Two experiments were conducted to examine the effects of exogenous GnRH on luteal function in hysterectomized (HYST) and unilaterally hysterectomized (UHYST) ewes. In each experiment, crossbred ewes were assigned randomly in equal numbers into four groups in a 2^2 factorial arrangement. Treatments consisted of two levels of GnRH (0 and 100 μg/day) and two levels of hysterectomy (Exp. 1, none and UHYST; Exp. 2, none and HYST). On day 12 of an estrous cycle, all ewes in Exp. 1 (n = 16) were unilaterally ovariectomized and in eight ewes the uterine horn adjacent to the remaining ovary was removed, while in Exp. 2 (n = 20) the entire uterus was removed from one-half of the ewes. In each experiment CL in the remaining ovary or ovaries were enucleated. After subsequent estrus, one-half of the control and UHYST or HYST ewes were injected i.v. with GnRH on days 2 and 3 (Exp. 1) or day 2 only (Exp. 2) while the remaining ewes were injected similarly with saline. Jugular blood was collected for 60 min after injection for analysis of serum LH (Exp. 1) and periodically thereafter for analysis of serum progesterone (P_4) (Exp. 1 and 2), and plasma oxytocin (OT) (Exp. 1). In Exp. 1, catheterization of the caudal vena cava was performed on all ewes on day 4 and periodic plasma samples were collected for OT and prostaglandin F_2α (PGF_2α) analysis. In Exp. 1 injection of GnRH increased serum concentrations of LH within 60 minutes compared
with those of saline-treated ewes (P = .01). The GnRH-induced secretion of LH in intact and UHYST ewes on day 2 was greater than on day 3 (P = .05). Treatment with GnRH did not alter jugular or vena cava concentrations of OT in intact or UHYST ewes on d 5 to 10 after estrus. However, mean jugular plasma concentrations of OT on days 12 and 14 were greater in all intact vs all UHYST ewes (P = .006), with levels of OT in saline-treated intact ewes being significantly greater than those of the other groups. Overall, vena cava levels of PGF$_{2\alpha}$ did not differ significantly among treatment groups; however, on days 10 to 14 those intact ewes receiving GnRH had higher concentrations of PGF$_{2\alpha}$ compared to those ewes of other groups (P = .07). Treatment with GnRH was without effect on serum concentrations of P$_4$ but levels of this steroid were greater in all UHYST ewes compared with those in intact ewes (P = .09). In Exp. 2, HYST ewes that were injected with GnRH had lower serum levels of P$_4$ on days 4 to 12 (P = .04) than GnRH-treated intact or saline-treated intact and HYST ewes. Therefore, while luteal P$_4$ production by intact and UHYST or HYST ewes was not consistently altered by exogenous GnRH, levels of this hormone were affected by the presence or absence of the uterus.
Corpus Luteum Function in Hysterectomized and Unilaterally Hysterectomized Ewes Treated with Gonadotropin-Releasing Hormone

by

Diana L. Whitmore

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I understand that my thesis will become part of the permanent collection of Oregon State University Libraries. My signature below authorizes release of my thesis to any reader upon request.

Diana L. Whitmore, Author
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INTRODUCTION

The corpus luteum is a transient endocrine gland that is required for the maintenance of pregnancy in mammals (Niswender and Nett, 1988; Niswender et al., 1994). The primary function of this ovarian structure is to produce progesterone, which prepares the uterine endometrium for the implantation of the fertilized ovum and maintains early pregnancy. In domestic animals, fertilization rates are typically high and approximately 70% of ovulations give rise to live births (Roberts et al., 1990). The loss of embryos typically occurs within the first three weeks of pregnancy and at least some of this loss appears to be due to inadequate corpus luteum function (Niswender and Nett, 1988). Reproductive failure has been implicated as one of the most costly and limiting factors in the livestock industry with the overall economic impact well into the billions of dollars (Gerrits et al., 1979). Therefore, efforts to understand the regulation of the life span and function of the corpus luteum is of the utmost importance for improving reproductive efficiency in domestic animals. The following is an attempt to review the literature pertaining to the factors regulating the life span of the corpus luteum of several mammalian species and more specifically the effects of injecting gonadotropin-releasing hormone on the luteal function of the ewe.
LITERATURE REVIEW

THE ESTROUS CYCLE: AN OVERVIEW

Most sexually mature mammalian females are subject to cyclic changes in reproductive activity. These changes may be coordinated with alterations in the seasonal environment so that young are born at a time when both climate and food availability are optimal. Seasonal breeding occurs in most wild animals, whereas only some domesticated animals, such as sheep, goats and horses, exhibit breeding seasons while cattle and swine do not (Hafez, 1987). Ewes and goats are "short-day" breeders, meaning they become sexually active in the fall when daylight hours are decreasing, thus parturition occurs in early spring when temperatures are warm and forages are plentiful. The mare, in contrast, is a "long day" breeder, becoming sexually active in the spring, but due to a year long gestation period, birth also occurs in the spring. In addition to the external factors mentioned, the presence of a male can also influence onset of estrus in several species (Hafez, 1987).

In numerous mammals, sexual behavior is closely linked to ovulation and is dependent on ovarian production of estradiol to stimulate estrous behavior (Asdell et al., 1945). It is only during this phase of the estrous cycle that the female is sexually receptive to the male. In contrast, most species of non-human primates do not limit sexual activity to any one phase of the menstrual cycle, although peaks in sexual activity are still observed midcycle, around the time of ovulation (Hadley, 1992a).

Duration of the estrous cycle varies among species as well as individual animals. The estrous cycle of the ewe is approximately 16-18 days, the cow, sow, and doe 20-21 days, and that of the mare is 20-24 days. Internal factors regulate the character of the estrous cycle, including hormones emanating from the hypothalamus, pituitary, ovary and uterus.
The estrous cycle is divided into four stages: estrus, metestrus, diestrus and proestrus. Based upon ovarian structures present, the estrous cycle can also be divided into two phases, luteal and follicular; both phases are somewhat overlapping in domesticated animals. The luteal phase encompasses the period of maximal functional activity of the corpus luteum (CL) and lasts 14-15 days in the ewe and 16-17 days in the cow and sow. The follicular phase coincides with proestrus and lasts 2-3 days in the ewe and doe and 3-6 days in the cow and sow (Hafez, 1987).

Estrus is characterized by behavioral changes, the most obvious being the female standing for the male to mount. The period of behavioral estrus lasts approximately 24-36 hours in ewes, 32-40 hours in does, 18-19 hours in cows, 48-72 hours in sow and 4-8 days in mare. Behavioral estrus is closely coordinated with ovulation and is the only time in which the female is sexually receptive to the male. Ovulation of the ovum occurs primarily as a result of a peak release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary, which are under the control of gonadotropin-releasing hormone (GnRH) from the hypothalamus.

During metestrus, the granulosa and theca cells lining the walls of the recently ovulated follicle undergo a luteinization process to become the luteal cells. As the corpus luteum continues to develop, two distinct cell types emerge that are termed small and large luteal cells based on their different sizes. In the ewe, progesterone secretion begins to rise to significant levels by 30 hours post-ovulation due to the formation of the functional CL (Caldwell et al., 1972). The increase in luteal progesterone is thought to be under the control of LH (Niswender and Nett, 1988).

Diestrus is characterized by maximal luteal secretion of progesterone. The CL formed from the granulosa and theca cells is now fully functional and capable of secreting increased levels of progesterone. Estradiol of ovarian origin is capable of increasing the sensitivity of the hypothalamic GnRH pulse generator to progesterone negative feedback, which indirectly suppresses the release of LH and prevents ovulation (Karsch, 1987).
Progesterone is also capable of directly inhibiting the secretion of LH from the pituitary in an estradiol-dependent manner as demonstrated in pituitary stalk-sectioned ewes subjected to periodic boluses of GnRH (Girmus and Wise, 1992).

Proestrus, characterized by declining serum concentrations of progesterone, occurs around days 13-14 of the ovine estrous cycle due to the regression of the existing CL (Caldwell et al., 1972). Identification of the uterine hormone prostaglandin F$_{2\alpha}$ (PGF$_{2\alpha}$) as the substance that brings about the demise of the CL was first demonstrated in pseudopregnant rats (Pharriss and Wyngarden, 1969), but soon after was shown to occur in other species such as cattle, sheep, goats, horses and guinea pigs (Chaichareon et al., 1974; Douglas and Ginther, 1972; Lauderdale, 1972; McCracken et al., 1970b; Ott et al., 1980). The manner in which PGF$_{2\alpha}$ exerts its effect on the CL varies between species. In ruminants such as cattle and sheep, the luteolytic effect of PGF$_{2\alpha}$ occurs locally as described by McCracken and coworkers (1972), whereas in horses the effect is via systemic circulation.

Prostaglandin F$_{2\alpha}$ has also been shown to stimulate secretion of oxytocin from the CL in ruminants (Flint and Sheldrick, 1982; Lamsa et al., 1989). Conversely, oxytocin has been shown to cause premature regression of the CL by stimulating the release of uterine PGF$_{2\alpha}$ (Flint and Sheldrick, 1983). Therefore, it has been hypothesized that in these species uterine PGF$_{2\alpha}$ and luteal oxytocin comprise a positive feedback loop, where PGF$_{2\alpha}$ is released from the uterine horn and acting through a counter-current transfer mechanism, acts upon the adjacent ovary to stimulate oxytocin secretion from the CL. This luteal oxytocin then acts back upon the uterine horn to further increase the secretion of PGF$_{2\alpha}$, which causes the eventual regression of the CL (Flint and Sheldrick, 1983; McCracken et al., 1984; Sheldrick and Flint, 1984). The ovarian steroids progesterone and estradiol-17β are also believed to be involved in regulating the secretion of PGF$_{2\alpha}$, possibly by regulating the concentration of oxytocin receptors in the endometrium of the uterus (Roberts et al., 1976; Robinson et al., 1976; McCracken et al., 1984).
The concentration of estrogen secreted from surrounding follicles begins to rise coincidental with the decline in progesterone levels around day 13 of the ovine estrous cycle, and attains maximal levels 24-48 hours later (Bjersing et al., 1972). Reduction in progesterone and the increased production of estradiol results in increased pulsatile secretion of LH and FSH (Goding et al., 1970; Scarmuzzi et al., 1971). It is this rise in estradiol that causes expression of behavioral estrus and stimulates the pulsatile secretion of LH and therefore ovulation.

SEASONAL ANESTRUS

During the time of seasonal anestrus the normal cyclicity of the female reproductive system ceases. Ovarian and follicular activity is minimal thereby preventing ovulation as well as behavioral estrus. The extent of seasonal anestrus varies with the species, breed and physical environment. Anestrus occurs in such domestic species as sheep, goats and horses as well as wild species such as deer, elk and several other members of the cervidae family. In most of these animals, anestrus occurs during the spring, summer and in some instances the fall, such that parturition occurs at an environmentally desirable time. In temperate zones, spring is usually the time for delivery of the young because food is more abundant and there is less environmental stress.

The Gonadotropin-Releasing Hormone Pulse Generator

The central mechanism controlling both external and internal cues for reproductive activity is the GnRH pulse generator (also referred to as LH pulse generator). The GnRH pulse generator produces an episodic pattern of GnRH release from hypothalamic nerve terminals. These pulses in turn stimulate pulses of LH release from the anterior lobe of the pituitary. Normally, the pulse of LH has a high amplitude but a low frequency of release. However, near the time of ovulation, LH pulse frequency increases from one pulse every 3
to 4 hours to one pulse every 30 minutes (Goodman and Karsch, 1981). The overall concentration of LH, as well as pulse frequency, increases as the time of ovulation approaches while the amplitude of the individual LH pulse actually gets smaller. However, if this sustained increase in pulsatile LH secretion is blocked, ovulation will be prevented (Goodman and Karsch, 1980; Legan and Karsch, 1979). During seasonal anestrus the pattern of LH release stays relatively constant with LH secretion having a low frequency of release and a relatively high amplitude.

Ovarian steroids also seem to have an effect on the ability of the GnRH pulse generator to modulate LH secretion. It has been well established that removal of circulating gonadal steroids by ovariectomy results in a major increase of both the frequency and the amplitude of LH pulses and that addition of exogenous estradiol and/or progesterone can lower LH to normal levels (Foster and Ryan, 1979; Foster et al., 1986; Goodman and Karsch, 1980). Goodman and coworkers (1982) studied both ovariectomized (OVX) anestrous and ewes supplemented with an estradiol implant (2.7 pg/ml) 16 hours after ovariectomy. These authors found that in OVX cycling ewes, the administration of estradiol had no effect on LH pulse frequency but did decrease pulse amplitude and mean serum LH concentrations. In OVX anestrous ewes, the presence of exogenous estradiol decreased mean serum LH concentrations and obliterated both LH pulse frequency and amplitude over an 8 hour sampling period, suggesting that estradiol is an extremely powerful inhibitor of LH secretion. This seasonal response of ewes to the negative feedback action of estradiol was also observed by Karsch et al. (1993) who found that positioning an implant containing a low dose of estradiol (0.3 pg/ml) into ovariectomized anestrous or cycling ewes produced a differential response. Anestrous ewes responded with an increase in GnRH pulse size but a decrease in both GnRH and LH pulse frequency due to estradiol implants, while cycling ewes exhibited no effect of low exogenous estradiol on either frequency or magnitude of GnRH or LH pulses. Taken
together, these results indicate that there is an increased sensitivity of the GnRH neurosecretory system to the negative feedback actions of estradiol during anestrus.

The way in which estradiol elicits its effect on gonadotropin secretion has been studied so extensively that its proposed mechanism of action is referred to as the "gonadostat hypothesis". This hypothesis states that concentrations of circulating gonadotropins are low in the immature or anestrous animal because the hypothalamus and(or) pituitary are very sensitive to the inhibitory feedback action of ovarian estradiol. However, as breeding season approaches, there is a decrease in sensitivity to estradiol, allowing gonadotropin secretion to increase and reproductive activity to commence (Foster, 1988). However, the exact mechanism by which estradiol exerts its control is still an enigma.

Some researchers have postulated that estradiol may act directly on GnRH-secreting neurons to depress secretion (Dyer et al., 1980) and(or) pulse amplitude of GnRH (Dreifuss et al., 1981; Lincoln et al., 1985). Immunocytochemical techniques, however, have shown that the target cells for estradiol in the hypothalamus are different than the GnRH-producing neurons, although the distribution of these neurons do overlap (Shivers et al., 1983). Despite this, McEwen et al. (1982) suggested that the absence of identified binding sites for estradiol in GnRH neurons does not exclude these cells as targets because steroid hormones may elicit certain neural effects through pathways other than classical receptor-mediated genomic mechanisms.

The other possible way in which estradiol may modulate GnRH and its control over LH and FSH secretion is by its action on the pituitary. Some researchers postulated that the negative feedback effect of estradiol in the ewe is exerted directly at the level of the anterior pituitary gland, and that the pituitary is more sensitive to the negative effects of estradiol during the anestrous season (Clarke and Cummins, 1984; Goodman and Karsch, 1980; Nett et al., 1984). The negative feedback of gonadal steroids on the anterior pituitary, during the anestrous season, may occur by modulating GnRH membrane
receptor availability and thereby controlling the amount of gonadotropin release (Hauger et al., 1977).

Gregg and Nett (1989) used ovariectomized ewes to investigate the possibility that estradiol may be controlling pituitary GnRH receptors. Ewes were either subjected to a pituitary stalk-section or left intact, and all ewes were treated with GnRH in the presence or absence of estradiol. These researchers found that the presence of estradiol caused an initial up-regulation of pituitary GnRH receptors, but only intact ewes responded with an increase in LH secretion. This suggested that while GnRH is necessary for the secretion of the gonadotropins, its action may be contingent upon estradiol-induced regulation of GnRH receptors in the pituitary. Similar research was performed by Girmus and Wise (1992) who treated ovariectomized and pituitary stalk-sectioned ewes with GnRH in the presence or absence of estradiol, progesterone or both steroids. These researchers found that only a combination of progesterone and estradiol lowered GnRH-induced LH surges most efficiently. It is through this complex interaction of positive and negative control by ovarian steroids that the GnRH pulse generator and thus ovulation is regulated.

The GnRH pulse generator in the ewe is highly susceptible to variation in the external environment; particularly olfactory cues and changes in light patterns. For example, introduction of pheromones from an unfamiliar ram to a group of anestrous ewes results in an immediate induction of hourly LH pulses (Martin et al., 1983; Poindron et al., 1980). However, this response may vary according to breed of sheep. Minton et al. (1991) exposed both Polypay and Suffolk anestrous ewes to an unfamiliar ram, and while both groups of ewes responded with an increase in serum LH, only Polypay ewes exhibited serum progesterone profiles indicative of consecutive estrous cycles, while the Suffolk ewes ovulated but had only one full length luteal phase or exhibited a short luteal phase. Conversely, constant exposure to a familiar ram throughout the breeding season and anestrus does not seem to have a significant effect on the timing of anestrus or cyclicity in mature ewes. Ewes that have either constant exposure or no exposure to
males, have a specific timing of anestrus and reproductive seasons, which appears to be regulated by photoperiod (Karsch et al., 1984). This suggests that the changes in photoperiod associated with seasonality are probably the major contributor in controlling seasonal breeding.

