Reproductive Technology for Dairy Cattle

Contents

1. Anatomy of Reproductive Tract

Fig.1 shows the diagram sketch of reproductive tract of cow. Although this illustration was a simplified one, before somone starts to inseminate, to make rectal examination, or to treat the reproductive disorders, the anatomy of reproductive tract should be well understood. For example, when you pass some instrument thorough the vagina, you should direct the device upperward. If not, downwardly directed device could be inserted into the bladder or the blind pouch (suburethral diverticulum). – this is also important when you collect the urine using a catheter.

Fig.1 Diagrammatic sketch of the reproductive tract

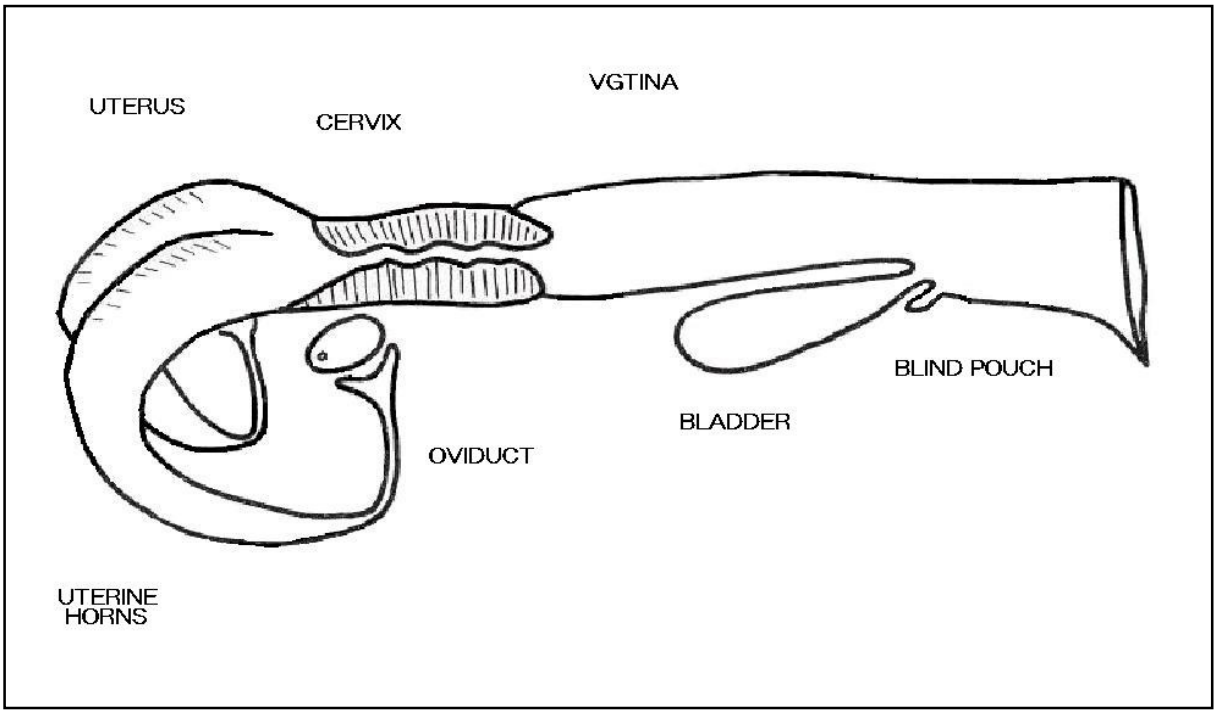
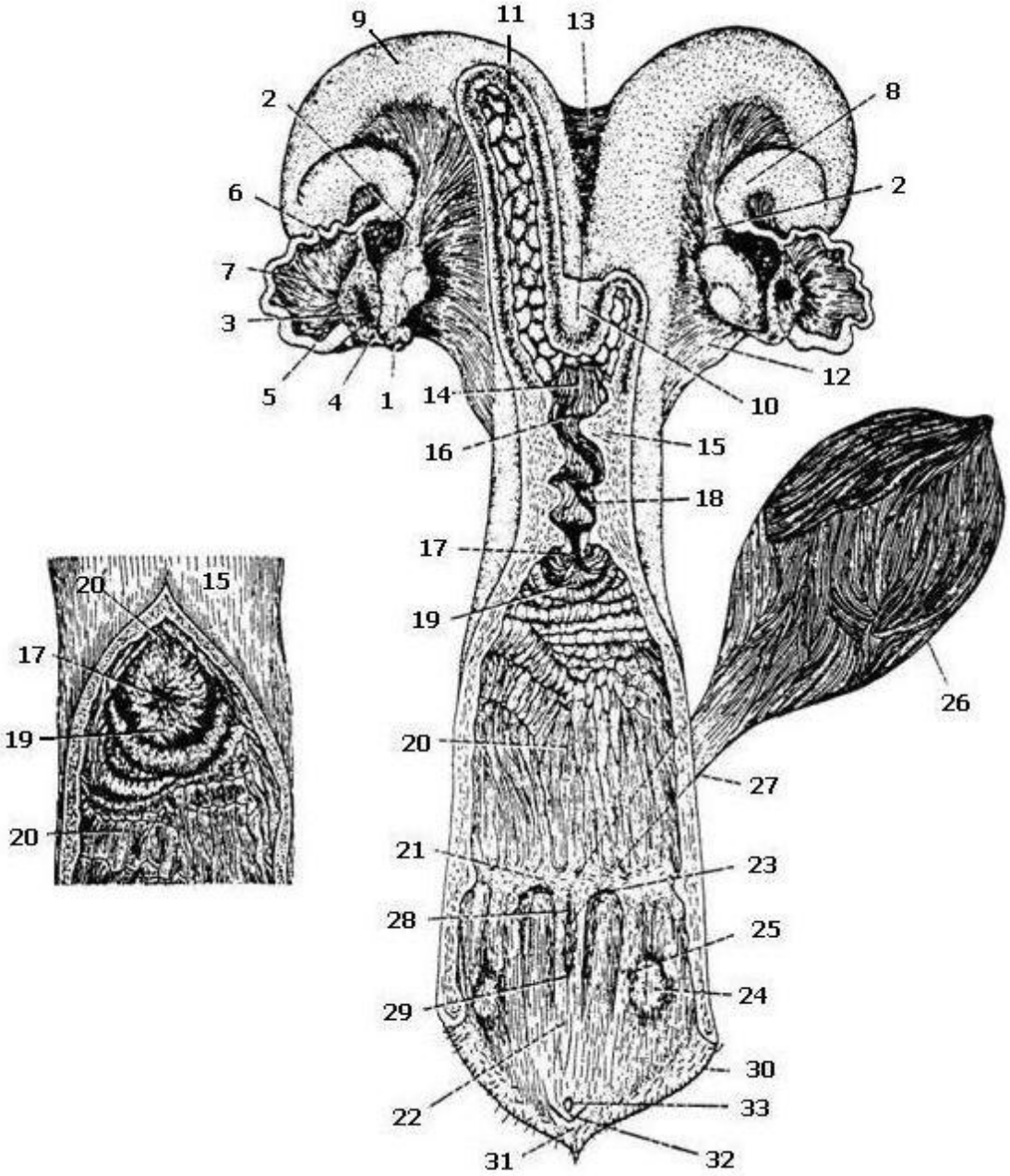


Fig. 2 shows more detailed anatomy of the uterus and ovary. However, the condition/size of these organ will dramatically change depending on the estrus cycle, gestation, parturition, nutrution etc. Therefore , it is important not only to know the anatomy but also to know each cow's condition as well.

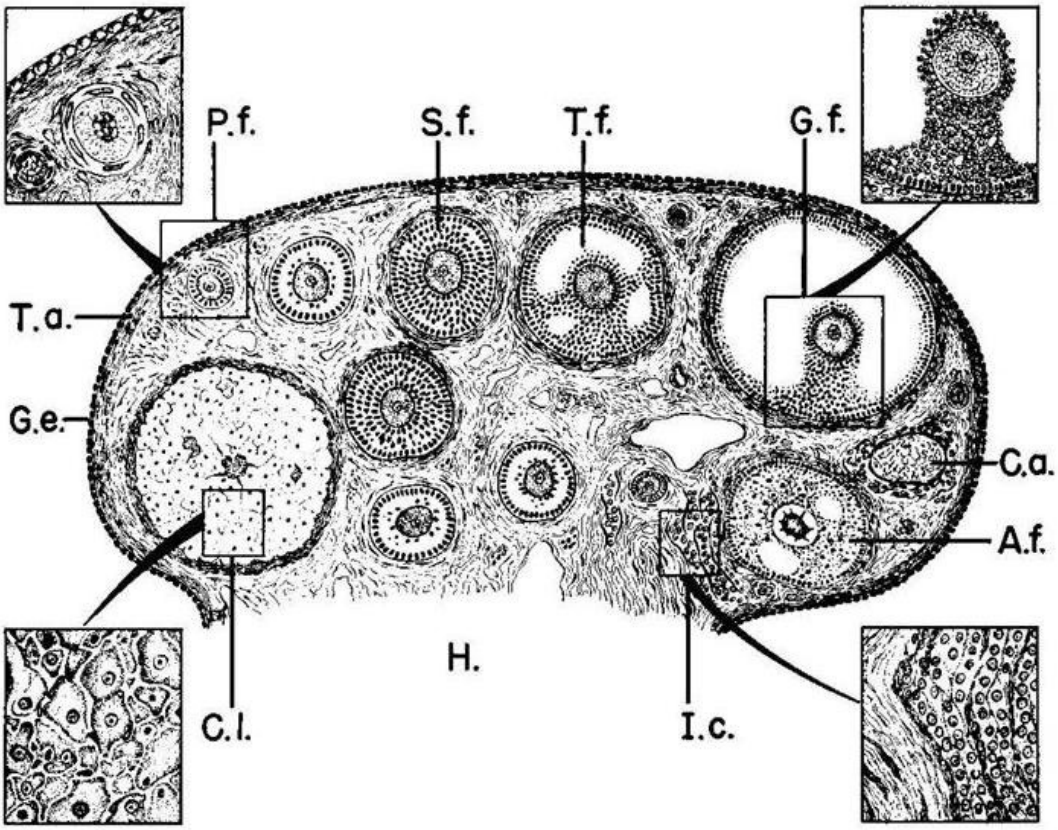
Fig.2 Anatomy of Reproductive Tracts in Cattle



