heritability estimate of milk yield is 0.25-0.30 and there seems to be great genetic variance.

4 Other characteristics from the perspective of reproductive physiology

In addition to age and body weight at first calving and calving interval, the following are breed characteristics of Zebu cattle. Compared to European breeds, great differences in environmental conditions between tropical and temperate regions pose a problem in considering this subject. Humid and arid regions in the tropical zone also have significantly different effects on the productivity and productivity of cattle. Thus, distinguishing differences in reproductive performance due to the effects of breeds from differences due to environmental effects is difficult. Environmental effects on reproduction will be described in other sections in more detail.

i. Anatomy of reproductive organs

The reproductive organs of Zebu cattle are generally smaller than those of the European breeds naturally because of the difference in body size. From the author's experience of embryo transfer in Red Sindhi, Brahman, Nelore and Gir, the cervix was quite thin and twisted. The protrusion of the external uterine orifice into the vagina was remarkable especially in Red Sindhi and Gir. Anatomical differences from the European species were apparent in these points but no detailed reports on this matter are available.

ii. Endocrinology

The intervals between estrus onset and LH surge and LH surge and ovulation were determined under the same environmental conditions in Brahman, Brahman × Hereford and Hereford. The results are shown in Table 5-3 (p.148). The intervals were shorter in Brahman than in other breeds. An increase in estrogen levels immediately before estrus was detected 24, 16 and 8 hours before estrus onset in the above three breeds respectively.

In a comparative study of Brahman and Hereford × Holstein, the weight of the corpus luteum was lower in Brahman and there was a difference in the response of cultured luteal tissue to LH between these breeds (Rhodes et al., 1982).
Table 5-3  Hormonal changes around estrus in Brahman, Brahman × Hereford and Hereford (Randel et al., 1976, 1977, 1980)

<table>
<thead>
<tr>
<th></th>
<th>Estrus onset-LH surge (hr)</th>
<th>LH surge-ovulation (hr)</th>
<th>Estrogen peak before estrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brahman</td>
<td>2.0 ± 1.3</td>
<td>18.5 ± 3.1</td>
<td>24 hours before estrus</td>
</tr>
<tr>
<td>Brahman × Hereford</td>
<td>3.0 ± 1.0</td>
<td>22.2 ± 2.6</td>
<td>16</td>
</tr>
<tr>
<td>Hereford</td>
<td>6.5 ± 1.8</td>
<td>23.3 ± 2.1</td>
<td>8</td>
</tr>
</tbody>
</table>

(5) Upgrading of Zebu cattle through the introduction of improved breeds

Red Sindhi, Sahiwal, Gir, Kankrej and Ongole are relatively improved breeds among other Zebu dairy breeds. These breeds have therefore been used to improve milk production by increasing tick and heat resistance in various parts of the tropical and subtropical zones. This method has been used to increase milk yield especially in the Indian subcontinent because quality dairy breeds are available. In Kenya, Sahiwal were used for 25 years to improve East African Zebu and milk yield improved at various ratios (31-64%) of Sahiwal. Compared to East African Zebu, there were increases in milk yield and lactation length and a slight increase in calving interval (Mahadevan et al., 1962). Sahiwal was also demonstrated to be adaptable to highlands and heavy-rain regions.

(6) European dairy breed

European breeds have been introduced to tropical regions because of their high milk producing ability but most of the attempts were without satisfactory results. The causes of this include climatic stresses, diseases to which resistance was not established, parasites and nutritional deficiency. In the tropical regions of Israel and Australia, however, the utilization of pure Holsteins is possible if nutrition, diseases and the environment are favorably controlled. Pure European cattle are also utilized on corporate farms provided with favorable feeding and management and summer heat mitigation measures in other tropical countries. In highland regions in Java, Indonesia, small-scale farmers are attempting to develop a pure Holstein breed because summer heat is not that intense.

Wilkins et al. (1979) reported on the result of a study in Bolivia concerning the effect of feeding forms on the productivity of European dairy cattle introduced to tropical regions. The result shown in Table 5-4 (p.149) indicates that feeding forms have significant effects on productivity.

As described below, European dairy cattle are used as the sources of crossbreeding with local breeds to improve milk producing ability rather than as pure breeds. Of the introduced breeds such as Holstein, Brown Swiss, Jersey, Guernsey, Simmental, Red Dane, Ayrshire and Shorthorn, Holstein-Friesian is the most suitable for crossbreeding for its high-milk yield capacity. These breeds are not described in detail here.
Table 5-4  Productivity of European dairy cattle by feeding forms in tropical lowlands of Bolivia (Wilkins et al., 1979)

<table>
<thead>
<tr>
<th>Feeding forms</th>
<th>Breeds</th>
<th>Milk yield (kg/year)</th>
<th>Calving interval (days)</th>
<th>Mortality rates of calves (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Pasturing, concentrate, twice milking</td>
<td>Holstein</td>
<td>3,041</td>
<td>417</td>
<td>5.3</td>
</tr>
<tr>
<td>Pasturing, concentrate, twice milking</td>
<td>Holstein</td>
<td>2,043</td>
<td>470</td>
<td>23.1</td>
</tr>
<tr>
<td></td>
<td>Brown Swiss</td>
<td>2,348</td>
<td>403</td>
<td>16.1</td>
</tr>
<tr>
<td>Pasturing, concentrate, once milking</td>
<td>Brown Swiss</td>
<td>1,470</td>
<td>408</td>
<td>14.8</td>
</tr>
<tr>
<td>Pasturing, twice milking</td>
<td>Brown Swiss</td>
<td>1,950</td>
<td>369</td>
<td>26.5</td>
</tr>
<tr>
<td>Pasturing, once milking</td>
<td>Holstein</td>
<td>543</td>
<td>514</td>
<td>41.7</td>
</tr>
</tbody>
</table>

* Calves were maintained on restricted nursing in the case of once milking and were weaned within a week of calving in the case of twice milking.

(7) New breeds developed through crossbreeding with European breeds (See Photograph 5-2 (p.151.).)

1. Australian Friesian Sahiwal: AFS

AFS is a dairy breed developed in Queensland, Australia, by crossing male Sahiwal with female Holstein-Friesian and selecting over several generations. This breed is 50% related to Sahiwal and Holstein-Friesian by blood. A pure Holstein-Friesian produces 3,670 kg of milk and 141 kg of milk fat while an AFS produces 2,749 kg of milk and 115 kg of milk fat in Queensland (Tierney, 1985). Milk production in AFS improves in the humid tropical climate and AFS produces 124% of milk and 141% of milk fat compared to Bos taurus herds in the same environment. This indicates that AFS is superior to B. taurus in hot and humid environments in Australia and other tropical countries. Genetic improvement by progeny tests is continuing in Australia.

2. Australian Milking Zebu: AMZ

AMZ was developed by the Commonwealth Scientific & Industrial Research Organization (CSIRO) in Australia and is 20-40% related by blood to B. indicus (Sahiwal and Red Sindhi) and 60-80% to Jersey. There is no significant difference in milk yield at first calving between Jersey (1,944 kg) and AMZ (1,917 kg) in a favorable feeding environment. The heritability of milk yield and milk fat in AMZ is 0.23 and 0.27, respectively, and suggests effectiveness in selection. Milk yield and milk fat were comparable among AMZ (3,304kg, 146 kg), Guernsey (2,913 kg, 124 kg) and Friesian (4,165kg, 138 kg). There was a difference in heat resistance between AMZ and B. taurus breeds. If Friesian was exposed to a temperature of 36°C and AMZ to 40.5°C, milk yield decreased by less than 5% in AMZ compared to 30% in Friesian (Hayman, 1977).

3. Illawara

In Australia, a variety of dairy breeds have been introduced since colonization. This breed was created from Jersey, Kerry Dexter, Friesian, Shorthorn and Ayrshire. It was previously called Illawara Shorthorn but is now called simply Illawara. In Australia, some regions have a wide spectrum of temperature ranging from 0 to 40°C and this
breed survives this severe climate even in pasturing. It does not need assistance for calving, either. Some cows have a milk yield of more than 10,000 kg. Milk fat content is medium but milk protein content is considerably high.

7 Jamaica Hope: JH

JH was developed in Jamaica and is 80% related by blood to Jersey, 15% to Sahiwal and 5% to Holstein. Its body shape is similar to Jersey but its body size is larger. The hair is pale yellowish brown to brown or black. This breed was developed to be a dairy breed suitable for humid tropical weather. Average 305-day milk yield is 2,737 kg, milk fat is 130 kg and milk fat content is 5%. On one farm, milk yield reached 3,218 kg. Milk yield varies depending on feeding and management practices but reports showed that the highest, middle and lowest levels were 2,905, 2,000 and 1,623 kg, respectively. Average age at first calving is 33.1-42.4 months and calving interval 371-466 days. The heritability and repeatability of milk yield are 0.35 and 0.77, respectively. This breed has strong resistance to Anaplasmosis and Proplastmosis caused by ticks and is exported to other Caribbean and Latin American countries.

5 Karan Fries

This breed was created at the Dairy Experimental Station in Karnal, India, as a result of crossbreeding between Tharparkar and Holstein-Friesian. It is 50% descended from Holstein but is quite gentle in nature. Average age at first calving is 30-32 months, milk yield 3,700 kg, milk fat 3.8-4.0% and calving interval 400-430 days.

5 Karan Swiss

Karan Swiss was created at the Dairy Experimental Station in Karnal, India, as a result of crossbreeding between female Sahiwal or Red Sindhi and male American Brown Swiss. It is 50% related by blood to Brown Swiss. The hair is reddish grayish brown. Its body size and general appearance are similar to Sahiwal and the dewlap also hangs. The hump is negligible and the body is long and deep. The udder is sufficiently large and is broad, deep and long. The teats are medium-sized. Milk veins are well developed and twisted. Males have strong shoulders. Average age at first calving is 32 months, milk yield at first calving 2,564.7 kg and milk fat 4.2-4.4%. Average milk yield per lactation from pooled after pooling is 3,257.3 kg and calving interval 395.5 days.

7 Siboney

This breed is 5/8 Holstein-Friesian and 3/8 Zebu by blood and was created in Cuba. Average milk yield is 2,897 kg. Average age at first calving is 31.3 months and calving interval 405 days.