Effect of Photoperiod on Anestrus

The way in which photoperiod affects ovarian cyclicity has been studied by numerous scientists. In an experiment conducted by Goodman et al. (1982), serum LH levels from ovariectomized ewes were analyzed over the course of a year under natural photoperiod. These authors found that during the long daylight hours of summer, LH pulses were of a high amplitude but of low frequency. As hours of daylight decreased, however, the secretory pattern of LH switched such that there was an increase in LH pulse frequency but a decrease in pulse amplitude. Similar results were achieved in ovariectomized ewes that were kept under artificial light conditions (Bittman et al., 1982; Robinson, 1989).

As previously mentioned, however, the negative effects of ovarian hormones also have a differential effect depending on season. This is demonstrated best by a series of experiments by Goodman et al. (1982) who fitted ovariectomized anestrous and cycling ewes with an estradiol implant. These authors found that while estradiol was capable of reducing LH pulse frequency and amplitude in anestrous animals, the same dose was ineffective in cycling ewes. A series of experiments by Legan and Karsch (1980) demonstrated that the timing of the shift to increased sensitivity to estradiol negative feedback during anestrus was driven by photoperiod rather than temperature. These researchers kept intact and ovariectomized ewes (with estradiol implants) indoors and exposed them to an artificial photoperiod, which alternated between "long" and "short" days every 90 days. In both groups, ewes responded with increased overall concentrations of LH normally associated with natural breeding season during the artificial "short" days.
This particular response was totally out of phase with the natural seasonal reproduction. Therefore these researchers postulated that reproductive seasons are driven by changes in photoperiod (Goodman et al., 1982). Effects of photoperiod on seasonal reproduction appear to be mediated through the pineal gland rather than exerted directly on the GnRH pulse generator.

The Pineal Gland and Melatonin

In both "short" and "long" day breeders, researchers have proposed that the pineal gland mediates reproductive response to photoperiod cues through its pattern of melatonin secretion (Bittman et al., 1983; Glass and Lynch, 1981). Melatonin is an indoleamine that is secreted only at night such that the duration of melatonin release is roughly proportional to the hours of darkness. The pathway for transmission of photoperiod cues, which regulates or affects the annual reproduction cycle in ewes, includes the retinal photoreceptors, a monosynaptic retino-hypothalamic tract, the superchiasmatic nuclei, the superior cervical ganglia, the pineal gland and finally the secretion of melatonin (Foster, 1988).

It has been hypothesized that it is the seasonal change in duration of melatonin secretion that coordinates reproductive activity (Wayne et al., 1988). In the ewe, as hours of daylight decrease, the duration of melatonin secretion at night increases. This increased duration in melatonin secretion causes a change in the sensitivity of the GnRH pulse generator to the negative feedback of estradiol, such that the pulse generator becomes more refractory to the inhibitory effects of ovarian steroids (Foster, 1988), thus causing a resumption in reproductive activity. It is important to note that the patterns of melatonin production can be similar in both "short" and "long" day breeders but the response can be markedly different. For example, just as an increase in melatonin secretion can trigger the onset of the breeding season in the ewe, it can also cause a cessation of reproductive activity in the mare.
The influence of the pineal gland and melatonin secretion has been extensively researched in the ewe. In an experiment conducted by Bittman et al. (1983), ewes with intact ovaries were pinealectomized or left intact in early spring and subjected to artificial photoperiods with 90 day shifts between "long" and "short" days for a 2 year period. These researchers found that intact ewes responded to the artificial photoperiod consistently such that reproductive activity commenced upon exposure to decreasing light. In contrast, the pinealectomized ewes exhibited a reproductive pattern out of synchrony with the imposed photoperiod while retaining some natural seasonality; however, this endogenous seasonality became asynchronous with time. Bittman et al. (1983) also performed a similar experiment on estradiol-treated ovariectomized and pinealectomized ewes. Pinealectomized ewes retained an LH profile that was similar to the normal seasonal reproductive cycle, however, this endogenous timing of seasonality eventually became incongruent as well (Bittman et al., 1983). Based upon the results of these experiments, it seems that the pineal gland must be present to mediate the effect of photoperiod on ovarian cyclicity in the ewe. This function is likely to be affected through changes in potency of estradiol in the negative feedback inhibition of tonic LH secretion.

Probably the most compelling evidence for the role of melatonin in seasonal reproduction was demonstrated in an experiment conducted by Bittman and Karsch (1984) in which pinealectomized ewes were kept reproductively suppressed by exposure to "long" day melatonin infusion along with an artificial "long" day photoperiod. Ewes were subsequently transferred to the inductive "short" day photoperiod, where control ewes received concurrent "short" day melatonin infusion and the treated ewes received a "long" day melatonin infusion (out of synchrony). As expected, all control ewes had elevated serum LH concentrations typical of the secretory profile of this hormone during normal "short" days. However, the group receiving the "long" day melatonin treatment continued to have an attenuated LH secretory pattern even after being transferred to short day photoperiod thus resulting in a LH secretory profile typical of long days. This
research provides compelling evidence that the circadian rhythm of melatonin secretion has not merely a permissive role on the reproductive response to photoperiod, but rather determines it.

FOLLICULOGENESIS

Ovaries of the mammalian female at birth, contain all the oogonia that the animal will be allotted for its entire life. The ovaries contain a pool of primordial follicles, each consisting of an oocyte arrested in Prophase I of meiosis, and surrounded by a single layer of flattened granulosa cells (Hadley, 1992a; Fortune, 1994). In the case of most mammals this pool develops during the fetal life, while in others such as rodents, mink, and rabbits, the pool develops in the early neonatal period (Hirshfield, 1991; Marion and Gier, 1971). Primordial follicles remain in this arrested state of development until puberty, when there is an associated increase in LH production from the anterior pituitary (Hadley, 1992a).

Beginning at puberty, follicles gradually but continually leave the resting pool to begin growth that eventually will result in either ovulation or atresia of the follicle. There are a series of developmental stages through which follicles pass to attain an ovulatory size; the first is the primordial follicle, followed by primary follicle, secondary follicle, early tertiary follicle, and finally the Graafian follicle (Erickson et al., 1985; Hafez, 1987). Granulosa cells of the primary follicle become more cuboidal in shape and surround the mature oocyte in a single layer. These granulosa cells secrete glycoproteins which will eventually form the zona pellucida (Erickson et al., 1985; Hadley, 1992a; Hafez, 1987). Secondary follicles are characterized by further proliferation of the granulosa cells, completion of the zona pellucida, an increase in blood supply, and the early development of theca cells that are delineated from the granulosa cells by a basement membrane (Erickson et al., 1985; Hadley, 1992a; Hafez, 1987). Follicular development, up to the secondary follicle stage, can occur independent of gonadotropins, and most likely is
stimulated by a signal generated by the follicle itself (Erickson et al., 1985). However, further development and antrum formation is dependent on LH and FSH (Hafez, 1987). The early tertiary follicle is characterized by formation of a fluid-filled antrum, completion of the theca interna, and beginning development of the theca externa. The final stage of follicular development is the Graafian follicle, which contains a large antrum filled with follicular fluid, and completed development of the theca externa (Erickson et al., 1985; Hadley, 1992a; Hafez, 1987).

Through careful examination of blood hormone profiles, ultrasonographic techniques, surgeries and laparoscopy, characterization of follicular development (folliculogenesis) has been attempted in many domesticated animals. Sirois and Fortune (1988) conducted an extensive investigation of folliculogenesis in heifers, generally monovular animals, using real-time ultrasonography of ovaries daily throughout the estrous cycle. Their findings confirmed some earlier reports that follicular growth occurs in three distinct waves (Fogwell et al., 1985; Ireland and Roche, 1987). These researchers found that during each wave one follicle is selected to become dominant, while the remaining follicles undergo atresia. This dominant follicle will either ovulate or undergo atresia, depending on the stage of the estrous cycle during which it is dominant (Sirois and Fortune, 1988).

While the theory of follicular development occurring in waves in cattle has now become central dogma, information on folliculogenesis in the ewe (a polyovular species) remains controversial. In fact, two recent studies have been conducted, one following follicular development by daily transrectal ultrasonography (Schrick et al., 1993), the other by performing daily laparoscopy (Noel et al., 1993). The results of these studies are conflicting.

Schrick et al. (1993) found that while there were two periods during the ovine estrous cycle characterized by increased follicular activity, there was not an emergence of a dominant follicle during these times. In fact, these researchers support the theory that
follicular activity is characterized by a continuous entry, growth and atresia of follicles, which they believe fits with a lack of dominance. In essence, this allows for the presence of several ovulatory-sized follicles necessary for multiple ovulations.

In contrast, Noel et al. (1993) examined ewes daily using non-traumatic laparoscopy (5 mm optic fibre and manipulation probe under local anesthesia), and concluded that follicular development occurs in three distinct waves, each lasting approximately 6 days. They reported that during each wave, follicles ≥ 2 mm develop from a pool, and that a few of these follicles will continue to undergo maturation and growth to eventually become dominant follicles. These follicles either ovulate (dominant ovulatory follicle) or become atretic (dominant non-ovulatory follicle). Therefore the results of this study do not support the concept of continuous turnover of ovarian follicles, but rather a pattern of follicular growth and turnover similar to that observed in the bovine.

OVULATION OF THE DOMINANT FOLLICLE

The preovulatory surge of LH sets in motion a series of morphological and biochemical changes resulting in the differentiation of the mature follicle into the CL (Niswender and Nett, 1988; Niswender et al., 1994). Under stimulation of LH, the preovulatory follicles of the ewe are capable of synthesizing progesterone (Murdoch and Dunn, 1982) and prostaglandins (Murdoch et al., 1981). It has been suggested in other species that progesterone and/or prostaglandins may be mediators for follicular collagenase activity responsible for the degradation of the basement membrane between the theca interna and the membrana granulosa, which leads to eventual ovulation (Espey, 1974, 1980; Rondell, 1974).

It was not until an elaborate study performed by Murdoch and coworkers (1986), that the role of these hormones in ovine ovulation was elucidated. Ovine follicles were
isolated from ovaries at different intervals after the initial surge of LH and analyzed for concentrations of progesterone, prostaglandins and collagenase activity. They then performed the same measurements in the presence a progesterone antagonist or indomethacin (a prostaglandin biosynthesis inhibitor). These researchers found that in the presence of either or both of these substances ovulation would not occur. Their findings suggested that progesterone acts as an intermediate by influencing prostaglandin synthesis, primarily PGF$_{2\alpha}$, from the preovulatory follicle. They also found that PGF$_{2\alpha}$ is essential for increased collagenase activity and ovulation.

During luteinization, the granulosa and theca cells rapidly increase in number and any clotted blood in the follicular cavity is reabsorbed. Theca cells migrate into the follicular cavity and become dispersed among granulosa cells (O'Shea et al., 1980; Hadley, 1992a). Granulosa cells exhibit a smooth endoplasmic reticulum, their mitochondria become rounded with tubular cristae, and granules form within the cytoplasm (Niswender and Nett, 1988; Niswender et al., 1994). Mitotic activity will continue to occur in theca cells, but as the granulosa cells accumulate large amounts of cholesterol after ovulation, cell division virtually ceases (McClellan et al., 1975; Hadley, 1992a). It is this luteinization process that leads to the formation of the functional CL.

CHARACTERISTICS OF THE CORPUS LUTEUM IN THE NONPREGNANT ANIMAL

The corpus luteum, or "yellow body", is a transient endocrine gland that is required for the maintenance of pregnancy in mammals (Niswender and Nett, 1988; Niswender et al., 1994). After the ovum is ovulated, the cells of the recently ovulated follicle luteinize to form the CL and if the ovum is fertilized, the presence of an embryo within the uterus results in the maintenance of the CL (Niswender et al., 1985). The CL produces progesterone, which prepares the uterine endometrium for the implantation of
the fertilized ovum and maintains early pregnancy. The CL must remain functional for a certain period of gestation in order for a successful pregnancy, however, this duration of time varies with species. In rabbits (Browning et al., 1980), sows (Ellicott and Dzuik, 1973) and goats (Hafez, 1987), the CL are required for maintenance of pregnancy throughout gestation. The CL in cows must be present for the first 200 days of gestation and thereafter the adrenal gland is able to secrete enough progesterone to maintain pregnancy (Wendorf et al., 1983). In the ewe, the CL must remain functional for only 55 days of gestation at which time the placenta assumes the role as the primary source of progesterone and is responsible for the maintenance of pregnancy (Casida and Warwick, 1945; Cowie et al., 1963). In the mare, Holtan et al. (1979) reported that ovariectomy after days 50-70 of gestation did not terminate pregnancy, nor did it affect gestation length, incidence of placental retention, mammary development or milk production. Evidently the placenta assumes the role of the CL to maintain pregnancy after this time. These authors later discovered that the progesterone produced by the placenta is rapidly metabolized to other progestins (Holtan et al., 1991). If fertilization or implantation does not occur, ovulation will occur at the subsequent estrus after regression of the CL (Auletta and Flint, 1988).

Morphology and Biochemistry of the Corpus Luteum

The corpus luteum is composed of at least two morphologically and biochemically distinct steroidogenic cell types (Corner, 1919; 1945; Warbritton, 1934; Mossman and Duke, 1973; Niswender et al., 1985, Niswender and Nett, 1988). The most obvious difference between these two cell types is their size, leading to their designation as small and large luteal cells. The precise size range used for each type of cell varies among species and researchers. In general, ovine small luteal cells are 10-20 µm in diameter and account for 12-18% of total volume of the CL whereas large luteal cells range from 20-35 µm and account for 25-35% of the total luteal volume (Nett et al., 1976; Rodgers et al.,
1984; Stormshak et al., 1987). At the time of maximum progesterone secretion, during the luteal phase, vascular elements account for more than 11% of luteal volume, connective tissue 22-29%, and fibroblasts 7-11% of total volume (Nett et al., 1976; Rodgers et al., 1984).

The structural appearance of the two cell types is also distinct and has been reviewed at length by numerous researchers (Hansel et al., 1987; Niswender and Nett, 1988; Niswender et al., 1994; O'Shea et al., 1979; Rothchild, 1981; Stormshak et al., 1987). Small cells appear spindle-shaped with darkly stained cytoplasm and large lipid droplets. The nuclei are irregular in shape and convoluted, with about 10% of the small cell nuclei appearing to contain inclusions of cytoplasm (Rodgers and O'Shea, 1982). The Golgi complex is located along the periphery of the cell and is associated with the rough endoplasmic reticulum. Small cells contain numerous amounts of smooth endoplasmic reticulum and mitochondria with tubular and lamelliform cristae, both of which are characteristic of steroid secreting cells (Christensen and Gillim, 1969; Niswender et al., 1994).

Large cells are probably the most readily distinguishable luteal cells. They possess a spherical or polyhedral shape and contain a distinct nucleolus. These cells also contain elements of steroid secreting cells, abundant mitochondria and smooth endoplasmic reticulum, as well as stacks of rough endoplasmic reticulum (Christensen and Gillim, 1969; Niswender et al., 1994). In addition, large luteal cells contain electron dense secretory granules that are said to contain oxytocin and relaxin in some species (Niswender et al., 1994). Within the cytoplasm, there are some areas of clustering and exclusion of mitochondria (Enders et al., 1973). There is also an extensive Golgi complex located at one side of the nucleus and some indication that the smooth endoplasmic reticulum may be in direct communication with the Golgi cisternae (Fawcett et al., 1969).

Large and small cells have been hypothesized to have differentiated from granulosa membrana and theca interna cells, respectively. Donaldson and Hansel (1965) found that
while large luteal cells have a limited ability to divide, small luteal cells retained the capacity to multiply and therefore these researchers believed it to be conceivable that small cells are capable of growing into large luteal cells under the influence of gonadotropins. This hypothesis was supported by Niswender et al. (1985) who found that by treating ewes with human chorionic gonadotropin (hCG) that there was an increased number of large luteal cells by midcycle.

By using monoclonal antibodies against theca specific and granulosa cell specific antigens, Alila and Hansel (1984) set out to determine the origin of large and small cells of the bovine CL during the estrous cycle and early pregnancy. They found that during the early stages of the estrous cycle, days 4-6, the majority of large luteal cells bound granulosa antibody while small cells bound theca antibody. However, as the age of the CL increased, the number of large cells binding granulosa antibody decreased and more large cells were observed binding theca antibody. In fact, by day 100 of gestation luteal cells only bound theca antibody, suggesting that small luteal cells differentiate into large luteal cells as the CL ages (Alila and Hansel, 1984).