1: Ovary
2: *Ligamentum Ovarii Proprium*
3: Abdominal orifice of uterine tube
4: *Fimbria ovarica*
5: Ampulla of uterine tube
6: Ithmus of uterine tube
7: Mesosalpinx
8: anterior end of uterine horn
17: *Ostium uteri externum*
18: Uterine cervical canal
19: *Portio vaginalis cervicis*
20: Vagina, 20': Vault of vagina
21: Hymenal rudiment
22: Vaginal vestibule
23: *Ductus epoophori longitudinalis*
24,25: *Grandulae vestibulares majores*

9: Uterine horn, Uterine cavity
10: *Velum uteri*
11: Caruncle
12: Mesometrium
13: *Ligamentum intercornuale*
14: Uterine body
15: Uterine cervix
16: *Ostium uteri internum*
26: Bladder
27: Urethra
28: External urethral orifice
29: *Suburethral diverticulum*
30: Pudendal lip
31: *Commisura labiorum ventralis*
32: *Glans clitoridis*
33: *Grandulae vestibulares minores*

Fig.3 Anatomy of Ovary



A.f., Atretic follicle; *C.a.*, corpus albicans; *C.l.*, corpus luteum; *G.e.*, germinal epithelium; *G.f.*, graafian follicle; *H*, hilus; *I.c.*, interstitial cells; *P.f.*, primary follicle; *S.f.*, secondary follicle; *T.a.*, tunica albuginea; *T.f.*, tertiary follicle. (Partly adapted from Turner, (1948) General Endocrinology. Philadelphia, W. B. Saunders Co.)

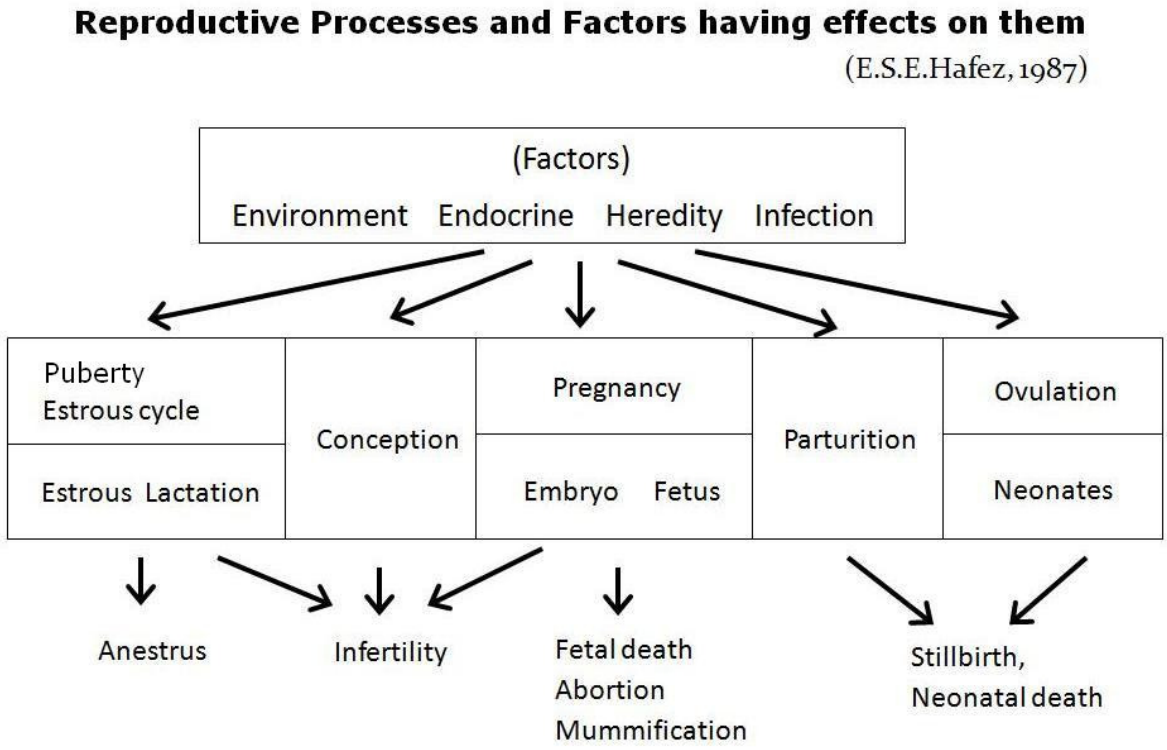
The important structures in the ovary are “follicles” and “corpus luteum” . Both will change their conditions according to the estrous cycle. Especially there are many and variable developmental stages of the follicles co-exist. The important thing to keep in mind is the ovary’s features change due to the estrus cycle. The details will be discussed in the chapter, ”Reproductive Phisiology”.

*Follicle development : Primordial follicle ⇒ Primary follicle ⇒Secondary follicle ⇒

Tertiary follicle $\Rightarrow$ Graafian follicle

2. Basic Reproductive Phisiology

Fig.4.



Before learning the reproductive phisiology, it is important to know that there are many steps and many affecting factors in the reproduction.

Of course, the final objective of the reproduction is "to obtain a healthy calf". However the reproductive processes consist of many factors as shown in Fig.3. This figure shows that several factors such as Environment, Endocrine, Herdity & Infection affect the whole processes of the reproduction.

Environment includes inner environment such as Nutrition. Also there are some correlations between the factors.

2-1. Puberty

Puberty is defined as the process/time in which the young female become sexually maturated and capable of reproduction. In case of cattle, the onset of the first ovulation is considered as the time of puberty. Well-grown Holstein heifer will shows puberty 10-12 months of age. However the time for the first insemination should be decided according to their body growth. Too early (young) pregnancy will cause distocia at the time of delivery, because of the narowness of the birth canal.

In Japan the recommended standards for the first insemination is body weight-350kg in pure Holstein. If the heifer reached this body weight at 15-month age and was pregnant, we can respect the first delivery at 2-year (24 months) of age.

2-2. Hormonal Control

Fig.5 Schematic of stages of the estrous cycle, serum progesterone concentrations, and serum luteinizing hormone (LH) concentrations.

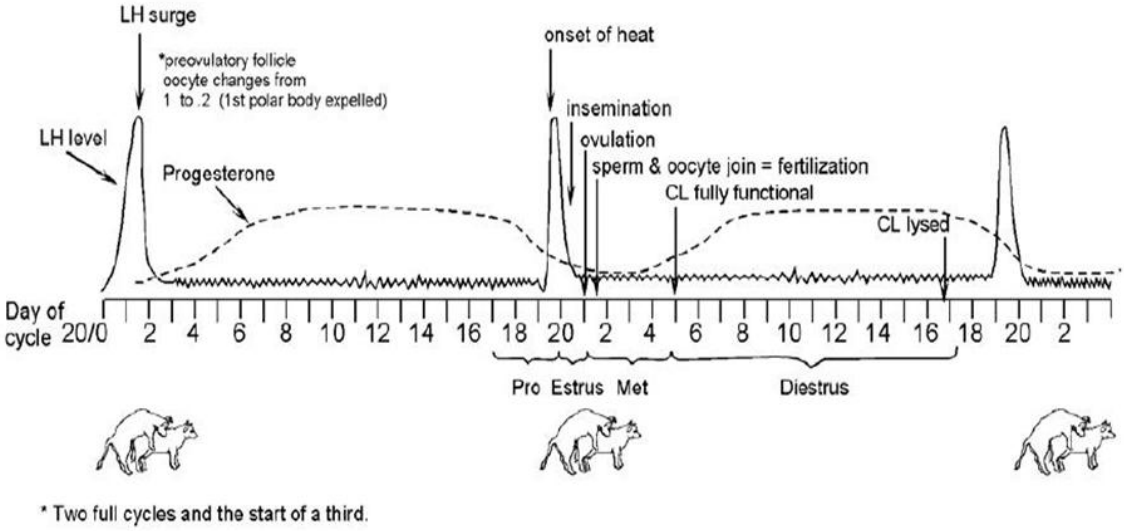
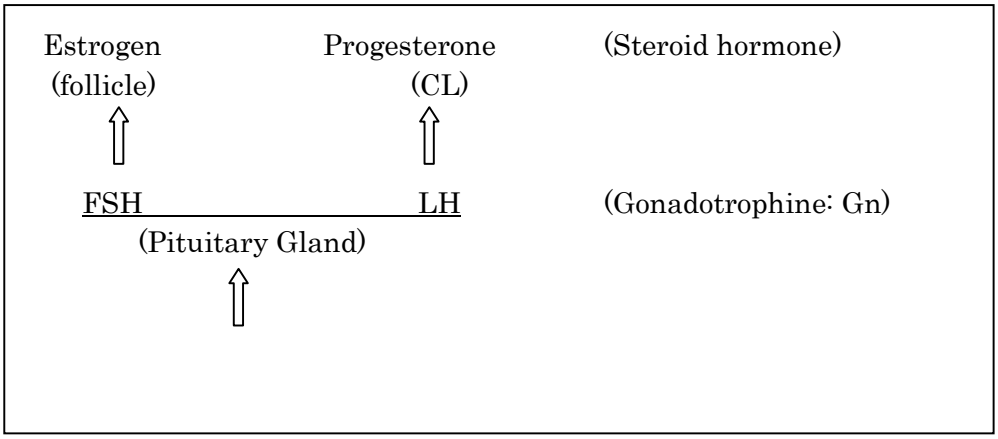


Fig. 5 is a schematic explanation of estrous cycle stage, serum levels of progesterone and LH, which includes two full cycles and the start of a third. The stages of the estrous cycle include Pro-estrus, Estrus, Met-estrus and Di-estrus. Corpus Luteum (CL) is functional during Day5* to Day17, and the progesterone level is higher at this period. LH surge is essential for the developed follicle to ovulate. Before the ovulation LH level shows a transient high peak like this.

