# Characterization of seasonal reproduction in Virginia Tech Selection Line, St. Croix, and Suffolk ewes

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## (ABSTRACT)

This dissertation research contained three studies. The first two studies were conducted to investigate the ability of ewes to rebreed while lactating during seasonal anestrus. Breeds studied included the Virginia Tech Out-of-season (OOS) Line, which is a wool line genetically selected to lamb in the fall, and the St. Croix, a hair breed of tropical origin thought to be lowly seasonal. When January-lambing ewes were exposed to rams while lactating in April, significantly more OOS than St. Croix ewes were marked by rams in the first 21 d and total 39 d of ram exposure (58.3 vs. 8.7%, $P$ = 0.0003 and 95.8 vs. 43.5%, $P < 0.0001$). Percentages of ewes diagnosed pregnant (53.2%) and percentages of ewes lambing (41.3%) were not different between breeds. When March-lambing OOS ewes were exposed to rams while lactating in May, 52.9% of ewes were marked though only 20% of ewes exposed to rams gave birth to viable lambs. Both OOS and St. Croix ewes appear to be well suited to accelerated production systems involving 7 to 8 mo lambing intervals. However, reduction of lambing intervals to 6 to 7 mo appeared to have detrimental effects on fetal survival in OOS ewes.

In a third study, alterations in endocrine profiles associated with differing degrees of hypothalamic sensitivity to estradiol-negative feedback and changing daylength in OOS, St. Croix, and Suffolk ewes in the absence of rams were investigated for 1 yr. The results show for the first time that based on progesterone profiles from intact ewes, St. Croix ewes do not have shorter anestrous periods than ewes of wool breeds, as previously thought. Based on luteinizing hormone profiles from ovariectomized ewes treated with estradiol implants, the duration of luteinizing hormone inhibition was shorter in OOS than Suffolk ewes (68 vs. 170.2 d, $P$ = 0.02), but was not different from that found in St. Croix ewes (124.8 d). Specific roles for thyroxine and prolactin in timing the breeding season could not be assigned. This study was the first known use of the ovariectomized, estradiol-implanted ewe model to compare degree of reproductive seasonality in different breeds.

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# INTRODUCTION

In 1784, the Italian Biologist Lazzaro Spallanzani wrote, “It is well known that almost all animals, except man, have a stated season for the propagation of their species”. Thus, the domestic ewe exhibits a seasonal breeding pattern timed by photoperiod whereby maximum breeding behavior and fertility occur during the fall (McKenzie and Terrill, 1937). In the Northern Hemisphere, ewes generally begin cycling in late summer and continue sexual behavior through mid-winter. During the spring and summer, under the influence of increasing daylength, most ewes undergo a period of low to non-existent ovulatory and estrous activity known as seasonal anestrus (Hulet et al., 1974). When seasonal anestrus coincides with lactational anestrus in the early postpartum period, a significant block to successful pregnancy ensues. The changes in reproductive activity observed during anestrus are consequences of changes at the hypothalamic-pituitary-gonadal axis, specifically a decrease in the frequency of secretion of gonadotropin releasing hormone from the hypothalamus, due to increased sensitivity of the hypothalamus to negative feedback effects of estradiol, and a resultant decrease in secretion of luteinizing hormone from the anterior pituitary gland (Karsch et al., 1984).

The length of the breeding season in ewes is affected by numerous factors, including genetic composition of individual ewes, age, nutrition, lactational status, and notably, geographic origin of breeds. Breeds of tropical origin, those from the Mediterranean region, and those with Merino ancestry have breeding seasons of longer durations than breeds originating from temperate and higher latitudes (Whisnant and Inskeep, 1992).

The inability of most ewes to cycle in the spring has limited sheep producers to one crop of lambs per year. Understanding the genetic, physiologic and endocrine differences among ewes in their ability to remain reproductively active during times of the year traditionally thought to be the non-breeding season may allow future manipulation of seasonal and lactational reproduction in the ewe. Sheep producers would then benefit from reduced costs per offspring reared, increased net returns, increased production per dollar of capital investment, a more uniform supply of lamb throughout the year, and more consistent lamb prices.

The goals of this dissertation were 1) to investigate the effect of breed on the ability of ewes to conceive while lactating during anestrus, 2) to observe rates of reproductive loss that occur in ewes exposed to rams while lactating during seasonal anestrus and the early postpartum period, and 3) to investigate endocrine profiles of ewes exposed to natural photoperiods for 1 yr. Breeds studied included the Suffolk, a highly seasonal breed, the St. Croix, a lowly seasonal tropical breed, and the Virginia Tech Selection Line. The VT Selection Line was developed by selection beginning in 1988 for fertility in May and June matings (Al-Shorepy and Notter, 1996), and is a three-way cross of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding. Overall, this dissertation was aimed at shedding light on how physiological and neuroendocrine responses to changing daylength manifest themselves in breeds that occupy different positions on the continuum of reproductive seasonality.

# CHAPTER 1

## REVIEW OF LITERATURE

### The ovine estrous cycle

Domestic ewes are classified as being seasonally polyestrus, meaning that ewes have clusters of estrous cycles that occur only during a portion of the year, commonly referred to as the breeding season, and followed by a distinct period of non-cyclicity or anestrus. The estrous cycle lasts 14 to 19 d in 95% of ewes (McKenzie and Terrell, 1937). Most breeds of sheep in the Northern Hemisphere begin to cycle as daylight decreases in the fall and continue to cycle during the short days of winter. Estrous cyclicity ceases as day length increases in spring and anestrus continues through the long days of summer. Biologically, this rhythm, in combination with the approximately 5-mo gestation length in sheep, ensures that lambs are born in the spring when environmental conditions are most favorable for lactation and lamb survival. Although the general rule is for the breeding season to occur in the fall and winter, reproductive patterns may vary dramatically due to a variety of factors including breed and environment.

#### *Anatomy of the Hypothalamic-Pituitary Axis*

Ovine reproduction is under the control of the hypothalamic-pituitary-gonadal (HPG) axis. The hypothalamus is the interface between the nervous and endocrine systems. The hypothalamus is derived embryologically from an area of the diencephalon that forms the floor of the third ventricle and includes the optic chiasma, tuber cinereum, mammillary bodies, and median eminence (Greco and Stabenfeldt, 2002). The hypothalamus itself is divided into clusters of nerve cell bodies that make up the

hypothalamic nuclei and have different functions and stimulatory conditions. The hypothalamic nuclei include the supraoptic nucleus, paraventricular nucleus, periventricular nucleus, arcuate nucleus, and preoptic area (Page, 2006). In general, the hypothalamus produces peptides and amines that directly cause biologic effects in tissues or influence the pituitary gland to produce either tropic hormones which in turn influence the production of hormones by peripheral target endocrine tissues or hormones that directly cause a biologic effect in tissues. The hypothalamus has two extensions into the pituitary gland, the infundibulum and the neurohypophysis. The pituitary gland consists of the adenohypophysis, or anterior lobe, and neurohypophysis, or posterior lobe.

The adenohypophysis was formed during embryonic development when Rathke's Pouch, an out-pocketing of the roof of the embryonic oral ectoderm, extended upward to meet the neurohypophysis. The neurohypophysis extends downward as an out-pocketing of neural ectoderm from the third ventricle. The neurohypophysis is predominantly composed of axons which have a neural origin from within the supraoptic and paraventricular nuclei of the hypothalamus and which produce anti-diuretic hormone and oxytocin, respectively. This connection is known as the hypothalamic-hypophyseal tract. Secretory products from these hypothalamic/neurohypophyseal neurons are secreted into the systemic blood and can elicit physiological action at distances greatly removed from the neuron.

Unlike the neurohypophysis, which is connected to the hypothalamus through direct neural input, the glandular tissue of the adenohypophysis is connected to the hypothalamus through a vascular system. The hypothalamus produces regulatory hormones that are transported first through the median eminence, which is common to the

neurohypophysis, and then on to the adenohypophysis by the hypophyseal portal venous system. These hypothalamic regulatory hormones control the release of various anterior pituitary hormones. The hypophyseal portal system is divided into the primary capillary plexus in the infundibulum into which the hypothalamic hormones are secreted and the secondary capillary plexus which delivers the hormones to the adenohypophysis. This discussion will first focus on the functions of the individual components of the HPG axis and will then integrate these functions together to explain the components of the estrous cycle.

*GnRH, LH, and FSH Release Patterns*

The estrous cycle can be divided into two major phases based upon the predominant ovarian structures. The follicular phase is dominated by the presence of follicles on the ovary and the associated production of estradiol ($E_2$), while the luteal phase is characterized by production of progesterone ($P_4$) by the corpus luteum (CL) or multiple corpora lutea. This literature review will first explore the individual hormones that have a role in reproduction and then describe how these hormones interact to control the succession of events defining the ovine estrous cycle. The central control of the HPG axis is in the hypothalamus, which secretes gonadotropin releasing hormone (GnRH) along with other peptide hormones that act on the adenohypophysis. Numerous studies have clearly established that GnRH results in the release of luteinizing hormone (LH) from the anterior pituitary, and that GnRH release by the hypothalamus and LH secretion by the anterior pituitary are highly correlated (Senger, 2003). Two distinct modes of LH secretion have been identified in the ewe, as well as in the rat, primate, and other

livestock species, and each of these modes controls a different aspect of ovarian function (Goding et al., 1970). Tonic LH secretion occurs in a pulsatile fashion at low levels throughout the estrous cycle and is important for ovarian steroidogenesis. However, an LH surge occurs simultaneously with estrous behavior, inducing ovulation and CL formation. These two modes of LH secretion are controlled by different feedback mechanisms, thereby accounting for the observed differences in release patterns.

Tonic LH secretion is inversely related to luteal function; therefore LH concentrations decline from d 1 to 9 of the estrous cycle as $P_4$ secretion from the CL rises and then tonic levels of LH increase during the follicular phase following luteolysis, or regression of the CL (Karsch et al., 1979). Significant variation in the pattern of pulsatile GnRH secretion exists, however. The luteal phase is characterized by low frequency and high amplitude pulses, but following luteolysis, the frequency of GnRH pulses increases and the amplitude decreases (Clarke and Cummins, 1982). Because GnRH pulses drive LH pulses, LH pulse frequency follows the same pattern, but LH pulse amplitude does not differ between luteal and follicular phases (Karsch et al., 1983).

The LH surge is a transient, massive release of LH from the anterior pituitary. Concentrations rise rapidly to a peak of 100 to 200 ng/mL, which is 50 to 100 times basal levels, and then decline just as rapidly so that the surge lasts approximately 12 h (Goodman and Inskeep, 2006). The LH surge is closely coupled with estrus and the duration of time from onset of the LH surge to ovulation is usually 22 to 26 h. Although the initial phases of the pre-ovulatory GnRH and LH surges are relatively synchronous, elevated GnRH secretion is maintained for many hours after termination of the LH surge (Moenter et al., 1992).

In addition to controlling the release of LH, GnRH also plays a role in timing the release of follicle stimulating hormone (FSH) from the anterior pituitary. During the follicular phase, FSH concentrations decline to a low on the day before the LH surge. A surge of FSH then occurs coincidentally with the LH surge and FSH subsequently falls in parallel with LH. A second peak of FSH occurs 20 to 28 h after the first (Baird et al., 1981). Small increases in FSH concentrations are also associated with follicular development and are often observed at 3 to 6 d intervals throughout the luteal phase (Ginther et al., 1995).

*Ovarian Dynamics*

The third component of the HPG axis, in addition to the hypothalamus and pituitary, is the gonads. In adult ewes, higher levels of follicular activity occur in association with a functional CL (Dailey et al., 1982). These periods are characterized by variation in numbers of total follicles with day of the estrous cycle and are known as follicular waves. Studies have shown that there are between two and four follicular waves per estrous cycle and that this number differs with breed (Smeaton and Robertson, 1971 and Dailey et al., 1982). Follicular waves continue during the anestrous period, but do not lead to ovulation.

The ovaries produce different hormones depending on what ovarian structures are present. Inhibin is produced by granulosal cells of antral follicles (Engelhardt et al., 1993). Large follicles are the most important source of inhibin, but smaller follicles also appear to contribute significantly. In contrast to $E_2$ secretion, there is no change in either peripheral inhibin concentrations or secretion rate from the ovary during the follicular

phase (Souza et al., 1997). Inhibin begins to decline within a few hours after the LH surge and reaches a minimum about 24 h later. This decline corresponds to the aforementioned second peak of FSH. Inhibin secretion then increases between d 1 and 3 of the luteal phase followed by a slight decline over the next 1 to 2 d (Souza et al., 1997). No further changes are observed in inhibin secretion during the luteal phase.

Most studies on the secretion of ovarian steroids have focused on $P_4$ and $E_2$. *In vivo*, the follicles are the source of $E_2$, and $P_4$ is secreted into the blood exclusively by the CL, which is formed after the walls of the ruptured post-ovulatory follicles remodel and luteinize.

The principal $E_2$ rise in the ewe occurs during the 2 to 3 d of the follicular phase. Estradiol begins to rise after the first drop in $P_4$ at luteolysis and increases five- to ten-fold over the next few days (Karsch et al., 1979). Estradiol peaks at the start of the pre-ovulatory LH surge and then rapidly declines to basal concentrations. Secretion of $E_2$ during this time comes predominantly from the follicle that is destined to ovulate. An additional peak in $E_2$ concentrations occurs around d 4 after ovulation and corresponds to the first follicular wave (Baird et al., 1976b). Some secretion of $E_2$ occurs during the rest of the luteal phase, but mean concentrations remain relatively low and show no consistent pattern. When measured at frequent intervals in individual sheep, $E_2$ concentrations show an intermittent secretory pattern. This episodic secretion is thought to be driven by LH pulses because secretion of $E_2$ is always preceded by LH pulses (Baird et al., 1976a). In contrast, $P_4$ pulses are not correlated with LH pulses.

The physiological changes that occur in the follicles and lead to ovulation include changes in steroidogenesis, secretion of prostaglandins, microcirculation, and production

of cytokines and enzymes. An immediate effect of the LH surge is to increase cAMP, which blocks aromatase and thus the synthesis of $E_2$ from $P_4$ via testosterone (Murdoch et al., 1986). This blocking allows $P_4$ to increase in the follicular tissue and leads to increased secretion of prostaglandins, which is obligatory for follicular rupture. Murdoch and McDonnel (2002) proposed the following sequence of events in the ovulatory follicle following the LH surge: (1) LH stimulates secretion of urokinase-type plasminogen activator from ovarian epithelium; (2) this increase stimulates the conversion of plasminogen to plasmin; (3) this conversion activates latent collagenases in the follicular wall and results in release of tumor necrosis factor-α; (4) the resulting collagenolysis weakens and thins the apical follicular wall leading to stigma formation and rupture.

Progesterone concentrations are generally undetectable early in the cycle and then rise gradually from d 2 to 8 to reach a maximum ranging from 1.5 to 3 ng/mL depending on the breed (Quirke et al., 1979). Concentrations of $P_4$ remain relatively constant from d 8 to 14 and then fall to undetectable levels over the next 1 to 2 d as luteolysis occurs. Although actual levels of $P_4$ secretion vary between breeds, the pattern of secretion is remarkably constant.

Prostaglandin secretion from the uterus is not part of the HPG axis, per se, but is an important factor controlling ovarian steroid secretion. Prostaglandin $F_2\alpha$ ($PGF_2\alpha$) is luteolytic in ewes (McCracken et al., 1970), and its secretion results from the binding of oxytocin to receptors on endometrial cells (Senger, 2003). From d 2 through 10 of the estrous cycle, there is little $PGF_2\alpha$ secretion from the uterus. Episodes of secretion first appear between d 11 and 13, and the frequency of these episodes increases over the next 2 to 3 d (Baird et al., 1976a). Mean concentrations of $PGF_2\alpha$ increase late in the luteal

phase, plateau during early follicular phase, and then increase again to a peak after $P_4$ declines.

**Endocrine control of components of the estrous cycle in the ewe**

*Control of estrous behavior*

When approached by a ram, a ewe in estrus will stand still with her head lowered, wag her tail slightly, and possibly look over her shoulder as the ram nudges her flank or anogenital region (Goodman and Inskeep, 2006). Courtship behavior is usually followed by mounting and copulation. In general, behavioral estrus lasts 24 to 36 h in the ewe (Quirke et al., 1979). However, the duration of estrus can be influenced by ovulation rate and ram pheromones. The onset of estrus is coupled with the LH surge so that ovulation occurs on average 24 to 30 h after the onset of behavioral estrous (McKenzie and Terrill, 1937). The first day of estrus, and therefore the LH surge, is designated d 0 of the estrous cycle.

Under physiological conditions, both $P_4$ and $E_2$ are necessary for induction of estrus. Treatment with $E_2$ alone can induce estrus, but in the absence of $P_4$ pretreatment, doses higher than physiological ranges of $E_2$ are necessary (Karsch et al., 1980). Pretreatment with $P_4$ increases sensitivity of the hypothalamus to positive effects of $E_2$ and increases intensity of behavioral estrus (Fabre-Nys and Martin, 1991). Sensitization of the hypothalamus to $P_4$ generally requires at least 6 d of $P_4$ exposure, although treatments as short as 5 d may be effective in anestrous ewes (Knights et al., 2001). Low $E_2$ concentrations (3 pg/mL) induce estrus in virtually all ewes while higher concentrations decrease the latency to estrus and ram seeking behavior of the ewe (Fabre-

Nys and Martin, 1991). Both high concentrations of $P_4$ prior to $E_2$, and maximal secretion of $E_2$ are necessary for synchronization of estrus with the LH surge (Karsch et al., 1980). The temporal relationship between $P_4$ and $E_2$ during the estrous cycle is critical for initiation and cessation of estrus. When $P_4$ is given simultaneously with $E_2$, it blocks estrus (Karsch et al., 1980). Treatment with $E_2$ is highly effective, but only if given for no more than 1 to 2 d after cessation of $P_4$ treatment (Fabre-Nys and Martin, 1991). Thus, the pattern of these hormones observed during the follicular phase serves as the model for the ideal protocol of steroid treatment for induction of estrus: a prolonged period of elevated $P_4$ followed by a fall in $P_4$ and a rise in $E_2$ over the next 1 to 2 d. The physiological importance of both steroids is further emphasized by the absence of estrus at the transition into the breeding season from anestrus, which will be discussed below.

*Control of tonic GnRH and LH secretion*

Two ovarian steroids have been implicated in control of tonic LH secretion: $E_2$ and $P_4$. Quantitatively, $P_4$ is more important than $E_2$ in determining the pattern of tonic LH secretion. Studies in ovariectomized ewes demonstrated that $P_4$ produces a strong inhibition of pulsatile LH secretion due to suppression of GnRH and LH pulse frequency while $E_2$ produces only a weak inhibition of mean LH concentrations during the breeding season (Karsch et al., 1980 and Legan et al., 1977). The inhibitory effects of $E_2$ are not dose-dependent and are relatively constant throughout the cycle. In contrast, $P_4$ exerts a dose-dependent inhibition of tonic LH secretion so that changes in $P_4$ are responsible for the pattern of LH secretion (Goodman et al., 1980). During the early luteal phase, tonic LH decreases because $P_4$ increases and suppresses GnRH and LH pulse frequency. This

inhibition is maintained as long as $P_4$ is elevated due to presence of the CL. The fall in $P_4$ at the time of regression of the CL allows the frequency of the pulse generator to increase, an effect that is further enhanced by $E_2$.

The mechanisms of $E_2$ negative feedback are much more complex that that of $P_4$, which simply inhibits GnRH pulse frequency. Estradiol produces four different effects on pulsatile GnRH/LH secretion. During the luteal phase, it increases the ability of $P_4$ to inhibit frequency of GnRH pulses. During the follicular phase, it decreases GnRH/LH pulse amplitude, increases pulse frequency, and changes the shape of pulsatile release (Evans et al., 1995). The primary negative feedback effect of $E_2$ is to inhibit LH pulse amplitude by acting at both the hypothalamus and pituitary and this effect is much more pronounced during the anestrous period than during the breeding season.

*Control of the LH surge*

As reviewed by Goodman (1994), the rise in $E_2$ during the follicular phase is the ovarian signal that induces the pre-ovulatory GnRH and LH surges. This is different from the situation in many other animals, such as the rat, where $P_4$ augments the LH surge. Induction of the LH surge does not require the full pre-ovulatory $E_2$ rise and an increase to 3 to 4 pg/mL is sufficient, but greater amounts increase the magnitude of the surge and synchronize it with the onset of estrus (Goodman et al., 1981). As mentioned previously, if $P_4$ is elevated during $E_2$ stimulation, the GnRH and LH surges are blocked (Karsch et al., 1980). The sites of action of $E_2$ in triggering these surges are still being investigated, but it is clear that stimulatory effects impact both the hypothalamus and pituitary. In addition to $E_2$, at least seven different neurotransmitters have been

implicated in control of the GnRH surge. These neurotransmitters, including norepinephrine, β-endorphin, neuropeptide Y, glutamate, GABA, dopamine, and somatostatin modulate the effects of $E_2$ on the hypothalamus in a triphasic manner (Evans et al., 2002).

*Control of ovarian dynamics*

A model proposed by Scaramuzzi and colleagues (1993) and generally accepted today, dictates that once a follicle begins to grow, it becomes subject to atresia. If atresia does not occur, and growth continues, development of an antrum and growth to up to 2 mm can happen in the absence of pituitary gonadotropins. Once a follicle has at least 100,000 granulosal cells, it is considered committed, does not return to a quiescent state, expresses LH receptors in differentiated thecal cells, and is gonadotropin-responsive. Under the influence of LH and FSH, the follicle acquires aromatase and becomes gonadotropin-dependent when it acquires LH receptors on granulosal cells. These gonadotropin-dependent follicles are subject to high rates of atresia, especially during the luteal phase when $P_4$ limits LH pulse frequency. Ovulatory-sized follicles become atretic if an LH surge does not occur within 72 h (Goodman and Karsch, 1980).

Ovulation rate is subject to control by hormonal, environmental, and genetic factors. The influence of season and lactation will be considered later in this review. Independent of nutritional, metabolic, and seasonal effects, low $P_4$ concentrations in peripheral blood can increase ovulation rate. Prolific breeds of ewes had lower concentrations of $P_4$ than non-prolific breeds and anestrous ewes pretreated to produce $P_4$ levels lower than those in the luteal phase before introduction of rams had greater

ovulation rates than ewes just introduced to rams (Bartlewski et al., 1999; Knights et al., 2003). This increase in ovulation rate with $P_4$ pretreatment was equal to that obtained with treatment with FSH at $P_4$ withdrawal and ram introduction (Knights et al., 2003). Several individual genes are known to influence ovulation rate and vary in the extent to which ovulation rate is increased and in their associated physiological characteristics (Montgomery et al., 2001). Physiological characteristics that are influenced by the gene, such as inhibin A or FSH concentrations, or LH receptor expression, give clues as to the exact site of action of the gene. Examples of genes that have been shown to have an effect on ovulation rate include the Booroola (FecB), Inverdale ($FecX^I$) , and Hanna ($FecX^H$) genes (Montgomery et al., 2001).

Functional lifespan and demise of the CL are controlled by luteotropic and luteolytic factors from the anterior pituitary and uterus, respectively. Both of these types of factors are regulated by $P_4$. A key factor in luteal development is angiogenesis since extensive blood flow is required for luteal activity. Vascular endothelial growth factor is a cytokine that is involved in angiogenesis. In the ewe, its mRNA levels are greatest early in the estrous cycle and least during luteal regression (Redmer et al., 2001). Another factor that has received recent attention in this area is stem cell factor, but no specific functional role in luteal development or luteolysis has been established thus far (Gentry et al., 1996). Estrogen, acting through the estrogen receptor β, may pay a role in activating secretion of $P_4$ by the early CL even though tonic $E_2$ is required for luteal regression. Oxytocin, generally known for its involvement in luteolysis, also may be necessary for luteal development and function (Goff et al., 2004). As discussed earlier in this review, $PGF_2\alpha$ is the uterine luteolysin in the ewe (McCracken et al., 1970).

## Characterization of seasonal reproduction in the ewe

Maximum reproductive activity in the ewe is associated with short-day photoperiods, and the percentage of ewes displaying estrus is highest during the late summer, fall and early winter (McKenzie and Terrill, 1937; Hulet et al., 1974). Nearly all Targhee, Hampshire, Rambouillet, Suffolk, Polled Dorset, and Columbia ewes displayed estrus during September through March in Wisconsin, after which the percentage of ewes displaying estrus declined from April through June (Mallampatti et al., 1971; Lax et al., 1979), and then gradually increased as the breeding season approached once again. Ovulation rate follows an annual pattern similar to that of estrous cyclicity; the number of ovulations is highest during the breeding season and lowest during the non-breeding season (Mallampati et al., 1971; Hulet et al., 1974). The non-breeding season, also referred to as anestrus or the anestrous period, can therefore be defined as a period of low to non-existent ovulatory and estrous activity.

The peak in breeding activity of the ewe occurs from September to November in the Northern Hemisphere and is reflected in a subsequent peak in lambing activity from February to April. The breeding and lambing patterns are reflected in seasonal availability of lamb and fluctuations in price. The induction of fertile matings outside the normal breeding season would therefore allow a more consistent supply and more uniform lamb prices.

### *Endocrine basis for seasonal reproduction*

The changes in reproductive activity observed during anestrus are consequences of changes at the hypothalamic-pituitary axis, specifically a decrease in the frequency of

secretion of GnRH from the hypothalamus and a resultant decrease in secretion of LH from the pituitary. This decrease in frequency of secretion of GnRH is attributed to an increase in the sensitivity of the hypothalamus to negative feedback effects of $E_2$ (Legan et al., 1977). Karsch and colleagues (1993) found that during anestrus, $E_2$ at physiological concentrations inhibited LH secretion through suppression of the frequency of GnRH pulses. However, during the breeding season, the same concentration of $E_2$ was not effective in inhibiting LH pulse frequency (Karsch et al., 1993). Therefore, the main endocrine event responsible for the anestrous period in ewes is the increase in the negative feedback effect of $E_2$ on pulsatile secretion of GnRH and LH.

Although changes in temperature can be associated with changes in the reproductive activity of ewes, the dominant environmental signal that cues and synchronizes the breeding season in sheep is photoperiod (Hafez, 1952; Wodzicka-Tomasezewksa et al., 1967; Karsch et al., 1984). Marshall (1937) demonstrated that when ewes are transferred across the equator, the annual reproductive cycle of those ewes shifted in accordance with the new photoperiod.

Photoperiodic information is conveyed through several neural relays from the retina to the pineal gland, where the light signal is translated into a hormonal signal, melatonin. The mechanism by which daylength is perceived was described by Karsch et al. (1984). Briefly, a retinohypothalamic tract projects from photoreceptors in the retina to the suprachiasmatic nucleus, and then to the paraventricular nucleus and the superior cervical ganglion, which in turn innervates the pineal gland. The pineal gland responds to darkness with an increase in melatonin secretion and to light with a decrease in secretion, resulting in high concentrations of melatonin during the night and low

concentrations during the day. These differences in the pattern of melatonin secretion translate the photoperiodic signal to the neuroendocrine axis. This pathway was elucidated by studies that involved lesioning parts of the neural relay system that link the retina to the pineal gland. Blinded ewes exhibited estrous and anestrous periods, but these were no longer synchronous with the normal seasonal patterns in sighted ewes (Karsch et al., 1984). Melatonin release during short daylengths in ewes likely causes a decreased responsiveness of GnRH neurons to the negative feedback effects of $E_2$ through inhibition of dopamine release from the hypothalamus and decreased signaling to two dopaminergic cell populations (Williams and Helliwell, 1993). Destroying dopaminergic neurons A-14 and A-15 in the hypothalami of ovariectomized ewes during seasonal anestrus decreased the inhibitory effects of $E_2$ on reproductive activity (Havern et al., 1994). Given these results, many studies have shown that melatonin may be a useful tool in advancing the onset of the breeding season in this economically important food-producing species. However, these cell groups are almost certainly not the only neural relay involved in mediating sensitivity to $E_2$ negative feedback because $E_2$ receptors have not been found in these locations.

The recently elucidated KiSS-1/GPR54 system shows evidence of being a potent stimulator of the HPG axis with the products of the KiSS-1 gene, kisspeptins, acting directly on GnRH neurons solely through the GPR54 receptor. Studies have shown this system to be important in regulating reproductive activity during times when GnRH neuron activation is required, such as puberty. To determine a potential role for the KiSS-1/GPR54 system in seasonal breeding, Revel and colleagues (2007) used the Syrian hamster. In the hamster, a common model for seasonal breeding, exposure to short days

(SD) results in a dramatic inhibition of reproductive activity manifested by a decrease in serum LH, FSH, and prolactin, and accompanied by a dramatic reduction of gonadal hormone production (Goldman, 2001). Mean levels of KiSS-1 mRNA and the number of KiSS-1 expressing neurons were decreased in the anteroventral periventricular nucleus and arcuate nucleus (ARC) of adult sexually active male hamsters when they were placed in SD after being raised in long days (LD) compared to animals kept in LD (Revel et al., 2007). This study also showed that this decrease in KiSS-1 activity was manifested by a reduction in testicular weight. Because decreased testicular weight occurs coincident with decreased circulating sex steroids, KiSS-1 mRNA levels in control and testosterone-treated SD hamsters were compared. After 4 wk of testosterone treatment, the low level of KiSS-1 expression was not significantly changed and remained comparable to that of control hamsters.

To test whether the downregulation of KiSS-1 expression in the ARC of SD hamsters was mediated by melatonin, Revel and colleagues (2007) pinealectomized hamsters prior to transfer to SD. After 10 wk, these hamsters did not undergo sexual inactivation and failed to show SD-induced decreases in KiSS-1 mRNA levels in the ARC in contrast to sham-operated hamsters. This result suggests that in Syrian hamsters, melatonin mediates the SD-induced down-regulation of KiSS-1 expression. Smith and colleagues (2007) recently reported that KiSS-1 mRNA expression is also modulated by the time of year in sheep, another seasonal breeder. In contrast to hamsters, reproductive activity in sheep is promoted by SD photoperiods and inhibited by LD photoperiods. Interestingly, KiSS-1 expression was higher at the onset of the breeding season compared to the anestrous season. Therefore, KiSS-1 mRNA in the ARC appears to be

differentially regulated by photoperiod in LD compared to SD breeders, perhaps due to an opposite interpretation of the same melatonin signal by the KiSS-1 neurons of the ARC. These results implicate the KiSS-1/GPR54 system as being an important modulator of $E_2$ sensitivity at the hypothalamus acting either upstream of, or in concert with, other modulators such as dopamine release. Whether melatonin receptors are expressed in the same neurons expressing KiSS-1 mRNA and whether melatonin acts on these neurons directly remains to be elucidated.

*Factors affecting length of the breeding season*

In addition to photoperiodic signals, many other factors, including breed, and its geographic origin, individual genetic makeup, age, and nutritional and lactational status, influence the duration and timing of the breeding season in ewes. These factors were discussed in detail in reviews by Whisnant and Inskeep (1992) and Knights (2001) and will be summarized briefly. The effects of lactation on seasonal reproduction will be discussed in detail in a subsequent section. Data pertaining to the length of the breeding season have been reported in a variety of ways including the number of estrous periods per year, the proportion of ewes showing estrus or ovulating each month, the duration of the season in weeks, and the number of estrous periods in the season.

Breeds and their geographic origins

Breeds of tropical origins, those from the Mediterranean region, and those with Merino ancestry have breeding seasons of longer durations than breeds originating from temperate and higher latitudes (Whisnant and Inskeep, 1992). This association, however, is not absolute: the Finnish Landrace and Romanov breeds have extended breeding

seasons despite their origins in northern Europe. Popular U.S. breeds with extended breeding seasons include the Dorset, Rambouillet, Finnsheep, and crosses with and among these breeds. Breeds with breeding seasons of intermediate-length include the Corriedale, Columbia, and Targhee, while the Suffolk, Hampshire, Oxford, Southdown, Shropshire, and Cheviot are breeds with short seasons. However, data from studies comparing the lengths of the breeding seasons of different breeds have been confounded by the fact that not all breeds have equal proportions of ewes exhibiting behavioral estrus in association with ovulation. For example, Quirke et al. (1988) noted that Rambouillet ewes had a tendency to ovulate without estrus at the beginning and end of the breeding season. Additionally, there is great variation in the duration of the breeding season among locations and years and within breeds. These facts are further confounded by factors such as individual genetics, time since last lambing, nutritional and lactational status, age, and the presence of rams. Marshall (1937) observed that the latitude in which the study is conducted can have a large impact on the duration of the breeding season. Researchers and producers alike are able to take advantage of breed differences in the degree of seasonality to improve the ability of ewes to breed out-of-season (Notter et al., 1992).

Genetic Selection

Heterosis, or hybrid vigor, is important for many reproductive traits and this appears true for length of breeding season. In studies reviewed by Notter (1992), DLS sheep, which are a mix of Dorset, Leicester and Suffolk stock, had a longer breeding season than any of the component purebreds. Likewise, the breeding season of crosses of the Dorset, Rambouillet, and Finnsheep breeds averaged 9 d longer than those of the

parent purebreds (Quirke et al., 1988). The duration of first breeding season of Finnsheep x Dorset ewe lambs was 131 d, compared to 127 d in Finnsheep and 87 d in Dorsets (Quirke et al., 1985). Based on these results, heterosis may be beneficial to selection for long breeding seasons; studies on other traits associated with out-of-season breeding, such as conception rates in various seasons, have been inconclusive. However, the benefits of heterosis have been realized in the production of special lines selected for extended breeding seasons. The Virginia Tech Selection Line is one such example and will be discussed in a later section.

Few studies have objectively assessed the opportunities for within-breed genetic improvement in traits associated with out-of-season breeding. Several experimental populations with desirable out-of-season breeding characteristics have been developed through a combination of crossbreeding and selection, but it has generally not been possible to separate effects of initial breed composition and non-genetic adaptations to the imposed management from the effects of selection. Furthermore, heritability estimates for seasonal reproductive traits ranges from .03 to .32 (Al-Shorepy and Notter, 1996). This adds another level of complexity to the improvement of out-of-season breeding traits through selection. Results of selection experiments indicate that some level of genetic control of the seasonality of reproduction exists, but few controlled experiments appear to have resulted in large, documentable changes in the seasonal breeding pattern within breeds.

Age

The breeding season is shorter in ewe lambs than in mature ewes (Cole and Miller, 1935; Hafez, 1952). Dyrmundsson (1973) concluded that the first breeding

season of ewe lambs is shorter because it both begins later and ends earlier. Although genetic selection extended the breeding season in mature ewes, these improvements were not achieved in ewe lambs or yearlings (Notter, 1992).

Nutrition

The precise mechanisms by which nutrition influences reproduction are not well understood. However, it is clear that body condition directly affects hypothalamic activity and GnRH secretion and that effects on reproductive performance are mediated through changes in ovarian hormones or hypothalamic-pituitary sensitivity to ovarian hormones (Rhind et al., 1989). Conception and pregnancy rates generally are depressed when ewes are kept on a low plane of nutrition before mating (Coop, 1966). The percentage of ewes responding to introduction of rams with ovulation and the percentage of ewes with spontaneous ovulations in the following spring were greater in ewes on a high than a low plane of nutrition during autumn and early winter (Oldham and Fisher, 1992). When a low plane of nutrition is superimposed on lactation during the time of rebreeding, severe negative effects on reproduction may be observed.

**Characterization of lactational anestrus in the ewe**

The response to estrous induction procedures is generally lower in lactating than in non-lactating ewes during anestrus. Conception and pregnancy rates generally are depressed when ewes are mated while lactating during the anestrous season. Therefore, lactation creates another level of complexity in relation to expectations for reproductive performance of the ewe in different seasons. The effects of lactation on various lambing-related variables were reviewed by Cognie and colleagues (1975). In contrast to results observed in cows, restoration of the uterus after lambing took longer in lactating than in

non-lactating ewes (Foote et al., 1971). The elimination of cellular debris and the return to normal uterine weight took longer in spring than in autumn and after lambing in the spring, more lactating than non-lactating ewes still had cellular debris in the uterus at 24 d postpartum (30 vs. 0%). Greater dosages of equine chorionic gonadotropin were required to induce ovulation in lactating than in non-lactating ewes. The number of ovulations was more variable and ovulations were spread over a longer period in lactating than in non-lactating ewes (Cognie et al., 1975). A high proportion of uterine contractions originating near the oviducts and moving toward the cervix, rather than the other direction, was cited as one possible cause for low conception rates in ewes bred during the early postpartum period (Kiesling et al., 2000). A more likely explanation is that the fertility problem begins even before mating, as lactating ewes have reduced ovulatory responses and lower ovulation rates.

Lactating ewes have longer intervals to first estrus and conception than non-lactating ewes (Whiteman et al., 1972; Pope et al., 1989). In fall-lambing ewes, the percentage of ewes that showed estrous behavior by d 67 postpartum was greater in non-lactating than lactating ewes (89 vs. 33%; Call et al., 1976). When seasonal anestrus is combined with lactation, a significant block to successful pregnancy ensues.

There are numerous physiological reasons for lowered fertility in lactating anestrous ewes. During lactation, serum concentrations of prolactin are elevated and are related inversely to the concentrations of LH and FSH in serum. However, in ovariectomized ewes, elevated concentrations of prolactin in serum did not directly inhibit the pituitary's ability to respond to GnRH (Moss et al., 1980). Findings using the postpartum suckled cow may aid in understanding the related endocrine events in the

postpartum ewe. Mean concentrations of LH and the frequency and amplitude of episodic LH peaks are lower in suckling dairy cows (Williams et al., 1982). Whether suckling-mediated events decrease basal LH secretion through actions at the hypothalamic level, pituitary level, or both, is still unclear. Beef cows suckling a calf released less LH in response to GnRH on d 5 postpartum than cows not suckling a calf. Further, GnRH-induced LH release was lower in pituitary explants from dairy cows suckling calves at d 14 postpartum compared to explants from cows that were not suckling calves. The authors suggested that pituitary gonadotrophs of early postpartum suckled cows were either somewhat refractory to GnRH stimulation or contained a smaller readily releasable pool of LH than those of non-suckled cows. Separately, GnRH and $E_2$ successfully induced release of LH in suckled cows between 17 and 60 d postpartum. The characteristics of these releases were similar to those seen in ovariectomized heifers and milked dairy cows at 2 wk postpartum. The authors concluded that adequate amounts of releasable LH are available early in the postpartum period of suckled cows. Therefore, normal synthesis and storage of pituitary LH may occur even if the frequency and amplitude of GnRH release are inadequate to sustain normal tonic LH secretion.

In sheep, Moss et al. (1980) reported that the resumption of estrous behavior following parturition was associated with increasing pituitary stores of LH and FSH, but not with altered hypothalamic content of GnRH or changes in the pituitary response to GnRH. Similar to the findings of Williams and colleagues (1982), they failed to demonstrate any effect of suckling on readily releasable pools of LH between 1 and 30 d postpartum.

Adding to the complexity of the effects of lactation on reproduction is the fact that there appear to be interactions between lactation and season. Ford (1979) found that serum concentrations of LH increased between d 10 and 30 postpartum in ovariectomized ewes. Ewes that lambed in the fall and did not nurse any lambs had higher concentrations of LH than ewes that nursed one or two lambs. Further, 8 d after ovariectomy, concentrations of LH were higher in ewes that lambed in the fall than in ewes that lambed in the spring (Ford, 1979).

**Introduction of rams as a method to interrupt seasonal and lactational anestrus in the ewe**

One method used to achieve breeding activity during the non-breeding season is to join previously isolated anestrous ewes with rams. Numerous studies have shown that the introduction of rams to seasonally or lactationally anovulatory ewes before the start of the normal breeding season can result in ovulation. Underwood and colleagues (1944) and Schinckel (1954) showed that anestrus in Merino ewes could be interrupted by introduction of rams due to induced ovulation. This method is commonly referred to as the "ram effect" or "male effect". In order to get a reproductive response to the introduction of rams, it is common practice to isolate the ewes from rams (including sight, sound, and smell) for a period of time before introduction. There is evidence, however, that isolation may not be essential if novel rams are used. Cushwa and colleagues (1992) found that introduction of novel rams evoked similar responses from ewes that were isolated from rams (housed 1.5 km away) and ewes that were adjacent to rams (either in pens 15 m away or in adjacent pastures separated only by a fence).

As discussed previously, release of GnRH from the hypothalamus controls release of LH from the anterior pituitary. Therefore, the pattern of release of LH is generally similar to that of GnRH (Clarke and Cummins, 1982). In anestrous ewes, GnRH and LH pulses occur very infrequently compared to pulses in ewes during the breeding season. This decrease in frequency is due to the increased sensitivity of the hypothalamus to the negative feedback effects of $E_2$ (Legan et al., 1977). Initial studies suggested that introduction of rams might directly cause the preovulatory LH surge (Knight et al., 1978); however, it is now apparent that the first effect of introduction of rams is an increase in tonic LH secretion, causing the onset of a typical follicular phase (Martin et al., 1983). The increase in LH pulse frequency drives follicular development, resulting in a rise in the circulating concentrations of $E_2$ (Goodman, 1994). The observation by Martin and colleagues (1983) that ram-induced increases in LH pulse frequency do not occur in the absence of ovaries supports this hypothesis. The increase in circulating concentrations of $E_2$ has two effects: in the first 2 to12 h, it reduces concentrations of FSH and amplitude of LH pulses; in 12 to 48 h, it induces preovulatory surges of both LH and FSH. The LH surge induces ovulation and the formation of CL (Martin et al., 1986).