Although the research by Alila and Hansel (1984) is compelling evidence that small luteal cells may differentiate into large luteal cells, some controversy remains. For example, research conducted by O'Shea and coworkers (1986) compared cellular morphology of the CL by ultrastructural morphometry on days 9 and 13 of the cycle. These researchers found no difference in luteal volume, plasma progesterone concentration, or volume density of any component of the luteal tissue between days 9 and 13, suggesting that there is no transformation of small to large luteal cells. This supported their earlier findings in which they compared CL from day 10 of the estrous cycle and days 15, 25, 50, 100, 125 and 140 of gestation. By using light and electron microscopy the ultrastructure of the various CL were analyzed but no evidence was found that would suggest transformation of small to large luteal cells (O'Shea et al., 1979).
Hormones Synthesized by the Corpus Luteum

Small and large luteal cells are capable of synthesizing and secreting both steroid and protein hormones. In fact, the primary function of the CL is considered to be the production of the steroid hormone progesterone, which prepares the uterine endometrium for implantation and is responsible for the maintenance of early pregnancy (Auletta and Flint, 1988). In some species estrogen, another steroid hormone, is also manufactured by the CL and is important for up-regulating progesterone receptors in target tissues. Protein hormones, such as oxytocin, are produced by the large luteal cells in most species, and are essential for interacting with uterine hormones that regulate the life span of the CL.

Steroid Hormones

Steroid hormones are synthesized by complex multiple-enzyme systems located in the mitochondria, smooth endoplasmic reticulum and cytoplasm (Hadley, 1992a; Miller, 1988). The initial substrate for luteal steroidogenesis is cholesterol, which may be extracted from low or high density lipoproteins (LDL and HDL) in the circulation, released from intracellular stores of cholesterol esters or synthesized de novo from acetate. Transfer of cholesterol to the mitochondria appears to involve the cytoskeleton and may be stimulated by cAMP and activation of protein kinase A (Wiltbank et al., 1993). It is within the inner mitochondrial membrane where the rate-limiting step in progesterone biosynthesis occurs: the cleavage of the cholesterol side chain by the cytochrome P450 side-chain cleavage enzyme (Niswender et al., 1994). Further conversion of cholesterol occurs within this organelle with the aid of various enzymes for the production of pregnenolone. Pregnenolone is then transported to the endoplasmic reticulum, where it is converted to the progesterone. Depending upon the stimulation and the stage of the estrous cycle, progesterone may be the end product or further converted by lyase to testosterone and then aromatized to estradiol-17β (Savard, 1973). These steroid hormones are then passively transported out of their cell of origin and are bound to
specific plasma proteins to protect the steroid hormones from premature degradation.

Upon reaching the target tissue, steroid hormones diffuse through the membrane and cytosol of the cell to bind to their receptors located within the nucleus. These steroid-receptor complexes dimerize, bind to steroid hormone response elements (HRE) on DNA and influence gene transcription (Hadley, 1992a).

There are species differences in the biosynthetic potential of the CL. For example, whereas the human CL will secrete progesterone, androgens and estrogens, the bovine CL appears to lack both lyase and the aromatizing enzyme system so that no significant amounts of either androgens or estrogens are produced (Miller, 1988; Savard, 1973). The CL of the sow (Cook et al., 1967) and ewe (Kaltenbach et al., 1967) appear to produce 17α-hydroxyprogesterone and 4-androstenedione in addition to progesterone, but not estrogens. The two steroid hormones that will be discussed in detail in this review are progesterone and estrogen.

**Progesterone**

It was once believed that secretion of progesterone from the CL was an active process rather than a passive one. Gemmell and Stacy (1979) found that upon treatment of the ovine CL with cyclohexamide, an inhibitor of protein synthesis, there was an inhibition of the formation and secretion of densely stained granules of the luteal cells with an associated decrease in progesterone secretion. Because of these findings these researchers hypothesized that progesterone is stored in secretory granules from the Golgi bound with a high affinity to a protein and released from the cells by exocytosis.

However, this hypothesis was challenged by Rodgers and coworkers (1983) when they separated small and large luteal cells and measured the concentration of progesterone produced by each cell type under the stimulation of LH. These scientists found that under the influence of LH, small luteal cells appear to be the major source of progesterone, secreting about three times more than large luteal cells. They concluded that because
small luteal cells are virtually devoid of any secretory granules, progesterone must be stored and secreted in some other manner.

The work of Hirst et al. (1986) further supported the hypothesis that progesterone was not stored in granules when they found that oxytocin, not progesterone, was associated with and actively released by luteal secretory granules. This was demonstrated by an in vitro incubation of luteal cells with or without calcium and by measuring changes in progesterone or oxytocin secretion. Their results indicated that oxytocin secretion is calcium dependent, which is indicative of exocytotic events, while progesterone is not. Similar results were found by Rice et al. (1986) when they separated particle-associated progesterone and oxytocin and subjected these hormones to treatments that stimulate release from granules. Their results indicated that progesterone is not stored in a protein-bound form in luteal secretory granules, but rather particle-associated progesterone may intercalate into cell membranes for ultimate release.

Secretion of progesterone is required for the growth and development of the uterus in preparation for the fertilization of the ovum as well as to suppress further ovulation (Hadley, 1992a). In order to fulfill this role, the CL must remain fully functional for a specific period of time. In some species such as sheep and primates, the placenta will take over the role as the major source of progesterone for the remainder of the pregnancy (Auletta and Flint, 1988); in cattle the adrenal gland assumes this role (Wendorf et al., 1983), whereas in others such as the pig, goat and rabbit, the CL remains the sole source of progesterone throughout gestation (McCracken, 1984). To suppress ovulation, progesterone is capable of inhibiting pituitary LH secretion either directly or through the hypothalamus by reducing GnRH release (Girmus and Wise, 1992, Hadley, 1992b; Karsch et al., 1987).

Progesterone administered early in the estrous cycle, prior to when it normally reaches biologically active levels, has been shown to induce premature secretion of PGF$_{2\alpha}$ and luteolysis in both cows and sheep (Garrett et al., 1988; Ginther, 1970; Ottobre et al.,
Ginther and Woody (1970) demonstrated that the detrimental effect of progesterone administration early in the estrous cycle was mediated by the uterus when they treated unilaterally hysterectomized ewes and cows with progesterone. Upon morphological analysis of the ovaries on day 14 of the cycle they found that only the ovary ipsilateral to the remaining uterine horn responded with a decrease in luteal weight, whereas the CL in the ovary opposite the remaining horn remained unchanged. Oxytocin has been shown to stimulate uterine secretion of PGF$_{2\alpha}$ in ovariectomized ewes and cows only after the animal has been exposed to progesterone for 7-10 days (Homanics and Silvia, 1988; LaFrance and Goff, 1988). Therefore, the action of progesterone on PGF$_{2\alpha}$ secretion may be indirect by affecting uterine secretory responsiveness to oxytocin during the estrous cycle. Vallet et al. (1990) reinforced the indirect action of progesterone for normal luteolysis when they reported that oxytocin receptor concentrations increased after treatment of ovariectomized ewes with exogenous progesterone followed by estradiol. Zhang et al. (1992) suggested that while progesterone priming is important for luteolysis in the ewe, the increase in estradiol concentration after the withdrawal of progesterone actually causes the increase in oxytocin receptor numbers. Further, active immunization of nonpregnant ewes against progesterone has been shown to result in an erratic cycle and is frequently associated with prolonged maintenance of the CL (French and Spennetta, 1981; Thomas et al., 1985). In addition to the effects of progesterone on oxytocin receptor development, this steroid also appears to affect uterine tissue more directly by changing the uterine secretory responsiveness to oxytocin. The accumulation of lipid droplets in uterine epithelial cells of the ewe has been demonstrated to be a progesterone-dependent process (Brinsfield and Hawk, 1973). Further, the amount of immunoreactive prostaglandin H endoperoxide synthase (PGH synthase) activity, the primary enzyme required for PGF$_{2\alpha}$ production in uterine epithelial cells, is increased when ovariectomized ewes are treated with progesterone (Raw et al., 1988). Further, treatment of intact ewes with progesterone early in the estrous cycle induces a premature increase in the
concentrations of mRNA coding for PGH synthase in uterine tissue as well as premature luteolysis (Eggleston et al., 1990). These data imply that progesterone may act both directly by stimulating synthesis of PGH synthase and indirectly by upregulating OT receptors on the uterine endometrium to increase PGF$_{2\alpha}$ production.

In contrast to the luteolytic effects of progesterone, late administration of this steroid can actually prolong the duration of the estrous cycle (Sirois and Fortune, 1990). Ginther (1970) demonstrated that exogenous progesterone administered on days 8 to 11, 12 to 15 or 16 to 19 of the bovine estrous cycle significantly prolonged the cycle when compared to controls. Further, progesterone supplementation has been used by sheep and cattle producers to compensate for inadequate luteal function in young animals or "repeat-breeder" animals (animals that must be bred several times to achieve pregnancy), and has been shown to increase fertility in some cases (McMillan, 1987; Robinson et al., 1989). However, it is important to note that progesterone supplementation does not increase the life span of the CL. Ginther (1971) found that progesterone supplementation over a 4 day period either early or late in the ovine estrous cycle caused a decrease in number of large follicles at necropsy, regardless of the time during the cycle supplementation occurred. Therefore, administration of progesterone late in the estrous cycle does not appear to prolong the life span of the CL, but rather acts at the level of the hypothalamus and(or) the pituitary to suppress release of the gonadotropins necessary for ovulation (Karsch et al., 1987; Girmus and Wise, 1992).

**Estrogen**

Just as progesterone is the major steroid hormone secreted by the ovary during the luteal phase, estrogen is the primary steroid produced during the follicular phase. In most species estrogens are produced primarily by ovarian follicles and high estrogen levels reflect periods of increased follicular growth. However, in the human and non-human
primate, the corpus luteum contains all the enzymes needed for the both progesterone and estrogen synthesis (Niswender and Nett, 1988).

During follicular growth, estradiol production results from the coordinated steroidogenic activity of the theca interna and the granulosa cells. This was first demonstrated by Falck (1959) in rat ovarian follicles and was later shown to be the case in the follicle of pigs (Evans et al., 1981), cows (Fortune, 1986; Fortune, 1994), ewes (Armstrong et al., 1981) and primates (McNatty et al., 1979). Various in vitro research led to the formation of the "two-cell theory" of follicular estrogen synthesis, which states that LH stimulates the production of androgens from the theca interna, which are then transported to the granulosa cells where they are converted to estrogens under the influence of FSH. According to this hypothesis, theca cells lack aromatase activity and are unable to convert androgens to estrogens, while the granulosa cells are incapable of producing androgens. Therefore, these cells must work together in order to produce estrogens. In vitro research with bovine follicles further suggests that progestins produced by the granulosa cells, primarily pregnenolone, may act back upon the theca cells and act as a precursor for androgen production, thereby regulating estrogen synthesis (Fortune, 1986).

While there seems to be no doubt that these two follicular cell types interact extensively for the production of estrogen, this interaction seems to vary somewhat among different species. Evans and coworkers (1981) demonstrated that porcine theca cells, in addition to granulosa cells, are capable of producing some estrogens in vitro. Armstrong et al. (1981) reported similar findings in ovine medium or large preovulatory follicles. However, regulation of granulosa estrogen production may still be regulated by theca cells. Furthermore, human granulosa cells in culture have been shown to be capable of de novo androgen synthesis although interaction between the two follicular cell types still exists (McNatty et al., 1979).
An appropriate endocrine balance is essential for ovarian follicular development as well as female sexual behavior. Behavioral estrus begins around the time of maximal estradiol-17β production and the coincident preovulatory LH surge (Thatcher and Chenault, 1976), and estrogens have been implicated as having an essential role in sexual behavior. In fact, sexual behavior is inhibited by ovariectomy in domestic animals, but can be restored in the cow and sow by progesterone pretreatment followed by estrogen administration (Hafez, 1987). Further, administration of estradiol benzoate alone has been shown to induce sexual receptivity in ovariectomized heifers and cows (Cook et al., 1986). In the ewe and the sow there appears to be a linear dose-response relationship between the duration of estrus and the logarithm of the dose of estrogen (Hafez, 1987). In cattle, however, this does not seem to be the case, although estrogens still appear to be responsible for estrous behavior (Glencross et al., 1981).

Saumande and Lopez-Sebastian (1982) reported that serum concentrations of estradiol-17β, along with other estrogens, were elevated in association with increased ovulation rate. This increase in estrogen production may be associated with the essential role of estradiol in triggering the preovulatory gonadotropin surge (Legan and Karsch, 1979). In fact, active immunization of ewes against estradiol blocked the preovulatory LH surge in addition to inhibiting behavioral estrus (Rawlings et al., 1978).

The effects of estradiol on ovulatory events occur at both the level of the hypothalamic-hypophyseal axis, as mentioned previously, and at the level of the ovary itself. At the level of the hypothalamic-hypophyseal axis, estradiol exerts a biphasic effect on the secretion of LH (Clarke and Cummins, 1985; Herman and Adams, 1990). Initially, administration of estradiol reduces serum concentrations of LH and pituitary responsiveness to GnRH (Herman and Adams, 1990; Nett et al., 1990). After the initial inhibitory period, estradiol stimulates pituitary GnRH receptor formation (Clarke et al., 1988; Gregg and Nett, 1989; Gregg et al., 1990) and frequency of GnRH pulses (Moenter et al., 1990), thereby inducing a preovulatory-like surge of LH (Nett et al., 1984). At the
level of the ovary, estradiol has been shown to enhance the FSH stimulated LH-receptor formation on rat granulosa cells in culture (Rani et al., 1981). Goldenberg et al. (1972) examined the effects of diethylstilbestrol (DES) in hypophysectomized rats and found that administration of DES alone significantly increased ovarian weights and follicular antrum formation and that concomitant injection of FSH further enhanced ovarian development. Further, Welsh and coworkers (1983) found that while LH or FSH increased production of progesterone in the rat granulosa cell in vitro, addition of either DES or estradiol also enhanced the ability of the granulosa cells to produce progesterone.

The effects of estradiol are not restricted to follicular and ovulatory events, but can also influence the life span of the corpus luteum. Researchers determined that injection of estradiol-17β during the ovine luteal phase resulted in a significant decrease in luteal weight indicative of premature luteal regression (Bolt and Hawk, 1975; Stormshak et al., 1969). However, ewes that were totally hysterectomized did not respond to the luteolytic effect of injected estradiol (Stormshak et al., 1969), demonstrating that estradiol may act through the uterus. This theory was further supported by the research of Akbar et al. (1971) who determined that in estradiol-treated unilaterally hysterectomized ewes, CL in the ovary adjacent to the intact uterine horn were significantly smaller in size than the CL of the other ovary. Destruction of the ovarian follicles, the source of estrogen synthesis, has been shown to delay luteal regression in sheep (Karsch et al., 1970). Howland et al. (1971) treated ewes with a single injection of estradiol on either day 4 or 11 of the estrous cycle. They found that while injection of estradiol on day 4 increased CL weight, treatment on day 11 decreased the weight of the CL significantly over controls. Caldwell et al. (1972) found that injection of exogenous estradiol to progesterone-primed ovariectomized ewes caused increased plasma PGF$_{2\alpha}$ concentrations. Similarly, infusion of physiological levels of estradiol into the arterial supply of an autotransplanted uterus during the late luteal phase of the cycle resulted in elevated PGF$_{2\alpha}$ release within 60-90 min of administration. Further, increased synthesis of PGF$_{2\alpha}$ from uterine tissue in vitro
has been found after estradiol treatment of progesterone-primed ewes. Similarly, treatment of ovariectomized ewes with estradiol has been shown to increase uterine synthesis of PGF$_{2\alpha}$ (Homanics and Silvia, 1988; Ottobre et al., 1984; Sharma and Fitzpatrick, 1974; Vincent and Inskeep, 1986).

The action of estradiol on luteolysis is most likely by facilitating the action of oxytocin. This is supported by Holtorf et al. (1989) who found that there was a high correlation between the amount of oxytocin secreted by bovine granulosa cells and the estrogen concentration in the corresponding follicular fluid. In fact, both estradiol and progesterone appear to stimulate uterine prostaglandin production indirectly by controlling the availability of endometrial oxytocin receptors, as demonstrated by Zhang et al. (1992), in progesterone-primed ovariectomized ewes treated with estradiol. Estradiol alone also has been implicated to increase oxytocin receptor formation (McCracken et al., 1978). These investigators demonstrated that when estradiol was infused into the systemic circulation for 12 hours, formation of oxytocin receptors occurred within 6 hours, as evidenced by a significant increase in oxytocin-induced PGF$_{2\alpha}$ secretion. This was confirmed by Hixon and Flint (1987) who found that induction of oxytocin receptors occurs within 12 hours of estradiol administration in nonpregnant ewes. Burgess et al. (1990) also found that long-term infusion of estradiol into ewes enhanced uterine responsiveness to oxytocin, possibly by increasing uterine oxytocin receptor concentrations.