* When describing the days of cycle, Day5 means 5 days after estrus. Day0 = the day of estrus

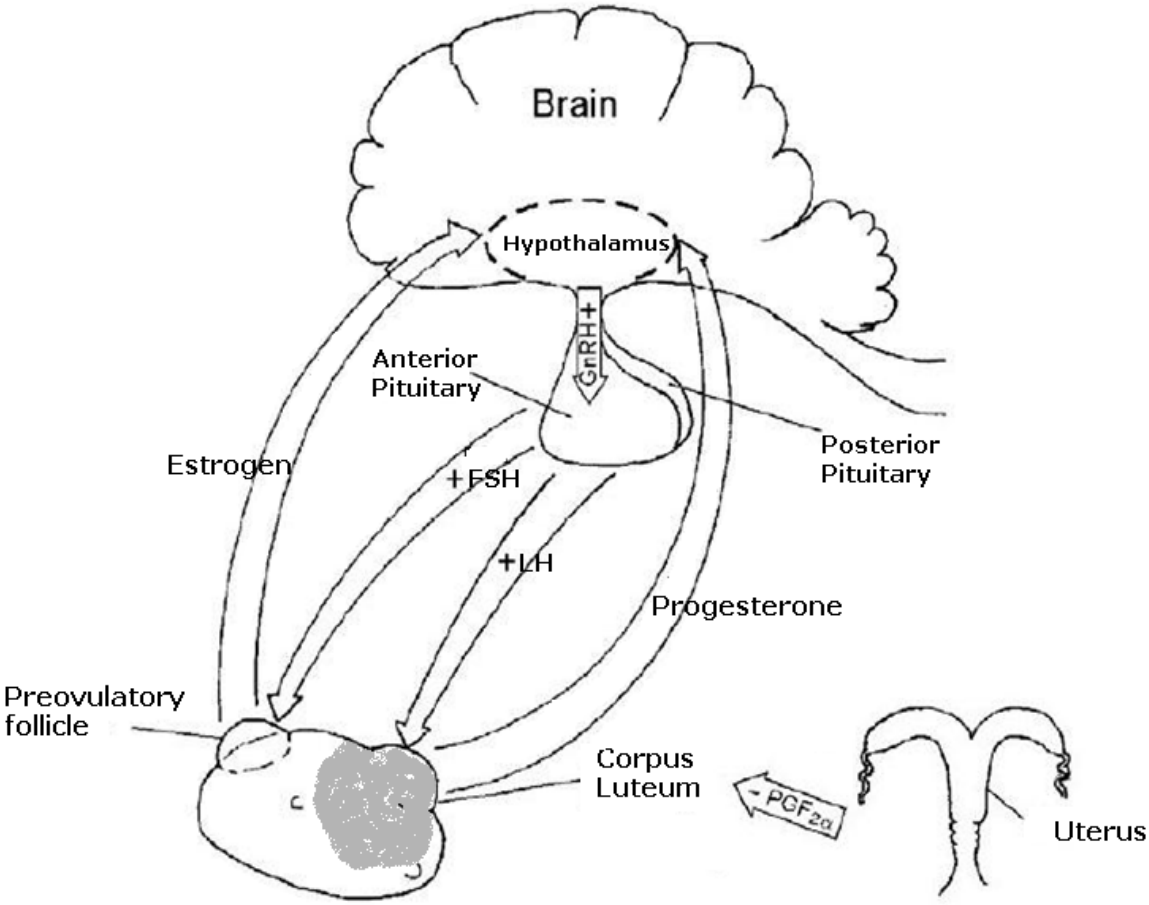
(Hormonal Control of Estrus Cycle)



GnRH (Gn Releasing Hormone)
(Hypothalamus)

The estrous cycle is controlled by hormones, and it is 3-step control like above.

Fig.6 Interaction of hypothalmic, anterior pituitary, ovarian, and uterine hormones on the control of reproduction



The relation of this 3-step control is shown as Fig.6. In this figure, another hormone Prostaglandin F2α (PGF2α) is added. PGF2α is produced in uterine endometrium and have a important role for regressing CL.

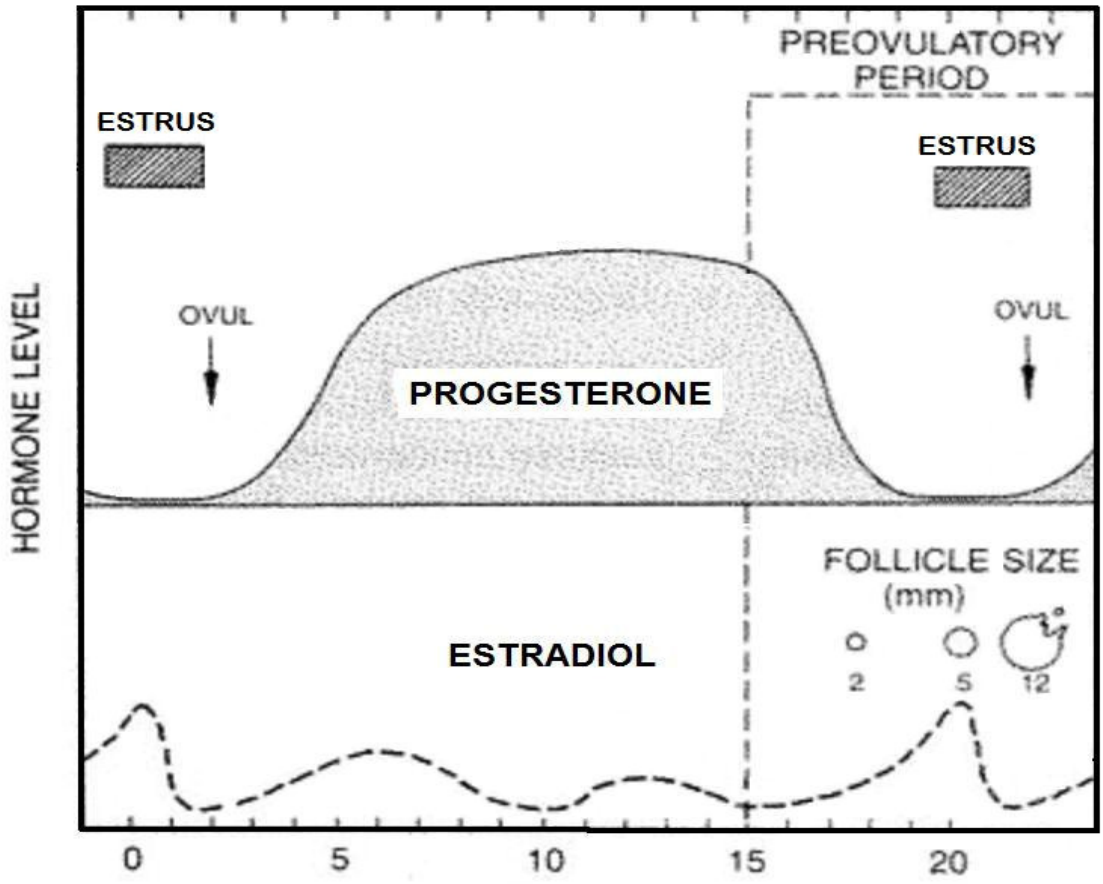
Another important point is “negative feedback” of Estrogen and Progesterone. Note that the arrows of Estrogen and Progesterone are directed to Hypothalamus, which means that the

information about these hormone's level is sent to Hypothalamus, and when these hormones become too high Hypothalamus will reduce the secretion level of GnRH.

2-3 Estrus Cycle

At first we have to know the functions of the steroid hormones, Estrogen and Progesterone. Because these 2 hormones are changed according to the estrius cycle and have direct effects to reproductive organs and the female's sexual behavior.

