EFFECTS OF BREED AND RAM EXPOSURE ON SPRING ESTROUS BEHAVIOR
AND SUMMER FERTILITY IN DOMESTIC EWES

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Unlike swine and cattle, the domestic sheep (<u>Ovus aries</u>) exhibits a seasonal breeding pattern whereby maximum breeding behavior and fertility occurs during the fall. Ewes generally begin cycling in late summer (July or August) and continue sexual behavior through mid-winter (January). During the spring most, if not all, ewes have an anestous period when ovulation is absent and no behavioral estrus occurs. Rams also show some seasonal fluctuation in libido and semen quality, but the overall seasonal effect is less drastic than with the female.

The inability of most ewes to cycle in the spring has limited the sheep producer to one crop of winter or spring born lambs per year. If genetic differences among ewes in their ability to breed out of season were identified and exploited, circumvention of the anestrous period could be possible through selection to produce a line of domestic ewes which show either a limited, short anestous or no anestrous period at all. This could in turn double the number of lambs produced per ewe per year.

The objective of the two studies described within this thesis was to determine the extent of breed differences in out of season breeding ability. One study dealt with Hampshire and Dorset purebred ewes and differences between these breeds in their ability to cycle spontaneously or after

ram introduction in late spring. A genetic difference between these breeds would indicate the possibility for genetic improvement in breeding season length through selection.

The other study described herein involved breed differences between Dorset and Suffolk rams in their ability to stimulate Suffolk x Rambouillet ewes to exhibit estrous cycles in early summer. The rams' ability to sire lambs early in the summer breeding period was used as an indication of breeding behavior and fertility. Libido and fertility during the spring and summer may vary between breeds even though the ram never attains a complete nonbreeding state similar to that of the ewe.

Overall, the comparison of the whitefaced and blackfaced sheep in these studies would indicate the extent of variation in spring breeding ability between and within the breeds used. This information is the first step needed toward selecting for a ewe line which could lamb twice per year.

REVIEW OF LITERATURE

Endocrinology of the Ewe

Domestic sheep have a seasonal breeding pattern. The ewe generally exhibits behavioral estrus during the fall and subsequently experiences an anestrous period in the spring. The actual time and duration of the breeding season depends upon the breed.

The generally-accepted model of endocrine control of sexual behavior in ewes involves a negative feedback effect of estradiol on luteinizing hormone (LH) pulse frequency. Secretion of LH is pulsatile and the pulses are more frequent during the breeding season than during anestrous. Pulses of LH are followed by pulses of ovarian estradiol and seasonal changes in the sensitivity of the hypothalamus to negative feedback effects of estradiol may affect the frequency of LH pulses and thereby determine whether the ovary is active or inactive (Legan et al., 1977).

The LH pulse generator appears to be the focal point for regulation of the sexual state of the ewe. The generator controls the pulsatile release pattern of gonadotrophin releasing hormone (GnRH) which in turn regulates the release of LH by the anterior pituitary (Martin et al., 1983a). The pattern of release of LH and the sensitivity of the ovary to the LH determines whether the ewe is in the cyclic or anestrous state.

The existence of a neural oscillator (or pulse generator) was first inferred from the typical pulsatile pattern of serum LH concentrations observed in blood samples collected at frequent intervals (Goodman and Karsch, 1981). Levels of LH increased abruptly within a few minutes and then decayed exponentially until the beginning of the next pulse. This pattern reflects discrete bursts of secretory activity by the hypothalamus followed by a relatively long (1 h) period of little or no secretion during which LH concentrations decline as the hormone is metabolized (Dierschke et al., 1970; Butler et al., 1972). Such episodic secretion of LH has been observed in many species including monkeys, humans, sheep, deer, cows, rabbits, rats and quail (see Goodman and Karsch, 1981, for review).

Neural control of the estrous cycle is exerted primarily by the secretion of GnRH from the hypothalamus. By action on the pituitary gland, this neuropeptide plays an obligatory role in the synthesis and release of the gonadotrophins, LH and follicle stimulating hormone (FSH). For ovulation to occur, the frequency of LH pulses must be high enough to provide gonadotrophic drive to the developing ovarian follicle. As the follicle matures, a sustained increase in the levels of estradiol release from the follicle is observed. The rising estradiol is required for both estrus

4

behavior and the LH surge which causes ovulation (McNatty, 1981; McNeilly, 1982).

The episodic release of LH is apparently controlled by corresponding episodic release of GnRH from the hypothalamus. Belchetz et al. (1978) showed that intermittent but not continuous administration of GnRH was able to maintain typical patterns of LH secretion following destruction of the hypothalamus. Thus, the episodic nature of GnRH secretion and the consequent pulsatile secretion of LH may be required for normal functioning of the hypothalamo-hypophyseal axis. Generally, the pulse generator is believed to be located in the medial basal hypothalamus (Krey et al., 1975) and is assumed to act through control of GnRH release. The arcuate nucleus appears to be involved, since lesions in this area abolish LH pulses (Plant et al., 1978).

Progesterone (P_4) is the primary negative feedback hormone during the breeding season (Baird and Scaramuzzi, 1976). The fall in serum P_4 concentration at luteolysis initiates the sequence of events which culminates in the LH surge which produces ovulation. The sequence begins with low LH pulse frequencies during the luteal phase of the estrous cycle due to the inhibitory action of P_4 from the corpus luteum (CL). The estradiol that is present at ovulation and estrus appears to sensitize the pulse generator to P_4 and increase its ability to suppress the frequency of pulsatile

LH release (Martin et al. 1983a). When the CL regresses, the progestational blockade of the LH pulse generator is lifted, and pulse frequency increases markedly (Baird and Scaramuzzi, 1976; Karsch et al., 1983). This LH increase stimulates ovarian follicular development and estradiol secretion which again elicits estrus behavior and the preovulatory LH surge (Karsch et al., 1980). Thus, P_4 controls the timing of ovulation during the breeding season. When it is elevated, tonic LH secretion is held in check and ovulation does not occur. When P_4 levels fall, LH rises and ovulation follows within 4 d.

Goodman and Karsch (1981) showed that both P_4 and estradiol had an effect on LH secretion. Progesterone implants in ovariectomized ewes during the breeding season reduced the frequency of LH pulses and inhibited the LH surge whereas estradiol reduced only the amplitude of the pulses. They suggest that P_4 acts on the brain to reduce the frequency of episodic GnRH release whereas estradiol acts upon the anterior pituitary to decrease its response to GnRH and thus to limit LH pulse amplitude (Goodman and Karsch, 1980). Estradiol appears to be able to limit LH pulse frequency only during anestrous but increases LH pulse frequency at the time of luteolysis or removal of P_4 implants (Karsch et al., 1983).

Plasma FSH levels remain relatively constant throughout the luteal phase of the estrous cycle. During the follicular phase FSH levels fall gradually. At the time of the preovulatory LH surge there is a concomitant FSH surge (reaching levels of 80 to 150 ng/ml) and then a second FSH rise occurs 18 to 24 h later (Salamonsen et al., 1973).

During the anestrous season, LH pulses are very infrequent (approximately once every 10 h vs once per h during breeding season). This low frequency of pulses occurs in the absence of P_4 from the CL. The low LH pulse frequency prevents follicular development and thereby inhibits the estradiol rise and subsequent LH surge which would occur during the breeding season. Seasonal changes in the ability of estradiol to elicit negative feedback on LH production have been shown in a number of breeds (Legan et al., 1977; Webster and Haresign, 1983; Martin et al., 1983a). These seasonal changes in the negative feedback potency of estradiol are large and reflect a dramatic change in the role of estradiol in control of pulsatile LH secretion (Legan and Karsch, 1983; Goodman et al., 1982; Martin et al., 1983a). Legan et al. (1977) used an ovariectomized, estradiol-implanted ewe to show that constant estradiol levels throughout the year had varying effects on LH secretion. During the breeding season LH pulse frequency was high (one

pulse per h) while the same estradiol levels caused pulse frequency to drop to one per 10 h during anestous.

During the breeding season, the negative feedback effect of estradiol is low and physiological levels of the hormone do not reduce the frequency of LH pulses (Goodman and Karsch, 1980; Karsch et al., 1983; Martin et al., 1983a). Thus LH pulse frequency and estradiol can rise in parallel between luteal regression and the onset of the preovulatory LH surge. As the transition to anestrous starts, estradiol has an increasing ability to inhibit tonic LH secretion. During the anestrous period estradiol has a powerful negative feedback effect on LH secretion (Goodman et al., 1982; Martin et al., 1983a). The existence of this highly effective negative feedback loop between estradiol and LH explains why LH pulse frequency remains minimal during anestrous and why follicular estradiol production is suppressed. Hence during anestrous, rising LH causes increased estradiol secretion which now inhibits further LH release. Once the estradiol levels have dropped LH rises again, continuing a futile loop of rising and falling levels. However, as the ewe begins transition back to the normal breeding season, the negative feedback effect of estradiol weakens, allowing increases in LH pulse frequency and the preovulatory estradiol rise and estrous cyclicity is restored.

In summary, estradiol inhibits LH pulse frequency in anestrous and also decreases the amplitude but not the frequency of LH pulses during the breeding season. In addition, estradiol treatment of ovariectomized ewes does not suppress pituitary response to GnRH in anestrous. From these observations, Goodman et al. (1982) concluded that estradiol is a more potent inhibitor of LH secretion in anestrous because it gains the capacity to suppress the frequency of hypothalamic GnRH discharges. Also, seasonal changes in pulsatile LH secretion that are independent of steroid feedback may also contribute to the low LH pulse frequency in anestrous ewes. LH pulses in untreated ovariectomized ewes were 40% less frequent in anestrous than during the breeding season. Legan et al. (1985c) also found that the negative feedback action of estradiol prevents cycles in anestrous by suppressing the frequency of the hypothalamic pulse generator. Similarly, Montgomery et al. (1985) found that there was an inherent difference in LH pulse frequency between the breeding and non-breeding season using Ile-de-France ewes. By using untreated ovariectomized ewes, Montgomery et al. found that LH pulses occurred more frequently during the breeding season.

Recent studies (Brooks et al., 1986a,b) have investigated the possible opioid modulation of LH secretion in Suffolk-cross ewes. These preliminary experiments indicate

that endogenous opioid peptides may modulate gonadotrophin secretion during some phases of the estrous cycle with possible interactions with P_4.

Effects of Photoperiod

Photoperiod is the major environmental factor regulating seasonal reproduction (Hafez, 1952). In ewes maintained under natural light conditions, onset of the breeding season is associated with decreasing photoperiod in late summer and early fall. The onset of anestrous occurs with increasing daylengths in late winter and early spring. Legan and Karsch (1978) demonstrated that photoperiod governs the marked seasonal changes in pulsatile LH release in the estradiol-implanted, ovariectomized ewe. In their study, LH was elevated in short photoperiods which permitted estrous cycles; conversely LH was suppressed to undetectable levels in long photoperiods which inhibited estrous cycles. These findings led to the conclusion that the profound seasonal change in negative feedback effects of estradiol on tonic LH secretion is photoperiod-induced. Legan and Karsch (1979) were able to drive ewes through two breeding and anestrous seasons in one year by using alternative 90 d periods of 16 h dark and of 8 h dark. Hence, photoperiod apparently had some control over the cyclicity of ewes.

The pineal gland has been shown to mediate the control by daylength of seasonal reproduction in ferrets, voles, horses, and two species of hamsters (see Bittman, 1983a, for references). These mammals are all long day (spring and summer) breeders. This mediation appears to be regulated

through the nocturnal release of melatonin. Generally, circulating melatonin levels are elevated during hours of darkness and fall during daylight. Pinealectomy renders ewes insensitive to both inhibitory effects of long days and stimulatory effects of short days (Bittman et al., 1983b). Serum melatonin rose during the night in all pineal-intact ewes in this study, and melatonin patterns in pineal-intact ewes housed in artificial photoperiods resembled those seen in ewes maintained outdoors during the corresponding natural daylengths. Pinealectomy eliminated the night-time rise in melatonin. In these ewes, melatonin levels were equal to or lower than melatonin levels of intact ewes sampled during daylight hours, regardless of photoperiod.

Exposure of pineal-intact ovariectomized estradiol implanted ewes to long days beginning on December 23 (late natural breeding season) was followed by a decline in serum LH levels to less than 1 ng/ml within 36 d. A return to short days in March (early natural anestrous season) reduced the negative feedback potency of estradiol upon LH secretion. Pinealectomized ovariectomized ewes with estradiol implants also experienced a decline of LH after introduction to long days on December 23, a change that occurred regardless of whether or not melatonin was infused during the night-time hours. In the absence of melatonin infusion, however, subsequent exposure to short days during natural anestrus failed

to induce a rise of LH in the pinealectomized ewes. Without exception, nightly infusions of melatonin reversed this effect of pinealectomy. LH levels rose to a maximum of 13.5 ± 3.9 ng/ml within 67 d; these values did not differ significantly from those of pineal-intact ewes. The protracted time course of the increase in LH may be unique to photoperiodic activation of the hypothalamo-hypophyseal axis since castration or exposure of ewes to rams during anestrous allows LH to increase within days (Oldham et al., 1979a). These results confirm the importance of the pineal gland and its associated hormone, melatonin, in photoperiodic control of reproduction in the ewe.

In order to test the hypothesis that photoperiodic signals are perceived by retinal photoreceptors and transmitted to the pineal gland and then to the hypothalamus, Legan and Karsch (1983) performed two experiments to determine whether the eyes are necessary for photoperiodic control of reproduction in ewes. In one experiment intact and estradiol-treated ovariectomized ewes were housed in each of two photoperiodically controlled rooms with a vasectomized ram and subjected to 90 d alternations between long (16 h of light) and short (8 h of light) days. Before blinding, long days initiated anestrous in intact ewes and a suppression of serum LH levels in ovariectomized ewes. Short-day exposure caused the expected opposite result. After 1.5 yr of this

photoperiodic scheme, all ewes were surgically blinded. Photoperiodic control was lost following blinding in ewes without a ram present but circannual alternations between cyclicity and anestrous or high and low LH levels were observed for the following 2.5 yr. In a group of estradiol-implanted ovariectomized ewes, serum LH levels remained synchronized to the 90 d shifts in photoperiod as long as a sighted intact ram was present with the ewes. When the sighted ram was removed, these ewes began a circannual rhythm of LH levels that was independent of photoperiod. This result suggested that the blind ewes were receiving photoperiodic cues from the sighted ram.