**Peptide Hormones: A Focus on Oxytocin**

The majority of vertebrate hormones are peptide (protein) in nature, meaning they are made up of specific amino acid sequences. In peptide secreting tissues, hormone biosynthesis occurs in the rough endoplasmic reticulum, specifically by membrane attached ribosomes. Ribosomes attach to the mRNA of the protein hormone and translates the nucleotide sequence into amino acids until the precursor protein hormone is complete
Most polypeptide hormones and neuropeptides are synthesized as inactive protein precursors, called pre-pro-hormones, from which the active hormone is derived.

The nascent pre-pro-hormone is released from the ribosome and transported into the cisternae of the rough endoplasmic reticulum where the pre-piece, consisting of the signal peptide, is cleaved off. The pro-hormone is then transported to the Golgi where it is packaged with proteolytic enzymes and pinched off the terminal cisternae into secretory vesicles which are then distributed within the cytoplasm. The conversion of the pro-hormone to the active peptide hormone begins within the Golgi complex and continues in the secretory vesicles (Sheldrick and Flint, 1989; Wallis et al., 1985).

Secretion of protein hormones occurs by calcium-dependent exocytosis. With an influx of calcium, secretory granules are transferred from the cytoplasmic vesicle pool to the plasma membrane, where the vesicle fuses with the plasma membrane and releases its contents. The vesicle can then be recycled back into the Golgi where it may be utilized again (Wallis et al., 1985). The mammalian corpus luteum is the source of several peptide hormones; the most significant is the peptide hormone oxytocin.

Oxytocin originates from a larger precursor which contains a signal sequence, the nonapeptide oxytocin, the protein neurophysin I, and another peptide of unknown function. Post-translational processing leads to production of the mature peptide and its associated neurophysin I, which is thought to act as a carrier for oxytocin (Ivell and Richter, 1984). This complex is packaged into membrane bound granules, which contain proteolytic enzymes that will cleave oxytocin from neurophysin I (Ivell et al., 1985).

Oxytocin synthesis was originally believed to take place only in the hypothalamus and transferred down axons to the posterior pituitary where it was subsequently stored and released into the systemic circulation. Functions usually associated with oxytocin include the stimulation of uterine contractions during parturition and the milk ejection reflex (Ivell et al., 1985). However, with research indicating that oxytocin may cause
luteolysis in cattle if administered early in the estrous cycle (Armstrong and Hansel, 1959), along with research demonstrating that immunization against oxytocin prolongs the duration of the ovine estrous cycle (Sheldrick et al., 1980), the importance of oxytocin in luteal function was realized. Further evidence that oxytocin plays a role in luteal function was provided by McCracken (1980). He proposed that oxytocin acts by stimulating uterine PGF$_{2\alpha}$ production and that steroid hormones, such as progesterone and estradiol, may regulate PGF$_{2\alpha}$ secretion by controlling the number of oxytocin receptors in uterine endometrial tissue (McCracken, 1980; Roberts et al., 1976).

It was not until an observation made by Wathes and Swann (1982), when testing peptide extracts of the ovine CL, that a second storage site for oxytocin was revealed. Testing ovine luteal peptide extracts for relaxin-like activity on rat uterine strips, they observed that some fractions were stimulatory rather than inhibitory. Upon further investigation these researchers found that a similar observation was reported for cows (Fields et al., 1980). Wathes and Swann (1982) tested the ovine luteal extract for cross-reactivity with oxytocin and found that the luteal fractions did indeed contain oxytocin. However, it was still not known at this time whether oxytocin was produced or simply sequestered and stored in the CL. By examining luteal cells via immunocytochemical techniques, it was determined that neurophysin I and oxytocin are found together in granules within the large luteal cells of the ovine (Rodgers et al., 1983) and bovine corpus luteum (Guldenaar et al., 1984; Schams et al., 1985b). This colocalization reflects de novo synthesis of oxytocin and neurophysin I.

Since the initial observation that the ovine CL contained oxytocin, this peptide has been shown to be produced in the CL of many other species such as humans (Fields et al., 1983), non-human primates (Khan-Dawood et al., 1984; Khan-Dawood, 1986), pigs (Jarry et al., 1990) and goats (Kiehm et al., 1989). Ovarian oxytocin is synthesized and secreted primarily by the large luteal cells and in smaller quantities by the granulosa cells of preovulatory follicles (Guldenaar et al., 1984; Schams et al., 1985a). Oxytocin is
secreted into the ovarian vein (Flint and Sheldrick, 1982) where it may act via systemic route on the adjacent uterine horn to regulate luteal life span (McCracken et al., 1972; Sheldrick and Flint, 1983).

The capacity of these ovarian cells to synthesize oxytocin changes dramatically at different stages of the estrous cycle (Wathes et al., 1992). In both bovine and caprine preovulatory follicles, oxytocin is detectable in significant amounts only when sampled after the gonadotropin surge (Kiehm et al., 1989; Voss and Fortune, 1991). Furthermore, Voss and Fortune (1991) found that bovine granulosa cells isolated before the LH/FSH surge responded to gonadotropins added to culture with increased oxytocin secretion, suggesting a role for oxytocin in the regulation of luteinization and ovulatory events.

Luteal concentrations of oxytocin in cattle and sheep increase during the early part of the luteal phase and decline thereafter (Ivell et al., 1985; Sheldrick and Flint, 1981; Wathes et al., 1984). In the ovine CL, mRNA concentrations of oxytocin and its prohormone peak around day 3 (Ivell et al., 1990; Jones and Flint, 1988); however, peak luteal concentrations of oxytocin do not occur until day 6 of the cycle (Sheldrick and Flint, 1989). Similarly, in the bovine CL, the maximum amount of mRNA is attained around day 3 of the cycle and the oxytocin content peaks around day 8 (Abdelgadir, 1987; Ivell et al., 1985). After this time the concentrations of oxytocin and its mRNA decline, such that low levels are present just prior to luteolysis. Flint and Sheldrick (1983) found when taking frequent blood samples from ewes around the time of luteolysis that there are small episodic releases of luteal oxytocin. They surmised this pulsatile release of oxytocin may be in response to uterine release of PGF$_{2\alpha}$ (Flint and Sheldrick, 1983).

Luteal oxytocin has been postulated to have two major actions: a paracrine action on luteal tissue to regulate steroid secretion and an endocrine action on the uterus to increase secretion of PGF$_{2\alpha}$ and hence hasten luteolysis. In porcine luteal cell cultures, there is a dose-dependent inhibition of progesterone secretion but a stimulation of estradiol release in response to oxytocin. The inhibitory effect of oxytocin on
progesterone secretion was abolished in the presence of an oxytocin antagonist, which suggests a receptor-mediated effect (Pitzel, 1988). However, in other species the evidence of a direct effect of oxytocin on luteal steroid secretion has been conflicting.

Pretreatment of caprine luteal tissue treated in vitro with oxytocin significantly inhibited hCG-enhanced secretion of progesterone production. However, treatment with an oxytocin antagonist early in the estrous cycle also inhibited progesterone production in this species (Homeida and Al-Eknah, 1992). Studies of progesterone production by dispersed bovine and ovine luteal cells have failed to demonstrate any inhibitory action of oxytocin alone or with LH and hCG (Rodgers et al., 1985). Further, oxytocin receptors have been reported in porcine, bovine and ovine luteal tissue, but research has been equivocal as to time of development, and control or action of these receptors. Luteal oxytocin receptors have been shown to be present in the porcine and bovine CL during the early luteal phase and decline thereafter (Jarry et al., 1990; Okuda et al., 1992). However, this was contradicted earlier by reports of Fuchs et al. (1990b) who found that in bovine luteal tissue the oxytocin receptor concentration was highest late in the estrous cycle. In addition, luteal oxytocin receptors have been found in pregnant but not cycling ewes (Sernia et al., 1989). While evidence for the paracrine action of oxytocin in regulating steroidogenesis remains unclear, its endocrine action and involvement in luteolysis have been well characterized, especially in ewes and cows.

As mentioned previously, exogenous oxytocin has been shown to shorten the estrous cycle in cows if administered between days 3-6 of the cycle (Armstrong and Hansel, 1959; Hansel and Wagner, 1960). However, if oxytocin is given either earlier or later it appears to have no effect on development of the CL or cycle length (Donaldson et al., 1965; Hansel and Wagner, 1960; Harms et al., 1969). Similar findings were demonstrated in the goat, with the daily administration of oxytocin early in the estrous cycle resulting in the premature demise of the CL, whereas active immunization against oxytocin prolonged the luteal phase (Cooke and Homeida, 1985). Active immunization
against oxytocin also increased the length of the estrous cycle in ewes (Sheldrick et al., 1980; Wathes et al., 1989). These observations provide strong evidence that oxytocin may act as a luteolytic factor late in the estrous cycle. Furthermore, oxytocin administration late in the estrous cycle of ewes has been shown to increase uterine secretion of PGF$_{2\alpha}$ (Fairclough et al., 1984), a substance which has been identified as the primary luteolysin (McCracken et al., 1972). Similarly, exogenous PGF$_{2\alpha}$ has been shown to stimulate luteal oxytocin and its associated neurophysin release from the ovine CL (Flint and Sheldrick, 1983; Watkins and Moore, 1987).

Oxytocin receptors have been reported to exist in the uterine endometrium and myometrium of many mammals. Oxytocin has been reported to enhance myometrial activity in the form of uterine contractions (Fitzpatrick, 1960; Roberts and McCracken, 1976). The overall concentration of bovine and ovine uterine oxytocin receptors increase over the late luteal phase and peak on the day of behavioral estrus (Fuchs et al., 1990a; Roberts et al., 1976; Sheldrick and Flint, 1985). In sheep, the increase in oxytocin receptor concentrations occurs in the endometrium, caruncular stroma and myometrium, and appears to be under the influence of estradiol because there is a strong positive correlation between oxytocin and estradiol receptor development (Ayad et al., 1991; Wathes and Hamon, 1993). However, in cows the concentrations of uterine oxytocin receptors in myometrium show little variation over the estrous cycle while endometrium concentrations do increase in association with estradiol (Fuchs et al., 1990a). The development of oxytocin receptors in the endometrium during the time of luteolysis is of particular interest because the endometrium has been shown to be the site of prostaglandin synthesis, further substantiating the role of oxytocin in luteal regression (Cerini et al., 1979; Salamonsen and Findlay, 1990).

Development and concentration of uterine oxytocin receptors seems to be regulated primarily by the steroid hormones estrogen and progesterone. Treatment with estradiol-17β has been shown to promote uterine secretion of PGF$_{2\alpha}$ in response to
oxytocin stimulus in ewes (McCracken et al., 1984; Sharma and Fitzpatrick, 1974) and to increase the number of specific binding sites for oxytocin in the uterus of ewes (Hixon and Flint, 1987), rabbits (Nissenson et al., 1978) and rats (Soloff, 1975). Presence of progesterone has been demonstrated to be initially inhibitory to ovine oxytocin receptor formation, but near the end of the luteal phase it becomes facilitory (Levitt et al., 1983; Vallet et al., 1990). In fact, progesterone pretreatment is necessary before estrogen can maximally stimulate oxytocin receptor production in the ewe (Homanic and Silvia, 1988; McCracken et al., 1984; Vallet et al., 1990; Zhang et al., 1992). Additionally, maximal responses to oxytocin in terms of PGF$_{2\alpha}$ secretion in vivo are elicited by administration of estrogen after prolonged treatment of progesterone (McCracken et al., 1981). Therefore, progesterone alone or in the presence of estradiol causes a decrease in uterine oxytocin receptors, while estradiol given after progesterone withdrawal increases the number of oxytocin receptors (Zhang et al., 1992). This increase in oxytocin receptors also coincides with an increase in the pulsatile release of PGF$_{2\alpha}$ from the uterus (Sheldrick and Flint, 1988) and is similar to the hormone profile observed around the time of luteolysis.

Beard and Hunter (1994) injected estradiol into ovariectomized ewes for 2 days, one-half of which had undergone progesterone pretreatment for 10 days, then subsequently injected these animals with both estradiol and progesterone for either 0, 2, 3, or 4 days. Following injections with estradiol and progesterone ewes were treated with an oxytocin challenge and endometrial tissue oxytocin receptor numbers were analyzed. These researchers found that both groups of ewes were able to form endometrial oxytocin receptors, regardless of progesterone pretreatment, but that the number of endometrial oxytocin receptors in the progesterone pretreated ewes declined rapidly after day 0 while those non-pretreated ewes had a slower decline in oxytocin receptor concentration. These authors hypothesized that the slow decline of endometrial oxytocin receptors in ewes, not pretreated with progesterone, may induce premature luteolysis. This is because the oxytocin-PGF$_{2\alpha}$ positive feedback mechanism that induces luteolysis may be able to act
earlier in these animals. Further, they postulated it is because of this mechanism that the first estrous cycle during seasonal anestrous or the post-partum period, when progesterone concentrations are low, often result in premature luteolysis.

**HYPOTHALAMIC-HYPOPHYSSEAL HORMONES INFLUENCING THE CORPUS LUTEUM DURING THE ESTROUS CYCLE**

The life span and function of the mammalian corpus luteum are regulated by complex interactions between the hormones secreted from the hypothalamus, pituitary, uterus, as well as the ovary and CL themself. The hypothalamus secretes gonadotropin-releasing hormone (GnRH) which indirectly influences the life span of the CL in domesticated animals by regulating the secretion of the pituitary gonadotropins. These gonadotropins then act directly on the ovary and participate in regulating the development of the CL. Additionally, ovarian hormones such as oxytocin may act to stimulate the release of uterine hormones, which may bring about the ultimate demise of this transient endocrine organ.

**Hypothalamic Hormones: An Overview of Gonadotropin-Releasing Hormone**

Of all the hypothalamic hormones known to exist, only GnRH has been implicated in affecting ovarian function. This well conserved mammalian decapeptide hormone is synthesized and released from the hypothalamus (Hadley, 1992b). The actions of GnRH are essential for normal follicular growth, ovulation and the maintenance of luteal functions via the control of progesterone synthesis by luteinizing hormone.

Many questions surround the mechanism by which GnRH regulates the secretion of LH and FSH from gonadotropes at different times during the estrous cycle. At the time of ovulation, both LH and FSH are secreted in response to GnRH stimulation. Shortly after the initial surge of both gonadotropins, FSH has a secondary release that seems to be important to increase follicle growth and possibly ensure ovulation in cyclic ewes (Wallace
This rebound release of FSH appears to be independent of GnRH secretion because treatment with a GnRH antibody failed to inhibit its release (Narayana and Dobson, 1979). As the estrous cycle continues to progress, FSH secretion controls follicular development and recruitment without the concomitant release of LH.

Because of the differential control over the pituitary gonadotropes by GnRH, it was initially hypothesized that the release of LH and FSH were controlled by two different substances. These substances were termed luteinizing hormone-releasing hormone (LH-RH) and follicle stimulating hormone-releasing hormone (FSH-RH), respectively (Schally and Kastin, 1970). However, with improved protein purification technology, porcine LH-RH was isolated and found to be capable of stimulating the release of both LH and FSH in vivo or in vitro (Schally and Kastin, 1970). This purified hypothalamic extract was also shown to stimulate the release of both LH and FSH in rats, chimpanzees and humans (Schally and Kastin, 1970).

It is now fairly well accepted that GnRH is the sole hypothalamic peptide hormone that induces the secretion of both LH and FSH. Nonetheless, the differential release of these hormones suggests that other modulators, such as steroid hormones or hypothalamic neurotransmitters may assist in controlling the release of LH and FSH (Hadley, 1992b). The hormones that have been implicated in controlling the action of GnRH on gonadotropin secretion are the ovarian steroid hormones progesterone and particularly estradiol.

The mechanism of action by which GnRH affects ovarian function and luteal life span may be either indirectly, through the regulation of the pituitary gonadotropes, or may be a direct effect on the ovary. Rippel and Johnson (1976) were among the first researchers to provide evidence that GnRH injection may act directly to alter luteal function in rats. These researchers found that treatment of immature hypophysectomized rats with GnRH inhibits hCG-induced uterine and ovarian weight gain. Additional research demonstrating the direct effects of GnRH was conducted on adult
hypophysectomized diethylstilbestrol-treated female rats that were induced to ovulate with injection of LH after FSH priming. Luteal function in these animals was subsequently maintained by daily injections of prolactin, which increased serum progesterone level; however, if GnRH was injected in addition to prolactin, there was a dose-dependent decrease in progesterone concentration as well as ovarian LH receptor content (Jones and Hsueh, 1980). These authors also conducted experiments on rat luteal cells in vitro, incubating luteal cells with FSH, LH and prolactin in the presence or absence of a GnRH-agonist. The control cells, incubated without GnRH-agonist, responded with a threefold increase in progesterone secretion (in the presence of the pituitary hormones) while those incubated in the presence of GnRH-agonist responded with a dose-dependent decrease in progesterone secretion (Jones and Hsueh, 1980). Furthermore, receptors for GnRH have been detected in rat luteal tissue, thus supporting the hypothesis that this peptide acts directly at the level of the ovary to affect luteal function in this species (Clayton et al., 1979; Hsueh and Jones, 1983).