In anestrous ewes, $E_2$ is capable of inducing an LH surge within as little as 6 to 8 h after treatment, but the average is nearer 18 h. As noted by Knights (2001), responsiveness of follicles to gonadotropic stimulation is reduced in anestrous ewes, which may limit the synthesis of $E_2$ that can be attributed to ram-induced increases in the synthesis of gonadotropins. Additionally, Martin and colleagues (1986) observed that the period from introduction of rams to the LH surge (approximately 36 h) is shorter than the normal follicular phase in cycling ewes. The authors proposed that these early surges are

a result of exaggerated stimulation of tonic LH secretion by introduction of rams. It is possible that an increase in the sensitivity of the LH surge mechanism to $E_2$ rather than an actual increase in the concentration of $E_2$ might contribute to triggering the early LH surges observed in some animals.

The precise mechanism through which the introduction of novel rams results in increased secretion of LH in anestrous ewes is not clearly understood. Because the ram-induced increase in pulse frequency of LH is observed in ovariectomized, $E_2$-treated, but not control ewes, disruption of the $E_2$ negative feedback system seems a likely explanation (Martin et al., 1983). Estradiol negative feedback effects are probably mediated by catecholaminergic neurons (Havern et al., 1994). The suppression of these catecholaminergic neuronal systems might explain why the ram effect induces an increase in tonic LH secretion. The introduction of rams induces a follicular phase in anestrous ewes by blunting the actions of long photoperiod, allowing ewes to revert transiently to the reproductive condition found during the breeding season.

Limited data exist on the pattern of growth and development of follicles following introduction of rams to anestrous ewes. An increase in the number of small, large, and total follicles has been observed during the first 40 h after introduction of rams (Atkinson and Williamson, 1985). Most ewes ovulate within 50 h after introduction of rams (Martin et al., 1986). Martin and colleagues (1986) reported increased ovulation rates in seasonally anestrous ewes introduced to rams compared to rates of ewes that spontaneously ovulated during the same time. However, results from studies investigating the ovulation rates in response to introduction of rams are inconsistent,

possibly due to differences in nutritional status and the method of selection of experimental animals.

Because of the lack of exposure to $P_4$ prior to the ram-induced increases in $E_2$, the first ovulation is not associated with behavioral estrus. However, breed differences might affect the proportion of ewes showing estrus in conjunction with ovulation at the onset of the breeding season (Quirke et al., 1988). Oldham and Martin (1978) reported that the CL resulting from the first ram-induced ovulation can either experience a normal lifespan or be short-lived and prematurely regress. Ewes with a normal CL will ovulate in conjunction with estrus 17 d later. Corpora lutea that are short-lived regress 5 to 6 d after ovulation and are usually followed by another ovulation without estrus. The length of the second luteal phase usually is normal with estrus and ovulation occurring about 17 d later (Oldham and Martin, 1978). Thus, the estrous activity of the flock is spread over 10 d with two peaks; the first around d 18 and the second around d 24 after rams are introduced.

Knight and Lynch (1980) demonstrated that the scent from the male was the most important sensory cue in inducing ovulation in anestrous ewes. They found that the wool and wax of rams contained odoriferous substances, pheromones, which stimulated 48% of a group of ewes to ovulate within 5 d of introduction, a response similar to that in ewes in contact with rams. Surprisingly, ram urine was not a major source of the pheromone (Knight and Lynch, 1980). There are two olfactory systems, the main and vomeronasal systems, which conduct sensory inputs to the central nervous system. In the ewe, the main olfactory system alone is capable of conducting the pheromonal stimuli to the central nervous system. Indeed, vomeronasal cauterization and nerve section that spared

the main olfactory system did not inhibit the increased LH response of ewes exposed to the odor of males (Cohen-Tannoudji et al., 1989). The induction of increased secretion of LH by the fleece of rams alone supports the concept that visual and physical components of perception of the ram are not essential (Knight and Lynch, 1980). Once the bulbs of the main olfactory system sense a pheromonal stimulus, the message is sent on to the olfactory cortex, from which efferent fibers branch out, innervating the hypothalamus via the amygdala and fornix (Knights, 2001). Thus, the pheromonal stimulus can be mediated through the hypothalamus to stimulate the secretion of GnRH.

There is a wide range of variation in responses of ewes to introduction of rams. The factors affecting magnitude of the response were reviewed by Oldham and Fisher (1992). Isolation of ewes from the rams for at least 1 mo prior to introduction is the generally accepted practice, suggesting that a process of habituation occurs, whereby rams lose their ability to stimulate increased secretion of LH from ewes after joining. If habituation occurs after the first induced estrus, then ewes become anovulatory again before ever displaying estrus (Pearce et al., 1985). The stage or depth of anestrus, as reflected by the percentage of spontaneously ovulating ewes in the flock, also influences the response to introduction of rams. Oldham and Fisher (1992) showed that the percentage of ewes ovulating in response to introduction of rams was positively correlated with the proportion of the flock ovulating spontaneously at the time. Additionally, the length of the postpartum interval at introduction, breed differences of both the rams and ewes, sexual activity level of the rams, and nutritional status of the ewes, as discussed previously, affect the magnitude of the response of anestrous ewes to introduction of rams.

Treatment with progestogens in conjunction with introduction of rams has been used, with varying degrees of success, to synchronize the estrous cycle in ewes. Progesterone was first used to synchronize estrus 60 yr ago (Dutt and Casida, 1948), and fertile estrus was induced in anestrous ewes with progesterone and equine chorionic gonadotropin (Dutt, 1953). Progestogens are important to many processes that make out-of-season breeding possible, including display of behavioral estrus and the maintenance of the first ram-induced CL. A multitude of treatment combinations have been developed using progestogens and gonadotropins at different dosages and times. A limitation to the use of progesterone in out-of-season breeding approaches is that it is not readily available to sheep producers.

As discussed earlier, the first ram-induced ovulation in anestrous ewes is not accompanied by behavioral estrus. However, estrus is normally exhibited at subsequent ovulatory events. In early studies, evidence was obtained that $P_4$ blocks the initiatory effects of $E_2$ on estrus (Dutt and Casida, 1948). The stimulatory roles of $P_4$ pre-treatment on sexual behavior have since been demonstrated and reviewed (Knights, 2001). The occurrence of estrous behavior in conjunction with ovulation in response to introduction of rams during anestrus is dependent on the presence and age of functional CL on the ovary at the time of treatment (Robinson, 1950). In a study on maiden ewes during seasonal anestrus, Robinson (1955) observed estrous behavior in all $P_4$ pre-treated ewes receiving $E_2$ or $E_2$ and equine chorionic gonadotropin. Ewes receiving similar dosages of $E_2$ and/or equine chorionic gonadotropin but without $P_4$ pre-treatment did not show behavioral estrus. Ewes expressed estrus in response to $E_2$ even though they were last treated with $P_4$ 8 d previously (Fabre-Nys and Martin, 1991). When $P_4$ was present at the

time of $E_2$ treatment, progesterone inhibited the stimulatory effect of $E_2$, but this effect disappeared as soon as the $P_4$ was withdrawn. Thus, it is not necessary or desirable for $P_4$ to be present immediately prior to administration of $E_2$, but rather there is a requirement for some pre-exposure to $P_4$.

The data on duration of progestogen treatment to allow ewes to show behavioral estrus at the first ram-induced ovulation indicate a minimum requirement of 5 to 6 d to allow for adequate sensitivity to be developed in the behavioral brain centers to the amounts of $E_2$ secreted as a result of gonadotropin treatment and or introduction of rams (Knights, 2001).

Corpora lutea from the ovulation resulting from introduction of rams or administration of GnRH or LH to anestrous ewes were short-lived in at least 50% of all ewes (Knights, 2001). Treatment with $P_4$ prior to the induction of ovulation prevented premature regression of CL. The $P_4$ pre-treatment may be given in the form of a long-term regimen beginning 10 to 14 d before ovulation (McLeod, et al., 1982) or in the form of a single i.m. injection at the time of introduction of rams or treatment with GnRH, LH, or FSH (Oldham et al., 1985). Each of these methods of administration elevated serum concentrations of $P_4$ to greater than 1 ng/mL for at least 30 h, which appears to be the minimal duration of $P_4$ exposure needed for normal luteal lifespan (Knights, 2001). In conclusion, a single injection of $P_4$ at the time of introduction of rams did not affect the proportion of ewes that ovulated or displayed estrus, but ensured that all CL that resulted from introduction of rams persisted for the period of a normal estrous cycle (Oldham and Fisher, 1992; Pearce et al., 1985).

Earlier studies led to the suggestion that inadequate luteal function in anestrous ewes induced to ovulate might be due to reduced response to the LH surge, probably due to problems in the final maturational stages of the ovulatory follicle (Hunter et al., 1986). However, Southee and colleagues (1988) showed that uterine-derived $PGF_2\alpha$ was responsible for the premature regression of CL induced in anestrous ewes without $P_4$ pre-treatment. Hunter and colleagues (1989) also concluded that premature release of $PGF_2\alpha$ was the cause of early luteal regression. Thus, $P_4$ pre-treatment might protect CL from early regression by causing an early rise in $PGF_2\alpha$ prior to ovulation or before CL become susceptible to the luteolytic effects of $PGF_2\alpha$ (Knights, 2001).

Progestogens used for the control of the estrous cycle during the breeding season and induction of non-cycling ewes include progesterone, SC-9880 (fluorogestone acetate), medroxy progesterone acetate (MAP), SC-9022, SC-21009 (Norgestomet), and melengestrol acetate (MGA). Although potencies and dosages vary markedly, there is little difference in the efficacy of the various progestogens to induce fertile estrus in anestrous ewes. The choice of a particular progestogen may therefore be related more to other factors such as availability, ease of use, and approval by regulatory agencies.

Knights (2001) reviewed the methods of progestogen administration, including i.m. injection, progestogen-impregnated intravaginal or subcutaneous pessaries, orally active feed additives, ear implants, and controlled internal drug release dispensers (CIDRs). As with the particular progestogen used, there are limited differences in the efficacy of the various methods of administration, with the exception that intake can vary when the hormone is delivered in feed or drinking water.

A variable and generally lower conception rate relative to cycling ewes has been associated with synchronization of estrus with progestogens (Dutt and Casida, 1948). The threshold dosage of progestogen beyond which fertility is compromised is lower for induction of fertile estrus in anestrous ewes than for synchronization of estrus during the breeding season.

**Effect of thyroid hormones on seasonal reproduction in the ewe**

Multiple studies indicate that thyroid hormones are essential for maintenance of seasonal reproductive changes in a variety of species including the sheep, ground squirrel, starling, quail, red deer, and American tree sparrows (Karsch et al., 1991). To elucidate the role of the thyroid gland in seasonal reproduction in the ewe, researchers at the University of Michigan did a series of elegant experiments that were published as a three-part paper in 1991. The first study, conducted by Moenter and colleagues (1991) tested the hypothesis that the thyroid is needed for endogenous seasonal suppression of the neuroendocrine mechanism that regulates pulsatile LH secretion. Ewes were thyroidectomized in summer, or remained thyroid intact. All ewes were ovariectomized, had an $E_2$ implant administered to standardize $E_2$ feedback affecting LH secretion, and were housed in a simulated natural photoperiod until the winter solstice, after which time they remained on 10 h light and 14 h dark. During the midbreeding season, both thyroidectomized and thyroid-intact ewes exhibited frequent LH pulses with no difference between groups. However, at the end of the breeding season (February 3) LH fell to basal values in thyroid-intact ewes while levels remained elevated in thyroidectomized ewes until the end of the study (April 26). These observations

supported previous evidence that thyroidectomy prevents the transition to anestrus in ewes maintained in a fixed daylength. The authors concluded that the thyroid is necessary for endogenous suppression of neuroendocrine mechanisms that generate LH pulses (Moenter et al., 1991)

The second paper in this series consisted of four experiments to further examine the phenomenon that the thyroid gland is required for the breeding season to end in ewes (Webster et al., 1991b). Experiment 1 tested the hypothesis that the thyroid is required because of its secretion of $T_4$. Thyroidectomized ewes received either $T_4$ replacement or no further treatment. All animals were ovariectomized and kept in constant photoperiod as described in the previous paper, and seasonal reproductive shifts, as evidenced by changes in LH, were compared to thyroid-intact controls. Thyroidectomy did not alter onset of the breeding season, but blocked its end in the absence of $T_4$ replacement, but not in thyroidectomized ewes that received $T_4$ replacement.

Experiment 2 tested the hypothesis that the thyroid is required only until the onset of the breeding season for reproductive activity to end at its normal time. Ewes thyroidectomized after the onset of the breeding season experienced sustained elevated LH levels, indicating that the thyroid is indeed required after the onset of reproductive activity for the breeding season to end. Experiment 3 tested the hypothesis that thyroidectomy causes a widespread disruption of steroid feedback responses. The authors found no effect of thyroidectomy on the ability of an $E_2$ rise to elicit an LH surge, or the ability of $E_2$ or $P_4$ to suppress LH secretion in the breeding season. Importantly, thyroidectomy did alter the seasonal shift in the potency of $E_2$ negative feedback. Experiment 4 examined circulating $T_4$ levels in thyroid-intact ewes over a 2-year period.

Serum $T_4$ peaked in winter, during the late breeding season, and reached a nadir in summer during late anestrus. The authors concluded that secretion of $T_4$ after the onset of reproductive activity is required for an endogenously generated change in the neuroendocrine axis that leads to intensified $E_2$ feedback on the hypothalamus and an end to the breeding season (Webster et al., 1991b).

The last paper in this series tested the hypothesis that the role of the thyroid gland in initiation of changes in LH secretion that lead to the end of the breeding season in the ewe is mediated via the GnRH neurosecretory system (Webster et al., 1991a). Pulsatile secretion of GnRH into hypophyseal portal circulation and LH into peripheral circulation, and the neuroanatomical distribution and morphology of GnRH neurons were compared among thyroid-intact anestrous ewes and thyroidectomized ewes that failed to enter the anestrous period. All ewes were ovariectomized and had been administered $E_2$ implants. High frequency pulses of GnRH and LH were evident in thyroidectomized ewes that failed to transition out of the breeding season while pulsatile release of these hormones was not observed in thyroid-intact anestrous ewes. This effect in thyroidectomized ewes was not associated with changes in total number, distribution, or morphology of GnRH neurons in the hypothalamus and preoptic area. The authors concluded that the thyroid gland is required for the endogenously generated switch in function of the GnRH neurosecretory system that leads to transition to anestrus in ewes (Webster et al., 1991a).

The interaction between the thyroid and HPG axes changes dramatically throughout the year. Thyroid hormones affect only neuroendocrine processes that lead to transition into anestrus. They are not required for maintenance of anestrus or for transition into the breeding season (Thrun et al., 1997a). Studies by Thrun and

colleagues (1996 and 1997b) assessed the influence of thyroidectomy, with or without $T_4$ replacement for specific durations and at different times of the year, on the transition to anestrus in ovariectomized ewes with estradiol implants. Thyroidectomy in mid-December, just before the putative period of thyroid hormone action, prevented the development of the anestrous season as evidenced by persistent elevated LH levels. Thyroxine replacement for 90 d beginning in late December overcame the blockade of anestrus with the minimum effective duration of exposure to thyroid hormones estimated at 60 to 90 d. Exposure to $T_4$ for 60 to 90 d beginning in late December was found to be the only time of year that thyroid hormones were required to maintain seasonal changes in reproductive neuroendocrine activity. Importantly, replacement of $T_4$ for 90 d beginning in August failed to provoke development of neuroendocrine anestrus in thyroidectomized ewes (Thrun et al., 1996, 1997b). These results built on previous knowledge of the interaction between the thyroid and HPG axes and indicated that thyroid hormones are necessary only during a limited interval late in the breeding season to promote seasonal reproductive suppression in the ewe and that the reproductive neuroendocrine axis is not equally responsive to thyroid hormones at all times during the year. More recent studies confirm these results and specify that responsiveness to $T_4$ is lost gradually during the mid to late anestrous season (Billings et al., 2002).

In addition to the studies already mentioned, numerous others have demonstrated that thyroid hormones are required for maintenance or entrainment of the endogenous reproductive rhythm in the ewe (Anderson et al., 2002; Viguie et al., 1999; Dahl et al., 1995; and O'Callaghan et al., 1993). However, all these studies were done using highly seasonal Suffolk ewes. In fact, a thorough search of the literature indicates that every

paper published on the interaction between seasonal reproduction and thyroid hormones in the ewe used Suffolk ewes except one (Hernandez et al., 2003). Further studies are needed to investigate how breed composition and characteristics affect this interaction.

**Effect of prolactin on seasonal reproduction in the ewe**

In the ewe, as the duration of melatonin secretion increases under shortening daylength in fall, concentrations of circulating prolactin decrease (Thimonier et al., 1978). The same inverse relationship between melatonin and prolactin is observed in spring when duration of nocturnal melatonin secretion decreases and prolactin increases. The suppressive effect of melatonin on prolactin secretion is predominantly caused by a direct action of melatonin on the pituitary gland since hypothalamo-pituitary disconnected rams continue to show changes in the secretion of prolactin in response to alterations in photoperiod or administration of melatonin (Lincoln and Clarke, 1994). The earliest studies on the 24-h rhythm of prolactin in the sheep showed that a major peak occurs at the beginning of the dark phase throughout the year, but other studies only found this to be true in summer (Ravault and Ortavant, 1977; Walton et al., 1980). The circadian rhythm of prolactin in the ewe may be controlled by a mechanism mediated through the pineal gland. Barrell and Lapwood (1978) found that pinealectomy prevents the evening surge of prolactin found in sham-operated animals. However, daylength is not the only factor influencing prolactin release. Karsch and colleagues (1989) documented the existence of an endogenous circannual rhythm of prolactin secretion in the ewe; ovariectomized ewes administered an estradiol implant and held in short daylengths for 5 yr experienced unambiguous cycles of prolactin release.

Thus, the regulation of prolactin secretion is a complex process which involves various hypothalamic factors, much like the secretion of LH. It is well known that catecholamines influence the secretion of prolactin and that dopamine is the major prolactin-inhibiting-factor. Interestingly, in anestrous ewes, the period of high prolactin secretion is also characterized by an increase in activity of the dopaminergic system in the hypothalamus (Theiry, 1991). Nocturnal increases in prolactin concentration during the longest days in summer are thus likely evoked at least in part by an inhibitory action of melatonin on the dopaminergic pathway (Misztal et al., 1997).

There is also evidence that genetic factors may influence prolactin release in different species of sheep. Santiago-Moreno and colleagues (2000) studied seasonal changes in ovulatory activity and prolactin concentrations in Mouflon (*Ovis gmelini musimon*) and Manchega (*Ovis aries*) ewes. Mouflon ewes are wild sheep, while Manchega are domesticated and resemble breeds commonly used in the U.S. sheep industry. In this study, conducted in Spain, Mouflon ewes cycled from October to April while Manchegas cycled from July to March. While prolactin concentrations in Manchegas were high when they started cycling, concentrations were at their lowest in Mouflons when they started cycling. Overall, mean prolactin concentrations were higher in Mouflons than Manchegas throughout the year (Santiago-Moreno et al., 2000).

A very recent study by some of these same researchers investigated whether an endogenous rhythm controls seasonal reproductive activity in Mouflon and Manchega ewes and how photoperiod might affect the rhythm (Gomez-Brunet et al., 2008). The study examined the ovulatory activity and prolactin release of ewes subjected to either a constant photoperiod of long days (16L:8D) or natural changes in photoperiod for 16 mo.

The two species showed changes in reproductive activity under the conditions of constant long photoperiod, suggesting an endogenous cycle of reproduction. The domesticated Manchega ewes showed much greater sensitivity to the negative effects of long days on reproductive cyclicity than the Mouflon ewes. A circannual rhythm of plasma prolactin concentrations was seen in both species and under both photoperiodic conditions, although in both species, the amplitude of prolactin release was always lower in the long-day animals. Under natural photoperiodic changes, the Manchega ewes, which are more similar to ewes in the present study than Mouflon ewes, had higher prolactin levels in spring and summer months (67.8 ± 9.8 and 101.9 ± 5.8 ng/mL, respectively) and lower levels in fall and winter months (9.4 ± 1.4 and 10.5 ± 1.1 ng/mL, respectively; Gomez-Brunet et al., 2008). These results indicate that prolactin is not only affected by daylength, but also follows an endogenous rhythm, as previously proposed by Karsch and colleagues in 1989. Furthermore, this study provides the first evidence that genetic factors might play a role in modulating the release of prolactin. Temperature was not controlled in this study, but the authors hypothesized that it is unlikely to dictate seasonal changes in prolactin, at least in Mouflon ewes, because changes in the ambient temperature between summer and winter in the natural habitat of these ewes are not very pronounced.

While these studies have helped shed light on the complex interaction of prolactin release and seasonal reproduction in the ewe, much work remains to be done. No studies have looked directly at the influence of steroid negative feedback on prolactin release during different times of the year. Additionally, differences in prolactin release among domesticated breeds of ewes that show differences in degree of reproductive seasonality

remain to be elucidated. Closing these two gaps in current knowledge of this subject is the aim of one of the present studies.

**History of the Virginia Tech Out-of-season mating line**

The Virginia Tech out-of-season (OOS) mating line was developed by selection beginning in 1988 for fertility in May and June matings (Al-Shorepy and Notter, 1996). These months are the deepest part of anestrus for most North American sheep breeds. Three-way crosses of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding were used to start the line and after at least one generation of fall matings among these three-way crosses, the base population was divided into three populations including genetic control, selection, and environmental control lines in 1987. The genetic control line was composed of 45 ewes selected at random from a pool of 225 ewes to remain in the spring lambing system. The remaining 180 ewes were transferred to a fall lambing system and were randomly divided into a selection flock of 125 ewes and 10 rams and an environmental control flock of 55 ewes and 5 rams. The selection line was established in an annual spring mating system by mating these ewes in single-sire breeding pastures starting May 1, 1988 (Notter and Cockett, 2005). The environmental control flock was bred in spring concurrently with the selection flock and the genetic control flock continued to be bred in fall. Up to one third of ewes and one half of rams were replaced each year in the selection and environmental control lines (Al-Shorepy and Notter, 1996).

Phase 1 of the experiment to develop the OOS line lasted from 1988 through 1993, with selection of replacements based on mean fertility of the ewes. During this

phase of the experiment, ewes were exposed to vasectomized rams for 2 wk before breeding to take advantage of the ram effect and induce ovulation and estrus (Al-Shorepy and Notter, 1996; Martin et al., 1986). Phase 2 of the experiment lasted from 1994 through 1998. During this time, teasing with vasectomized rams was discontinued as mean fertility values increased and a system of breeding value estimation using best linear unbiased prediction was implemented. Under this system, ewes were assigned a binomial score of 1 or 0 at each mating denoting whether they did or did not lamb during the fall.

The selection experiment ended in 1998, at which time the environmental control and genetic control flocks were terminated, but selection in the OOS line continued. Fertility was variable in the early years of the experiment, but by 1995, a significant difference was evident between environmental control and selection flocks and this difference increased until the end of the experiment (Notter et al., 1998).

The timing of the breeding season and duration of anestrus were evaluated in the OOS ewes by using estimated breeding values for fertility. High and low breeding value ewes were housed with vasectomized rams from January through the following July and their mating behavior was monitored (Vincent et al., 2000). Only ewes that had lambed the previous fall were used to ensure physiological comparability and the same rams were used throughout the study to avoid elicitation of the ram effect due to the introduction of novel rams. In ewes evaluated in 1992, 1993, and 1995, the seasonal anestrus period of high fertility ewes in the selection and environmental control flocks averaged 28.4 d and was significantly lower than the 70.1 d observed in low fertility ewes (Notter and Cockett, 2005). An additional 13 OOS ewes were evaluated in 1997; all exhibited nearly

continuous cyclicity during spring and summer, with a mean period of anestrus of only 11.3 d (Vincent et al., 2000).

Ewes in the selection flock were also shown to have lower nocturnal levels of circulating melatonin and higher levels of prolactin than environmental control ewes (Notter and Chemineau, 2001). Therefore, there is some evidence that the endocrine profiles of ewes that are extremely proficient at breeding during times of year traditionally thought to be part of the non-breeding season may be different than the profiles of ewes that are highly reproductively seasonal. The OOS ewes may provide a model for understanding genetic control of the secretion of reproductive hormones affecting seasonality and how these breed differences affect successful out-of-season breeding.

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## CHAPTER 2

### Ability of two selected breeds of ewes to rebreed while lactating in April

**ABSTRACT:** Because sheep are seasonally polyestrus, an attempt to mate at a frequency greater than once per year will require one breeding during anestrus. Without intervening treatments, little ovarian and estrous activity is anticipated during anestrus, and pregnancy rates are low, especially if the out-of-season breeding coincides with the early postpartum period. The present study was designed to investigate the effect of breed on ability of 24 Virginia Tech Selection Line/Out-of-season (OOS) Line and 23 St. Croix ewes to conceive while lactating during seasonal anestrus. The OOS Line is a unique three-way cross of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding and has been genetically selected since 1988 to lamb in the fall. Rams were introduced to previously isolated ewes on 21 March when OOS ewes averaged 62.2 d and St. Croix ewes averaged 51.5 d postpartum ($P < 0.001$). Ewes were checked for marks and blood sampling was completed twice weekly for 39 d following ram introduction. Percentages of ewes marked in the first 21 d and total 39 d of ram exposure were greater among OOS than St. Croix ewes (58.3 vs. 8.7%; $P = 0.0003$ and 95.8 vs. 43.5%; $P < 0.0001$, respectively). However, there was no difference between breeds in the percentage of ewes diagnosed pregnant 90 d after ram introduction and the percentage of ewes that lambed. After adjustment for lambing date, the differences in percentages of ewes marked in the first 21 d and in the total 39 d of ram exposure were still significant ($P = 0.005$ and $P = 0.02$, respectively). Neither percentage of ewes pregnant nor percentage of ewes lambing were significantly different between breeds once adjustment for lambing date was completed. Analysis of progesterone profiles and marking data revealed that 10 OOS ewes experienced a silent ovulation shortly after ram introduction followed by a subsequent ovulation accompanied by estrous behavior. Similar numbers of St. Croix ewes showed no ovulatory activity until the last week of ram exposure at which time a silent ovulation occurred. In fact, all OOS ewes except one showed ovulatory and/or mating activity in the first 21 d of ram exposure while 17 of the 23 St. Croix ewes remained inactive. Thus OOS ewes appeared to be able to reinitiate estrous behavior and ovarian cyclicity more quickly after lambing in winter than St. Croix ewes. However, when ewes are bred while lactating during seasonal anestrus, significant fetal loss may occur in OOS but not St. Croix ewes.

**KEYWORDS:** fertility, lactation, season, sheep

## INTRODUCTION

Peak breeding activity in ewes begins in late summer and early autumn in the Northern Hemisphere and is reflected in a subsequent peak in lambing activity in late winter. Breeding and lambing patterns are reflected in seasonal availability of lamb and fluctuations in lamb price. Incentives to breed ewes more than once per year include reduced costs per offspring reared, increased net return, increased production per dollar of capital investment, a more uniform supply of lamb throughout the year, and more consistent lamb prices. Because sheep are seasonally polyestrous, an attempt to mate at a frequency greater than once per year will require one breeding during or near anestrus. Without intervening treatments during anestrus, little ovarian and estrous activity is anticipated, and pregnancy and conception rates are low, especially if the out-of-season breeding coincides with the early postpartum period (Cognie et al., 1975; Ford, 1979).

One method used to achieve breeding activity during the non-breeding season is to expose previously isolated anestrous ewes to mature rams. Numerous studies have shown that the introduction of rams to seasonally or lactationally anovulatory ewes before the start of the normal breeding season can result in ovulation. Underwood and colleagues (1944) and Schinckel (1954) showed that anestrus in Merino ewes could be interrupted and ovulation induced by introduction of rams. This method is commonly referred to as the "ram effect." In order to get a reproductive response to introduction of rams, it is common practice to isolate the ewes from rams (including sight, sound, and smell) for a period of time before introduction. The precise mechanism through which the introduction of novel rams results in increased secretion of luteinizing hormone, and therefore follicular development and secretion of estradiol, in anestrous ewes is not

clearly understood. Ram introduction most likely induces a follicular phase by blunting the actions of long photoperiod, allowing ewes to revert transiently to the reproductive condition found during the breeding season (Martin et al., 1983). Ewes that are in an anestrous state, whether because of season or lactation, have not been ovulating, and therefore have not been forming corpora lutea (CL). Because of the lack of exposure to progesterone ($P_4$) prior to the ram-induced increases in estradiol, the first ovulation after ram introduction is usually not associated with behavioral estrus. However, breed differences might affect the proportion of ewes showing estrus in conjunction with ovulation at the onset of the breeding season (Quirke et al., 1988).

The response to estrous induction procedures, including the introduction of rams during anestrus, is generally lower in lactating than in non-lactating ewes. Lactating ewes have longer intervals to first estrus and conception than non-lactating ewes (Whiteman et al., 1972; Pope et al., 1989). In fall-lambing ewes, the percentage of ewes that showed estrous behavior by d 67 postpartum was greater in non-lactating than lactating ewes (Call et al., 1976). When seasonal anestrus is combined with lactation, a significant block to successful pregnancy ensues. Therefore, lactation creates another level of complexity in relation to expectations for reproductive performance of the ewe in different seasons.

The present study was designed to investigate the effect of breed on ability of ewes to conceive while lactating during seasonal anestrus. The two breeds chosen for the study were St. Croix and the Virginia Tech Selection Line. The St. Croix breed first arrived in the U.S. from the Virgin Islands in 1975. As noted by Whisnant and Inskeep (1992), breeds of tropical origin have breeding seasons of longer durations than breeds

originating from temperate and higher latitudes. Accordingly, two lambings per year is not uncommon for the St. Croix breed. Other breeds with extended breeding seasons include the Dorset, Rambouillet, and Finnish Landrace. The Virginia Tech Selection Line, also called the Out-of-season (OOS) Line, was first created in 1988 and takes advantage of the favorable reproductive properties of these breeds (Notter and Cockett, 2005). Studies on these ewes, three-way crosses of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding, indicate that they exhibit nearly continuous cyclicity during spring and summer, with a mean period of anestrus of only 11.3 d (Vincent et al., 2000). If ewes of selected wool breeds are as adept as hair breeds of ewes at breeding more than once per year, producers may take advantage of increased efficiency not only due to more lambs per year, but also because of the more desirable carcass characteristics of wool breeds (Brown and Jackson, 1995).

## MATERIALS AND METHODS

### *Animals*

This study was conducted at Virginia Tech's Copenhaver Sheep Center in March, April, and May 2006. All procedures were approved and carried out in accordance with Institutional Animal Care and Use Committee of Virginia Tech. A total of 47 ewes was included in the study and were of St. Croix (n = 23) or VT Selection Line/OOS (n = 24) breeding. St. Croix ewes ranged in age from 4 to 7 yr with an average of 5.0 ± 0.3 yr. OOS ewes ranged in age from 2 to 9 yr with an average of 4.75 ± 0.3 yr. Ewes of the two breeds did not differ significantly in age.

All 47 ewes lambed during the winter preceding the study. St. Croix ewes lambed between January 14 and 21, 2006 and averaged 51.5 ± 1.2 d since lambing at commencement of the study. OOS ewes lambed between January 4 and 30, 2006 and averaged 62.2 ± 1.2 d since lambing at commencement of the study. Thus OOS ewes lambed earlier than St. Croix ewes ($P < 0.0001$). All 48 ewes were nursing either one or two lambs for the duration of the study. The mean number of lambs nursed per ewe was 1.83 ± .09 and 1.63 ± .09 for St. Croix and OOS ewes, respectively. The number of lambs nursed per ewe was not different between breeds.

Ewes and lambs were held on pasture and received water ad libitum and supplemental grain daily.

*Experimental design*

At the beginning of the study, two blood samples were taken 3 d apart and assayed for concentration of $P_4$ to assess ovarian status prior to ram introduction. Ewes that had $P_4$ concentrations < 1 ng/mL were considered to be in a state of anestrus.

Ewes had been isolated from rams for at least 2 mo before commencement of the study. Five intact rams (3 of which were fitted with crayon marking harnesses) had passed breeding soundness exams and were introduced to ewes on March 24 (d 0). Crayon color was changed every 2 wk. Ewes, lambs, and rams were gathered into pens and rams and lambs were separated from ewes while observation and sampling was conducted. Blood samples were taken and ewes were checked for crayon marks on their rumps each Tuesday and Friday for 39 d following ram introduction. Crayon marks were

categorized as heavy, indicating high probability of standing estrus, or light, indicating that the ewes may not have been in standing estrus.

Pregnancy was checked 90 d after introduction of rams. Ultrasonographic scanning for diagnosis of pregnancy was performed transabdominally using an Aloka 500 console (Corometrics Medical Systems, Inc., Wallingford, CT) and 3.5 MHz probe.

*Blood sampling and assay procedures*

Blood samples (5 mL) were acquired using the Vaccutainer system via jugular venipuncture and stored at 4 ºC to allow for clotting. Samples were then spun in a centrifuge at 3,000 rpm for 20 min. Serum was collected within 24 hr and frozen at -20 ºC in plastic tubes.

Serum was assayed for $P_4$ concentration using the Coat-A-Count Progesterone Kit (Siemens Healthcare Diagnostics, Inc., Los Angeles, CA) as described and validated by Minton et al. (1991). Order in which serum samples were assayed was chosen using a random number generator to minimize effects of inter-assay variability. All samples were assayed in duplicate. The assay was sensitive to 0.1 ng/mL and the intra- and inter-assay coefficients of variation were 9.5% and 14.5%, respectively. Progesterone concentrations $\geq$ 1 ng/mL were used to indicate presence of a CL and therefore ovulatory activity.

*Statistics*

Proportions of ewes that were marked in the first 21 d of exposure to rams, marked during the total 39 d of exposure, diagnosed pregnant 90 d after introduction of

rams, and that lambed were tested for significance of breed effects with a Chi-square analysis using the Frequency procedures of SAS (SAS Institute, Inc., Cary, North Carolina, US).

Because mean lambing date for the lambing before commencement of the study was significantly different between breeds, the Generalized Linear Models procedure of SAS was used to compare breed effects on these response variables after including lambing date as a covariate in the analytical model.

## RESULTS

Percentages of ewes marked by rams in the first 21 d after ram introduction were higher for OOS than St. Croix ewes (58.3 vs. 8.7%; $P = 0.0003$; Table 2-1). The same was true for percentages of ewes marked by rams at any time during the observation period (95.8 vs. 43.5%; $P < 0.0001$). The difference between percentages of ewes diagnosed pregnant by ultrasound 90 d after introduction of rams to OOS and St. Croix ewes approached significance (66.7 vs. 39.2%; $P = 0.06$), but there was no difference in percentage of ewes that lambed of all ewes exposed to rams (47.8 vs. 34.8%).

Because the number of days postpartum at commencement of the experiment was significantly greater for OOS than St. Croix ewes, the dependent variables for each breed were reexamined after adjusting for lambing date. After adjustment, the difference in percentage of ewes marked in the first 21 d after ram introduction was still significantly higher in OOS than St. Croix ewes ($P = 0.005$), as was the difference in the total percentage of ewes marked ($P = 0.02$). Neither the percentage of ewes pregnant nor

percentage of ewes lambing were significantly different between breeds once adjustment for lambing date was completed.

Assays of $P_4$ concentrations during the study yielded a unique profile for each ewe (see appendix). Based on the observations discussed above, and the $P_4$ profiles, each ewe was put into one or more of four categories for d 0 to 21 and d 22 to 39 following introduction of rams (Tables 2-2 and 2-3). The categories were: (1) ewes that were marked and diagnosed pregnant, (2) ewes that were marked, diagnosed pregnant, and lambed, (3) ewes that were not marked, but ovulated (with a silent ovulation and normal luteal phase, or silent ovulation and short luteal phase), and (4) ewes that were not marked and did not ovulate. Statistical analysis of category frequencies was generally not possible because numbers in many breed x category classes were too small (< 5) to permit calculation of informative Chi-square statistics.

## DISCUSSION

Significantly more OOS than St. Croix ewes were marked by rams in the first 21 d after ram introduction and during the total 39 d of breeding. Thus OOS ewes appear to be able to reinitiate estrous behavior more quickly after lambing in winter than St. Croix ewes. However, the lack of differences between breeds in ewes diagnosed pregnant and lambing indicates that substantial numbers of OOS ewes are not able to establish or maintain pregnancies resulting from these breedings. Circulating $P_4$ levels were not determined following the end of breeding, so it was not possible to assess luteal function in OOS ewes that were marked but not diagnosed pregnant. The marks may not have

been associated with standing estrus, mating may have occurred but did not result in pregnancy, or the pregnancy may have been lost before pregnancy diagnosis occurred 90 d after introduction of rams. Estimates of embryonic and fetal loss in the sheep have averaged approximately 30% (Bolet, 1986). Most embryonic loss has been reported to occur before d 18 (Hulet et al., 1956; Moore et al., 1960; Quinlinvan, 1966). Complete losses from d 18 to lambing were estimated at 9.4% (Hulet et al., 1956), and late embryonic or fetal losses from d 30 to term were only 1 to 5% (Quinlivan, 1966). In contrast to these findings, substantial fetal loss occurred between the time of pregnancy diagnosis and lambing in OOS ewes. This phenomenon was not seen in St. Croix ewes. Embryonic and fetal losses generally increase in association with increasing ovulation rates (Knights et al., 2003; Kleemann and Walker, 2005). However, multiparous ewes such as the St. Croix and OOS more commonly lose one rather than all lambs (Dixon et al., 2007). Even though OOS ewes were not as capable as St. Croix ewes at maintaining pregnancies that occur soon after parturition in lactating ewes, the lambing outcome of these ewes did not differ between the two breeds. According to Casas et al. (2005), fertility during spring mating is an important constraint of the sheep industry. This study demonstrates that with regards to the economically important outcome of percentage of ewes lambing, both breeds studied demonstrated a substantial capacity to mate and conceive while lactating during the long days of spring.

The $P_4$ profiles for each ewe during the observation period provide more information than that which can be gleaned from marking, pregnancy, and lambing data alone. Based on patterns of elevated $P_4$, as well as observational data, ewes were classified into activity groups for two different time periods. Only 2 St. Croix ewes were

marked by rams while 13 OOS were marked, ewes apparently exhibiting estrous behavior (Table 2-2). Only 1 OOS ewe did not exhibit some level of ovulatory activity during the first 21 d of ram exposure. This is in contrast to 17 St. Croix ewes with apparently completely inactive ovaries. Among all ewes that were not marked, but did ovulate, only one ewe (a St. Croix) experienced a short luteal phase. Oldham and Martin (1978) reported that the CL resulting from the first ram-induced ovulation can experience a normal lifespan but is often short-lived and prematurely regresses. Ewes with a normal CL following their first ovulation often do not exhibit estrus but normally will ovulate in conjunction with estrous behavior 17 d later. Ovulations that result in short-lived CL are generally not accompanied by estrus, the resulting CL regresses 5 to 6 d after ovulation, and luteal regression is usually followed by another silent ovulation. The length of the second luteal phase usually is normal, with estrus and ovulation occurring about 17 d later (Oldham and Martin, 1978). Because of the lack of exposure to $P_4$ prior to the ram-induced increases in $E_2$, the first ovulation is often not associated with behavioral estrus, and this is presumably what happened in the 10 ewes that ovulated without being marked. Accordingly, four ewes appeared to be already cycling at commencement of the experiment (3 OOS and 1 St. Croix), and would therefore have had prior exposure to $P_4$ and none of these ewes were among those that experienced silent ovulations. As noted by Oldham and Martin (1978), some ewes experience a shortened luteal phase after the first ram induced ovulation. Three ewes showed evidence of this phenomenon and, interestingly, all were St. Croix ewes. It has been postulated that before the first postpartum ovulation, a low concentration of preovulatory $E_2$ may result in the early generation of a luteolytic mechanism during the subsequent luteal phase because of

impaired inhibition of oxytocin receptors that allow early release of prostaglandin $F_2\alpha$ (Mann and Lamming, 2000, Sakaguchi et al., 2004). The incidence of a shortened luteal phase early in the experiment apparently did not have a negative impact on fertility since all three of these ewes were diagnosed pregnant and lambed.

Three OOS ewes could not be categorized based on activity because they had $P_4$ concentrations between 1 and 6 ng/mL for at least 35 d. A likely explanation for the profiles of these 3 ewes is that they had persistent luteal cysts. In addition to having a negative impact on follicular development and display of sexual receptivity, luteal activity in the early postpartum period may also negatively impact involution of the uterus after parturition (Lewis, 1997).