A second experiment confirmed these results. Ovariectomized estradiol-implanted ewes did not have 90 d LH fluctuations once they were blinded. These results are consistent with the hypothesis that ewes need their eyes for perception of photoperiod but that they have an endogenous circannual rhythm of reproduction and(or) their reproductive state can be controlled by other environmental signals in the absence of photoperiodic input.

Bittman and Karsch (1984) used ovariectomized estradiol-implanted ewes to check effects of melatonin independent of day length. Ewes were pinealectomized and intravenously infused with melatonin to restore the nightly melatonin rise. On December 11, ewes were moved from long

to short days and concurrently changed from short to long day infusions of melatonin. LH dropped in all ewes within 58.6 ± 11 days; a similar result occurred in a group of pineal intact control ewes that was treated similarly. In a second group of ewes exposed to short day light levels but infused with long day melatonin levels, LH levels fell to baseline (<0.4 ng/ml). The opposite effect was seen when a third group of ewes was kept in short days and infused with short-day melatonin patterns.

In a second experiment, ewes were initially exposed to a long-day lighting regime and LH levels subsequently fell to baseline. These pinealectomized ewes were subsequently transferred to short day lengths (8 h) for 90 d. Five ewes continued to receive long-day melatonin infusions (8 h of infusion during the last half of the 16 h period). A second group consisting of three ewes was infused with short day melatonin patterns. LH levels remained baseline in ewes receiving long day melatonin patterns. In contrast, the ewes receiving short day melatonin patterns had an induced rise in LH levels after approximately 2 mo. Similarly, exposure of pineal intact controls to short days induced a marked rise in LH which exceeded 1 ng/ml within 67.0 ± 6.1 d. These data indicate that the nighttime melatonin rise mediates reproductive responses to inhibitory as well as stimulatory photoperiods, and they further suggest that the duration of

this rise controls suppression of LH under long days. Hence, rather than being strictly pro- or antigonadal, the pineal gland itself participates in measuring daylength.

A similar finding was reported by Arendt et al. (1983). In that study, Suffolk- cross ewes were fed physiological amounts of melatonin characteristic of short days in constant summer light (16 light:8 dark). Melatonin was fed during the second 8 hr of light treatment. These ewes cycled 2 to 8 wk earlier than untreated controls in natural light. This data indicated that melatonin alone in physiological quantities is sufficient to induce early onset of the ovine breeding season. Yellon et al. (1985) also found that melatonin patterns determine both inhibition and stimulation of reproduction regardless of photoperiod.

Hafez (1952) noted that the domestic sheep is a short day breeder but that its reproductive season is skewed with respect to photoperiod. During 8 yr of observations on Suffolk ewes, Robinson and Karsch (1984) observed a mean day of September 3 (14.0 h of light per day) for the beginning of estrous cycles as measured by behavioral estrus and P_4 profiles and a mean day of February 15 (11.5 h of light per day) for beginning of the anestrous period. Despite the fact that the ewes are short day breeders, they began cycling when days were longer than when they terminated cycling.

In order to test whether ewes were becoming anestrous due to increasing daylength or because they were losing the ability to respond to short daylengths (refractoriness), three groups of ovariectomized estradiol-implanted ewes were placed in controlled photoperiodic conditions beginning on the winter solstice (December 21). Group number 1 (6 ewes) was maintained in an outdoor pen with rams and a cycling flock of 13 intact ewes. A second group was maintained indoors on a daylength equivalent to the shortest day at this latitude (solstice hold; 10 h light). The final group of 6 ewes was placed indoors and light was increased daily to mimic the natural daily light increases of the onset of spring. Estrous cycles ceased in the cycling flock of intact ewes maintained outdoors on February 10 ± 3 d when daylength was at 11.4 h of light. At a similar time there was a dramatic decrease in LH levels in the ovariectomized estradiol implanted ewes being held in natural daylengths outdoors. Levels of LH in this group were consistently below 1 ng/ml on February 10 ± 3 d. Similarly, LH levels in the "natural simulate" group indoors and the "solstice hold" groups fell together to reach values consistently below 1 ng/ml on January 26 ± 5 d and January 24 ± 6 d, respectively. Therefore, the low LH levels were attained approximately 3 wk in advance of that in animals maintained in a natural environment with rams (P<0.001). These results provide strong support for the

conclusion that Suffolk ewes normally cease breeding because they are no longer able to respond to prevailing short days, not because they are actively inhibited by increasing photoperiod. Hence the asymmetry between the annual cycles of photoperiod and reproduction can be explained at least in part by photorefractoriness, or the loss of response to a daylength which was once stimulatory.

Similarly, Robinson et al. (1985a,b) found that refractoriness to inhibitory daylengths initiates the breeding season of the Suffolk ewe. When ovariectomized estradiol-implanted ewes were held at summer solstice (16.5 h daylight) conditions and compared to ovariectomized estradiol-implanted ewes held under natural lighting conditions, all ewes showed increasing LH levels at the same time.

Karsch et al. (1986) hypothesized that the photorefractoriness reflects a deficit in the postpineal processing of the photoperiodic message since ovariectomized estradiol-implanted pinealectomized ewes held in either short or long photoperiods became photorefractory even though daily melatonin patterns remained unchanged. To check this, Karsch et al. (1986) infused ovariectomized estradiol implanted pinealectomized ewes with short day melatonin regimens which were initially reproductively stimulatory but later became unable to support reproduction at about the same time intact control ewes were entering anestrus.

Prolactin concentrations in sheep show marked seasonal fluctuations in concert with daylength. Prolactin is high during long days and low during short days. Webster and Haresign (1983) examined seasonal changes in LH and prolactin levels in ewes under natural daylength conditions. They found that breeds that differed in time of onset of breeding season (Dorset Horn with long breeding season vs Welsh Mountain with short breeding season) showed identical temporal changes in prolactin levels. Worthy and Haresign (1983) housed both Dorset Horn and Welsh Mountain ewes in controlled lighting on December 12. Ewes were exposed to either 16 h dark or 8 h dark per day. Ewes which were switched to long days stopped cycling earlier than control ewes in natural lighting. The mean day of the end of the last cycle for long-day treated ewes vs control ewes was March 3 and April 3, respectively, for Dorset ewes and March 12 and April 11, respectively, for Welsh Mountain ewes. For ewes held on short days, cessation of estrous activity occurred at approximately the same time as in controls. The different daylength treatments had an effect on prolactin levels, as expected, but no breed differences were apparent. The differences in response to photoperiod-induced changes in prolactin concentrations allowed the possible role of prolactin as a mediator of the seasonal change in negative feedback responses to estradiol to be examined. Since ewes

in short daylengths stopped cycling at the same time as controls even though they exhibited low prolactin concentrations, it was concluded that prolactin is not a major vehicle controlling seasonal changes in hypothalamic responsiveness to estradiol negative feedback. This does not, however, rule out a minor or facilitating role of prolactin.

Bittman et al. (1983b) notes that while the pineal gland is related to seasonality in sheep, the persistence of seasonality in the absence of the pineal might result from annual changes in temperature, food quality, or other environmental cues. Alternatively, it might reflect persistence of some endogenous annual rhythm which remains intact and functional after photoperiodic synchronization to the environment has been eliminated. Robinson et al. (1985a) suggests that a changing daylength does not normally drive either of the seasonal reproductive transitions of the Suffolk ewe and that blinded or pinealectomized ewes (Bittman et al., 1983a,b; Legan and Karsch, 1983) show seasonal cycles which are no longer synchronized with animals perceiving day length. Thus, rather than actively driving reproductive changes, that daylength apparently ensures the appropriate timing of the breeding season of the ewe by entraining an endogenous circannual rhythm of reproduction.

In conclusion, the pineal gland exerts its effect as timer of reproduction and favorable environment (i.e., en-

suring lambs are born in the spring) through secretion of melatonin. The melatonin in turn modulates a key determinant of estrous cyclicity: the capacity of estradiol to inhibit tonic LH secretion. Melatonin is thus a blood borne photoperiodic hormone which drives seasonal changes of reproductive activity in the ewe. The ability of melatonin to control the capacity of estradiol to inhibit gonadotrophin secretion is critical in determining the seasonal reproductive state and can fully account for pineal mediation of the effects of stimulatory and inhibitory photoperiods in the ewe.

Endocrinology of the Ram

In the ram, many parallel seasonal changes in reproduction occur as in the ewe, but the changes are less striking. During spring, LH and testosterone levels decrease, as does the size of the testicles as measured by scrotal circumference or diameter. These changes do not result in the total cessation of reproductive activity. Most, if not all, rams retain some level of fertility throughout the year. Research into the endocrinological changes associated with reproductive seasonality in the ram is much less extensive and well organized compared with that associated with the ewe. The following review includes much that has been done with the domestic ram, but does not offer the clear cut conclusions that were obtained with the ewe.

Throughout the year, LH and testosterone are released into the blood in a pulsatile pattern. Each peak of LH from the pituitary is followed by a peak of testosterone from the testes (Lincoln, 1976; Pelletier et al., 1982). Generally, the number of LH and subsequent testosterone peaks increases when the animals pass from the nonbreeding season to the breeding season (Sanford, 1974, 1977; Schanbacher and Ford, 1976; Wilson and Lapwood, 1978). Pelletier et al. (1982) report that a testosterone peak was associated with an LH peak 97.5% and 94.0% of the time with Prealpes de Sud and Ile-de-France rams, respectively. In this French experiment

the frequency of LH and associated testosterone peaks increased between December and June for rams of both breeds but there was only a significant increase from December to February for Prealpes de Sud rams. Hence, there is apparently a genotype by season interaction effect on the physiological sexual response to photoperiod. Sanford et al. (1974) observed similar results using both crossbred and Finnish Landrace rams.

Sanford et al. (1977) measured levels of LH, FSH and testosterone in Finnish Landrace and Managra synthetic throughout the breeding season (August through December). As in previous studies, pulses of LH were consistently followed by elevations in testosterone which peaked within 60 min. As the breeding season advanced, LH peaks became more frequent and decreased in amplitude while the frequency and amplitude of the testosterone peaks increased. FSH fluctuated very little from baseline concentrations (<100 ng/ml). Moderate increases (up to 200 ng/ml) in FSH occurred in September but were not consistently associated with LH peaks.

Schanbacher and Ford (1976) bled Suffolk-Hampshire crossbred rams every 30 min for 24 h during September and May. They found similar results to other studies reported herein for LH and testosterone levels. This study failed to provide evidence for any relationship between estradiol and LH or testosterone secretion. Estradiol varied widely be-

tween and within rams and months with levels ranging from 2 to 16 pg/ml. Wilson and Lapwood (1978) saw similar results with Romney rams while Katongole et al. (1974) also observed similar seasonal fluctuations with Suffolk rams. Sanford et al. (1976) found fairly constant FSH levels during a 24 h period but found lower mean levels of FSH in January as compared to August in Finnish Landrace rams.

Schanbacher and Kinder (1984) reported that the pituitary-testicular axis was more active (in terms of testosterone production) during short days as compared with long days and that the magnitudes of changes of serum LH, FSH and testosterone concentrations were greater for the two most seasonal breeds used in this study: Finnish Landrace and Suffolk (other breeds used were Rambouillet and Polled Dorset). During long days, there were no differences between the four breeds. These results indicate that breed differences exist in mature rams with regard to hormone secretory profiles. Breed differences in serum gonadotropin and testosterone levels may be only apparent during short days when the hypothalamo-pituitary-testicular axis in rams is considered most active.

Once the general seasonal fluctuations in LH levels and subsequent change in frequency of testosterone pulses were detected, researchers tried to mimic these changes by treatments with releasing hormones to determine the extent of

hypothalamic control of the seasonal fluctuations. Also, further experiments tested mutual feedback effects involving these hormones. Bolt (1971) showed that testosterone injections could depress the episodic fluctuations of plasma LH levels in rams through a negative feedback effect. Sanford et al. (1976) reported that the injection of GnRH had no effect on FSH levels but increased LH levels within 15 min with an accompanying rise in testosterone levels. Bolt (1971) also found that injection of estradiol or P_4 causes a marked depression of plasma LH in Rambouillet rams.

Lincoln (1979) was able to mimic seasonally-induced endocrine changes in Soay rams by injecting GnRH (100 or 500 ng) for 60 s every 2 h for 33 to 57 d during the spring (non-mating season). The GnRH treatment resulted in growth of the testes and development of the sexual skin flush. These effects were lost when treatment was stopped. This result indicated that seasonal effects could be reversed by releasing-hormone treatment.

Katongole et al. (1974) concluded that the ram, like the ewe, should be regarded as a seasonal breeder even though it never lapses into a period of complete sexual quiescence and infertility at any time of the year. Both Katongole and Wilson and Lapwood (1978) suggested that rams have a changing hypothalamic sensitivity to steroid feedback inhibition dur-

ing the year which is probably mediated by photoperiod as it is in the ewe.

Each individual LH discharge from the pituitary results in a transient stimulation of the testes which then releases the associated testosterone pulse. As the fall breeding season approaches, LH discharges become more frequent such that stimulation of the testes increases and the gonads grow in size and increase their output of testosterone. Lincoln (1976) found a five-fold increase in the volume of the testes in adult Soay rams from the regressed (spring) to the most active (fall) state. In controlled lighting conditions, only a slight growth of the testes occurred during long days (16 L:8 D) but this growth was accelerated when the lighting was changed to short days (8 L:16 D). Maximum testes size was achieved under these short days. Rapid regression of the testes occurred following the return to long days. Blood samples revealed marked changes in the concentrations of LH and testosterone that were correlated with the growth changes in the testes. Initially, when the testes were regressed, the plasma levels of both LH and testosterone were generally very low (<1 ng/ml). As LH levels began to increase with short days, the peak values of testosterone increased (mean peak maximum was 9.45 ± 1.7 ng/ml). At this point, the testes were growing slowly and plasma testosterone concentrations were gradually increasing. After 7 to 10 wk on 8 L:16 D the

gonads were fully grown and testosterone levels were at a maximum (24 to 28 ng/ml). LH levels had tended to decline by the time the gonads were fully grown. After the rams were switched back to long days of 16 L:8 D, a rapid reduction in blood levels of both LH and testosterone paralleled the regression of gonadal volume. Early in the study when the testes were regressed, plasma LH levels were generally very low with an occasional peak in the concentration which increased the basal values for 60 to 90 minutes. These surges in LH levels occurred about once every 6 h and each was associated with an increase in plasma testosterone. The increase in testosterone concentrations began 15 to 30 min after the peak level of LH and rose to a maximum 45 to 75 min later. As the development of the testes continued, the response to LH peaks was increased. The first testosterone increase was within 15 min, with a peak at 30 to 60 min after LH peak. Also, the maximum testosterone concentration increased 2 to 3 fold. At maximum testes volume, the large surges of LH were no longer apparent but instead the plasma showed a series of small LH peaks that occurred far more frequently. At this point, plasma testosterone levels were greatly increased compared with the earlier periods and the small fluctuations in LH levels were associated with large and immediate increases in plasma testosterone (rising 0 to 15 min after LH peak and reaching a peak 30 to 44 min later).

