Reproductive Endocrinology and Hormonal Control of the Estrous Cycle

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Abstract

Maximizing reproductive efficiency depends upon whether a heifer attains puberty before the start of the breeding season, conceives early in the breeding season, calves unassisted, raises the calf to the time it is marketed, and the heifer/cow must conceive to calve early during the subsequent calving seasons. Failure to do so constitutes reproductive loss, which is estimated to cost the US beef industry around $500 million annually. Estrous synchronization and artificial insemination are among the most powerful and applicable technologies for genetic improvement of beef herds. Development of new and improved methods of synchronizing estrus and ovulation depends on an understanding of the physiological and hormonal mechanisms controlling the estrous cycle and the initiation of estrous cyclicity in non-cycling heifers and postpartum cows. It is imperative for veterinarians and producers to familiarize themselves with commercially available estrous synchronization products and the physiologic mechanisms by which these products synchronize estrus and (or) ovulation in cattle.

Key words: estrous cycle, follicle, corpus luteum, hormonal control

Résumé

Le rendement de la reproduction peut se maximiser si les taures atteignent la puberté avant la période de saillies, si elles conçoivent tôt dans la période de saillies, si elles vêlent sans aide et si elles élèvent le veau jusqu'au moment de la mise en marché. De plus, les taures/vaches doivent concevoir pour vêler plus tôt dans les périodes de saillies subséquentes. Lorsque ces conditions ne sont pas rencontrées, il en résulte une perte de reproduction pour l'industrie américaine du bœuf que l'on estime à près de 500 millions de dollars annuellement. La synchronisation de l'œstrus et l'insémination artificielle sont parmi les technologies les plus puissantes et pertinentes pour l'amélioration génétique des troupeaux de boucherie. Le développement de nouvelles méthodes pour mieux synchroniser l'œstrus et l'ovulation dépend de la connaissance des mécanismes physiologiques et hormonaux qui contrôlent le cycle œstral et l'initiation de la cyclicité chez les taures non-cycliques et les vaches postpartum. Il est essentiel que les vétérinaires et les producteurs se familiarisent avec les produits de synchronisation de l'œstrus présentement disponibles sur le marché et les mécanismes physiologiques qui permettent à ces produits de synchroniser l'œstrus et/ou l'ovulation chez les bovins.

Introduction

Reproductive efficiency is the most important factor impacting the economics of a cow-calf operation. The economic value of reproduction for commercial beef producers was reported to be five times greater than calf growth.[70] Maximizing reproductive efficiency depends upon successful completion of the following events: a heifer must reach puberty before the start of the breeding season, conceive early in the breeding season, calve unassisted, raise the calf to the time it is marketed, and the heifer/cow must conceive to calve early during the subsequent calving season. Any interruption in the preceding cycle will constitute reproductive loss, which is estimated to cost the US beef industry around $500 million annually.[6] Therefore, minimizing reproductive loss needs to be a high priority.

Recent years have witnessed rapid development of technologies utilized to increase reproductive efficiency and (or) improve the genetic merit of a herd. Some of these technologies include estrous synchronization, artificial insemination, gender-selected semen, *in vitro* embryo production, embryo transfer, ultrasonography, transgenics, and cloning. Of the preceding reproductive technologies, estrous synchronization and artificial insemination are among the most powerful and applicable technologies for genetic improvement of beef herds.[68] Development of new and improved methods of synchronizing estrus and ovulation depends on an

understanding of the physiological and hormonal mechanisms controlling the estrous cycle and the initiation of estrous cyclicity in prepubertal heifers and postpartum cows. Although estrous synchronization products and protocols have changed over time, the basic physiological principles underlying how these products work have not. An understanding of the bovine estrous cycle and how estrous synchronization products work will facilitate the application of these technologies in groups of cycling and anestrous females. This article reviews the endocrine regulation of the estrous cycle with specific emphasis on the regulation of growth of a dominant follicle and the lifespan of the corpus luteum (CL). In addition, emphasis will be given to commercially available estrous synchronization products, and the physiologic mechanisms by which these products synchronize estrus and (or) ovulation in cattle.

Principles of the Bovine Estrous Cycle

Characteristics of the estrous cycle

In cattle, the estrous cycle normally varies from 17 to 24 days and the duration of estrus is generally 10 to 18 hours; however, considerable variation exists among individual animals (range < 8 to > 30 hr).[56] The primary sign of estrus in cattle is standing to be mounted, and secondary signs of estrus include frequent mounting, watery mucus from the vulva, and restlessness. A number of estrous detection aids are available to assist producers, including pressure mount detectors, tail chalk/paint, androgenized cows, and teaser bulls rendered sterile by vasectomy, epididectomy, and (or) penile deviation. However, the HeatWatch electronic estrous detection system is the most effective estrous detection aid, and provides precise information on the onset, intensity, and duration of estrus. Rorie *et al*[64] utilized the HeatWatch system with 500 Angus cows to evaluate the effect of the intensity of estrus on pregnancy rate. Estrus was synchronized with the Select Synch protocol (gonadotropin releasing hormone [GnRH] followed seven days later with an injection of prostaglandin $F_{2\alpha}$). Length of estrus ranged from 0.5 to 24 hours and there was no effect of length of estrus on pregnancy status. However, cows that became pregnant were mounted more times per estrus than cows that did not conceive. These data are similar to another study with Angus cows in which cows that became pregnant were mounted more times per estrus than cows that did not become pregnant.[39]

A seasonal effect on estrous behavior has been reported in Angus x Hereford cows located in Oklahoma.[74] In that study, the length of estrus was greater in summer compared to winter or spring; however, cows were mounted more frequently per estrus in winter compared to summer or spring. Therefore, estrous detection may need to occur more frequently in winter compared to

spring or summer, whereas in summer, estrous detection may need to occur for a longer duration at each check. In this study, there was no effect of season on the interval from the onset of estrus to ovulation (mean = 31 hr). In Florida, an increase in the temperature-humidity index (THI) decreased the number of mounts per estrus.[41]

The number of mounts per estrus increases as the number of females in estrus increases.[31,41] This is likely due to the formation of sexually active groups of cattle, which is known to increase the number of mounts per female.[20,33] In non-synchronized cattle there will be fewer sexually active groups (or fewer animals per group) and less mounting activity. Therefore, improved estrous detection efficiency is an advantage of an estrous synchronization program. However, it is also true that frequent animal handling and restraint are stressors,[12] and that increased handling and restraint of heifers during a synchronized estrus decreased the number of mounts per estrus.[42] Depending upon the estrous synchronization protocol, a fixed-time insemination protocol should reduce the amount of animal handling associated with sorting estrual heifers at the time of insemination.