Several species, including rats (Nelson, 1929), mice (Crew and Mirskaia, 1930), hamsters (Krehbiel, 1952), and cattle (Donald, 1943), have been documented to show symptoms of both physiological and psychological heat during pregnancy. Williams and colleagues (1956) reported that 22% of a group of 103 pregnant ewes mated one or more times during early pregnancy and 62.5% of a group of 24 pregnant ewes mated during late stages of pregnancy. This phenomenon appears to be responsible for the marking by rams of 4 ewes on the present study after they were already pregnant (3 OOS and 1 St. Croix). Relevant to the US sheep industry, this physiological phenomenon may result in culling of pregnant ewes believed to be open because of continuing mating behavior (Williams et al., 1956).

To address the significant differences between the breeds in days postpartum at commencement of the experiment, Table 2-2 also gives the number of St. Croix ewes in each category for d 0 through 32. On d 32 after ram introduction, St. Croix ewes were on

average 86 d postpartum, the same as OOS ewes on d 21 after ram introduction. When those additional 10 d are included, similar percentages of ewes lambed, but among those ewes that were not marked, 15 St. Croix ewes still showed no ovulatory activity compared to only 1 OOS ewe. Thus it would appear that even if the two breeds of ewes had been similar in days postpartum at commencement of the experiment, the results would have been the same. Indeed, lambing date was not a factor in the differential breed responses to the introduction of rams. Effects of lambing date were not significant; the analysis showed that for every 10 d later a ewe lambed, she was 14% less likely to be marked by rams during the observation period, but only 5% less likely to lamb. Even so, similar percentages of OOS and St. Croix ewes were diagnosed pregnant and lambed after 39 d of exposure to rams.

One phenomenon that cannot be explained is the incidence of apparent fetal loss among OOS ewes that were impregnated during d 0 – 21 compared to d 22 – 39 after ram introduction. During the first 21 d, 10 OOS ewes experienced matings that resulted in pregnancies, and 8 of these ewes later lambed as a result of these matings. During the last half of the observation period, 6 OOS ewes experienced matings that resulted in pregnancies, but only 2 of the ewes lambed. Furthermore, a greater percentage of OOS ewes marked but were not diagnosed pregnant during the second half of the study than during the first half. It seems that greater embryonic and fetal loss should occur in ewes mated in the first half of the observation period since they would have been fewer days postpartum than during the second half. This draws attention to the difficulty of explaining physiological phenomena with low animal numbers. It is quite likely that the understanding of how breed differences affect the ability to rebreed while lactating in

spring could be furthered by conducting similar studies with dramatically increased sheep numbers.

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Table 2-1. Frequencies of OOS and St. Croix ewes marked by rams in the first 21 d after ram introduction, marked by rams during 39 d of exposure, diagnosed pregnant, and lambing.

| | Breed | |
|---|---|---|
| | **OOS** | **St. Croix** |
| **Variable** | | |
| Ewes marked in first 21 d after ram introduction [a] | 58.3 ± 10.3% (14/24) | 8.7 ± 6.0% (2/23) |
| Ewes marked during 39 d of exposure to rams [b] | 95.8 ± 4.2% (23/24) | 43.5 ± 10.6% (10/23) |
| Ewes diagnosed pregnant | 66.7 ± 9.8% (16/24) | 39.1 ± 10.4% (9/23) |
| Ewes lambing | 47.8 ± 10.6% (11/23[c]) | 34.8 ± 10.2% (8/23) |

[a]Breeds differ $P = 0.003$.
[b]Breeds differ $P < 0.0001$.
[c]One OOS ewe died before lambing after being diagnosed pregnant.

Table 2-2. Classification of ewe reproductive status based on marking observations, pregnancy diagnosis, lambing data, and $P_4$ profiles during d 0 through 21 for OOS and St. Croix ewes and d 0 through 32 for St. Croix ewes.

| | **Ewe Breed** | | |
|---|---|---|---|
| | **OOS** | **St. Croix** | |
| **Classification** | **d 0-21** | **d 0-21** | **d 0-32 [a]** |
| Total number | 24 | 23 | 23 |
| Number marked | 13 | 2 | 6 |
| Number diagnosed pregnant | 10 | 0 | 6 |
| Number lambing | 8 | 0 | 6 |
| Number not marked | 11 | 21 | 17 |
| Number ovulated | 7 | 4 | 2 |
| Number with no ovulatory activity | 1 | 17 | 15 |
| Other | 3 [b] | 0 | 0 |

[a] St. Croix ewes lambed an average of 11 d later than OOS ewes. Extension of the "early" postpartum period to d 32 after ram introduction allows comparability between OOS and St. Croix ewes in days postpartum at the end of the period.
[b] Three OOS ewes had elevated $P_4$ concentrations at every sampling point indicating possible persistent luteal cysts.

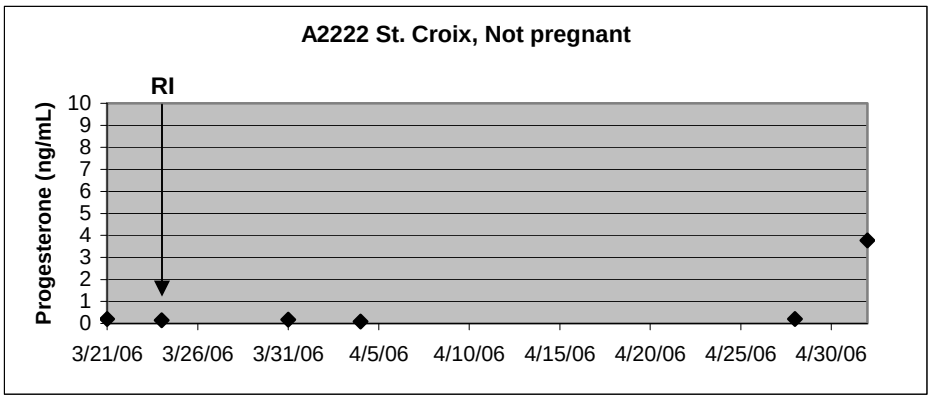
Table 2-3. Classification of ewe reproductive status based on marking observations, pregnancy diagnosis, lambing data, and $P_4$ profiles during d 22 through 39 for OOS and St. Croix ewes.

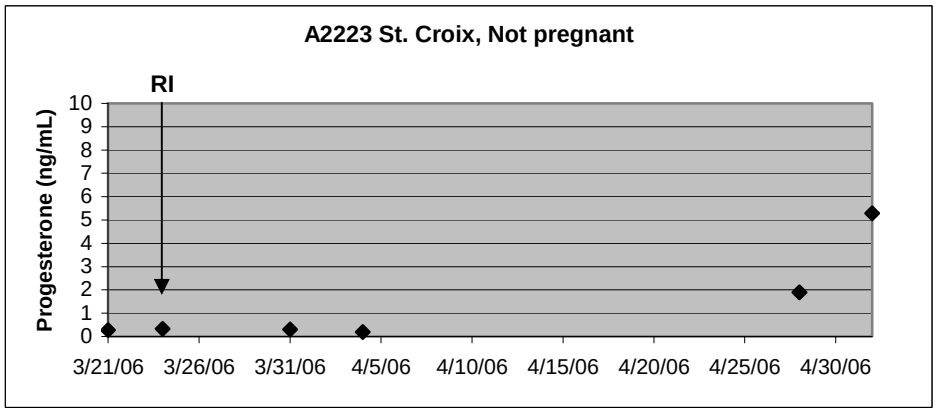
| | Breed | |
|---|---|---|
| | **OOS** | **St. Croix** |
| **Classification** | | |
| Total number [a] | 14 | 23 |
| Number marked | 11 | 9 |
| Number diagnosed pregnant | 6 | 9 |
| Number lambing | 2 | 9 |
| Number not marked | 3 | 14 |
| Number that ovulated | 2 | 11 |
| Number with no ovulatory activity | 1 | 3 |

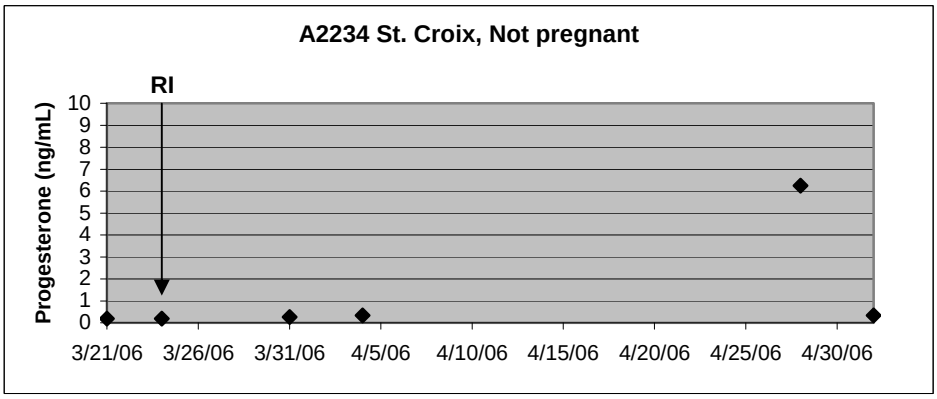
[a] Total number includes only ewes that did not become pregnant during the first 21 d of exposure to rams.

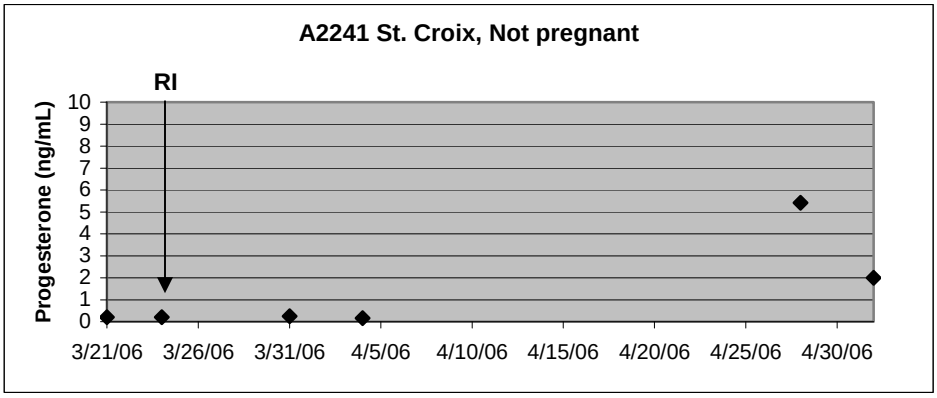
# APPENDIX

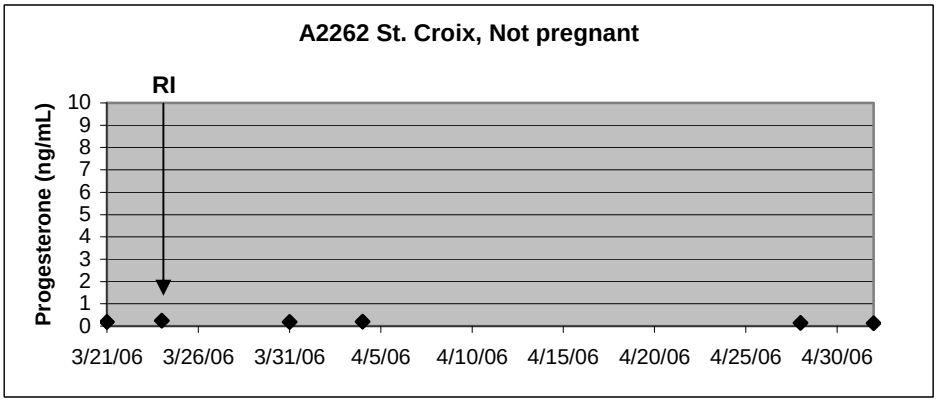
This appendix contains the $P_4$ profiles of individual ewes given as ng/mL for each sampling date. Ewe number and breed as well as pregnancy outcome is given at the top of each graph. Lambing dates are given for those ewes that were diagnosed pregnant and lambed. If the ewe was diagnosed pregnant, but did not lamb, her designation is "Diagnosed pregnant". The first blood sample was taken 3 d before ram introduction and rams were put in with ewes on the day of the second blood sample (March 24) after sampling was complete. Lambing dates were used to determine if a mating resulted in a pregnancy. Once pregnancy was achieved, assessment of $P_4$ concentrations was halted. RI = ram introduction; P = mating that most likely resulted in pregnancy based on lambing date

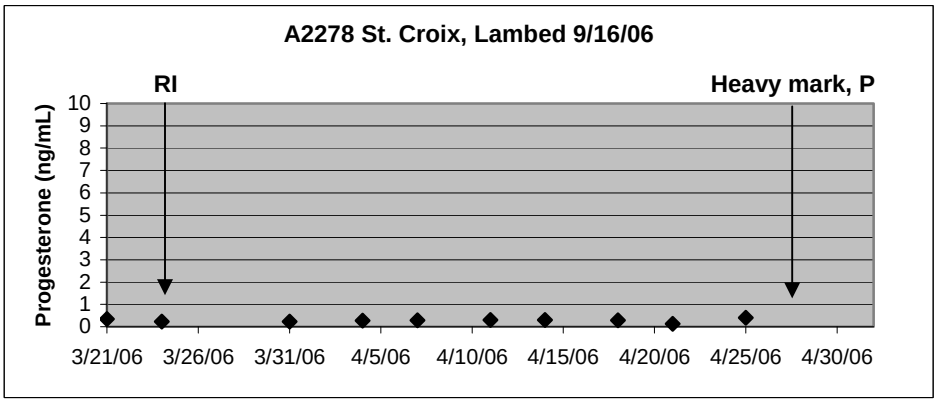


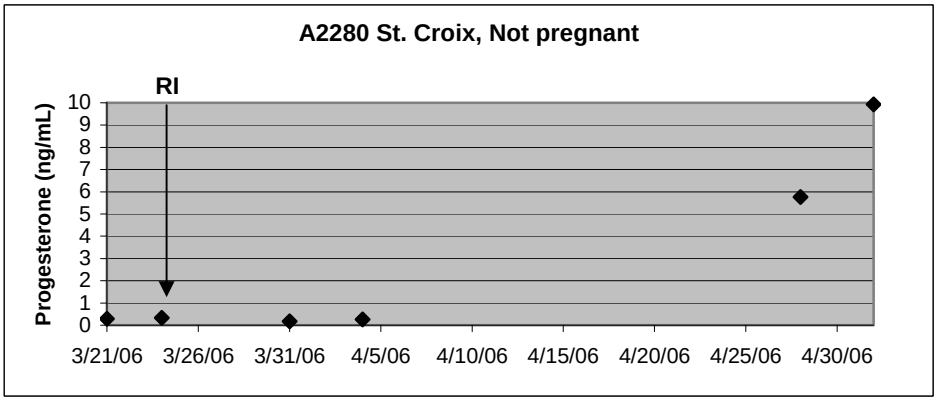



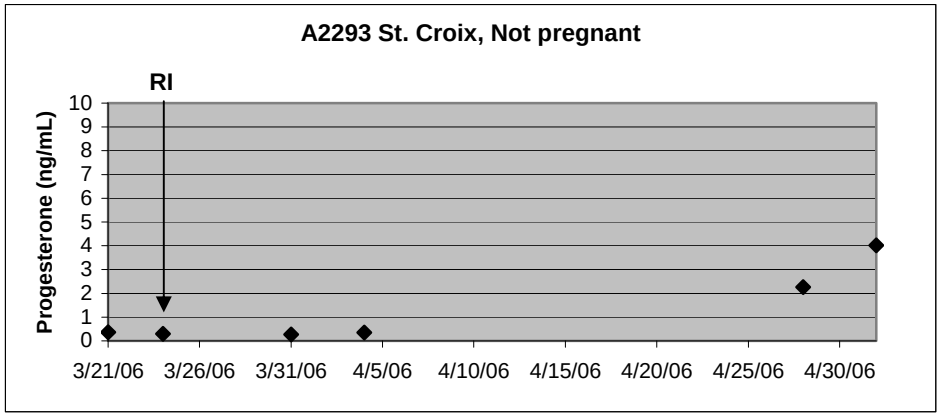



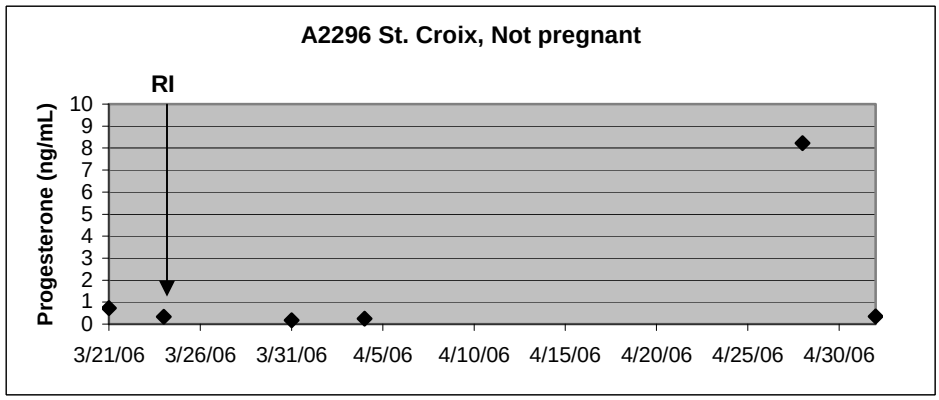

A2241 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

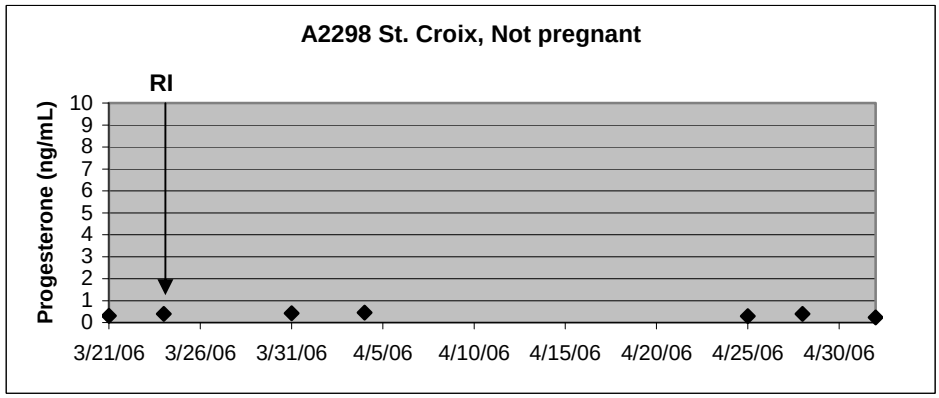

A2262 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

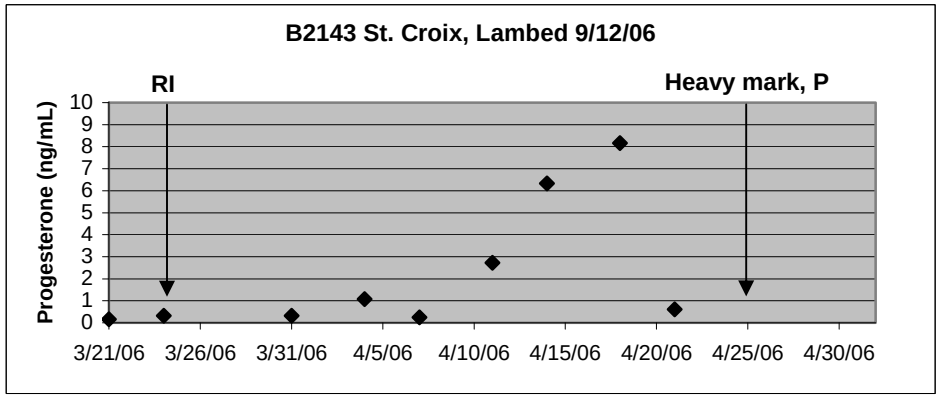

A2278 St. Croix, Lambed 9/16/06
RI
Heavy mark, P
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

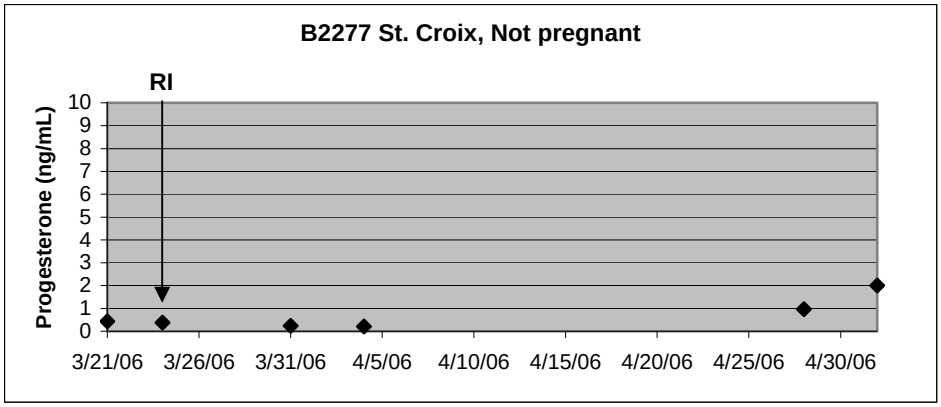

A2280 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

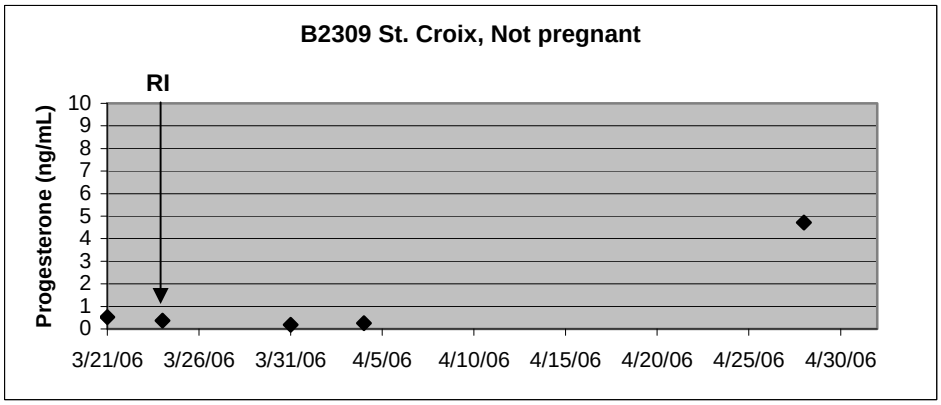

A2293 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

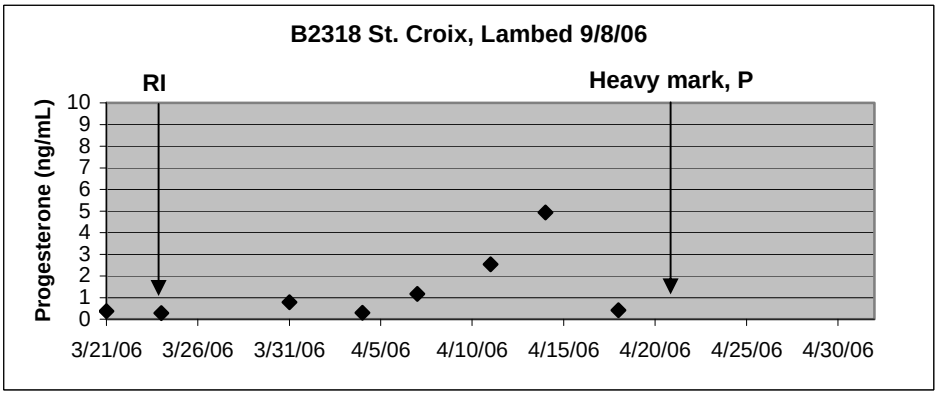

A2296 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

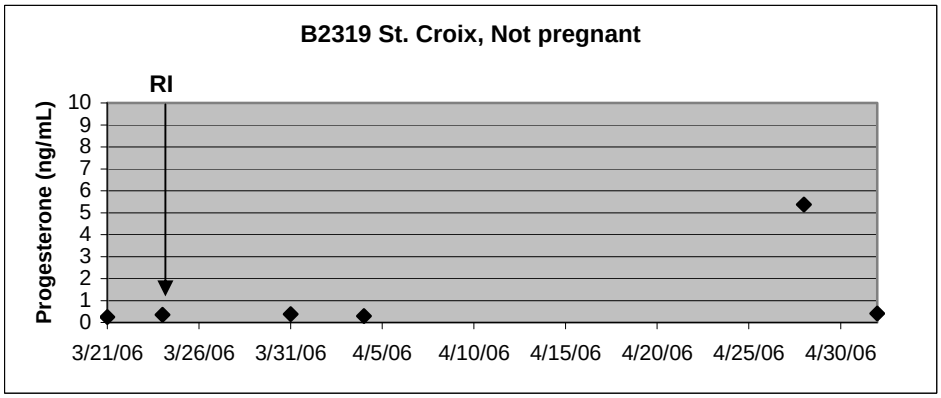

A2298 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

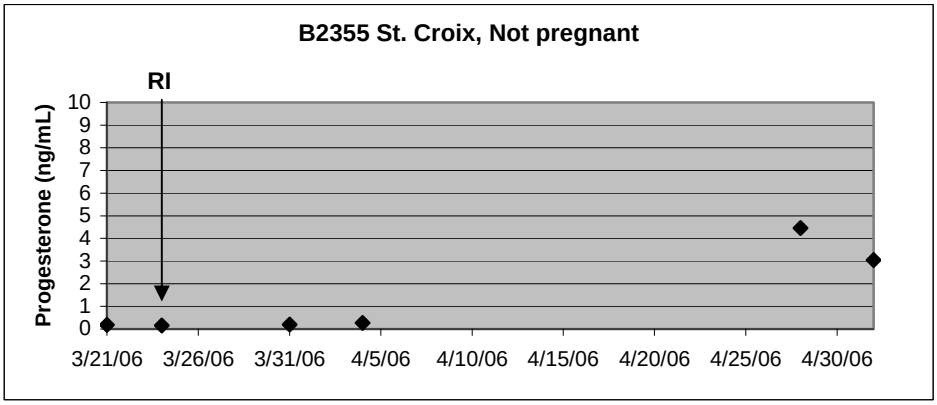

B2143 St. Croix, Lambed 9/12/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

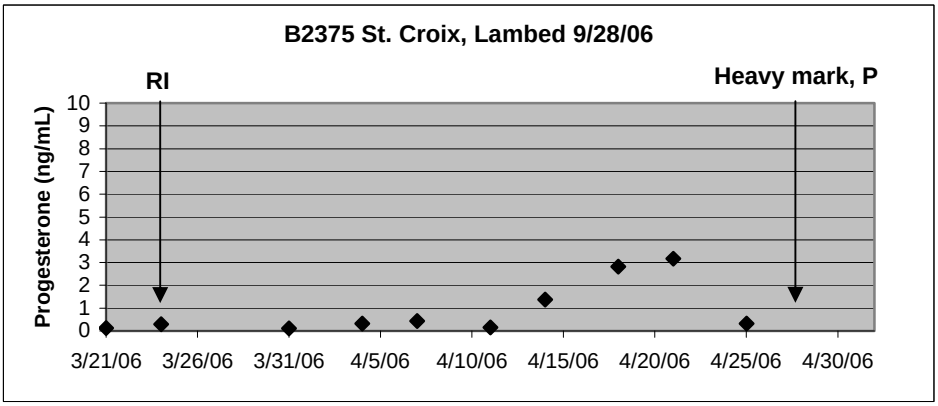

B2277 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

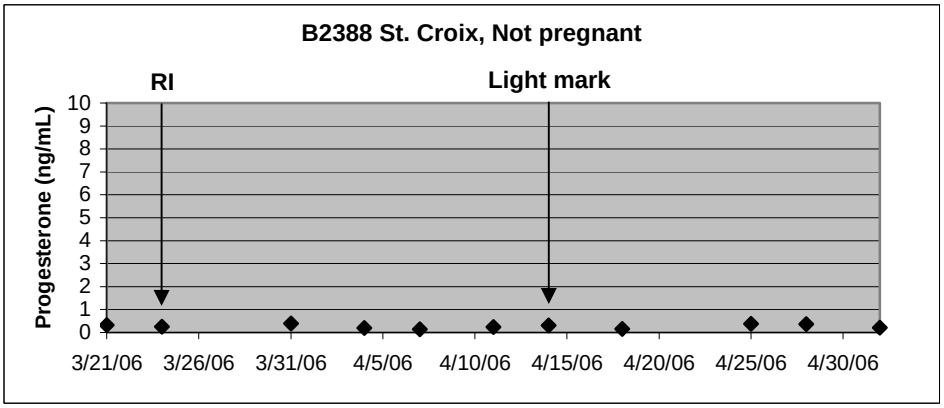

B2309 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

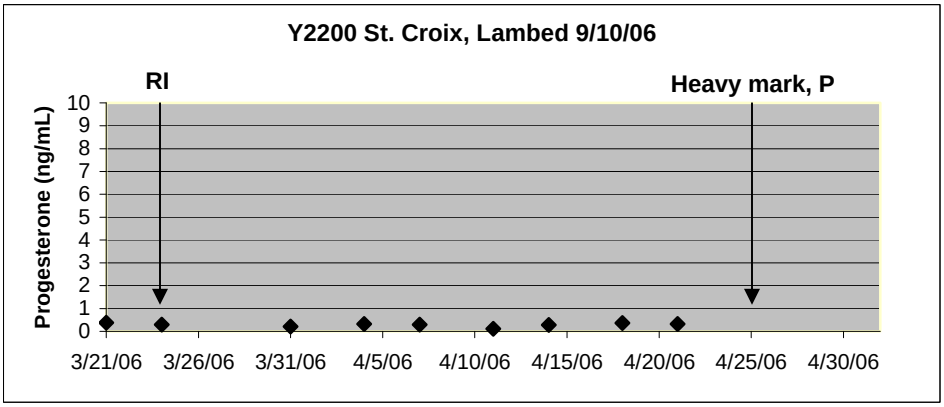

B2318 St. Croix, Lambed 9/8/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

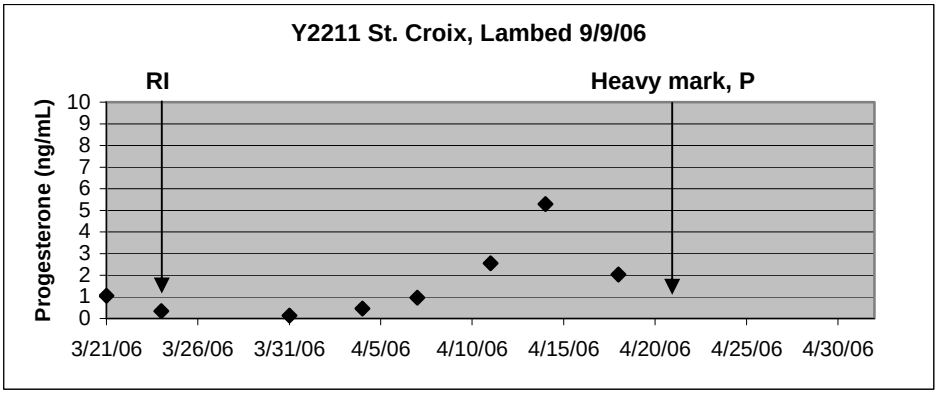

B2319 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

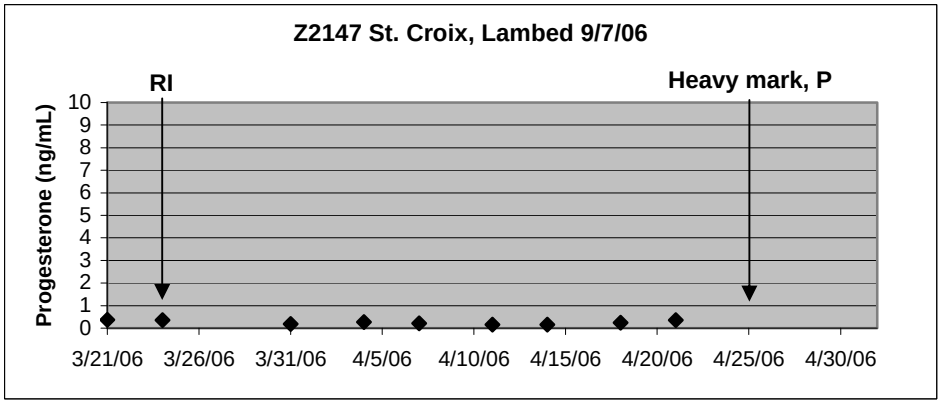

B2355 St. Croix, Not pregnant
RI
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

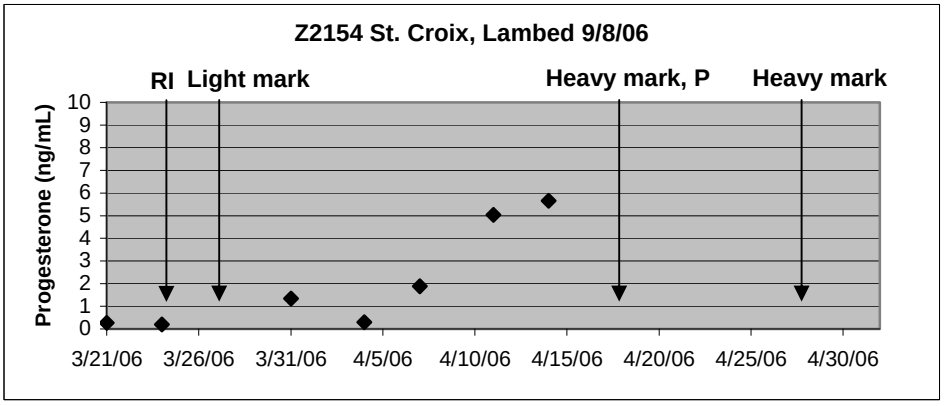

B2375 St. Croix, Lambed 9/28/06
RI
Heavy mark, P
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

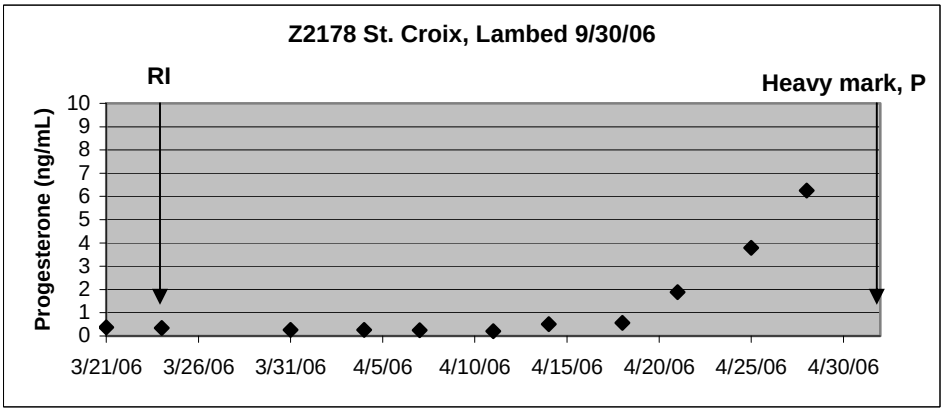

B2388 St. Croix, Not pregnant
RI
Light mark
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

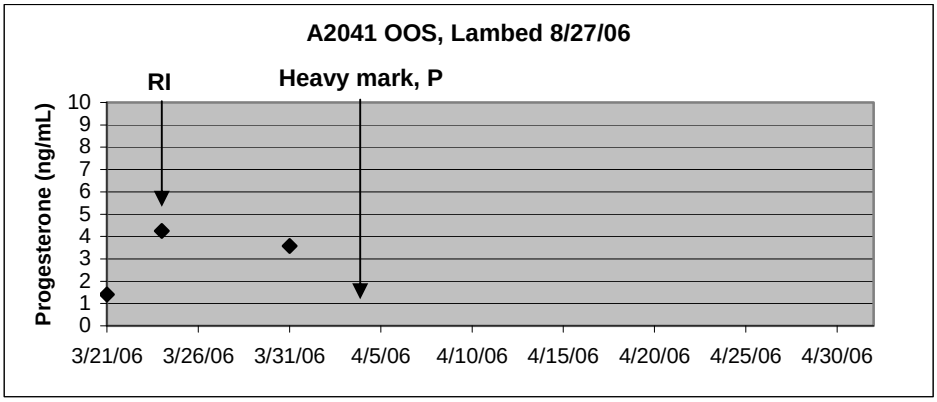

Y2200 St. Croix, Lambed 9/10/06
RI
Heavy mark, P
Progesterone (ng/mL)
10
9
8
7
6
5
4
3
2
1
0
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

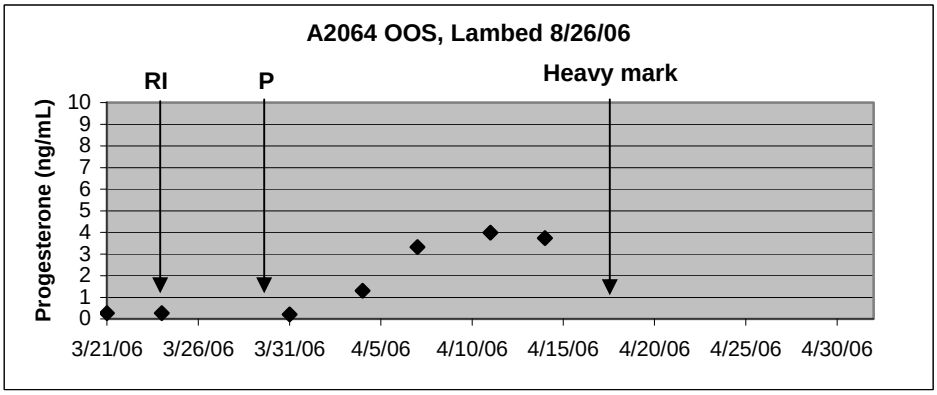

Y2211 St. Croix, Lambed 9/9/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

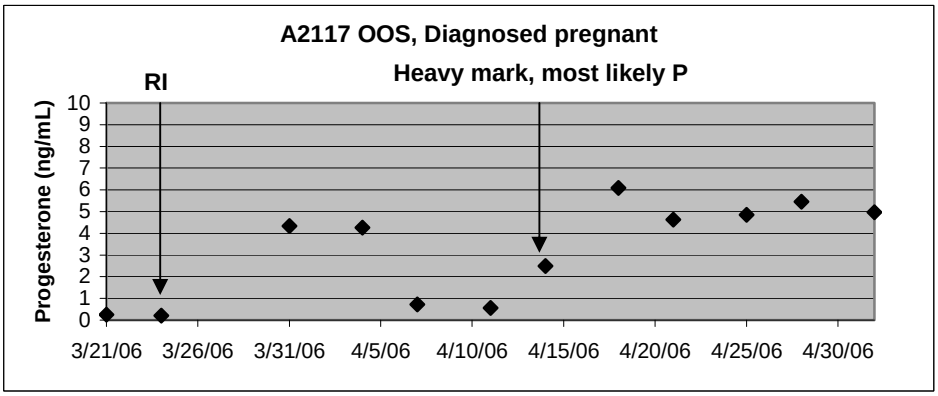

Z2147 St. Croix, Lambed 9/7/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

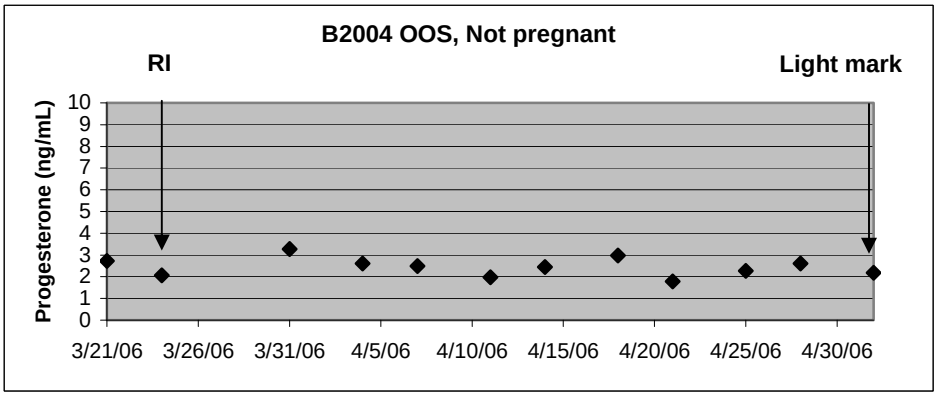

Z2154 St. Croix, Lambed 9/8/06
RI
Light mark
Heavy mark, P
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

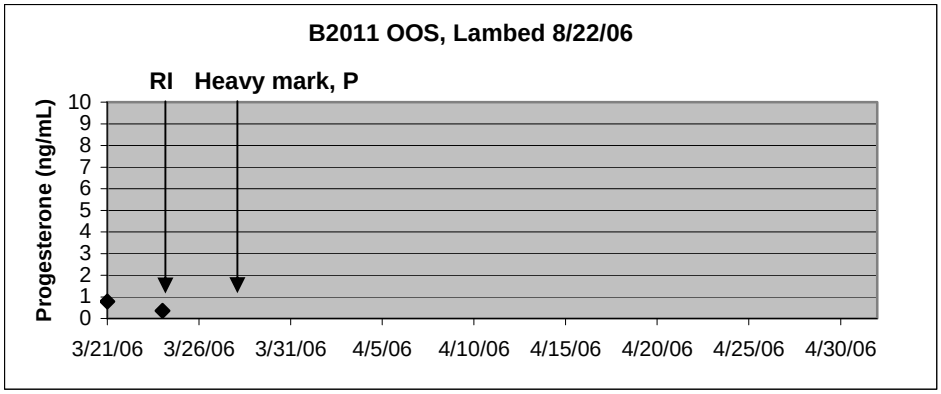

Z2178 St. Croix, Lambed 9/30/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