8 Sunandini

In an Indian-Swiss project that started in 1963 in Kerala, a local nondescript type of breed was crossed with male Brown Swiss. This produced 50%, 75% and 62.5% Brown Swiss crossbreds. At the farmer level, a 305-day milk yield of 1,351 kg was recorded. Progeny tests were performed on a total of 323 Sunandini males by field tests and 42 were proved.
Photograph 5-2  Tropical dairy crossbreds

1. Australian Friesian Sahiwal: AFS
2. Australian Milking Zebu: AMZ
3. Illawara
4. Jamaica Hope: JH
5. Karan Fries
6. Karan Swiss
7. Siboney
8. Sunandini
Frieswal

This is a new breed created by crossing among crossbreeds of Friesian and Sahiwal that were 3/8 and 5/8 Friesian by blood. Designated mating between proved semen of Holstein-Friesian and Friesian crossbreeds that were 3/8 Friesian by blood produced male Friesian that was 5/8 Friesian by blood. This crossbreeding program was a joint program between the Indian Agricultural Research Council and a military farm and aimed to perform progeny tests of 40 bulls every year. Average milk yield for 326 days was 2,729.9 kg, maximum milk yield 12.2 kg and average body weight at first calving 381 kg.

Jersindh

At the Agricultural Experimental Station in Naini, Allahabad, India, indigenous breeds such as Kankrej, Gir, Hariana, Sahiwal and Red Sindhi were crossed with Holstein-Friesian, Brown Swiss, Jersey and Guernsey. The purpose was to improve the milk yield of these indigenous breeds. As a result, crossbreeding between Red Sindhi and Jersey was the most suitable for the Indian climate. Crossbreeds 3/8 to 5/8 related to Jersey by blood were interbred and the progeny was named Jersindh. Similarly, crossbreeds 3/8 to 5/8 related to Brown Swiss by blood were interbred with Red Sindhi and the progeny was named Brownsindh. Jersindh showed a milk yield at first calving of 1,557-1,861 kg. Because this breed was limited in number and confined in the station, the population declined over time.

Mambi

This breed was created in Cuba by crossing Holstein-Friesian with Zebu. It is 3/4 Holstein-Friesian and 1/4 Zebu by blood. Milk yield is about 2,500 kg. Average age at first calving is 32 months and calving interval 439 days.

Pitanqueiras

This breed is 5/8 Red Pole and 3/8 Zebu by blood and was created in Brazil. Average age at first calving is 35 months and calving interval 414 days. Average milk yield is 2,780 kg and the body weight of a mature female is 422 kg.

(8) Reproductivity and productivity of crossbreds between Zebu and European breeds

European breeds have been crossed with local Zebu breeds in many countries. The most extensive studies were conducted by military farms and the National Dairy Experimental Station in India. On these farms, various types of crossbreeding experiments have been performed since the beginning of the 20th century. Introduced breeds included Shorthorn, Ayrshire and, later, Friesian. Over a certain period, male Bos taurus and B. indicus were used alternately (cross-breeding). Many researchers have statistically analyzed data from decades of study conducted on the military farms.

Amble and Jain (1966) collected data on 1,000 cattle from experiments performed for 22 years on 9 military farms and reported the result as shown in Table 5-5 (p.153).

The result indicates that Friesian breeds that were 1/2 and 5/8 Friesian by blood outperformed the others in milk yield and death rates.
Khatpatal (1970) analyzed data from 521 Sahiwal × Friesian crossbreds in a military farm. The ratios of Holstein-Friesian by blood ranged from 5/16 to 15/16. Using the least squares method, medium-level crossbreds were the most superior in growth rate as well as in milk production.

Bhat et al. analyzed data from 1,800 Sahiwal × Holstein-Friesian crossbreds (1/16-63/64 Holstein by blood) in 8 military farms over 30 years. The age at first calving decreased as the ratio of Holstein by blood increased up to 50% but remained at the same level thereafter. Milk yield at first calving and lactation length increased until the ratio increased to 38/64 but the lowest age at first calving and the highest milk yield were shown by the 63/64 Holstein breed.

The purpose of crossbreeding between native tropical breeds and temperate dairy breeds is to combine the high-lactation ability and early maturation of European breeds with the robustness, disease resistance and adaptability of local breeds. However, early crossbreeding experiments did not produce satisfactory results. This was due to the occurrence of fatal diseases to which European breeds did not have resistance such as cattle plague. In India, Pakistan, Sri Lanka, eastern Africa, the West Indies, southern US and Australia, crossbreeding experiments were not possible before vaccines were used to prevent these diseases. These experiments have demonstrated that crossbreds have higher milk yield than native tropical breeds and higher adaptability to tropical climates than pure European breeds. These crossbreeding experiments were designed to answer the questions as to the important role of native tropical breeds in crossbreeding, superior European breeds, ideal genetic ratios of European breeds and the effects of crossbreeding and heterosis. The results of crossbreeding experiments in various tropical countries have been discussed by many researchers (Taneja and Bhat, 1986; Cunningham and Syrstad, 1987; Syrstad, 1989; McDowell et al., 1996).

Taneja (1999) drew the following conclusions by analyzing the results of various crossbreeding experiments.

- Significant decreases in the age at first calving and calving interval were observed in crossbreds. Milk yield (both at first calving and during lifetime) in crossbreds was 2-3 times higher than in native tropical breeds and the degree of increase

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Table 5-5  Lactation performance by crossbreeding level between *Bos taurus* and *B. indicus* (mainly Friesian: F × Sahiwal) (Amble and Jain, 1966)

<table>
<thead>
<tr>
<th>Genetic group</th>
<th>Age at first calving (months)</th>
<th>Milk yield at first calving (kg)</th>
<th>First calving interval (days)</th>
<th>Mortality rates up to the first calving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahiwal</td>
<td>38.3</td>
<td>1,772 ± 83</td>
<td>421</td>
<td>28</td>
</tr>
<tr>
<td>1/4 F</td>
<td>37.5</td>
<td>1,550 ± 177</td>
<td>417</td>
<td>33</td>
</tr>
<tr>
<td>3/8 F</td>
<td>38.5</td>
<td>2,114 ± 104</td>
<td>445</td>
<td>41</td>
</tr>
<tr>
<td>1/2 F</td>
<td>36.2</td>
<td>2,538 ± 99</td>
<td>432</td>
<td>4</td>
</tr>
<tr>
<td>5/8 F</td>
<td>35.9</td>
<td>2,415 ± 104</td>
<td>433</td>
<td>3</td>
</tr>
<tr>
<td>3/4 F</td>
<td>36.3</td>
<td>2,393 ± 52</td>
<td>468</td>
<td>17</td>
</tr>
<tr>
<td>7/8 F</td>
<td>36.0</td>
<td>2,268 ± 70</td>
<td>462</td>
<td>32</td>
</tr>
<tr>
<td>15/16 F</td>
<td>37.0</td>
<td>2,111 ± 77</td>
<td>442</td>
<td>37</td>
</tr>
<tr>
<td>31/32 F</td>
<td>36.8</td>
<td>1,837 ± 192</td>
<td>463</td>
<td>47</td>
</tr>
</tbody>
</table>
depended on the breed used, the genetic ratio of the European breed used, feeding and management conditions and climatic conditions.

- Crossbreds between Holstein and improved native tropical breeds (Sahiwal, Red Sindhi, Gir, Tharparkar, etc.) were superior to those between Holstein and nondescript type breeds so improved native tropical breeds seemed to play some role in crossbreeding.

- The crossbreds of Holstein were the most superior in growth and milk production among other temperate dairy breeds while the crossbreds of Jersey exhibited more satisfactory reproductive performance. Holstein showed the best milk producing ability followed by Brown Swiss, Red Dane and Jersey.

- A genetic ratio of European breeds of 50% was the best in terms of growth, lactation and reproduction. This result was contrary to the theoretical presumption that higher genetic ratios would produce higher milk yields. This means that replacing completely with European breeds will not necessarily result in high productivity.

- The lower milk yield in F₂ than in F₁ was due partially to reduced heterosis. However, great reductions shown by some experiments were due to the low ability of the F₁ bulls used. Although proven sires were used for the production of F₁ in most cases, little attention was paid to the selection of F₁ bulls. Improvement plans based on efficient crossbreeding require careful attention and accurate documentation.

- Crossbreds on ad libitum feeding showed an increase in milk yield of 30-60% compared to other cattle under ordinary management conditions. This indicates the necessity for reasonably good feeding and management to draw out the abilities of crossbreds sufficiently.

(9) **Examples of breeding and improvements of dairy breeds in tropical countries**

As seen above, introducing European breeds is essential in increasing the productivity of dairy cattle in tropical countries. However, the proper ratios of European breeds in terms of blood has not been established and seem to depend on local climatic conditions, feeding and management levels and socioeconomic conditions. Methods of sustainable crossbreeding of dairy cattle are unknown to researchers who are only familiar with pure line breeding such as Japanese researchers. Attention must be directed not only to milk yield but also to productivity in a broad sense including reproductivity. Obtaining as much accurate information as possible from small-scale farmers is critical in developing new breeds in developing countries.

Breeds and breeding methods employed by various countries are summarized below. Information on Indonesia and Thailand should be referred to when proceeding to Chapter 5 Section 2 on case reports.

1 Indonesia

In Indonesia, which had been under Dutch rule for a long time, the Dutch imported Holsteins and ran dairy farms from the end of the 19th century. These Holsteins were
crossed with Ongole, which was also imported from the Netherlands, and provided a prototype of a breed called Local Friesian. Later, as the artificial insemination service was begun in the 1970s, more than 0.12 million Holstein cows were imported mainly from New Zealand and Australia.

The consistent policy of the government regarding dairy breeds was to aim for pure breeding of Holstein. This was exceptional for a tropical country even if the Javanese highland areas with a relatively cool climate were mainly used for dairy farming. No information about the ratios of Local Friesian or Holstein in terms of blood is available but mating of dairy cattle is now performed using only pure Holstein semen.