In contrast to other livestock species, cattle ovulate following the end of estrus, approximately 28 to 32 hours after the onset of estrus or 12 to 20 hours following the end of estrus. Although characteristics of the estrous cycle are similar among most beef breeds, important differences have been reported between *Bos taurus* and *Bos indicus* breeds.[19,35] In general, it is more difficult to detect estrus in *Bos indicus* females compared to *Bos taurus* females. This is likely because *Bos indicus* females are reported to have a shorter duration of behavioral estrus compared to *Bos taurus* females.[8,60] In addition, *Bos indicus* females had a decreased interval from onset of estrus to ovulation,[68] decreased magnitude of the pre-ovulatory luteinizing hormone surge,[68] smaller corpora lutea,[36] and lower luteal phase concentrations of progesterone[3] than *Bos taurus* females.

Hormonal patterns during the estrous cycle

The estrous cycle is divided into three stages: follicular phase, estrus, and luteal phase. It is regulated by hormones secreted by the hypothalamus (GnRH), anterior pituitary gland (follicle stimulating hormone [FSH] and luteinizing hormone [LH]), ovary (estradiol and progesterone), and uterus (prostaglandin $F_{2\alpha}$ [$PGF_{2\alpha}$]). The preceding hormones serve as chemical messengers that travel in the blood to specific target tissues which contain receptors that are hormone specific and regulate the phases of the estrous cycle. The combination of hormone secretion and metabolism (liver, kidneys, and lungs) maintain the correct hormonal balance during the follicular phase, estrus, and luteal phase of the cycle. For a list of hormones, their biological functions, their role in estrous synchronization, and product names see Table 1.

Table 1. Reproductive hormones, their functions during the estrous cycle, roles in estrous synchronization, product name, dosages, and route of administration.

Hormone	Endocrine gland	Function of hormone	Biological action in estrous sync.	Product name	Dosage	Route of administration
Progesterone	Corpus luteum	Inhibit estrus Inhibit ovulation Prepares animal for pregnancy Maintenance of pregnancy	Inhibit estrus Inhibit ovulation Induce cyclicity Dominant follicle turnover	Melengestrol acetate (MGA®) EAZI-BREED CIDR®	0.5 mg/hd/day 1 CIDR per animal (1.38 g prog)	Feed Vaginal insert
Prostaglandin F$_{2\alpha}$	Uterus	Induce luteal regression	Induce premature luteal regression	Lutalyse® ProstaMate® In Synch® Estrumate® estroPLAN®	5 mL 5 mL 5 mL 2 mL 2 mL	IM inject IM inject IM inject IM inject IM inject
GnRH	Hypothalamus	Controls secretion of LH Induces gonadotropin surge	Synchronize follicle wave Induce ovulation	Cystorelin® Factryl® Fertagyl® OvaCyst®	2 mL 2 mL 2 mL 2 mL	IM inject IM inject IM inject IM inject
Follicle stimulating hormone (FSH)	Anterior pituitary gland	Initiation of a follicular wave	Superovulation	Follitropin®	Depends on application	IM inject
Luteinizing hormone (LH)	Anterior pituitary gland	Stimulated by GnRH Induction of ovulation Oocyte maturation Luteal tissue formation	Synchronize follicular wave Induction of ovulation	N/A	N/A	N/A
Estradiol	Ovarian follicle	Estrous behavior Induction of gonadotropin surge Sperm transport	Dominant follicle turnover Estrous behavior	N/A	N/A	N/A

GnRH = gonadotropin releasing hormone; prog = progesterone; CIDR = controlled internal drug release device; N/A = not applicable

A pre-ovulatory follicle and the subsequently formed CL are the two primary ovarian structures that regulate the estrous cycle through secretion of estradiol and progesterone, respectively. Changes in a pre-ovulatory follicle and CL, patterns of secretion of LH, estradiol and progesterone, and changes in ovarian blood flow during the ruminant estrous cycle are depicted in Figure 1.