Lincoln broke the testes growth pattern down into three phases: regressed, developing and active. The regressed phase generally occurs in spring and early summer when testicular volume is at a minimum and LH and testosterone pulse frequencies are low. The developing stage occurs in late summer and early fall when LH pulse frequency is at a maximum and testicular volume is increasing. Accordingly, testosterone secretion is increasing. Finally, the active stage is during the fall breeding season. LH pulse frequency has already decreased somewhat and testicular volume and testosterone secretion is at a maximum. Injection of GnRH into rams resulted in the greatest response, as measured by LH increase, during the developing stage. The next greatest response occurred during the regressed phase. Hence, it appears that the frequency, amplitude and duration of LH peaks and the basal concentrations of LH are all affected by season and photoperiod. Lincoln also concluded that there was a seasonal change in the pituitary responsiveness to a standard dose of GnRH (5 ug).

Wethers (castrate rams) have appreciably higher basal LH concentrations than do rams, providing evidence for the importance of negative feedback of gonadal steroids on gonadotrophins (D'Occhio, 1982a). Wethers also show frequent rhythmic pulses of LH, whereas pulses of LH in rams occur relatively infrequently. D'Occhio (1982a) castrated Finnish

Landrace rams and then immediately implated them with Silastic capsules filled with crystalline testosterone. The size and number of capsules implanted was varied in order to produce graded concentrations of circulating testosterone. LH secretory patterns together with pituitary responses to exogenous LHRH were characterized after 6 wk of hormone treatment. Control wethers had significantly higher basal LH concentrations than rams as well as more frequent rhythmic LH pulses. The frequency of LH peaks in wethers receiving large testosterone implants (1.11 ng vs .54 ng/ml) was significantly lower than in wethers receiving the smaller testosterone implants and also lower than the control wethers with no implant (.22 ng/ml). An increase in serum testosterone of .89 ng/ml above castrate control levels, therefore, caused an increase in the interval between LH pulses without an effect on mean LH concentrations. These results indicate that as the concentration of testosterone in the serum is increased from castrate to intact levels, at least two distinct changes in the pattern of LH secretion are observed. Testosterone concentrations that were intermediate between those seen in wethers and rams caused a decrease in the frequency of rhythmic LH pulses without an effect on mean LH concentrations. As serum concentrations of testosterone approached levels characteristic of rams, there was a dramatic decrease in both the frequency of LH pulses and also

basal concentrations. A decrease in the frequency of rhythmic LH pulses suggests a change in the frequency of LHRH discharge into the hypophyseal portal vessels. Wethers implanted with large testosterone implants showed a reduced LH response (a low peak value of 13.1 ng/ml vs a high peak of 36.3 ng/ml for controls) to exogenous LHRH indicating a direct suppressive action of testosterone on the pituitary. This observation together with the marked decrease in the incidence of LH pulses in these animals suggest a dual action of testosterone at both the hypothalamic and pituitary level in rams. Hence, the negative feedback on LH by testosterone is not an all-or-none phenomenon. The first effect of testosterone is to decrease the frequency of rhythmic LH pulses. As testosterone levels approach physiological concentrations there is a further dramatic decrease in LH pulses accompanied by a decline in basal LH.

Estradiol can have a similar negative feedback effect on LH secretion. Exogenous estradiol inhibits LH secretion in the ram primarily by reducing LH pulse frequency and secondarily (or consequently) by reducing basal and mean LH secretion. Physiological levels of estradiol are capable of suppressing the high circulating levels of LH that is found in castrated males (Schanbacher, 1984).

Testosterone also exhibits negative feedback on FSH secretion. FSH levels increased 4 to 5 fold in Finn rams after

castration. Testosterone implants at physiological levels in wethers brought FSH to equivalent levels of intact rams (D'Occhio et al., 1982a).

By examining the nature of LH secretion in testosterone-implanted castrated rams given pulsatile infusions of LHRH, Schanbacher and D'Occhio (1984) concluded that the hypothalamus rather than the pituitary is a principal site for the negative feedback of androgens in rams. This study showed that a rhythmic pulse pattern in LH secretion is observed in wethers and develops quickly after castration (within 24 h). This castrate pattern of LH results from an increased frequency of LHRH input to the pituitary. Testosterone would, therefore, appear to prevent the post-castration rise in LH by suppressing activity of the presumptive LHRH pulse generator. This may be the principal mode of action of testosterone since a 'castrate-like' pattern of LH secretion is seen in wethers treated with testosterone and LHRH. To eliminate LHRH without performing a hypothalectomy, Lincoln et al. (1986) infused Soay rams with 50 µg buserelin/day (LHRH agonist) for 21 d and successfully blocked LH, FSH and testosterone secretion as expected. Within 7 d after the treatment was stopped, blood hormone concentrations returned to normal.

As with the ewe, many experiments in the ram have dealt with either controlled lighting conditions or melatonin in-

fusion of pinealectomized rams or both to uncover the effects of day length and melatonin patterns on hormonal levels and subsequent seasonal sexual cycles. Some photoperiodic refractoriness was observed by Almeida and Lincoln (1982). Rams which were exposed to long days of 16 L:8 D showed regression of testicular diameter and sexual skin flush after 12 wk but if held on this lighting regimen, the testes began to redevelop after 16 to 20 wk. These researchers suggest a circadian model for photoperiodic time measurement with melatonin acting as one of the principal hormones in the relay of photoperiodic effects upon reproduction. Rams exposed to light cycles of 24 h or multiples of 24 h, i.e., 8 L:16 D or 8 L:40 D, acted as expected with increased sexual activity whereas rams exposed to 8 L:28 D were similar to rams held in long days of 16 L:8 D. The rams held in 8 L:28 D also showed melatonin peaks with no daily rhythm. Peaks sometimes occurred during the light period.

Adult Romney rams were either pinealectomized or sham-operated by Barrell et al. (1985) to eliminate pineal activity. The rams were then split into two groups: one was given artificial lighting mimicing natural conditions while the second group was exposed to a lighting regimen which was 6 mo out of phase from the natural conditions. Weekly blood samples were taken and plasma FSH concentrations were determined. Mean plasma FSH levels in all rams subjected to

normal lighting were elevated (up to 7-fold) during the fall when daily photoperiod was decreasing. In the control rams exposed to the reversed lighting regime, plasma FSH levels fell abruptly in correspondence with the increasing daily photoperiod. This did not occur in pinealectomized rams under the reversed lighting conditions. Instead, plasma FSH levels followed a pattern of changes similar to that recorded from rams on the normal lighting regime. This study revealed that removal of the pineal gland uncoupled FSH secretion in rams from photoperiodic control so that this hormone could not respond to artificially-imposed changes in lighting. The plasma FSH secretion pattern in pinealectomized rams still showed a strong seasonal component with increased levels in the fall even when the lighting cycle was reversed. This apparent endogenous rhythm of FSH secretion in rams probably persists in the absence of environmental cues.

Almeida and Lincoln (1984) found that Soay rams become reproductively photorefractory to light in a similar fashion to ewes as reported by Robinson and Karsch (1984). In an experiment in France using "6 month years", decreasing day length stimulated LH pulsatility and testicular growth while increasing day length was inhibitory (Lindsay et al., 1984). Lincoln and Ebling (1985) implanted intact rams with constant-release melatonin implants and found that the rams experienced a rapid initiation of reproductive redevelopment

if held in long days. Also, the melatonin implants prevented rams from responding to normal changes to photoperiod. Eventually, the rams returned to cyclic reproductive behavior by becoming refractory to constant melatonin. Pelletier (1986) also showed that decreasing day length increased the frequency of LH pulses.

Hence, findings with the ram paralleled those in the ewe. Decreasing daylength in the fall stimulates pulsatile LHRH release by the hypothalamus which sets off a cascade of events which brings about sexual redevelopment. This effect is mediated through nightly melatonin release. Any interruption of normal lighting patterns or melatonin secretion causes an endogenous circannual reproductive cycle to take effect. Also, similar to the ewe, there is possible opioid regulation of the sexual redevelopment; Ebling and Lincoln (1985) showed that an endogenous opioid mechanism is involved in the tonic inhibition of LH secretion and that this mechanism is most active during the breeding season in Soay rams. Regulation of endogenous opioids in the hypothalamus may be part of the mechanism by which environmental factors modulate steroid negative feedback control of LHRH and thus LH secretion in seasonally breeding mammals.

Ram Effect

Most ewes are acyclic during the spring of the year because of negative feedback inhibition of LH by estradiol, but, depending on the breed, a proportion of animals can be stimulated to ovulate and possibly show behavioral estrus through the acute introduction of rams into the ewe flock. This phenomenon, whereby ewes are stimulated by rams to show a relatively synchronized estrus during the usual anestrous period, is termed the ram effect.

Ewes that are continuously exposed to rams will exhibit a natural breeding season and anestrous. However, if ewes are preconditioned by a period of isolation from rams, subsequent reintroduction of males has been shown to stimulate ovulation and estrus at an earlier date than that observed for ewes which are continuously exposed to rams (Watson and Radford, 1960; Martin and Scaramuzzi, 1983; Oldham, 1980). Watson and Radford isolated Merino ewes from rams for 4 mo prior to acute ram exposure (termed teasing) of some ewe groups. Behavioral estrus was indicated by the transfer of paint placed on the brisket of rams to the ewes' backs during sexual activity. Significantly more of the ewes which were exposed to rams showed behavioral estrus during the first 18 d after ram introduction. Martin and Scaramuzzi (1983) indicate that ewes of a variety of breeds can be stimulated to ovulate after ram introduction. Among the breeds already

observed are Merino, Awassi (Israel), Romney Marsh (New Zealand), Merinos D'Arles, Ile de France, Prealpes du Sud, Berrichon, and Romanov (all in France).

The fact that ewes must be isolated from rams prior to reexposure in order to express the ram effect is well established, but the exact length of isolation has yet to be determined. Oldham (unpublished) in 1980 has shown that 34 days is certainly sufficient and possibly a period as short as 17 d is sufficient for seasonally anovular Merino ewes during October in Western Australia. Also, the response of seasonally anovular Ile-de-France ewes isolated from rams for 21 d prior to teasing in June in Nouzilly, France, was the same as that of flockmates isolated from rams for more than 4 mo.

Apparently, ewes do not need to be in direct visual or physical contact with rams in order to be stimulated to ovulate. Watson and Radford (1960) designed an experiment to determine if the ram stimulation was reaching the ewe through visual, olfactory, auditory or tactile perception. Merino ewes were isolated from all ram contact for 4 mo prior to onset of the experiment. Following isolation, anestrous ewes were exposed to different degrees of ram contact and then estrus behavior was monitored. One group of ewes acted as a control and was exposed to vasectomized rams continuously during the experimental period. Ewe groups were ex-

posed to either to a combination of auditory and olfactory or of auditory, olfactory and visual stimulation or were isolated from rams. Results indicated that all groups exposed to rams showed estrus at an earlier date than the group which remained isolated. The smell and(or) sound of rams was sufficient stimulus to exert full influence on the occurrence of estrus. Although this experiment did not separate out the effects of sight or smell of the ram, it showed that actual physical contact with a ram was not needed for a ewe to ovulate in response to teasing.

Morgan et al. (1972) showed that ewes with an impaired sense of smell were significantly less responsive to the introduction of rams than were intact ewes or ewes that had an impaired sense of touch or hearing. In this experiment Border Leicester x Merino ewes had their senses of hearing, touch around the mouth, and(or) smell surgically impaired. Ewes were then joined with rams for 8 wk during the spring. Mating behavior of rams was monitored through crayon marking harnesses. Six of 15 ewes with sense of smell impaired, all 16 with senses of touch or hearing impaired, and 34 out of 36 intact ewes were marked, indicating that they had been mated. The proportion of ewes with impaired sense of small that mated was significantly less than for the other groups. These results suggested that rams stimulate estrous activity in non-cycling ewes through olfactory receptors in the ewe.

This result has led to the hypothesis that the ram effect is mediated through the release of pheromones by the ram. Pheromones are odiferous substances which are secreted to the outside of an individual and received by a second individual of the same species in which they elicit a specific action. Pheromones have been shown to be involved in sexual and social activities of many mammals including deer, rabbits, rats, dogs, and badgers (Stoddart, 1976) as well as in insects (Buschinger, 1975).

One or more pheromones which induce ovulation in seasonally anovular ewes appear to be produced by the ram (Knight and Lynch, 1980a,b). Border Leicester-Romney ewes were first isolated from rams for 5 mo. After laparoscopic examination to confirm acyclicity, ewes were divided into 3 groups. One group was sprayed with urine collected from entire Dorset rams while a second group was sprayed with water as a control. The remaining ewes were exposed to intact Dorset rams. Significantly more of the ewes (43%) with the entire Dorset rams ovulated than in the other two groups. The proportion of ewes ovulating in the latter two treatment groups was not significantly different (22% for urine vs 0% for water). In a second experiment (Knight and Lynch, 1980a), Romney ewes which had also been isolated from rams for 5 mo were split into 3 groups. The first group of ewes had wax collected from the flanks and freshly shorn wool of

intact Dorset rams smeared on and held over their noses, re-spectively. The second group was exposed to intact Dorset rams and the third group remained isolated as a control. There was no difference in the proportion of ewes ovulating in groups exposed to either the ram (50%) or its wool and flank wax (48%). Both were significantly higher than the isolated control (7%). These two experiments indicated that a pheromone secreted by the ram is probably the active factor which drives a ewe's ovulatory response to teasing. The exact source of the putative pheromone(s) remain unknown but they may be released into the wool from glands over the entire body or released with wax from special sebaceous glands on the flanks. The urine which stimulated 22% of ewes in this group to ovulate was speculatively said to have been contaminated with the pheromone from the wool and not to be a natural concentrated source of the compound(s). This may or may not be the case.