In domesticated animals, however, the mechanism of action of GnRH in altering luteal function appears to be indirect via the pituitary gonadotropes. The most compelling evidence for such action was provided by Brown and Reeves (1983) who found that luteal tissue from ewes, cows and sows were devoid of GnRH receptors. Injection of GnRH has been demonstrated to induce ovulation in seasonal anestrous ewes by inducing a preovulatory increase of LH (McLeod et al., 1982). Similarly, an injection of estradiol benzoate can also cause a GnRH-induced surge of LH in anestrous animals (Clarke, 1988). In addition, Rodger and Stormshak (1986) found that injection of GnRH into beef cows on either day 2 or 10 of the estrous cycle caused an immediate increase in LH secretion and a subsequent decrease in serum progesterone without affecting estrous cycle length. These authors also discovered that the administration GnRH not only caused an increase in serum LH, but also caused a down-regulation of luteal LH receptors. Slayden and Stormshak (1990) found that administration of GnRH to ewes on day 2 of the estrous
cycle resulted an immediate increase in serum LH and a decrease in serum progesterone by day 6 of the estrous cycle. This effect could be mimicked by injecting ovine LH alone on day 2 of the estrous cycle. Therefore, the effect of GnRH on luteal function in domesticated animals appears to be indirect via the pituitary hormone LH.

To determine how GnRH may act through LH to compromise luteal function Martin et al. (1990) administered GnRH to beef cows on days 2 and 8 of the estrous cycle and similarly found that its administration increased LH while attenuating progesterone. However, when the CL were removed and incubated on day 10 of the estrous cycle, these authors found that luteal tissue from cows treated with GnRH had higher basal secretion of progesterone but had a reduced response to LH when the gonadotropin was added to the culture. A similar study in which GnRH was injected into dairy cows 12 hours after the onset of behavioral estrus resulted in an increase rather than a decrease in serum progesterone levels (Mee et al., 1993). Corpora lutea collected on day 10 from treated and control cows had similar basal secretion of progesterone; however, when tissue was incubated in the presence of LH luteal tissue from GnRH-treated cows responded with lower production of progesterone compared to tissue from control cows. Upon morphological examination, the CL from those cows treated with GnRH had a higher proportion of large luteal cells than those of untreated cows, and a lower number of small luteal cells, which contain receptors for LH (Mee et al., 1993). Therefore, it has been postulated that LH secretion, stimulated by exogenous GnRH, may act on luteal tissue to cause the transition of small to large luteal cells.

The quantity of LH release from the pituitary is presumably controlled, not only by the amount of GnRH released from the hypothalamus, but also by the number of GnRH receptors on the pituitary gonadotropes themselves. Nett et al. (1981) demonstrated the ability of GnRH to regulate its own pituitary receptor by infusing ovariectomized ewes with low levels of GnRH for either 12 or 24 hours. These authors found that ewes that were exposed to a constant low level of GnRH experienced a down-regulation of pituitary
receptors. Further, if these ewes were challenged with a high amount of GnRH after infusion, release of LH would occur but at attenuated levels. Gonadotropin-releasing hormone receptors may also be down-regulated when exposed to a GnRH antagonist. Treatment with an antagonist results in a reduction in GnRH receptor availability, however, this reduction does not affect overall LH secretion rates unless the number of pituitary GnRH receptors are reduced by more than 50% (Wise et al., 1984). This finding comes as no surprise because in many target tissues only a small percentage of receptors need to be occupied in order to achieve maximal stimulation. The relationship between a high number of receptors available and a maximal response achieved by binding only a fraction of the total receptors, is termed the spare receptor hypothesis. This allows sensitivity of the cell to low amounts of circulating hormone such that even a small quantity of hormone can provoke a biological effect. Estradiol also seems to play a role in GnRH receptor availability. Gregg et al. (1990) found that cultured ovine pituitary cells treated with various concentrations of estradiol increased the number of GnRH receptors in a dose-dependent manner. Similar findings have been found in vivo (Clarke et al., 1988). Taken together, these results indicate that GnRH pulse frequency and steroid concentrations are important factors in regulating GnRH receptor concentration and regulation of gonadotropin secretion.

Pituitary Hormones

The pituitary gland has been implicated as the source of luteotropic substances that regulate luteal development and function. The importance of the pituitary gland as a source of luteotropins in the ewe was demonstrated by Kaltenbach et al. (1968). These researchers found that when ewes were hypophysectomized on the day after normal ovulation a functional CL failed to form, while hypophysectomy on day 5 of the estrous cycle caused the regression of the partially formed corpus luteum. Additional research by Denamur et al. (1973) demonstrated that when hysterectomized ewes having maintained
CL were hypophysectomized, the CL regressed. However, hypophysectomy or hypophyseal stalk-section in some rodents shortly after ovulation does not alter the normal life span of the corpus luteum (Greenwald and Rothchild, 1968; Illingsworth and Perry, 1971), while hypophysectomy during early pregnancy, before day 10 in the mouse, results in the termination of luteal function and pregnancy (Choudary and Greenwald, 1969). These studies clearly indicate the pituitary gland as the source of luteotropic hormones.

Although there are numerous hormones produced by the pituitary, there are three in particular that have been implicated in affecting luteal function, these include prolactin, LH and FSH. Prolactin, while being the primary hormone regulating luteal function up to implantation in many rodents (Greenwald, 1973; Smith and Neill, 1976), appears to have no significant effect after implantation in rodents or in regulating luteal function during the estrous or menstrual cycles of domestic animals and primates (Stormshak et al., 1987). Oxytocin secreted from the posterior pituitary has been implicated in influencing reproduction, however, the extent of this influence on luteal function is unclear. In the following section, the discussion will primarily focus on the gonadotropins, LH and FSH, and their role in regulating luteal life span. However, a brief discussion on the influences of oxytocin and prolactin will also be presented.

Luteinizing hormone and FSH are glycoprotein hormones produced and secreted from the gonadotropes of the anterior pituitary under the stimulation of GnRH. Both of these peptide hormones are composed of an α and a β subunit; the α subunits of LH and FSH are virtually identical whereas the amino acid sequence of the β subunit is distinct and determines the biological activity of the hormone (Hadley, 1992b). The actions of FSH have been implicated in follicular growth and recruitment, while LH is normally associated with ovulation of the ovum and stimulation of progesterone secretion. However, it is the coordinated activity of both LH and FSH during the follicular phase of the estrous cycle that regulates estrogen production, follicular growth and ovulation.
Luteinizing Hormone

Luteinizing hormone is essential for normal ovulation and maintenance of the corpus luteum in many mammalian species (Leers-Sucheta and Stormshak, 1991). Administration of LH to hypophysectomized rats has been shown to cause luteinization of granulosa cells (Richards et al., 1976). Similarly, pulsatile injections of LH has been shown to induce ovulation in anestrous ewes (McNeilly et al., 1982). Kaltenbach et al. (1968) demonstrated that constant infusion of crude pituitary extracts, containing LH and FSH, maintained luteal function in both pregnant and non-pregnant hypophysectomized ewes. Furthermore, ewes (Karsch et al., 1971) and cows (Donaldson and Hansel, 1965) injected with LH have exhibited prolonged estrous cycles with an extended CL life span compared with controls.

The relationship between LH and progesterone secretion is complex. Luteinizing hormone concentrations increase only when progesterone levels are low and estrogen levels are rising (Karsch et al., 1983, Schallenberger et al., 1984). However, it also has been established that LH can increase progesterone synthesis by luteal cells either in vitro or in vivo (Niswender and Nett, 1988; Niswender et al., 1994). To further complicate this relationship, during the luteal phase of the ovine estrous cycle when progesterone concentrations are high, there appears to be little correlation between the episodic peaks of serum LH and systemic progesterone concentrations (Baird et al., 1976).

This obscure relationship can be explained in part by examining the characteristics of the CL during the estrous cycle. During the luteal phase, more than 80% of the total progesterone secreted is from large luteal cells (Rothchild, 1981) which contain only 10% of the total number of LH receptors (Niswender et al., 1985). However, if LH is added to ovine luteal cells in culture, it is the small luteal cells which respond with an increase in progesterone secretion while large luteal cells exhibit little or no response (Lemon and Loir, 1977; Rodgers et al., 1983; Ursely and Leymarie, 1979). Similarly, when LH was introduced into the culture medium containing the luteal cells of cows (Ursely and
Leymarie, 1979) and sows (Lemon and Loir, 1977), the small luteal cells had a much greater response as measured by progesterone secretion.

One possible mechanism by which LH may affect luteal function was first proposed by Donaldson and Hansel (1965) who stated that this gonadotropin may regulate the differentiation of small to large luteal cells in cattle. Mee et al. (1993) found that cows that had been treated with GnRH 72 hours after the onset of estrus had an immediate increase in LH secretion, and when the CL was examined on day 10 of the estrous cycle it contained a higher proportion of large luteal cells. A similar shift from small to large luteal cells has been observed in ewes treated with either hCG (a known LH-agonist) or LH on days 5 to 10 of the estrous cycle when compared with controls (Farin et al., 1988; Niswender et al., 1988). Taken together, this research suggests that while secretion of progesterone by large luteal cells is not directly regulated by LH; the number of large luteal cells may depend, at least in part, on the actions of LH.

Another possible way in which LH may influence luteal steroidogenesis is by regulating the availability of the luteal LH receptors. Diekman et al. (1978a) were among the first researchers to follow the profile of LH receptor availability throughout the ovine estrous cycle. These researchers found that the total number of LH receptors per CL increased approximately 40-fold from day 2 to 10 of the cycle, while the number of occupied receptors increased sixfold. The number of luteal LH receptors and secretion of progesterone during the cycle also have been shown to be correlated in cows (Garverick et al., 1985; Spicer et al., 1981), sows (Ziecik et al., 1980), mares (Roser and Evans, 1983) and monkeys (Cameron and Stouffer, 1982). In these species, the number of occupied and unoccupied LH receptors are highly correlated with the weight of the corpus luteum and progesterone secretion. Therefore, in these species, it is the LH receptor availability that regulates the secretion of progesterone rather than the circulating levels of this hormone.
Because the role of LH and LH receptors in controlling luteal cell steroidogenesis has been fairly well characterized, researchers set out to determine whether the mechanism of PGF$_{2\alpha}$-induced luteolysis is controlled by a reduction in the number of luteal receptors for LH or by some other mechanism. Diekman et al. (1978b), induced luteolysis in ewes by injecting PGF$_{2\alpha}$ during the luteal phase of the estrous cycle, and found that serum concentrations of progesterone decreased before significant changes were detected in luteal concentrations of LH receptors. Spicer et al. (1981) similarly found that PGF$_{2\alpha}$-administered to cows during midcycle was followed by a significant reduction in luteal progesterone secretion, which attained basal levels by 12 hours post-treatment. However, specific binding of labeled hCG to luteal homogenates remained unchanged at 12 hours and did not reach basal concentrations until 24 hours after initial injection. However, when these same authors examined the correlation between LH receptors and progesterone concentrations in cows undergoing spontaneous luteolysis, they found that they decreased concomitantly. Research with sows also indicates that when luteolysis is induced (Barb et al., 1984) the reduced concentrations of serum progesterone and LH receptors are highly correlated. However, when luteolysis occurs spontaneously (Ziecik et al., 1980) a depression in luteal LH receptors actually precedes a decrease in serum progesterone. Thus, while it is tempting to deduce that the decrease in luteal LH receptors is the prelude to the events leading to the ultimate destruction of the CL, clearly more research is needed to determine the exact relationship between these factors in different species as well as in spontaneous and induced luteolysis. However, it is feasible that a decrease in LH receptors is not the primary determinant for the reduction in luteal function, especially since the small luteal cells contain the majority of LH receptors, while the large cells possess the majority of PGF$_{2\alpha}$ receptors, and hence are the more likely targets for the luteolytic action of PGF$_{2\alpha}$. 
**Follicle Stimulating Hormone**

While the function of FSH in regulating the life span of the CL is not fully understood, its role in follicular dominance, growth and ovulation that leads to the eventual formation of the CL, has been well documented. As discussed above, it is the intimate relationship between LH and FSH that has been primarily indicated in controlling follicular maturation.

In ewes, there is a functional relationship between surges in circulating concentrations of FSH and the emergence of follicular waves. Adams et al. (1992; 1993) found that each follicular wave in the ovine estrous cycle was preceded in 1-2 days by a significant surge in serum FSH. Numerous other species also appear to require FSH to stimulate follicular growth and recruitment. Rats exhibit a secondary surge of FSH on the day of estrus, just before the next cohort of follicles begin development (Smith et al., 1975). In primates, basal FSH levels increase slightly at the beginning of the follicular phase and the subsequent duration of the FSH rise influences the number of follicles stimulated (Goodman et al., 1977; Zeleznik and Kubik, 1986). In cattle, a secondary surge of FSH on the day of ovulation precedes the first follicular wave of the cycle (Dobson, 1978; Walters and Schallenberger, 1984) but slight elevations in serum FSH have also been shown to precede the second and third waves (Adams et al., 1992).

There seems to be a temporal correlation between higher FSH secretion and recruitment of follicles. Abolishing the preovulatory surge of FSH in ewes and cows by injection of bovine follicular fluid, after prostaglandin-induced luteolysis, caused a delay in the onset of behavioral estrus. McNeilly (1984) similarly demonstrated that ewes injected with charcoal-treated bovine follicular fluid delayed the onset of estrus and ovulation by selectively inhibiting FSH secretion without altering LH secretion. Turzillo and Fortune (1993) investigated the importance of FSH for the development of the dominant follicle during the first follicular wave in cows by injecting charcoal-extracted bovine follicular fluid on days 6 and 7 of the cycle, followed by PGF$_{2\alpha}$ to induce luteolysis. These
researchers found that all heifers treated with follicular fluid had arrested follicular development, additionally one-half of those treated exhibited a depression in estradiol production and a delay in the onset of estrus (Turzillo and Fortune, 1993). Results of several studies suggest the suppression of FSH by follicular fluid may be independent of short-term GnRH input, because follicular fluid seems to have no effect on LH secretion (McNeilly, 1984; Brooks et al., 1992). Treatment with a GnRH-antagonist has no effect on the response of FSH to follicular fluid treatment (Brooks et al., 1993).

The substance in the follicular fluid that is responsible for suppressing FSH release, is the protein hormone inhibin. Inhibin is produced by the granulosa cells of the follicle but little is known about its control and release during the estrous cycle (Hafez, 1987). In an effort to increase ovulation rates in domestic animals, some researchers have attempted the immunoneutralization of inhibin. Wheaton et al. (1992) found that either active or passive immunoneutralization in ewes resulted in an increase in FSH secretion as well as increasing the ovulation rate.

Follicular recruitment and development occurs in waves under the influence of FSH, but why is it that only one follicle (in monovulatory species) or a few (in polyovulatory species) of the follicles from the original cohort ovulate while the others undergo atresia? There are two main hypotheses to explain the mechanism by which the dominant follicle of the cohort suppresses the growth of the remaining follicles. The dominant follicle may either secret a substance that directly impairs further growth and development of the subordinate follicles or the dominant follicle could cause the atresia of subordinates indirectly by a negative feedback mechanism (Fortune, 1994). Most evidence seems to suggest that the dominant follicle exerts its control indirectly by secreting estradiol and(or) inhibin which would decrease FSH to levels that are unable to support further growth of the subordinate follicles. Zeleznik and Kubik (1986) demonstrated that in cynomologous monkeys the plasma concentration of FSH necessary to maintain preovulatory follicular maturation is less than that necessary to initiate
preovulatory growth. By this mechanism, the maturing follicle inhibits the growth of the less mature follicles by decreasing FSH concentrations, but does not succumb to its own inhibitory influences.

Ability to continue growth and development in the presence of lower FSH levels may be due to the increased vascularization of the dominant follicle or because of the increased number of LH receptors in the granulosa cells (Zeleznik, 1993). However, low levels FSH appear to remain critical to the dominant follicle, because experimental reduction of plasma FSH during the dominance phase in cattle is correlated with cessation of growth and sometimes the demise of the dominant follicle (Turzillo and Fortune, 1993). Therefore, while FSH does not seem to be critical in regulating the life span of the existing CL, its role in follicular development leading up to the formation of the CL is essential.

**Oxytocin**

Oxytocin, as mentioned previously, is a peptide hormone made from a larger precursor hormone that is synthesized by both the hypothalamus and the ovary. Hypothalamic oxytocin is produced primarily within the cell bodies of the paraventricular and supraoptic nuclei where it is packaged into secretory vesicles (Sawchenko and Swanson, 1985; Zimmerman et al., 1974). Once packaged, oxytocin is transported down the axon of these magnacellular neurons, through the median eminence, and stored in the posterior pituitary (neurohypophysis). Under the proper stimulation, oxytocin is released from nerve terminals in the neurohypophysis and into the blood stream by exocytosis (Ivell and Richter, 1985).