Most imported cattle were the products of pregnancy of primiparae but the delay in the return of estrus after the primiparous calving is a problem compared to Local Friesian. Quite small-scale farmers are involved so improvements in feeding and management techniques are expected. However, the animal health care system maintained by the dairy farmers’ association in this country is stronger than those in other developing countries so improvements in the qualifications of field veterinarians and inseminators are expected to produce results.

(2) Thailand

Dairy farming in Thailand started in 1962 with the Thai-Danish Farm supported by the Danish Government. Red Dane was imported from Denmark and crosses with Red Sindhi and Sahiwal imported from India and indigenous Thai breeds were attempted. The Thai-Danish Farm was later reorganized to the Thai Dairy Farming Promotion Organization (DPO), which changed its policy at the beginning of the 1980s and started to import Holstein semen.

The Thai Government established an artificial insemination center at the end of the 1970s with the help of the Netherlands and started to produce frozen semen. From 1979 to 1994, 34,000 dairy crossbred cows were imported from Australia and New Zealand. These crossbreds included Australian Friesian Sahiwal and Australian Milking Zebu from Australia and Sahiwal Friesian (25-50% Sahiwal and 50-75% Holstein) from New Zealand.

The government recommends the use of 75% Holstein breeds for small-scale farmers and is reported to be planning to create a line of Thai Milking Zebu by fixing at this level, though the details are not known. About 70% of dairy cattle raised by farmers are reported to be at this level. However, 80% of semen distributed to farmers is of pure Holstein blood so the Holstein blood is thickening. This raises the concern that heat and disease resistance may decrease and requires the improvement of feeding and management techniques of small-scale farmers.

(3) Philippines

In the Philippines, dairy farming using pure Holsteins is profitable for corporate farms but dairy farming as a whole in this country is on the decline. Dairy cattle total no more than 31,000 (1986) and the country relies on imports for most of its demand for milk and dairy products. A large-scale dairy development project was once planned but failed, partially because some imported Holsteins died during quarantine. Several problems were pointed out including improper response of the receiving organizations
and shortage of technical specialists as well as adaptability problems of imported cattle to the tropical climate. Holsteins have been crossed mainly with superior dairy Zebu breeds such as Red Sindhi, Sahiwal and Tharparkar.

A project to cross indigenous swamp type water buffaloes with imported river type Murrah water buffaloes has been implemented mainly at the Carabao Center to allow small-scale farmers raising water buffaloes to obtain cash income. A cooperative project with JICA was begun in 2000 for this purpose.

4 Nepal

The indigenous breeds of Nepal were produced as a result of fixation of crossbreds between *Bos indicus* and *B. taurus* and have been used largely for the purpose of draught. Similarly as the national land is divided into flat land, hilly and mountainous areas, indigenous breeds have been used to suit respective areas. Dairy farming is concentrated in subtropical flat land areas and relatively large-size breeds such as Hariana and its crossbreds were imported for the purpose of draught. These breeds have been crossed with Jersey and Holstein and used as dairy breeds. Brown Swiss was presented by Switzerland and is now recommended to be used in the highlands. The government recommends the ratio of these European breeds in terms of blood of 50-62.5%. Red Sindhi and Sahiwal were introduced from India and Pakistan to be used as dairy Zebu breeds.

Water buffalo milk accounts for two thirds and 90% of the total milk production is consumed by the farmers themselves. The self-sufficiency rate of milk is quite high (98.5%, 1993).

5 Vietnam

The indigenous breed of Vietnam is Vietnam Yellow Cattle, which is small in size and has a body weight of 230-250 kg for mature males and 180-200 kg for females. The *F*₁ of this breed crossed with Holstein has a milk yield of 2,900-3,300 kg. Crossing with other dairy breeds such as Red Sindhi and Sahiwal was also attempted.

6 Tanzania

Tanzania is proceeding with dairy development by crossing the indigenous cattle East African Zebu with Jersey. The method is as follows.

Individuals showing good lactation performance (400-600 kg/year) are selected from indigenous cattle and crossed with male Jersey by artificial insemination to produce *F*₁ of 50% Jersey blood. This *F*₁ is crossed with male Jersey to produce females of 75% Jersey blood. This cow is crossed with an *F*₁ bull to produce crossbreds of 62.5% Jersey blood. This level of blood can tolerate summer heat. However, the *F*₁ of 50% Jersey blood is more suitable for regions lacking a veterinary service and this cow is crossed with an *F*₁ bull.

7 Chile

Frison Negro, Frison Rojo and Holstein-Friesian are the three most important dairy breeds of Chile. The former two breeds are crossbreds between Holstein and dual-
purpose breeds Obero Negro and Obero Colorado, which were imported from German about a century ago and naturalized, and cattle of 50% Holstein blood are most commonly used by small-scale farmers. While improvements in feeding and management are considered to allow thicker Holstein blood and higher productivity, the important role of these indigenous cattle has been recognized anew and efforts to conserve these genetic resources are continuing.

2. Cases in Indonesia, Thailand and China and actions being taken

Japan has provided technical cooperation in dairy development for Indonesia, Thailand and China. The following are reports on reproductive disorders in dairy cattle by specialists dispatched to these countries and describe problems present in local areas and how they coped with and provided guidance on these problems. This manual was prepared mainly to help control reproductive disorders in tropical regions. China is not exactly a tropical country but is included here because China may serve as a valuable example of the control of reproductive disorders in a developing country.

Kazuhisa Hosokawa describes the case in (1) Indonesia and Mitsuo Oto the cases in (2) Thailand and (3) China.

(1) Case in the Bandung region in Indonesia

(The following are based on experiences from March 1997 to February 2000 when the author was dispatched as a specialist to be involved in the JICA's project “Dairy Technology Improvement Project.”)

① Overview of dairy farming in Indonesia

The introduction of dairy farming dates back to Dutch rule and the governmental promotional policy starting from the 1970s resulted in an import of about 120,000 dairy cattle (Holsteins) from New Zealand, Australia and the US and the subsequent establishment of full-scale dairy farming. Dairy farming is concentrated in the Javanese highlands (an elevation of 800-1600 m) which account for about 70% of the population and 7% of the national land area. About 300,000 dairy cattle are raised by about 80,000 farmers. An average dairy farmer does not have sufficient fields for producing forage crops (“Even if they don't have fields for producing rice or vegetables, they can be dairy farmers.”) so an average of 3-4 cattle tethered in a slotted floor barn just in front of the house are fed with cut wild grass and low-quality formula feed as well as bean curd residue, banana leaves and rice straw. Cows are milked manually and feces are washed off into a nearby water canal. The average annual milk yield of Holstein is not more than 3,000 kg because of the delay in breed improvement, quantitative and qualitative deficiencies of feed and improper management.

② Problems concerning reproduction

The economic breeding goal of Holstein is an age at first calving of 24 months and a calving interval of 12-13 months. In Indonesia, the average age at first insemination is 18-24 months or more and average calving interval is 15-16 months. The causes of this inferiority include the following.
Feeding and management can be improved

Average dairy farmers in Indonesia do not have sufficient fields for ensuring roughage so wild grasses on hill slopes and roadsides are used for feed. During a dry season, grass volume decreases dramatically, roughage intake decreases considerably and some farmers are even obliged to use grass roots. Rice straw is produced in large quantities but is used at low proportions except in some regions and is burnt in most cases. The preparation of silage for dry seasons has been begun lately as a result of technical guidance and its spread is necessary. Low-nutrient formula feed is fed at the rate of half the milk yield as a supplement to roughage. However, formula feed given in a liquid formulation by adding water is not good for cattle in terms of digestive physiology. There was a fear that deficiencies in mineral contents in formula feed and accumulation of nitrates in forage grasses on farms to which feces were returned might have adverse effects on reproduction. Less attention is paid to the management of rearing cattle that do not produce milk compared to lactating cows. Thus, calves have difficulty feeding on formula feed and roughage immediately after weaning because of poor acclimation to such feed during the nursing period and they are also not able to take in necessary nutrients because of the quantitative and qualitative insufficiency of feed. Their growth is often delayed especially when diarrhea and internal parasitosis due to improper hygienic management are combined. The standard body weight, height and chest girth for the first insemination of Holstein cows in Indonesia are 275 kg, 115 cm and 150 cm, respectively.

In Indonesia, dairy cattle are contained in barns from birth to slaughter except those for trading and this results in disorders of the extremities such as arthritis of the knee and overgrown hooves and shortening of the breeding longevity.

Sanitary problems

Although temperate dairy breeds, Holsteins, are used in Indonesia, they do not have tick fever because they are raised in highland areas 800-1,600 m above sea level where ticks do not exist. No occurrence of trichomoniasis or campylobacteriosis of the reproductive organs has been reported because of a saturation rate of artificial insemination of approximately 100%. Brucellosis occurs sporadically. Information about the prevalence of other infectious reproductive diseases such as leptospirosis and viral diseases was not available. One of the causes of the delay in age at first insemination is intestinal parasitosis during the rearing period but the high prices of imported anthelmintics prevent proper parasite control measures. Table 5-6 (p.159) shows the results of a study of the adverse health effects of the combination of feed deficiency and parasitosis on rearing cattle and the results improved by taking countermeasures against them using hematocrit (Ht) as the indicator.
Table 5-6  Results of a parasite control study in rearing cattle at the Breeding and Forage Center in Bumiaksh of the Provincial Livestock Services of West Java

<table>
<thead>
<tr>
<th>Cattle number</th>
<th>Age in month</th>
<th>Sex</th>
<th>March 30, 1998</th>
<th>April 21, 1998</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ht</td>
<td>EPG</td>
</tr>
<tr>
<td>No car tagging</td>
<td>?</td>
<td>♀</td>
<td>18</td>
<td>900</td>
</tr>
<tr>
<td>70281</td>
<td>13</td>
<td>♀</td>
<td>24</td>
<td>100</td>
</tr>
<tr>
<td>70416</td>
<td>11</td>
<td>♀</td>
<td>12</td>
<td>400</td>
</tr>
<tr>
<td>70719</td>
<td>8</td>
<td>♀</td>
<td>16</td>
<td>2,200</td>
</tr>
<tr>
<td>61223</td>
<td>15</td>
<td>♀</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>70423</td>
<td>11</td>
<td>♀</td>
<td>22</td>
<td>200</td>
</tr>
<tr>
<td>7022</td>
<td>13</td>
<td>♀</td>
<td>25</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: After treating with an Ivermectine preparation to control parasites after an EPG (number of parasite eggs per 1 gram of feces) test on March 30, 1998, cows were fed for three weeks with formula feed that was increased from 2 kg/cattle/day to 4 kg/cattle/day.

iii. Reproductive management condition

Detection of estrus at the farmer level is easy if clear estrous signs are present. However, it is often difficult because of feeble estrus, silent heat and delayed estrous cycle and some cows could not be inseminated because no estrous signs were present due to ovarian dysfunction. Few farmers refer to insemination slips, the copies of which are handed out to individual farmers at the time of insemination, to determine the date of the next estrus. If estrus is detected, a liaison card to the inseminator is posted into a liaison box at the time of shipping milk to the intermediate milk collection center in the morning or evening and insemination will be performed within 5-6 hours. In an interview with inseminators, they responded that there was no problem with the supply of liquid nitrogen and frozen semen but in a survey of farmers, some responded that inseminators did not come because of lack of straws.