Follicular phase

The follicular phase (proestrus) begins with the initiation of CL regression (luteolysis) and ends with the onset of estrus. Luteolysis is accompanied by a rapid decrease in progesterone resulting in a decrease in the negative feedback on pituitary LH secretion. As circulating concentrations of progesterone decrease, LH pulse frequency increases followed by a rapid increase in follicular estradiol secretion. The production of follicular estradiol results from the coordinated actions of LH and FSH on theca and granulosa cells, respectively.[14,15] The follicle wall consists of two distinct cell layers (granulosa and thecal cells) that are separated by a basement membrane. Granulosa cells are located in the compartment with the oocyte, whereas theca cells surround the granu-
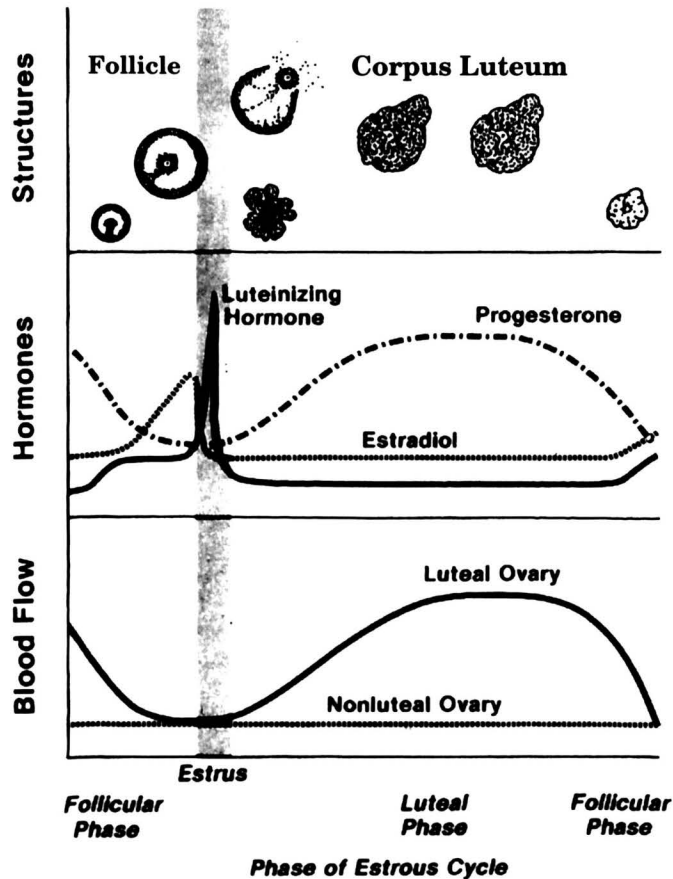
Figure 1. Changes in ovarian structures (pre-ovulatory follicle and corpus luteum), hormones (luteinizing hormone, estradiol, and progesterone), and ovarian blood flow (ovary containing [luteal ovary] or not containing [non-luteal ovary] a corpus luteum) during the three phases of the estrous cycle (follicular, estrus, and luteal phase). (Modified from Garverick HA, Smith MF: Female reproductive physiology and endocrinology of cattle. *Vet Clin North Am Food Anim Pract* 9:223-247, Copyright Elsevier 1993, used with permission.)

losa cells and are in close association with a wreath of capillaries. Theca cells have membrane receptors that bind LH, resulting in the synthesis of androgens that subsequently diffuse through the basement membrane into granulosa cells. Following FSH binding to membrane receptors on granulosa cells there is an increase in aromatase activity that converts androgens to estradiol. Increased circulating concentrations of estradiol initiate estrous behavior and induce the pre-ovulatory gonadotropin surge, which is essential for ovulation. In addition, estradiol can act within granulosa cells to increase LH receptor concentration and thereby prepare the pre-ovulatory follicle to respond to the gonadotropin surge.[63]

Regulation of follicular waves. Two general patterns of antral follicular development are present in mammals. In cattle, sheep, and horses, dominant ovulatory-sized follicles develop in sequential waves during both the follicular and luteal phases of the cycle. In primates, pigs, and rodents, however, dominant ovulatory follicles only develop during the follicular phase of the cycle.[16] The bovine estrous cycle usually consists of two to three follicular waves, and each wave begins with the recruitment of a cohort of antral follicles from a pool of growing small follicles. One follicle is subsequently selected from this cohort for continued growth and becomes dominant. The remaining follicles in the cohort become atretic. During a non-ovulatory follicular wave, the dominant follicle eventually becomes atretic and a new follicular wave is initiated. A viable dominant follicle present at luteolysis will generally become the ovulatory follicle.[2] The estrous cycle length of cows that have three follicular waves is generally longer (20-24 days) compared to cows with two follicular waves (18-20 days).

In cattle, follicular waves can be detected during most reproductive states, including the prepubertal period, estrous cycle, gestation, and postpartum anestrous period.[2] The only exception to the continuous growth and development of follicular waves in cattle is during the last 21 days of gestation. During this time, follicles greater than 6 mm in diameter have not been detected.[23] Following parturition, follicular waves resumed following a rise in circulating concentrations of FSH,[67] and the first dominant follicle appeared between days 7 and 15 postpartum in both beef and dairy cows.[10,51]

Follicular waves have been studied most extensively in cattle and consist of the following three stages: recruitment, selection, and dominance.

Recruitment. Recruitment of a cohort of follicles, around 3 mm in diameter, is stimulated on each ovary by a transient rise in FSH. Inhibition of both FSH and LH arrested follicular growth at 2 to 4 mm. However, when physiological levels of FSH were infused for 48 hours, follicular growth from 5 to 8 mm was stimulated.[29] Peak concentration of FSH occurred when the future dominant follicle attained a mean diameter of approximately 4 mm, after which concentrations of FSH declined,[24] and were at basal concentrations by the time follicular selection occurred.[27] The mechanism responsible for the initial decline in FSH concentration is unknown, however, estradiol and inhibin are follicular products that probably play a major role in the decline of FSH.[2]

Selection. Follicular selection is the process by which a single follicle from the recruited cohort is selected to continue to grow and become dominant, while

the remaining follicles of the cohort undergo atresia. With the decline in circulating FSH concentrations, small follicles are presumably unable to continue growth and the selected follicle (dominant follicle) may shift its dependency from FSH to LH.[24] The decreased circulating concentrations of FSH at the time of selection are likely important for the selection of a single dominant follicle. The decline in circulating concentrations of FSH is presumably driven by increasing concentrations of estradiol (and perhaps inhibin) produced by the cohort of recruited follicles.[28] Increased concentrations of estradiol and inhibin may feed back on the hypothalamic-pituitary axis to selectively suppress FSH secretion.[43] At follicular deviation, the selected follicle continues to grow while the subordinate follicles enter atresia.[24] In cattle, deviation usually occurs when the largest follicle reaches a diameter of approximately 8 mm, approximately 2.7 days after the initiation of a follicular wave,[25,26] or 61 hours after the LH surge.[40]