Classically, hypothalamic oxytocin has been implicated in the milk ejection reflex, maternal recognition of pregnancy and even parturition. At parturition, oxytocin of maternal and fetal pituitary origin as well as oxytocin from the CL has been implicated in the induction of uterine contractions in numerous species (for review see Fuchs, 1985). However, the role of hypothalamic oxytocin in regulating the life span the corpus luteum
is uncertain. Some researchers have postulated that it is the initial stimulation of oxytocin from the neurohypophysis that stimulates the positive feedback loop between oxytocin and PGF$_{2\alpha}$ during the terminal stages of the estrous cycle (Silvia et al., 1991). However, this hypothesis seems somewhat unlikely because a single CL can produce approximately 250 times more oxytocin mRNA than a single bovine hypothalamus (Ivell and Richter, 1985). Further, the posterior pituitary seems to release only minute quantities of oxytocin after injection with prostaglandins whereas the CL of the ovary responds precipitously to an injection of PGF$_{2\alpha}$ (Schams et al., 1985c). These findings were confirmed by Orwig et al. (1994) who found that while administration of cloprostenol, a PGF$_{2\alpha}$ analog, caused a significant increase in plasma oxytocin in an intact cow; those cows that received an injection of cloprostenol at the time of CL removal, did not exhibit a release of oxytocin that was significantly greater than baseline.

**Prolactin**

Prolactin is a peptide hormone that is produced and released from the anterior pituitary (adenohypophysis). Prolactin is a species specific hormone because there is only about 40% homology in amino acid sequence among different species as well as in its three dimensional structure (Wallis et al., 1985b). Prolactin contains between 197 to 199 amino acids depending on the species studied (Hadley, 1992b). Prolactin, like pituitary oxytocin, is typically associated with mammary gland growth and lactation. However, its role as a luteotropin has been extremely well documented in rodents.

Prolactin has long been recognized as a luteotropin in the rat. Prolactin secreted by the pituitary can, by itself, sustain basal levels of progesterone during the first week of pregnancy in the rat (Albarracin et al., 1994). During pregnancy, prolactin is secreted from the pituitary as two pulses per day for the first 10 days of gestation (Gunnet and Freeman, 1983). From the day of implantation to day 10 of pregnancy, the corpus luteum is under the combined control of pituitary prolactin and placental hormones (Kelly et al.,
1976; Morishige and Rothchild, 1974). Gafvels et al. (1992) found that prolactin had a stimulatory effect on luteal LH receptor mRNA. These researchers also demonstrated that blocking prolactin secretion by injecting bromocriptine during early pregnancy resulted in abortion; however, if exogenous prolactin was given concomitant with bromocriptine until the time of implantation pregnancy was carried to term (Gafvels et al., 1992). Inhibition of prolactin secretion has been shown to induce luteolysis by decreasing LH and hCG receptor binding capacity (Chan et al., 1980; Holt et al., 1976) and by induction of luteal 20α-hydroxysteroid dehydrogenase activity, which results in the conversion of progesterone to 20α-hydroxyprogesterone, a reduced steroid with little progestational activity (Albarracin et al., 1994). Administration of prolactin to rats has been shown to decrease 20α-hydroxysteroid dehydrogenase activity (Albarracin et al., 1994). In addition to maintaining the corpus luteum during pregnancy and stimulating luteal progesterone production, prolactin promotes luteolysis of the CL from the previous reproductive cycle (Malven and Sawyer, 1966). Clarke and Linzer (1993) found that the mouse ovary contains mRNA encoding four different forms of the prolactin receptor and these authors suggested that it is through stimulation of different prolactin receptors that this hormone can exhibit both luteotropic and luteolytic effects.

In contrast with the luteotropic effects in the rodent, prolactin appears to play no significant role in regulating the luteal life span in most domestic animals and primates. Injections of the prolactin inhibitor bromocriptine into ewes reduced serum concentrations of prolactin by more than 95% for the period of an estrous cycle without affecting either estrous cycle length or serum progesterone concentrations (Niswender, 1974). Additionally, when excess quantities of prolactin antiserum were administered in conjunction with bromocriptine there was still no effect on the serum concentrations of progesterone (Niswender et al., 1976). Further, constant infusion of prolactin into intact ewes had no significant effect on luteal function (Karsch et al., 1971). However, it is
noteworthy that in the sow, prolactin may be luteotropic during the terminal stages of gestation.

Kraeling and Davis (1974) reported that hypophysectomy during late gestation terminates pregnancy in pigs, but when prolactin was administered immediately following hypophysectomy the pregnancy was maintained (du Mesnil du Buisson, 1961; Denamur et al, 1973). Yangfan et al. (1989) found that when hysterectomized pigs containing CL of 110 days are hypophysectomized, serum prolactin levels decline and the CL regress. However, if porcine prolactin is administered from the time of hypophysectomy, these hysterectomized and hypophysectomized gilts will maintain serum progesterone levels as well as morphology of the aging CL during the period of prolactin administration. Similarly, injection of prolactin between days 110-120 in hysterectomized gilts caused an enhancement in serum progesterone concentrations when compared with saline-treated controls (Felder et al., 1988). Hyperprolactinaemia is frequently associated with an inhibition of luteal function, such as lactational anestrus, and is commonly associated with infertility in humans (Ginsburg, 1992). To determine whether overproduction of prolactin could be detrimental to pregnancy, Szafranska and Tilton (1993) induced hyperprolactinaemia in gilts by administration of haloperidol during the second one-half of gestation and found that although there was a drastic suppression of LH secretion, pregnancy was maintained in these animals.

The way in which prolactin affects steroidogenesis and luteal function may be by the active role it plays in cholesterol homeostasis within the luteal cell. One of the functions of prolactin is to increase uptake and utilization of lipoprotein-born cholesterol, and in the few species studied, this hormone acts by increasing cellular high density lipoprotein (HDL) binding. Prolactin maintains or increases HDL receptor numbers in the CL (Murphy and Silavin, 1989). Menon et al. (1985) reported that incubation of rat luteal cells with prolactin increased progesterone synthesis in the presence of HDL. Further, incubation of pig luteal cells (Rajkumar et al., 1985) or luteinized granulosa cells
(Rajkumar et al., 1988) with HDL resulted in increased progesterone accumulation in the incubation medium. Additionally, Murphy and Rajkumar (1985) demonstrated that dissociated luteal cells isolated from pregnant pigs demonstrated increased uptake of $^{125}$I-LDL in response to prolactin. However, the mechanism by which prolactin increases lipoprotein uptake is not completely understood.

ROLE OF THE UTERUS IN REGULATING LUTEAL LIFE SPAN

For a number of years, researchers have been attempting to elucidate the role the uterus may have in regulating luteal life span and function. One method that has been employed is the surgical removal of the uterus (hysterectomy). During a hysterectomy, the uterine horns and the body of the uterus are removed while leaving the ovarian vasculature intact. In this fashion, researchers are able to demonstrate if luteal and ovarian structures can function in the absence of the uterus. Another method to examine the function of the uterus, at least in species with bipartite or bicornuate uteri, is by the surgical procedure known as a unilateral hysterectomy. This technique results in the removal of one uterine horn to the level of internal bifurcation of the uterus, without impairing ovarian blood flow or the integrity of the remaining uterine horn. The following sections will examine the role of the uterus in regulating luteal function in various species as well as examine the role of the uterus in luteolysis.

Effects of Hysterectomy

When experiments were first initiated in the late 1800's to elucidate the role of the uterus in luteal function, contradictory results were obtained. It was not until the experiments by Loeb (1923) that a clear understanding as to the purpose of this structure began to be defined. Loeb (1923) found that removal of the uterus in the guinea pig during mid-gestation resulted in the maintenance of the CL for more than 90 days; normal
gestation in this species is only about 63 days. This extension of luteal life span indicated that luteolysis is dependent upon the uterus in the guinea pig.

Since this discovery by Loeb, researchers have been able to compile a body of evidence that better explains the role of the uterus in a variety of species. The extent in which the uterus may aid in luteolysis, appears to be a species specific process. The uterus appears to be most important in regulating luteal life span in ungulates and guinea pigs and of little importance in humans and non-human primates (Anderson, 1973). To briefly summarize the results of these research projects, removal of the uterus in rabbits (Loeb and Smith, 1936; Tenny et al., 1955) and rats (Anderson et al., 1967; O'Shea, 1970) does not affect the length of the estrous cycle, but does prolong the duration of pseudopregnancy. Uterine removal in guinea pigs (Loeb, 1923; 1927), sheep (Hu et al., 1991; Moor and Rowson, 1966; Southee et al., 1988; Wiltbank and Casida, 1956), pigs (Felder et al., 1988; Staigmiller et al., 1972) and cows (Anderson et al., 1962; Brunner et al., 1969; Ginther et al., 1967; LaVoie et al., 1975) result in the prolongation of the estrous cycle that may equal or exceed the duration of gestation for that species. In the mare, hysterectomy does delay luteal regression, however, the secretory ability of the CL is significantly impaired (Squires et al., 1974). Conversely, hysterectomy has no significant effects on the duration of luteal life span in humans (Beling et al., 1970) and non-human primates (Neill et al., 1969).

As mentioned previously, the role of the uterus in regulating luteolysis in domestic animals is quite extensive. To further examine the utero-ovarian relationship in this group of animals, Anderson et al. (1962) performed an elegant study examining luteal function in groups of heifers. These researchers removed portions of uterus leaving the following structures intact: group 1) none (horns, body and cervix removed); group 2) cervix and body; group 3) one-quarter of the uterine horns closest to the ovaries; and group 4) one-half of the uterine horns closest to the ovaries. Only those heifers in which the entire uterus was removed remained anestrus for the duration of the study, approximately 270
days. Upon morphological analysis at the conclusion of the study, the ovaries from the group of totally hysterectomized heifers were found to have their original CL. This confirmed earlier work by Armstrong and Hansel (1959) who found that removal of the uterine body and horns in heifers prevented oxytocin-induced luteolysis. Felder et al. (1988) found by comparing serum progesterone concentrations in pregnant gilts at 100 days gestation that the integrity of the CL was not compromised in hysterectomized pigs with aging CL. However, when these gilts were injected with porcine LH, hysterectomized gilts responded with an increase in luteal progesterone secretion whereas there was no significant effect on the luteal function in pregnant gilts. Upon morphological analysis, the regression of the original CL and formation of new CL had developed in the hysterectomized gilts. This suggests that during pregnancy the conceptus and(or) uterine-derived luteotropins may override the effect of LH in inducing ovulations and regression of preexisting CL (Felder et al., 1988).

The first evidence suggesting that the CL of domestic animals may be influenced by the uterus in a local manner was obtained by du Mesnil du Buisson (1961) from experiments with unilaterally hysterectomized pigs. Ginther et al. (1967) similarly found that the local interaction between the uterine horn and its adjacent ovary is essential for luteolysis in cows. In this latter work, the uterine horn adjacent (ipsilateral) to or opposite (contralateral) to, the ovary bearing the CL was removed and animals were challenged with an injection of oxytocin. While oxytocin was effective in inducing luteolysis in both intact and contralateral hysterectomized heifers, oxytocin was ineffective in inducing premature luteolysis in heifers that had the ipsilateral uterine horn removed (Ginther et al., 1967). Similarly in sheep, unilaterally ovariectomized ewes have been demonstrated to have a normal estrous cycle unless the uterine horn ipsilateral to the remaining ovary was removed (Moor and Rowson, 1966) Further, in unilaterally hysterectomized ewes having intact ovaries, with each bearing CL, treatment with a luteolytic dose of estradiol-17β resulted in the reduction of luteal weight in the ovary ipsilateral to the remaining uterine
horn as compared with the weight of the CL in the contralateral ovary (Akbar et al., 1971).

**Utero-ovarian Functional Interrelationships and Luteolysis**

From the above studies it seems clear that in domestic animals the uterus and the ovary bearing the CL must be in close proximity in order for normal luteolysis to occur. Numerous researchers set out to investigate further the functional relationship of the uterus and the ovary in luteolysis. These projects were carried out primarily with sheep, because the reproductive system in this species had been extremely well characterized in comparison to other experimental models (McCracken et al., 1972). The purpose of these studies was to determine if the relationship between the uterus and the ovary in luteal demise was dependent upon some other local factors in the area of the reproductive tract or solely regulated by the uterus and the ovary.

Surgical procedures in sheep where the ovary is transplanted to the neck, leaving the uterus in its original position in the abdomen, results in the persistence of luteal function (Baird et al., 1968; Goding et al., 1967b). Further, researchers found that transplantation of the uterus to the neck, while leaving one ovary in the abdomen, also results in luteal retention (Goding et al., 1967a). However, when the ovary and its adjacent uterine horn is transplanted into the neck of the ewe as a unit, normal luteal function and regression occurs (McCracken et al., 1970a). Thus, it was clearly established that luteal function was controlled by the local effects of the uterine horn on the adjacent ovary, regardless of position of this unit in the body.

To determine if the effects of the uterus on the adjacent ovary bearing the CL were by the release of some substance into the blood system late in the estrous cycle, ligation of various vascular connections between the reproductive structures was conducted. Kiracofe et al. (1966) demonstrated that ligation of the uterine arteries and veins resulted in the extension of luteal function while ligation of the uterine artery alone was without
effect. McCracken et al. (1972) later prepared a series of elegant cross-vascularization experiments, to examine whether the uterine venous blood contained the luteolytic factor. In these experiments, the donor animal had a utero-ovarian transplant in the neck and the recipient ewes had only the ovary transplanted into the neck. Utero-ovarian venous blood was transfused from the donor animal on day 15 of the cycle to the recipient ewe which resulted in a decrease in progesterone secretion in the recipient ewe within 48 hours. However, regression of the CL did not occur when recipient ewes were infused with utero-ovarian blood from donor ewes that were at either day 2 or 10 of the estrous cycle (McCracken et al., 1972). These results suggest during the terminal stages of the estrous cycle that a luteolytic factor is secreted by the uterus into the uterine vein to cause the demise of the CL.

The luteolytic factor from the uterus was suggested to be PGF$_{2\alpha}$ because this hormone is known to be a potent vasoconstrictor (Ducharme et al., 1968) as well as being abundant in the uterus (Pickles, 1966). Pharris and Wyngarden (1969) were able to demonstrate that in the rat, PGF$_{2\alpha}$ administration induced a significant reduction in the length of pseudopregnancy. However, because PGF$_{2\alpha}$ is rapidly degraded to its inactive metabolite, PGFM, with just one pass through the lungs (Piper and Vane, 1969), the delivery of this hormone from the uterus to the ovary would have to be local. While there are no direct vascular connections between the uterus and the ovary, uterine vein and the ovarian artery are closely apposed to one another. Because of the close apposition of these vessels, Barrett et al. (1971) hypothesized that there may be some kind of counter-current exchange mechanism by which the luteolytic substance passes from the uterine vein to the ovarian artery. To test this hypothesis, these researchers surgically separated the ovarian artery from the surface of the utero-ovarian vein and subsequently found that complete separation of these two vessels and inserting a portion of broad ligament as a barrier between them resulted in maintenance of the CL (Barrett et al., 1971). These results confirmed the hypothesis that a counter-current exchange mechanism was
responsible for the luteolytic substance to travel to the ovary. However, it was still yet to be determined whether the proposed luteolysin, PGF$_{2\alpha}$, could travel via this countercurrent exchange mechanism. McCracken et al. (1972) set out to determine whether PGF$_{2\alpha}$ could travel by this mechanism by infusing [³H]-PGF$_{2\alpha}$ into the uterine vein of a ewe on day 14 of the cycle. By comparing the radioactivity in the plasma collected from the ovarian artery to plasma and the adjacent iliac artery, these researchers found that the levels of [³H]-PGF$_{2\alpha}$ was at least 30 times higher in the ovarian arterial plasma than the iliac arterial plasma. Taken together, this research supports the hypothesis that the luteolysin PGF$_{2\alpha}$ is transported from the uterus to the adjacent ovary by a countercurrent exchange mechanism which induces luteolysis.

**Action of Prostaglandins**

Prostaglandins belong to a family of chemically related substances known as eicosanoids. These substances are synthesized from a common precursor, arachidonic acid, which is liberated from the plasma membrane by phospholipase activity. Once liberated, arachidonic acid may be further converted in the cytosol of the cell to prostaglandins. Stimulation by hormones, such as oxytocin, or other stimuli may initiate calcium-mediated phospholipases to liberate arachidonic acid that will eventually serve as substrate for prostaglandin biosynthesis. The rate-limiting step in the biosynthesis of prostaglandins appears to be the availability of this substrate, the precursor arachidonic acid (Lapetina et al., 1978).