Although there are regional differences, each of the inseminators who are the staff members of the dairy cooperatives or provincial dairy offices is in charge of 300-600 breeding cows, visit individual farmers by motorcycle and inseminate an average of 3 cows daily. An inseminator collects liaison cards from the liaison box placed in the intermediate milk collection center in the morning and afternoon, drops in the office of the relevant dairy cooperative (often called “livestock hygiene post”) and brings the necessary quantity of frozen semen placed in a portable thermos bottle with liquid nitrogen to the farmer needing it. In some cases, clean water was not available in the field, alcohol soaked cotton swabs were not used and the handling of sheath covers was not hygienic. However, near best care possible in the outdoor environment seemed to be taken.

Veterinarians, reproductive assistants and assistant veterinarians of the dairy cooperatives are responsible for the treatment of diseases including reproductive disorders. Graduates of universities are put on probation for half to one year before starting their careers as independent veterinarians. After experiencing work as inseminators, breeding assistants go through two weeks of training and a certain period of probation before starting their professional careers. Assistant
veterinarians start their careers after a certain period of probation under experienced persons. Basically, the types of work performed by these professions do not differ but intractable cases are treated by veterinarians. For instance, in the Kertasari district within the jurisdiction of the South Bandung dairy cooperative, one veterinarian and two assistant veterinarians are in charge of about 3,700 cattle (about 3,000 of which are breeding cattle). They do not refer medical or breeding records for treatment and few technicians carry stethoscopes and clinical thermometers. After seemingly inadequate diagnosis, metabolic activator (vitamins and minerals) and antibiotics are generally administered. Intrauterine administration with antibiotics is performed to treat reproductive disorders but hormone therapy is not widely used except for PGF2α being used to treat persistent corpus luteum because of lack of technical know-how and high prices. A periodic reproductive inspection system to detect reproductive disorders early has not yet been established due to lack of staff members and so forth. To treat retained placenta, manual removal is usually performed on the day of calving or on the following day at the latest but this method may have adverse effects on the uterus.

### 3 Local breeding condition and the prevalence of reproductive disorders

Tables 5-7 and 5-8 show the summary of reproductive performance and cumulative results of reproductive diagnosis at the Cikole Breeding and Forage Center of the Provincial Livestock Services of West Java located in the suburbs of Bandung.

### Tables 5-7 Summary of reproductive performance of the cows at the Breeding and Forage Center of the Provincial Livestock Services of West Java (April 1997-March 1999)

<table>
<thead>
<tr>
<th></th>
<th>Breeding and Forage Center of the Provincial Livestock Services of West Java</th>
<th>Sample standards in Japan</th>
</tr>
</thead>
<tbody>
<tr>
<td>JMR (cow)</td>
<td>25 - 76</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Days open (days)</td>
<td>169 - 271</td>
<td>≤ 115</td>
</tr>
<tr>
<td>Calving interval (months)</td>
<td>15 - 18</td>
<td>12 - 13</td>
</tr>
<tr>
<td>Number of insemination per conception (cow)</td>
<td>2.3 - 2.7</td>
<td>1.3 - 1.8</td>
</tr>
<tr>
<td>Calving to first insemination time (days)</td>
<td>91.4 - 132</td>
<td>50 - 80</td>
</tr>
<tr>
<td>Conception rate at first insemination (%) (cow)</td>
<td>0 - 31.8</td>
<td>≥ 50 - 65</td>
</tr>
<tr>
<td>Age at first insemination (months)</td>
<td>24 - 30</td>
<td>14</td>
</tr>
<tr>
<td>Incidence of retained placenta (cumulative %)</td>
<td>29.3</td>
<td>≤ 5 - 8</td>
</tr>
</tbody>
</table>

Note: JMR is the abbreviation for the French words Jours (days), Moyen (mean) and Retard (retard) and was developed in Canada in 1987 to represent the delay in conception in cow herds.

### Tables 5-8 Cumulative results of reproductive diagnosis in cows at the Breeding and Forage Center of the Provincial Livestock Services of West Java (April 1997-March 1999)

Pregnant cows 96 (39.7%), physiologic non-pregnant cows 70 (28.9%), cows with reproductive disorders 76 (31.4%)

Details of reproductive disorders

(Endometritis 36.8%, ovarian dysfunction 36.8%, follicular cyst 11.8%, silent heat 7.9%, persistent corpus luteum 2.6%, anovulation 1.3%, repeat breeder 2.6%)

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Major health problems on this farm included malnutrition (especially growth retardation in heifers and postpartum BCS reductions in cows) in tethered cattle and intestinal parasitosis in calves and heifers. Reproductive problems included high age at first insemination, delay in first insemination after calving and prolonged calving intervals due to retained placenta, endometritis and ovarian dysfunction. Average milk yield decreased to 6 kg/day/cow in some seasons due to shortage of feed but was restored to 12 kg/day/cow as a result of improvements in feeding and management.

Data on reproduction and artificial insemination in two dairy farming regions of the Bandung Province, Java, Indonesia, are shown in Tables 5-9 and 5-10. These represent reproductive conditions in ordinary dairy farms.

Table 5-9  Mean monthly number of cases of treatment of reproductive disorders and number of AI within the jurisdiction of the Sarwamukti Agricultural Cooperative and South Bandung Dairy Cooperative

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of cattle raised</th>
<th>Number of cases of treatment of reproductive disorders</th>
<th>Number of cases of treatment of diseases other than reproductive disorders</th>
<th>Number of AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarwamukti</td>
<td>5,436</td>
<td>78 (18.8%)</td>
<td>337</td>
<td>435</td>
</tr>
<tr>
<td>South Bandung</td>
<td>13,000</td>
<td>407 (18.4%)</td>
<td>1,803</td>
<td>1,251</td>
</tr>
</tbody>
</table>


Note: Figures in the parentheses represent the ratios of the number of cases of treatment of reproductive disorders to the number of cases of treatment of all the diseases.

Table 5-10  Details of treatment of reproductive disorders within the jurisdiction of the Sarwamukti Agricultural Cooperative and South Bandung Dairy Cooperative

<table>
<thead>
<tr>
<th>Region</th>
<th>Sarwamukti Agricultural Cooperative</th>
<th>South Bandung Dairy Cooperative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of calvings</td>
<td>1,985</td>
<td>7,998</td>
</tr>
<tr>
<td>Retained placenta</td>
<td>474* (51.1)</td>
<td>1,538** (31.5)</td>
</tr>
<tr>
<td>Endometritis/pyometra</td>
<td>147 (15.8)</td>
<td>709 (14.5)</td>
</tr>
<tr>
<td>Vaginitis</td>
<td>-</td>
<td>178 (3.7)</td>
</tr>
<tr>
<td>Abortion</td>
<td>73*** (7.8)</td>
<td>759**** (15.6)</td>
</tr>
<tr>
<td>Dystocia (including assisted cases)</td>
<td>47 (5.1)</td>
<td>787 (16.1)</td>
</tr>
<tr>
<td>Mummification/fetal maceration</td>
<td>11 (1.2)</td>
<td>202 (4.1)</td>
</tr>
<tr>
<td>Persistent corpus luteum</td>
<td>10 (1.1)</td>
<td>232 (4.8)</td>
</tr>
<tr>
<td>Uterine/vaginal prolapse, uterine torsion</td>
<td>10 (1.1)</td>
<td>119 (2.4)</td>
</tr>
<tr>
<td>Silent lact</td>
<td>8 (0.9)</td>
<td>168 (3.4)</td>
</tr>
<tr>
<td>Ovarian cyst/repeat breeder</td>
<td>-</td>
<td>53 (1.1)</td>
</tr>
<tr>
<td>Ovarian dysfunction</td>
<td>1 (0.1)</td>
<td>122 (2.5)</td>
</tr>
<tr>
<td>Others</td>
<td>147 (15.8)</td>
<td>17 (0.3)</td>
</tr>
</tbody>
</table>


Note: Incidence of retained placenta *23.9%, **19.2%, incidence of abortion ***3.5%, ****8.7% Figures in the parentheses represent the ratios of the number of cases to the number of cases of treatment of all the reproductive diseases.
The figures in Table 5-10 (p.161) may be smaller than actual numbers since they represent the number of treatment cases. However, the values for the number of calvings, retained placenta, abortion and uterine prolapse are considered to reflect the actual status because approximately 100% of cases are supposed to be reported. The incidence of retained placenta is considerably high in both regions and this explains the high incidence of abortion within the jurisdiction of the South Bandung Dairy Cooperative.

4. Effect of summer heat

Although major dairy farming regions around Bandung are located in the tropical zone (at latitude 6°30'-7°30'S), heat is not very intense in shaded areas in spite of the strong sunlight because they are 800-1,600 m above sea level. In the Cikole district of Lembang, Bandung (located at the Breeding and Forage Center of the Provincial Livestock Services of West Java, 1,000 m above sea level), the minimum and maximum monthly temperatures were 11.6 and 26.3°C, respectively, average monthly temperature 14.1-20.7°C and average monthly humidity 74.6-87.7% in 1997. Other regions have similar climatic conditions. Excepting cases in which cattle are raised in barns that are low roofed and susceptible to sunlight, summer heat is considered to have negligible effects on milk production and reproduction.