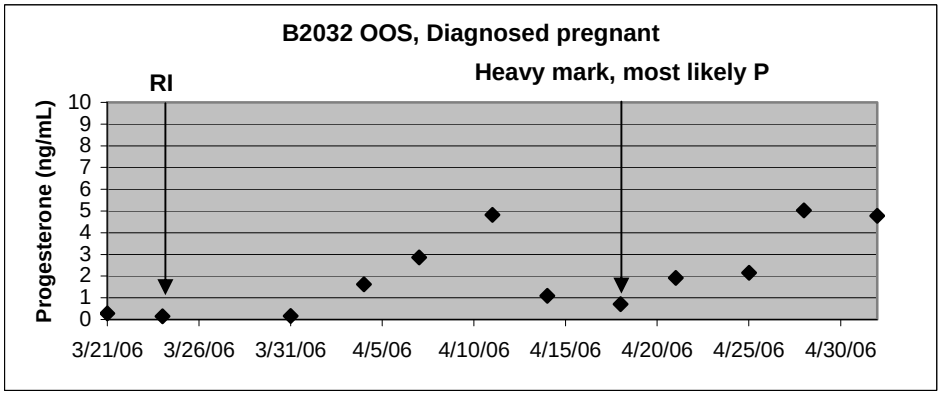

A2041 OOS, Lambed 8/27/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

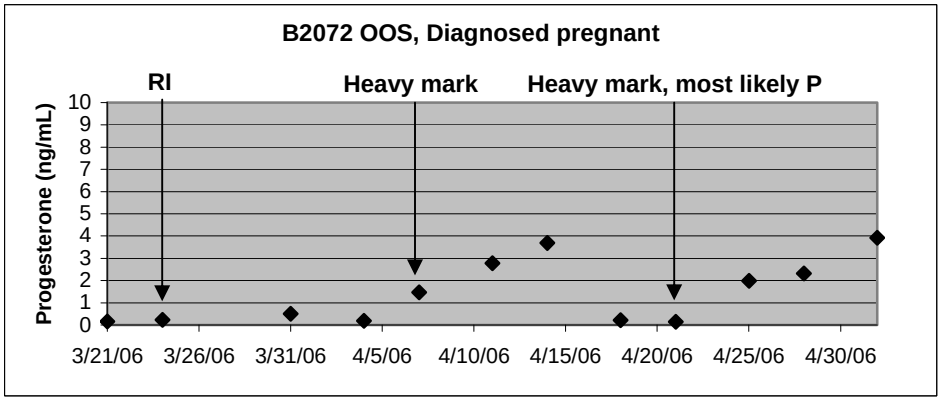

A2064 OOS, Lambed 8/26/06
RI
P
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

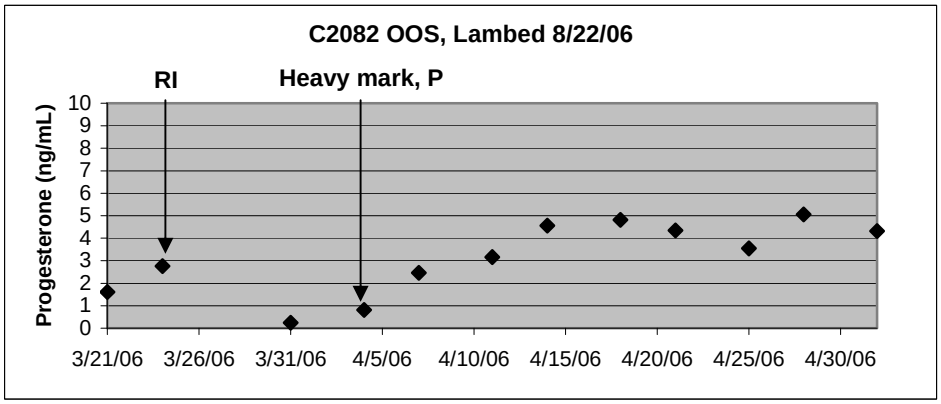

A2117 OOS, Diagnosed pregnant
RI
Heavy mark, most likely P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

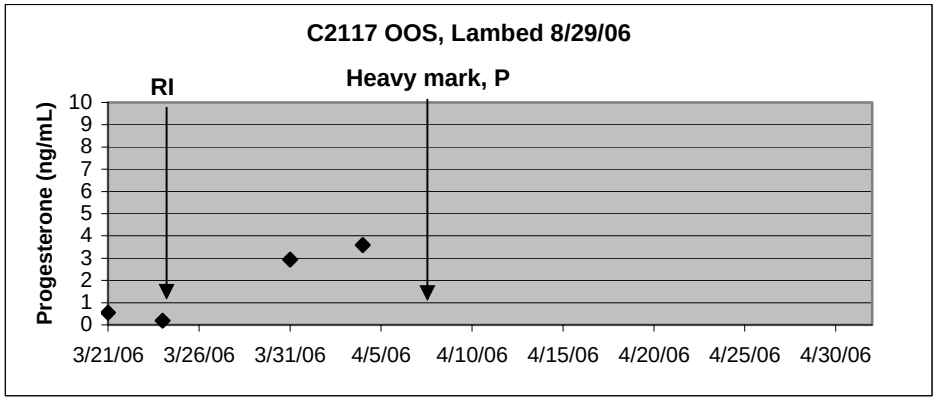

B2004 OOS, Not pregnant
RI
Light mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

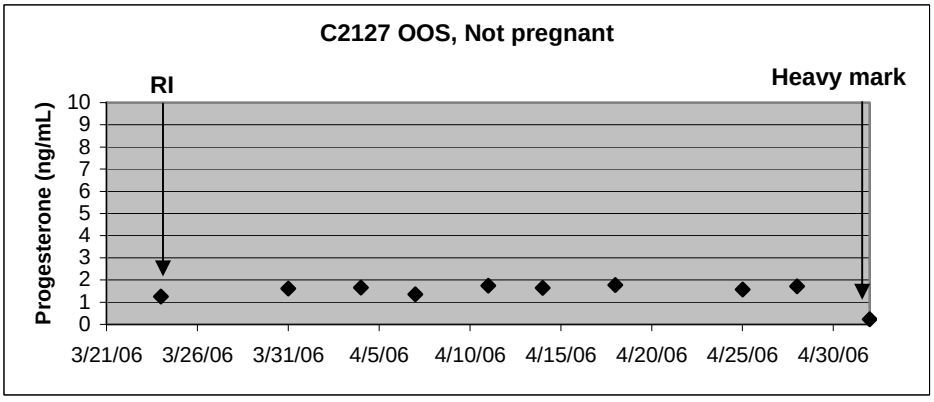

B2011 OOS, Lambed 8/22/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

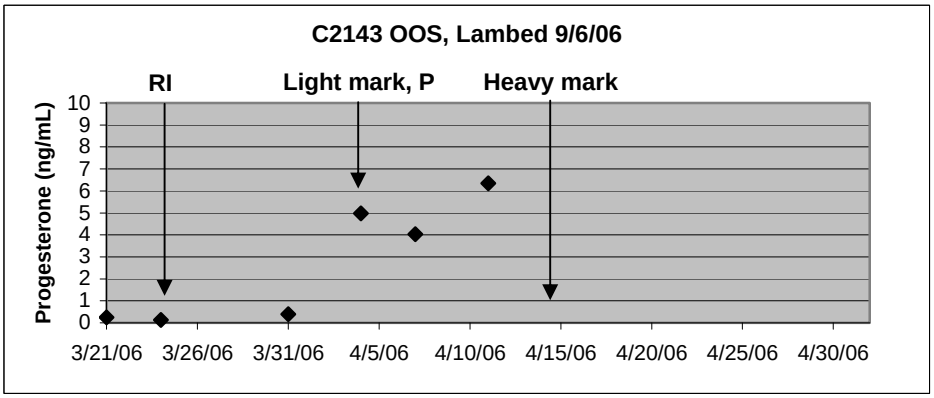

B2032 OOS, Diagnosed pregnant
RI
Heavy mark, most likely P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

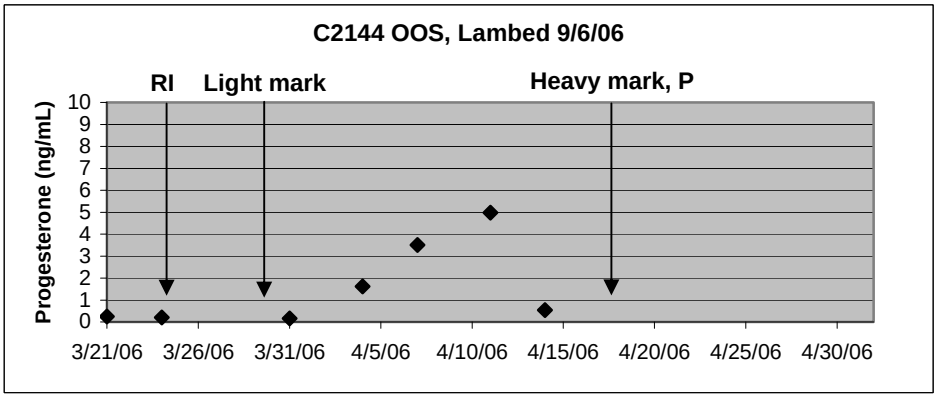

B2072 OOS, Diagnosed pregnant
RI
Heavy mark
Heavy mark, most likely P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

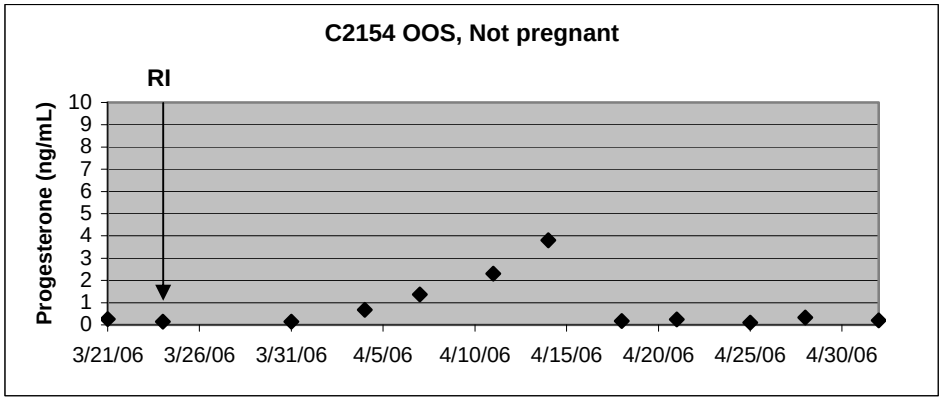

C2082 OOS, Lambed 8/22/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

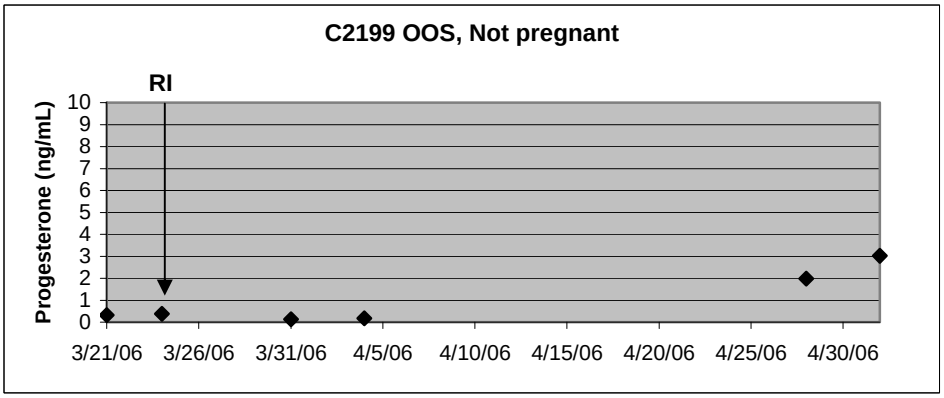

C2117 OOS, Lambed 8/29/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

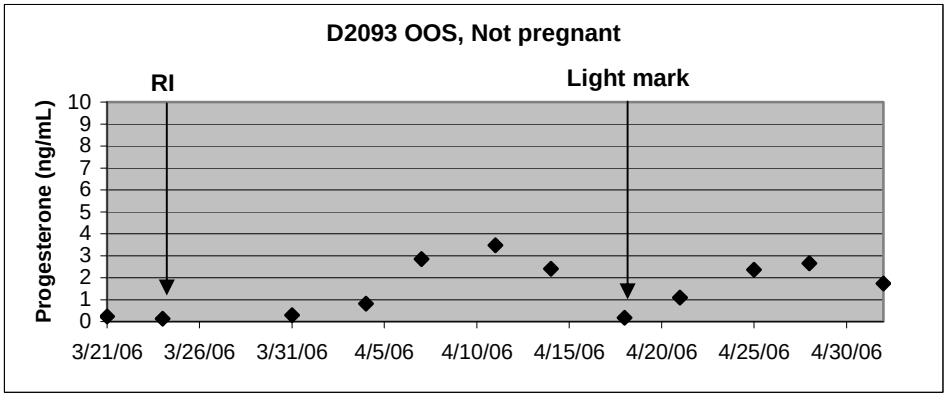

C2127 OOS, Not pregnant
RI
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

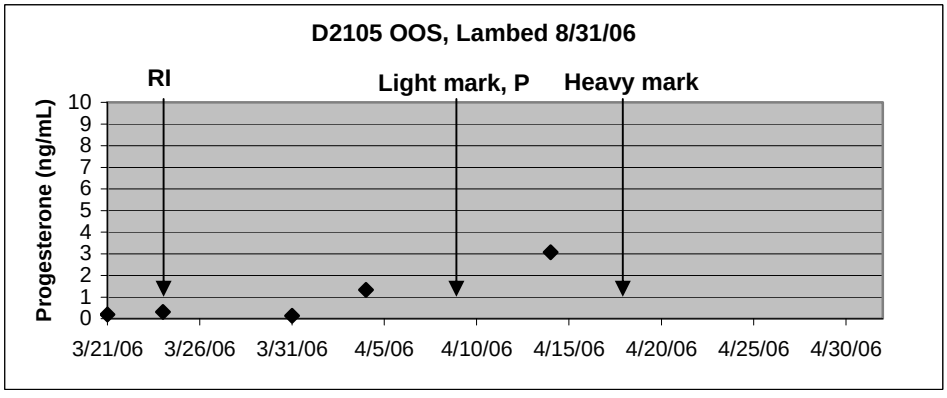

C2143 OOS, Lambed 9/6/06
RI
Light mark, P
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

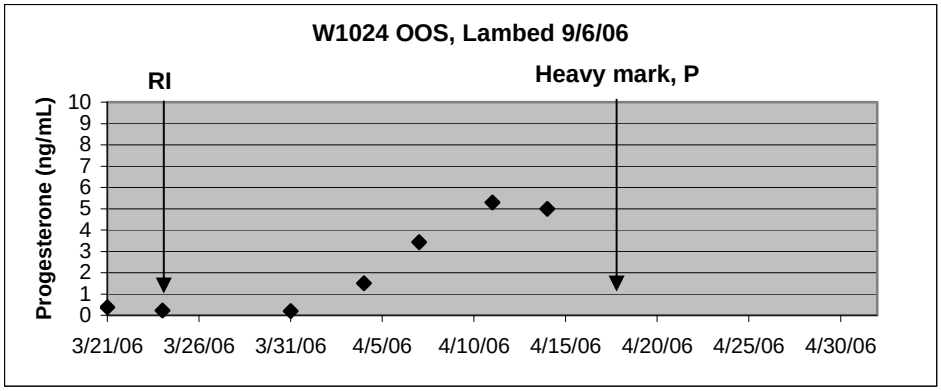

C2144 OOS, Lambed 9/6/06
RI
Light mark
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

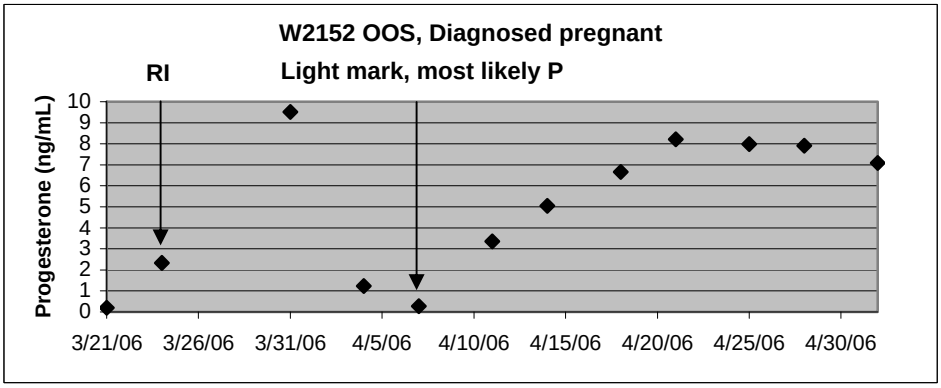

C2154 OOS, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

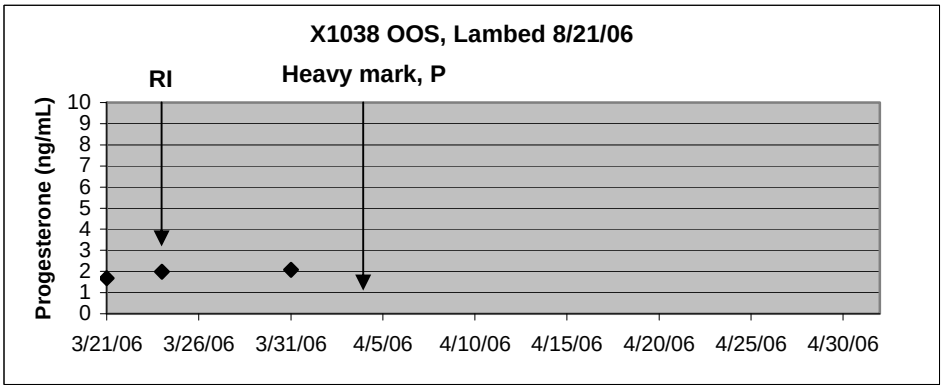

C2199 OOS, Not pregnant
RI
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

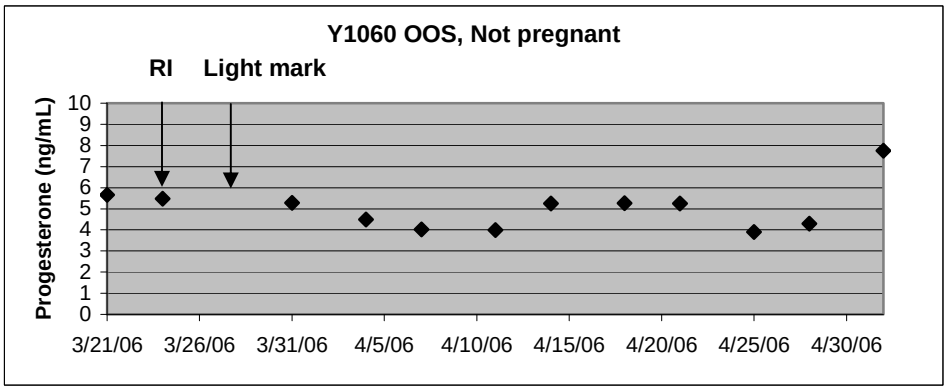

D2093 OOS, Not pregnant
RI
Light mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

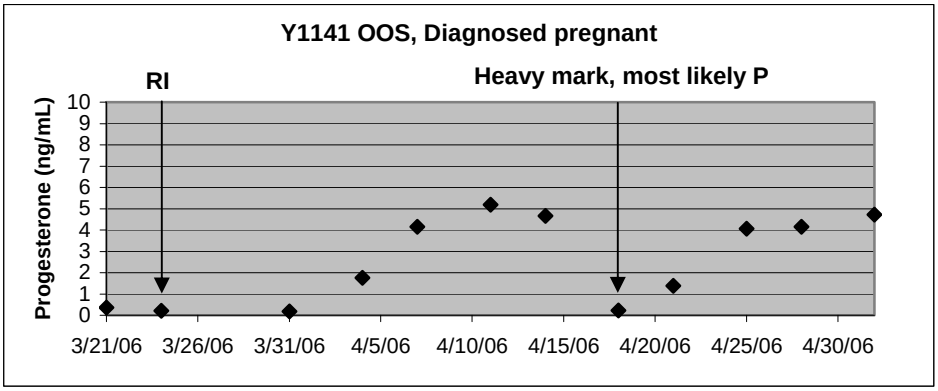

D2105 OOS, Lambed 8/31/06
RI
Light mark, P
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

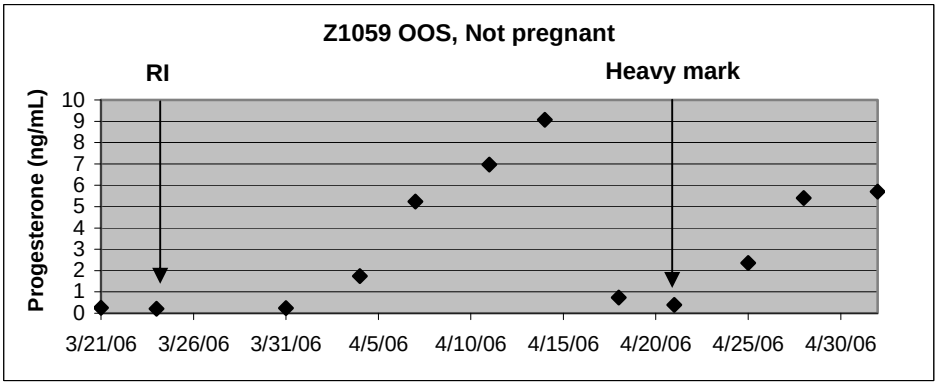

W1024 OOS, Lambed 9/6/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

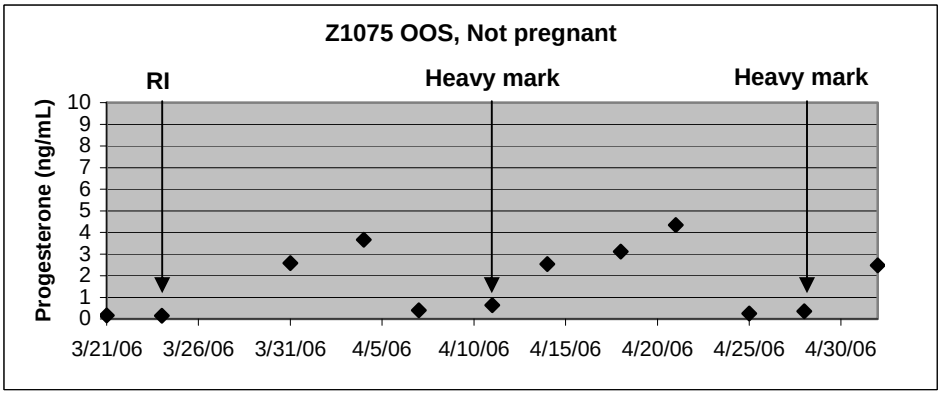

W2152 OOS, Diagnosed pregnant
RI
Light mark, most likely P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

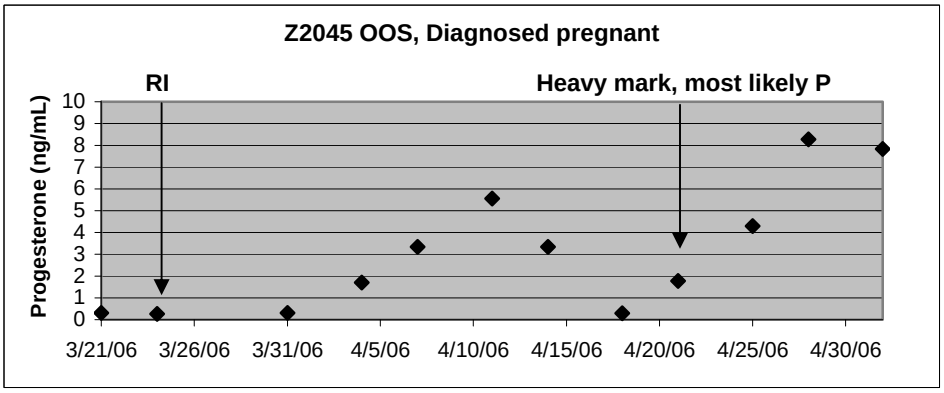

X1038 OOS, Lambed 8/21/06
RI
Heavy mark, P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06


Y1060 OOS, Not pregnant
RI
Light mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06


Y1141 OOS, Diagnosed pregnant
RI
Heavy mark, most likely P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06


Z1059 OOS, Not pregnant
RI
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06


Z1075 OOS, Not pregnant
RI
Heavy mark
Heavy mark
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06


Z2045 OOS, Diagnosed pregnant
RI
Heavy mark, most likely P
Progesterone (ng/mL)
0
1
2
3
4
5
6
7
8
9
10
3/21/06
3/26/06
3/31/06
4/5/06
4/10/06
4/15/06
4/20/06
4/25/06
4/30/06

## CHAPTER 3

### Ability of selected ewes to rebreed while lactating in May

**ABSTRACT:** The Virginia Tech Selection Line/Out-of-season (OOS) Line began in 1988 as an initiative to develop a line of wool ewes that were adept at breeding during the traditional anestrous period. Studies on these ewes, three-way crosses of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding indicate that they exhibit nearly continuous cyclicity during the spring and summer and that they can be as successful as St. Croix ewes, a lowly seasonal breed of tropical origin at participating in accelerated production systems involving 7 to 8 mo lambing intervals. The present study was designed to observe the reproductive success of OOS ewes exposed to rams while lactating in May, generally thought to be the deepest part of anestrus in the Northern Hemisphere. Rams were introduced on 3 May when ewes (n = 34) averaged 39.6 d postpartum. Rams remained with ewes for the duration of the 39 d observation period and ewes were checked for marks twice weekly. Of the 34 ewes, 12 were marked in the first 21 d following ram introduction, and 18 were marked during the total 39 d of exposure. Of the marked ewes, 13 were diagnosed pregnant by ultrasonography 127 d after ram introduction indicating that some marks may not have been associated with mating, some ewes that did mate failed to conceive, or some ewes experienced early embryonic loss. Only 10 ewes maintained their pregnancies through September, indicating that 3 ewes likely experienced fetal loss. Of those ewes that maintained pregnancies, 7 gave birth to lambs of normal birth weight while the other 3 ewes produced underweight, presumably premature lambs that did not survive. Therefore, even though 52.9% of ewes were marked, only 20% of ewes exposed to rams gave birth to viable lambs. The decreased success of OOS ewes in the present study compared to previous data is most likely due to the shortened postpartum interval at ram introduction and the associated decreased time for uterine recovery and evacuation of uterine debris and increased milk production early in the postpartum period. These results suggest that OOS ewes that are able to conceive while lactating during seasonal anestrus are often unable to carry the resulting lambs to term. Although OOS ewes appear to be well suited to accelerated lambing systems involving 7 to 8 mo lambing intervals, reduction of lambing intervals to 6 mo appears to have detrimental effects on fetal survival in these ewes.

**KEYWORDS:** fertility, lactation, season, sheep, reproductive loss

## INTRODUCTION

Decreasing the interval between lambings and hence increasing the number of young born per female per year is an attractive option for improving the efficiency of sheep production. However, because ewes are seasonally polyestrous with the peak in breeding activity occurring from September to November in the Northern Hemisphere, an attempt to mate at a frequency greater than once per year will require one breeding during or near the seasonal anestrus. In addition, lambs from the previous lambing must be weaned at young ages or the ewe must exhibit estrus, ovulate, and conceive while lactating.

Greater understanding of postpartum reproduction may provide methods to achieve a fertile estrus in lactating ewes within 40 d of parturition. Literature on this topic has been reviewed by Hunter (1968). Estrus is observed in some ewes within the first 24 hr after parturition, but this estrus is anovulatory (Barker and Wiggins, 1964). Reports vary greatly in regard to the interval from parturition to first ovulatory estrus, and such variation is likely the result of differences in breed and season. Additionally, Whiteman et al. (1972) observed that lactation extended postpartum anestrus during spring. Some evidence has shown that removal of lambs for a period of time or early weaning of lambs will shorten the postpartum anestrus interval (Hunter, 1968); however, it is generally not a management system that will maximize production efficiency.

The Virginia Tech Selection Line, also called the Out-of-season (OOS) Line, was started in 1988 as part of a genetic selection experiment (Notter and Cockett, 2005). Studies on these ewes, three-way crosses of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding, indicate that they exhibit nearly continuous cyclicity during

spring and summer, with a mean period of anestrus of only 11.3 d (Vincent et al., 2000). Since ewes in the VT Selection Line have been shown to be superior to other wool breeds in their ability to breed out of season, they are promising participants in an accelerated lambing system that shrinks lambing intervals down to 6 to 7 mo without compromising lamb growth by early or temporary weaning. The present study was designed to observe the reproductive success of OOS ewes exposed to rams for 39 d in spring during the early postpartum period.

## MATERIALS AND METHODS

*Animals*

This study was conducted at Virginia Tech's Copenhaver Sheep Center in April, May, and June 2007. All procedures were approved and carried out in accordance with Institutional Animal Care and Use Committee of Virginia Tech. A total of 34 ewes was included in the study and were of VT Selection Line/OOS breeding. Ewes ranged in age from 2 to 8 yr and averaged 4.18 ± .26 yr.

All 34 ewes lambed during the winter preceding the study; ewes lambed between March 12 and 31, 2007 and averaged 39.6 ± 1.0 d since lambing at commencement of the experiment. All ewes were nursing 1, 2, or 3 lambs for the duration of the study. The mean number of lambs nursed per ewe was 1.88 ± .07. Ewes and lambs were held on pasture and received water ad libitum and supplemental grain daily.

*Experimental design*

Before commencement of the study, ewes had been isolated from rams for at least 2 mo. Three intact rams which had passed breeding soundness exams were fitted with crayon marking harnesses and introduced to ewes on May 3 (d 0). Crayon color was changed every 2 wk. Ewes, lambs, and rams were gathered into pens and rams and lambs were separated from ewes while observation was conducted. Ewes were checked for crayon marks on their rumps each Tuesday and Friday for 39 d following ram introduction. Crayon marks were categorized as heavy, indicating high probability of standing estrus, or light, indicating that the ewes may not have been in standing estrus.

Pregnancy was checked 127 d after introduction of rams. Ultrasonographic scanning for diagnosis of pregnancy was performed transabdominally using an Aloka 500 console (Corometrics Medical Systems, Inc., Wallingford, CT) and 3.5 MHz probe.

## RESULTS

Table 3-1 shows the frequencies of the responses for Experiment 2. Of the 34 ewes, 12 were marked in the first 21 d following ram introduction, and 18 were marked during the total 39 d of exposure. Of the marked ewes, 13 were diagnosed pregnant 127 d after ram introduction, but only 10 ewes maintained their pregnancies through September. Of those ewes that maintained pregnancies, 7 ewes gave birth to lambs of normal birth weight. The other 3 ewes produced underweight, presumably premature lambs that did not survive. Therefore, even though 52.9% of ewes were marked, only 20.6% of ewes gave birth to viable lambs.

## DISCUSSION

Both the depth of anestrus and lactational status of the ewe affect the ovulatory response to introduction of rams. In a study conducted in 2006 that was similar to the present study, 95.8% of OOS ewes were marked by rams during 39 d of exposure in March and April. This rate of mating is considerably higher than the 52.9% marked by rams during 39 d of exposure during the present study. The main difference in the studies and the most likely culprit for the decreased response to ram introduction in the present study was the amount of time that lapsed between lambing and rebreeding. In the earlier study, the ewes lambed between January 4 and January 30 and were exposed to rams on March 24, making them an average of 62.2 ± 1.2 d postpartum at commencement of the study. In the present case, ewes lambed between March 12 and March 31 and were exposed to rams on May 3, making them an average of only 39.6 ± 1.0 d postpartum at commencement of the study. The 22 fewer d that ewes were postpartum in the present study were likely detrimental to fertility for a number of reasons including decreased time for uterine recovery and evacuation of uterine debris (Foote, 1971) and increased milk production, and therefore draws on metabolic stores, early in the postpartum period (Cognie et al., 1975; Dawe and Fletcher, 1976.).

Estrus and ovulation can be successfully induced during the early postpartum period, but the greatest challenge involves conception and/or early embryo survival. This emphasizes the importance of knowing the state of preparedness of the uterus to support pregnancy. Some reports have suggested that the uterus has completed involution in ewes by 30 d after lambing, depending on season (Kiracofe, 1980). However, histological evaluation of the luminal contents and endometrium at this time revealed that

the number of lymphocytes and plasma cells exceeded those found in ewes further from lambing (Akinbami, 1989). Foote (1971) found that the uteri from spring lambing ewes measured at 17 or 24 d postpartum approached the size of the uterus from the cycling ewe but remained slightly heavier, suggesting the continued presence of debris. There was, however, some indication that lactation retarded uterine involution in spring lambing ewes. In these ewes, uteri containing debris were found in 30% of ewes slaughtered and in nearly every case this was judged to be sufficient to interfere with conception (Foote, 1971). The presence of uterine debris therefore appears to be an important deterrent to fertility in the ewe in the early postpartum period. Furthermore, Kiesling et al. (2000) found a high proportion of uterine contractions originating near the oviducts and moving towards the cervix in d 32 postpartum ewes and suggested that these could be an additional cause for low fertility in ewes bred during the postpartum period.

Conception rates during lactation are reportedly low (Hulet and Stormshak, 1972; Cognie et al., 1975; Shevah et al., 1975). Ford (1979) found that conception rate was only 40% when lactating ewes were exposed to rams from d 11 to 36 postpartum in the fall; all ewes that mated did so on d 36. This decreased fertility of lactating ewes is most likely due to absence of endocrine signals necessary for successful ovulation, mating, and establishment of pregnancy to occur. Cognie et al., (1975) found that the endocrine balance which precedes and follows ovulation is different in nursing compared to dry ewes. The pre-ovulatory discharge of estrogens, which reflects follicular stimulation, does not evolve in the same way or reach the same concentrations in nursing ewes. The level of plasma progesterone is correlated with the level of estradiol during the 24 h before the LH surge, and the concentration of progesterone between d 10 and 14 of

gestation in ewes that do become pregnant is lower in nursing ewes than in dry ewes. The lowered levels of progesterone, especially, probably have a detrimental impact on maintenance of pregnancy in lactating ewes that are bred during the early postpartum period, as in the present experiment.

The time of year during which lactating ewes are exposed to rams may also have an impact on reproductive responses. Rams were with ewes for a few days in March, all of April, and a few days in May in the previous study and were with ewes from early May until June 11 in the present study. It is well documented that May is the deepest part of anestrus for ewes in the northern hemisphere. Although changes in temperature can be associated with changes in reproductive activity of the ewe, the dominant environmental signal that cues and synchronizes the breeding season in sheep is photoperiod (Hafez, 1952; Wodzicka-Tomasezewska et al., 1967). This does not, however, preclude the possibility that higher temperatures in addition to longer daylengths may have contributed to the decreased ovulatory responses of ewes to the introduction of rams in the present study compared to the 2006 study.

It has been well documented that lactating, early postpartum ewes have trouble conceiving. A result of the present study that was not anticipated was the high incidence of embryonic and fetal loss that occurred. Ewes in the 2006 study were 62.2 ± 1.2 d postpartum at commencement of the study while ewes in the present experiment were 39.6 ± 1.0 d postpartum at commencement of the study. In the earlier study, 62.5% of ewes that were diagnosed pregnant gave birth to viable lambs, while only 53.8% of ewes diagnosed pregnant in the present study gave birth to viable lambs. Of all ewes exposed to rams in 2007, only 20.6% gave birth to viable lambs. This low number reflects

pregnancy losses that occurred before pregnancy diagnosis in addition to those ewes that aborted late in gestation or gave birth to still-born or low birth weight lambs that did not survive. Most embryonic loss has been reported to occur before d 18 (Moore et al., 1960; Quinlivan, 1966). Complete losses from d 18 to lambing were estimated at 9.4% (Hulet et al., 1956), and late embryonic of fetal losses from d 30 to term were only 1 to 5% in the New Zealand Romney Marsh breed when ewes were mated while dry during the normal breeding season (Quinlivan, 1966). It would thus appear that lactation and season increase the incidence of pregnancy loss, especially late in gestation (Dixon et al., 2007).

Taken together, these results suggest that OOS ewes are able to become pregnant while lactating during seasonal anestrus, but are often unable to carry the lambs to term when the time from lambing to conception is short. Although OOS ewes appear to be well suited to accelerated lambing systems involving 7 to 8- mo lambing intervals, reduction of lambing intervals to 6 to 7-mo appears to have detrimental effects on fetal survival.

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Table 3-1. Frequencies of OOS ewes marked by rams in the first 21 d after ram introduction, marked by rams during 39 d of ram exposure, diagnosed pregnant, and lambing viable offspring.

| **Variable** | **Percentage (number of ewes)** |
|---|---|
| Ewes marked in first 21 d after ram introduction | 35.3 ± 8.3% (12/34) |
| Ewes marked during 39 d of exposure to rams | 52.9 ± 8.7% (18/34) |
| Ewes diagnosed pregnant | 38.2 ± 8.5% (13/34) |
| Ewes that lambed viable offspring | 20.6 ± 7.0% (7/34) |

## CHAPTER 4

### Characterization of annual hormonal patterns underlying differences in seasonality of reproduction in three selected breeds of ewes

**ABSTRACT:** The changes in reproductive activity observed during seasonal anestrus in ewes are consequences of changes associated with the hypothalamic-pituitary-gonadal axis, specifically a decrease in the frequency of release of hypothalamic gonadotropin releasing hormone and a resultant decrease in secretion of luteinizing hormone from the pituitary, which is attributed to an increase in the sensitivity of the hypothalamus to negative feedback effects of estradiol. This study was designed to investigate alterations in endocrine profiles associated with differences in timing of hypothalamic sensitivity to estradiol-negative feedback and changing daylength in three breeds of ewes in the absence of rams. Breeds studied included the Virginia Tech Out-of-season (OOS) Line, which has been selected to lamb in fall, the St. Croix, a hair sheep breed of tropical origin that is thought to be lowly seasonal, and the Suffolk, a highly seasonal wool breed. Twice weekly blood samples were collected from intact and ovariectomized ewes kept outside in natural photoperiod for a year. Evaluation of progesterone profiles of intact ewes revealed that OOS ewes (n = 9) were anestrous for 56.7 d, which was significantly shorter than the anestrous periods of both St. Croix (n = 9; 132.7 d) and Suffolk ewes (n = 9; 140.3 d; $P < 0.0001$). The anestrous period both began later and ended earlier in OOS ewes compared to St. Croix and Suffolk ewes ($P < 0.004$). Based on investigation of luteinizing hormone profiles in ovariectomized ewes that received estradiol implants, ewes of OOS breeding (n = 8) had luteinizing hormone concentrations that were inhibited for 68 d, which was shorter than the period observed for Suffolk ewes (n = 9; 170.2 d; $P = 0.02$), but not different from that found in St. Croix ewes (n = 9; 124.8 d). In summary, this study provides novel information on the endocrine responses of 3 breeds of ewes to differing photoperiods in the absence of rams. The results showed for the first time that St. Croix ewes do not have shorter anestrous periods than wool breeds, as previously thought. There were significant effects of breed and season, but not treatment, on thyroxine and prolactin concentrations, but more research is needed before a role in determining timing of seasonal reproduction in the ewe can be assigned to these hormones. This study was the first known use of the ovariectomized, estradiol-implanted ewe model to compare degree of reproductive seasonality in different breeds.

**KEYWORDS:** luteinizing hormone, progesterone, sheep, season

## INTRODUCTION

The ewe is a seasonally polyestrous animal that displays regular estrous activity during a defined breeding season. Reproductive activity in ewes is under the control of the hypothalamic-pituitary-gonadal (HPG) axis. During the follicular phase of the estrous cycle, increasing secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus drives secretion of luteinizing hormone (LH) from the anterior pituitary gland. The increasing concentrations of LH in the blood stimulate the final stages of growth and maturation of follicles on the ovary and the associated increase in the production of estradiol ($E_2$). The rise in concentration of $E_2$ initiates two important events in the estrous cycle of the ewe. First, the increasing concentration of $E_2$ acts on the behavioral centers of the brain to induce estrous behavior. The peak in $E_2$ also stimulates a surge in GnRH/LH release, which causes ovulation, the release of the oocyte from the follicle. The period of time from the beginning of luteolysis until ovulation is called the follicular phase and normally lasts 2 to 3 days. The duration of estrus in the ewe generally is between 1 to 1.5 days, with an average of 35 hours (McKenzie and Terrill, 1937; Hafez, 1952).