Fig.7 The levels of Estrogen and Progesterone during estrous cycle

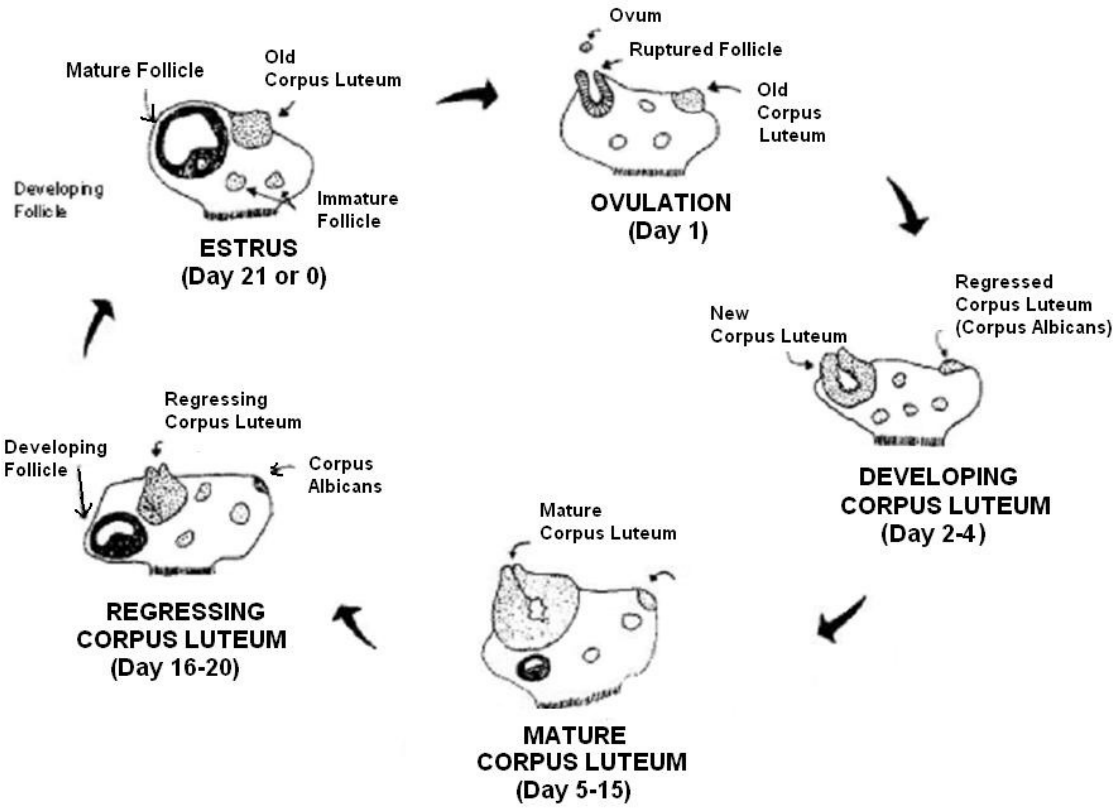


During the estrus cycle, Progesterone level shows dramatic decrease around the time of estrus, meanwhile Estrogen level shows wavy changes. This wavy changes is because of the "Follicular Wave", which will be explained later.

The stage of the estrus cycle is usually divided to 4 stages like below.

Stage	Cycle day	Duration	Events
Estrus	0	10-12 hr.	Mature follicle high levels estrogen LH surge
Metestrus	1-3	5-7 days	Ovulation (w/i 12-18 hrs) formation of CL no response to prostaglandin
Diestrus	5-18	10-15 days	Mature corpus luteum high levels progesterone
Proestrus	19-21	3 days	CL regressing maturing follicle rising estrogen

Fig.8 Schematic changes of the ovary during the estrus cycle



2-4 Follicular Wave

Recent development of the ultrasonographic machine made it possible to examine the follicular development inside the ovary more precisely. As a result we could know the existence of “Follicular Wave” in many animals. **(Fig.9 Ultrasonographic Machine)**

Ultrasonographic Diagnostic Machine such as shown in the right, can give us a cross-sectioned real-timed image of any organs.

In the field of reproductive physiology research, very accurate diagnosis of reproductive tracts become possible, such as pregnancy diagnosis, CL formation and follicular development.

Fig. 10 Ultrasonographic Image of Ovary

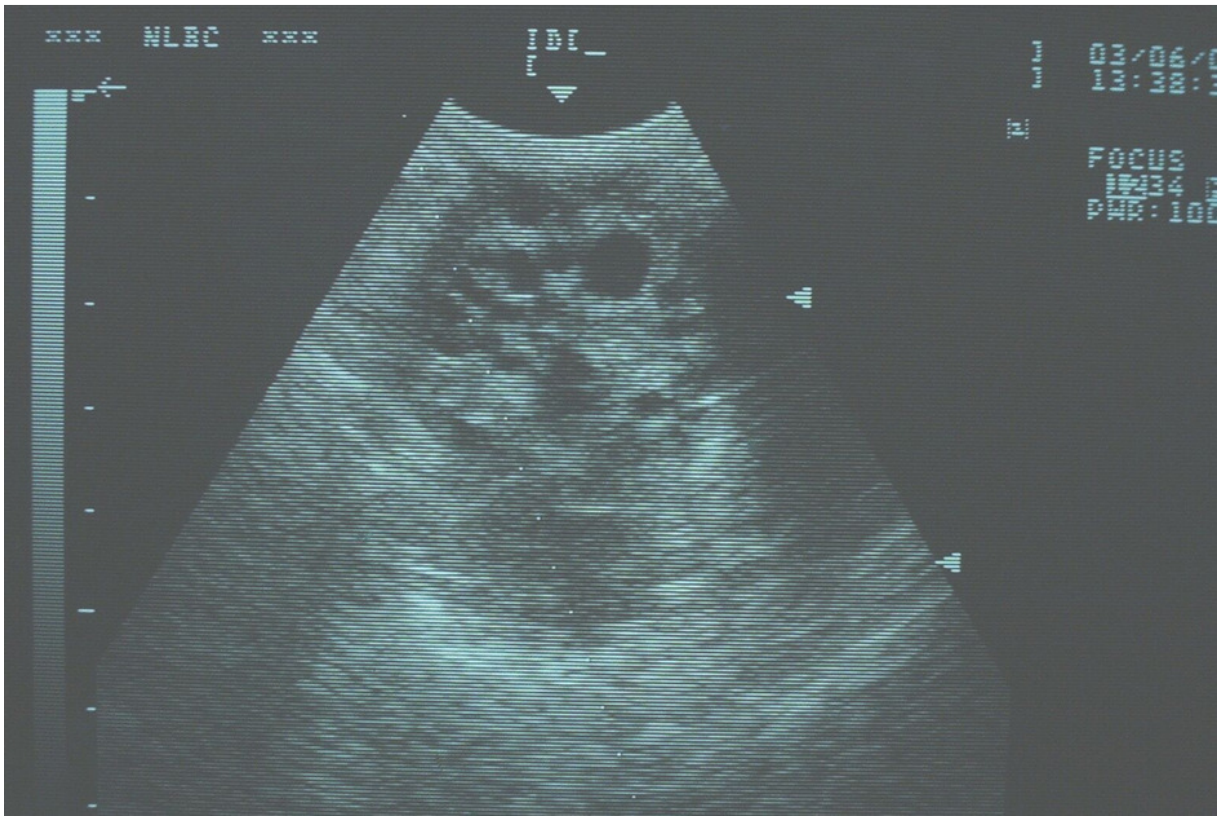
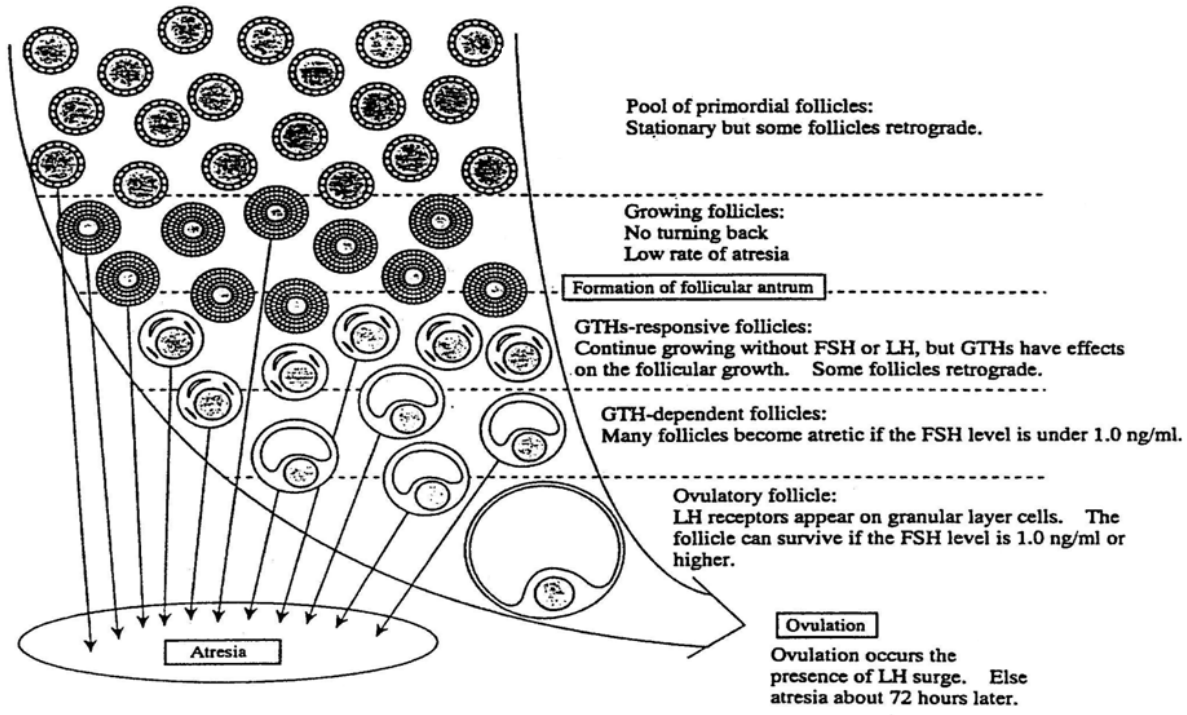


Fig.10 shows one example of the ultrasonographic image of cattle ovary. We can notice that there are many black circles inside the ovary. These circles are "Follicles". (Because the ultrasonic wave can pass through the fluid, any fluid-containing parts look black. Meanwhile hard tissues such as bone look white.). Also we can know the exact size of the organ by left-side scale or a measuring function usually available in most of the machine.