5. Actions taken in local regions

i. Actions taken by the Breeding and Forage Center in West Java

The Breeding and Forage Center of the Provincial Livestock Services of West Java adopts the following measures to improve reproduction conditions.

(i) Preparing forms for recording data on individual cattle to identify the reproduction conditions of individual cattle and herds and determining the reproductive performance of herds and individual cattle every month based on these data.

(ii) Monitoring estrous signs and abnormal signs (e.g. contamination of leaked mucus with pus) at least twice a day every day including holidays. Using a reproduction calendar useful for identifying the expected date of return of estrus in order to detect estrus.

(iii) Trying to ensure early (timely) examination and early treatment based on evidence to identify the reproduction conditions of individual cattle.

(iv) Improving and establishing treatment policies (for instance, avoiding early manual removal of retained placenta, and in the case of cattle with normal ovarian function, performing intraruminal administration of antibiotics after the insemination of the third cycle and, in the presence of the return of estrus, uterine irrigation and, in promising cases, administering hormone preparations)

(v) (most importantly) improving the management of weaned calves and rearing cattle and the feeding and management to prevent reductions in body condition scores (BCS) after calving especially in the case of this center.
Although it is a rare case in Indonesia, this farm has meadows and manure is returned to them to increase grass production and prevent pollution of rivers. However, there seems to be a need to control the input of manure by monitoring the accumulation of nitrate nitrogen in grass.

ii. Actions taken at the farmer level (pilot areas)

To improve reproductive hygienic management at the farmer level, three pilot areas have been established in the Kertasari district of Bandung Province, Cisurupan district of Garut Province and Tanjungsari district of Sumedang Province and dairy farmers and a specialist group mainly consisting of veterinarians belonging to the dairy cooperatives in charge of individual areas are engaged in the model breeding improvement activities described below. The numbers of breeding cattle and farmers (15, 9 and 18 farmers) are those that will allow twice-monthly reproductive examination.

(i) Recording reproductive information on individual cattle

To establish the habit of recording, steady efforts have been made; seminars were held in local regions to provide guidance for farmers and the items to be entered in the recording form were minimized. Information on reproductive performance in the target farmers of reproductive examination in the pilot areas is now obtainable. A recording form (Table 5-11.) and a plastic sheet to prevent dirt are affixed to a A4-size plywood board with masking tape, the board is placed near the cattle avoiding dirt, and a new recording form is added after calving so that reproduction records can be maintained. (A strong wind may turn over the plastic sheet and the form becomes wet.)

Table 5-11 [Reproduction recording form for individual cows now in use in local areas (a reduced version)]

<table>
<thead>
<tr>
<th>Farmer’s name</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code name of cow</td>
<td>Dam</td>
</tr>
<tr>
<td>Date of birth</td>
<td></td>
</tr>
<tr>
<td>Last date of calving</td>
<td></td>
</tr>
<tr>
<td>Calving history</td>
<td>Presence/absence of retained placenta at last calving</td>
</tr>
<tr>
<td>Date of insemination after last calving</td>
<td>Name of bull</td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Expected date of calving</td>
<td></td>
</tr>
<tr>
<td>Special remarks</td>
<td></td>
</tr>
</tbody>
</table>
(ii) Using an estrus detection calendar

Estrus detection calendars are not widely used but local technicians are promoting them. An estrus detection calendar without printing the year is covered with a polyethylene sheet, necessary items are entered using a marker, the polyethylene sheet is replaced with a new one at the turn of the year, and thus the original calendar is used for many years. This is the idea of a local specialist. An average dairy farmer owns three breeding cattle so whether or not estrus detection calendars are necessary for these small-scale farmers must be considered. The possibility of failing to detect estrus on days other than the expected date of estrus by overestimating these calendars must also be considered because local cattle are somewhat underfed and often fail to show a normal estrous cycle (21 days).

(iii) Improving the farmers’ knowledge of reproduction

Some dairy farmers had the wrong notion that insemination should be performed after letting satisfactory estrus pass twice after calving. To improve the farmers’ knowledge of reproduction, seminars on reproduction physiology encompassing estrus detection, optimum time for insemination and estrus and reproduction management parameters have been held by local specialists using a manual.

(iv) Improving techniques of specialists

Guidance for veterinarians is continuing concerning the identification of reproductive status by rectal palpation and necessary procedures (including early pregnancy diagnosis and BCS scoring system), including items in reproductive medical records for individual cattle, evaluation of herd reproductive performance (using JMR) by treating the farmers targeted for reproductive examination as a farm, and the method of selecting cattle that should undergo reproductive examination.

Recording of reproductive information of individual cattle and the experimental use of estrus detection calendars started on the farms in the pilot areas and the knowledge of farmers about reproduction has been improving. Through model periodic reproductive examinations, veterinarians now understand the method of identifying reproductive performance of the cattle in the pilot areas targeted for reproductive examination, their skills in the diagnosis and treatment of reproductive disorders and early pregnancy diagnosis by rectal palpation have improved and they are now able to serve as instructors for seminars on reproductive hygiene management.

Dairy techniques in Indonesia have been steadily improving such as skills relating to feeding and management, milking hygienic management and roughage production and use.
Case report from the central region of Thailand

Overview of dairy farming

There are about 300,000 dairy cattle in Thailand, 200,000 of which in the central part (flat land areas around Bangkok), 70,000 in the northeastern part (plateaus bordering Laos and Cambodia), 20,000 in the northern part (mountainous regions) and 5,000 in the southern part (Malay Peninsula). The feeding and management circumstances and reproduction conditions of dairy cattle in Thailand are introduced below focusing mainly on the central part.

As is common to southeastern Asian countries, the history of dairy farming is much shorter compared to Western countries. In Thailand, the number of dairy farmers engaged in milking cows began to increase only recently. The majority of dairy farmers started dairy farming in the 1990s by purchasing cattle and milking facilities with the help of public financing.

An average dairy farmer raises 5-10 adult cows without employing workers other than family members (a husband and wife). Few dairy farmers raise dozens of cows. Most dairy farmers own various sizes of pastures and the cows are at pasture for most of the time except the milking time. Cows are tethered and milked with a bucket milking machine in most cases. Mechanical milking is widely used and few dairy farmers milk cows by hand. Roughage is fed through the feed bunk on the pasture and concentrate is fed while the cows are tethered for milking. Roughage is derived from wild grasses and sometimes from cultured forage crops. Dairy cooperatives purchase concentrate in bulk from feed companies and resell it to members in most cases.

Milking is conducted twice daily, once in the morning and once in the evening but not at a 12-hour interval. For reasons of working hours of the staff, most milk collection centers are open from 7 AM through 5 PM. Thus, dairy farmers milk cows from 6 AM in the morning and from 4 PM in the evening. The annual milk yield of an average dairy cow (50-80% Holstein by blood) in Thailand is about 4,000 kg and daily milk yield about 10 kg.

Circumstances related to artificial insemination

Artificial insemination status

In dairy cattle in particular, artificial insemination using frozen straw semen is widely used throughout Thailand. The national artificial insemination center raises bulls of Holstein, Brahman and other crossbreds and produces and distributes frozen straw semen on a routine basis. It also distributes imported semen.

A livestock office has been established in every province of Thailand (75 offices in all) and each office has a district livestock office in each district. Each province has 5-10 district livestock offices and each district livestock office is in charge of a region similar in area to a town or village in Japan. Several inseminators are stationed in each district livestock office and perform artificial insemination at the request of the farmers. In areas where dairy farming is a major industry, district livestock offices have branch offices in which inseminators are stationed. Large-scale dairy cooperatives have several dedicated inseminators and carry out artificial
insemination as part of their business. In addition, some dairy cooperatives are actively striving for the improvement of cow herds and independently established routes for importing frozen straw semen from abroad.

Telephones are not widely used in rural regions so some methods of asking for artificial insemination have been devised. Dairy farmers residing near the district livestock office ask for artificial insemination in person. Some dairy farmers use the telephones of nearby homes and shops or public telephones to contact local livestock offices. In most cases, however, milk cans are used.

Milk cans are brought to the milk collection center every morning where there is a tank with a cooling system for storing milk. If an estrous cow is detected, the farmer hands in an “artificial insemination request form” by attaching it to the lid of a milk can. A staff member of the livestock office visits the milk collection center twice daily around the time of milk collection (8 AM and 5 PM) and brings the forms received back to the office. Some time later, inseminators visit the farmers who requested artificial insemination. Most inseminators carry frozen semen in a portable small semen tank (containing liquid nitrogen and a required number of straw semen tubes) placed in a cloth bag and visit individual farmers by motorcycle.

ii. Training of inseminators

The national artificial insemination center of the Livestock Services Bureau holds 5 or 6 seminar courses every year to train inseminators. Each course lasts 1-2 months and trainees are accommodated in a training facility. These training courses are usually intended for dairy farmers and persons who wish to be inseminators but also sometimes for students.

A certificate of completion every trainee receives at the end of seminars is not a “livestock inseminator's license.” Actually, however, the certificate serves like an inseminator’s license because it certifies the completion of training at a national organization. In many cases, farmers who took these training courses perform the artificial insemination of their own cows and the cows of nearby farmers because they can purchase frozen straw semen and plastic covers (sheath covers) used in artificial insemination from local district livestock offices and dairy cooperatives. Insemination by the farmers cannot be helped to some extent because making contact with inseminators is difficult and the number of inseminators is not sufficient to cover all the dairy farmers within the jurisdiction.

iii. Bulls and semen used

The central part of Thailand has a low elevation and the entire region belongs to the tropical zone. The higher milk yield of Holsteins compared to other breeds is well known to dairy farmers in Thailand. Being a native of northern Europe, however, pure Holsteins, and especially large-size Holsteins of the North American type, are almost unable to be adapted to the summer heat environment. Researchers at the national artificial insemination center have long tried to develop new breeds with heat resistance and high milk yield by crossing native tropical breeds such as Brahman and Sahiwal with Holstein. The researchers of the Reproduction and Breeding Department of the center have formulated various crossing plans to create
bulls to be used in producing dairy cows and implemented some of them, however few follow-up studies of the daughter cows have been conducted. Almost no records of the growth, reproductive performance, milk yield or health conditions of the daughter cows born on ordinary dairy farms are available. Thus, there is insufficient evidence to determine which bulls are suitable for dairy farming in tropical regions.