Ovulation marks the transition from the follicular to the luteal phase, which lasts approximately 14 to 15 days. After ovulation, LH remodels the remainder of the follicle wall to form a transitory endocrine gland called the corpus luteum (CL). The CL is the major site for the synthesis and secretion of progesterone ($P_4$), which suppresses the tonic release of GnRH and LH, and in so doing, indirectly suppresses ovulation and estrous behavior. Progesterone also plays a major role in preparation of the reproductive tract for pregnancy and is the major hormone that supports and maintains gestation. If conception

does not occur, prostaglandin $F_2\alpha$ ($PGF_2\alpha$), produced by the uterus, initiates the regression of CL, halting the production of $P_4$. Decreasing $P_4$ signals the end of the luteal phase and the start of the new follicular phase. However, if an embryo is present in the uterus, the CL does not regress, but is maintained and continues to secrete $P_4$ throughout the pregnancy (reviewed by Bazer and First, 1983).

The ewe has a pattern of seasonal reproduction with maximum reproductive activity associated with short-day photoperiods. Accordingly, the percentage of ewes displaying estrus is greater during the late summer, fall and early winter months (McKenzie and Terrill, 1937; Hulet et al., 1974). Nearly all Targhee, Hampshire, Rambouillet, Suffolk, Polled Dorset, and Columbia ewes displayed estrus during September through March in Wisconsin, after which the percentage of ewes displaying estrus declined from April through June (Mallampatti et al., 1971; Lax et al., 1979), then gradually increased as the breeding season approached once again. Ovulation rate follows an annual pattern similar to that of estrous activity with the number of ovulations being highest during the breeding season and lowest during the non-breeding season (Mallampati et al., 1971; Hulet et al., 1974). The non-breeding season, also referred to as anestrus or the anestrous period, can therefore be defined as a period of low to non-existent ovulatory and estrous activity.

Although changes in temperature can be associated with changes in the reproductive activity of the ewe, the dominant environmental signal that cues and synchronizes the breeding season in sheep is known to be photoperiod (Hafez, 1952; Wodzicka-Tomasezewksa et al., 1967; Karsch et al., 1984). Marshall (1937)

demonstrated that the annual reproductive cycle of ewes shifted in accordance with the new photoperiod when ewes were transferred across the equator.

The changes in reproductive activity observed during anestrus are consequences of changes at the HPG axis, specifically a decrease in the frequency of secretion of GnRH from the hypothalamus and a resultant decrease in secretion of LH from the pituitary. This decrease in frequency of secretion of GnRH is attributed to an increase in the sensitivity of the hypothalamus to the negative feedback effects of $E_2$ (Legan et al., 1977). Karsch and colleagues (1993) found that during anestrus, $E_2$ at physiological concentrations inhibited LH secretion through suppression of the frequency of GnRH pulses. However, during the breeding season, the same concentration of $E_2$ was not effective in inhibiting LH pulse frequency (Karsch et al., 1993). Therefore, the main endocrine event responsible for the anestrous period in the ewe is the increase in the negative feedback effect of $E_2$ on pulsatile secretion of GnRH and LH.

A landmark study published in 1977 by Legan et al. and entitled "The Endocrine Control of Seasonal Reproductive Function in the Ewe: A Marked Change in Response to the Negative Feedback Action of Estradiol on Luteinizing Hormone Secretion", first explained how the hormonal interactions observed in the physiologic setting of the estrous cycle were fused with the occurrence of seasonal reproductive function by means of a variation in the response to $E_2$ feedback. In order to uncover the changing sensitivity of the hypothalamus to $E_2$-negative feedback, the authors bilaterally ovariectomized (OVX) ewes, thereby removing their endogenous source of $E_2$. Each ewe was then administered an $E_2$ implant. This standardized circulating levels of $E_2$ between ewes, since endogenous concentrations normally vary with the number and size of follicles on

the ovary. More importantly, the implants standardized the amount of $E_2$ that each ewe was exposed to throughout the year. The authors could therefore document that the changes they observed in LH concentrations over the seasons were due to changes in sensitivity to $E_2$-negative feedback at the level of the hypothalamus. Since that groundbreaking study in 1977, the "Karsch Model", so named for the principal investigator of the study, Dr. Fred J. Karsch of the University of Michigan, has been used countless times to further the understanding of the neuroendocrine mechanisms involved in the control of reproductive events in the ewe. For example, the Karsch Model has been used to elucidate how $E_2$ enhances luteinizing hormone pulse frequency during the follicular phase (Karsch et al., 1983), how pineal melatonin secretion drives the reproductive response to daylength (Bittman et al., 1983), how nightly duration of pineal melatonin secretion determines the reproductive response to inhibitory day length (Bittman et al., 1984), how refractoriness to inductive day lengths terminates the breeding season (Robinson and Karsch, 1984), how refractoriness to inhibitory day lengths initiates the breeding season (Robinson et al., 1985), and how steroid feedback inhibits pulsatile secretion of GnRH (Karsch et al., 1987). Although this list of advances in understanding is impressive, there is one caveat. All of these studies were conducted using only Suffolk ewes as experimental models.

The current study is unique in that it uses the Karsch Model, in addition to ovary-intact ewes, to investigate breed differences in reproductive endocrine responses to changing daylength in the ewe. Breeds of tropical origins, those from the Mediterranean region, and those with Merino ancestry have breeding seasons of longer durations than breeds originating from temperate and higher latitudes (Whisnant and Inskeep, 1992).

This association, however, is not absolute: the Finnish Landrace and Romanov breeds have extended breeding seasons despite their origins in northern Europe. Popular US breeds with extended breeding seasons include the Dorset, Rambouillet, Finnsheep, and crosses with and among these breeds. Breeds with breeding seasons of intermediate-length include the Corriedale, Columbia, and Targhee, while the Suffolk, Hampshire, Oxford, Southdown, Shropshire, and Cheviot are breeds with short breeding seasons. Breeds chosen for the present study include the Suffolk, a highly seasonal breed, the St. Croix, a lowly seasonal tropical breed, and the Virginia Tech Selection Line. The VT Selection Line, also called the out-of-season, or OOS Line, was developed by selection beginning in 1988 for fertility in May and June matings (Al-Shorepy and Notter, 1996), and are three-way crosses of 50% Dorset, 25% Rambouillet, and 25% Finnish Landrace breeding. By standardizing steroid feedback conditions and observing and comparing endocrine responses, we may shed light on how neuroendocrine responses to changing daylength manifest themselves in breeds that occupy different positions on the continuum of reproductive seasonality.

In addition to seasonal changes in the relationship between $E_2$ and the hypothalamus, other hormones may interact with seasonal reproductive activity. In the ewe, as the duration of melatonin secretion increases under shortening daylength in fall, concentrations of circulating prolactin (PRL) decrease (Thimonier et al., 1978). The same inverse relationship between melatonin and PRL is observed in spring when duration of nocturnal melatonin secretion decreases and PRL increases. The suppressive effect of melatonin on PRL secretion is predominantly caused by a direct action of melatonin on the pituitary gland since hypothalamo-pituitary disconnected rams continue

to show changes in the secretion of PRL in response to alterations in photoperiod or administration of melatonin (Lincoln and Clarke, 1994). Karsch et al. (1989) and later Gomez-Brunet et al. (2008) showed that PRL is not only affected by daylength, but also follows an endogenous rhythm. While these studies have helped shed light on the complex interaction of PRL release and seasonal reproduction in the ewe, much work remains to be done. No studies have looked directly at the influence of steroid negative feedback on PRL release during different times of the year. Additionally, differences in PRL release among domesticated breeds of ewes that show differences in degree of reproductive seasonality remain to be elucidated.

Numerous studies have used parts of the Karsch Model to investigate the role of thyroid hormones in the maintenance of seasonal reproductive changes. Moenter and colleagues (1991) found that the thyroid gland is necessary for endogenous suppression of neuroendocrine mechanisms that generate LH pulses. Webster and colleagues (1991b) found that secretion of thyroxine ($T_4$) after the onset of reproductive activity is required for an endogenously generated change in the neuroendocrine axis that leads to intensified $E_2$ feedback on the hypothalamus and an end to the breeding season. The same group also concluded that the thyroid gland is required for the endogenously generated switch in function of the GnRH neurosecretory system that leads to transition to anestrus in the ewe (Webster et al., 1991a). Additionally, others have found that thyroid hormones affect only neuroendocrine processes that lead to transition into anestrus. They are not required for maintenance of anestrus or for transition into the breeding season (Thrun et al., 1997a). Despite the numerous studies done on the relationship between thyroid hormones and seasonal reproduction in the ewe, most of these studies were done using

highly seasonal Suffolk ewes. In fact, a thorough search of the literature indicates that every paper published on the interaction between seasonal reproduction and thyroid hormones in the ewe used Suffolk ewes except one (Hernandez et al., 2003). In that study, Rambouillet ewes that were treated with the thyroid-blocking compound propylthiouracil experienced lowered circulating $T_4$ levels and failed to enter anestrus. Further studies are needed to investigate how breed composition and characteristics affect the interaction of thyroid hormones and seasonal reproduction.

The objective of this study was to observe endocrine profiles, including LH, $P_4$, $T_4$, and PRL, of ewes exposed to natural photoperiods for one year.

## MATERIALS AND METHODS

*Animals*

This study was conducted at Virginia Tech's Copenhaver Sheep Center in 2006 and 2007. All procedures were approved and carried out in accordance with Institutional Animal Care and Use Committee of Virginia Tech. A total of 55 sexually mature ewes were included in the study and were of St. Croix (n = 19), VT Selection Line/OOS (n = 18), and Suffolk (n = 18) breeding. All ewes were at least 214 d postpartum at commencement of the observation period.

Ewes were identified for the study in August 2006 and housed in isolation from rams after this time. Approximately half of the ewes of each breed (St. Croix n = 10, OOS n = 9, Suffolk n = 10) were randomly chosen to be ovariectomized between 28 Sept and 30 Oct, 2006. On 21 Nov, all OVX ewes except one from each breed received a

subcutaneous implant containing $E_2$. One OVX ewe from each breed remained unimplanted to serve as controls. The remainder of the ewes (St. Croix n = 9, OOS n = 9, Suffolk n = 8) remained ovary-intact. One intact Suffolk ewe and 2 OVX ewes (an OOS and a Suffolk ewe) died before completion of the study. Their hormone profiles were not included in the analysis.

For the duration of the observation period, 21 November, 2006 through 19 November, 2007, ewes were housed separately from rams and brought into pens twice weekly for blood sampling. Breeds were housed separately with no fence-line contact with each other or other sheep from 25 January, 2007 through the end of the observation period.

In addition to twice-weekly blood sampling, a series of 3 bleedings, taken one hour apart beginning at 9 am, were completed on or near the solstices and equinoxes, specifically on 18 December, 2006, 5 April, 2007, 21 June, 2007, and 18 September, 2007.

All ewes were maintained under natural environmental conditions in Blacksburg, Virginia. Their diet consisted of pasture grass from May to November and hay for the remainder of the year.

*Ovariectomy procedure*

A paravertebral nerve block was performed using a 1% lidocaine solution on the left side at the level of the last thoracic vertebrae and first 2 lumbar vertebrae to achieve anesthesia of the left paralumbar fossa. The left paralumbar fossa and the left abdominal wall were surgically prepared by shaving and scrubbing the area three times with an

iodine surgical scrub and alcohol. A vertical incision was made midway between the last rib and the tuber coxae. The incision was made through the skin and the external oblique. The internal oblique and transverse abdominus muscles were dissected using blunt scissors resulting in exposure of the peritoneal surface which was then nicked with the scalpel. Once the opening into the peritoneal cavity was expanded, the hand was introduced. Upon locating the ovaries and the uterus, a LigaSure hemostat was inserted and clamped across the oviduct. Application of heat pulses though the LigaSure unit resulted in cauterization of the oviduct and related blood vessels and separation of the ovary from the rest of the reproductive tract. The ovary was caught in the hand and removed through the incision site. The LigaSure procedure was repeated for the other ovary. Once both ovaries were removed, they were visually inspected to ensure complete removal of all ovarian tissue from the animal. The muscle layers were closed using a simple continuous suture pattern and absorbable suture (0 chromic gut). A Ford Interlocking suture pattern was used to close the skin using surgical nylon. Ceftiofur (Excenel®) was administered at 1mg/kg subcutaneously and flunixin megulamine (Banamine®) was administered post-surgery. Sutures were removed 10 days post surgery.

*Implants*

Implants were manufactured and administered with instruction from Dr. Fred Karsch of the University of Michigan (also see Karsch et al., 1973). Briefly, silastic tubing that was 0.183 in outer diameter and 0.132 in internal diameter was cut to 3.8 cm length. Medical grade silastic adhesive was used to plug .4 cm of one end of the tubing.

The tube was then packed tightly with estradiol-17β (Sigma) and adhesive used to fill the remaining .4 cm. All implants contained approximately .1655 g of $E_2$.

Implants were soaked in water for 20 minutes prior to implantation. Immediately before implantation, implants were dipped in 70% ethanol. Implants were administered subcutaneously with forceps after making an incision in the axillary area of the right foreleg after disinfecting the skin with ethanol and iodine. After insertion, the incision was closed with wound clips. Implants were palpated to confirm retention at the solstices and equinoxes. These implants release 7 – 8 μg $E_2$/day and produce a concentration of serum $E_2$ (3 – 5 pg/mL) comparable to that of intact ewes during the luteal phase of the estrous cycle for at least 1 yr (Karsch et al., 1973; Legan et al., 1977; Karsch et al., 1980).

*Blood sampling and assay procedures*

Blood samples (5 mL) were obtained using the Vaccutainer system via jugular venipuncture and stored at 4 ºC to allow for clotting. Samples were then spun in a centrifuge at 3000 rpm for 20 minutes. Serum was collected within 24 hours and frozen at -20 ºC in plastic tubes.

Serum from intact ewes was assayed for $P_4$ concentration using the Coat-A-Count Progesterone Kit (Siemens Healthcare Diagnostics, Inc., Los Angeles, CA) as described and validated by Minton et al. (1991). Order in which serum samples were assayed was chosen using a random number generator to minimize effects of inter-assay variability. The assay was sensitive to 0.1 ng/mL and the intra- and inter-assay coefficients of variation were 9.5% and 14.5%, respectively. Progesterone concentrations ≥ 1 ng/mL were used to indicate presence of a CL and therefore ovulatory cyclicity. Twice-weekly

samples were assayed on all ewes from 2 January through 16 March, 2007, which was hypothesized to include transition into the anestrous period. From 22 March through 20 July, 2007, which was hypothesized to be composed of the anestrous period, once weekly samples were assayed for $P_4$ concentrations of all intact ewes. From 23 July through 4 October, 2007, hypothesized to include transition into the breeding season, twice-weekly samples were again assayed for all intact ewes. Twice-weekly samples from ewes that had not yet experienced two luteal phases by 4 October were assayed through the end of the observation period, 19 November, 2007. For $P_4$ determination, only one blood sample was used on the days that intensive blood sampling occurred.

Serum samples from all OVX ewes on each sampling date were assayed in random order in duplicate aliquots for concentrations of circulating LH using the radioimmunoassay of Niswender et al. (1969). The assay was sensitive to 1 ng/mL and the intra- and inter-assay coefficients of variation were 11.8% and 12.6%, respectively. Because of vast differences expected in LH concentrations, volumes of serum assayed were adjusted depending on season. For samples taken from 29 November, 2006 through 29 March, 2007 and 3 September through 19 November, 2007, 100 μL of serum was assayed, yielding a 1 ng/mL limit of detection. For samples taken from 2 April through 31 August, 2007, 200 μL of serum was assayed, yielding a 0.5 ng/mL limit of detection. When samples yielded unquantifiable concentrations of LH, their concentration was set to the appropriate limit of detection for the volume assayed. For LH concentration determination, only one blood sample was assayed on the days that intensive blood sampling occurred.

For $T_4$ concentration assessment, the 3 blood samples obtained from each ewe on 18 December, 2006, 5 April, 2007, 21 June, 2007, and 18 September, 2007, were assayed and results were averaged within each date and ewe. Blood samples were assayed in duplicate aliquots in random order using the Coat-A-Count Total $T_4$ Kit (Siemens Healthcare Diagnostics, Inc., Los Angeles, CA) as described and validated by Thrun et al., 1997a). The assay was sensitive to 10 ng/mL and the intra- and inter-assay coefficients of variation were 3.3% and 8.1%, respectively.

The three hourly blood samples from the intensive sampling dates were physically pooled for determination of circulating PRL concentrations. The pooled samples were assayed at random in duplicate using a double antibody radioimmunoassay. The limit of sensitivity was .48 ng/mL and the intra- and inter-assay coefficients of variation were 8.6% and 14.0%, respectively. When samples yielded unquantifiable concentrations of PRL, their concentration was set to the limit of detection.

*Analysis*

Progesterone profiles were used to determine the dates of onset and cessation of anestrus as well as duration of anestrus for each individual intact ewe. Anestrus was considered to start at the first sampling date that $P_4$ concentrations dropped below 1 ng/mL and remained there for at least 17 d. Anestrus was considered to stop at the first sampling date that $P_4$ concentrations rose above 1 ng/mL. The total length of anestrus was the number of days between beginning and end of anestrus minus 17 d, the length of the average ovine estrous cycle. The dependant variables “day of year that anestrus began”, “day of year that anestrus ended”, and “duration of anestrus”, were compared

between breeds using the General Linear Models (GLM) procedure of SAS (SAS Institute, Inc., Cary, North Carolina, US).

Luteinizing hormone profiles were used to determine the duration of LH inhibition for each OVX ewe. For each ewe, the LH concentration on each sampling date, $LH_i$, was considered relative to the concentrations of LH on adjacent dates and the critical value of 1 ng/mL. Thus, on day "i", ewes were considered to have inhibited LH concentrations if:

$$LH_i < 1 \text{ ng/mL } \textbf{and } (LH_{i-1} < 1 \text{ ng/mL } \textbf{or } LH_{i+1} < 1 \text{ ng/mL})$$

**OR**

$$LH_i \geq 1 \text{ ng/mL } \textbf{and } (LH_{i-1} < 1 \text{ ng/mL } \textbf{and } LH_{i+1} < 1 \text{ ng/mL})$$

Thus, in order to have an inhibited LH concentration, a ewe must have had a concentration of LH below 1 ng/mL at time "i" and at least one adjacent concentration of LH also below 1 ng/mL, **OR** the ewe may have had a concentration of LH above 1 ng/mL at time "i", but only if both adjacent concentrations of LH were below 1 ng/mL. Because samples were taken twice weekly, the number of sampling dates a ewe was found to have inhibited LH was multiplied by 3.5 to get the total number of LH inhibition for the year. The above technique was also used to quantify duration of LH inhibition using critical values of concentrations of LH of 2 ng/mL and 5 ng/mL, but a critical value of 1 ng/mL appeared to result in the most accurate quantification of duration of LH inhibition based on visual observation of LH profiles for each ewe. The dependant variable "total days of LH inhibition" was compared between breeds using the GLM procedure of SAS.

The GLM procedure was also used to test for significant effects of breed and treatment (OVX or intact), as well as interactions of breed by treatment on circulating concentrations of $T_4$ and PRL. Because of significant breed by date interactions for $T_4$ levels and large variances in PRL concentrations between dates, these analyses were conducted separately for each date.

Correlations at each sampling date between $T_4$ and PRL concentrations, $T_4$ and LH concentrations, and PRL and LH concentrations were also determined using the Multivariate ANOVA option of the GLM procedure in SAS.

## RESULTS

*Duration and timing of anestrus*

Figure 4-7 shows the average duration of anestrus in days and the average date that anestrus began and ended for intact ewes in each breed based on $P_4$ profiles. Ewes of OOS breeding had significantly shorter anestrous periods than both St. Croix and Suffolk ewes ($P < 0.0001$), which were not different. Ewes of OOS breeding started anestrus later (19 May) than both St. Croix (12 Apr; $P = 0.001$) and Suffolk (23 Mar; $P < 0.0001$) ewes. There was a tendency for Suffolk ewes to start anestrus earlier than St. Croix ewes ($P = 0.06$). In addition to starting anestrus later than the other breeds, anestrus ended earlier for OOS (2 Aug) than for St. Croix (8 Sept; $P < 0.0001$) and Suffolk (27 Aug; $P = 0.004$) ewes, which were not different from one another.

*Duration of LH inhibition*

Figure 4-8 shows the average duration of LH inhibition in days for OVX ewes in each breed based on LH profiles. Ewes of OOS breeding experienced significantly fewer days of LH inhibition than Suffolk ewes (68 ± 28.4 vs. 170.2 ± 26.6 d; $P = 0.02$), but were not different than St. Croix ewes (124.8 ± 25 d). Days of LH inhibition in St. Croix and Suffolk ewes did not differ.

*Thyroxine and Prolactin*

Table 4-1 gives means and standard errors of $T_4$ concentrations for each breed on the 4 sampling dates. Treatment, either OVX or intact, did not significantly affect $T_4$ at any time, so the treatments have been averaged together. Breed significantly affected $T_4$ concentrations on 18 December, 2006 when St. Croix ewes had significantly higher concentrations than OOS ewes ($P = 0.04$) and Suffolk ewes ($P = 0.003$). Ewes of OOS breeding were not different from Suffolk ewes in terms of $T_4$ concentration on this date. Breed did not have significant effects on $T_4$ concentrations on 5 April, 2007; however, St. Croix ewes tended to have higher concentrations than Suffolk ewes ($P = 0.06$). Again, breed did not have a significant effect on $T_4$ concentrations on 21 June, 2007; however, OOS ewes tended to have higher concentrations than Suffolk ewes ($P = 0.06$). Breed did have a significant effect on $T_4$ concentrations on 18 September, 2007 when Suffolk ewes had lower concentrations than both St. Croix ewes ($P = 0.002$) and OOS ewes ($P = 0.01$), which were not different from one another.

Table 4-2 gives the means and standard errors of the PRL concentrations for each breed on the 4 sampling dates. Treatment did not have significant effects on PRL

concentrations at any time point, so values for OVX and intact ewes were averaged. Breed significantly impacted PRL concentrations on 18 December, 2006 when St. Croix ewes had significantly higher concentrations than both OOS ($P$ = 0.01) and Suffolk ewes ($P$ = 0.002), which were not different. Concentrations of PRL in OOS ewes increased dramatically on 5 April, 2007 when OOS ewes had higher concentrations than both St. Croix ($P$ = 0.003) and Suffolk ewes ($P$ = 0.01), which were not different from one another. On 21 June, 2007, Suffolk ewes had higher PRL concentrations than both St. Croix ($P$ = 0.0003) and OOS ewes ($P$ = 0.04). There was a tendency for OOS ewes to have higher PRL than St. Croix on that date ($P$ = 0.08). There was no significant effect of breed on PRL concentrations on 18 September, 2007.

There was no significant correlation between $T_4$ and PRL or $T_4$ and LH concentrations on any sampling date. Although there were no significant correlations between PRL and LH, the correlation coefficients were very consistent for the 18 Dec, 2006, 5, April, 2007, and 18 September, 2007 sampling dates (-0.21; P = 0.3).

## DISCUSSION

The hypothesis for the feedback control of seasonal breeding in the ewe is based on a marked change in responsiveness of the system governing tonic LH secretion to the negative feedback effects of $E_2$. During the breeding season, the response to $E_2$ is low. At transition to anestrus, the responsiveness increases and remains high until onset of the breeding season, when response to $E_2$ negative feedback again diminishes. The most compelling evidence for a seasonal shift in the capacity of $E_2$ to inhibit secretion of LH

was obtained from a study in which OVX ewes were treated with $E_2$ implants which maintained physiological serum $E_2$ concentrations (Legan et al., 1977). In the presence of stable $E_2$ levels circulating LH was elevated during the breeding season but generally undetectable during the anestrous period. The seasonal shifts in LH secretion were abrupt, coincided with transitions between breeding and non-breeding seasons in intact ewes, and did not occur in the absence of $E_2$ implants (Karsch et al., 1980). This study, using the previously described Karsch Model, was conducted using Suffolk ewes, as were other studies modeled after it (Malpaux et al., 1988 and others). Minton (1990) used the Karsch Model in anestrous Rambouillet x Dorset ewes and got the same response. The current study, which used the Karsch Model on St. Croix, OOS, and Suffolk ewes, resulted in LH profiles that were also similar to those from previous studies and showed that seasonal shifts in LH were abrupt, coincided with transitions between breeding and non-breeding seasons in intact ewes, and did not occur in the absence of $E_2$ implants. This study is the first reported use of the Karsch Model to compare the feedback basis of seasonal breeding in 3 different breeds of ewes.

The 3 breeds chosen for this study were thought to represent a broad range of reproductive seasonality. Based on numerous published and unpublished studies, we hypothesized that OOS ewes would be the least reproductively seasonal breed, Suffolk ewes would be most seasonal, and St. Croix ewes would be intermediate. Tropical hair sheep breeds, like the St. Croix, have long been believed to be superior to wool breeds in terms of their ability to breed during the traditional non-breeding season. At an out-of-season breeding symposium in 1992, Whisnant and Inskeep explained that breeds of more tropical origin have longer breeding seasons than breeds of temperate and higher

latitudes (Whisnant and Inskeep, 1992). Surprisingly, a thorough search of historical literature reveals almost no objective studies to back up this widely held belief. In contrast, Suffolks have been clearly shown to have a relatively short breeding season (Nugent et al., 1988). Therefore, one of the most surprising results of the present study was that both the duration and the timing of the onset of anestrus did not differ between St. Croix and Suffolk ewes (Figure 4-7).

The disparity between the present findings and previously held beliefs regarding the ability of breeds to become pregnant during the traditional non-breeding season can most likely be attributed to two factors. First, the proportion of ewes displaying behavioral estrus in association with ovulation may differ among breeds (Whisnant and Inskeep, 1992). Quirke et al. (1988) have emphasized the tendency for Rambouillet ewes to ovulate without estrus at both the beginning and end of the breeding season. Furthermore, Nugent et al. (1988) compared the proportion of Dorset and Hampshire ewes that displayed estrous behavior in association with ovulation when exposed to rams during May and June and found that 83% of Dorset but only 28% of Hampshire ewes that ovulated showed estrus.

Although St. Croix and Suffolk ewes appear to be ovulating for approximately the same amount of time each year, Suffolk ewes may not show estrus as frequently as St. Croix ewes during spring and summer, and therefore not get pregnant at the same rate as hair ewes. Secondly, the present study did not take into account the effect of ram introduction on the reproductive activity of these 2 breeds during anestrus. Sensitivity to ram introduction may differ between these 2 breeds, resulting in the appearance of a shorter anestrous period in St. Croix ewes compared to Suffolk ewes. There is some

evidence that the response to ram introduction is related to the percentage of females that are ovulating spontaneously at the time of ram introduction (Oldham and Fisher, 1992). For example, Pearce and Oldham (1988) reported that 98% of Merino ewes ovulated in response to ram introduction in late spring while Nugent et al. (1988) reported that 96% of Dorset and 72% of Hampshire ewes ovulated when exposed to rams in May and June. If the ram effect is more pronounced in St. Croix ewes than Suffolk ewes, that would result in a greater ability of St. Croix ewes to lamb in the fall.

In a production setting, ovulating ewes cannot conceive without showing behavioral estrus, therefore many studies have been done on the ability of exogenous $P_4$ pretreatment to improve the percentages of ewes that are mated after exposure to rams during anestrus. Robinson (1955) observed estrous behavior in all $P_4$ pretreated Romney Marsh ewes that received $E_2$ during the non-breeding season. Ewes that received similar doses of $E_2$ without $P_4$ pretreatment did not show estrous behavior. Fabre-Nys and Martin (1991) confirmed these results when they observed that $P_4$ pretreatment increased the proportion of OVX ewes showing estrus, decreased the time from $E_2$ treatment to onset of estrus, increased the duration of estrous behavior, and increased the intensity of the receptivity to rams displayed by ewes. These studies indicate that if methods, such as exogenous $P_4$ pretreatment, were employed in Suffolk ewes in conjunction with ram introduction, they might be comparable to St. Croix ewes in ability to become pregnant during the traditional anestrous period.

The OOS ewes clearly had a shorter anestrous period than both St. Croix and Suffolk ewes. Twice-yearly lambing of ewes is an attractive proposal for improving sheep production efficiency since a decrease in the interval between lambings rapidly

increases the number of young born per female over her lifetime. Further efficiency may be realized by producers if more lambs per ewe can be marketed in wool breeds instead of just in hair breeds as previously thought. This is because wool breeds have more desirable carcass characteristics than hair breeds and therefore yield more pounds of marketable product (Brown and Jackson, 1995).

Selected $P_4$ profiles from intact St. Croix, OOS, and Suffolk ewes are shown in Figures 4-1, 4-2, and 4-3. Each figure includes the ewes that were anestrus for the shortest and longest durations as well as one ewe close to the breed average. The $P_4$ profiles of the remainder of the intact ewes are given in Appendix A. The general shape of the profiles was what was expected based on historical literature on seasonality of reproduction in the ewe (Karsch et al., 1984). Generally, peaks of $P_4$ occurred about every 17 days from beginning of the experiment until an abrupt cessation of $P_4$ peaks. Progesterone remained baseline until cyclicity began and $P_4$ peaks were observed about every 17 days once again. Although all ewes followed this general pattern, there were two OOS ewes that did not stop cycling at all and the $P_4$ profile of one of these ewes is shown in Figure 4-2. The timing of the beginning and end of the anestrous period varied with ewes and most notably with breed, consistent with a study that found that photoperiod synchronizes an endogenous rhythm of reproductive activity and that differences in photoperiodic synchronization of this rhythm are found between breeds (O'Callaghan et al., 1992).

The $P_4$ profiles of individual ewes, presented in Appendix A, document an interesting trend. As ewes approached the anestrous period, the amplitude of $P_4$ secretion during luteal phases appeared to become progressively lower, eventually reaching a nadir

just before the cessation of cyclicity. This result agrees with results published by Bartlewski et al (1999a) indicating that maximal serum concentrations of $P_4$ during the luteal phase appeared to be lower and the plateau phase of $P_4$ secretion appeared to be shorter during the last luteal phase of the ovulatory season in comparison with the mid-breeding season in Western white-faced ewes. Similarly, in the one OOS ewe that remained cyclic throughout the year (A2066), $P_4$ levels corresponding to luteal phases appeared to be depressed during the period that corresponded to anestrus in other OOS ewes. Likewise, Wheeler and Land (1977) found that $P_4$ secretion was affected by the stage of the ovulatory season in ewes, with higher serum $P_4$ concentrations in the middle than at the beginning or end of the breeding season. These decreased $P_4$ levels could be indicative of insufficient CL function (Kaulfuss et al, 2006) and may explain why high levels of early fetal loss apparently occurred in lactating OOS ewes bred in spring in experiments 1 and 2.

For some ewes (notably B2355 St. Croix, B2361 St. Croix, and A227 Suffolk), the first peak in $P_4$ secretion after the anestrous period appeared to be notably higher than $P_4$ peaks during subsequent cycles. Bartlewski et al. (1999b) noted that an increase in $P_4$ concentrations preceding the first ovulation of the breeding season may be produced by luteinized unovulated follicles and/or interstitial tissue of unknown origin. Without ultrasonographic inspection, we cannot say whether the increases in $P_4$ in these ewes were due to extra-luteal secretion or the ovulation of immature ovarian follicles and therefore short-lived CL.

An interesting observation is that all ewes were either cyclic or acyclic for extended periods of time. There were no breakthrough ovulations during anestrous

periods in any breed. This is not consistent with results of earlier studies, especially unpublished data on OOS ewes. A probable explanation is the lack of ram exposure in the current study.

One OOS ewe, B2103, that appeared to be permanently acyclic based on $P_4$ profiles. Because she was an outlier and apparently suffered from some physiological abnormality, she was not included in any of the analyses of duration and timing of anestrus.

Analysis of the duration of LH inhibition based on LH profiles of OVX ewes revealed that St. Croix ewes had inhibited LH concentrations for the same amount of time as Suffolk ewes, which were inhibited for a longer period of time than OOS ewes. These results were thus similar to those from analysis of $P_4$ profiles. What was different than the results from the $P_4$ profile analysis was that St. Croix and OOS ewes were inhibited for similar amounts of time. Because of breakthrough periods of activity during the transition periods, it was impossible to determine start and stop dates for LH inhibition. Because LH release is controlled, in part, by $E_2$-negative feedback on hypothalamic release of GnRH, and all these ewes had standardized $E_2$ inputs due to their implants, differences in LH levels were due to severity of negative-feedback effects on the hypothalamus (Karsch et al., 1980). Legan et al. (1977) hypothesized that a heightened response to $E_2$ negative feedback terminates ovarian cyclicity at the transition into anestrus by preventing the sustained increase in tonic LH secretion. Therefore, because their duration of LH inhibition was the longest, Suffolk ewes experienced this heightened response to $E_2$ negative feedback for the longest period of time of the 3 breeds in the current study. St. Croix ewes fell in the middle in terms of sensitivity, and OOS ewes

were the least sensitive to $E_2$ negative feedback, leading to a short period of LH inhibition. Modifying the hypothesis set forth by Legan et al. (1977), we had postulated that breeds of sheep with reproductive activities that are more negatively affected by increasing daylength would experience an increased duration of sensitivity to $E_2$ negative feedback. The differences between OOS and Suffolk ewes support this postulation. Although the Karsch Model has been used in two different breeds, neither study evaluated duration of LH inhibition, so a precedent for these findings is unavailable (Minton, 1990; Legan et al., 1977). However, considering the degree to which increasing daylength affected St. Croix compared to Suffolk ewes based on their $P_4$ profiles, the duration of LH inhibition supports the hypothesis for this comparison as well, although it was not anticipated that these breeds would be so similar in their durations of cyclicity.

The one breed comparison that does not fit with the hypothesized model that breeds of sheep with reproductive activities that are more negatively affected by increasing daylength would experience an increased duration of sensitivity to $E_2$ negative feedback is the activity of St. Croix versus OOS ewes. Using the hypothetical model, based on duration of anestrus, OOS ewes should have statistically shorter periods of LH inhibition than St. Croix ewes, which was not the case. One possibility is that the breeds have differing thresholds for LH requirements leading to ovulation. In other words, even though OOS ewes had LH concentrations that were inhibited for a similar length of time to St. Croix ewes, they were still able to ovulate for at least part of this time, leading to a shorter duration of anestrus for OOS ewes. Although there is no evidence in the literature of differing LH requirements leading to ovulation, there is evidence that different breeds of ewes have different $E_2$ requirements for the induction of estrus behavior and the LH

surge, which would have the same consequences mentioned above. Ben Said et al. (2007) found that a moderate $E_2$ signal, which was varied by $E_2$ implant size and duration of treatment, induced an LH surge in 10/10 Ile de France ewes while a larger $E_2$ signal was able to induce the surge in only 4/10 Romanov ewes. This provides clear evidence that there is a difference in the $E_2$ requirement for the induction of the LH surge between breeds of ewes. Note that the Ile de France ewe is known for having single ovulations while the Romanov has multiple ovulations. Studies of $E_2$-induced release of LH in OVX ewes have shown that breeds with high ovulation rates are less sensitive to the positive and negative feedback effects of $E_2$ than those with lower ovulation rates (Baird and Campbell, 1998; Land et al., 1973). Because St. Croix ewes usually undergo multiple ovulations, it is likely that these ewes require more $E_2$ to undergo an LH surge and therefore ovulation, leading to the disconnect in comparisons between breeds in days of LH inhibition and days anestrus.

There are 2 matters that complicate correlation of duration of anestrus with LH inhibition. The first is that the threshold of 1 ng/mL was used to evaluate LH inhibition. This level has been used in many studies evaluating reproductive activity (Karsch et al., 1980), but the fact remains that it is an arbitrary value. Current analysis was done with 1, 2, and 5 ng/mL as the threshold and 1 ng/mL was chosen because it resulted in fewer breakthroughs and was more consistent with duration of anestrus than any other value. It is possible, however, that a ewe does not become inhibited until she is lower than 1 or that each breed or even each individual ewe has her own threshold of inhibition when it comes to LH. The second matter that complicates comparisons of the two durations is that $P_4$ profiles are taken from intact ewes while LH profiles are from OVX ewes.

Therefore, it is impossible to correlate a specific LH pattern with overall ovulatory outcome.

Selected LH profiles from OVX St. Croix, OOS, and Suffolk ewes are shown in Figures 4-4, 4-5, and 4-6. Each figure includes the ewes that had concentrations of LH that were inhibited for the shortest and longest durations as well as one ewe close to the breed average. The LH profiles of the remainder of the OVX ewes are presented in Appendix B. Previous studies on OVX ewes kept in natural photoperiods found that long days increased the negative feedback potency of $E_2$ (Bittman et al., 1983). Although there were differences in the degree to which LH concentrations were suppressed among ewes and especially among breeds, the previous findings explain the observed periods of baseline LH secretion during the spring and/or summer in most ewes in the current study. Investigation of individual LH profiles, presented in Appendix B, yielded some interesting observations. St. Croix ewes had durations of LH inhibition of 0, 14, 66.5, 105, 136.5, 161, 182, 203, and 255.5 d. Ewes of OOS breeding had durations of LH inhibition of 0, 0, 31.5, 70, 115.5, 126, and 133 d. Suffolk ewes had durations of LH inhibition of 0, 150.5, 154, 196, 206.5, 213.5, 220.5, and 220.5 d. So, there were 14 d and 0 d between the 2 lowest values for St. Croix and OOS ewes, respectively, while there were 150.5 d between the 2 lowest values for Suffolk ewes. Therefore, the 0 d Suffolk appears to be an outlier. Although the results presented included that Suffolk, when she is excluded from the analysis, the durations of LH inhibition in St. Croix and Suffolk ewes are no longer similar, and there is only a tendency for St. Croix and OOS ewes to have similar durations of LH inhibition. Although it is impossible to know if the Suffolk that had 0 d of LH inhibition is an oddity or is in fact in line with breed

characteristics, excluding her from breed assumptions brings the results more into line with differences between breeds seen in durations of anestrus.

The final limiting factor in drawing assumptions from LH profiles lies in the physical differences between the breeds studied. Because the Karsch Model has never been used to compare breeds, there was no precedent for adjusting implant $E_2$ content to account for differences in body size among breeds, and even among ewes. The St. Croix Breeders of North America state that a mature weight for a St. Croix ewe is around 68 kg, but the ewes in the current study were generally much smaller. Both OOS and Suffolk ewes are much heavier than St. Croix ewes at maturity. This may have affected the concentration of $E_2$ in circulation that resulted from the implants. Brunner Huber and Hogue (2005) found that implanted contraceptives do not work as well in women who are overweight and obese compared to women with normal body mass indices. This may be due to decreased circulating concentrations of steroids, probably a physiological consequence of altered body composition. The differences in circulating steroids in overweight and normal women are similar to the dynamics that may have affected the heavier sheep breeds in the current study.

The mean $T_4$ concentrations indicate that Suffolks have concentrations lower than St. Croix ewes in December, 2006 and both St. Croix and OOS ewes in September, 2007. Multiple, elegantly planned studies have shown that $T_4$ is necessary for 1) endogenous suppression of neuroendocrine mechanisms that generate LH pulses (Moenter et al., 1991); 2) endogenously generated changes in the neuroendocrine axis that leads to intensified $E_2$ feedback on the hypothalamus and an end to the breeding season (Webster et al., 1991b); and 3) the endogenously generated switch in function of the GnRH

neurosecretory system that leads to transition to anestrus (Webster et al., 1991a). The critical period for $T_4$ action is limited to late in the breeding season, beginning around late December (Thrun et al., 1996). Therefore, if the significant differences in $T_4$ concentrations on 12/18/06 had anything to do with the reproductive outcomes of the current study, Suffolk ewes, which had lower $T_4$ than St. Croix ewes, should have had trouble transitioning into anestrus. In fact, this supposition is not in line with the findings regarding cyclicity of the breeds. The differences observed in $T_4$, therefore are probably indicative of differences in metabolic rates of the 3 breeds. Most likely there is a threshold level of $T_4$ that must be met in order for normal transition into the anestrous period to occur and apparently all 3 breeds met that threshold. Since $T_4$ concentration varied with breed and ewe in the current study, it is impossible to make any conclusions about $T_4$ requirements for transition into anestrus. A study that involves thyroidectomy of different breeds accompanied by $T_4$ replacement at various levels may help shed light on the threshold differences between breeds.

Analysis of variance in the current study revealed a significant effect of season on serum PRL concentrations. Likewise, Gomez-Brunet et al. (2008) found the same marked seasonal changes in PRL concentration; highest PRL concentrations were observed around the summer solstice and the lowest around the autumnal equinox and winter solstice. It is well known that catecholamines influence the secretion of PRL and that dopamine is the major prolactin-inhibiting-factor. Nocturnal increases in PRL concentration during the longest days in summer are thus likely evoked at least in part by an inhibitory action of melatonin on the dopaminergic pathway (Misztal et al., 1997). Since Suffolk ewes had significantly higher PRL concentrations than the other breeds on

the 21 June sampling date, it seems probable that their neuroendocrine pathways responsible for dopamine production are more sensitive to negative effects of long-daylengths than are the neuroendocrine pathways of other breeds. This change in PRL release with breed and season is merely associated with seasonal reproduction and not implicated as the cause of it. Gomez-Brunet et al. (2008) investigated PRL concentrations in two species of sheep, one domestic and one wild. They found that PRL concentrations were high in domestic ewes when they began cycling in June and July, but were low in wild ewes in mid-October when they began ovulatory cyclicity. Therefore, the pattern of PRL secretion appears to be endogenously generated and neither the present study nor previously published reports provide evidence of a causative effect of PRL concentration on seasonal reproduction in the ewe.