Knight and Lynch (1980b) further tried to isolate the source of possible pheromones. The pheromone was again found present in the flank wax and in water and petroleum spirits which had been used to wash the wool on an intact ram's back. It was concluded that the pheromone is probably secreted with the suint.

Knight and Lynch (1980b) reported that teasing with Dorset rams will stimulate ewes to ovulate (55%) but that

teasing with testosterone-treated wethers (0%) and testosterone-treated ewes (10%) failed to elicit an ovulatory response in anestrous ewes. By contrast, Fulkerson et al. (1979) are reported by Knight and Lynch (1980b) to have induced 42% of a group of ewes to ovulate by teasing with Merino wethers treated with testosterone in a similar way. Thirty-two percent of ewes teased with Merino rams responded while 5% of the ewes exposed to untreated wethers responded with ovulation. Croker et al. (1982) reported that Merino wethers and ewes treated with testosterone injections were as successful as vasectomized rams in inducing ovulation and cyclic activity in ewes. Eighty-one percent of ewes exposed to rams were marked compared with 74% of ewes exposed to treated wethers and 76% ovulated within 20 d after exposure when teased by the treated ewes.

Fulkerson et al. (1981) found that estrogen- or testosterone-treated wethers were as effective as rams in stimulating ewes to ovulate in the spring. Forty-two percent of ewes exposed to testosterone-treated wethers, 42% of ewes exposed to estrogen-treated wethers and 29% of ewes exposed to vasectomized rams (no significant difference) ovulated after 6 d of teasing compared with 5% ovulating when exposed to untreated Merino wethers. Hence, there appear to be conflicting results in the literature as to whether or not steroid-treated castrates and females can be used as teasers.

Generally, more studies support the idea that they can be used. Accordingly, Pearce and Oldham (1984) reported that the use of testosterone-treated wethers to induce ovulation in ewes has received widespread commercial acceptance in Australia.

Tervit et al. (1977) showed that Romney ewes joined with entire Dorset rams just prior to onset of natural estrus activity showed estrus, ovulated and conceived significantly earlier than ewes exposed to Romney rams. In the 14 d interval following ram introduction, 83% of ewes with Dorset rams cycled compared with 62% of the ewes with Romney rams. Meyer (1979) exposed a variety of ewe breeds and crosses to either Dorset, Finn x Romney, or Romney vasectomized rams 10 wk prior to actual mating. Dorset rams stimulated the onset of seasonal estrus about 3 wk earlier than did the teasers of the other two breeds. Fifty percent of the ewes exposed to Dorset teasers were cycling within 6 wk of exposure compared to 8 wk and 9 wk required for 50% of ewes in the Finn x Romney and Romney teaser ram groups, respectively, to begin cycling.

Using intact purebred rams, Tervit and Peterson (1978) have demonstrated a breed difference in teasing ability. Romney ewes were exposed to either Dorset or Romney rams in early summer. Testosterone levels were determined in the rams and all mating marks were recorded. The Romney ewes

joined with Dorset rams showed estrus earlier than those with Romney rams. Romney rams had higher mean testosterone levels in the latter part of the study even though they were less effective than Dorset rams in stimulating the ewes to cycle. This result indicates that absolute androgen level does not correspond directly with teasing ability but, keeping in mind the previous work cited, some threshold level of testosterone is apparently required to elicit the ram effect, possibly through its affect on pheromone production.

The effect of ram introduction on the endocrinology of the anestrous ewe has been studied fairly extensively. As has been discussed earlier, basal secretion of LH in the ewe is pulsatile and the pulses are more frequent during the breeding season than during anestrus. LH pulses are followed by rises in ovarian estradiol and seasonal changes in the sensitivity of the hypothalamus to negative feedback effects of estradiol may limit the frequency of LH pulses and thereby determine whether the ovary is active or inactive (Legan et al., 1977). Upon the introduction of rams to seasonally anovular Merino ewes, Martin et al. (1980) observed a rapid rise in LH from basal levels (<1 ng/ml) to a peak (>3 ng/ml) within 10 min. This LH peak was followed by an exponential decline over the next 2 to 10 h. Generally, the amplitude of the pulses varied within and between ewes. The frequency of the pulses was highly variable between ewes ranging from

one pulse every 70 min to one every 9 h but was consistent for each animal. Of the seven ewes exposed to rams, only one did not display an immediate release of LH in response to the introduction of rams. Two of the six ewes that responded had only minimal LH rises (to approximately 3 ng/ml) and did not ovulate. The four ewes with larger LH peaks (up to 20 ng/ml) did ovulate. Presumably the rising LH concentrations induced follicular development and secretion of estradiol by the ovary which in turn induced the preovulatory surge of LH and ovulation.

Chesworth and Tait (1974) noticed a similar rise in LH (mean LH of 2.3 vs 0.51 ng/ml for exposed vs control, respectively) within 1 h of introduction of rams. These researchers only bled the ewes once per hour; presumably LH levels could have risen much faster than they could detect, possibly within the 10 min observed by Martin et al. While Martin et al. carried on their experiment during the spring ("deep" anestrous), Chesworth and Tait sampled and teased their Border Leicester x Scottish Blackface ewes in the late summer (just prior to onset of natural breeding season). The ram effect can apparently induce ewes to ovulate both outside the normal season and just prior to the commencement of natural cyclicity.

The rapid rise in LH levels in response to ram introduction suggests a direct effect that is independent of a

change in the sensitivity of the hypothalamus to estradiol. Possibly the estrogen-induced pre-ovulatory surge of LH may be replaced by the ram-mediated release. A neural mechanism for this ram-induced preovulatory surge has been suggested (Knight et al., 1978; Oldham et al., 1978). The complex of ram stimuli, acting through diverse cerebral routes presumably converge on the hypothalamus where they bypass the effectiveness of the negative feedback by estradiol. This would increase the frequency of GnRH pulses and hence LH. Martin et al. (1983) suggests that the ram pheromone influences LH in the ewe through neural pathways that connect the accessory olfactory bulbs and the anterior hypothalamus. The observed response in LH would then be controlled by GnRH from the anterior hypothalamus.

Knight et al. (1978) stimulated 39 to 70% of anestrous Romney ewes to ovulate with acute Dorset ram exposure at the beginning of the natural breeding season. No rise in basal LH occurred until 12 h after ram introduction with 3 h blood sampling intervals. No rise in estrogen levels were found prior to 9 h before the LH surge indicating again that initial rises in LH levels above baseline were independent of estrogen stimulation of the hypothalamus.

Martin et al. (1983) determined if the ram stimulus induces ovulation by overcoming the inhibitory actions of estradiol and initiating an immediate increase in LH pulse

frequency. Using Merino ewes which were ovariectomized and estrogen implanted, or left unimplanted as controls, rams were acutely introduced and blood samples were drawn to assess LH levels. Before ram introduction, basal and mean levels as well as pulse frequency of LH were all lower in ewes with estradiol implants than in control ewes due to negative feedback. After the introduction of rams, 8 of 10 implanted ewes and 0 of 5 control ewes responded with increased LH pulse frequency (0.5 pulse/h before and 0.9 pulse/h after ram introduction for estradiol-implanted ewes vs 1.50 pulse/h and 1.34 pulse/h for control ewes). Hence the ovariectomized ewe treated with estradiol and the intact ewe have virtually identical responses to the introduction of rams during the anestrous season: a rapid increase in LH pulse frequency with associated increase in mean and basal LH levels. Apparently the ram effect works at least in part by inhibiting the negative feedback exerted by estradiol. In this same experiment, ewes implanted with estradiol + androstenedione reacted in the same way to ram introduction as ewes implanted only with estradiol. Thus, androstenedione had no apparent contribution. Contrary to the almost immediate increase in LH release after ram introduction, levels of FSH appear to remain unchanged (Poindron et al., 1980). Thus, the role of FSH appears primarily permissive (Martin et al., 1980b).

As LH pulses increase in a cycling ewe, estradiol levels increase until a positive feedback mechanism results in a massive LH surge which causes ovulation. In teased Merino ewes, the preovulatory LH surges began 27 ± 4 h (range 6 to 52 h) after ram introduction in the spring (Oldham et al., 1978). These researchers concluded that generally ewes will ovulate in response to rams within 54 h while Knight et al. (1978) found that ewes ovulated 65 to 72 h after ram introduction. Oldham et al. carried out their teasing in the spring while Knight et al. introduced their rams just prior to the onset of normal breeding. This discrepancy could have affected the time from introduction to ovulation. Martin (1984) and Oldham et al. (1978) suggest that the LH surge is due to the normal positive feedback mechanism or possibly to stimulation of the hypothalamus by direct sensory input or inputs from higher centers of the central nervous system.

The first ovulation following teasing is generally not accompanied by behavioral estrus. Oldham and Martin (1978) were able to induce ovulation in 74 of 91 seasonally anovular Merino ewes with the ram effect. Subsequent laparoscopy revealed that 38 of 74 corpora lutea persisted normally while the remaining 36 regressed prematurely. In 32 of the latter 36 ewes, premature regression was followed by a second ovulation (again without heat) within 6 d of ram introduction. Hence, two peaks of estrus activity generally occur

in ewes responding to teasing. The first occurs approximately 18 d after ram introduction and the second peak occurs about 6 d later. Following the ram-induced "silent" ovulation (not accompanied by behavioral estrus), some ewes develop corpora lutea (CL) with normal life spans of about 14 d. These ewes comprise the first peak in estrus activity because they ovulate a second time (with estrus) about 18 d after teasing. The second peak of activity occurs around day 24 and is comprised of ewes that experienced the premature regression of the initial CL followed by a second silent ovulation with a CL of normal life span. Finally these ewes ovulate a third time with estrus about 24 d after ram introduction (Oldham and Martin, 1978; Knight et al., 1981; Fulkerson et al., 1981). Knight et al. (1981) observed two peaks in estrus activity, the first 19 and the second 23 d after ram introduction into a flock of Romney ewes in the spring.

This dual-peak effect of mating behavior following teasing occurs because of a lack of an ovarian luteal phase with its associated elevated P_4 level prior to ram introduction (Oldham et al., 1978). Legan et al. (1985b) observed that most ewes experience a transient increase in serum P_4 concentrations for 1 to 2 d just prior to their first estrous cycle of the new breeding season. Progesterone levels rise to about 1 ng/ml during this time. Adult Suffolk and Suffolk

crossbred anestrous ewes were administered synthetic GnRH for 3 d in a pulsatile fashion which resulted in mimicking of the pattern of LH secretion during the preovulatory period of the breeding season. As further treatment, part of the ewes received P_4 by injection or implant and subsequent luteal phase lengths were measured. The induced LH surges caused full-length luteal phases in 10 of 10 ewes pretreated with P_4 and in only 8 of 18 ewes that were not pretreated. Thus, this study supports the idea that a ewe needs a small level of P_4 prior to ovulation to assure a CL of normal duration in the following cycle. In the breeding ewe, this P_4 is provided by the regressing CL of the prior cycle but in the anestrous ewe, frequently a short, 6-d CL is required. Though the short cycle is frequently observed, it does not appear to be essential in all ewes. Oldham et al. (1985) used increasing doses of P_4 from 0 to 20 mg to increase the proportion of ewes with CL's of normal life span when ovulation was induced in anovulatory ewes by the introduction of testosterone-treated wethers. Only 54% (19/35) of the ewes had normal CL's following 0 mg P_4 while 100% (34/34) had normal CL's following injection of 20 mg P_4 immediately before ram introduction.

The injection of P_4, however, did not affect the induction of ovulation. While 95% (130/136) of the anestrous ewes ovulated, a low proportion displayed behavioral estrus after the treated wethers were introduced (47% or 92/196).

Also, many ewes that did not display estrus also failed to ovulate again (70% or 37/53). Again, many ewes do not show estrus even though they ovulate in response to teasing and a large proportion returns to anestrous after one induced cycle.

Pearce et al. (1985) divided a flock of anovulatory Merino ewes into three treatment groups: one group received a single 20 mg injection of P_4, the second group received the injection of P_4 plus a series of GnRH injections which mimicked the ram induced preovulatory LH surge, and the third group received no hormone treatment. All ewes were then exposed to rams. The frequency of CL's with short life spans (5.1 ± 0.9 d) was 72% for control ewes, 58% for ewes treated with P_4 and GnRH and 0% for ewes receiving P_4 alone. The injection of P_4 alone also delayed the preovulatory surge of LH by 45.8 h. The progesterone alone mimiced a CL which inhibited a rise in LH until progesterone levels subsided. In ewes receiving P_4 and GnRH, the GnRH caused increased LH resulting in ovulation. The presence of the injected progesterone still primed the ewes's system for normal CL development. The control group had many short CL's due to lack of a prior progestational phase and its priming effect. This study supports the theory that P_4 assures normality of corpora lutea by lengthening the period of gonadotrophin priming of follicles before ovulation.

Intravaginal sponges can also be used to increase the number of normal CL's and the incidence of estrus at first ovulation. Cognie et al. (1982) treated anestrous ewes with either intravaginal sponges impregnated with 30 mg fluorogestone acetate for 12 d prior to ram exposure, a 20 mg P_4 injection just prior to ram exposure, or ram exposure only. Of the 35 Merino ewes in each group 34, 32, and 27 ovulated in response to rams, respectively. However, 34 of 34 ewes which ovulated exhibited estrus at the first ovulation when treated with the sponges while only 7 of 32 and 5 of 27 showed estrus at first ovulation in the P_4 injection and control ewe groups, respectively. Hence, while the injection limits the number of short CL's, longer P_4 pretreatment is needed to assure behavioral estrus at the first ovulation. It is not known whether the injection fails to increase incidence of estrus at the first ovulation due to failure to produce sufficiently high concentrations of P_4 or a sufficient period of elevated P_4.

Martin (1979) checked the effects of a 100 µg injection of estradiol just prior to ram introduction on the incidence of short cycles or behavioral estrus. Estradiol had no effect on expression of estrus (10/33 vs 7/33 in controls) or the frequency of short cycles (16/33 vs 15/33 for controls). Thus, the lack of estrus and formation of a CL with a short

life span are apparently not due to a deficiency of estradiol.

