

Physiology and Endocrinology of the Estrous Cycle

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Introduction

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

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

The period of estrus is short, lasting from 6-30 hours. It is the only time that a cow will allow herself to be mounted by a bull or other cows. The behavioral changes that occur at this time are used as the primary indicators of estrus. It has been shown that 70% of mounting activity occurs between 6 p.m. and 6 a.m. In addition, about 25% of cows have estrous periods of less than 8 hours.

The high incidence of mounting activity at night and short estrous periods make estrous detection difficult, partially explaining why estrous detection is the number one management problem and infertility is the major reason cows are culled. Low heritability of fertility suggests that causes of infertility are management related and accentuates the need to understand how hormones affect behavior and later the reproductive tract. Recognizing the behavioral changes is the key to estrous detection. Understanding the physiology and endocrine control of reproduction can be useful in correlating management practices with hormonal changes that occur throughout the estrous cycle.



IRM-2

Dairy Integrated Reproductive Management

Hormones and Reproduction

The events of the estrous cycle and pregnancy are regulated by chemical messengers called hormones. They are produced in endocrine tissues such as the ovaries, pituitary, hypothalamus, embryo and uterus. Hormones are generally released into the blood to be transported to their site of action, or target tissue, where they are recognized by special receptors and cause a change in function of the tissue. See Table 1 for the hormones involved in reproduction, the origin and actions.

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

Proestrus

Ovarian activity during proestrus is initiated by regression of the CL of the previous cycle. Progesterone levels are low and growth of the ovulatory follicle takes place. During this period the follicle destined to ovulate grows from a microscopic structure to a large fluid filled, blister-like structure 3/4 inch to 1 inch in diameter.

While several large follicles may develop during this period, only 1 (2 or 3 in the case of twins or triplets) will be selected to ovulate. This dominant follicle differs from others in that it is stimulated by follicle stimulating hormone (FSH) and luteinizing hormone (LH) to produce estrogens.

Table 1. Hormones That Regulate Reproduction.

Site of Production	Hormone Produced	Action
Hypothalamus	Gonadotropin Releasing Hormone (GnRH)	Releases FSH and LH from pituitary.
Pituitary	Follicle Stimulating Hormone (FSH)	Stimulates follicle development and estrogen production.
	Luteinizing Hormone (LH)	Induces ovulation, development of the corpus luteum, and progesterone production.
Follicles	Estrogens	Induce estrous behavior. Alter fluid production and muscular activity of oviducts, uterus, cervix and vagina. Cause surge in release of LH from pituitary during estrus.
Corpus Luteum	Inhibin	Selectively depresses FSH release.
	Progesterone	Prepares uterus for pregnancy. Prevents recurrence of estrous cycles by depressing FSH & LH release.
	Relaxin	Expansion of uterus during pregnancy and relaxation of cervix at parturition.
Uterus	Prostaglandins	Promote luteal regression at end of estrous cycle or pregnancy.
	Embryo proteins	Provide signal that promotes maintenance of CL during early pregnancy.

Estrogens are produced by cells that form the wall of the developing follicle. The outermost layers of cells are the thecal cells while the inner layers are the granulosa cells. These two cell types work cooperatively during development of the follicle to produce estrogens. Thecal cells bind LH and produce androgens which are then converted to estrogens by granulosa cells that have been stimulated by FSH.

Estrus

Continued estrogen production by the developing follicle results in a surge in the release of LH and FSH from the pituitary which stimulates maximum estrogen production by the follicle. The high levels of estrogen are responsible for behavioral signs of estrus. They also increase contractions of the reproductive tract to facilitate sperm and egg transport.

Estrogen also influences the amount and type of fluid produced by the oviducts, uterus, cervix and vagina. The stringy, clear mucus discharge seen at estrus is secreted from the cervix and is thought to assist the migration of sperm through the cervix.