As mentioned in the preceding section, PGF$_{2\alpha}$ is the known luteolysin in a number of non-pregnant animals. Pharriss and Wyngarden (1969) were the first to demonstrate that PGF$_{2\alpha}$ could induce luteolysis by injecting this substance into pseudopregnant rats and causing subsequent luteal regression. Since this discovery, PGF$_{2\alpha}$ has been demonstrated to be the luteolytic factor in rabbits (Pharriss, 1970), sheep (Caldwell et al.,
1972; McCracken et al., 1970), cows (Hafs et al., 1974; Thatcher and Chenault, 1976) and horses (Douglas and Ginther, 1972; Hafs et al., 1974).

During luteolysis in domestic animals, PGF$_{2\alpha}$ is released from the uterus in a pulsatile fashion with a total of approximately 5-8 discrete pulses (Fredriksson et al., 1984; McCracken et al., 1984; Peterson et al., 1976). Variability exists among species as to the duration and magnitude of pulses, however, they typically occur at 6-8 hour intervals (Silvia et al., 1991). Luteal tissue appears to be particularly sensitive to the luteolytic effect of PGF$_{2\alpha}$ when administered in a pulsatile fashion versus constant infusion (Schramm et al., 1983). Therefore, it is likely that it is the onset of the pulsatile secretion of PGF$_{2\alpha}$ that may induce the onset of luteolysis.

Prostaglandin F$_{2\alpha}$ has also been shown to stimulate secretion of oxytocin from the ruminant CL both in vivo (Flint and Sheldrick, 1982; Lamsa et al., 1989) and in vitro (Abdelgadir et al., 1987; Chegini and Rao, 1987). Additionally, large luteal cells, which have been identified to be the source of ovarian oxytocin, have been demonstrated to possess PGF$_{2\alpha}$ receptors. During luteolysis, pulses of PGF$_{2\alpha}$, and its metabolite, are highly correlated to levels of oxytocin when sampled in the utero-ovarian vein (Hooper et al., 1986) as well as systemic circulation (Flint and Sheldrick, 1983). Lamsa et al. (1992) demonstrated that the PGF$_{2\alpha}$ receptor in the ovine CL is rapidly desensitized upon stimulation with hormone, and that a minimum rest period of 6 hours was required to restore sensitivity. These authors hypothesized that it is the desensitization and recovery of the PGF$_{2\alpha}$ receptor that accounts for the pulsatile nature of oxytocin during luteolysis (Lamsa et al., 1992). Similarly, refractoriness of endometrial oxytocin receptors have also been characterized. After acute exposure to oxytocin, refractoriness is maintained for a period of approximately 6 hours (Sheldrick and Flint, 1986). Therefore, transient uterine refractoriness to oxytocin as well as luteal refractoriness to PGF$_{2\alpha}$, may account for the pulsatile nature of PGF$_{2\alpha}$. 
Due to the strong correlation between luteal oxytocin secretion and uterine PGF$_{2\alpha}$ secretion during luteolysis, the secretion of PGF$_{2\alpha}$ may be partially dependent upon ovarian oxytocin. Indeed, oxytocin administration during the latter part of the estrous cycle has been shown to cause premature regression of the CL by stimulating the release of uterine PGF$_{2\alpha}$ (Flint and Sheldrick, 1983; Kieborz et al., 1991). Therefore, it has been hypothesized that in domestic animals uterine PGF$_{2\alpha}$ and luteal oxytocin comprise a positive feedback loop, where PGF$_{2\alpha}$ synthesized in the uterus is released into the uterine vein and acting through a counter-current transfer mechanism, acts upon the adjacent ovary to stimulate oxytocin secretion from the CL. This luteal oxytocin then acts back upon the uterine horn to further stimulate the secretion of PGF$_{2\alpha}$, which causes the ultimate regression of the CL (Flint and Sheldrick, 1983; McCracken et al., 1984; Sheldrick and Flint, 1984).

**Role of Oxytocin**

The mechanism by which oxytocin induces PGF$_{2\alpha}$ secretion from the uterus is not well understood. The ability of oxytocin to regulate uterine PGF$_{2\alpha}$ release from the uterus may be dependent on several factors. Some of the factors that have been indicated in regulating PGF$_{2\alpha}$ release include: the availability of arachidonic acid as a precursor for prostaglandin synthesis in uterine tissue (Silvia et al., 1991); the quantity of prostaglandin H endoperoxide synthase (cyclooxygenase) enzyme, which is essential for prostaglandin synthesis; and probably most importantly, the uterine oxytocin receptor itself.

Oxytocin receptors have been reported to exist in the uterine endometrium and myometrium of many mammals. These two receptor locations seem to have a dual action in the uterus: a uterotonic action on myometrial cells to stimulate contractions and a prostaglandin-releasing action on endometrial cells (Fuchs et al., 1982). Availability and formation of the oxytocin receptors on the uterus are regulated by the concentration of progesterone and estradiol throughout the estrous cycle (Sheldrick and Flint, 1985; Zhang
et al., 1992). Number of endometrial oxytocin receptors increases with the approach of luteolysis and becomes maximal on the day of behavioral estrus (Ayad et al., 1991; Sheldrick and Flint, 1985). The pattern of development of oxytocin receptors in the endometrium is of particular interest because the endometrium has been shown to be the site of prostaglandin synthesis, further substantiating the role of oxytocin in luteal regression (Cerini et al., 1979; Salamonsen and Findlay, 1990).

It has been hypothesized that the initiation of luteolysis may begin at the level of the uterus, because PGF$_{2\alpha}$ secretion increases before any significant rise in luteal oxytocin occurs (Moore et al., 1986). Stimulation for the initial pulse of PGF$_{2\alpha}$ is still unknown, but oxytocin secretion from the posterior pituitary has been implicated (Silvia et al., 1991). Regardless, this release of PGF$_{2\alpha}$ stimulates the release of luteal oxytocin which then can bind its receptor in the uterus to further stimulate PGF$_{2\alpha}$ secretion.

**Luteotropic Mechanism**

While PGF$_{2\alpha}$ has been identified as the uterine luteolytic hormone, other prostaglandins of uterine origin have been implicated in luteotropic processes. For example, prostacyclin (PGI$_2$) which is a metabolite of the cyclic endoperoxide PGH$_2$. Milvae and Hansel (1980) found that injection of PGI$_2$ directly into the bovine CL at midcycle produced a striking increase in systemic plasma progesterone concentrations and that PGI$_2$ stimulated progesterone biosynthesis by dispersed luteal cells in vitro. Later these researchers found that incubated luteal cells in the presence of LH and arachidonic acid, had a higher rate of secretion of PGI$_2$ for CL collected on day 5 as compared with CL collected on days 10, 15 or 18 of the bovine estrous cycle (Milvae and Hansel, 1983). Additionally, progesterone production during the 2 hour incubation period decreased as the age of the CL advanced and as concentrations of PGI$_2$ declined. These findings suggest that PGI$_2$ may play an important role particularly during the first 10 days of the bovine estrous cycle. It is noteworthy that although PGI$_2$ can be manufactured in the
uterine tissues, the bovine CL has high PGI$_2$ synthase activity (Sun et al., 1977) implicating an autocrine or paracrine action of this hormone to maintain luteal function.

Prostaglandin E$_1$ (PGE$_1$) is another prostaglandin that has been found to possess luteotropic properties. Huie et al. (1981) found that intrauterine infusions of PGE$_1$ resulted in the prolonged maintenance of luteal function in ewes when infused into the uterine horn ipsilateral to the ovary bearing the CL. However, treatment of ewes with PGE$_1$ by infusion into the contralateral uterine horn failed to have a significant effect on luteal maintenance or the interestrous interval as determined by estrus observations and serum progesterone profiles. These data suggest that PGE$_1$ can act in a localized manner to maintain luteal integrity, and thus may be an important hormone during early pregnancy because it is capable of delaying luteal regression (Huie et al., 1981).

Weems et al. (1985) found that injection (333 µg) of either PGE$_1$ or prostaglandin E$_2$ (PGE$_2$) into the ovary of nonpregnant ewes resulted an increase in progesterone secretion compared with that of nontreated controls at 24 hours after injection. Only those ewes treated with PGE$_1$, however, had elevated progesterone concentrations at 48 hours after administration, suggesting that PGE$_1$ may be a more potent luteotropin than PGE$_2$.

Wilson et al. (1972) identified PGE$_2$ in the homogenate from the endometrium of nonpregnant ewes. In fact PGE$_2$ infused along with PGF$_{2\alpha}$ into the artery of ovaries transplanted into the neck in ewes, prevented the reduction of progesterone observed when PGF$_{2\alpha}$ was infused alone (Henderson et al., 1977). Silvia et al. (1981) utilized indwelling catheters in the utero-ovarian vein in pregnant and nonpregnant ewes. These authors found that levels of PGE$_2$ rose dramatically on day 13 in pregnant ewes and remained high through day 14, whereas there was no change in secretion in nonpregnant ewes. Conversely, Lewis et al. (1978) compared the level of this hormone in uterine venous plasma of nonpregnant and pregnant ewes on day 14 and found that the levels of PGE$_2$ were not significantly different, but did tend to be lower in the nonpregnant versus
pregnant ewes. These observations suggest that there may be a critical period during early pregnancy that the increase in PGE₂ is necessary in order to prevent luteolysis, and after this critical period, the levels of this luteotropic substance may return to normal levels. However, it was impossible to determine from these experiments whether the increased secretion of PGE₂ was of uterine or conceptus origin. Blastocysts in sheep (Hyland et al., 1982), cattle (Lewis et al., 1982), pigs (Geisert et al., 1982) and rabbits (Harper et al., 1983) also have been shown to produce PGE₂ in vitro.

Weems et al. (1993) suggested that after day 55 of gestation of the ewe, the primary source of PGE₂ becomes the placentome which continues to act to regulate steroidogenesis of the placenta as well as maintaining luteal function. It is therefore possible that during early pregnancy, both the uterus and the conceptus are sources of the luteotropin PGE₂, which act together to maintain pregnancy. Later on in gestation, however, the conceptus may be the primary source of this luteotropic prostaglandin.
STATEMENT OF THE PROBLEM

Approximately 30% of all embryos are lost during early development (Roberts et al., 1990) and at least some of this loss has been attributed to inadequate luteal function (Niswender and Nett, 1988). To producers, this can be a substantial financial loss particularly when dealing with repeat-breeders. These animals must be bred several times before maintaining a pregnancy possibly due to delayed ovulation and(or) embryonic death. Producers have adopted strategies to improve fertilization and early pregnancy rates, one of the most common strategies is artificial insemination along with hormone treatments to time luteolysis and ovulation. The administration of a GnRH analog at the time of artificial insemination has been met with moderate success to improve conception among repeat breeders (Hansel and Fortune, 1978; Lucy and Stevenson, 1986; Stevenson et al., 1984). Reasons for improved fertility after GnRH treatment are not clear (Lucy and Stevenson, 1986). However, GnRH administration after PGF$_{2\alpha}$ treatment may synchronize the timing of ovulation by inducing LH secretion such that artificial insemination is more successful.

Research has indicated that treatment of beef heifers with GnRH (Ford and Stormshak, 1978; Rodger and Stormshak, 1986) as well as ewes (Slayden and Stormshak, 1990) causes an attenuation of luteal progesterone secretion. Lucy and Stevenson (1986) hypothesized that the improved fertility may be associated with delayed or slowly rising concentrations of luteal progesterone after ovulation. However, Mee et al. (1993) administered GnRH at the time of artificial insemination of cows and found an increase in serum progesterone concentrations later in the estrous cycle, along with improved fertility. However, when CL were enucleated and incubated in vitro, CL from cows treated with GnRH had lower progesterone production in response to LH challenge (Mee et al., 1993). These authors hypothesized that GnRH may act on the developing CL to promote the
conversion of small to large luteal cells (Mee et al., 1993). These results were later confirmed by the experiments of Bertrand and Stormshak (1994) who found that CL incubated in vitro, after injection of GnRH on day 2, had a lower progesterone response to LH than CL from saline-treated cows. The following experiments have been conducted to further examine the effects that GnRH may have on luteal function as well as to elucidate whether the actions of this decapeptide are mediated through the uterus.
EXPERIMENTS 1 AND 2: CORPUS LUTEUM FUNCTION IN HYSTERECTOMIZED AND UNILATERALLY HYSTERECTOMIZED EWES TREATED WITH GONADOTROPIN-RELEASING HORMONE

INTRODUCTION

Treatment of ewes with GnRH during metestrus increased serum concentrations of LH and lowered subsequent serum concentrations of progesterone (Slayden and Stormshak, 1990). It was hypothesized that GnRH acted indirectly via LH to alter luteal function because bovine, ovine and porcine ovaries lack GnRH receptors (Brown and Reeves, 1983). Slayden and Stormshak (1990) confirmed the validity of this hypothesis by demonstrating that LH injected into ewes during metestrus mimicked the GnRH-induced attenuation of progesterone (P₄) secretion. This hypothesis is also supported by the earlier research of Rodger and Stormshak (1986) who found that treatment of beef heifers with GnRH caused down-regulation of luteal LH receptors.

Uterine prostaglandin F₂α (PGF₂α) is the natural luteolysin in ewes (McCracken et al., 1972; Goding, 1974). Luteolysis in nonpregnant ewes is promoted by secretion of luteal oxytocin (OT) that acts upon the adjacent uterine horn to induce the release of PGF₂α (McCracken et al., 1984). Treatment of dairy heifers with GnRH 12 hours after first detected estrus increased the number of large luteal cells, which are the source of OT in the corpus luteum (Mee et al., 1993). Voss and Fortune (1991) reported that exposure of cultured granulosa cells to increasing quantities of LH increased secretion of OT by these cells. Thus, we hypothesized that GnRH-induced secretion of LH may cause the developing corpus luteum (CL) to increase production of OT, which may in turn stimulate uterine secretion of PGF₂α in sufficient quantities to suppress luteal function.
The present experiments were conducted to determine whether response of the ovine corpus luteum to exogenous GnRH differed between intact, unilaterally or completely hysterectomized ewes. Changes in systemic serum concentrations of LH, P₄, plasma OT and utero-ovarian plasma OT and PGF₂α were monitored after administration of GnRH.

MATERIALS AND METHODS

Experiment 1

Sixteen mature crossbred ewes were checked twice daily for behavioral estrus with vasectomized rams. Ewes exhibiting at least two consecutive estrous cycles of normal duration were assigned randomly to four groups (n = 4) in a 2² factorial arrangement. Treatments consisted of two levels of GnRH (0 and 100 μg/day) and two levels of hysterectomy (none and UHYST).

Ewes were fasted for 48 hours prior to surgery; the last 24 hours were without water. On day 12 of the estrous cycle (day of detected estrus = day 0) a midventral laparotomy was performed; anesthesia for laparotomy was induced by an i.v. injection of sodium thiamylal (Biotal 2.5%) and maintained by halothane-oxygen inhalation. Surgical procedures were conducted under aseptic conditions and all ewes were unilaterally ovariectomized, and when present, CL in the remaining ovary were enucleated. In eight ewes the uterine horn, ipsilateral to the remaining ovary, and a portion of the uterine body extending to the internal bifurcation were removed. The reproductive tract of the remaining eight ewes was externalized and manipulated before wound closure. Ewes exhibited behavioral estrus (x ± SE) 2.2 ± .4 d after surgery.

On each of days 2 and 3 of the subsequent cycle, one-half of the intact and one-half of the UHYST ewes were injected i.v. with 100 μg GnRH (2 ml) while the remaining intact and UHYST ewes were injected i.v. with saline (2 ml). Jugular blood (10 ml) was
collected from all ewes at 0 min (prior to injection) and 15, 30, 45 and 60 min post-injection for analysis of serum LH.

On day 4 of the cycle the caudal vena cava of each ewe was catheterized via the lateral saphenous vein as described by Benoit and Dailey (1991). Anesthesia for the saphenous vein catheterization consisted of an i.v. injection of sodium thiamylal to relax the ewe, and a subcutaneous injection of 2% lidocaine administered at the point of incision, approximately 5 cm dorsal to the hock and 3 cm lateral to the Achilles tendon. Correct placement was determined by threading the catheter into the vein and collecting blood samples at 2 cm intervals; serum was subsequently analyzed for serum concentrations of P₄ within 24 hours. The catheter tip location at which P₄ concentrations were at least three times that of the jugular sample was determined to be at the junction of the utero-ovarian vein with caudal vena cava. The catheter was adjusted to the appropriate position on day 5 after estrus. Patency of the catheter was maintained by flushing with a sterile 3.5% sodium citrate-2% liquamycin solution after each blood collection or once daily on non-sampling days.