In conclusion, the ram effect appears to be variably effective in stimulating anestrous ewes to ovulate. Factors adding to variation of results are ewe breed, ram breed, time of year, period of isolation from rams and probably nutrition and time of lambing, weaning and mating. Ewes which ovulate may develop a short-lived CL and(or) a silent ovulation following ram exposure due to the lack of a progestational phase prior to ram-induced ovulation. Also, a significant proportion of ewes may not respond at all to teasing or may exhibit only one or two cycles before returning to anestrous. Oldham and Cognie (1980) found that in both France and Western Australia, approximately 50% of successfully teased ewes (those which ovulated in response to rams) returned to anestrous before the start of the natural breeding season. The closer the teasing is to the onset of natural cyclicity, the more likely the ewe is to go anestrous after teasing, although this phenomenon has not been well documented. There must be a limit to this effect, however, since the onset of estrous activity can be hastened with ram exposure in the late summer. Hence, the ram effect is a partly unpredictable yet still useful tool.

Breed Differences in Breeding Season

Many studies have documented differences among breeds and crosses in their ability to cycle and mate throughout the year. Differences between breeds can probably be traced in part to the latitude of origin of a particular breed. The more temperate breeds tend to exhibit a shorter estrous period than the equitorial breeds. No attempt will be made in this review to explain why a given breed has a particular breeding period; instead, studies documenting breeding season and estrous characteristics will be cited and reviewed.

Hafez (1952) extensively reviewed the literature on the breeding season of ewes up until that time. He pointed out that in general, maximum sexual activity of sheep occurs in the autumn and early winter months irrespective of the hemisphere. This period in domestic breeds coincides with the primitive breeding season of their wild ancestors and allows for lambs to be born in spring and early summer. This pattern should result in maximum survival of lambs due to favorable forage and weather conditions during periods when the young are most vulnerable. Since Hafez reviewed what was known on the breeding of the ewe in 1952, many researchers have investigated the mating characteristics of specific breeds and their crosses.

Generally, studies of this type have been carried out either in the presence or absence of rams (through isolation

of ewes) throughout the measurement of breeding season because of possible confounding effects of ram introduction after the study has been initiated. Jenkins and Ford (1982) compared Finnish Landrace (Finn) crossbred ewes with purebred Morlam ewes by daily exposure to vasectomized rams equipped with marking harnesses. Finn crosses appeared to initiate estrous activity later in the fall than Morlam ewes, but continued cycling later in the spring. A greater number of Morlam ewes exhibited estrous activity in August (30% vs 4%) but more Finn cross ewes exhibited behavioral estrus in February through May (90 vs 70% in February decreasing to 46% vs 8% in May). The number of ewes cycling in the remaining months did not differ. Mean date of first estrus was September 6 for Morlam ewes and September 19 for Finn cross ewes. Mean date of last estrus was March 13 for Morlam and April 19 for Finn ewes. Hence, Finn ewes showed estrous activity for 211 d vs 187 d for Morlam ewes.

Dufour (1974) used three breeds (Dorset, Leicester and Suffolk) and a crossbred line from these breeds (DLS; 50% Dorset, 25% Leicester and 25% Suffolk) in a Canadian study of length of breeding season. Ewes in this study were also exposed daily to vasectomized rams wearing marking harnesses. Dorset ewes showed their first estrus on a mean date of August 8 and their last estrus on March 1. Leicester ewes started cycling September 13 and ended on February 16. The

breeding season for the Suffolk ewes ran on average from September 16 to January 24. The crossbred ewes cycled from July 28 through March 11. Hence the Dorset and the three-way cross started cycling earlier and ended cycling later than the other two breeds. The duration of estrous activity was 226.6, 206.4, 157.2 and 131.6 d for the DLS, Dorset, Leicester and Suffolk ewes, respectively. All ewes cycled during September through January in the DLS, during September through November in the Dorsets and Suffolks and during October through January in the Leicesters. Only the Dorset group had some percentage of ewes cycling in every month of the year (lowest was 10.5% in May).

Phillips et al. (1984) monitored the breeding season of Polled Dorset ewes in Australia for 16 mo using laparoscopic observations. The peak time of ovarian activity was in June when 93.6% of the ewes were cycling. As was seen with Dufour (1974), at least 10% of the ewes were cycling in every month of the year. October (spring in the Southern Hemisphere) appeared to be the month of least ovarian activity.

Lamberson and Thomas (1982) bred Suffolk ewes to North Country Cheviot, Dorset, Finn, Romney and Suffolk rams and checked estrous activity in the F_1 crossbred ewes using vasectomized rams and laparoscopy. Results indicated a significant breed-type x month interaction for incidence of estrus. Finn-sired ewes stopped cycling later in the spring

54

than ewes of all other breeds and the Cheviot-sired ewes had the longest anestrous. May and June were the months of lowest incidence of estrus across all breeds (8% and 2%, respectively). In October, 100% of the ewes of all breeds exhibited behavioral estrus. Only the Finn-sired ewes showed estrus in May (40%).

Quirke et al. (1985) monitored the ovarian activity of maiden ewes of four breeds and one cross by using the progesterone concentration of weekly blood samples and daily exposure to vasectomized rams. The mean number of days from first to last estrus for these ewe lambs was 127 for Finn, 131 for Finn x Dorset crossbreds, 87 for Dorset, 77 for Suffolk and only 34 for Rambouillet. As seen in previous studies, Finn ewes cycled later into the spring than ewes of the other breeds. The extended breeding season of the Finn and Finn x Dorset lambs was due to the fact that they cycled well into the month of February, several weeks later than the cessation of sexual activity in the other mating combinations.

Targhee ewes in Wisconsin showed high estrous activity from September through February (at least 91% ewes cycling throughout) in two consecutive years in the continuous presence of vasectomized rams. At least 10% showed estrus in every month from November, 1965 to October, 1967 (Mullampati et al., 1971). In another Wisconsin study, Lax et al. (1978)

monitored the length of the breeding season for eight breed groups: Texas Rambouillets, Montana Rambouillets, Wisconsin Hampshires, Beltsville Hampshires, Suffolks, Polled Dorsets, Targhees and Columbias. All breeds showed relatively low incidences of estrus in May through August. The Wisconsin Hampshires, Texas Rambouillets, Suffolks and Dorsets had similar levels of estrous activity over the year and were more sexually active than the Beltsville Hampshires, Columbias, Montana Rambouillets, and Targhees (listed in decreasing order of activity). June and July were the months of minimal estrus activity (4% and 0% of total ewes showing estrus, respectively).

Hulet et al. (1974) monitored Rambouillet ewes from both Idaho and Texas in each of the two locations. After one year of acclimatization, all the ewes showed estrus in Idaho from October through February with May through August being the months of least activity. In Texas, ewes showed the most estrus activity in July through January with March through May as the period of least estrus activity. This study shows the effect of geographical location on breeding season probably mediated through photoperiodic response. Hence the breeding season of a breed or cross can significantly interact with the location at which the sheep were observed.

Wheeler and Land (1977) monitored the breeding season of Finn, Merino, and Scottish Blackface ewes for 15 mo using

vasectomized rams, laparoscopy, and twice weekly blood samples which were then assayed for P_4. Finn ewes had a median date of October 7 for their first estrus and a median date of May 10 for their last estrus. Merino ewes had a breeding season of September 5 through February 14 while Blackface ewes showed estrus from October 9 through February 20. Hence, duration of the breeding season was 215, 162, and 141 days for Finn, Merino, and Blackface ewes, respectively.

In another Canadian study, Jeffcoate et al. (1984) studied the breeding season in five breeds in Saskatchewan. These researchers defined breeding season as the number of days between the first day and the last day that 80% of the ewes were cycling and showing estrus. Rambouillet ewes first showed estrus on September 6 and stopped on February 10 for a breeding season of 157 d. Columbia ewes had a breeding season of 154 d (September 6 through February 7). The Suffolk ewes had a considerably shorter breeding season of less than 126 d. These ewes began cycling September 28 and stopped sometime before February 1. Two crossbred groups were included in this study, Rambouillet x Finn and Columbia x Finn. The former group showed a breeding season of 210 d (August 19 through March 17) while the latter cycled for approximately 217 d (August 22 through March 27). We see in this study that the two crossbred groups exceeded any of the purebred groups in length of breeding season.

Different breeds and crosses clearly differ in length of the breeding season, and the season is not centered around the same day for all breeds. Hence, ewes of some breeds start and stop cycling in the presence of different amounts of daylength than do other breeds. Webster and Haresign (1983) demonstrated this result with P_4 samples taken from Dorset Horn and Welsh Mountain ewes throughout the year. Dorset ewes started cycling July 13 and ended April 3 for a total breeding season of 265 d while the Welsh ewes did not start cycling until October 12 but continued until April 11 for a total of 182 d. Hence, the mid-breeding season date was November 22 for Dorsets and January 10 for Welsh Mountain ewes.

In a French study, Romanov ewe lambs began cycling August 28 while Solognote ewe lambs began August 30. However, the Solognate ewes stopped cycling earlier (December 24) than the Romanov ewes (February 18). Hence, the duration of the first breeding season was 174 d for Romanov ewe lambs and 116 d for the Solognate. The following year the Romanov's began cycling August 1 and the Solognates on July 22 indicating that the second and subsequent breeding seasons are probably longer than the first.

A recent Australian study monitored the breeding season of Poll Dorset ewes continually exposed to rams (Hall, 1986). Four of these ewes (7% of total) cycled continuously throughout the 15 mo of the study. Except for these four

ewes, the average end of the breeding season was September 24 and the average start was December 7 (June 7 to March 24 breeding season corrected to northern hemisphere). The average breeding season duration for these ewes, counting the four which cycled continuously was 294 ± 6.1 d.

The studies reviewed in this section give some idea of the great variability in the timing and length of the breeding season of domestic sheep breeds. Besides actual genetic differences between the breeds, geographical location plays an important role through daylength differences and its effect on a ewe's reproductive ability. When comparing the different breeds and crosses, the length of the breeding season is not symmetrical around the same Julian date in all breeds. Rather, some breeds continue cycling later into the spring (i.e., Finn) while others cease cycling early but reinitiate cycles in early summer (i.e., Rambouillet). There appears to be some heterosis for breeding season length, although not many studies have addressed this directly. Overall, when continually exposed to rams, the whitefaced breeds (especially Finn, Merino, and Dorset) appear to have longer breeding seasons than do the blackfaced breeds (i.e., Suffolk, Hampshire).

While the studies reviewed so far have dealt with elucidation of natural breeding season, many other studies have dealt with testing ewes of different breeds for their ability

to mate and(or) conceive during periods usually considered to be outside the normal breeding season. Whiteman et al. (1972) compared Dorset, Rambouillet and Dorset x Rambouillet ewes for ability to lamb in spring vs fall (i.e., mid-October through mid-December breeding vs mid-April through mid-June breeding). In spring lambing, Rambouillet (89% ewes lambing) were superior to both Dorset x Rambouillet (85%) and the Dorset ewes (74%). In contrast, in fall lambing the crossbred ewes (39%) outperformed both Dorset (37%) and Rambouillet (29%) ewes. This study would indicate a possible heterotic effect for out-of-season breeding ability.

Thomas and Whiteman (1979) compared the effects of substitution of 1/4 Finn or 1/4 Dorset for Rambouillet breeding in fall-lambing ewes in Oklahoma. Ewes that were 1/2-Dorset, 1/2-Rambouillet had 82.4% fertility while 1/4-Dorset, 3/4-Rambouillet ewes had 78.9% fertility. Thus, an increase in Dorset breeding increased spring fertility. Ewes that were 1/4-Finn, 1/2-Dorset, 1/4-Rambouillet were 63.1% fertile compared to 59.2% for 1/4- Finn, 1/4-Dorset, 1/2-Rambouillet ewes. Again, the replacement of Rambouillet breeding with the Dorset breeding resulted in higher fertility in the spring. Likewise, use of 1/4-Dorset breeding at the expense of 1/4-Finn also increased spring fertility. Contrary to expectation, 1/4 Finn breeding at the expense of 1/4 Rambouillet resulted in lower spring fertility.

Notter and Copenhaver (1980) compared 1/2 Finn, 1/2 Rambouillet; 1/4 Finn, 3/4 Rambouillet; and 1/2 Suffolk, 1/2 Rambouillet ewes for April conception rates in an accelerated lambing system (lamb three times in 2 yr). Seventy-one percent of the 1/2 Finn ewes conceived compared with 51% for the 1/4 Finn. So, results in the Virginia study were opposite to those in Oklahoma (Thomas and Whiteman, 1979). Substitution of 1/4 Finn breeding for 1/4 Rambouillet breeding increased April conception rates, a more logical result according to previously reported studies on the comparative breeding season of Finn and Rambouillet ewes. The Suffolk x Rambouillet ewes showed a 38% April conception rate, which is not surprising because April is a time of low estrous activity for both Suffolk and Rambouillet ewes.

Notter and McClaugherty (1984) observed high summer conception rates (89%) in crosses composed of breed types not normally considered to be cycling at this time. Mature 1/2-Suffolk, 1/2-Rambouillet and 1/2-Suffolk, 1/4-Finn, 1/4-Rambouillet ewes were bred in June through August and about half of the ewes conceived in June. There was no difference among breed types.

Clarke et al. (1984) observed April pregnancy rates for yearling ewes of five mating types in Virginia. Pregnancy rates were 78% for Finn ewes, 37% for Dorsets and only 12% for Rambouillet. Two crossbred groups, Finn x Dorset and

Barbados Blackbelly x Dorset had intermediate conception rates of 57%. Conversely, for mature ewes, April pregnancy rates were highest for the Blackbelly x Dorset cross ewes (88%). There were significant differences among remaining breed groups, although conception rates were still fairly high (65 to 73%). When these whitefaced ewe groups were compared with the blackfaced Suffolk and Hampshire breeds in June and July matings, 56.8% of the whitefaced group showed estrus compared with only 2% of the blackfaced ewes. Again, blackfaced ewes generally have low reproductive behavior in late spring and early summer.

In another experiment where ewes were mated three times in two years, Finn x Dorset Horn ewes in Scotland had higher conception rates (73%) in February than did ewes from a Border Leicester x Scottish Blackface cross (26%). The latter cross had an increased pregnancy rate in August (55%) but still fell below that of the Finn x Dorset cross ewes (82%) (Speedy and Fitzsimmons, 1977).