Dominance. The dominance phase of the follicular wave occurs when a follicle has been selected and continues to grow at a faster rate than the largest subordinate follicle, and inhibits the emergence of a new follicular wave.[24] Following selection and establishment of a dominant follicle, follicular recruitment is inhibited until dominance is lost or ovulation occurs. Inhibition of follicular recruitment may be mediated by inhibiting the transient rise in circulating concentrations of FSH.[2] An alternative hypothesis is that the dominant follicle directly inhibits growth of small follicles through the secretion of a factor(s) that acts directly on other follicles in the ovary. Regardless of the mechanism, destruction or ovulation of a dominant follicle results in a transient rise in circulating concentrations of FSH and subsequent initiation of a new follicular wave.[1]

Estrous phase

Increasing circulating concentrations of estradiol following luteolysis initiate estrous behavior, increase uterine contractions (facilitate sperm transport), and induce the pre-ovulatory gonadotropin surge. The pre-ovulatory gonadotropin surge coordinates the following events that are critical to the establishment of pregnancy: resumption of meiosis within the oocyte, follicular rupture, and luteinization of follicular cells. LH is generally considered to be the primary gonadotropin that controls the preceding events; however, FSH also has been shown to cause ovulation and luteal tissue formation.[21] The end of the estrus phase of the cycle is marked by follicular rupture, which is the culmination of a complex cascade of events leading to the activation of proteolytic enzymes that digest the follicular wall and allows the egg (oocyte) to be released for fertilization. This process is similar to mechanisms associated with inflammation. Injection of GnRH will induce a surge of LH within two to four hours, and ovulation of a dominant follicle will occur 24 to 36 hours after injection.

Estrus and ovulation are not always linked and frequently occur as independent events. The incidence of anovulatory estrus in peripuberal heifers was 22% and 13% for years 1 and 2, respectively, and this phenomenon has been called nonpuberal estrus.[52,65] The incidence of nonpuberal estrus may be affected by age, breed, and photoperiod or season of the year.[52] Formation of a cystic follicle can also result in estrous behavior without ovulation; however, the incidence of cystic follicles is low in beef cattle. Cystic follicles are normally treated by injecting GnRH to luteinize the follicular tissue, followed by an injection of $PGF_{2\alpha}$ seven days later to regress the luteal tissue.

Alternatively, ovulation without estrus is not uncommon in beef cattle. The first ovulatory estrus in heifers and postpartum cows is preceded by a transient increase in progesterone (short luteal phase).[30] This is presumably due to ovulation without estrus. Increased concentrations of progesterone may be involved in preparation of the uterus for the possibility of pregnancy or in the establishment of patterns of gonadotropin secretion characteristic of cycling females. Short-term exposure of prepuberal heifers or anestrous postpartum beef cows to a progestin (melengestrol acetate [MGA] or controlled internal drug release [CIDR]) has been used extensively in estrous synchronization protocols to mimic this short period of progesterone exposure, and will be discussed in more detail later.

Luteal phase

The luteal phase spans the time of CL formation and maintenance which begins with ovulation and ends with luteolysis. Progesterone is the primary secretory product of the CL and is regulated by secretions of the anterior pituitary, uterus, ovary, and embryo.[53] The regulation of progesterone secretion is likely controlled by a balance of luteotropic (stimulate progesterone) and luteolytic (inhibit progesterone) stimuli, given that both types of stimuli are secreted concurrently during the estrous cycle. In ruminants, LH is considered to be the primary luteotropic hormone, and concentration of luteal LH receptors is positively correlated with changes in progesterone and luteal growth.[55] Corpora lutea receive the majority of the ovarian blood flow, and blood flow to the luteal ovary and progesterone secretion are highly correlated.[53] Progesterone has a central role in the regulation of the estrous cycle as it determines estrous cycle length and is required for maintenance of pregnancy.

In cattle, $PGF_{2\alpha}$ is the uterine luteolysin and is commonly used to synchronize estrus. In the absence of an embryo, uterine concentrations of $PGF_{2\alpha}$ increase during the late luteal phase and $PGF_{2\alpha}$ is secreted as

pulses into the uterine veins on days 17 to 20 following estrus (day 0 = estrus).[34] $PGF_{2\alpha}$ is transported from the utero-ovarian vein into the ovarian artery via a counter-current transfer mechanism[32,45] and is transported to the CL. $PGF_{2\alpha}$ may have both a direct and an indirect effect on a ruminant CL to cause luteolysis. In the presence of an embryo, pulsatile secretion of $PGF_{2\alpha}$ is reduced and the CL does not regress. Maintenance of high circulating concentrations of progesterone in pregnant animals prevents the expression of estrus and ovulation.

Follicular determinants of corpus luteum function

Corpora lutea are a continuation of follicular maturation. Consequently, changes in the hormonal stimulation of a pre-ovulatory follicle may have a subsequent effect on luteal progesterone secretion. The endocrine microenvironment of a pre-ovulatory follicle is unique relative to surrounding non-ovulatory follicles, and is important for preparation of follicular cells for luteinization and secretion of progesterone.[47] McNatty *et al*[48] suggested that development of a normal CL may depend upon a pre-ovulatory follicle meeting the following criteria: 1) an adequate number of granulosa cells, 2) an adequate number of LH receptors on granulosa and theca cells, and 3) granulosa cells capable of synthesizing adequate amounts of progesterone following luteinization. Furthermore, the ability of luteinized human granulosa cells to secrete progesterone increased when the cells were collected from follicles having increased follicular fluid concentrations of estradiol, compared to granulosa cells collected from follicles that had lower concentrations of estradiol.[48] Premature induction of ovulation in ewes was associated with luteal insufficiency.[50] These data are relevant to fixed-time insemination protocols in which physiologically immature dominant follicles are induced to ovulate at AI and the subsequent circulating concentrations of progesterone are lower than in cows in which a larger dominant follicle is induced to ovulate with GnRH.[59] Inadequate luteal function following induced ovulation may be due to a reduced number of follicular cells and (or) inadequate preparation of follicular cells for luteinization and secretion of progesterone.