In summary, this study provides novel information on the endocrine responses of 3 breeds of ewes to differing photoperiods in the absence of rams. The results show for the first time that St. Croix ewes do not have shorter anestrous periods than wool breeds, as previously thought. In fact, the results indicate that OOS ewes, a line selected for efficacy in out-of-season breeding, have shorter durations of ovulatory inactivity than St. Croix ewes. This trend was not mirrored in the durations of LH inhibition of these breeds suggesting that many factors confound the translation of neuroendocrine signals to reproductive responses. Based on the present study, neither $T_4$ nor PRL play a significant role in determining timing of seasonal reproduction in the ewe.

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Figure 4-1. Progesterone profiles from individual St. Croix ewes representing the longest, average, and shortest durations of anestrus. See Materials and Methods section for an explanation of sampling dates.

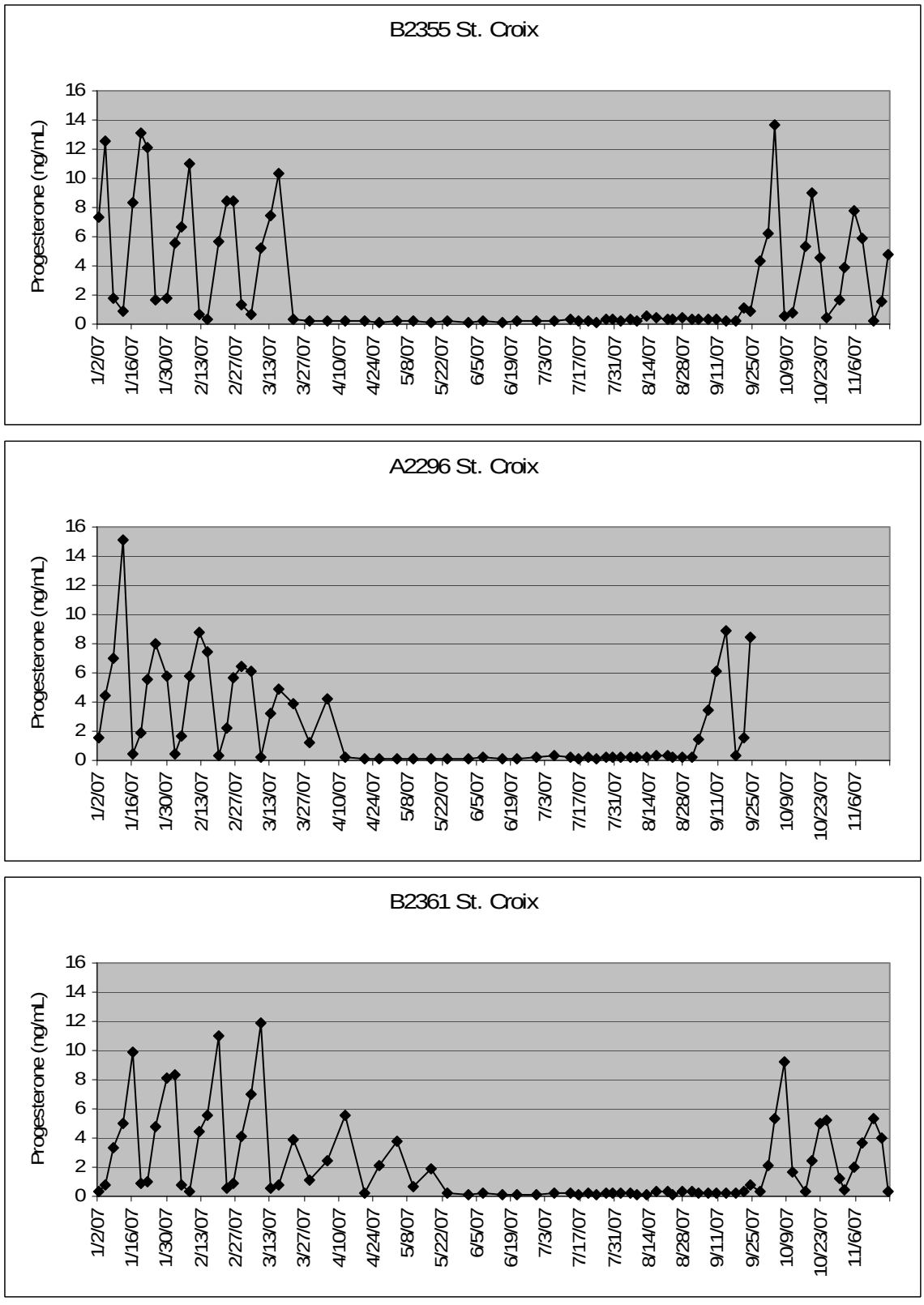
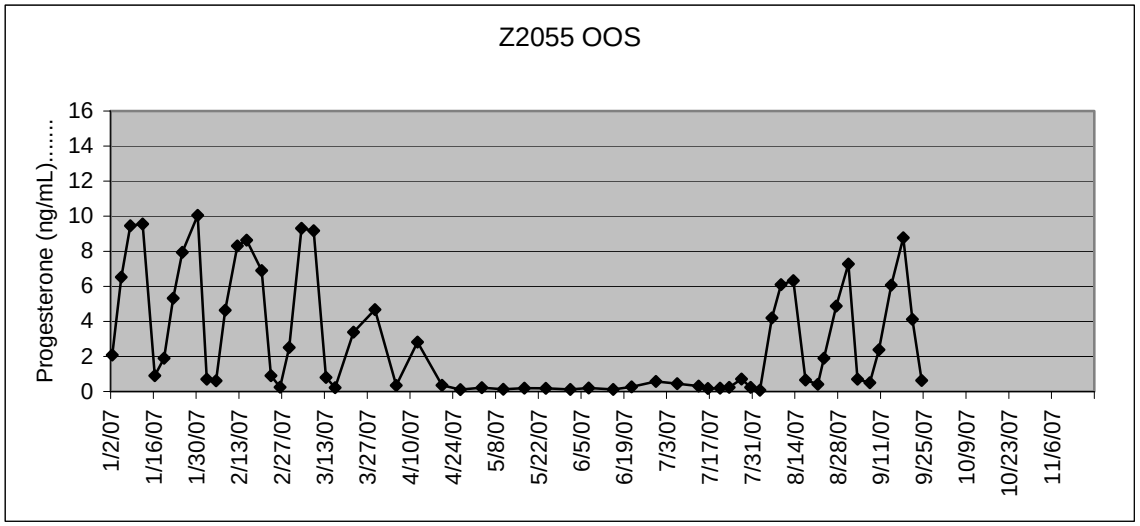
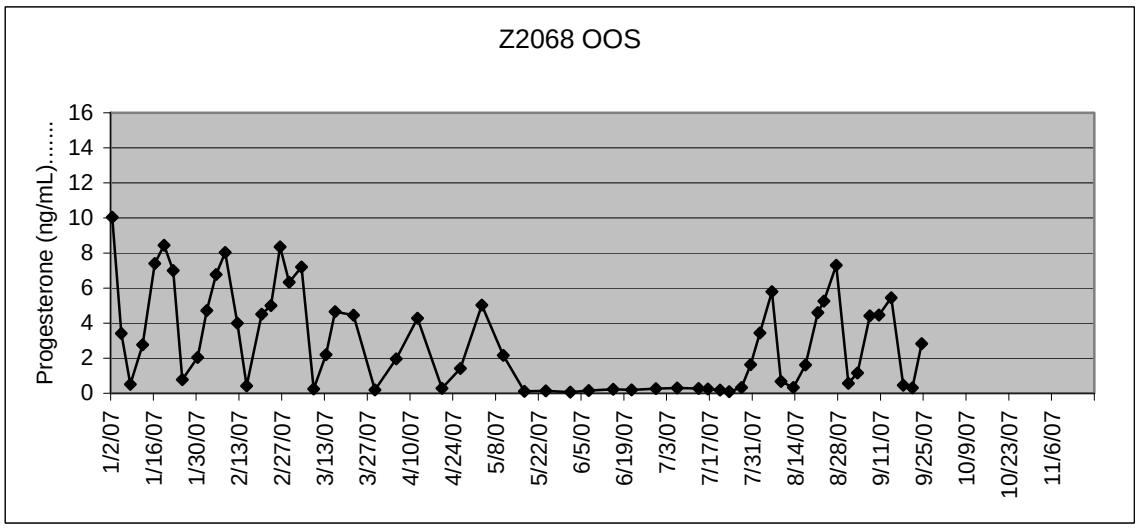


Figure 4-2. Progesterone profiles from individual OOS ewes representing the longest, average, and shortest durations of anestrus. See Materials and Methods section for an explanation of sampling dates.



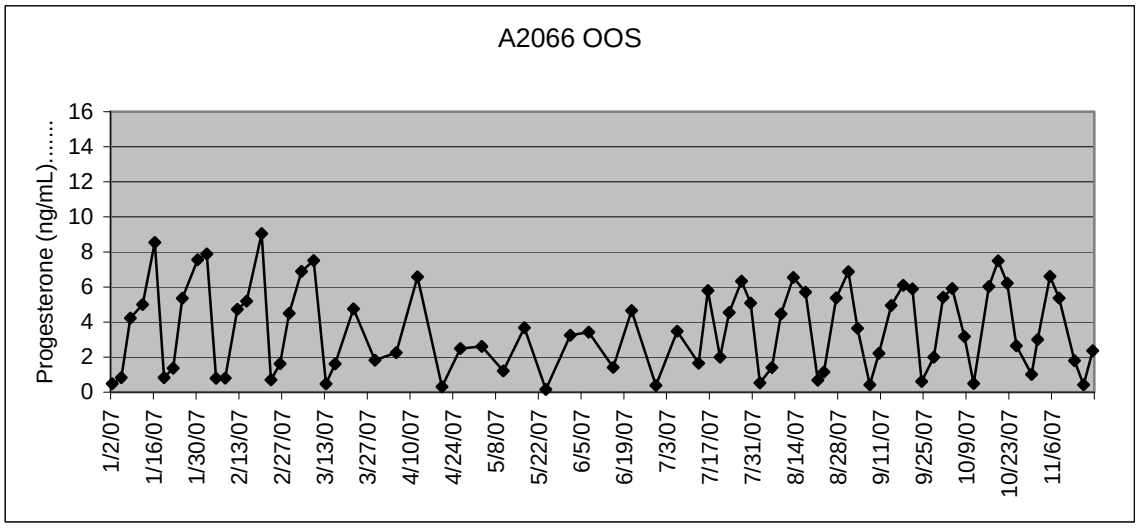
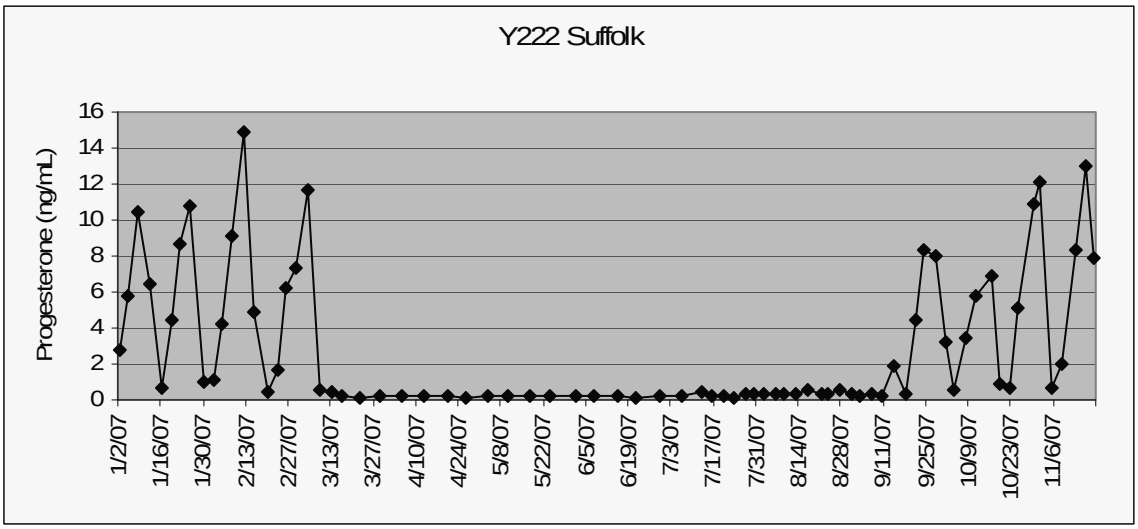
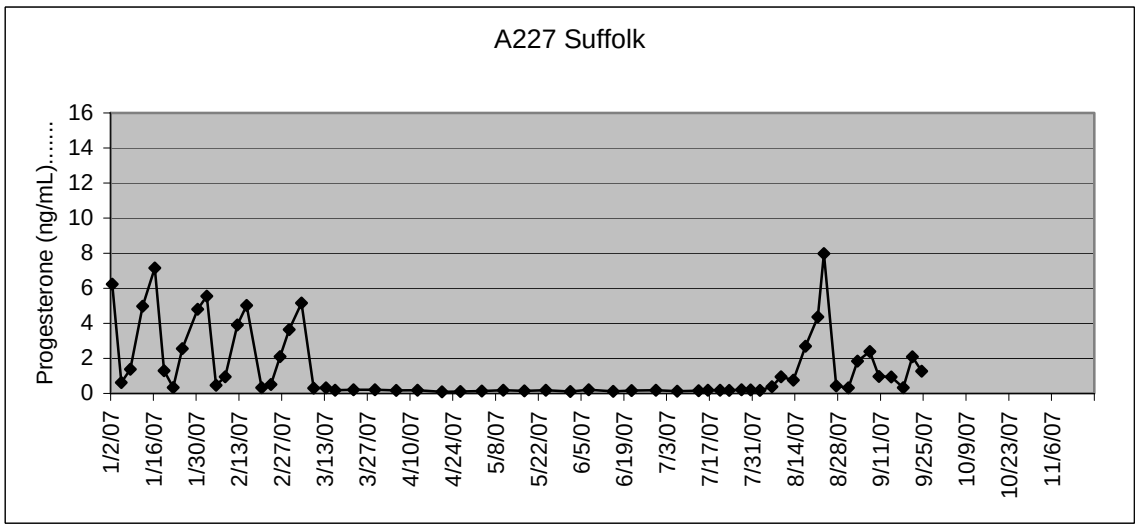



Figure 4-3. Progesterone profiles from individual Suffolk ewes representing the longest, average, and shortest durations of anestrus. See Materials and Methods section for an explanation of sampling dates.



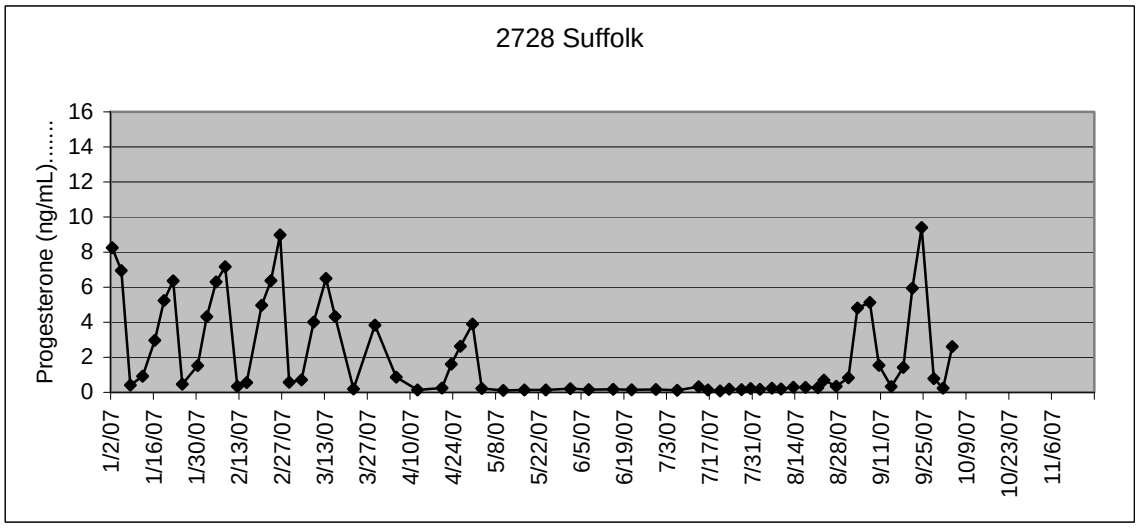



Figure 4-4. Luteinizing hormone profiles from individual St. Croix ewes representing the longest, average, and shortest durations of luteinizing hormone inhibition.

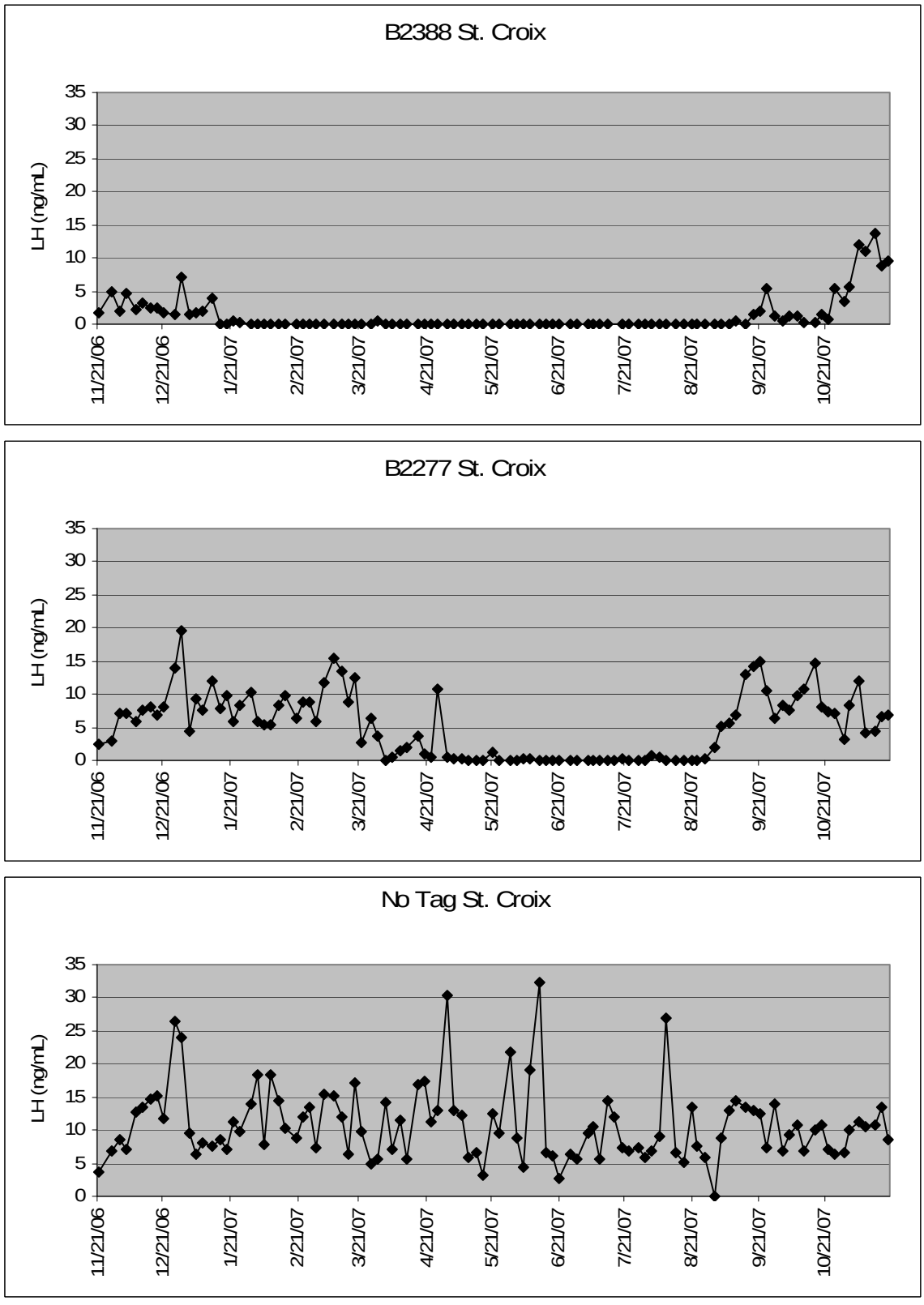


Figure 4-5. Luteinizing hormone profiles from individual OOS ewes representing the longest, average, and shortest durations of luteinizing hormone inhibition.

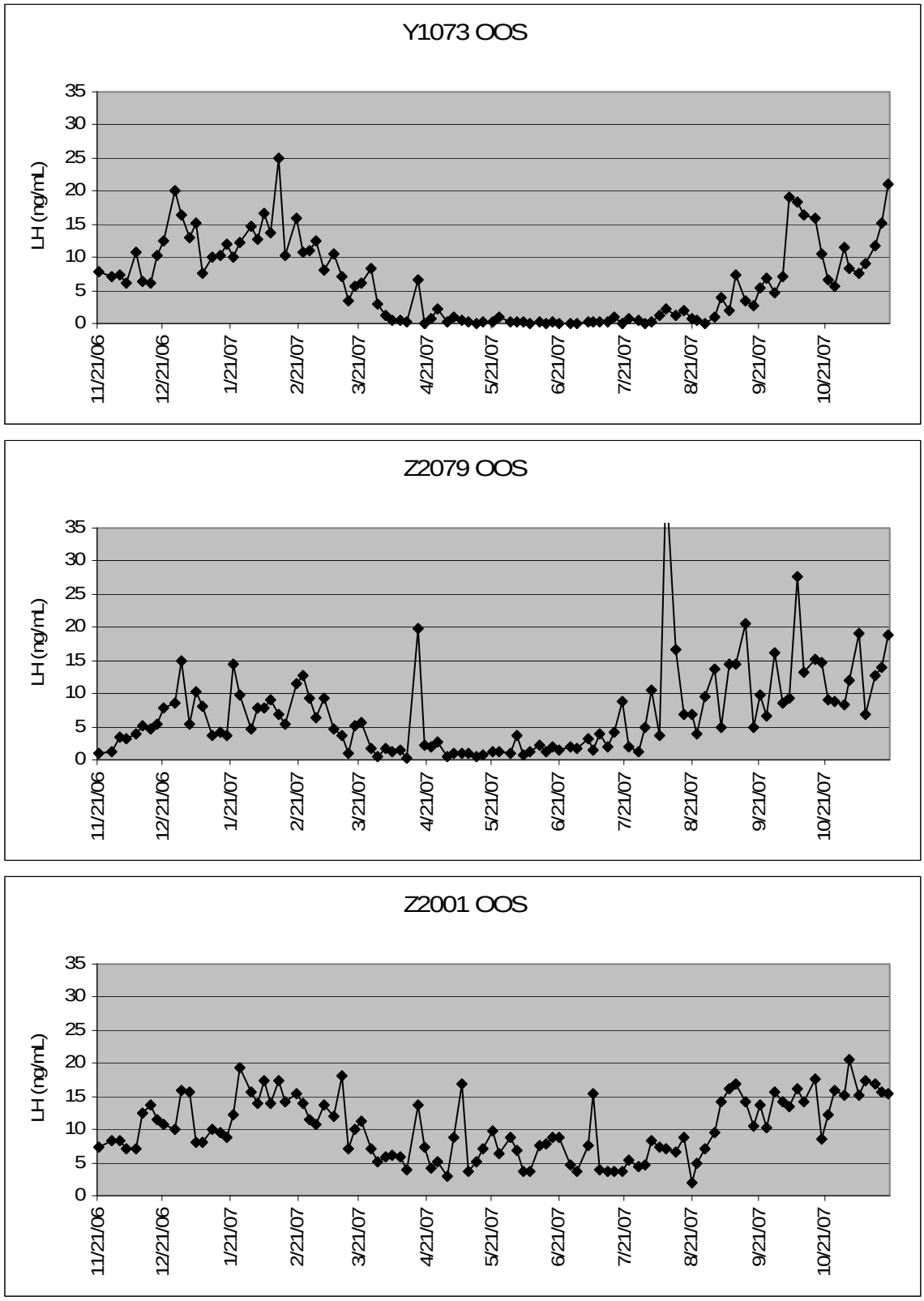


Figure 4-6. Luteinizing hormone profiles from individual Suffolk ewes representing the longest, average, and shortest durations of luteinizing hormone inhibition.

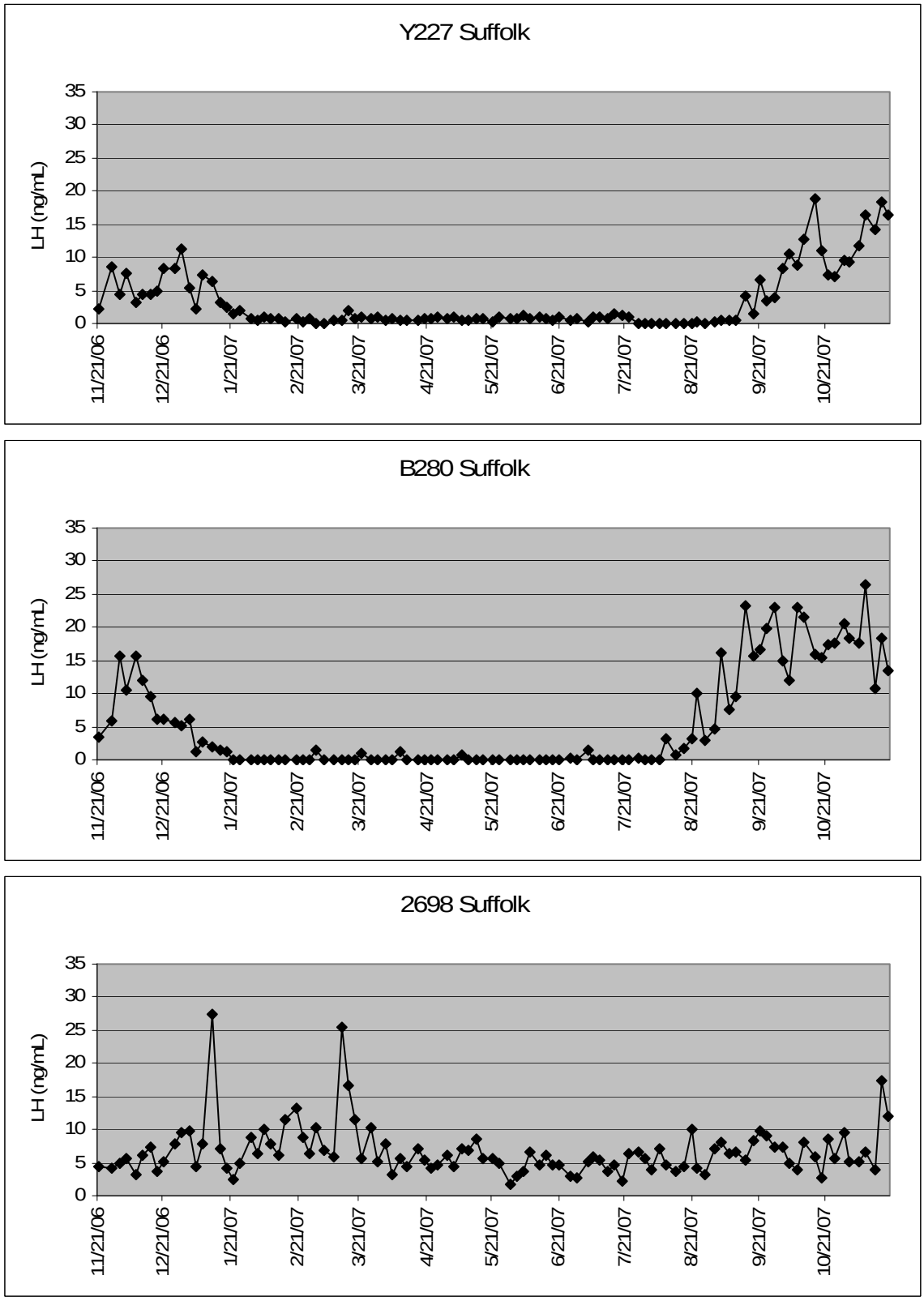


Figure 4-7. Mean day of the year anestrus started and stopped and duration of anestrus.
[ab] Breeds differ $P < 0.002$.
[cd] Breeds differ $P < 0.004$.
[ef] Breeds differ $P < 0.0001$.

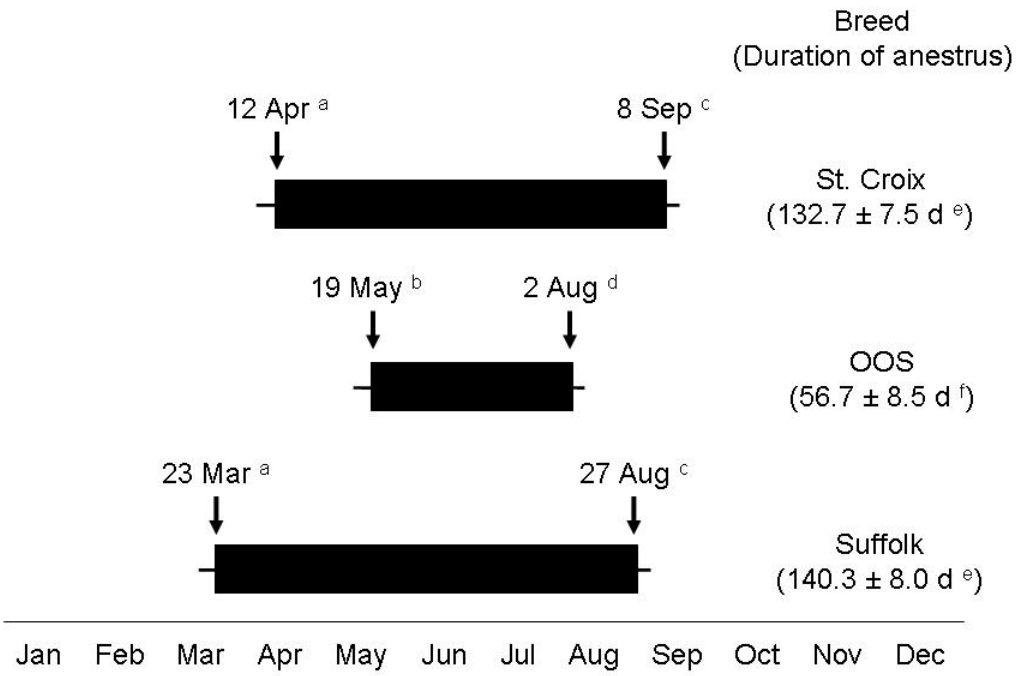


Figure 4-8. Mean duration of luteinizing hormone inhibition in days.
[ab] Bars with different superscripts differ $P < 0.02$.

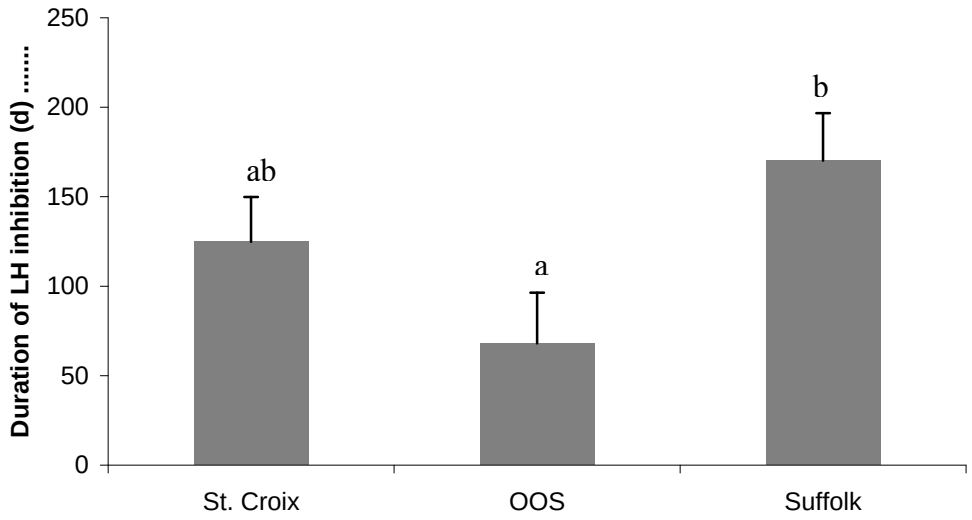


Table 4-1. Means and standard errors of $T_4$ concentrations on each of the 4 sampling dates given as ng/mL.

| | 12/18/06 | 4/5/07 | 6/21/07 | 9/18/07 |
|---|---|---|---|---|
| St. Croix | $62.48 \pm 2.60^{a}$ | $74.35 \pm 3.09$ | $66.95 \pm 2.47$ | $59.62 \pm 2.99^{c}$ |
| OOS | $54.60 \pm 2.60^{b}$ | $67.16 \pm 3.19$ | $68.92 \pm 2.55$ | $57.05 \pm 3.19^{c}$ |
| Suffolk | $50.93 \pm 2.60^{b}$ | $65.84 \pm 3.19$ | $62.09 \pm 2.55$ | $45.19 \pm 3.17^{d}$ |

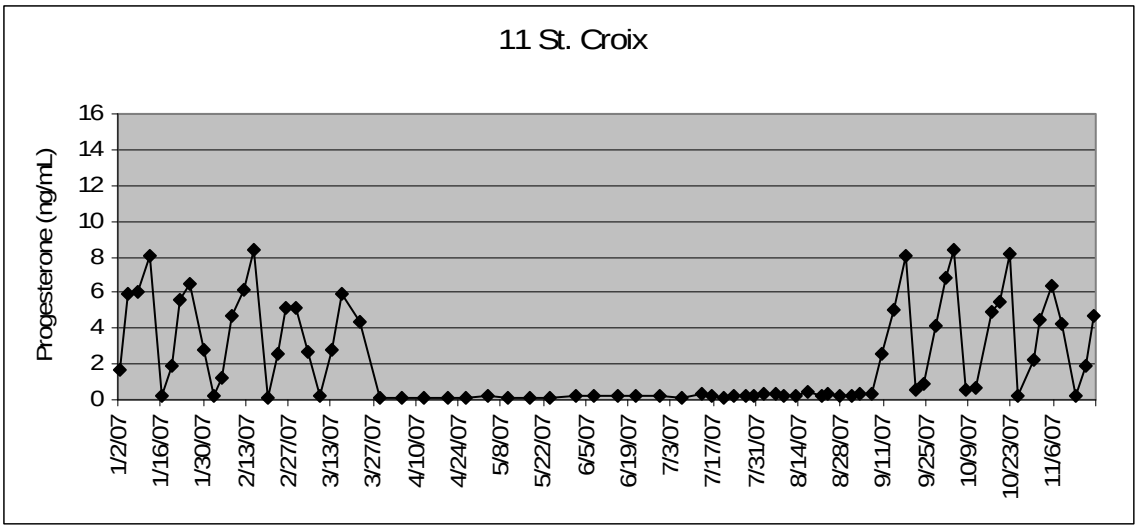
[ab] Differing superscripts indicate significant differences within column, $P < 0.04$.
[cd] Differing superscripts indicate significant differences within column, $P < 0.02$.

Table 4-2. Means and standard errors of PRL concentrations on each of the 4 sampling dates given as ng/mL.

| | 12/18/06 | 4/5/07 | 6/21/07 | 9/18/07 |
|---|---|---|---|---|
| St. Croix | $4.49 \pm 0.86^{a}$ | $11.72 \pm 2.68^{c}$ | $38.33 \pm 8.89^{e}$ | $4.93 \pm 0.92$ |
| OOS | $1.39 \pm 0.86^{b}$ | $23.68 \pm 2.76^{d}$ | $61.08 \pm 9.16^{e}$ | $2.08 \pm 0.98$ |
| Suffolk | $0.48 \pm 0.86^{b}$ | $13.74 \pm 2.76^{c}$ | $88.88 \pm 9.16^{f}$ | $3.20 \pm 0.97$ |

[ab] Differing superscripts indicate significant differences within column, $P < 0.015$.
[cd] Differing superscripts indicate significant differences within column, $P < 0.015$.
[ef] Differing superscripts indicate significant differences within column, $P < 0.05$.