About 70% of the frozen straw semen used in insemination by dairy farmers is of pure Holstein blood (100% Holstein blood) but the remaining 30% is of 75% or 87.5% Holstein blood. Local dairy farmers and inseminators believe that cattle of higher percentages of Holstein blood have higher milk yield but poorer tolerance to summer heat possibly resulting in accidents. Thus, while cattle of 75% or 87.5% Holstein blood are used, care has been taken to ensure that Holstein blood does not become too thick.

3. Reproduction overview

i. Reproduction condition in farmers

The age at first insemination of about 23 months and age at first conception of about 24 months of an average heifer are considerably later compared to leading dairy countries. The body weight goal for the first insemination is more than 250 kg and ordinary cows do not reach this body weight until they are 23 months old. Dairy farmers are not very aware of this slow rearing speed. Most farmers think that cattle will grow if only they are pastured on grassland. After three months of nursing, intense feeding and management is not conducted.

In addition to problems concerning rearing of heifer, there still are many points to be improved concerning the reproductive performance of cow. The results of a survey conducted in 1997 show that the interval between calving and the first insemination was 87 days, the interval between calving and conception 165 days and calving interval 438 days. The overall conception rate achieved by artificial insemination (the ratio of the number of conception to the total number of times of insemination) was 26% but the conception rate in heifer was 58%.

One calving per year is thought by leading dairy countries to be ideal in sound dairy farming but forcing this idea on dairy farming in Thailand is unreasonable. Identifying what really matters by analyzing the current status is necessary instead of simply paying attention to the figures. A calving interval of 438 days is indeed too long. About 40% of the total cow have achieved conception after the first or second artificial insemination without special treatment. Dairy farmers and inseminators regard the remaining 60% as cows with reproductive disorders.

ii. Actual status of reproductive disorders

Table 5-12 (p.168) shows the results of reproductive examination performed on dairy farms from July 1997 through April 1998. Cows examined were those that farmers reported as “anestrus” or “non-pregnant in spite of artificial insemination.”

The number of cows that the farmers mentioned above and inseminators reported as
cows with reproductive disorders is 116 and this accounts for 60% of the total cow. As a result of examination, cows without special abnormalities were included in a proportion of 31%. Cows identified as having reproductive diseases were 70% of the cows examined, or 40% of the total cow.

Of reproductive disorders, the number of cases of endometritis is the largest, followed by ovarian dysfunction, luteal hypoplasia and follicular cysts. Endometritis includes mucometra. Ovarian dysfunction often occurs in cows with undernutrition and may as well be regarded as a nutritional problem rather than a reproductive disorder.

Heat is especially intent in the dry season with the temperature rising up to 40°C every day. If the lactation peak falls in this season, some cows with good appetite and high feed intake may have a body temperature of 40°C. There are no apparent problems because they maintain vigor, vitality and normal milk yield but a body temperature of near 40°C has a significant effect on the conception rate as a result of artificial insemination. Increased body temperature is thought to deteriorate the uterine environment and reduce the fertilizing ability of sperm, and thus results in a decrease in the conception rate. Thus, summer heat causes a reproductive disorder although there are not sufficient statistical data to allow estimating accurate reductions in the conception rate.

### Table 5-12 Results of reproductive examination of 116 dairy cows in the central part of Thailand (July 1997-April 1998)

<table>
<thead>
<tr>
<th>Disorders</th>
<th>Number of Cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometritis</td>
<td>39 (33.6%)</td>
</tr>
<tr>
<td>Ovarian dysfunction</td>
<td>20 (17.2%)</td>
</tr>
<tr>
<td>Luteal hypoplasia</td>
<td>9 (7.8%)</td>
</tr>
<tr>
<td>Follicular cysts</td>
<td>6 (5.2%)</td>
</tr>
<tr>
<td>Others (pyometra, etc.)</td>
<td>6 (5.2%)</td>
</tr>
<tr>
<td>No abnormalities (including a failure to detect estrus)</td>
<td>36 (31.0%)</td>
</tr>
</tbody>
</table>

### iii. Current status of treatment of cows with reproductive disorders

Several veterinarians are stationed at the livestock office of each province but no veterinarians are stationed at any district livestock office. The staff members of district livestock offices who are graduates of animal husbandry vocational colleges and called livestock technical experts provide technical guidance for dairy farmers. Besides these livestock technical experts, other staff members such as inseminators and assistant inseminators are in charge of artificial insemination. Veterinarians from the livestock office are in charge of guiding and managing livestock technical experts of district livestock offices. However, they seldom visit the district livestock offices to provide guidance nor do they visit dairy farmers with livestock technical experts. Thus, livestock technical experts and inseminators are obliged to play a role as veterinarians. In fact, the personnel who are engaged in artificial insemination are regarded as veterinarians by dairy farmers irrespective of their qualifications or titles.

However, these personnel are not engaged in treating diseases at all as far as
reproductive disorders are concerned. They do not carry various medicines. They may carry some antibiotics and vitamin preparations but not high-price hormone preparations such as prostaglandin and GnRH. They no more than infuse antibiotics into the uterine of cows leaking opaque mucus or inject vitamin preparations to cows that are thin and have reduced ovarian function. The farmers pay for the medicines used but the technical fee is considerably low. Some dairy farmers have a stock of medicines and ask inseminators for treatment but these farmers do not have expensive medicines for reproductive disorders either. If an inseminator uses medicine for the cow of a cooperative member, the charge is deducted from the sales of milk.

Other than reproductive disorders, inseminators are engaged in assisting in the case of dystocia. However, they tend to draw fetuses forcibly regardless of the condition of the mother cow and fetus and this may lead to postpartum diseases and reproductive disorders. This is also true for retention of placenta; they often remove the placenta forcibly. This may lead to injuries of the uterine membrane and bacterial infection of the uterus and result in reproductive disorders.

4. New challenges in dealing with dairy farmers

i. Visiting reproductive examination team

As part of a dissemination activity in JICA's "The Dairy Farming Development Project in The Central Region of Thailand," a "visiting reproductive examination team," which consisted of a veterinarian from the livestock office, livestock technical expert at the district livestock office, an inseminator and an office staff member, was established in a certain province from 1997 to 1998 and started to visit particular dairy farms periodically (once monthly). All the team members that visited the dairy farmers, confirmed the calving and artificial insemination records of all the cattle raised and examined all the cows, pregnancy diagnosis of which was possible, and sterile cows by rectal palpation. The veterinarian was responsible for the overall operation of the visiting reproductive examination as well as for technical matters. The inseminator was responsible for the arrangement of the visiting day and other routine tasks. The office staff member was responsible for the recording of necessary items. The team members clarified where the responsibility lies.

The team did not give any special treatment but simply identifying pregnant and sterile cows and observing their status was useful. Cooperation between the livestock office and the district livestock office in fieldwork on a regular basis was exceptional.

ii. "Reproduction notebook"

Few dairy farmers record the exact details of reproduction. Some dairy farmers who recorded reproductive information accurately were once the staff members of the livestock offices, graduates of agricultural universities or colleges or veterinarians.

Inseminators usually leave some record of insemination by leaving the copy of an insemination slip or filling out the artificial insemination card. Otherwise, no
record of insemination is left in the hands of the farmer because ordinary dairy farmers are not in the habit of filling in insemination details. If an inseminator entered a wrong date or name of the cow or code of the bull, there would be no means of confirmation because there is no one to check them on the spot.

Using a "reproduction notebook" is recommended for dairy farmers. Recording of reproductive information was also recommended in the "visiting reproductive examination team" activity mentioned above. At that time, homemade notebooks were used. Notebooks of a unified form are desirable to be used in a wide area. In the first place, dairy farmers should be encouraged to form a habit of filling in reproductive information with the help of local inseminators.

The following items were recommended as essential information: ① cow name (number), ② date of birth, ③ date of calving plus number of times of calving, ④ date of insemination plus number of times of insemination, ⑤ bull name (code), ⑥ date of pregnancy diagnosis and pregnant/non-pregnant status and ⑦ expected date of calving. Items ① and ② are entered at the time of preparing the notebook. Item ⑤ is entered at calving and items ⑥ and ⑦ are entered at artificial insemination. If pregnancy is diagnosed, the expected date of calving is determined from the reproduction calendar and entered ⑦. Forming a new habit is a laborious task. Thus, assistance from local livestock specialists such as inseminators is necessary until the habit is established. In the first place, encourage the farmer write down the cow name and, if the farmer needs help, the inseminator provides help. If the dates of birth and calving are unclear, approximate dates are entered. Records may be incomplete. To avoid giving up, items should be entered where possible.

A young trusted inseminator willingly took the role of instructing dairy farmers to fill out the "reproduction notebook." At first the inseminator wrote down items and the dairy farmers followed him. Thus, at least five dairy farmers learned and continued to fill out the "reproduction notebook."

(3) Reproduction conditions in Tianjin, China

① Overview of dairy farming

Reproduction conditions in dairy farming in Tianjin are described based on two years of experience of technical guidance in controlling reproductive disorders as a expert for the JICA project "Tianjin Dairy Development Project."

The City of Tianjin is situated in lat. 39°N, the same latitude as the central part of Tōhoku Region, Japan. Located in a large plain of North China, Tianjin has a hot climate in summer and severely cold climate in winter. It does not have a large amount of snowfall. Cold winter is not problematic to cattle raising but summer heat must be prevented. However, dairy farmers have some difficulties during winter, especially with outdoor artificial insemination because thawed straw semen may refreeze. At -18°C or less, an estrous cow should be inseminated indoors instead of outdoors.

In Tianjin, there are 21 national dairy farms belonging to the Livestock Department of the Farm Administration Bureau of Tianjin. The City of Tianjin has an area larger
than an average prefecture of Japan. The City of Tianjin looks as if 21 dairy farms are distributed throughout a large prefecture. Some dairy farms are few km from the central part of the city and some 100 km.