Fig.11 shows "Model of Follicular Growth in Sheep", though there is no difference from cattle in this aspect. When female animals are born, their ovaries contains several hundred thousands of primordial follicles. It is called as "Pool of primordial follicles". The number of these follicle will never increased and only decreased during their life-time. In case of cattle, the number of the ovulating follicle is usually only one (Sometimes there are 2 ovulations, but not so often occur.) but which doesn't mean that only one follicle develope from the start. Actually, a group of follicles starts to develope at first, but most of them will regress (called "atresia"). One of the follicles developed to almost the size of the ovulatory follicle (which is called "Dominant Follicle") but also regress (except the case of one wave). These group-developments of the follicle are repeated usually 2-3 times (rare cases, but some female have 1 or 4 times) like waves. Finally the dominant follicle at the final wave will be the ovulatory follicle. This phenomenon is called "Follicular Wave".

It is very difficult to defined the waves in each female without the ultrasonographic machine and continuous examinations (at least once per 2 days cheking are necessary). Also each female shows different wave-type in each estrus cycle, which means there are is no 2-wave or 3-wave female. But with the knowledge about the "Follicular Wave", we can avoid the mistake in diagnosing the ovary's condition at rectal palpation. For example, before we know the exsistence of the follicular wave, we tended to consider that the coexistence of a large follicle and mature corpus luteum (CL) means something abnormal and maybe the function of CL is not enough. However the coexistence is definitely normal condition.

Fig. 11 Model of Follicular Growth in Sheep



Model of Follicular Growth in Sheep

Sensitivity of and dependency on gonadotropic hormones vary with the growth of ovarian follicles. (Scaramuzzi *et al.*, 1993; after slight modification)

The below is a summariation of "Follicular Wave". In each normal estrus cycle, the following phenomenon occurs after the ovulation.

Grouped development of small follicles

↓

One follicle is selected and become dominant

↓

Dominant follicle supresses small follicles' development

↓

Dominant follicle loses the dominancy

↓

(repeat)

Fig.12 The size-changes of largest and second-large follicle and corpus luteum during the estrous cycle.

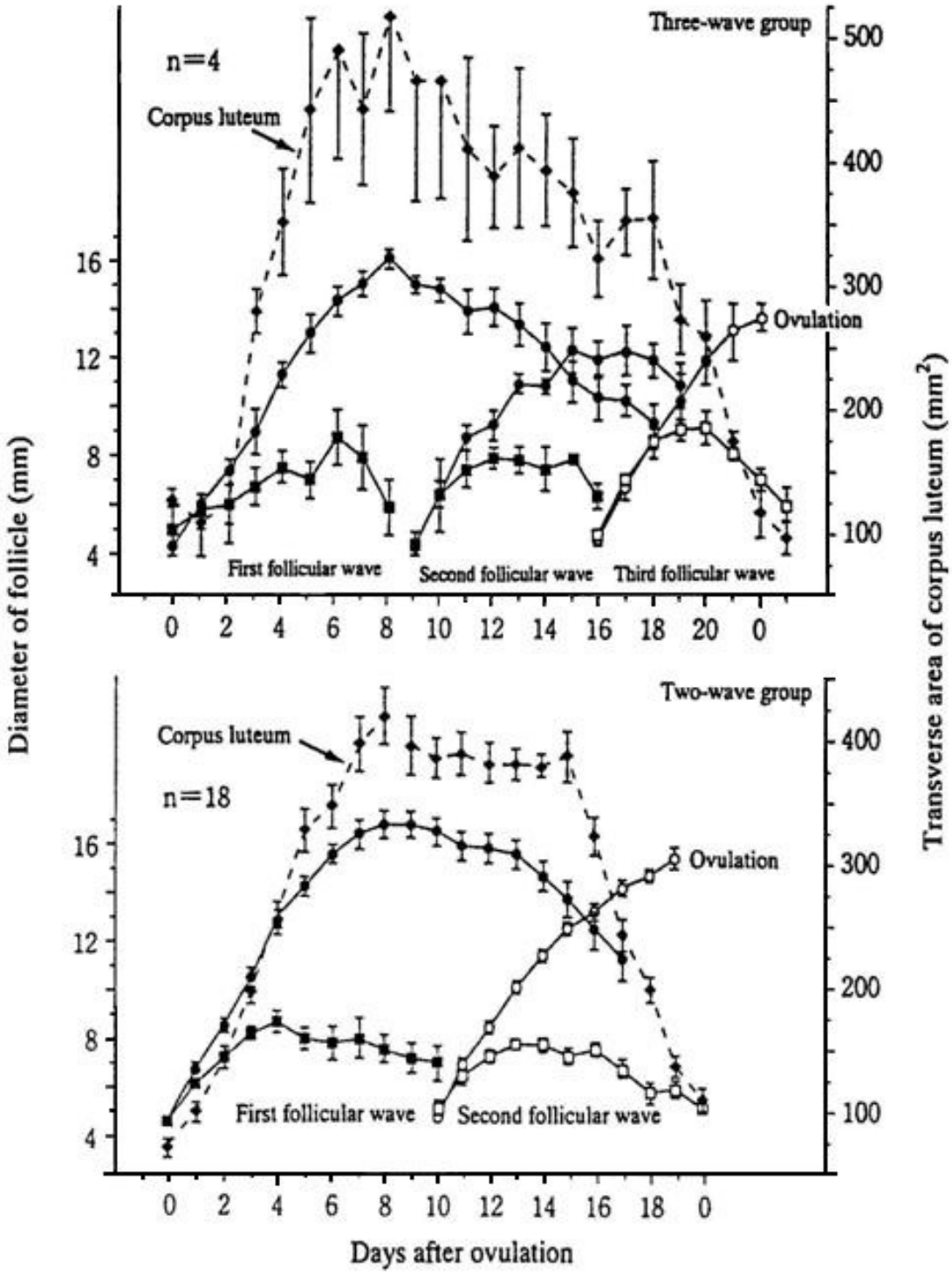


Fig.12 is showing the size-changes of largest and second-large follicle and corpus luteum during the estrous cycle of 2- and 3-wave cow.

According to the graph, we can know the following points about the follicular wave.

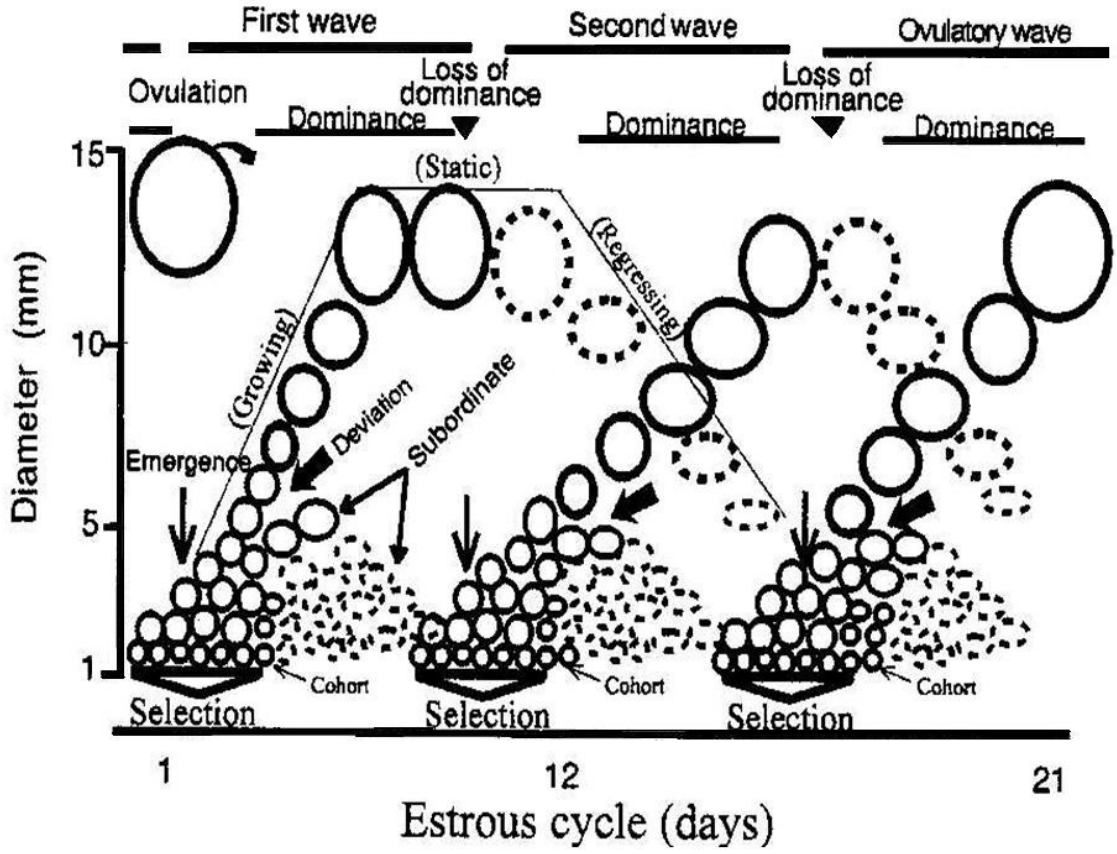
* The estrus cycle length is longer in 3-wave (21-22 days) than 2-wave (18-19 days).

* The dominant follicle of the last wave (2nd wave in 2-wave, and 3rd wave in 3-wave) will be the ovulatory follicle.

* The length of the first follicular wave is not different in both wave type. But we can notice that the length of the 2nd and 3rd follicular wave in 3 wave type is very short. In another word, the ovulatory follicle in 3-wave type developes very rapidly compared to 2-wave type. For example, supposedly we palpated ovaries of Day 16-18 cow and detected large follicle, if the cow is 2-wave type that follicle might be a ovulatory follicle, but if the cow is 3-wave type, the ovulatory follicle might not yet developed.