Hormonal Management of the Luteal Phase for Synchronization of Estrus

Successful estrous synchronization protocols require control of the timing of both dominant follicle development and luteal regression. During the estrous cycle when a CL is present and circulating concentrations of progesterone are high, standing estrus and ovulation are inhibited; however, when the CL regresses and progesterone concentrations decrease, circulating concentrations of estradiol increase and the animal returns to standing estrus. Progestins mimic the actions of progesterone produced by the CL and inhibit estrus/ovulation, which can delay the interval to estrus when luteal tissue is not present. Following the removal of the progestin, progesterone concentrations will be low and standing estrus and ovulation will occur.

Progestins

Two progestin products commercially available for estrous synchronization include MGA and the CIDR. In cycling cows and heifers, administration of MGA or CIDRs does not affect the time of CL regression. However, once CL regression has occurred, progestin administration can prevent a cow or heifer from showing estrus and ovulating. Consequently, progestin administration in cows that have experienced CL regression will delay the expression of estrus and ovulation until after progestin withdrawal.

Role of progestins in anestrus. At the start of a breeding season, most herds consist of a mixture of cycling and anestrous females. An effective estrous synchronization protocol must be able to induce a fertile estrus or ovulation in both anestrous and cycling heifers and cows. A short luteal phase usually occurs in prepuberal heifers and postpartum beef cows following the first ovulation.[57,73] This short exposure to progesterone is believed to be necessary for reprogramming the reproductive axis to resume normal estrous cycling. Therefore, in herds that have a large proportion of prepuberal heifers or anestrous cows, progestin pre-treatment before induction of ovulation can initiate estrous cycling status and eliminate or at least reduce the occurrence of short estrous cycles.

Administration of low levels of a progestin (i.e. MGA) in the absence of a CL can result in the formation of a persistent follicle (see below). However, the effect of progestin treatment on persistent follicle formation differs between cycling and anestrous animals. Administration of low concentrations of progestins did not induce persistent follicle formation in early postpartum anestrous dairy heifers[62] or anestrous postpartum beef cows.[58] It is not clear why persistent follicles did not form in anestrous cows.

Progestin administration and formation of persistent follicles. Persistent follicles are characterized by an extended dominant follicle life span and increased estradiol production.[17,69,76] Treatment of cycling heifers or cows with low levels of a progestin, following luteolysis, resulted in the formation of persistent follicles that had a large diameter, extended lifespan, and increased production of estradiol.[18,69,75] Administration of low (subluteal) concentrations of progestins to cattle, in the absence of luteal tissue, increased LH pulse frequency.[37,38,66] However, midluteal phase concentrations of progesterone de-

creased LH pulse frequency, and persistent follicles did not form.[66,69] Thus, the formation of persistent follicles has been associated with increased LH pulse frequency, and infusion of exogenous LH-induced persistent follicle formation.[13]

Insemination immediately following long-term progestin treatment and ovulation of a persistent follicle has been associated with decreased fertility.[49] No difference was reported in fertilization rate following ovulation of persistent follicles, but fewer zygotes developed into embryos containing 16 or more cells compared to ovulation of oocytes from control follicles.[4] Decreased fertility following formation and ovulation of persistent follicles may result from alterations in the uterine environment due to increased estradiol secretion[9] and (or) premature resumption of meiosis due to prolonged exposure to increased LH pulse frequency.[44]

Prostaglandin $F_{2\alpha}$

Prostaglandins are naturally occurring compounds produced by most cells in the body and have a variety of biological actions. $PGF_{2\alpha}$ is a naturally occurring luteolytic hormone utilized to synchronize estrus and induce abortion in cattle through induction of CL regression. In the absence of an embryo, uterine concentrations of $PGF_{2\alpha}$ increase during the late luteal phase. $PGF_{2\alpha}$ is secreted in pulses and transported to the CL via a counter-current mechanism. The mechanisms associated with $PGF_{2\alpha}$-induced luteolysis are not completely understood, however, $PGF_{2\alpha}$ probably has both a direct and indirect (decreased blood flow) action. Luteal cells are known to have $PGF_{2\alpha}$ receptors on the plasma membrane, and direct inhibitory effects of $PGF_{2\alpha}$ on luteal progesterone secretion have been demonstrated.[55] In addition, $PGF_{2\alpha}$ is known to reduce luteal blood flow due to vasoconstrictor activity.[54]

Administration of $PGF_{2\alpha}$ to domestic ruminants does not induce luteolysis during the early luteal phase. For purposes of estrous synchronization, injection of $PGF_{2\alpha}$ is only effective in cycling heifers and cows (approximately day 6 to 16 following estrus; day 0 = estrus). Although functional $PGF_{2\alpha}$ receptors and signal transduction mechanisms are present in developing ovine corpora lutea,[71,72] the acquisition of luteolytic capacity is not established until after day 4 postestrus.[71]

Injection of $PGF_{2\alpha}$ into prepuberal heifers or anestrous cows is not effective due to the absence of luteal tissue. Furthermore, $PGF_{2\alpha}$ treatment will not induce cycling activity in non-cycling cattle. Therefore, when using $PGF_{2\alpha}$ alone to synchronize estrus it is important to assess the proportion of cycling animals before initiating the treatment. In herds containing both cycling and non-cycling females, the most effective estrous synchronization protocols combine treatment with a progestin and an injection of $PGF_{2\alpha}$. In pregnant feedlot heifers,

$PGF_{2\alpha}$ is highly effective at inducing abortion before 100 days of gestation.

Hormonal management of follicular waves for synchronization of estrus

The development of effective protocols for fixed-time insemination is dependent upon the precise synchronization of follicular waves culminating in a fertile ovulation at a predetermined time. Two approaches used to synchronize bovine follicular waves include: 1) ovulating/destroying the dominant follicle and thereby initiating a new follicular wave, and 2) prolonging the lifespan of a dominant follicle (persistent follicle).