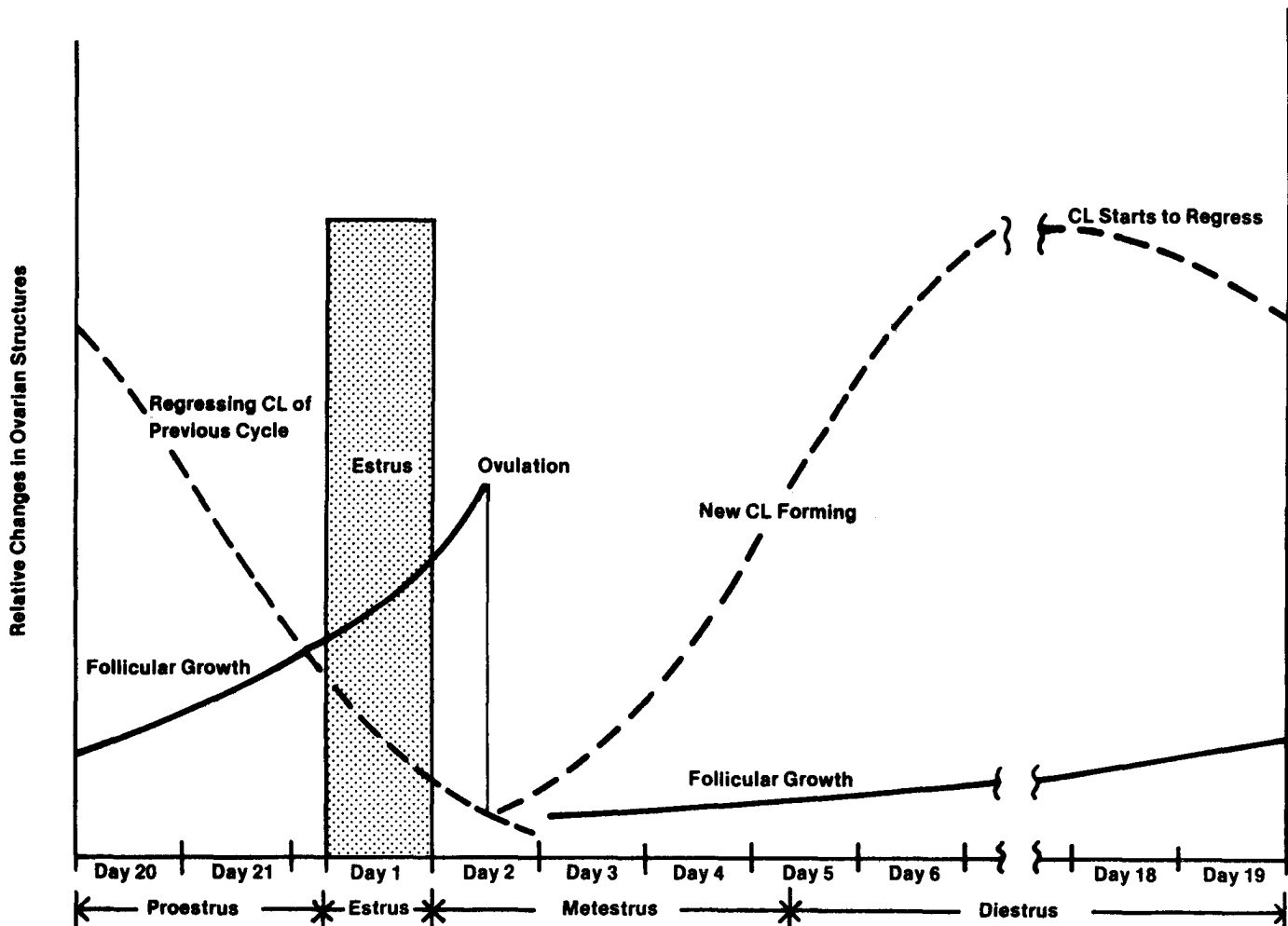
During estrus the granulosa cells also release inhibin, a hormone that prevents the release of FSH from the pituitary.

Thus during proestrus and estrus, follicular growth is completed, the egg is ready to be released at ovulation, and the cow is brought into estrus and can be bred. Synchrony between the endocrine events that allow an animal to be mated and the time of ovulation are essential to a successful mating.

Metestrus

The 3-4 days immediately following estrus are termed metestrus. The surge in LH and FSH during estrus leads to rupture of the follicle about 30 hours after the beginning of "standing estrus," or 10-14 hours after the end of estrus, and the release of the egg from within.

The granulosa and thecal cells lining the collapsed follicle become sensitive to LH and will form the CL or "yellow body" and begin to produce progesterone. This hormone is responsible for preparing the uterus for pregnancy and inhibiting estrous cycle activity.



- Estrous Cycle:** The interval from the first signs of sexual receptivity at estrus (heat) to the start of the next estrus.
- Length:** 18-24 days (Avg. 21)
- 4 Periods:**
1. **Proestrus:** 1-3 days prior to estrus
 2. **Estrus (heat):** 8-30 hours (Avg. 18)
Ovulation occurs 10-14 hours **after** estrus.
 3. **Metestrus:** 3-4 days after estrus. Some cows may have bloody discharge. Indicates she was in heat 2-4 days earlier, will be in heat again in 16-19 days.
 4. **Diestrus:** 12-15 days, active corpus luteum. Regresses if cow is not pregnant, maintained if she is.