* We can know that large follicles (more than 10mm of the diameter, 1 or 2) are always exisist inside the ovaries except just after the ovulation.

Fig.13 Schematic changes of the Follicular Wave (3-wave)



Sometimes we use Prostaglandin F2α (PGF2α) to regress the CL and to induce estrus. This drug is very effective for estrus synchronization. In average 3 days after the injection of PGF2α to the females, they will show estrus. However, actually the day of estrus is very variable, sometimes 2 days, sometimes 5 days. The reason of the variability of the responce is well explained by "Follicular Wave". It depends on the stage of the follicular wave when PGF2α was injected. As shown in Fig.13, in (1) case PGF2α was injected just after the new wave started, and 2-3 days after the injection the estrus was onset. Meanwhile, in (2) case PGF2α was injected when the dominant follicle was static or started to regress, then the estrus was onset 4-5 days after the injection. It takes longer time for the new wave to start.

Fig.14 Relation between Synchronization by PGF2α and Follicular Wave

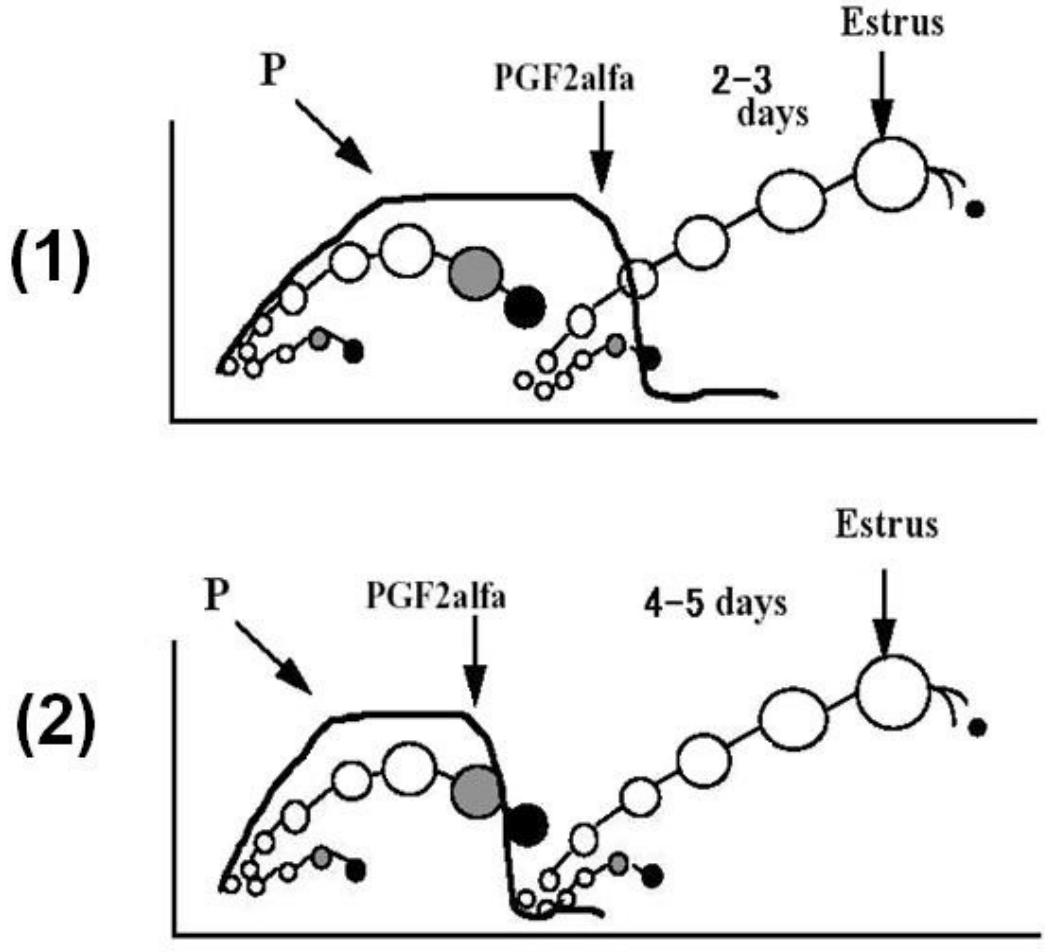
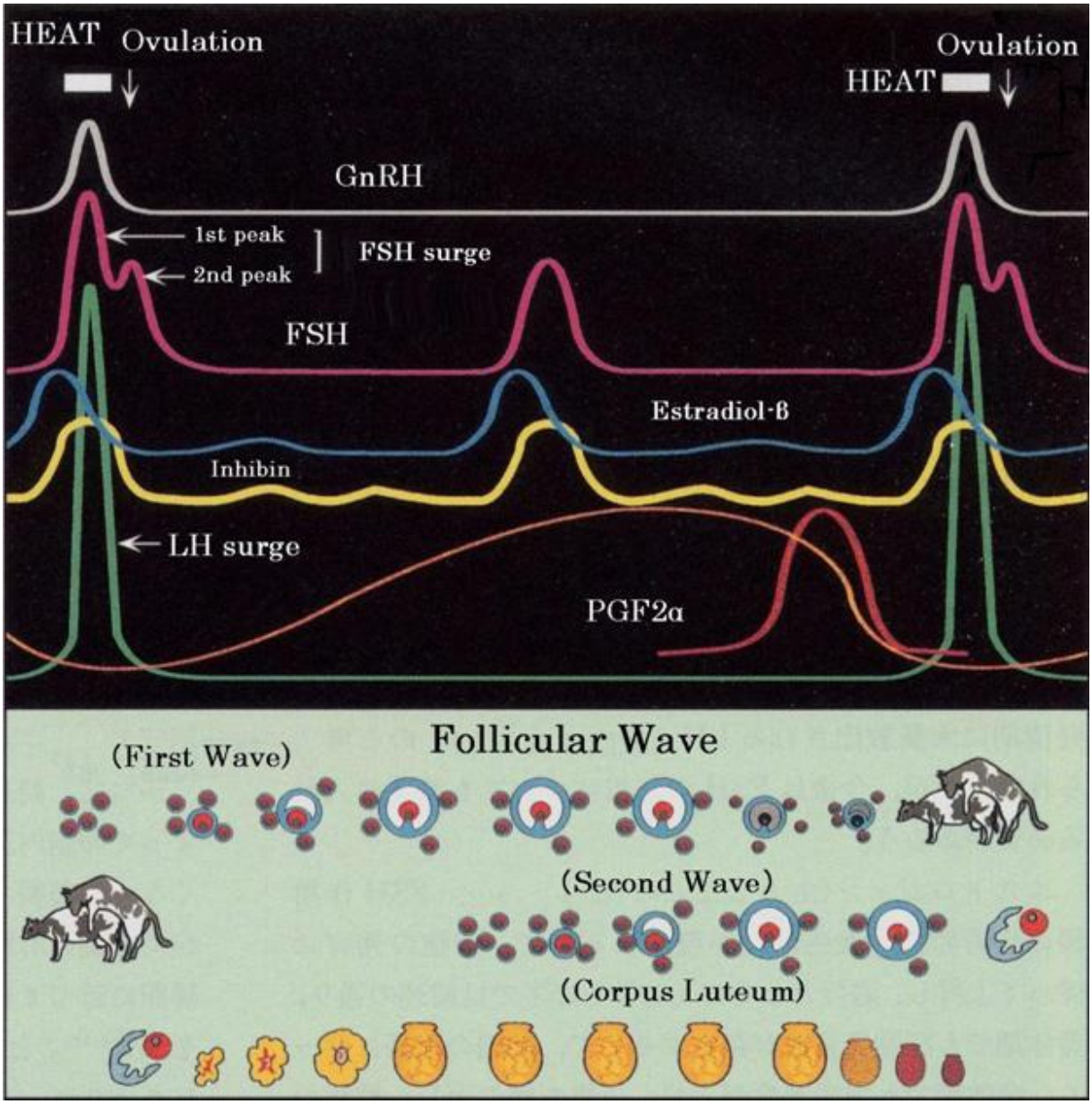


Fig.15 is showing the relation of the follicular wave and sexual hormonal changes in 2-wave type. Notice that there are increases of FSH and estrogen even in the middle of the estrus

cycle (Diestrus stage). This is because of thedevelopment of dominant follicle of the 1st wave.

Fig.15 Follicular Wave and Sexual Hormonal Changes during Bovine Estrus Cycle



2-5 Heat Detection

Heat detection is very important for daily reproductive management and also to define the optimal timing for AI.

In cattle the estrus is defined by the behavior of the female, "Standing Heat", which means that the female stay still when mounted by other female (or teaser bull). However there are many symptoms (signs) implying the estrus. At first we have to know what kind of estrus

signs the females show at estrus, and be careful, these signs will be changed as the estrus going on.

(Changes of Estrus Signs)

6-10 hrs before Estrus

- Get closer to other cows.
- Mounting to other cow.
- The vulva becoming swelled and wet.

At Estrus

- Standing Heat (stay still by other cow's mounting)
- Standing most of the time
- Discharged clear mucous from vagina
- Decreased appetite
- Decreased milk production (lessened Let-Down)
- Increased walking steps (in case of not-tied)
- Opened pupil
- Barking loud

12 hrs after Estrus

(still observed)

- Clear Mucous Discharge
- Swelled Vulva

(but no more behavioral signs)

- ~~Standing Heat~~
- ~~Increased walking~~

"Which Cow is in heat ?"