Initiation of a new follicular wave occurs following ovulation or turnover (atresia) of the dominant follicle. Administration of exogenous progesterone, estradiol, or GnRH have been utilized to turnover (progesterone and estradiol) or ovulate (GnRH) dominant follicles and to synchronize follicular waves in heifers and cows.[7,11] Follicular turnover (atresia) of persistent follicles can be accomplished through the administration of progesterone. Progesterone as a single injection[5] or administered over a 24-hour period[46] effectively regressed persistent follicles and initiated new follicular waves. Reduction of LH pulse frequency and amplitude following the administration of exogenous progesterone may be the mechanism by which persistent follicles are induced to undergo atresia.[46]

Conclusions

Understanding the basic principles of the bovine estrous cycle and how estrous synchronization products affect the cycle is essential when choosing the best protocol for heifers or cows and for determining what went wrong when pregnancy rates, following a synchronized estrus, are less than expected. Three general approaches that have been used to develop estrous synchronization protocols include: 1) inhibit ovulation following spontaneous CL regression (long-term progestin treatment); 2) induction of CL regression ($PGF_{2\alpha}$ treatment); and 3) a combination of 1 and 2. Most of the protocols utilized today can be categorized under the third approach. The ability to synchronize bovine follicular waves through an injection of GnRH has added a new and important dimension to estrous synchronization and has made fixed-time AI in cows a viable option. Many of the current protocols are able to synchronize the growth of a dominant follicle in addition to the time of CL regression.

Acknowledgements

References

1. Adams GP, Matteri RL, Kastelic JP, Ko JC, Ginther OJ: Association between surges of follicle-stimulating hormone and the emergence of follicular waves in heifers. *J Reprod Fertil* 94:177-188, 1992.

2. Adams GP: Comparative patterns of follicle development and selection in ruminants. *J Reprod Fertil* Suppl 54:17-32, 1999.

3. Adeyemo O, Heath E: Plasma progesterone concentration in *Bos taurus* and *Bos indicus* heifers. *Therio* 14:411, 1980.

4. Ahmad N, Schrick FN, Butcher RL, Inskeep EK: Effect of persistent follicles on early embryonic losses in beef cows. *Biol Reprod* 52:1129-1135, 1995.

5. Anderson LH, Day ML: Acute progesterone administration regresses persistent dominant follicles and improves fertility of cattle in which estrus was synchronized with melengestrol acetate. *J Anim Sci* 72:2955-2961, 1994.

6. Bellows DS, Ott SL, Bellows RA: Review: Cost of reproductive diseases and conditions in cattle. *Prof Anim Sci* 18:26-32, 2002.

7. Bo GA, Adams GP, Pierson RA, Mapletoft RJ: Exogenous control of follicular wave emergence in cattle. *Therio* 43:31-40, 1995.

8. Brewester J, Cole CL: The time of ovulation in cattle. *J Dairy Sci* 24:111, 1941.

9. Butcher RL, Pope RS: Role of estrogen during prolonged estrous cycles of the rat on subsequent embryonic death or development. *Biol Reprod* 21:491-495, 1979.

10. Crowe MA, Goulding D, Baguisi A, Boland MP, Roche JF: Induced ovulation of the first postpartum dominant follicle in beef suckler cows using a GnRH analogue. *J Reprod Fertil* 99:551-555, 1993.

11. Diskin MG, Austin EJ, Roche JF: Exogenous hormonal manipulation of ovarian activity in cattle. *Domest Anim Endocrinol* 23:211-228, 2002.

12. Dobson H, Kamonpatana M: A review of female cattle reproduction with special reference to a comparison between buffaloes, cows, and zebu. *J Reprod Fertil* 77:1-36, 1986.

13. Duffy P, Crowe MA, Boland MP, Roche JF: Effect of exogenous LH pulses on the fate of the first dominant follicle in postpartum beef cows nursing calves. *J Reprod Fertil* 118:9-17, 2000.

14. Fortune JE: Bovine theca and granulosa cells interact to promote androgen production. *Biol Reprod* 35:292-299, 1986.

15. Fortune JE, Quirk SM: Regulation of steroidogenesis in bovine preovulatory follicles. *J Anim Sci* 66:1-8, 1988.

16. Fortune JE: Ovarian follicular growth and development in mammals. *Biol Reprod* 50:225-232, 1994.

17. Fortune JE, Rivera GM: Persistent dominant follicles in cattle: basic and applied aspects. *Arq Fac Vet* 27:24-36, 1999.

18. Fortune JE, Rivera GM, Evans AC, Turzillo AM: Differentiation of dominant versus subordinate follicles in cattle. *Biol Reprod* 65:648-654, 2001.

19. Galina CS, Orihuela A, Duchateau A: Reproductive physiology in Zebu cattle. *Vet Clin North Am Food Anim Pract* 3:619-632, 1987.

20. Galina CS, Orihuela A, Rubio I: Behavioral characteristics of zebu cattle with emphasis on reproductive efficiency, in Fields MJ, Sands RS (eds): *Factors Affecting Calf Crop*. Boca Raton, CRC Press, 1994, pp 345-361.

21. Galway AB, Lapolt PS, Tsafriri A, Dargan CM, Boime I, Hsueh AJW: Recombinant follicle stimulating hormone induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. *Endocrinology* 127:3023-3028, 1990.

22. Garverick HA, Smith MF: Female reproductive physiology and endocrinology of cattle. *Vet Clin North Am Food Anim Pract* 9:223-247, 1993.

23. Ginther OJ, Kot K, Kulick LJ, Martin S, Wiltbank MC: Relationships between FSH and ovarian follicular waves during the last six months of pregnancy in cattle. *J Reprod Fertil* 108:271-279, 1996.

24. Ginther OJ, Wiltbank MC, Fricke PM, Gibbons JR, Kot K: Selection of the dominant follicle in cattle. *Biol Reprod* 55:1187-1194, 1996.