There are farmers who raise cattle, milk cows and sell milk but dairy farmers who are mainly engaged in dairy farming are few. There are also some public dairy farms (farms of armies and prisons) outside the jurisdiction of the Livestock Department of the Farm Administration Bureau, however the national dairy farms of the Farm Administration Bureau account for the major part of dairy farming in Tianjin.

There are a total of about 12,000 dairy cows in 21 national dairy farms, about 7,600 of which are cows. These dairy farms vary in size with the largest having a staff of 180 members and a herd of 1,200 cattle (including 650 cows), producing an average of 11,000 kg of milk daily. The smallest farm has a staff of 30 members and a herd of 140 cattle (90 cows) producing 1,200 kg of milk daily. (See Table 5-13.)

Table 5-13  Number of cattle raised and reproductive performance on national dairy farms

<table>
<thead>
<tr>
<th>Name of dairy farm</th>
<th>Total number of cattle</th>
<th>Number of cows</th>
<th>More than half year of non-pregnant condition</th>
<th>Calving interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gong nong liameng No.1</td>
<td>933</td>
<td>506</td>
<td>8.3%</td>
<td>412.7 days</td>
</tr>
<tr>
<td>Gong nong liameng No.2</td>
<td>686</td>
<td>362</td>
<td>4.1</td>
<td>417.8</td>
</tr>
<tr>
<td>Hong guang No.1</td>
<td>831</td>
<td>467</td>
<td>9.6</td>
<td>415</td>
</tr>
<tr>
<td>Hong guang No.2</td>
<td>754</td>
<td>429</td>
<td>1.9</td>
<td>383</td>
</tr>
<tr>
<td>Hong guang No.3</td>
<td>176</td>
<td>91</td>
<td>2.2</td>
<td>369.5</td>
</tr>
<tr>
<td>Dong jiao No.1</td>
<td>669</td>
<td>421</td>
<td>5.2</td>
<td>392</td>
</tr>
<tr>
<td>Dong jiao No.2</td>
<td>621</td>
<td>368</td>
<td>19.8</td>
<td>428</td>
</tr>
<tr>
<td>Jun lang cheng No.1</td>
<td>789</td>
<td>469</td>
<td>4.5</td>
<td>387</td>
</tr>
<tr>
<td>Jun lang cheng No.2</td>
<td>704</td>
<td>453</td>
<td>-</td>
<td>403</td>
</tr>
<tr>
<td>Bei da gang No.1</td>
<td>604</td>
<td>378</td>
<td>6.3</td>
<td>403.8</td>
</tr>
<tr>
<td>Bei da gang No.2</td>
<td>312</td>
<td>187</td>
<td>-</td>
<td>397.3</td>
</tr>
<tr>
<td>Bei da gang No.3</td>
<td>430</td>
<td>282</td>
<td>8.5</td>
<td>414</td>
</tr>
<tr>
<td>Shuang lin</td>
<td>951</td>
<td>566</td>
<td>6.0</td>
<td>385</td>
</tr>
<tr>
<td>Yang liu qing</td>
<td>644</td>
<td>411</td>
<td>5.1</td>
<td>403.8</td>
</tr>
<tr>
<td>Hong qi</td>
<td>546</td>
<td>322</td>
<td>6.8</td>
<td>420.4</td>
</tr>
<tr>
<td>Wu qing</td>
<td>392</td>
<td>311</td>
<td>-</td>
<td>409</td>
</tr>
<tr>
<td>Huang zhuang</td>
<td>432</td>
<td>315</td>
<td>7.0</td>
<td>408</td>
</tr>
<tr>
<td>Pan zhuang</td>
<td>418</td>
<td>263</td>
<td>4.6</td>
<td>461</td>
</tr>
<tr>
<td>Li zi hu</td>
<td>565</td>
<td>362</td>
<td>12.4</td>
<td>356.5</td>
</tr>
<tr>
<td>Tang lu</td>
<td>726</td>
<td>510</td>
<td>6.5</td>
<td>379.3</td>
</tr>
<tr>
<td>Xing lin</td>
<td>153</td>
<td>91</td>
<td>-</td>
<td>390 (1992)</td>
</tr>
<tr>
<td>Total</td>
<td>12,336</td>
<td>7,564</td>
<td>Mean 7.1%</td>
<td>Mean 401.4</td>
</tr>
</tbody>
</table>

Source: Reproduction statistics of dairy farms of Tianjin Farm Administration Bureau (1994)
2. Circumstances related to artificial insemination and inseminators

i. Artificial insemination status

Artificial insemination using frozen semen was performed at the national dairy farms in the proportion of 100%, though the procedures followed were quite different compared to Japan. Pellet semen was mainly used and frozen straw semen was used on about half of the dairy farms. Frozen straw semen was exclusively used on only two dairy farms. (See Table 5-14.)

Table 5-14 Results of surveys of reproduction-related operations on national dairy farms

<table>
<thead>
<tr>
<th>Dairy farm name</th>
<th>Artificial insemination</th>
<th>Medical examination, treatment (recovery rate)</th>
<th>Special remarks</th>
<th>Days after AI</th>
<th>Date of pregnancy diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gong nong lamang No.1</td>
<td>Pellet 50% Straw 50%</td>
<td>On and after the 60th day of calving</td>
<td>15th day of calving (50%)</td>
<td>≥ 60 days</td>
<td>on and after the 10th day of every month, simultaneous implementation</td>
</tr>
<tr>
<td>Gong nong lamang No.2</td>
<td>Pellet 100%</td>
<td>On and after the 45th day of calving</td>
<td>25th to 30th day of calving (30%)</td>
<td>≥ 60 days</td>
<td>on the 30th day of each month</td>
</tr>
<tr>
<td>Hong guang No.1</td>
<td>Pellet 70% Straw 30%</td>
<td>On and after the 45th day of calving</td>
<td>20th day of calving (almost recovered)</td>
<td>55-85 days</td>
<td>on the 25th day of each month (for instance, cows inseminated in March are examined on May 25)</td>
</tr>
<tr>
<td>Hong guang No.3</td>
<td>Pellet 100%</td>
<td>21st day of calving</td>
<td>Mainly the uterus is treated at first examination</td>
<td>≥ 60 days</td>
<td>on the 26th or 27th day of each month</td>
</tr>
<tr>
<td>Dong jian No.1</td>
<td>Pellet 70% Straw 30%</td>
<td>30th day of calving (20-30%)</td>
<td>In and after November 1992, inseminators give treatment</td>
<td>60 days</td>
<td>(Reexamination is performed if the result of rectal palpation is unclear (&gt; even in the case of 55 days)</td>
</tr>
<tr>
<td>Jun lang cheng No.1</td>
<td>Pellet 90% Straw 10%</td>
<td>On and after the 60th day of calving</td>
<td>8th day of calving (35%)</td>
<td>55-85 days</td>
<td>on the 24th day of each month (for instance, cows inseminated in March are examined on May 24)</td>
</tr>
<tr>
<td>Jun lang cheng No.2</td>
<td>Pellet 100%</td>
<td>On and after the 45th day of calving</td>
<td>26th day of calving (30%)</td>
<td>≥ 60 days</td>
<td>on the 25th or 26th day of each month (reconfirm before dry off)</td>
</tr>
<tr>
<td>Bei da gang No.1</td>
<td>Pellet 100%</td>
<td>On and after the 35th day of calving</td>
<td>20th day of calving (summer 40%, winter 10%)</td>
<td>80-115 days</td>
<td>on the 20-23rd day of each month (cows inseminated 2 month before are examined)</td>
</tr>
<tr>
<td>Bei da gang No.2</td>
<td>Pellet 70% Straw 30%</td>
<td>On and after the 45th day of calving</td>
<td>2-3 months</td>
<td>on the 23rd day of each month</td>
<td></td>
</tr>
<tr>
<td>Hong qi</td>
<td>Pellet 20% Straw 80%</td>
<td>On and after the 45th day of calving</td>
<td>25th day of calving (50% recovered)</td>
<td>≥ 60-90 days</td>
<td>on the 28th day of each month</td>
</tr>
<tr>
<td>Huang zhuang</td>
<td>Mostly straw</td>
<td>On and after the 45th day of calving</td>
<td>40th day of calving (10%)</td>
<td>≥ 45 days</td>
<td>once every 2-3 days</td>
</tr>
<tr>
<td>Pan zhuang</td>
<td>Pellet 100%</td>
<td>On and after the 60th day of calving</td>
<td>15th to 20th day of calving (30%)</td>
<td>≥ 60-90 days</td>
<td>on the 29-30th day of each month</td>
</tr>
<tr>
<td>Li zhi hu</td>
<td>Straw 100%</td>
<td>On and after the 45th day of calving</td>
<td>30th day of calving (30%)</td>
<td>Rectal palpation is performed on the 15th day of calving</td>
<td>≥ 60 days</td>
</tr>
<tr>
<td>Xing lin</td>
<td>Pellet 100%</td>
<td></td>
<td></td>
<td></td>
<td>Strain method will be used from December 1993</td>
</tr>
</tbody>
</table>
Pellet semen was used throughout China. This method involved thawing frozen pellet semen in a test tube with distilled water and transferring thawed semen into the uterus with a glass pipette. This pellet method is advantageous in that special equipment or expendables are not necessary in producing and using frozen semen. The straw method requires straw tubes, a straw printer, artificial insemination equipment, sheath covers and so forth. Thus, the straw method is not suitable for remote regions.

The pellet method also has some disadvantages. One of them is the identification problem. There is a possibility of making a mistake because marking each pellet is not feasible. Another is the hygiene problem. Various instruments including test tubes and glass pipettes must be thoroughly sterilized and handled hygienically. Ensuring that sufficient care is taken by inseminators as to this is difficult.

Considering the need for frozen semen in the country, the national artificial insemination center had to focus on the production and distribution of pellet semen. Thus, a considerable amount of pellet semen was produced from select bulls at large artificial insemination centers in Beijing, Harbin, Shanghai, Nanjing, etc. Pellet semen was preferable to straw semen especially for popular bulls. Thus, dairy farms that were using straw semen had to purchase certain proportions of pellet semen to ensure a sufficient quantity of semen taken from quality bulls. Under these circumstances, the pellet method was not replaced completely by the straw method. A full changeover to the straw method was possible on some advanced dairy farms that abandoned the policy to rely on domestic bulls and recognized the benefits of using imported frozen straw semen.

ii. Veterinarian’s license and inseminator’s license

From 2 to 4 veterinarians and inseminators are stationed at each dairy farm according to the herd size and are engaged in hygiene and reproduction.