Jugular and caudal vena cava blood samples were collected from all ewes on days 5, 6, 7, 8, 10, 12 and 14 after estrus for jugular serum P₄ and plasma OT analysis, as well as for vena cava plasma OT and PGF₂α. Samples for analysis of serum LH and P₄ (10 ml) were collected via jugular venipuncture and stored for 24 hours at 4°C before sera were separated by centrifugation. Jugular and vena cava blood samples (5 ml), for determination of plasma OT were collected via heparinized vacutainer tubes, placed on ice and treated with ethylenediaminetetraacetic acid (EDTA; .5 M, 10 μl) and 1,10-phenanthroline (5 mg/ml in ethanol, 5 μl) to prevent oxytocinase activity (Kumaresan et al., 1974). Similarly, vena cava blood samples collected for determination of plasma PGF₂α (5 ml) were placed on ice and 1,10-phenanthroline (5 mg/ml in ethanol, 5 μl) was added immediately to prevent PGF₂α degradation. To obtain plasma, all blood samples
were processed immediately upon returning to the laboratory. All sera and plasma were separated by centrifugation (500 x g) for 10 min at 4°C and stored at -20°C until analyzed.

Experiment 2

Twenty mature crossbred ewes were checked twice daily for behavioral estrus with vasectomized rams. Ewes exhibiting at least two consecutive estrous cycles of normal duration were assigned randomly to four groups (n = 5) in a 2 x 2 factorial arrangement. Treatments consisted of two levels of GnRH (0 and 100 µg) and two levels of hysterectomy (none and HYST).

On day 12 of the estrous cycle, all ewes were subjected to a midventral laparotomy as described for Exp. 1. The reproductive tract was externalized and manipulated and corpora lutea in both ovaries were enucleated. Ten ewes were HYST after ligating the uterine arteries, uterine veins and placing a ligature at the utero-cervical junction. The entire uterus (horns and body) was removed leaving the ovarian vasculature intact. Ewes exhibited behavioral estrus (x ± SE) 3.6 ± 1.4 days after surgery.

On day 2 of the subsequent cycle one-half of the intact and one-half of the HYST ewes were injected i.v. with 100 µg GnRH (2 ml) while the remaining intact and HYST ewes were injected similarly with 2 ml sterile saline. Only a single injection of GnRH was given in this experiment; a treatment regimen previously shown to be effective in altering luteal function in cows and ewes (Rodger and Stormshak, 1986; Slayden and Stormshak, 1990).

Jugular blood samples were collected from all ewes on days 4, 6, 8, 10 and 12 after estrus to examine the effect of injected GnRH on serum concentrations of P₄. Samples for analysis of P₄ (10 ml) were allowed to clot for 24 hours at 4°C. All samples were centrifuged (500 x g) for 10 min at 4°C and the resulting sera were stored at -20°C until assayed.
Radioimmunoassays

Radioimmunoassay for LH was performed according to the procedure of McCarthy and Swanson (1976) with some modifications using ovine LH that was iodinated by the chloramine-T method. Highly purified LH (LER-1056-C2 immunochemical grade ovine LH) was weighed and solubilized to a concentration of 10 µg LH/25 µl distilled water. Prior to iodination 25 µl of .5 M sodium phosphate buffer (PB) at pH 7.5 were added to the LH and mixed. Iodination was performed by the addition 5 µl (.5 mCi) ¹²⁵I (IMS.30, Amersham Corporation, Arlington Heights, IL) and 5 µL of chloramine-T (.2 mg/ml .05 M PB, pH 7.5) to the solubilized LH and the mixture was then gently agitated for 60 seconds. The iodination reaction was terminated with the addition of 20 µl sodium metabisulfate (.25 mg/mL .05 M PB, pH 7.5)

Separation of labeled hormone from free iodine was performed by filtration through an anion exchange column consisting of a 3 ml plastic syringe with a small glass wool plug, filled with Analytical Grade Anion Exchange Resin (AG 2-X8, 100-200 mesh, chloride form, Bio-Rad Laboratories, Hercules, CA). The resin bed was equilibrated with .5 M PB (pH 7.6) and coated with 2 ml of 5% bovine serum albumin (BSA) in .05 M PB (pH 7.5) and collected into a 12 x 75 mm tube containing 1 ml .01 M-0.1% gelatin PB (pH 7.5). To establish the concentration of purified labeled LH, 10 µl of the final solution were counted. Concentrations of LH were expressed using ovine LH standards (NIADDK-oLH-25).

All serum samples for LH were assayed in duplicate in a single assay. The intraassay coefficient of variation was 9.8%. This was determined from aliquots of a serum pool containing a concentration of LH that was near the midpoint of the standard curve. Sensitivity of the assay was .5 ng/tube.

Sera were assayed for P₄ in duplicate after hexane:benzene (2:1) extraction. Progesterone RIA was performed on extracted samples following the procedure of Koligian and Stormshak (1976). [³H]-progesterone (4000 cpm, NET-381, New England
Nuclear, Boston, MA) was added to a third tube containing an aliquot of the sample to
determine and correct for procedural loss due to extraction. Anti-progesterone-11-BSA
antibody (provided by Dr. Gordon Niswender, Colorado State University) was utilized in
all the assays. The intra- and interassay coefficients of variation were 8.3% and 18.4%
(Exp. 1) and 7.5% and 6.5% (Exp. 2), respectively. The mean extraction efficiency for
samples of Exp. 1 and 2 was 96 ± 4% and 91 ± 2%, respectively. Assay sensitivity was
10 pg/tube.

Oxytocin was extracted from plasma following the method of Schams et al. (1979)
using Waters Sep-pak C-18 cartridges (Schams, 1983). Plasma was assayed for OT by
RIA as described by Abdelgadir et al. (1987) using OT antibody generously provided by
Dr. Dieter Schams, Technical University of Munich, Freising-Weihenstephan, Germany.
Mean plasma OT extraction efficiency was 61 ± 5% as determined by the addition of [3H]-
OT (4000 cpm, NEX-187, New England Nuclear). Oxytocin values determined by RIA
were corrected for the added [3H]-OT and losses due to extraction. Plasma sample
volume was 25 μl per tube and all samples were adjusted to a final volume of 200 μl with
assay buffer (.05 M sodium phosphate, 50 mM EDTA and .5 mg/ml gelatin). Intra- and
interassay coefficients of variation were 9% and 8.6%, respectively. Sensitivity of the
assay was .25 pg/tube.

**Enzyme Immunoassay**

Prostaglandin F$_{2\alpha}$ was analyzed using an enzyme immunoassay kit (EIA)
purchased from Cayman Chemical (Ann Arbor, MI). Plasma was purified by following the
procedure described in the Cayman Kit using Waters Sep-pak C-18 Cartridges. Mean
plasma extraction efficiency was 78 ± 2% as determined by the addition of [3H]-PGF$_{2\alpha}$
(4000 cpm, NET-433, New England Nuclear). Values for PGF$_{2\alpha}$ determined by EIA
were corrected for losses due to extraction. Intra- and interassay coefficients of variation
were 1.9% and 13.2%, respectively. Sensitivity of the assay was 1.2 pg/well.
Statistical Analysis

Data pertaining to the effects of treatment on serum and plasma concentrations of LH, P₄ and PGF₂α were analyzed separately using a multi-way repeated measures analysis of variance. Because of heterogeneity of variance in the samples, data were subjected to natural log transformations for analysis but are presented in this paper using the nontransformed values. Plasma OT data were analyzed using the nontransformed data by a multi-way repeated measures analysis of variance. Correlation analysis between jugular and vena cava plasma levels of OT as well as individual day comparisons for plasma OT were performed using Statgraphics (1991).

RESULTS

Administration of GnRH to ewes on day 2 (Fig. 1a) and 3 (Fig. 1b) of the cycle (Exp. 1) increased serum concentrations of LH on each day compared with those of saline-treated ewes (P = .01). Secretion of LH in GnRH-treated animals differed between days with the quantity of LH released on day 2 being greater than on day 3 of the cycle (P = .05). On day 2, serum concentrations of LH were still increasing at 60 min post-injection of GnRH whereas on day 3, LH levels in GnRH-treated ewes were maximal by 15 min after injection and then plateaued.

Injection of GnRH failed to affect jugular plasma concentrations of OT in intact or UHYST ewes on days 5 through 10 of the cycle (Fig. 2). However, (x ± SE) jugular plasma concentrations of OT (ng/ml) on days 12 and 14 were greater (P = .006) in all intact (saline, 240.4 ± 23.2; GnRH, 195.2 ± 22) than in all UHYST animals (saline, 140.7 ± 5.9; GnRH, 157.7 ± 9.8). Further, averaged over both days, jugular plasma levels of OT in saline-treated intact ewes were significantly greater than GnRH-treated intact ewes (P = .03).
Failure of the saphenous vein catheter occurred in a total of four ewes; two ewes on day 5 (GnRH-treated intact and GnRH-treated UHYST), one ewe on day 10 (saline-treated intact), and one ewe on day 12 (GnRH-treated UHYST). Failure was due to either development of a blood clot or by the accidental removal of the catheter by the animals. This resulted in a reduced number of animals used in the analysis of caudal vena cava blood samples. Because of this, as well as the tremendous variation in vena cava plasma OT samples, there were no significant differences detected among the treatment groups in plasma OT concentrations (Fig. 3). However, it is noteworthy that ewes that were UHYST and received GnRH had lower levels of vena cava plasma OT during the terminal stages of the sampling period (days 12 to 14) than ewes in the other three treatment groups. Over all sampling periods the concentrations of OT in vena cava plasma were positively correlated with those in jugular plasma ($r = .52, P < .0001$).

Vena cava plasma PGF$_{2\alpha}$ did not differ significantly among treatment groups (Fig. 4). However, vena cava plasma concentrations of PGF$_{2\alpha}$ were greater in GnRH-treated intact ewes on days 10 through 14 compared with those of saline treated or all UHYST ewes ($P = .07$).

Treatment with GnRH was without significant effect on serum concentrations of P$_4$ in Exp. 1. However, serum levels of this steroid were greater in UHYST ewes compared with those in intact ewes ($P = .09$) regardless of whether they were treated with GnRH (Fig. 5). In Exp. 2, ewes that were HYST and treated with GnRH produced less P$_4$ on days 4 through 12 of the cycle ($P = .04$) than HYST ewes injected with saline or intact ewes treated with either GnRH or saline (Fig. 6). In both Exp. 1 and 2, UHYST and HYST ewes, regardless of treatment, maintained CL beyond the expected time of normal luteolysis.
DISCUSSION

Results of the present experiments failed to support the initial hypothesis that exposure of the developing CL to increased quantities of LH would provoke increased production of OT, which in turn would stimulate increased uterine production of PGF$_{2\alpha}$ and attenuate P$_4$ secretion. Administration of GnRH on each of days 2 and 3 after detected estrus did stimulate the release of LH. This response to GnRH was anticipated because injection of the decapeptide into heifers (Lucy and Stevenson, 1986; Rodger and Stormshak, 1986; Lamming and McLeod, 1988; Martin et al., 1990) and ewes (Nett et al., 1981; Slayden and Stormshak, 1990) early in the estrous cycle has previously been shown to provoke the release of LH. The quantity of LH released in response to GnRH was less on day 3 then on day 2, which may be attributable to a down-regulation of the GnRH receptors in the anterior pituitary or to depletion of releasable pituitary LH.

Overall, plasma OT concentrations in jugular and vena cava blood were similar. However, in previous reports OT concentrations were found to be greater in ovarian than systemic blood of cows (Schallenberger et al., 1984) and ewes (Sheldrick and Flint, 1983; Hooper et al., 1986). Catheterization of the caudal vena cava was performed in this study, and correct placement of the catheter was determined by the insertion distance at which P$_4$ concentrations were greatest. The catheter was initially anchored with suture. However, the position of the catheter may have changed during the 10 day sampling period due to mobility of the ewe and(or) its interaction with other ewes in the pen. This may account for the high variability of the vena cava plasma levels of OT among ewes. Regardless, the highly significant positive correlation between vena cava and jugular plasma concentrations of OT indicates that jugular sampling can be an accurate measure of ovarian OT secretion.

Treatment with GnRH did not significantly affect secretion of jugular plasma OT from days 5 to 10 of the cycle as determined by the measurement of plasma concentrations
of the neuropeptide. However, there are aspects of the secretory pattern of the jugular plasma OT that are noteworthy. First, OT levels in intact ewes on days 12 and 14 were significantly greater than in UHYST ewes. This may be due to lack of the local interrelationship between the ovary bearing the CL and the adjacent uterine horn in UHYST ewes, thus precluding PGF$_{2\alpha}$ stimulation of OT secretion that normally occurs in intact ewes. Additionally, intact ewes treated with saline had greater plasma concentrations of OT on days 12 and 14 than GnRH-treated intact ewes. This suggests the possibility that release of OT occurred earlier in GnRH-treated ewes thus depleting existing stores of luteal OT.

No significant differences in vena cava plasma PGF$_{2\alpha}$ concentrations were found among treatment groups. However, intact ewes treated with GnRH had a higher concentration of plasma PGF$_{2\alpha}$ than ewes of other treatment groups on days 10 to 14 after estrus. Because PGF$_{2\alpha}$ promotes the release of luteal OT these data are consistent with the lower plasma concentrations of OT detected in these same animals during days 12 to 14 of the cycle.

It has been previously reported that injection of GnRH into beef heifers (Ford and Stormshak, 1978; Rodger and Stormshak, 1986), as well as ewes (Slayden and Stormshak, 1986), subsequently resulted in attenuated P$_4$ secretion by the CL. However, in this study injection of GnRH failed to significantly suppress luteal P$_4$ secretion in either intact or UHYST ewes. Nevertheless, serum concentrations of P$_4$ in UHYST ewes were greater (P = .09) than serum levels of P$_4$ in the intact controls. Increased serum levels of P$_4$ in the UHYST ewes may be related to removal of the ipsilateral uterine horn, which is the source of PGF$_{2\alpha}$ that normally promotes luteolysis. This possibility is supported by the research of Robinson et al. (1976) who reported that the major site of prostaglandin synthesis in the ipsilateral nonpregnant uterine horn is the caruncles, removal of which resulted in a significant increase in serum concentrations of P$_4$ over that of intact controls. It is also possible that the difference in P$_4$ concentrations is the consequence of higher
ovulation rates due to compensatory hypertrophy of the remaining ovary, thus resulting in more CL in the UHYST than in the intact ewes.

Experiment 2 was conducted to determine whether administration of GnRH to HYST ewes would result in suppression of luteal function. Further, because UHYST resulted in markedly increased serum levels of P₄ it was deemed important to determine whether total hysterectomy would have a similar effect on luteal function. As in Exp. 1, an effect of GnRH alone on luteal function was not detected. However, in this experiment serum levels of P₄ in HYST ewes receiving GnRH were significantly less than in ewes of the remaining three treatment groups. These data are consistent with those of Southee et al. (1988) who hysterectomized anestrous ewes 2 weeks prior to P₄-priming and then treated ewes with low doses of GnRH. These authors found that while all ewes exhibited behavioral estrus, serum P₄ in hysterectomized animals was less than that in intact ewes, and the CL were maintained for a prolonged period of time. This research suggests that after hysterectomy, the CL is maintained but with a limited secretory capacity.

Data on serum concentrations of P₄ in UHYST ewes in Exp. 1 and HYST ewes in Exp. 2 cannot be directly compared. However, cursory inspection of these data suggest that P₄ levels in unilaterally ovariectomized-UHYST ewes are greater than in HYST ewes. Whether this is due to the presence of one ovary or absence of one uterine horn is unknown. To our knowledge no studies have been conducted in which luteal function in UHYST and HYST ewes are compared. Such an experiment is warranted, the results of which may shed additional light on the data of our experiments.

SUMMARY

The present research was conducted to further investigate the effects of GnRH on luteal function in intact, partially hysterectomized and totally hysterectomized ewes. Administration of GnRH during formation of the corpus luteum did not affect subsequent
luteal secretion of progesterone in control, partially hysterectomized or totally hysterectomized ewes. However, luteal function was altered by the absence of the adjacent uterine horn or the entire uterus.
FIGURE 1. Jugular serum concentrations of LH in intact or unilaterally hysterectomized (UHYST) ewes for 60 min after i.v. treatment with either saline or GnRH (100 μg/d) on d 2 (A) and 3 (B) of the cycle. Time 0 is just prior to injection.
FIGURE 2. Jugular plasma concentrations of OT in intact or unilaterally hysterectomized (UHYST) ewes treated with either saline or GnRH (100 µg on d 2 and 3) over d 5 to 14 of the cycle.
FIGURE 3. Vena cava plasma concentrations of OT in intact or unilaterally hysterectomized (UHYST) ewes, treated with either saline or GnRH (100 μg on d 2 and 3) over d 5 to 14 of the cycle.
FIGURE 4. Vena cava plasma concentrations of PGF$_{2\alpha}$ in intact or unilaterally hysterectomized (UHYST) ewes treated with either saline or GnRH (100 µg on d 2 and 3) over d 5 to 14 of the cycle.
FIGURE 5. Jugular serum concentrations of \( P_4 \) in intact or unilaterally hysterectomized (UHYST) ewes treated with either saline or GnRH (100 \( \mu \)g on d 2 and 3) over d 5 to 14 of the cycle.
FIGURE 6. Jugular serum concentrations of $P_4$ in intact or hysterectomized (HYST) ewes treated with either saline or GnRH (100 µg on d 2) over d 4 to 12 of the cycle.


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