# APPENDIX A


11 St. Croix
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
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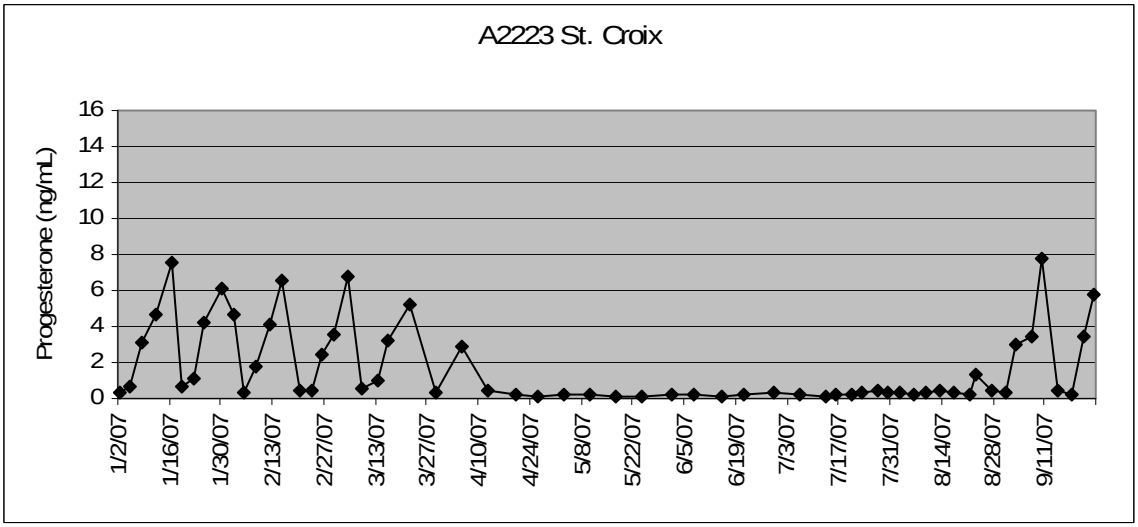
9/25/07
10/9/07
10/23/07
11/6/07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Progesterone (ng/mL)
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14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
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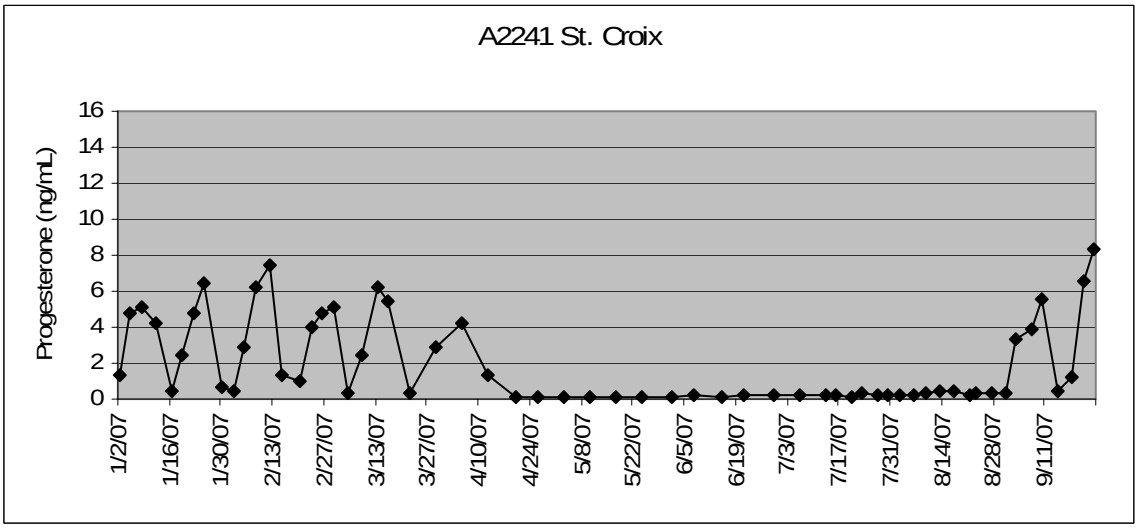
7/31/07
8/14/07
8/28/07
9/11/07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Progesterone (ng/mL)
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14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
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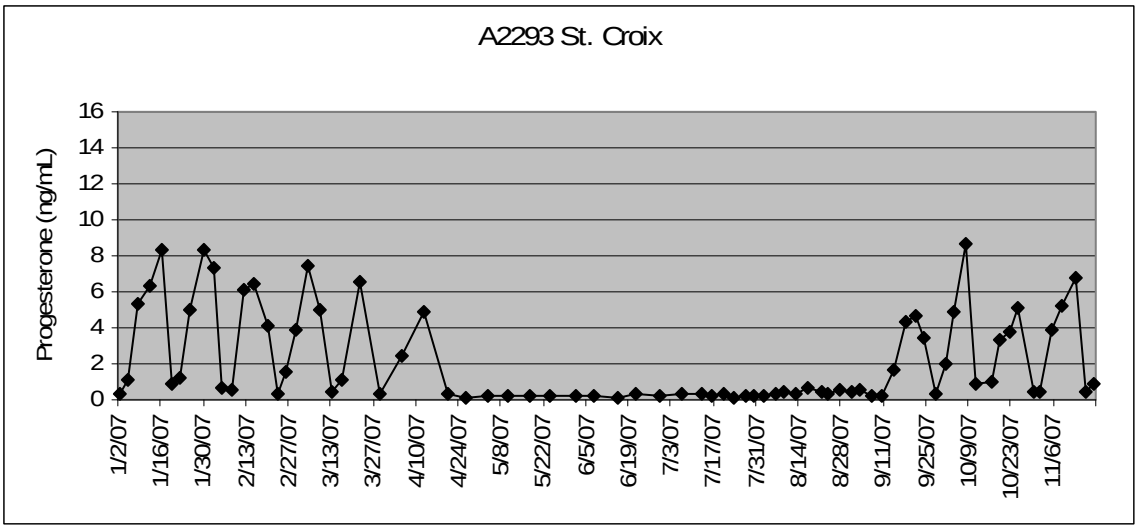
7/31/07
8/14/07
8/28/07
9/11/07


A2293 St. Croix
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
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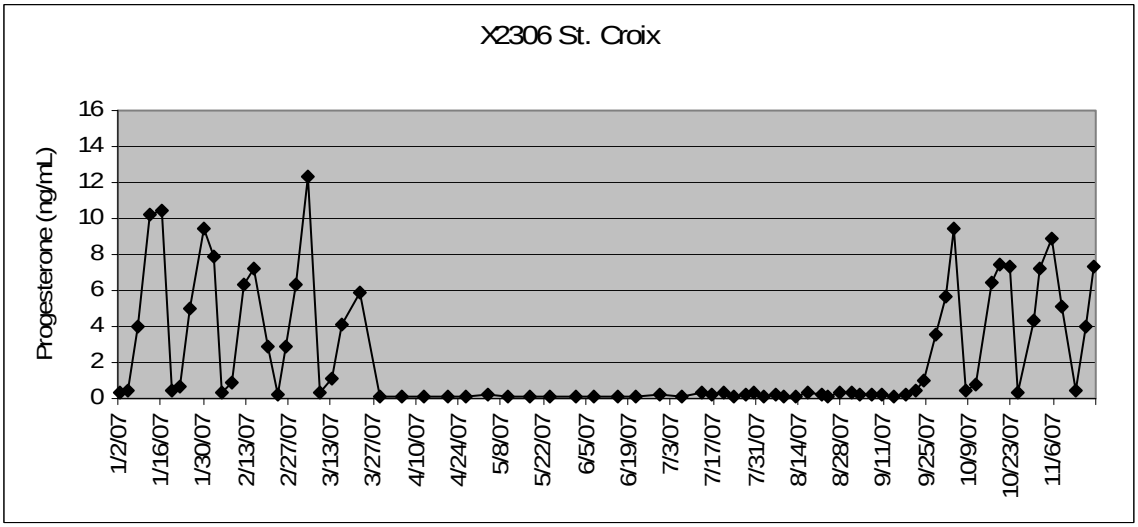
9/25/07
10/9/07
10/23/07
11/6/07


X2306 St. Croix
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
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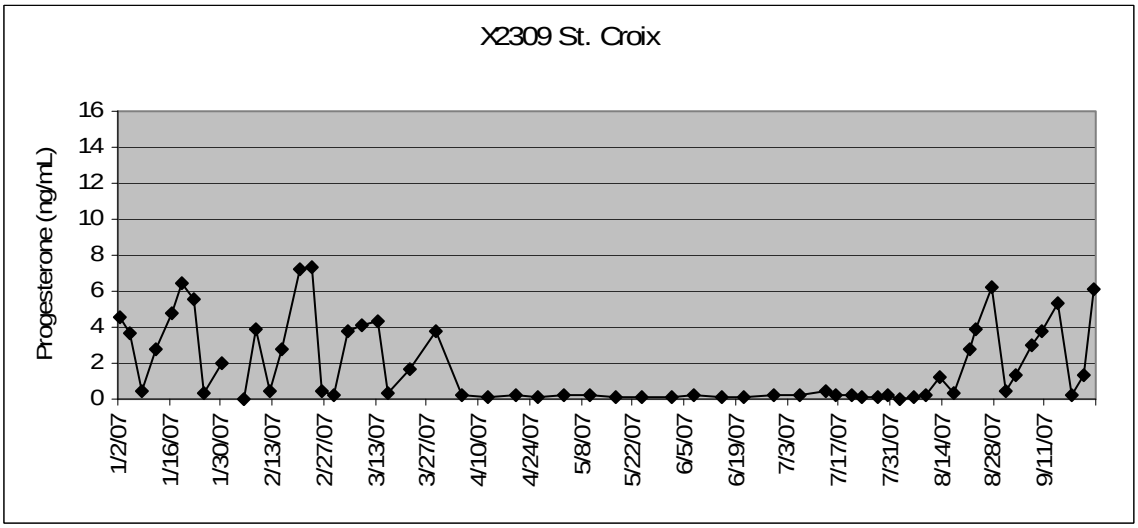
9/25/07
10/9/07
10/23/07
11/6/07


X2309 St. Croix
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
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2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
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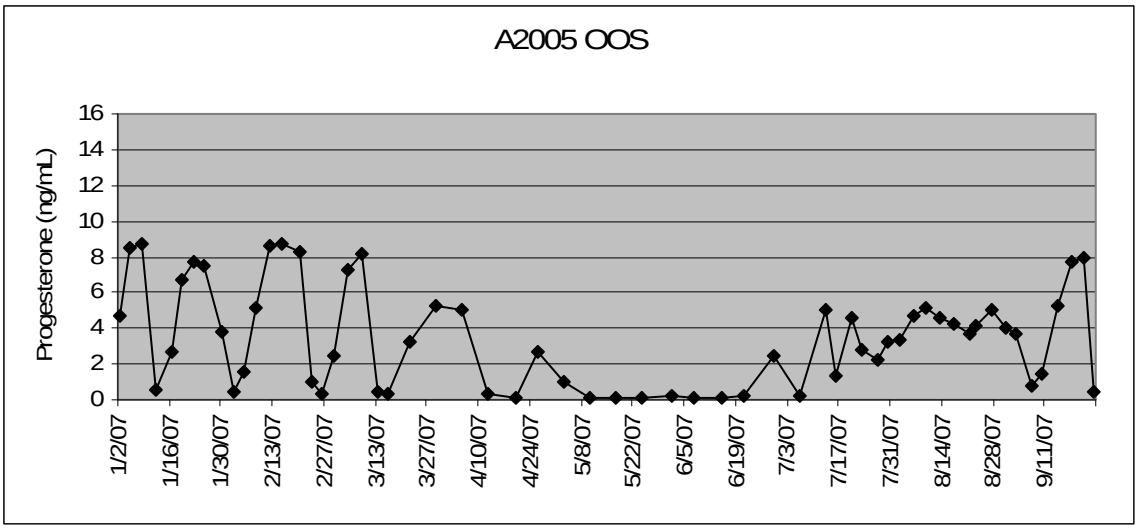
7/31/07
8/14/07
8/28/07
9/11/07


A2005 OOS
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
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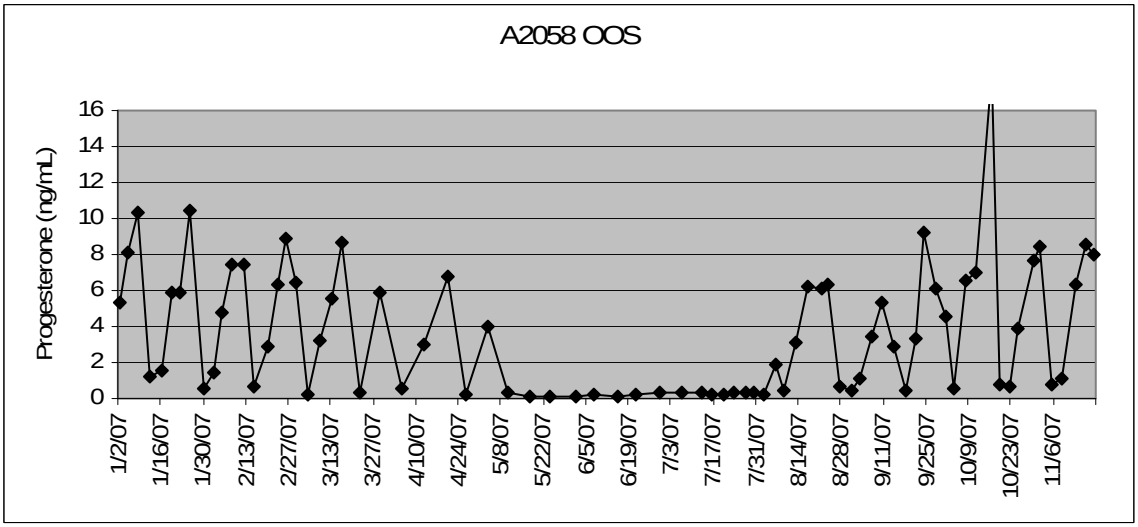
7/31/07
8/14/07
8/28/07
9/11/07


A2058 OOS
Progesterone (ng/mL)
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14
12
10
8
6
4
2
0
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1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
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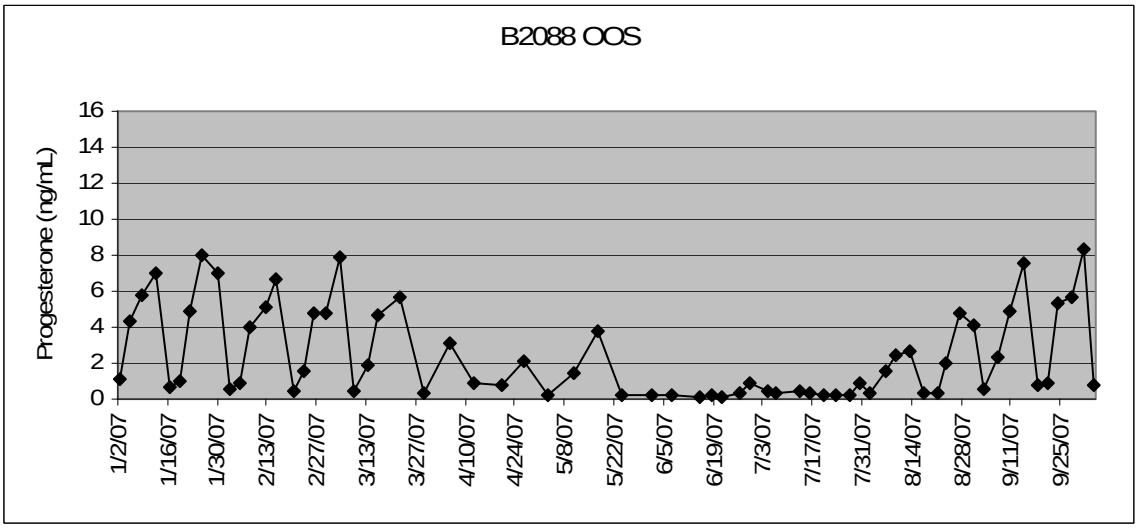
10/9/07
10/23/07
11/6/07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Progesterone (ng/mL)
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10
8
6
4
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1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
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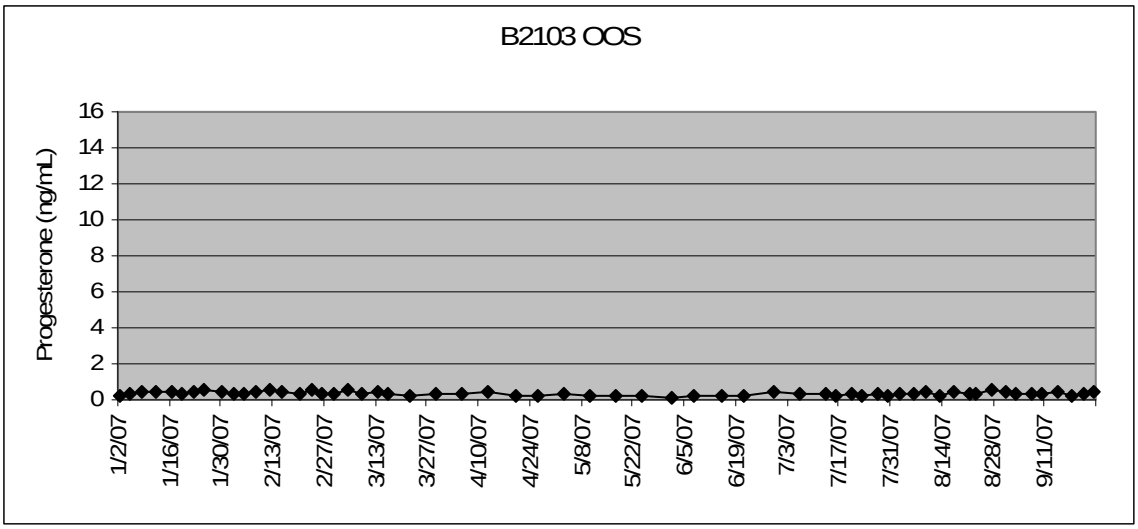
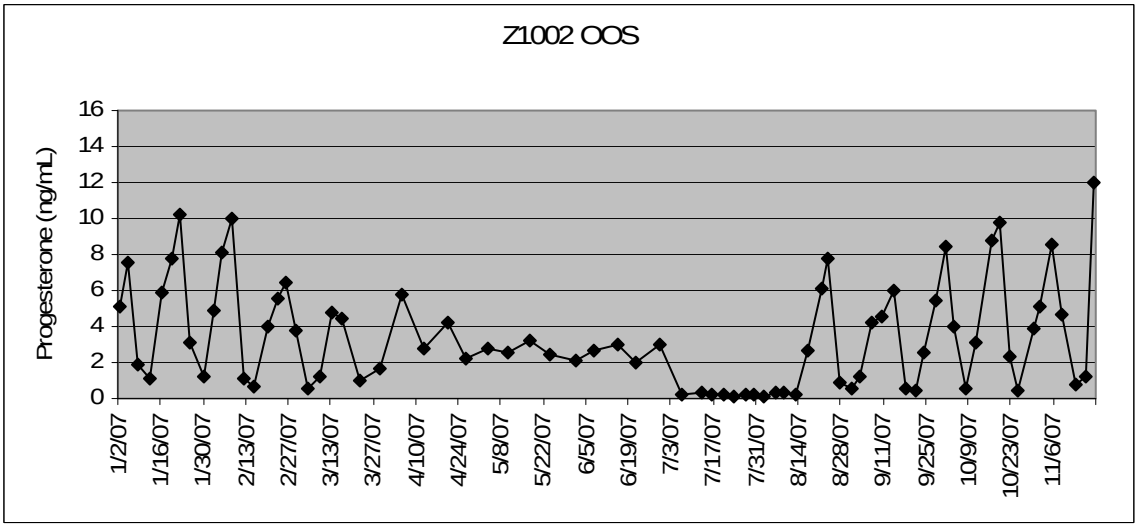
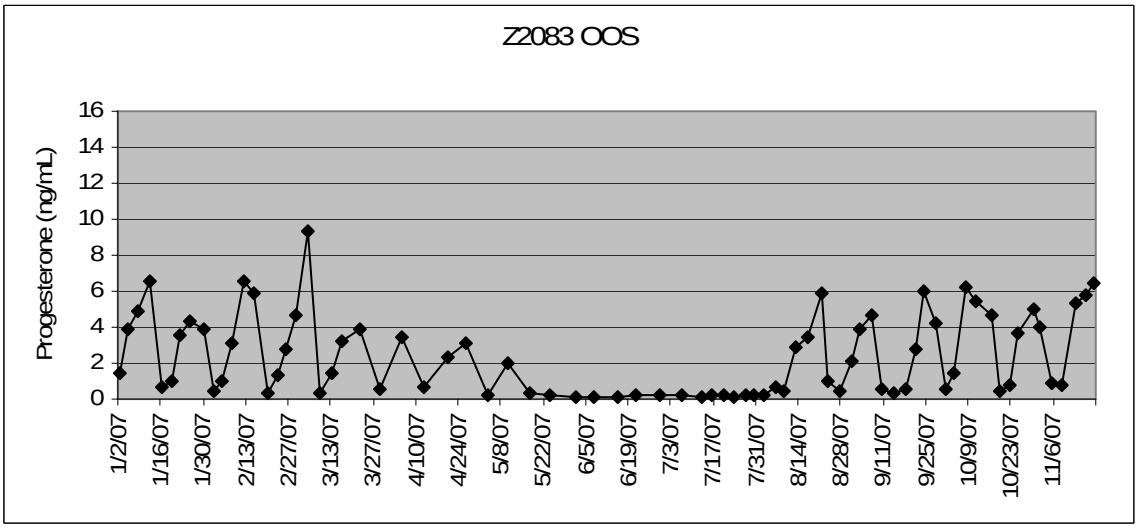
8/28/07
9/11/07
9/25/07


B2103 OOS
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
7/17/07
7/31/07
8/14/07
8/28/07
9/11/07


Z1002 OOS
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
7/17/07
7/31/07
8/14/07
8/28/07
9/11/07
9/25/07
10/9/07
10/23/07
11/6/07


Z2083 OOS
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
7/17/07
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8/14/07
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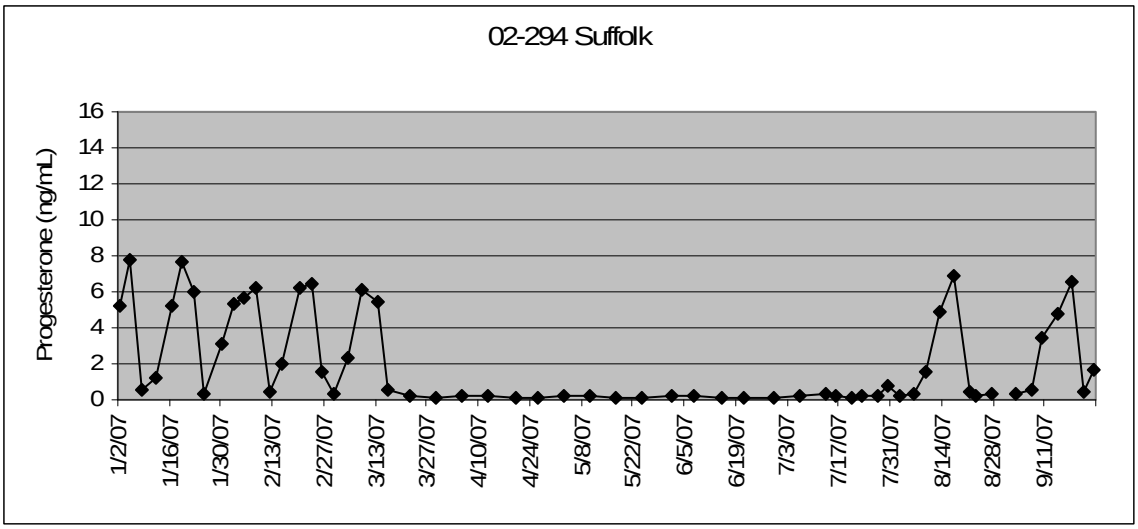
9/25/07
10/9/07
10/23/07
11/6/07


02-294 Suffolk
Progesterone (ng/mL)
0
2
4
6
8
10
12
14
16
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
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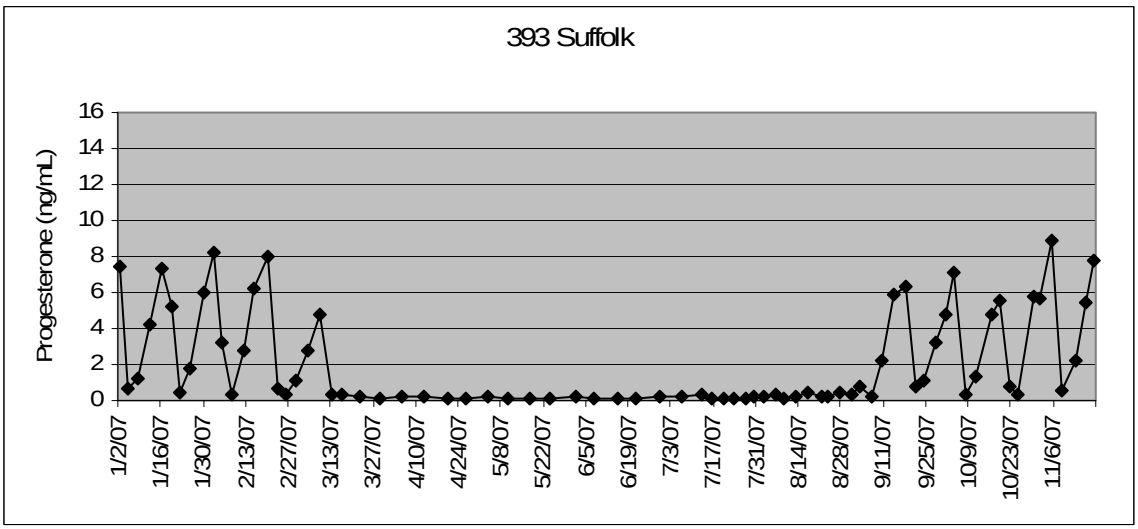
7/31/07
8/14/07
8/28/07
9/11/07


393 Suffolk
Progesterone (ng/mL)
0
2
4
6
8
10
12
14
16
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
7/17/07
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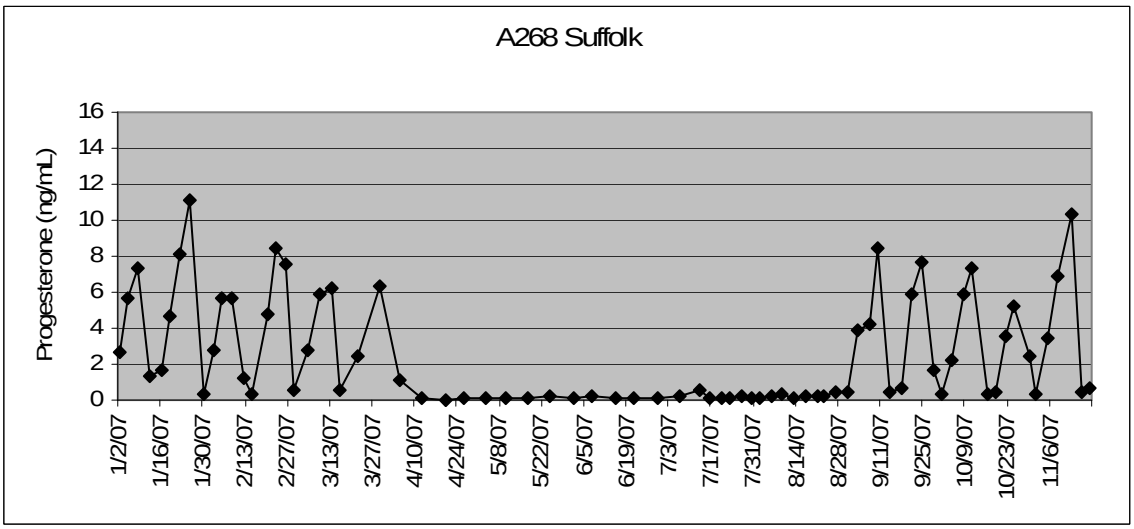
9/25/07
10/9/07
10/23/07
11/6/07


A268 Suffolk
Progesterone (ng/mL)
0
2
4
6
8
10
12
14
16
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
7/17/07
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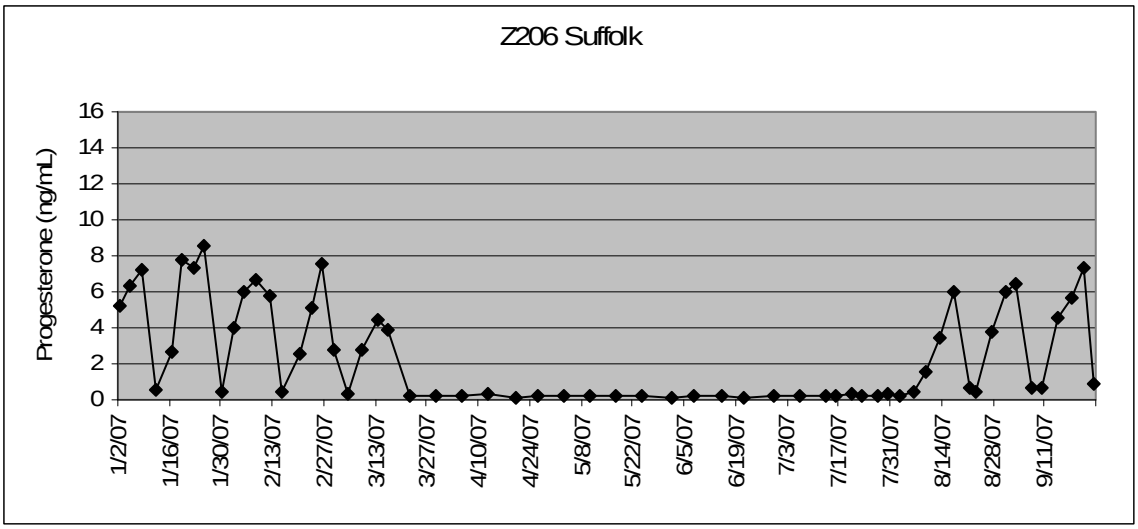
9/11/07
9/25/07
10/9/07
10/23/07
11/6/07


Z206 Suffolk
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
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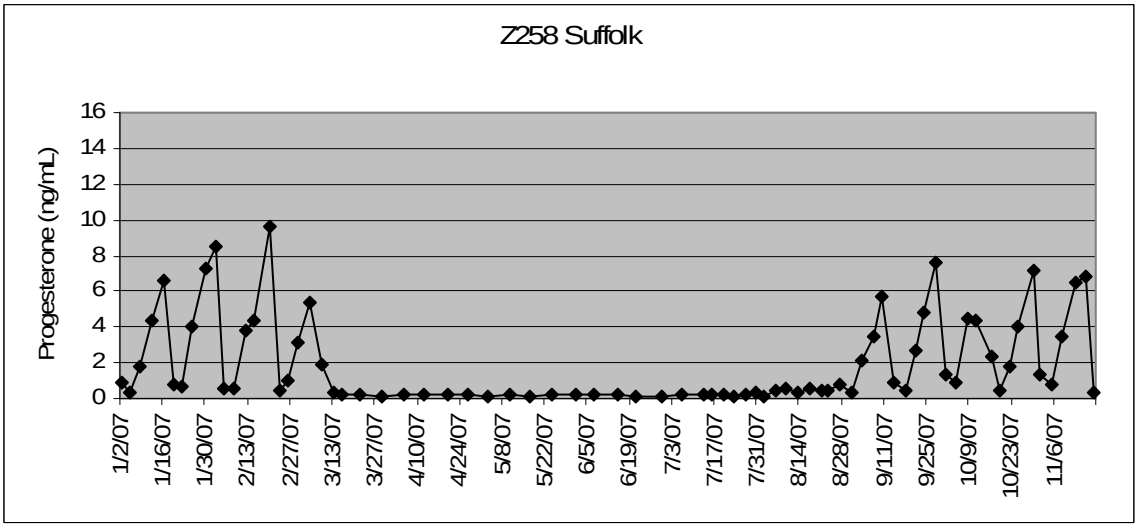
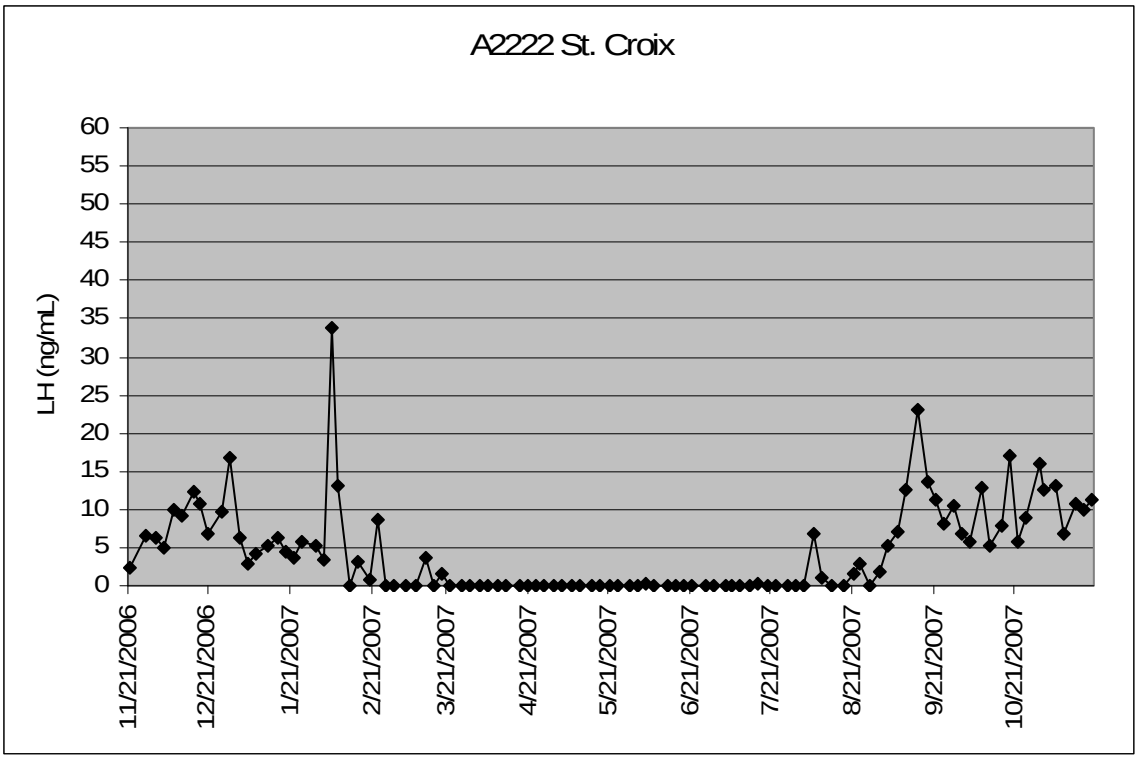
7/31/07
8/14/07
8/28/07
9/11/07


Z258 Suffolk
Progesterone (ng/mL)
16
14
12
10
8
6
4
2
0
1/2/07
1/16/07
1/30/07
2/13/07
2/27/07
3/13/07
3/27/07
4/10/07
4/24/07
5/8/07
5/22/07
6/5/07
6/19/07
7/3/07
7/17/07
7/31/07
8/14/07
8/28/07
9/11/07
9/25/07
10/9/07
10/23/07
11/6/07

# APPENDIX B


A2222 St. Croix
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/2006
12/21/2006
1/21/2007
2/21/2007
3/21/2007
4/21/2007
5/21/2007
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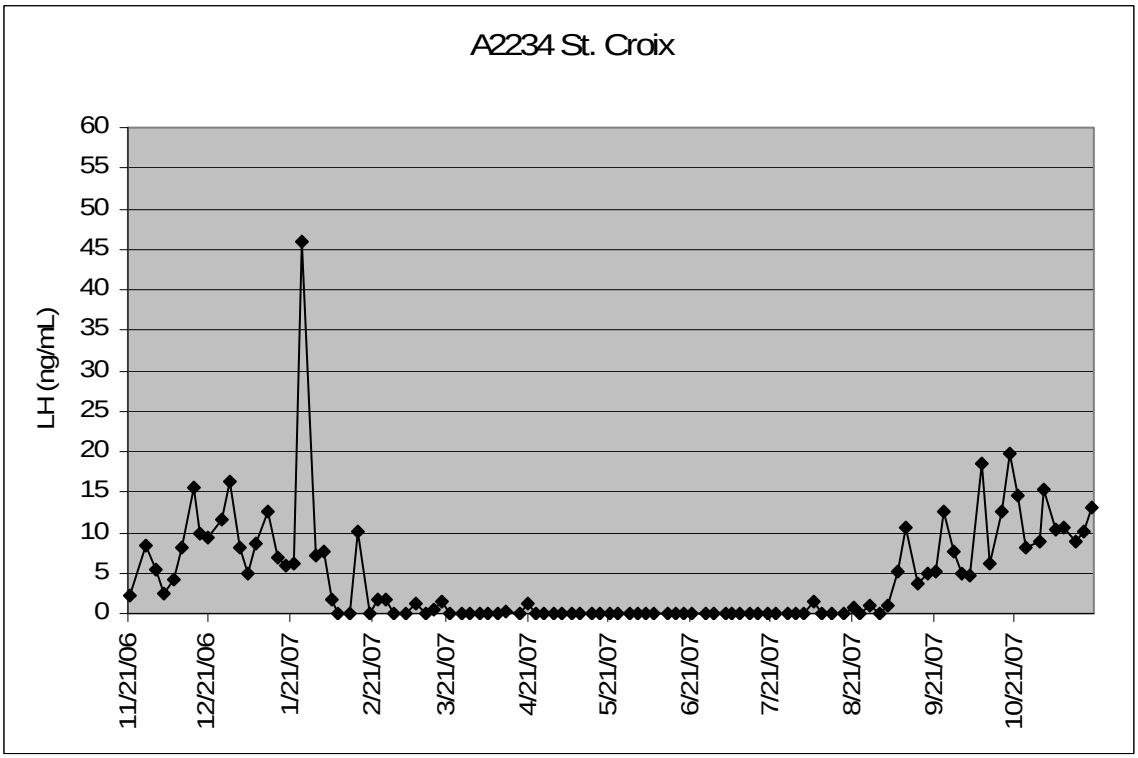
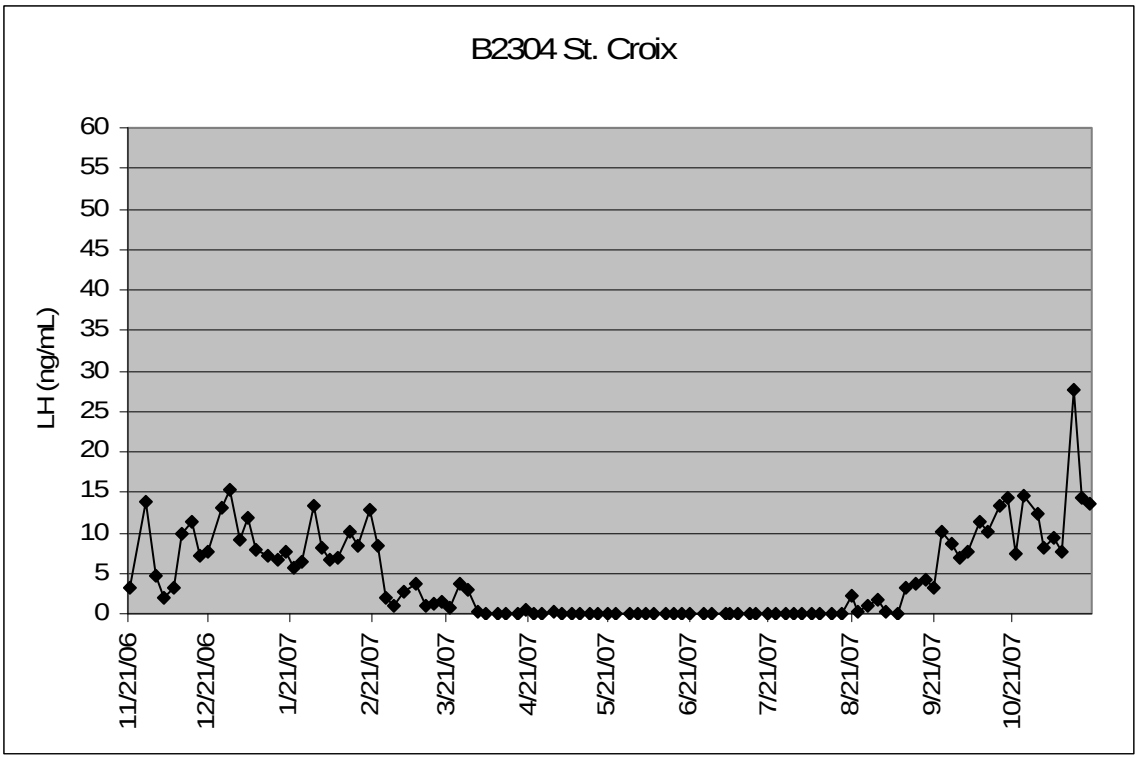
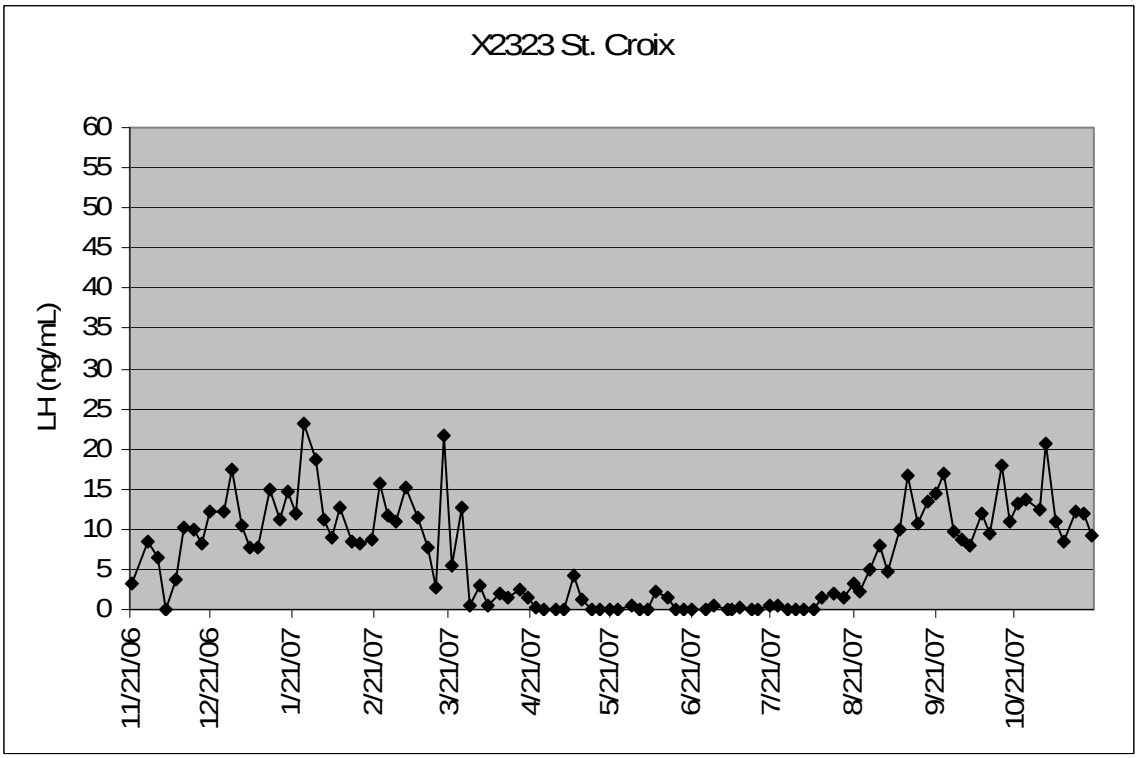
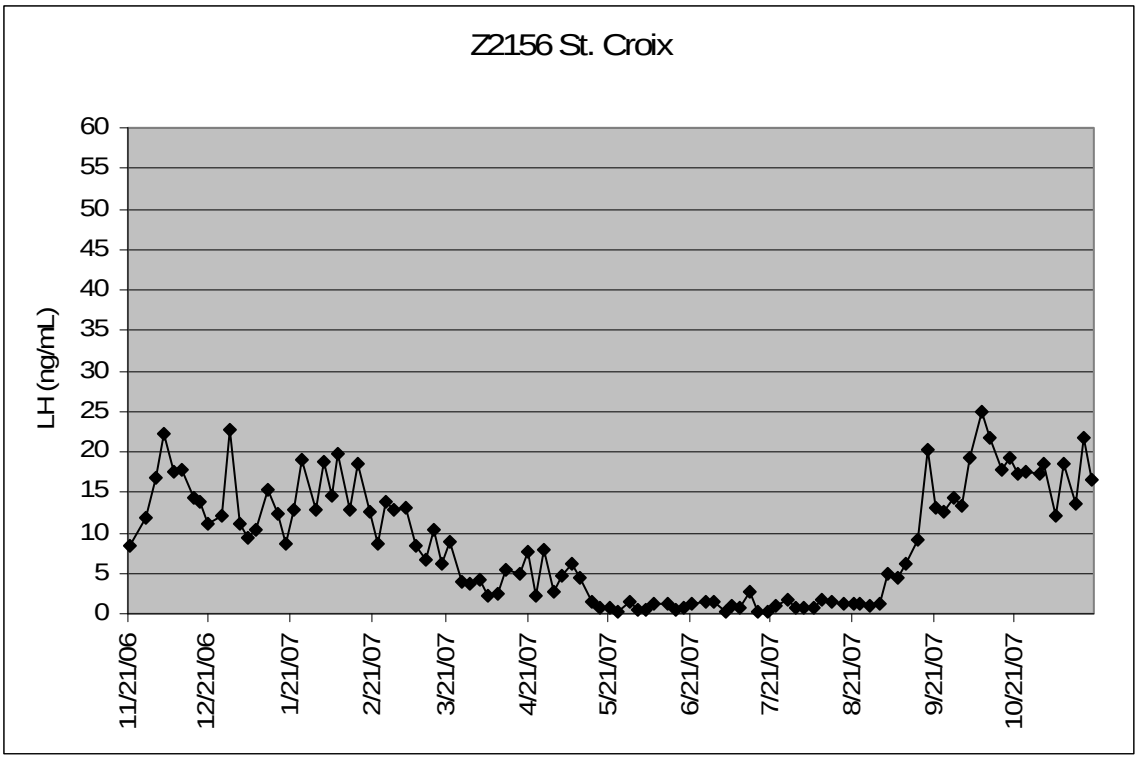
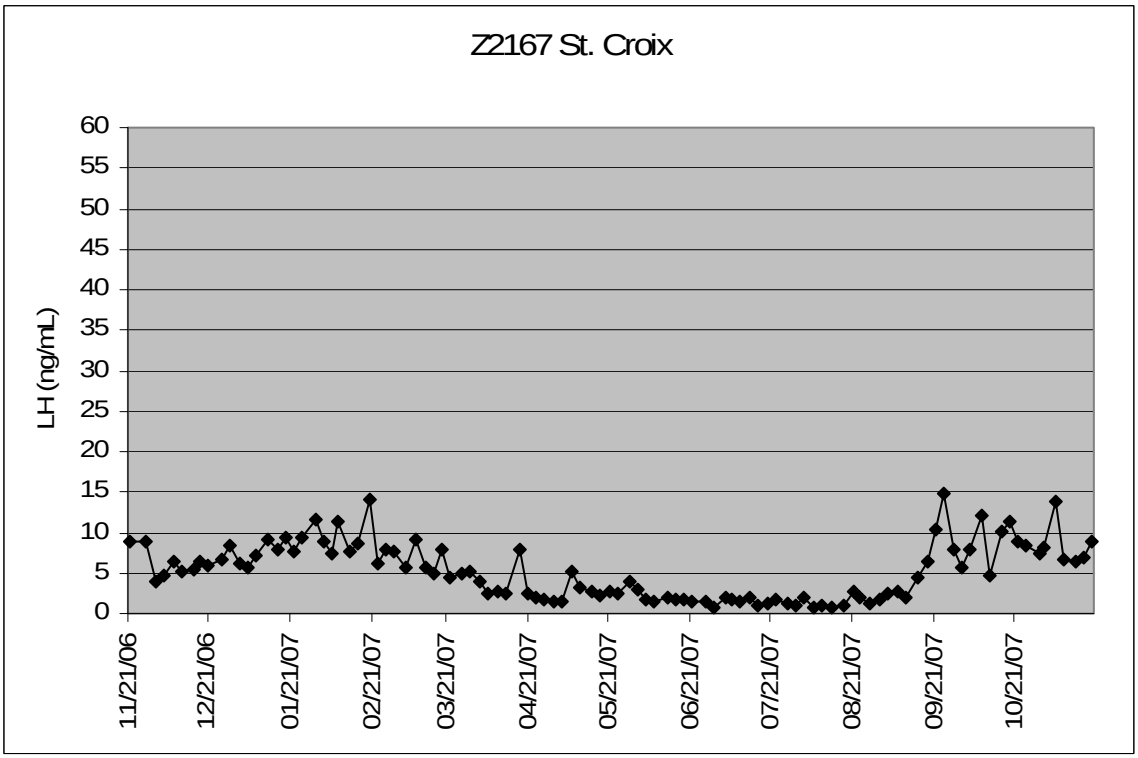
7/21/2007
8/21/2007
9/21/2007
10/21/2007