How to detect Estrus

- Careful observation (at least twice/day)
  more estrus occurs in evening to early morning than daytime.
- Release the cows in paddock
  Always tied cows cannot show "STANDING HEAT"
- Devices for detecting Estrus (no use for tied cows)
  Tail Painting
  Heat Marker
  Step Counter etc.

To detect the estrus, careful observation is necessary and at least twice/daily observation is necessary. Keep in mind that more estrus can be seen in evening to early morning than daytime. Because "Standing Heat" is a behavior with other cattle, if the female is always tied to cow shed, it is impossible to detect "Standing Heat". It is recommended to release the female to a paddock area in a certain time in the day. Otherwise, we have to rely on other signs such as clear mucus, swollen vulva, decrease of appetite/milk production etc.

Also, we have to keep in mind that some factors will affect the detection of estrus like below.

(Factors Affecting Estrus Behavior)

* Influence of Herdmates
* Environmental Temperature
* Footing Surface
* Foot and Leg Problems
* Nutrition and Level of Milk Production

When other cows are near heat, a cow in heat will be influenced by these other cows and shows clearer estrus signs. In case a male calf is in the herd, he will be a good detector of the estrus.

In hot time cows seldom show clear estrus. If cows are on the concrete floor, the cow's estrus behavior is less compared to on the ground or the bedded floor.

If females had problems in their hoof, they will seldom show clear estrus. Therefore, hoof management (periodical hoof trimming) is important also for this purpose.

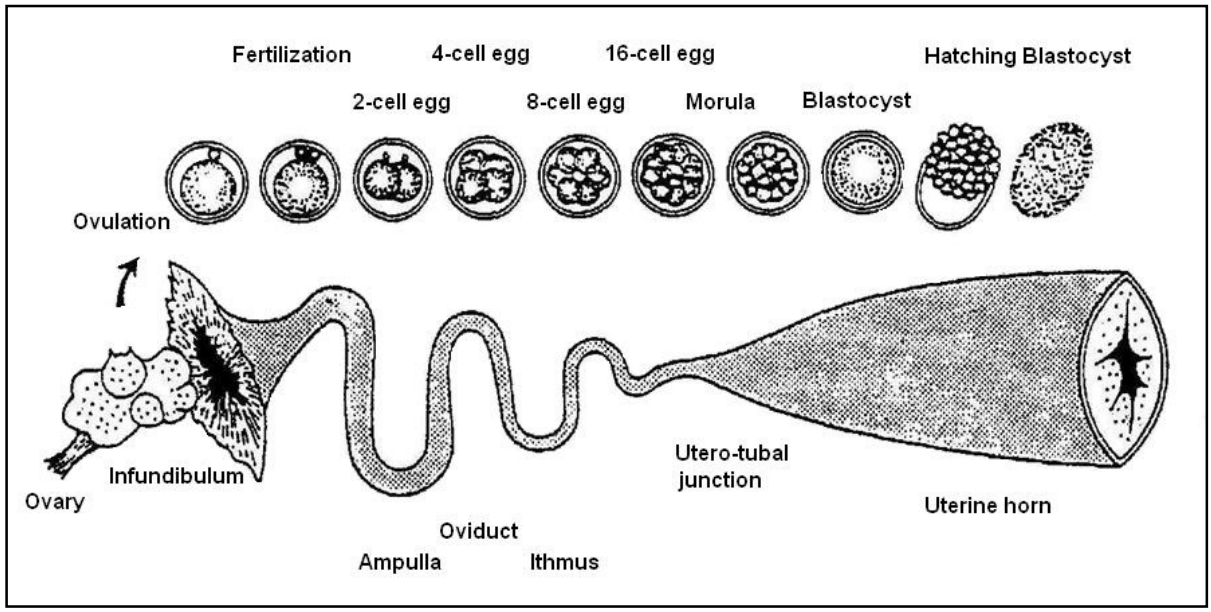
2-6 Fertilization & Embryo Development

After the ovulation, the ovium will be catched by the infundibulum of oiduct and enter into the oviduct. The infundibulum is a funnel-shaped open-end of the oviduct. The end of the infundibulum is called "fimbria of oviduct", a very thin film-like membrane. At the time of ovulation, the fimbria covers the ovary and catchs the ovulated ovium. If rectal palpation is

carried out just before the ovulation, the covering fimbria will be easily removed and the ovium might go into the abdominal cavity (not into the oviduct). Careful attention is necessary when we palpate the ovaries of final stage of estrus (near to ovulation) .

Fig.16 shows that the ovium is fertilized at the ampulla of the oviduct, and the fertilized egg develops moving down the oviduct. About 4-5 days after the fertilization, the embryo will enter into the uterus. Fig.17 shows real pictures of embryo development, though these are In Vitro Fertilized embryos.

Fig.16 Embryo movement and development



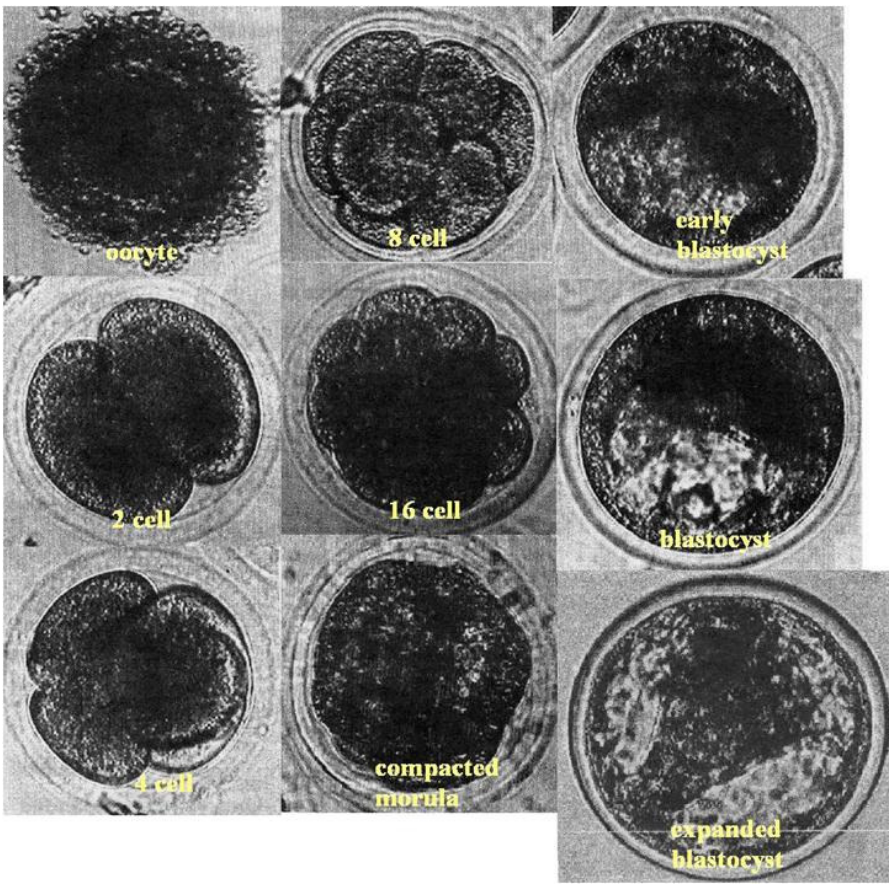


Fig.17 Developmental Stages of Embryo (IVF embryos)

2-7 Optimum Insemination Timing

Especially for Artificial Insemination (AI), optimum insemination timing is crucial for the fertilization and the pregnancy. Because the sperm's viability is less than un-frozen natural sperms.
The timing depends on "How normal sperm and ovium can meet at Ampulla of oviduct (date site)." There are 2 factors affecting this, which are the time to reach the site and the surviving time of both sperm and ovium. Usually these are considered as below:

	(time to reach date site)	(surviving time)
Sperm	2-12 min. after AI	24-48 hrs. (depend on quality)
Ovium	5-6 hr.	5-6 hr

* Sperm needs capacitation (for 3-4 hr.).

* Capacitation: Ejaculated sperms don't have the ability to fertilize ovium. The ability will be obtained when the sperms are put into the female's body (vagina or uterus) then moving inside the reproductive tract. This is called "capacitation".

It is considered that the high occurence rate of early embyonic death is due to the aging of sperm and/or ovium resulted from inappropriate timing of AI.

* Early Embryonic Death: Early embryo's death before implantation. Because this will be happened before pregnancy diagnosis, it has not yet fully investigated. Possible other causes of the early embyonic death might be embryo's chromosomal abnormality, mild endometritis or hormonal abnormality.