Bellinger and Mendel (1974) checked ovarian activity of Suffolk and Hampshire ewes in April and June by using cervical smears. In the absence of rams and under normal lighting conditions, four of six Hampshires but only one of six Suffolks cycled during these two months. Though the techniques were less than optimal and numbers very low, this study gives some indication that of the blackface breeds, the

Hampshire may be more likely to show estrus in the spring than the Suffolk.

In another accelerated lambing situation, Finn x Dorset ewes were mated four times in 2 yr (Land and McClelland, 1971). In summer breeding (August and September) 69% of yearling ewes showed estrus compared with 75% for mature ewes. Spring breeding (March and April) of yearlings showed 53% in estrus while 85% of mature ewes showed behavioral estrus. Thus, ewe age affects ability to mate out of season. Finally, in another situation where ewes were given the chance to breed and lamb twice in one year, mature Finn ewes in Canada showed a 61% conception rate in March and April (Walton and Robertson, 1974).

When comparing ewe breeds and crosses for ability to mate and conceive out of season, whitefaced breeds generally have much higher conception rates in these spring and early summer matings. Dorset and Finn ewes apparently have a very high ability to mate in spring, especially compared to Suffolk or Hampshire ewes. The Barbados Blackbelly also appears to have good potential for out-of-season breeding. Age of ewe also seems to have a major effect whereby yearling ewes have a reduced ability to breed out of season but as they mature these same ewes can have increased cyclicity in the spring depending upon breed. Also geographical location elicits an effect on any study measuring out-of-season

breeding due to different photoperiodic effects in different locations. Overall, the genetic differences in ability to cycle and mate out of season in ewes is readily apparent and demonstrable.

The seasonal effects on reproductive ability in rams is much less pronounced than in ewes but still is present with genetic differences between breeds. While ewe groups show a more discrete cyclic or non-cyclic state, rams generally show decreased libido and fertility in late spring and early summer yet still usually retain the ability to sire some lambs at any time of year. Mickelsen et al. (1981) observed scrotal circumference and sperm motility in Suffolk and Lincoln rams. Scrotal circumferences were highest in October and lowest in February for both breeds. Similarly, the percentage of morphologically normal sperm was highest in October (92%) and lowest in February (57%). Percentage motile sperm was highest for Suffolk rams in June (81%) and lowest in February (61%) and highest for Lincolns in August (87%) and lowest in February (67%). Overall in this study both breeds were very similar, although slight differences did exist.

Ducheux et al. (1981) studied four French breeds and crosses (Ile-de-France, Romanov, Prealpes du Sud and Romanov x Ile-de-France or x Prealpes du Sud) for spermatozoa production and secretion of rete testis fluid. Results indi-

cated that seasonal variation in daily sperm production of the testis was more pronounced in the Ile-de-France breed. Sperm concentration and production increased from nonbreeding to breeding season for all groups except Romanov, which had a constant sperm concentration. The crossbred group exceeded all three breeds in sperm concentration during the breeding season.

Barrell and Lapwood (1979) reported that semen from Romney, Merino, and Polled Dorset rams all showed regular seasonal changes in ejaculate volumes with peak values being observed during March (New Zealand study). The Polled Dorset rams were less seasonally variable in seminal plasma fructose concentration and concentration of spermatozoa per ejaculate than rams of the other two breeds. This result is in accord with the reduced seasonality of Dorset ewes relative to many other breeds.

Finally, Schanbacher and Lunstra (1976) demonstrated that exposure of a ram to estrus-induced ewes within an observation pen can be used to calculate a libido and mating index for a particular breed. The indices involved amount of time the ram spent sniffing, mounting, and engaging in intercourse with the ewes. Finnish Landrace and Suffolk rams were compared using this method every 2 mo for 1 yr as a measure of changing sexual drive and aggressiveness. The Finn rams exceeded the Suffolks in both indices at all months

of the year. Finn rams showed their highest libido index in January through March while Suffolk rams were highest in October. This study shows that inherent breed differences in willingness to mate are also present.

Overall, breed differences are clear in libido, semen quality, and scrotal circumference. Logically, ram breed differences should parallel those of ewe breed differences with whitefaced rams being more sexually active and competent than blackfaced rams in the spring. Again, age, geographical location, and nutrition would probably have some impact on any study addressing this point.

EFFECTS OF EWE BREED AND RAM EXPOSURE ON
ESTROUS BEHAVIOR IN MAY AND JUNE

Materials and Methods

<u>Animals</u>. Fifty purebred Hampshire (H) and 50 purebred
Dorset (D) ewes were used in this study. Dorset ewes ranged
in age from 1 to 12 yr and averaged 3.82 yr with a standard
deviation (SD) of 2.37 yr. Hampshire ewes ranged in age from
2 to 8 yr and averaged 4.24 yr (SD = 1.88 yr). The two breeds
did not differ significantly in age (P>.10 by t-test).

All 100 ewes lambed during the winter preceding the
study. Dorset ewes lambed between January 1 and February 15,
with an average lambing date of January 26 ± 2 d. Hampshire
ewes lambed between January 1 and February 11 with an average
lambing date of January 18 ± 1 d. Thus H ewes lambed earlier
(P<.01) than D ewes. Lambs were weaned on either April 11
or April 18, 1985. Forty-three H ewes had lambs weaned on
April 11 and 6 had lambs weaned on April 18. Twenty-nine D
ewes had lambs weaned on April 11 and 20 had lambs weaned on
April 18. Lambs from one D and one H ewe died before weaning.

Ewes were held on open pasture at either the Virginia
Tech Sheep Center or the Moore Barn on Price's Fork Road,
Blacksburg, VA (37 15' N latitude and 80 27' W longitude).
These facilities are separated by approximately 4 km.
Pasture at both locations was predominantly fescue. Water
was provided ad libitum and no supplemental feed was given.

The animals were sheared 1 wk prior to the onset of the study. All ewes were weighed and condition scored at the beginning (May 8) and end (July 15) of the study. Condition scores were based on a subjective scale of 1 to 9 with a score of 1 indicating an extremely thin ewe and a score of 9 indicating an extremely fat ewe. Scores were determined by palpation over vertebrae, ribs and tailhead.

Experimental Design. At the beginning of the study the 100 ewes were divided into two groups designated A and B. Each group consisted of 25 D and 25 H ewes. Ewes within each breed were allocated to treatments such that lambing dates were approximately equalized between the groups. Hence, the ewes were listed in chronological order of lambing date and every other ewe was put in group A. The remaining 25 ewes in each breed were assigned to group B.

On May 8, ewes of group A (held at the Virginia Tech Sheep Center) were exposed to three vasectomized teaser rams. Rams were picked from a group of vasectomized rams available from the sheep center. Breeding of the rams varied; Finnsheep, 1/2 Dorset x 1/2 Blackbelly, Rambouillet, and 1/2 Coopworth x 1/2 Dorset rams were available. Rams were equipped with crayon-equipped marking harnesses so that colored marks on the rumps of ewes could be recorded as an indication of mating activity. Colors of the crayons were changed every 17 d so that mating at consecutive estruses

could be detected. The use of vasectomized rams allowed for detection of behavioral estrus in ewes by mating but without the interference of pregnancy on the ewe's subsequent estrous activity.

On May 8, the ewes of group B were taken to the Moore Barn. These ewes were isolated from rams. No rams were kept within 1.9 km of the group B ewes while they were located at the Moore Barn.

Ewes remained at these locations from May 8 until June 11, 1985. Every Tuesday and Friday throughout this period the ewes of both groups were bled via jugular venipuncture and group A ewes were checked for incidence of mating. Crayon marks on the rumps of ewes were evaluated for intensity using a numerical scale of 1 to 3. A score of 1 indicated an intense mark such that the ewe clearly stood for the ram and was repeatedly mated. A score of 2 indicated a mark of lesser intensity whereby the ewe possibly allowed the ram to mount once. A score of 3 represents a small, inconclusive mark indicating a single attempt by the ram to mount a ewe that apparently did not stand. Hence, mark scores of 1 and 2 were generally considered to indicate that a ewe had mated.

Ewes were bled via the jugular vein using 10 ml evacuated blood collection tubes. Twenty-gauge, 2.54-cm needles were used and tubes had no interior coating except for a glycerin-coated stopper for easy removal. Actual amounts of

whole blood taken from the ewes ranged from about 5 to 8 ml. Ewes at the Moore Barn were always handled before ewes at the Sheep Center to prevent group B ewes from being exposed to the scent of rams that might have been transferred from the vasectomized rams to the isolated ewes via clothing and hands.

On June 11, 1985, group A ewes were removed from the vasectomized rams and trucked to the Moore Barn while group B ewes were in turn brought to the Sheep Center and exposed to the vasectomized rams. On Tuesdays and Fridays from June 11 to July 13 all ewes were again bled and group B ewes were checked for mating activity. Again, the isolated ewes were handled first.

Crayon colors on the ram harnesses were again changed every 17 d. The original vasectomized rams were replaced on May 24 and June 28 due to decreasing physical condition of the rams which had been with the ewes and to potentially provide additional mating stimulus from the introduction of new males.

Blood Handling and Hormone Assay. Immediately upon collection of blood, the tubes were stored together at room temperature and out of direct sunlight. The blood tubes were allowed to sit for approximately 1.0 h to allow samples to clot. Samples were then placed in an IEC Centra-7R centrifuge, cooled to 4 C and spun at 2800 revolutions/min

for 20 min. After centrifugation, plasma was decanted into 12 x 75 mm plastic tubes and frozen at -20 C for storage.

After all serum samples were collected, tubes were chosen at random and the plasma was assayed for progesterone (P_4) content using radioimmunoassay (RIA) techniques as described in Beal et al., 1986. All samples were run in duplicate and re-evaluated if final P_4 concentrations of the duplicates differed by more than 25% and .1 ng/ml. The intra-assay and inter-assay coefficients of variation were 11.4% and 13.7%, respectively. Exact procedures for the RIA are given in the appendix.

Progesterone Profile Interpretation. Generally, precise P_4 concentrations on a particular day were not required to evaluate the ovarian state of individual ewes. Rather, relative values within each animal from day to day gave the necessary information to determine whether a ewe was cycling or anestrus and whether cycles were of normal (17 d) length or shortened (4 to 7 d). There were three general types of P_4 profiles that were thought to be potentially distinguishable: uniform baseline values, short luteal phases, and normal luteal phases. Baseline P_4 values were considered to be those less than .2 ng/ml. Increases above baseline for three consecutive bleeding periods were considered indicative of a normal corpus luteum. These elevations returned to baseline 16 to 18 d after the closest previous baseline value

71

and were considered to represent a normal ovarian cycle of 16 to 18 d. Most ewes with premature regression of the corpus luteum (CL) exhibit a transient elevation in P_4 of 4 to 7 d before the prolonged P_4 elevation of a normal CL (Knight et al., 1981).

Statistical Analysis. Differences between breeds in frequency of occurrence of the various events (ovulation, mating, etc.) were tested by t-test. Binomial standard errors (SE) for each frequency were calculated as $\sqrt{p(1 - p)/n}$ where p is the frequency of occurrence of the event. The standard error of the difference (SE_d) between frequencies of D and H ewes was calculated as $\sqrt{SE_D^2 + SE_H^2}$. The t-statistic was calculated as $(p_D - p_H)/SE_d$ and compared to standard tabular values to determine significance.

For further evaluations of the P_4 profiles, each particular point was designated as being one of four types:

(1) B or baseline values that were generally less than .2 ng/ml with two exceptions where non-cycling ewes showed apparent baseline values of about .3 ng/ml,

(2) P or peak values that are the highest P_4 values observed during a normal 14 to 17 d elevation in serum P_4 concentrations. These values may not be the actual highest levels of P_4 reached during a particular cycle but they are assumed to be equally representative between the breeds,

(3) T or transient rise points are peak P_4 levels achieved other than during a normal 14 to 17 d luteal period and most likely represent levels during short 7 d luteal phases and

(4) P_4 values designated as N which are those which do not fit any of the other three categories.

After each particular P_4 concentration for a given ewe was tagged with B, P, T, or N, an analysis of variance for P_4 values was run for each type of point using the model:

$$Y_{ijklm} = \mu + B_i + G_j + BG_{ij} + E(BG_{ij}) + R_1 + BR_{il} + GR_{jl} + e_{ijkilm}$$

where $Y_{ijklm} = P_4$ concentration

B_i = breed of ewe; i = 1 = Dorset; i = 2 = Hampshire

G_j = group of ewe; j = 1 = A; j = 2 = B

$E(BG_{ij})_k$ = ewe within breed x group

R_l = whether the P_4 value was in the presence of a ram;

1 = ram present; 2 = ram not present

e_{ijklm} = random error

$B_i, G_j, and R_l$ were fixed effects while $E(BG_{ij})_k$ was assumed random. Effects of breed, group and their interaction were tested with the between-ewe mean square. All other effects were tested with residual error.

Analysis of variance for P_4 concentration using the specified model was only run for baseline and peak values. Due to bleeding regimen and resulting difficulty in definitely identifying short luteal phases, the analysis for

transient peak levels was not considered meaningful. Generally, these peaks were indicated by only one elevated P_4 level. Similarly, analysis of N values would be meaningless.

Results

Frequencies of Ovulation and Estrus. Frequencies of
ovulation and estrus are shown in tables 1 and 2 and in fig-
ures 1 through 4. In group A, more D ewes ovulated (P<.01)
in May and more of the ewes that ovulated mated (P<.01).
Hence, many more D ewes than H ewes mated in May (P<.01).
Ewes which first mated after 17 d of ram exposure were con-
sidered to have been induced to mate by the ram effect. Ewes
responding to this effect generally have a silent ovulation
followed by a normal ovulation with estrus some 17 to 24 d
later (see review of literature). Of the ewes which mated
in group A in May, there was no significant difference be-
tween breeds in frequency of induced matings. Thus, D ewes
were inherently more sexually active than H ewes during May.
However, when the ram was exposed to non-cycling ewes of each
breed (i.e., those which were baseline prior to ram intro-
duction), the response was equal. No H ewes which mated in
May ovulated in June after rams were removed, however, 65%
of the D ewes continued cycling after ram removal. Appar-
ently, the H ewes which responded to rams exhibited one or
two estrous cycles and then returned to anestrus.

Group B ewes showed similar differences in reproductive
activity between breeds to group A ewes except that in June
a somewhat larger proportion of H ewes ovulated and mated.
Before rams were introduced on June 11, almost half (44%) of

TABLE 1.