Fig. 1. The periods of the estrous cycle and the accompanying changes in the follicle and corpus luteum.

One to 3 days after estrus, blood may be detected in discharges from the vagina. This blood "oozed" through the lining of the uterus when levels of estrogen in circulation decreased. It indicates that estrus **has occurred** and estrus will most likely occur 18-20 days later. Therefore, it is a useful sign for predicting the time of the next estrous period.

Synchrony of estrus, mating and ovulation is critical. The lifespan of the egg, once it is ovulated, is only 10-12 hours, and sperm only survive for 24-48 hours after deposition into the female reproductive tract. While it may appear that timing of insemination is not critical, remember that the sperm must spend 4-6 hours in the female tract before they are capable of fertilizing an egg. This explains why conception rates are higher when cows are inseminated from mid to late estrus rather than after the end of estrus.

It is also worth noting a primary cause of infertility in cattle is "cystic follicles" (Fact Sheet IRM-25).

A cystic follicle is one that has failed to ovulate after reaching maturity. The egg is retained inside the follicle and fertilization cannot occur. The CL does not form and the cycle does not proceed. The exact cause of this failure is unknown. Treatment with LH or LH-like compounds, especially gonadotropin releasing hormone (GnRH, which will cause the release of LH), promotes resumption of cyclic activity by causing changes in the theca and/or granulosa cells that lead to the development of a CL-like structure which produces progesterone. These "induced cycles" are of normal length with most cows returning to estrus 18-23 days after treatment.

While follicular development and ovulation are under the control of FSH and LH from the pituitary, these events can be modified by injection of hormones. Cattle generally ovulate one egg during each cycle. However, the number of follicles that can be induced to develop in a given cycle can be influenced by injections of FSH or FSH-like substances, such as pregnant mare's serum gonadotropin (PMSG).

Ovulation of these follicles can then be induced by the injection of GnRH or LH-like substances such as human chorionic gonadotropin (hCG). This provides the basis for superovulation where several eggs may be obtained following injection of PMSG at the correct time of the cycle. However, the response to superovulation treatments varies widely between animals. While some animals produce few eggs others are overstimulated and the ovaries become extremely large. As a result, many eggs may be lost because they cannot be "picked up" by the oviducts.

Following ovulation the egg is "picked up" by the oviduct where it is fertilized. The upper end of the oviduct is funnel shaped. Under the influence of estrogen it envelops the surface of the ovary and sweeps the ovulated egg into the oviduct with fine hair like projections (cilia) that line its surface. It is here, in the upper part of the oviduct, that fertilization takes place.

Congenital defects or adhesions induced by aggressive rectal palpation of the ovaries or manually expressing the CL for treatment of "persistent CL" or infections can prevent contact between the ovary and oviduct. Such animals would be infertile since the eggs would not reach the site of fertilization.

After fertilization occurs the developing embryo is retained within the oviduct for 3-4 days where it develops into a ball of 16-32 cells (a morula). It is still surrounded by a sticky membrane, the zona pellucida. Retention of the egg within the oviduct is critical. Fertilized eggs that enter the uterus too early or too late fail to develop.

Early embryo development is thought to be regulated by changes in the fluids produced by the oviduct and uterus that are brought about by increasing levels of progesterone from the developing CL. The rising levels of progesterone also inhibit the uterine contractions that are so essential for sperm transport but are detrimental to retention of the embryo in the uterus. The multiple effects that progesterone exerts on the oviduct and uterus explain why successful embryo transfers are dependent upon synchronizing the estrous cycles of the donor and recipient animals.

Diestrus

The corpus luteum is the dominant structure on the ovary during diestrus. It develops mainly from the granulosa cells lining the walls of the collapsed follicle. In effect, the LH that induced ovulation is also responsible for the changes in the granulosa cells that form the CL. The CL reaches maximum size 8-10 days after ovulation. The levels of progesterone in blood parallel the growth of the CL. Maximum levels are reached around day 10 and maintained until day 16-18 of the cycle, if pregnancy does not intervene.

It should also be noted that follicles continue to develop during this period. A large follicle, similar in size to preovulatory follicles, develops around day 12 after estrus. It is frequently on the same ovary as the active CL. It does not ovulate and eventually regresses.