25. Ginther OJ, Kot K, Kulick LJ, Wiltbank MC: Emergence and deviation of follicles during the development of follicular waves in cattle. *Therio* 48:75-87, 1997.

26. Ginther OJ, Bergfelt DR, Kulick LJ, Kot K: Selection of the dominant follicle in cattle: establishment of follicle deviation in less than 8 hours through depression of FSH concentrations. *Therio* 52:1079-1093, 1999.

27. Ginther OJ, Bergfelt DR, Kulick LJ, Kot K: Selection of the dominant follicle in cattle: role of two-way functional coupling between follicle-stimulating hormone and the follicles. *Biol Reprod* 62:920-927, 2000.

28. Ginther OJ, Bergfelt DR, Kulick LJ, Kot K: Selection of the dominant follicle in cattle: role of estradiol. *Biol Reprod* 63:383-389, 2000.

29. Gong JG, Campbell BK, Bramley TA, Gutierrez CG, Peters AR, Webb R: Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotropin-releasing hormone agonist. *Biol Reprod* 55:68-74, 1996.

30. Gonzalez-Padilla E, Wiltbank JN, Niswender GD: Puberty in beef heifers I. The interrelation between pituitary, hypothalamic and ovarian hormones. *J Anim Sci* 40:1091-1104, 1975.

31. Helmer SD, Britt JH: Mounting activity as affected by stage of estrous cycle in Holstein heifers. *J Dairy Sci* 68:1290-1296, 1985.

32. Hixon JE, Hansel W: Evidence for preferential transfer of prostaglandin $F_{2\alpha}$ to the ovarian artery following intrauterine administration in cattle. *Biol Reprod* 11:543-552, 1974.

33. Hurnick JF, King GJ, Robertson HA: Estrous and related behavior in postpartum Holstein cows. *App Anim Ethology* 2:55-68, 1975.

34. Inskeep EK. Murdoch WJ: Relation of ovarian functions to uterine and ovarian secretion of prostaglandins during the estrous cycle and early pregnancy in the ewe and cow, in Greep RO (ed): *Reproductive Physiology III, International Review of Physiology*. Baltimore, University Park Press. 22:325-356, 1980.

35. Inskeep EK, Dailey RA, Rhodes RC: Some considerations on the value of hormonal assays and a knowledge of hormonal profiles to reproduction of red meat animals. *S Afr J Anim Sci* 12:85-101, 1982.

36. Irvin HJ, Randel RD, Haensley WE: Reproductive studies of Brahman cattle. III. Comparison of weight, progesterone content, histological characteristics, and 3β-hydroxysteroid dehydrogenase activity in corpora lutea of Brahman, Hereford and Brahman X Hereford heifers. *Therio* 10:417, 1978.

37. Kinder JE, Kojima FN, Bergfeld EG, Wehrman ME, Fike KE: Progestin and estrogen regulation of pulsatile LH release and development of persistent ovarian follicles in cattle. *J Anim Sci* 74:1424-1440, 1996.

38. Kojima FN, Chenault JR, Wehrman ME, Bergfeld EG, Cupp AS, Werth LA, Mariscal V, Sanchez T, Kittok RJ, Kinder JE: Melengestrol acetate at greater doses than typically used for estrous synchrony in bovine females does not mimic endogenous progesterone in regulation of secretion of luteinizing hormone and 17 beta-estradiol. *Biol Reprod* 52:455-463, 1995.

39. Kuhlmann KK, Shelby DR, Scott DB, May BJ, Engdahl GR: The use of an electronic estrous detection system to monitor estrous behavior in Angus females of various ages. *J Anim Sci* 81 (Suppl 1):271 Abstr, 1998.

40. Kulick LJ, Kot K, Wiltbank MC, Ginther OJ: Follicular and hormonal dynamics during the first follicular wave in heifers. *Therio* 52:913-921, 1999.

41. Landaeta-Hernandez AJ, Yelich JV, Lemaster JW, Fields MJ, Tran T, Chase Jr CC, Rae DO, Chenoweth PJ: Environmental, genetic, and social factors affecting the expression of estrus in beef cows. *Therio* 57:1357-1370, 2002.

42. Lemaster JW, Telich JV, Kempfer JR, Schrick FN: Ovulation and estrous characteristics in crossbred Brahman heifers treated with an intravaginal progesterone-releasing insert in combination with prostaglandin $F_{2\alpha}$ and estradiol benzoate. *J Anim Sci* 77:1860-1868, 1999.

43. Martin GB, Price CA, Thiery JC, Webb R: Interactions between inhibin, oestradiol and progesterone in the control of gonadotrophin secretion in the ewe. *J Reprod Fertil* 82:319-328, 1988.

44. Mattheij JA, Swarts JJ, Hurks HM, Mulder K: Advancement of meiotic resumption in graafian follicles by LH in relation to preovulatory ageing of rat oocytes. *J Reprod Fertil* 100:65-70, 1994.

45. McCracken JA, Carlson JC, Glew ME, Goding JR, Baird DT, Green K, Samuelson B: Prostaglandin $F_{2\alpha}$ identified as a luteolytic hormone in sheep. *Nature* 238:129-134, 1972.

46. McDowell CM, Anderson LH, Kinder JE, Day ML: Duration of treatment with progesterone and regression of persistent ovarian follicles in cattle. *J Anim Sci* 76:850-855, 1998.

47. McNatty KP, Hunter WM, MacNeilly AS, Sawers RS: Changes in the concentration of pituitary and steroid hormones in the follicular fluid of human graafian follicles throughout the menstrual cycle. *J Endocrinol* 64:555-571, 1975.

48. McNatty KP, Smith DM, Makris A, Osathanondh R, Ryan KJ: The microenvironment of the human antral follicle: interrelationships among the steroid levels in antral fluid, the population of granulosa cells, and the status of the oocyte in vivo and in vitro. *J Clin Endocrinol Metab* 49:851-860, 1979.