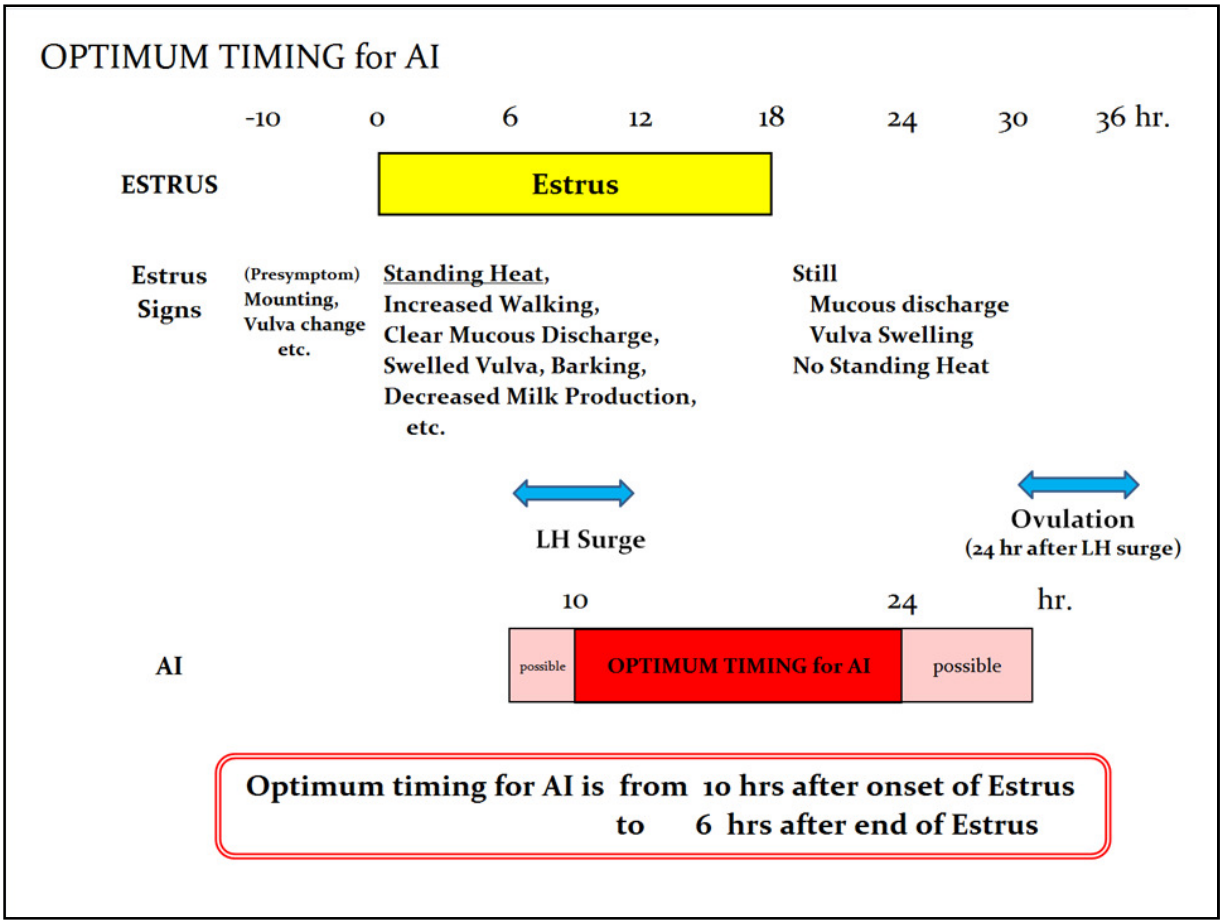
The following Fig.18 is a schematic explanation of the optimum insemination timing. Even though we followed the classical am-pm rule, there seems not much difference. However, the most important thing is to know the exact time of estrus onset. In many cases the insemination tends to be delayed than to be too earlier. Because when a farmer report the esrus to Inseminator, usually several hours are already passed. Anyway the technicians have to make efforts to define when the standing heat actually started.

* am/pm rule: a guideline for the insemination time according to the estrus onset. The following is the recommendation.

Cows First Showing Estrus	Should be Bred	Too late for Good Results
In the morning	That evening	Next day
In the evening	The next morning	After 3:00 p.m. next day

* Be careful, many cows show estrus from late evening to early morning.

Fig.



3. Reproductive Disorders

3.1 Etiological Classification of Reproductive Disorders

Etologically the reproductive disorders can be classified as followings

(1) Hereditary Disease

(2) Infectious Disease

(3) Endocrine Disease

(4) Others

Malnutrition, Injuries, Tumors, etc.

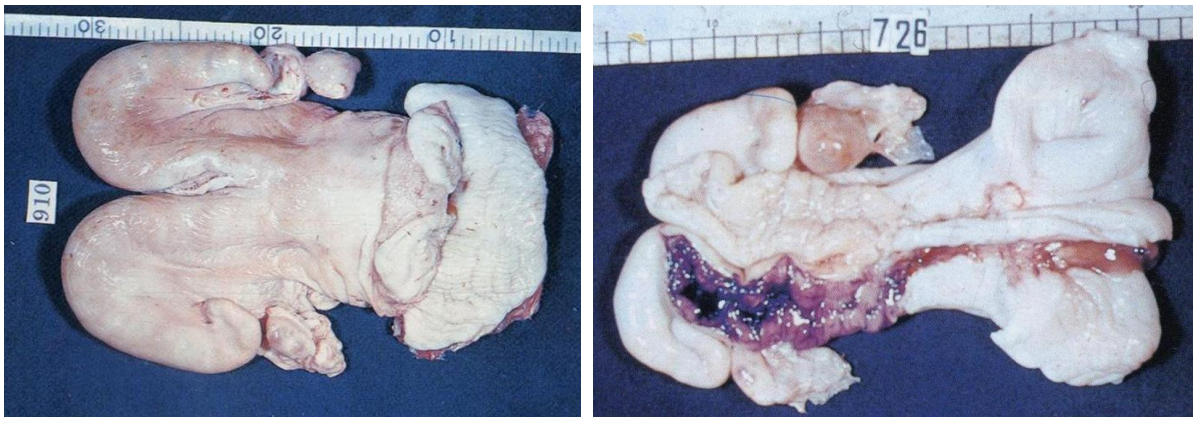
3-1-1. Hereditary Disease

About the hereditary disease, only heifers will be the objectives of attention. Because cows already had calvings, which means at least they don't have any infertile hereditary disease. Therefore, our main concern is if heifers have any hereditary diseases and if they are infertile or not. Most of hereditary disease, we don't have any treatment methods. And in case that these diseases are heritable, early diagnosis and ealy culling is recommended.

(1) Hypoplasia, Aplasia or Malformation of Reproductive Organs

Sometimes "Double external orifices of cervix" can be seen. It is a kind of malformation, though if the one of cervix is open to uterus, there is a possibility to be pregnant. There are many types of this malformation, sometimes completely cervix, uterine body and uterine horn are separated, or in another case only the external orifices of cervix are separated. In these case we have to check if the cervix is blind-end or not.

Fig. 19 One case of double external orifices of cervix



* A case of double external orifices of cervix. After sectioned (right), it is cleared that cervix, uterine body and uterine horn are completely separated.

(2) Freemartin

In case of twin or more pregnancy, if the gender of the fetuses were mixed (female and male), most of the female calves will lose their fertility. Infertile freemartin female's reproductive tract will be poorly developed, a kind of hypoplasia. This is because of the blood vessels of the female and male tied together (anastomosis) inside the uterus. More than 90% of such female become infertile, so early diagnosis is recommended.

* Female calf can be checked of its fertility by checking vagina depth. Inserts something like test tube (the tip should be round-shape) into vagina. If the vagina depth is less than 10cm, they can diagnosed as infertile. Normal female calf has deeper vagina depth.

Fig. 20 Twin Fetus (The fetal fluid of one fetus was stained)

3-1-2 Infectious Disease

Many infectious disease cause Abortion, Endometritis, Pyometra, Infertility, Stillbirth, Retained placenta etc. In case that similar type of abortions are frequently occured in some area, these infectious disease should be on the question. The clinical points are time of abortion, abnormality of the fetus or placenta, any other symptoms of pregnant female etc. However, the diagnosis of these infectious diseases are impossible only by clinical findings.

It is recommended to send necessary samples to the laboratory of a research institute. Infectious diseases that cause abortion and reproductive disorders are summarized in Tab.1.

Tab.1 Infectious disease causing abortion in cattle

Disease (Pathogen)	Effects on Female Reproduction	Infection Route	Countermeasures
(Bacterial)			
Brucellosis (Brucella abortus)	Abortion (6-9 month) Infertility Retained Placenta	Contamination of food or water with aborted fetus	Culling of positive animals Vaccination
Campylobacteriosis (Campylobacter fetus)	Embryonic Death Abortion (4-7 month)	Bull (Natural Mating) Contaminated Semen	Artificial Insemination Anitibiotics Treatment
Leptospirosis (Leptospira pomona, etc)	Abortion (Late Gestation) Agalactia Hemolytic Anemia	Contaminated food or water with urine from infected animals	Vaccination Anitibiotics Treatment
Listeriosis (Listeria monocytogenes)	Abortion (Late Gestation) Retained Placenta Endometritis Encephalitis	Contaminated food and environment	Cleaning & disinfection of environment
Epidemic bovine miscarriage (Chlamydia)	Abortion (Late Gestation), Stillbirth Retained Placenta	Bull (Natural Mating) Contaminated food	Artificial Insemination Anitibiotics Treatment Cleaning of environment
(Fungal)			
Mycotic abortion (Aspergillus fumigatus etc.)	Abortion (Middle to Late) Necrosis of placenta	Intake of moldy food	Proper storage of food Avoid using moldy food
(Viral)			
Infectious bovine rhinotracheitis:IBR (IBR virus)	Abortion (Middle to Late) Infertility Vulvovaginitis	Contaminated semen Contaminated environment	Vaccination Artificial Insemination
Bovine viral diarrhea mucosal disease :BVD-MD (BVD-MD virus)	Abortion (Middle to Late) Abnormal fetus	Contaminated semen Contaminated environment	Vaccination Artificial Insemination
Akabane disease (Akabane virus)	Abortion, Premature birth, Stillbirth Malformation of calves	Carried by blood-sucking insects	Vaccination Eradication of blood-sucking insects
Bluetongue disease (Bluetongue virus)	Abortion (Late Gestation) Central nervous disorders	Contaminated semen	Vaccination
(Protozoal)			
Trichomoniasis (Trichomonas fetus)	Abortion (1-4 month) Pyometra Infertility	Bull (Natural Mating)	Artificial Insemination Culling of positive animals
Toxoplasmosis (Toxoplasma gondii)	Abortion (Late Gestation) Stillbirth Retained placenta	Contaminated food or water	Avoid contaminated food or water