Days 16-18 of the cycle are critical to the maintenance of CL function. If the cow is not pregnant, the CL is induced to regress by the release of prostaglandin $F_{2\alpha}$ from the uterus. The substance is transported directly to the CL where it interferes with progesterone synthesis and decreases blood progesterone levels. This allows FSH to stimulate the development of a new follicle over the next 3-4 days. As the follicle matures, the level of estrogens rise and the cycle is repeated.

On the other hand, if the cow is pregnant, the CL is maintained, blood levels of progesterone remain high, and resumption of cyclic activity is blocked. The signal for maintaining the CL in pregnant cows is thought to come from the developing embryo itself. As discussed previously, the embryo reaches the uterus on day 3 or 4 of the cycle as a solid ball of cells enclosed within the zona pellucida.

Development from day 4 through 10-12 is characterized by the re-arrangement of the cells so that the embryo appears as a hollow ball with thicker walls on one end. This is the first major step in differentiation. The cells on the outer portion of the ball are destined to form the fetal placenta while those in the thickened area are to become the fetus. The embryo, at this development stage, is called a blastocyst and it remains enclosed in the zona pellucida.

Noticeable growth of the embryo begins between days 10-12 when it is released from the zona pellucida. Growth over the next 4-6 days is rapid as the placental membranes expand. During this rapid growth period, the embryo expands from a sphere of less than 1/6 inch in diameter to a hollow tube 1/8 inch in diameter and 6-8 inches long. It is thought that these embryonic cells are responsible for producing the signal (probably chemical) that blocks the regression of the CL at the end of the cycle.

It is clear that maintenance of pregnancy is dependent upon presence of the CL that is actively secreting progesterone and that the presence of a developing embryo is essential for CL maintenance. Death of the embryo during this critical period prolongs the length of the diestrus phase and may explain estrous cycles that last 25-35 days.

Ovarian Function After Calving

During pregnancy recurrence of estrous cycle activity is blocked by the continued secretion of high levels of progesterone from the CL. Three to 4 days prior to calving progesterone levels in the blood decline because the CL regresses.

At calving the CL has regressed and progesterone levels in the blood are low. This allows FSH to be released from the pituitary and follicular growth begins.

Most cows will ovulate between 10 and 20 days after calving. The majority of these early ovulations are associated with "silent estrus" and are not observed. The CL that forms is short lived. The cow may again ovulate 12-17 days later. The majority of these cows will show normal estrus but may have short, 15-18 day cycles also. The third ovulation after calving usually occurs within 30-35 days. Most of these cows show estrus and have normal estrous cycles.

The reason(s) for the "short cycles" and "silent estrus" after calving is (are) not known. It does appear, however, that the rate at which uterine repair proceeds affects the return to normal ovarian function. Uterine involution normally occurs during the first 45 days postpartum. Cows that clean easily and do not have uterine infections return to normal cycles earlier than those with retained placentas (Fact Sheet IRM-21) and infections (Fact Sheet IRM-22) in the uterus. The incidence of cystic follicles is higher during the early postpartum period. It has been suggested that the release of GnRH and LH is low in early lactation. Successful treatment of the condition with GnRH supports this idea. However, the "stress" imposed on the cow by high milk production during this period may predispose the cow to the condition.

This emphasizes the need to carefully monitor general health and the nutritional requirements of the cow to minimize the possible effects of stress (Fact Sheet IRM-15) on reproduction. While we generally emphasize the importance of monitoring uterine involution during the post calving period, we cannot overload the overall management of the cow during this period.

Summary

Reproduction in cattle is dependent upon the interaction of hormones from the hypothalamic area of the brain (GnRH), pituitary gland (FSH and LH), the follicle (estrogens), corpus luteum (progesterone), uterus (prostaglandins) and the developing embryo. Follicular development and ovulation are brought about by FSH and LH.

Estrogen from the developing follicle is responsible for synchronizing behavioral estrus and the release of LH that causes ovulation and the subsequent development of the CL.

Progesterone from the CL acts on the oviduct and uterus to provide a hospitable environment for the development of the embryo and at the same time prevents new ovulations from occurring. The embryo plays an active role in promoting its early survival by producing substances that prevent uterine prostaglandins from causing CL regression and resumption of cyclic activity. It is clear that all phases are closely synchronized and interdependent.

While ovulations occur within 20 days after calving, normal estrous cycle activity begins 30-45 days postpartum. The incidence of cystic follicles is higher during the early postpartum period. The rate of uterine involution and the stresses imposed on the cow by the demands of lactation influence the return to normal activity. These observations accentuate the need to closely monitor the general health and nutrition of the cow during this most critical period.

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