Although it is not a state examination system, the country has a similar qualification system for veterinarians. Graduates of veterinary universities are qualified as “veterinary technicians.” After about 10 years of experience of veterinary work, they will be qualified as “assistant veterinarians” and more than 10 years later as “veterinarians.” They may be qualified as “senior veterinarians” if they achieve prominent performance or are ranked high in society.

There is no qualification system for inseminators and the staff members who are engaged in artificial insemination tasks on dairy farms are virtually regarded as inseminators. Inseminators learn skills through their daily work as a workman does. Young staff members who are supposedly on probation are often seen working with inseminators on dairy farms. With a somewhat nervous look, these young staff members are working according to the directions of senior inseminators. Inseminators are trained under these circumstances and this creates an atmosphere that allows no one to interfere.

iii. The role of inseminators on dairy farms

Work on dairy farms is divided into several specific fields in China and some of them are out of the reach of veterinarians. Inseminators are responsible for the
period from calving to pregnancy diagnosis and veterinarians are responsible for
the gestation period. Veterinarians assist in calving in the case of dystocia but
inseminators treat retention of placenta. Inseminators are in charge of all the
important parts of reproduction such as the treatment of reproductive disorders,
artificial insemination and pregnancy diagnosis.

Most inseminators have not received education in technical fields related to
veterinary science or animal husbandry and are engaged in work based on years of
experience and ideas. Young inseminators are not experienced enough to
distinguish between correct and incorrect determinations. They do as directed by
senior inseminators. The situation is common to all dairy farms and reproduction-
related tasks are all left to senior inseminators rather than veterinarians.
Inseminators take pride in their work and resist incorporating new knowledge and
skills. They are confident that the methods they employ are correct regarding
reproductive examination, treatment of reproductive disorders, selection of bulls,
artificial insemination and pregnancy diagnosis. Even the heads of dairy farms
hesitate to interfere. The workplace of inseminators is their sacred place on dairy
farms.

Reproduction conditions

Reproduction conditions vary from farm to farm as shown in Table 5-13 (p.171).
The Livestock Department of the Farm Administration Bureau of Tianjin aimed at
achieving a calving interval of less than 400 days and 9 dairy farms attained this
goal at the time. One of them achieved "one calving per year." The longest
calving interval recorded was 461 days. Some improvements in feeding and
management may increase the number of farms that attain the goal. The average
calving interval of all the dairy farms was 401.4 days so an average calving interval
of less than 400 days seems attainable.

Examinations for pregnancy diagnosis are performed once monthly in most dairy
farms, as shown in Table 5-14 (p.172), but this may delay the detection of sterile
cows. Increasing the frequency to at least once a week should lead to early
detection of sterile cows as well as early treatment of reproductive disorders and
hence to shortened calving intervals. Examinations for pregnancy diagnosis are
performed no earlier than the 60th day of artificial insemination simply following
tradition with no scientific basis.

A surprising fact was that information about reproduction such as artificial
insemination and calving was well documented on every dairy farm. Upon
request, information about the number of times of artificial insemination, the types
of bull and calving history including abortion and premature birth were readily
searchable because such information was recorded in data books maintained by
inseminators. Personal computers were not available at the time so this was the
result of their steady and continuous efforts. In this respect, their strong
commitment to their work impresses everyone.
ii. Actual status of reproductive disorders

Table 5-15 shows reproductive diseases in cattle by diagnosis. Overall, uterine diseases accounted for 70% and ovarian diseases 30%. Mucometra and endometritis accounted for most of the uterine diseases and luteal hypoplasia accounted for most of the ovarian diseases. Of relatively intractable reproductive diseases, pyometra accounted for 1.8% and follicular cysts 1.4%. These data indicate that more than 90% of the reproductive diseases observed are not serious so that most cows considered to have reproductive disorders can give birth and lactate if they are treated appropriately by reproductive technical experts who are skilled in diagnosing and treating cows with reproductive disorders.

Table 5-15 Examination results of cows with reproductive disorders
13 dairy farms (March 1993-July 1994)

<table>
<thead>
<tr>
<th>Name of diagnosis</th>
<th>Number of cows</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine diseases</td>
<td>434</td>
<td>69.1%</td>
</tr>
<tr>
<td>Mucometra</td>
<td>218</td>
<td>34.7</td>
</tr>
<tr>
<td>Endometritis</td>
<td>203</td>
<td>32.3</td>
</tr>
<tr>
<td>Pyometra</td>
<td>11</td>
<td>1.8</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Ovarian diseases</td>
<td>195</td>
<td>30.9%</td>
</tr>
<tr>
<td>Luteal hypoplasia (cystic corpus luteum)</td>
<td>171</td>
<td>27.2</td>
</tr>
<tr>
<td>Retained corpus luteum</td>
<td>9</td>
<td>1.4</td>
</tr>
<tr>
<td>Follicular cyst</td>
<td>9</td>
<td>1.4</td>
</tr>
<tr>
<td>Ovarian dysfunction</td>
<td>4</td>
<td>0.6</td>
</tr>
<tr>
<td>Others</td>
<td>2</td>
<td>0.3</td>
</tr>
<tr>
<td>Number of cows examined</td>
<td>629</td>
<td></td>
</tr>
</tbody>
</table>

Inseminators stock medicine in their drug cabinets. Dairy farms seem to set aside a portion of the budget to purchase medicine so medicine is rarely out of stock except expensive hormone preparations. An interesting fact was that Chinese medicines were stocked such as internal medicine for retention of placenta and medicine for intrauterine administration.

iii. Current status of treatment of cows with reproductive disorders

The Health and Breeding Division of the Tianjin Dairy Cattle Health Center, which is under the control of the Farm Administration Bureau in Tianjin, is responsible for the hygienic and reproduction affairs of dairy farms. The staff of the Division are supposed to visit every dairy farm periodically for guidance. However, visiting all the 21 dairy farms scattered over a wide area is not possible for the staff consisting only of 3 young technical personnel (2 assistant veterinarians and 1 veterinary technical expert) however industrious they are.

Actually, inseminators of each dairy farm are engaged in treatment of reproductive disorders and the guidance from the staff of the Dairy Cattle Health Center does not seem to play a critical role. If the young staff of the Dairy Cattle Health Center visit old dairy farms, inseminators of the farms are doing nothing but seeing how
well they do. Inseminators do not seem to be trying to learn new knowledge and skills. To establish new techniques as standards in the field, the Livestock Department of the Farm Administration Bureau of Tianjin held a seminar on the control of reproductive disorders at the Tianjin Dairy Cattle Health Center.

iv. Seminar on the control of reproductive disorders

In October 1993, a 3-day seminar on the control of reproductive disorders was held at the Tianjin Dairy Cattle Health Center. The exceptionally large-scale seminar was cosponsored by the Livestock Department of the Farm Administration Bureau of Tianjin and JICA and was intended for reproductive technical experts from all the dairy farms in Tianjin.

Although anyone engaged in reproduction-related tasks on dairy farms was eligible to be enrolled in, the seminar was intended mainly for young inseminators and probationary inseminators and had an attendance of more than 30.

Experts from JICA and the technical staff of the Health and Breeding Department of the Tianjin Dairy Cattle Health Center served as lecturers and provided guidance in reproductive anatomy, reproductive physiology, rectal palpation, recording of uterine and ovarian findings, artificial insemination and early pregnancy diagnosis. Some reproductive organs (uterus and ovary) were used for practical training in recording palpation findings. Textbooks in Chinese were prepared beforehand. There was a lively discussion after the seminar. Although the young trainees were obedient to seniors on the dairy farms, they showed another youthful and lively look at the seminar.

Seminars were held twice a year from that time onwards and started to serve as a location for exchange among young reproductive technical experts. The seminars produced better results than were initially expected because new winds were infused into the dairy farms.

With the project over, counterparts are now in a higher position but are still engaged in reproduction-related work while guiding junior staff members.
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## Abbreviation list

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>English</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic Hormone</td>
</tr>
<tr>
<td>APG</td>
<td>Anterior Pituitary Gonadotropin</td>
</tr>
<tr>
<td>AU</td>
<td>Armour Unit</td>
</tr>
<tr>
<td>CCL</td>
<td>Cystic Corpus Luteum</td>
</tr>
<tr>
<td>CIDR</td>
<td>Controlled Internal Drug Release</td>
</tr>
<tr>
<td>CRH</td>
<td>Corticotropin Releasing Hormone</td>
</tr>
<tr>
<td>E</td>
<td>Estrogen</td>
</tr>
<tr>
<td>E₁</td>
<td>Estrone</td>
</tr>
<tr>
<td>E₂</td>
<td>Estradiol</td>
</tr>
<tr>
<td>E₃</td>
<td>Estriol</td>
</tr>
<tr>
<td>E₃S</td>
<td>Estrone Sulfate</td>
</tr>
<tr>
<td>eCG</td>
<td>equine Chorionic Gonadotropin</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal Growth Factor</td>
</tr>
<tr>
<td>EIA</td>
<td>Enzyme Immunoassay</td>
</tr>
<tr>
<td>ES Cell</td>
<td>Embryonic Stem Cell</td>
</tr>
<tr>
<td>FC</td>
<td>Follicular Cyst</td>
</tr>
<tr>
<td>GnRH</td>
<td>Gonadotropin Releasing Hormone</td>
</tr>
<tr>
<td>GTH</td>
<td>Gonadotropic Hormone</td>
</tr>
<tr>
<td>hCG</td>
<td>human Chorionic Gonadotropin</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like Growth Factor</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin-1</td>
</tr>
<tr>
<td>IU</td>
<td>International Unit</td>
</tr>
<tr>
<td>LC</td>
<td>Luteal Cyst</td>
</tr>
<tr>
<td>LH</td>
<td>Luteinizing Hormone</td>
</tr>
<tr>
<td>NGF</td>
<td>Neuro Growth Factor</td>
</tr>
<tr>
<td>OT</td>
<td>Oxytocin</td>
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