OVULATION AND ESTRUS IN EWES EXPOSED TO RAMS IN MAY

Item	Dorset	Hampshire
Ovulated/total	24/25 (96%)	18/25 (72%)*
Mated/ovulated	20/24 (83%)	5/18 (28%)*
Mated/total	20/25 (80%)	5/25 (20%)*
Induced/mated[a]	9/20 (45%)	4/5 (80%)
June ovulation/May mated	13/20 (65%)	0/5 (0%)*

[a]Includes only those ewes that first mated after 17 d of ram exposure and were therefore assumed to have been induced to mate by the ram effect.

*Breeds differ (P < .01).

TABLE 2.

OVULATION AND ESTRUS IN EWES EXPOSED TO RAMS IN JUNE

Item	Dorset	Hampshire
Ovulated in May/total	11/25 (44%)	2/25 (8%)*
Ovulated in June/total	23/25 (92%)	21/25 (84%)
Mated/ovulated	18/23 (78%)	11/21 (52%)**
Mated/total	18/25 (72%)	11/25 (44%)**
Induced/mated[a]	11/18 (61%)	11/11 (100%)*

[a]Includes only those ewes that first mated after 17 d of ram exposure and were therefore assumed to have been induced to mate by the ram effect.

*Breeds differ (P < .01).

**Breeds differ (P < .05).
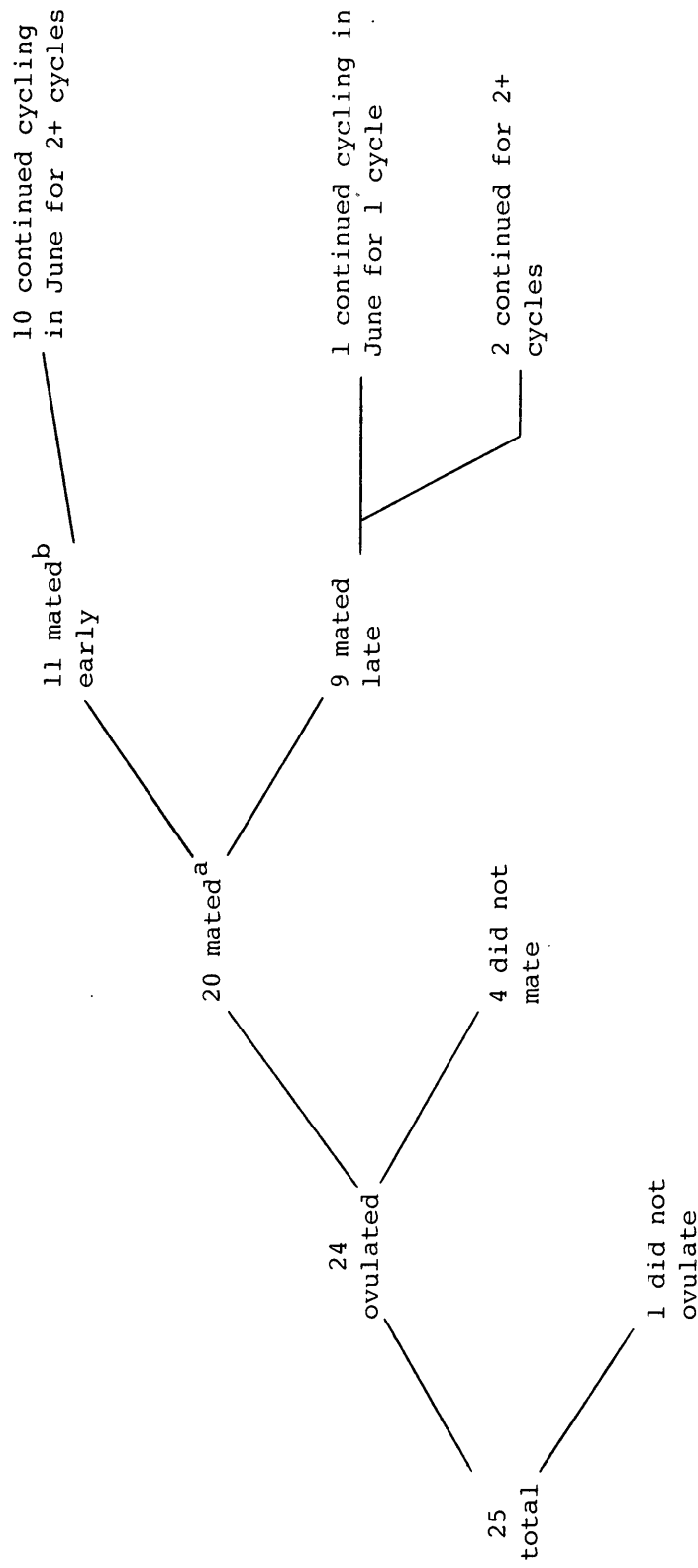
FIGURE 1. Dorset - group A.

[a] mark intensity = 1 or 2.
[b] within the first 17 d of ram exposure.

25
total

24
ovulated

1 did not
ovulate

20 mated[a]

4 did not
mate

11 mated[b]
early

9 mated
late

10 continued cycling
in June for 2+ cycles

1 continued cycling in
June for 1 cycle

2 continued for 2+
cycles

0 continued
cycling in June

1 mated[b]
early

4 mated late

5 mated[a]

13 did not
mate

18 ovulated

7 did not
ovulate

25
total
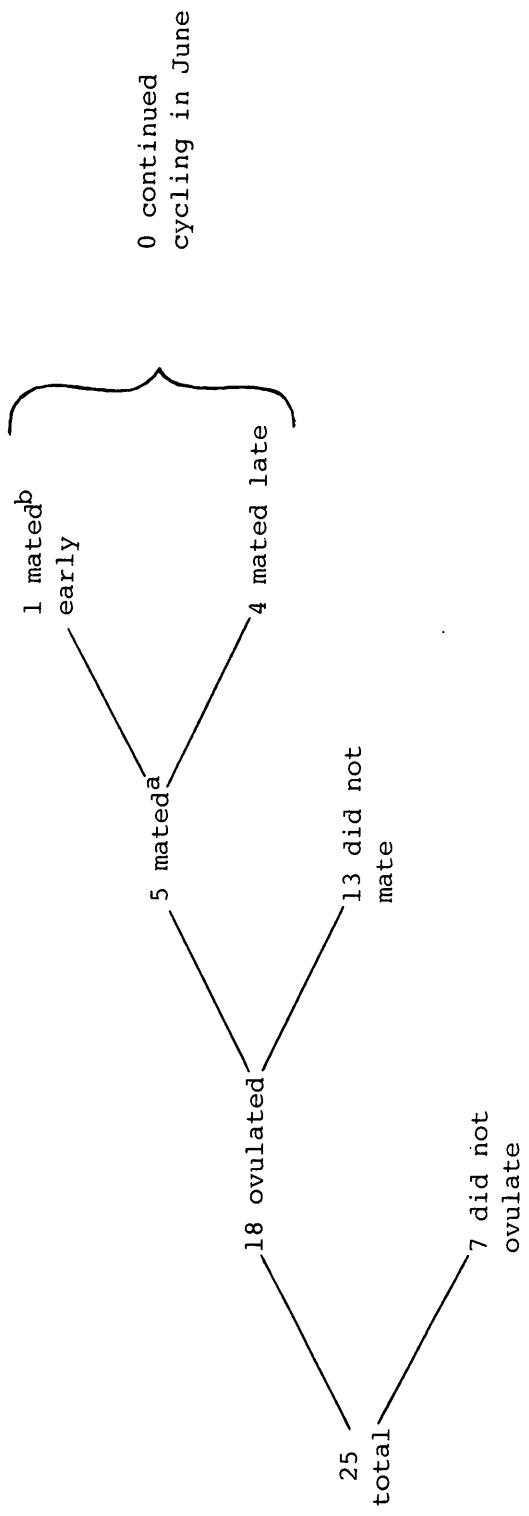
FIGURE 2.   Hampshire - group A.

[a]mark intensity = 1 or 2.
[b]within the first 17 d of ram exposure.
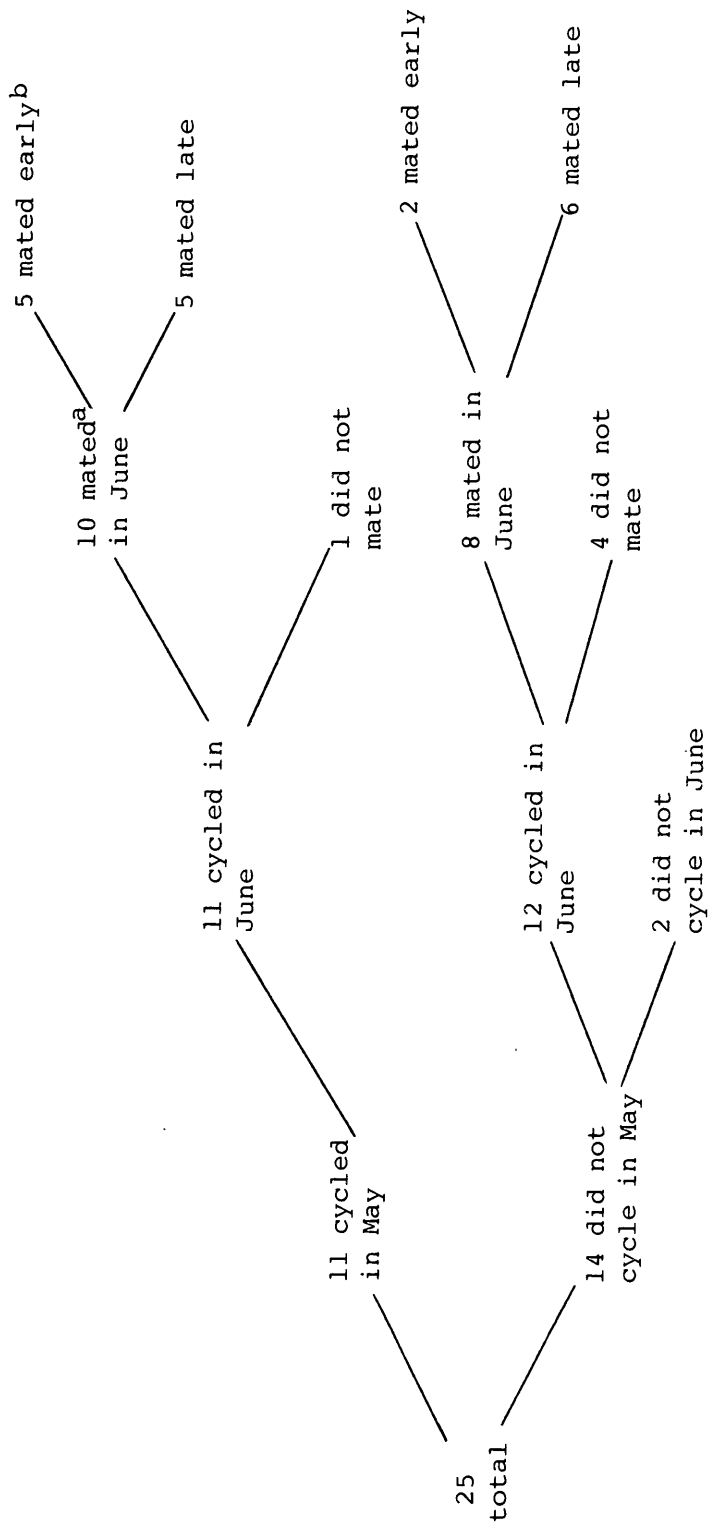
FIGURE 3. Dorset - group 2.

[a] mark intensity = 1 or 2.

[b] within the first 17 d of ram exposure.

25
total

2 cycled
in May

1 cycled in
June

1 mated in[a]
June

1 mated late[b]

23 did not
cycle in May

20 cycled
in June

10 mated
in June

10 mated late

10 did not
mate

3 did not
cycle in
June

FIGURE 4. Hampshire - group 2.

[a]mark intensity = 1 or 2.

[b]mated first after at least 17 d of ram exposure.

the group B D ewes had ovulated. Only 8% of the H ewes ovulated in May in the absence of the ram. After rams were introduced a large proportio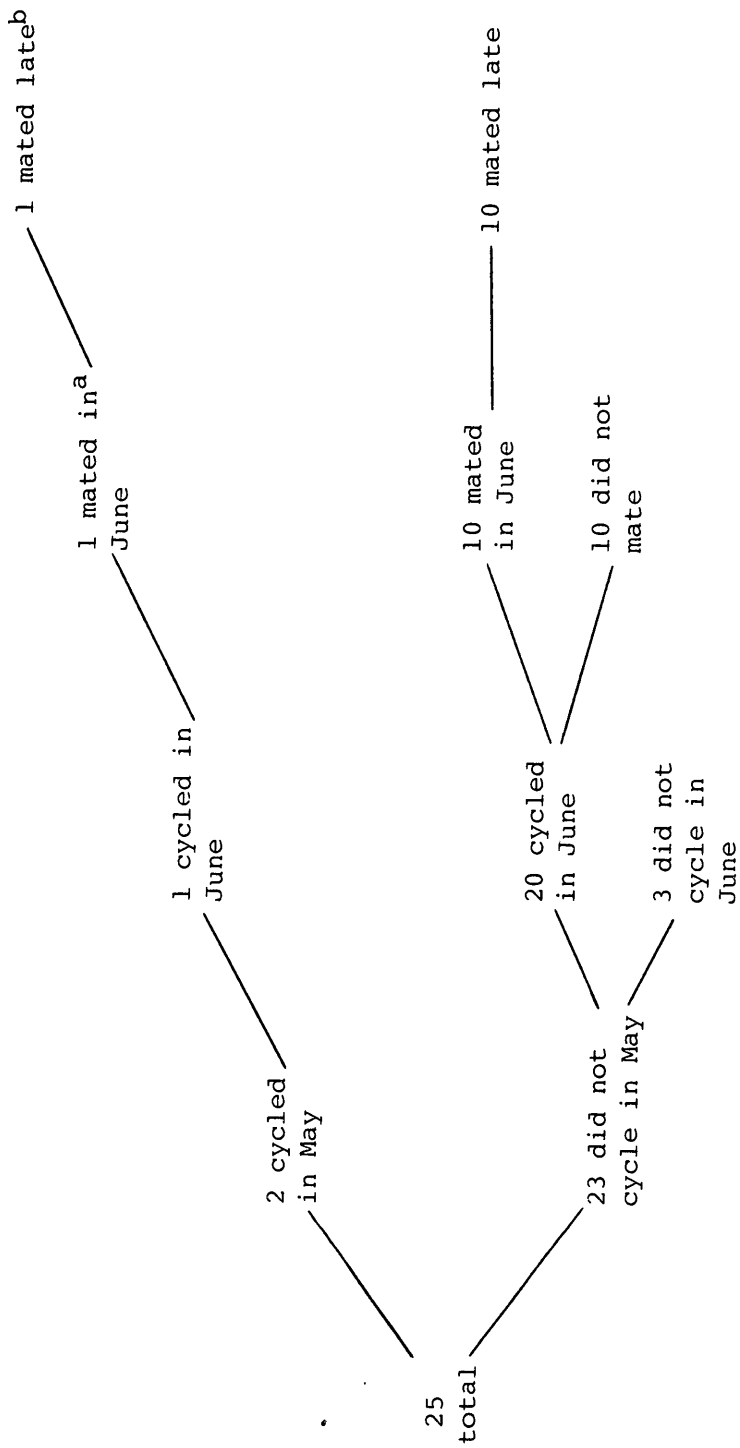n of the ewes of each breed ovulated. Also, the percentage of H ewes that mated (of those ovulating) was nearly double the amount observed in May. So compared with May, where most of the H ewes that ovulated had silent ovulations and did not show standing estrus, the ewes in June had ovulations that were accompanied by estrus. About the same percentage of D ewes mated in each group but again over twice as many H ewes mated in June compared to May. All the H ewes that mated in June were apparently induced to mate by the ram effect. Thus, there appears to be a trend toward increased estrous activity in June for H ewes whereas the D ewes were fairly consistent over the two months. The increased June activity of the H ewes may indicate that these ewes were approaching the onset of their natural breeding season and were more easily stimulated by the rams. This would lead one to believe that the anestrus in the Hampshire is deeper in mid-to-late spring than in early summer.