49. Mihm M, Baguisi A, Boland MP, Roche JF: Association between the duration of dominance of the ovulatory follicle and pregnancy rate in beef heifers. *J Reprod Fertil* 102:123-130, 1994.

50. Murdoch WJ, De Silva M, Dunn TG: Luteal phase insufficiency in the ewe as a consequence of premature induction of ovulation by intra-follicular injection of gonadotropins. *J Anim Sci* 57:1507-1511, 1983.

51. Murphy MG, Boland MP, Roche JF: Pattern of follicular growth and resumption of ovarian activity in postpartum beef suckler cows. *J Reprod Fertil* 90:523-533, 1990.

52. Nelsen TC, Short RE, Phelps DA, Staigmiller RB: Nonpuberal estrus and mature cow influences on growth and puberty in heifers. *J Anim Sci* 61:470-473, 1985.

53. Niswender GD, Riemers TJ, Diekman MA, Nett TM: Blood flow: a mediator of ovarian function. *Biol Reprod* 14:64-81, 1976.

54. Niswender GD, Nett TM: The corpus luteum and its control, in Knobil E, Neill JD, Ewing LL, et al (eds): *The Physiology of Reproduction*, vol 1. New York, Ravel Press, 1988, p 489.

55. Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW: Mechanisms controlling the function and life span of the corpus luteum. *Physiological Reviews* 80:1-29, 2000.

56. O'Connor ML, Senger PL: Estrus detection, in Youngquist RS (ed): *Current Therapy in Large Animal Theriogenology*. Philadelphia, WB Saunders, 1997, pp 276-285.

57. Perry RC, Corah LR, Kiracofe GH, Stevenson JS, Beal WE: Endocrine changes and ultrasonography of ovaries in suckled beef cows during resumption of postpartum estrous cycles. *J Anim Sci* 69:2548-2555, 1991.

58. Perry GA, Kojima FN, Salfen BE, Bader JF, Patterson DJ, Smith MF: Effect of an orally active progestin on follicular dynamics in cycling and anestrous postpartum beef cows. *J Anim Sci* 80:1932-1938, 2002.

59. Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, Roberts AJ, Geary TW: Relationship between follicle size at insemination and pregnancy success. *PNAS* 102: 5268-5273, 2005.

60. Plasse D, Warnick AC, Koger M: Reproductive behavior of *Bos indicus* in a subtropical environment. IV. Length of oestrous cycle, duration of oestrus, time of ovulation, fertilization, and embryo survival in grade Brahman heifers. *J Anim Sci* 30:63-72, 1970.

61. Randel RD: LH and ovulation in Brahman X Hereford and Hereford heifers (abstract). *J Anim Sci* 43:300, 1976.

62. Rhodes FM, Clark BA, Day ML, Macmillan KL: Can persistent ovarian follicles be induced in young postpartum dairy cows? *Proc Aust Soc Reprod Biol* 28:103, 1997.

63. Richards JS: Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation. *Physiol Rev* 60:51-89, 1980.

64. Rorie RW, Bilby TR, Lester TD: Application of electronic estrus detection technologies to reproductive management of cattle. *Therio* 137-148, 2002.

65. Rutter LM, Randel RD: Nonpuberal estrus in beef heifers. *J Anim Sci* 63:1049-1053, 1986.

66. Savio JD, Thatcher WW, Morris GR, Entwistle K, Drost M, Mattiacci MR: Effects of induction of low plasma progesterone concentrations with a progesterone-releasing intravaginal device on follicular turnover and fertility in cattle. *J Reprod Fertil* 98:77-84, 1993.

67. Schallenberger E, Prokopp S: Gonadotrophins and ovarian steroids in cattle. IV. Re-establishment of the stimulatory feedback action of oestradiol-17 beta on LH and FSH. *Acta Endocrinol (Copenh)* 109:44-49, 1985.

68. Seidel GE: Reproductive biotechnologies for profitable beef production. *Proc Beef Improvement Federation* p 28, 1995.

69. Sirois J, Fortune JE: Lengthening the bovine estrous cycle with low levels of exogenous progesterone: a model for studying ovarian follicular dominance. *Endocrinology* 127:916-925, 1990.

70. Trenkle A, Willham RL: Beef production efficiency: The efficiency of beef production can be improved by applying knowledge of nutrition and breeding. *Science* 198:1009-1015, 1977.

71. Tsai SJ, Wiltbank MC: Prostaglandin $F_{2\alpha}$ regulates distinct physiological changes in early and mid-cycle bovine corpora lutea. *Biol Reprod* 58:346-352, 1998.

72. Tsai SJ, Juengel JL, Wiltbank MC: Hormonal regulation of monocyte chemoattractant protein-1 messenger ribonucleic acid expression on corpora lutea. *Endocrinology* 138:4517-4520, 1997.

73. Werth LA, Whittier JC, Azzam SM, Deutscher GH, Kinder JE: Relationship between circulating progesterone and conception at the first postpartum estrus in young primiparous beef cows. *J Anim Sci* 74:616-619, 1996.

74. White FJ, Wettemann RP, Looper ML, Prado TM, Morgan GL: Seasonal effects on estrous behavior and time of ovulation in nonlactating beef cows. *J Anim Sci* 80:3053-3059, 2002.

75. Zimbelman RG, Smith LW: Control of ovulation in cattle with melengestrol acetate. II. Effects on follicular size and activity. *J Reprod Fertil* 11:193-201, 1966.

76. Zimbelman RG, Smith LW: Control of ovulation in cattle with melengestrol acetate. I. Effect of dosage and route of administration. *J Reprod Fertil* 11:185-191, 1966.

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1. Zimmerman, AD et al. Efficacy of bovine herpesvirus-1 inactivated vaccine against abortion and stillbirth in pregnant heifers. J Am Vet Med Assoc 2007;231(9):1386-1389.
2. Data on file at APHIS' Center for Veterinary Biologics.

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