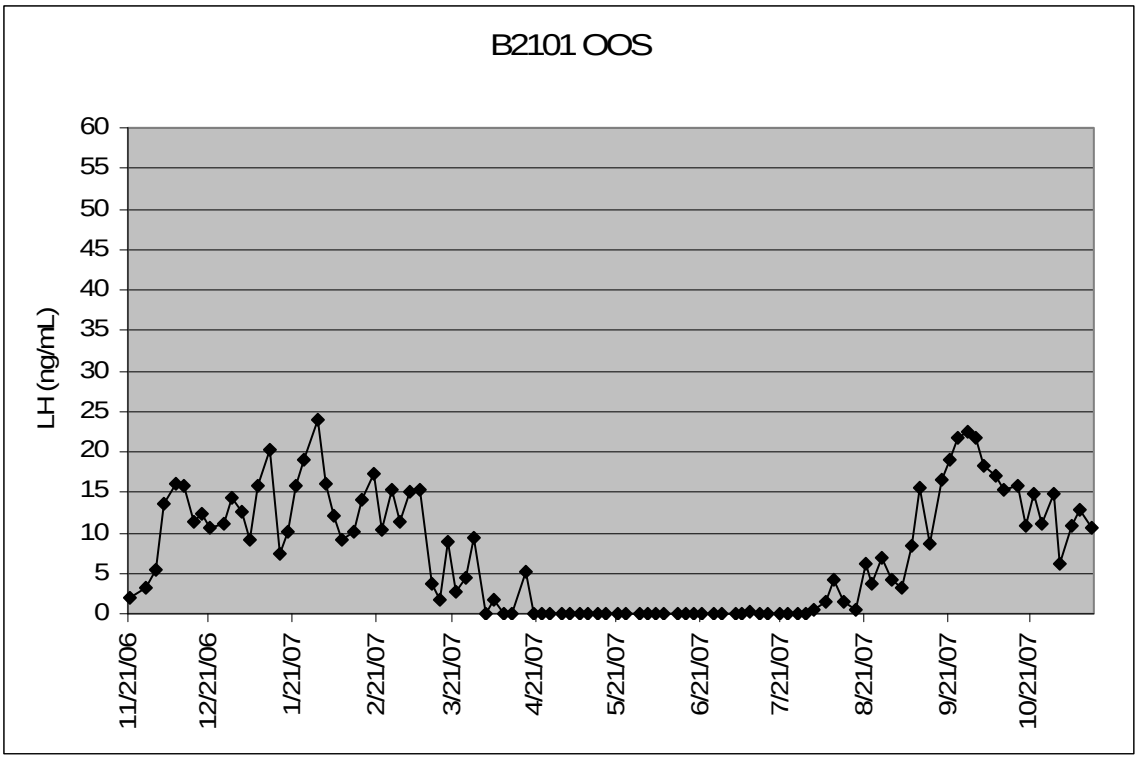

A2234 St. Croix
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

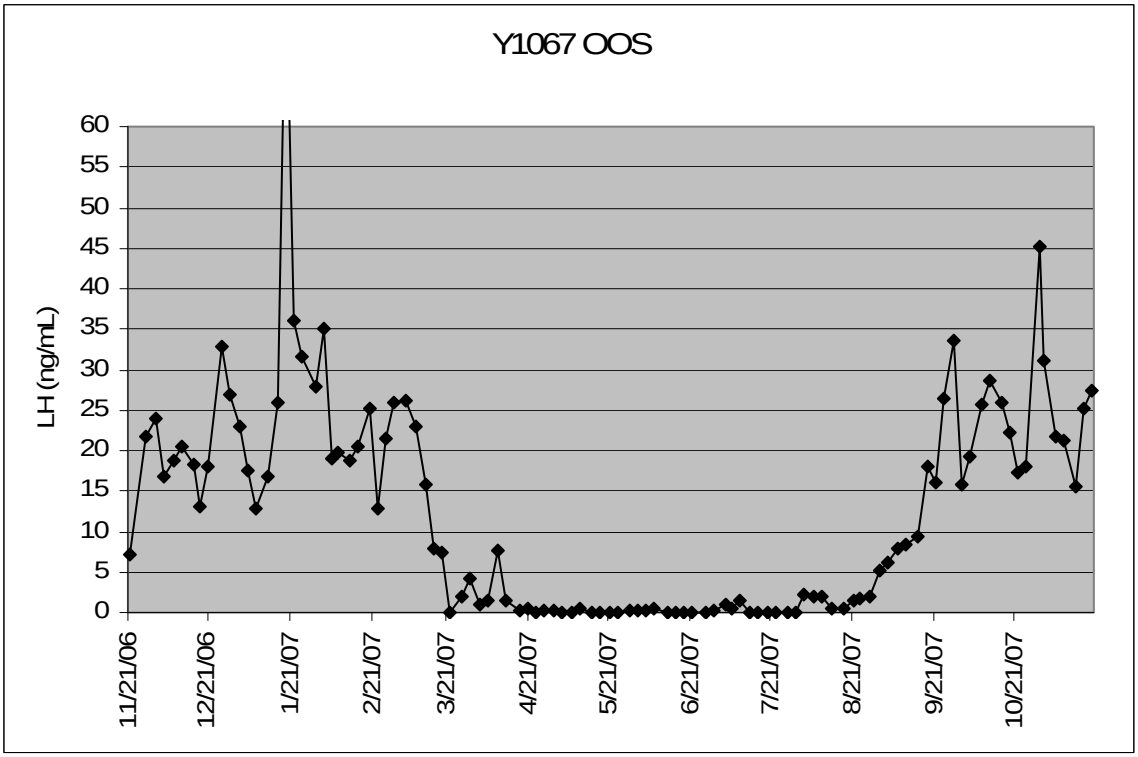

B2304 St. Croix
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

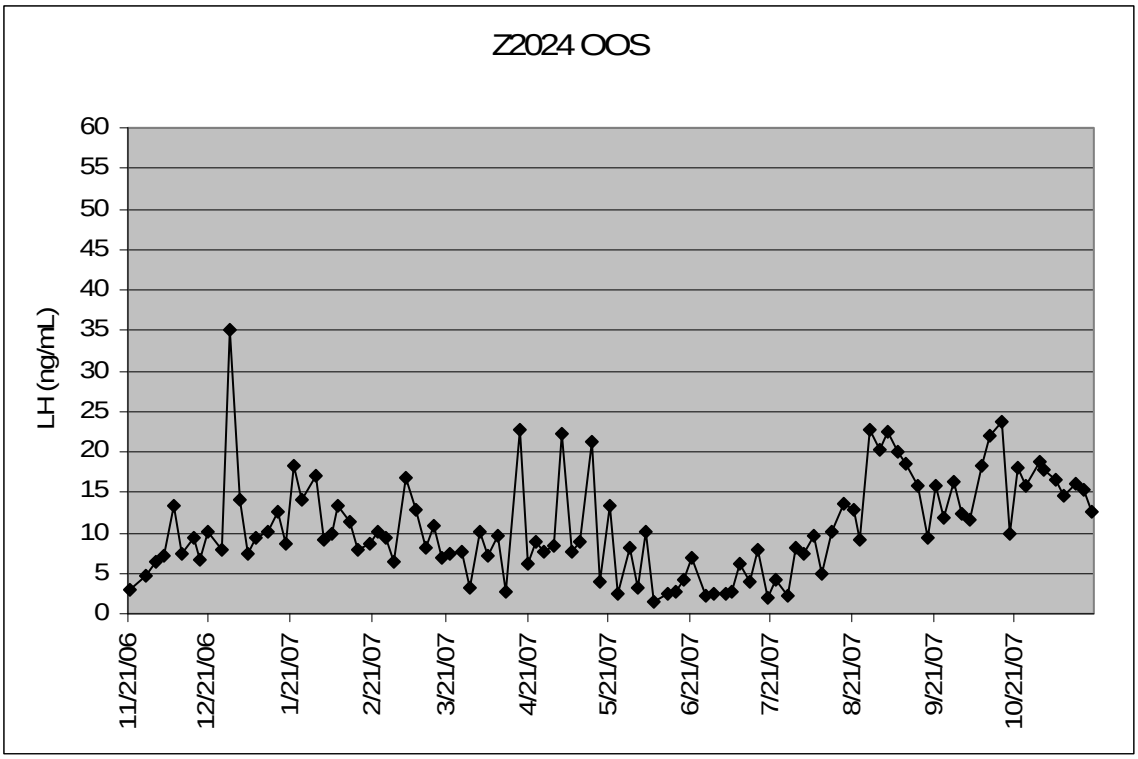

X2323 St. Croix
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

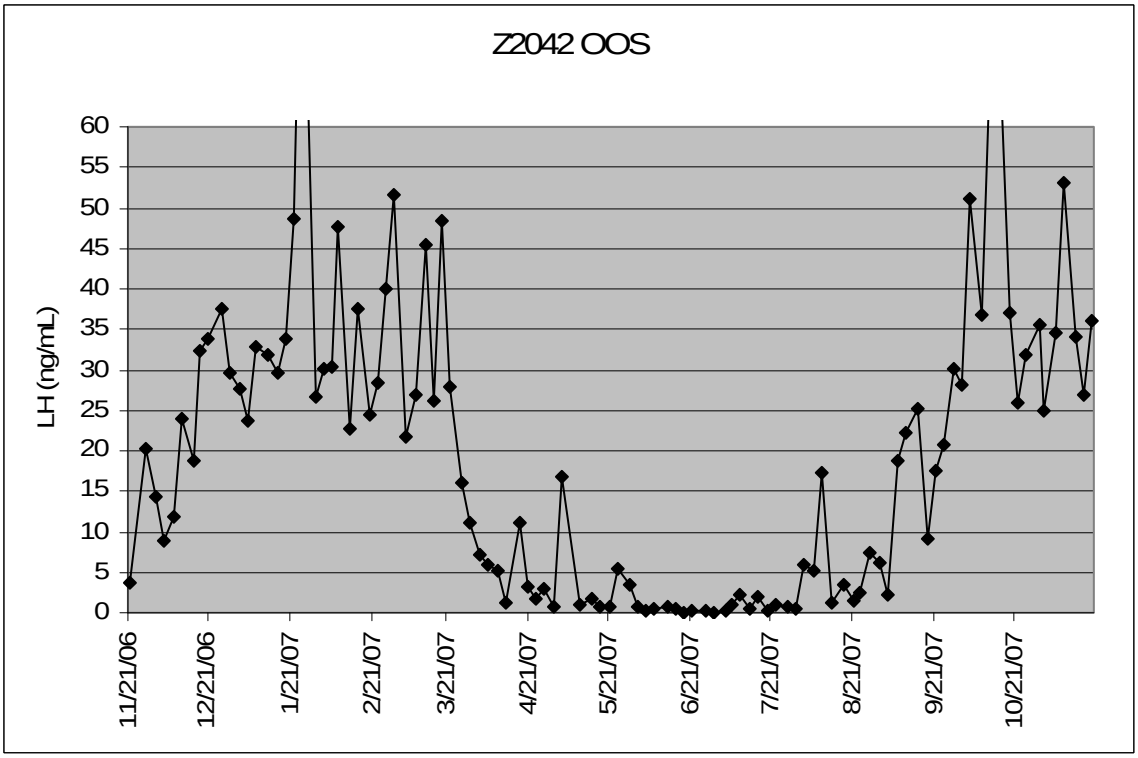

Z2156 St. Croix
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

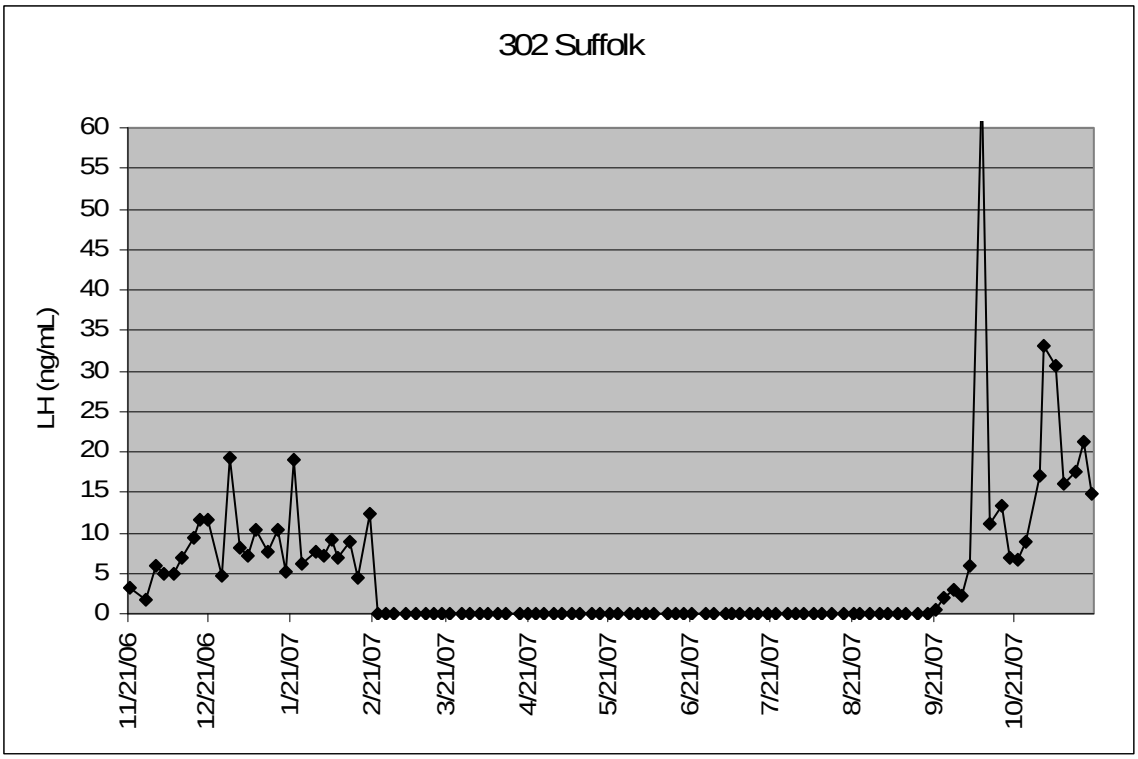

Z2167 St. Croix
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
01/21/07
02/21/07
03/21/07
04/21/07
05/21/07
06/21/07
07/21/07
08/21/07
09/21/07
10/21/07

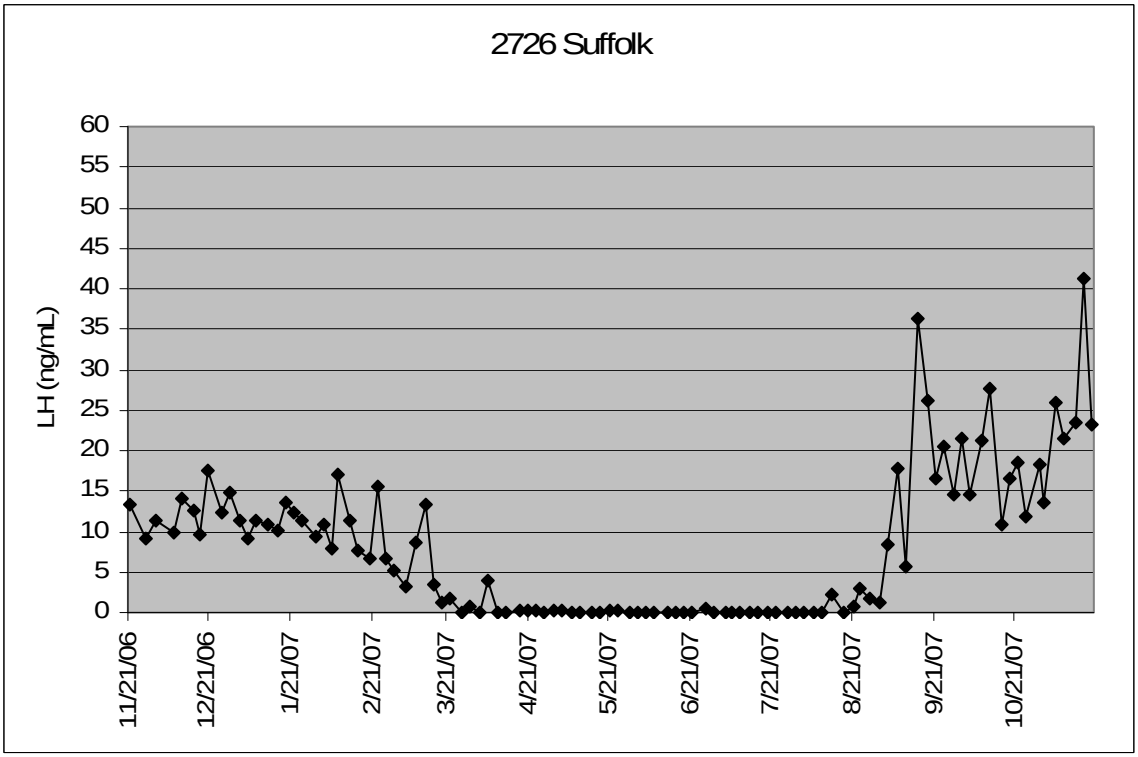

B2101 OOS
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

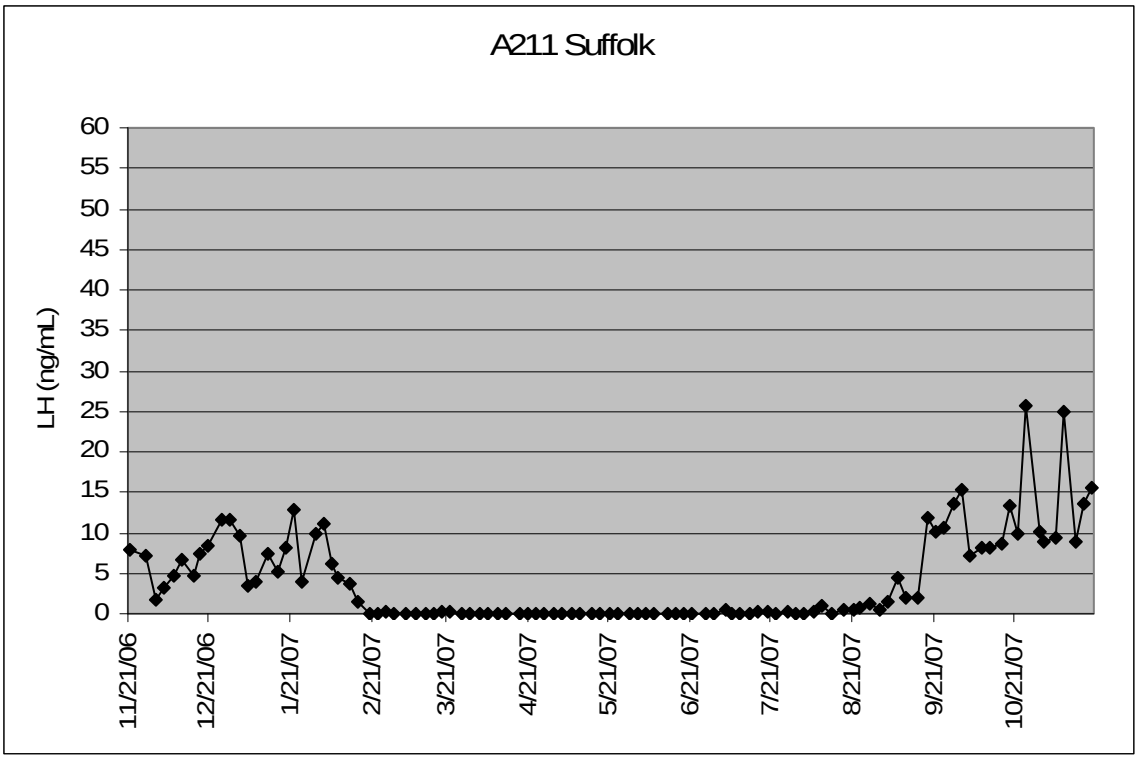

Y1067 OOS
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

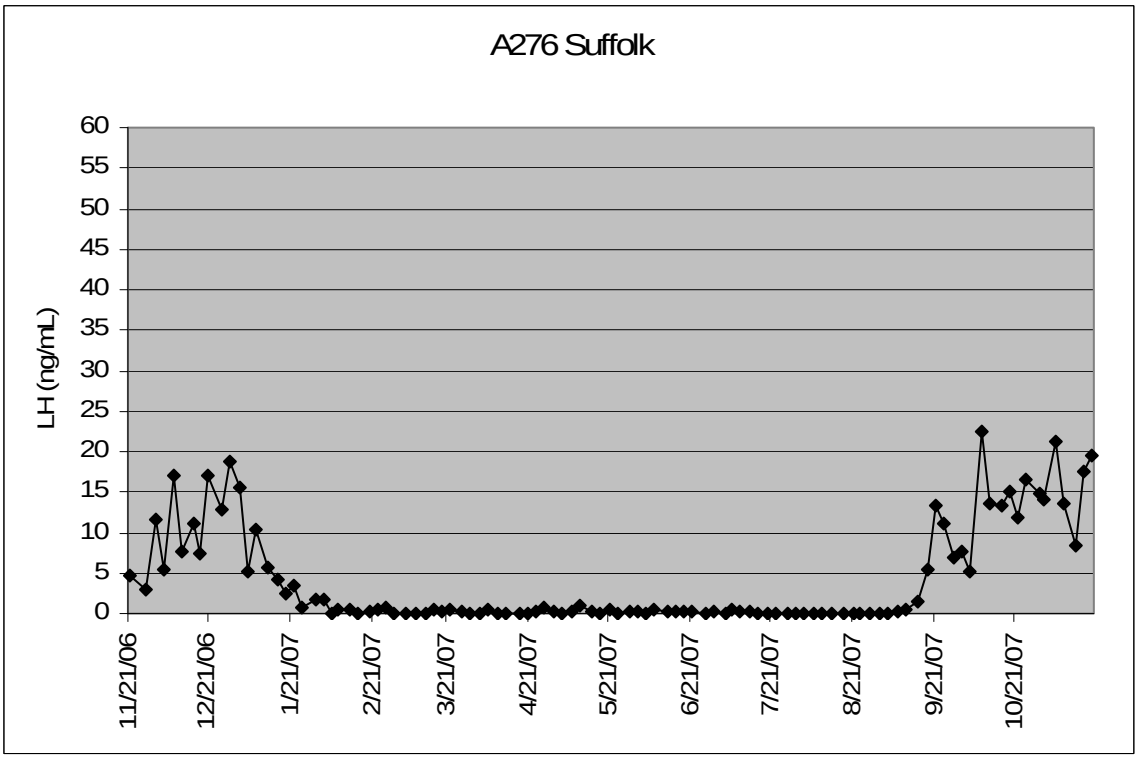

Z2024 OOS
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

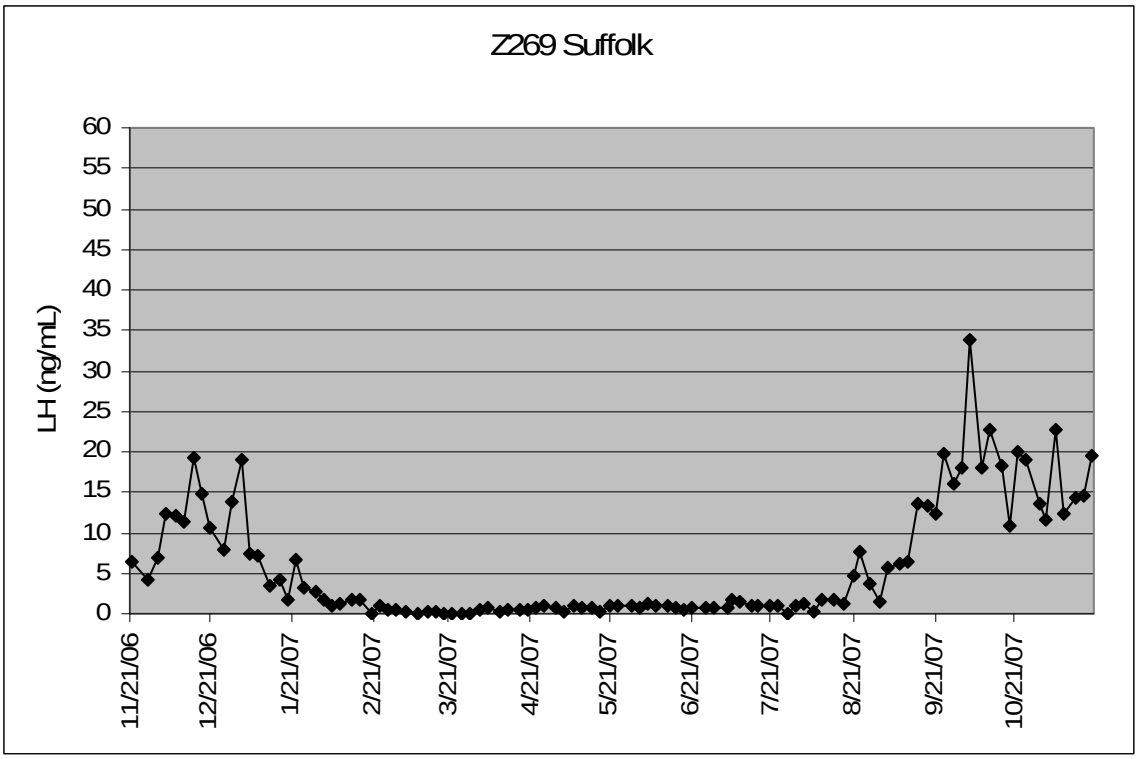

Z2042 OOS
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


302 Suffolk
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


2726 Suffolk
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


A211 Suffolk
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


A276 Suffolk
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


Z269 Suffolk
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
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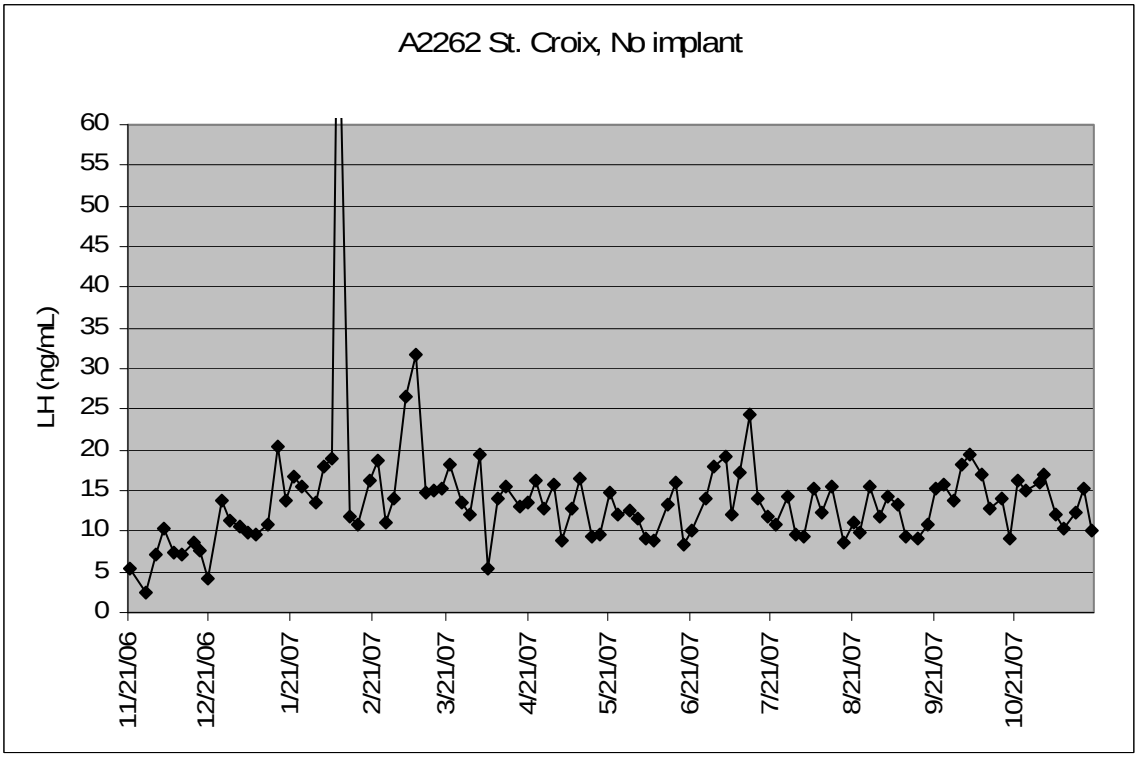
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


A2262 St. Croix, No implant
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
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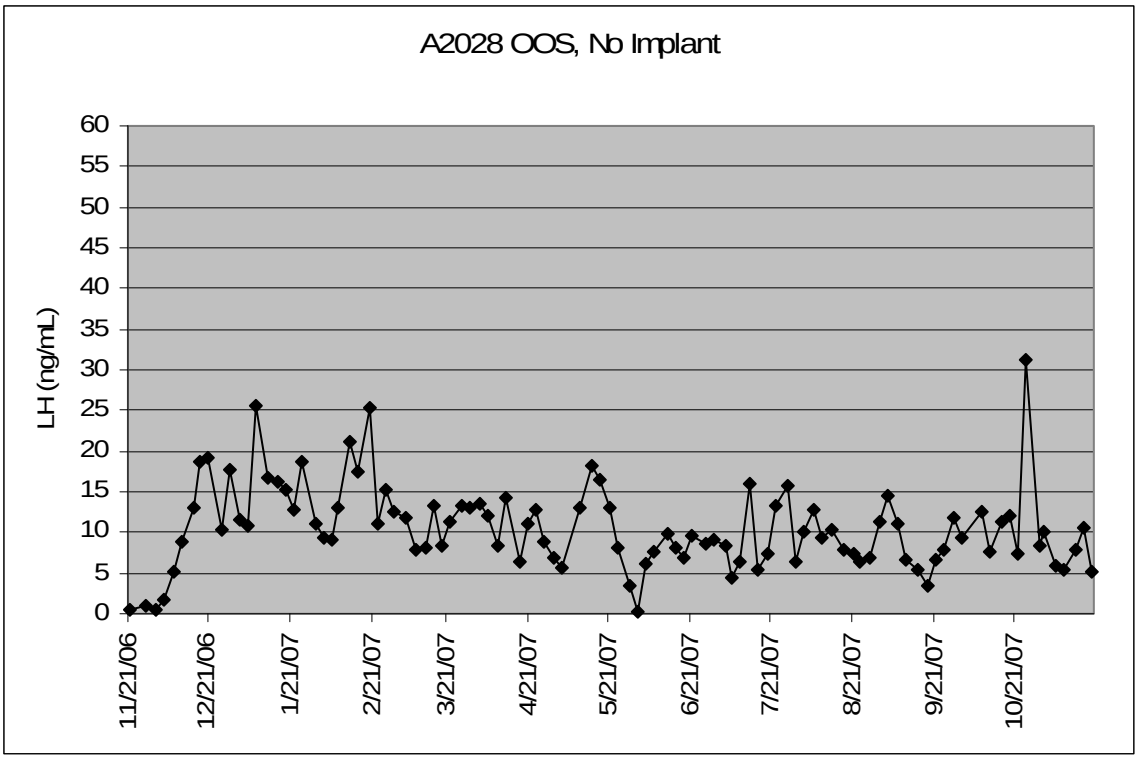
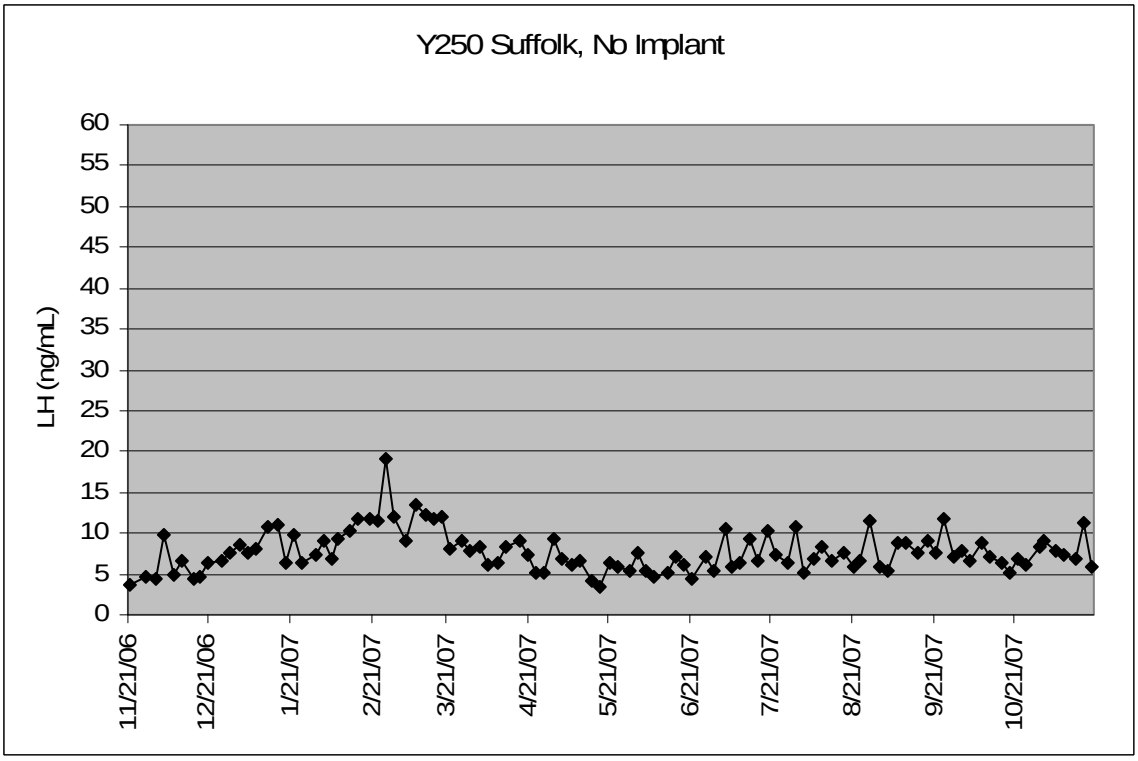
7/21/07
8/21/07
9/21/07
10/21/07


A2028 OOS, No Implant
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07


Y250 Suffolk, No Implant
LH (ng/mL)
60
55
50
45
40
35
30
25
20
15
10
5
0
11/21/06
12/21/06
1/21/07
2/21/07
3/21/07
4/21/07
5/21/07
6/21/07
7/21/07
8/21/07
9/21/07
10/21/07

## CHAPTER 5

### General discussion

Greater percentages of OOS than St. Croix ewes were marked by rams when exposed while lactating during April. Thus OOS ewes appeared to be able to reinitiate estrous behavior more quickly after lambing in winter than St. Croix ewes. However, the lack of differences between breeds in ewes diagnosed pregnant and lambing indicated that substantial numbers of OOS ewes were not able to establish or maintain pregnancies that resulted from these breedings. The marks may not have been associated with standing estrus, mating may have occurred but did not result in pregnancy, or the pregnancy may have been lost before pregnancy diagnosis occurred 90 d after introduction of rams. In contrast to findings that most pregnancy losses in sheep occur before d 18 of gestation, significant fetal loss appeared to have occurred between the time of pregnancy diagnosis and lambing in OOS ewes.

Even though significant reproductive losses occurred in OOS ewes that were mated while lactating in April, approximately 41% of OOS and St. Croix ewes lambed. This is impressive given that no labor intensive management procedures or costly pharmaceutical treatments were associated with this outcome. Given the success of the first study, the aim of the study described in Chapter 3 was to assess the possibility of reducing lambing interval even further in OOS ewes. The ewes in the second study were only 40 d postpartum at the time of ram introduction in May, which is thought to be the deepest part of anestrus in the Northern Hemisphere. A result of this study that was not anticipated was the increased incidence of embryonic and fetal loss that occurred in these ewes compared to ewes in the first study that were 62 d postpartum when rams were

introduced in April. Of all ewes exposed to rams in May, only 20% gave birth to viable lambs.

Taken together, the results from the first two studies suggest that OOS ewes were able to become pregnant while lactating during seasonal anestrus, but were often unable to carry the lambs to term when the time from lambing to conception was shortened. Both OOS and St. Croix ewes appeared to be well suited to accelerated lambing systems common to the U.S. sheep industry involving 7- to 8-mo lambing intervals. However, reduction of lambing intervals to 6- to 7-mo appeared to have detrimental effects on fetal survival in OOS ewes. Studies incorporating ultrasonographic imaging and endocrine profiling for the duration of gestation would be helpful in verifying rates and timing of financially detrimental reproductive losses in this unique line of sheep.

The changes in reproductive activity observed during seasonal anestrus are consequences of changes at the hypothalamic-pituitary-gonadal axis, specifically a decrease in the frequency of secretion of gonadotropin releasing hormone from the hypothalamus and a resultant decrease in secretion of luteinizing hormone from the pituitary. This decrease in frequency of secretion of gonadotropin releasing hormone is attributed to an increase in the sensitivity of the hypothalamus to the negative feedback effects of estradiol. In the third study, a common model involving ovariectomized ewes that had been administered estradiol implants was used in a novel way, to compare endocrine profiles of three selected breeds of ewes exposed to natural photoperiods and isolated from rams and each other for one year. The most surprising result of this study was that St. Croix ewes did not have shorter anestrous periods than wool breeds, as was previously thought. In fact, the results indicated that OOS ewes, had shorter durations of

ovulatory inactivity than St. Croix ewes. This was not mirrored in the durations of LH inhibition of these breeds suggesting that many factors confound the translation of neuroendocrine signals to reproductive responses. Neither $T_4$ nor PRL played a significant role in determining timing of seasonal reproduction in the ewe.

The comparison of ovariectomized estradiol-treated and intact ewes between breeds is a novel and useful model for investigating breed differences in endocrine responses to changing daylength. However, additional studies using this model are needed. Increasing the frequency of blood sampling to observe changes in luteinizing hormone pulse frequency, and including ewes that are in contact with rams in addition to ewes isolated from rams, as in the present study, will help elucidate how physiological and neuroendocrine responses to changing daylength manifest themselves in breeds that are reproductively seasonal to differing degrees.