Analysis of Serum Progesterone. The analysis of variance and means for baseline P_4 concentrations (tables 3 and 4) reveals that the only factor that significantly affected baseline P_4 levels was the random ewe effect. Most of the baseline P_4 points were from anestrus ewes, so it appears

TABLE 3.
ANALYSIS OF VARIANCE FOR
BASELINE PROGESTERONE CONCENTRATION

Source	df	MS	F-value
Breed	1	0.00001085	0.0105
Group	1	0.00132200	1.2812
Breed x group	1	0.00142406	1.3800
Ewe (breed x group)	96	0.00103186	5.0100**
Ram	1	0.00034674	1.6800
Breed x ram	1	0.00025270	1.2300
Ram x group	1	0.00012783	0.6200
Error	94	0.00020580	–

**$P < .01$.

TABLE 4.
MEANS AND STANDARD ERRORS FOR
BASELINE AND PEAK PROGESTERONE CONCENTRATIONS (ng/ml)

	Baseline P_4	Peak P_4
Dorset	0.0623 ± 0.0022	1.9029 ± 0.0629
Hampshire	0.0597 ± 0.0010	1.8308 ± 0.1239
Group A	0.0636 ± 0.0018	1.7556 ± 0.0711
Group B	0.0581 ± 0.0011	2.0613 ± 0.0899
Ram present	0.0649 ± 0.0020	1.9143 ± 0.0680
Ram absent	0.0585 ° 0.0012	1.7739 ± 0.0959
Overall	0.0607 ± 0.0010	1.8825 ± 0.0570

that the amount of P_4 in the serum of anestrus ewes varies among individuals. Breed of ewe did not influence baseline P_4 levels.

Means and analysis of variance for peak height concentration (tables 4 and 5) showed that some effects in the model did contribute significantly to variation in peak P_4. Ewes in group B had higher mean peak concentrations than ewes in group A. This may be the result of two confounded factors: the first is that more H ewes seemed to respond to rams in June than in May (see percentage H ewes mating in tables 1 vs 2). The H ewes may have been more responsive in June because the upcoming breeding season was closer. It appears from this data that once a ewe is cycling, P_4 production is greater than at the initiation of cyclic activity at the beginning of a new breeding season or during a short 4 to 7 d CL phase. Ewes which ovulated just once in response to rams (14 ewes) had lower peak P_4 values than did all other ewes which exhibited peaks (63 ewes) ($P<.05$). A second factor which affected peak heights was presence or absence of a ram ($P<.25$). In the presence of rams, the peak heights were greater. It appears that the ram is having an effect on hormone levels, but bleedings were too infrequent to allow clear conclusions to be drawn. The fact that levels were higher in the presence of a ram seems intuitively correct. Finally, again, the ewe herself influenced the peak P_4 level.

TABLE 5.
ANALYSIS OF VARIANCE FOR
PEAK PROGESTERONE CONCENTRATION

Source	df	MS	F-value
Breed	1	0.02409603	0.06
Group	1	2.01435385	4.72*
Breed x group	1	0.89809986	2.10
Ewe (breed x group)	74	0.42682273	2.28*
Ram	1	0.49287958	2.64
Breed x ram	1	0.01508671	0.08
Ram x group	1	0.00130433	0.01
Error	21	0.18701988	

*P < .05.

**P < .01.

Some ewes would seem to have higher P_4 production from their CL's than do others.

Mean body weights, condition scores and lambing dates are shown in table 6. Mean weights of the ewes increased by 3.7 kg over the course of the study. This was generally expected because they were on spring pasture. Mean condition scores decreased during the study period but this may have been a reflection of the subjective nature of the scores which could be influenced by the overall condition of the flock. Generally, a particular condition score is only comparable to the condition of other animals scored at the same time.

Simple correlations among condition scores, weights at both the beginning and end of the experiment, lambing dates, and mean baseline and peak P_4 concentrations are shown in table 7. Weights and condition scores had significant positive correlations and lambing date and weight at the end of the study had a negative correlation that approached significance ($P<.10$). Hence, ewes which lambed earliest weighed the most at the end of the study. Correlations involving baseline P_4 were uniformly nonsignificant, but correlations of lambing date, weights, and condition scores with mean peak P_4 levels tended to be significant ($P<.03$ to $P<.1$) and were positive.

TABLE 6.
LAMBING DATE, WEIGHT AND CONDITION SCORE
MEANS FOR BLACKSBURG EWES

Variable	Mean
Lambing date, Julian day	21.8 ± 1.2
Weight 1, kg	71.7 ± 1.2
Weight 2, kg	75.4 ± 1.2
Condition score 1[a]	5.4 ± .1
Condition score 2[a]	5.1 ± .1

[a] Scores range from 1 to 9. One is very thin; 9 is very fat.

TABLE 7.
CORRELATION COEFFICIENTS[a]

Lambing date	Weight 1	Weight 2	Cond Sc 1	Cond Sc 2	Mpeak[b]	Mbase[c]
Lambing date –	-.086 (.399)	-.180 (.074)	.018 (.862)	-.031 (.763)	.215 (.059)	.127 (.207)
Weight 1 .205 (.046)	–	.944 (.001)	.572 (.001)	.451 (.001)	.243 (.033)	.058 (.567)
Weight 2 .130 (.207)	.908 (.001)	–	.457 (.001)	.439 (.001)	.200 (.086)	.112 (.272)
Cond Sc 1 .066 (.526)	.601 (.001)	.528 (.001)	–	.742 (.001)	.217 (.056)	.029 (.775)
Cond Sc 2 .041 (.689)	.404 (.001)	.458 (.001)	.723 (.001)	–	.156 (.173)	.132 (.192)
Mpeak[b] .256 (.027)	.292 (.011)	.308 (.007)	.264 (.022)	.182 (.117)	–	.112 (.330)
Mbase[c] .151 (.143)	.099 (.336)	.195 (.057)	.047 (.646)	.147 (.154)	.141 (.228)	–

[a]Above diagonal are simple correlations, below diagonal are residual (within breed, group and ram exposure) correlations. Significance of the coefficients is given in parentheses.

[b]Based on 77 ewes.

[c]Based on 98 ewes.

Residual correlations in table 7 are calculated for each variable holding breed and group constant. Again, positive significant correlations existed between mean peak P_4 levels, weight, and body condition ($P<.01$ to $P<.12$). However, residual correlation between lambing date and weight 2 was not significant. Hence, the negative simple correlation discussed above may have been due to the earlier lambing, heavier H ewes. Similarly, mean baseline P_4 values were correlated with weight 2 ($P<.06$). The lighter ewes at the end of the study had lower mean baseline values. One explanation for this is that lighter ewes were probably more likely to remain baseline throughout the spring (Hulet et al., 1986). Heavier ewes which cycled still had values for baseline P_4 concentrations but the twice weekly bleeding schedule may not have detected their lowest P_4 level of a particular cycle. In other words, baseline ewes were uniformly low so any bleeding day was an accurate representation of baseline P_4 concentrations, but a cycling ewe had heavily fluctuating P_4 levels and what we considered baseline values may have only been a level somewhere between a peak and actual baseline.

For purposes of further analysis, the two weights on each ewe were combined into a mean weight during the study and the same was done for the two condition scores to arrive at a mean condition over the experiment period. An analysis

of variance for mean peak P_4 concentration originally was conducted that included effects of breed, group, breed x group interaction, lambing date, mean body weight, and mean body condition score as sources of variation. Mean body condition score and the interaction were found insignificant in preliminary analysis and were dropped from the model. The resulting analysis of variance is shown in table 8.

As discussed earlier, the breed effect was nonsignificant while group had a significant effect on mean peak height. Lambing date had a positive significant correlation with mean peak height. Mean peak P_4 values increased by .0125 ± .0059 ng/ml for each 1 d later lambing. It is possible that later lambing ewes were more likely to cycle in response to the rams. Finally, the mean body weight had a significant positive relationship with mean peak value such that heavier ewes had higher peaks. The resulting regression coefficient was .010 ± .0038 ng/ml/kg.

Finally, ewes which mated (mark intensity = 1 or 2) were compared to ewes which did not mate (mark intensity = 3 or absent) for average lambing date within breed and group, (table 9) The t-test of average lambing date between ewes which marked and those which did not within a breed and group revealed that the H ewes which marked had lambed earlier regardless of their group. No significant effect was found for D ewes although the same trend was apparent. So, whereas the

TABLE 8.
ANALYSIS OF VARIANCE FOR
MEAN PEAK PROGESTERONE CONCENTRATION

Source	df	MS	P-value
Breed	1	0.3450	0.3110
Group	1	1.9418	0.0180
Lambing date	1	1.5031	0.0366
Mean body weight	1	2.2603	0.0110
Error	72	.3315	—

TABLE 9.
AVERAGE LAMBING DATES AND STANDARD ERRORS
FOR EWES THAT DID OR DID NOT MATE[a,b]

	Mated	Did not
Hampshire group[a]	9.2 ± 3.4 (5)	19.9 ± 2.1 (20)*
Hampshire group[b]	13.3 ± 2.4 (11)	21.5 ± 2.4 (14)*
Dorset group[a]	24.0 ± 2.9 (20)	28.0 ± 3.3 (5)
Dorset group[b]	24.2 ± 2.9 (18)	33.0 ± 4.5 (7)

[a] Numbers in parenthesis indicate number of observations.
[b] Julian date.
*Means within a row differ (P < .05).

later lambing ewes had higher peak P_4 values, the earlier lambing ewes were more likely to show behavioral estrus in the presence of the ram. The earlier lambing ewes may have had less effects of lactation on their ability to cycle by the time rams were introduced.

Progesterone Profile Analysis. The assessment of bi-weekly serum P_4 concentrations throughout the study revealed that the P_4 patterns of the Dorset and Hampshire ewes fell into one of nine categories (sample profiles of each category are given in the appendix). The nine groups of P_4 patterns and the percentage of ewes in each breed (50 ewes per breed) in each category is in table 10. Profiles for specific ewes (referred to parenthetically in the discussion) are available as a separate appendix from the author.

The baseline category encompasses those ewes whose P_4 values remained below .2 ng/ml throughout the study. These ewes were apparently in an anestrus state and did not respond to the introduction of rams. Ten H ewes but only 2 D ewes remained at baseline levels of P_4 throughout the 68 d of the experiment. Blackface ewes have generally been shown to have a longer anestrus period than white-faced D ewes. Of the 2 D ewes, one was from group A and one from group B. Of the ten H ewes, seven were from group A. Three of the H ewes (G170, F128, D151) showed a small peak of P_4 at one blood sampling. This peak reached a level of .2 to .4 ng/ml and

TABLE 10.
EWE BREED PROGESTERONE PROFILE TYPES

| Profile type[a] | Dorset | | | Hampshire | | |
| | % | Group[b] | | % | Group[b] | |
		A	B		A	B
Baseline	4%	1	1	20%	7	3
One peak	4%	2	0	24%	12	0
Two peaks	12%	6	0	10%	5	0
Start cycling	2%	0	1	2%	0	1
Late induction	14%	0	7	28%	0	14
Ram induction	12%	0	6	12%	0	6
Cycling then stopped	6%	3	0	4%	1	1
Time off	22%	4	7	0%	0	0
Continuous cycles	24%	9	3	0%	0	0

[a]Profile types are defined in the text.

[b]Actual numbers of ewes.

then returned to below .2 ng/ml. These ewes showed no other increases and were hence categorized into the baseline group. One H (D156) and one D ewe (B376) were lightly marked by the ram (intensity of mark = 3) but apparently this mark indicated a single futile attempt by the ram to mount a noncycling ewe. Finally, one D ewe (C23) had a relatively constant baseline P_4 profile although the serum P_4 levels were consistently in the .2 to .35 ng/ml range. This gives some indication of the within breed variation in the baseline P_4 levels of anestrus ewes.

The second profile type under consideration is what has been termed 'one peak'. Again, this category has a relatively large proportion of H ewes and a small proportion of D ewes. All 12 H and 2 D ewes in this category were from group A and were exposed to rams from day 1 to 36 of the experiment. These ewes generally had initial baseline P_4 levels and first exhibited increasing P_4 levels 15 to 25 d after ram exposure. Generally P_4 levels remained elevated for three consecutive bleeding periods and returned to baseline for the remainder of the study. Hence these ewes showed one normal peak of P_4 in the presence of the ram and returned to baseline after being isolated from the rams. Two of the D (C97, F381) and six of the H (G193,G141,G124,E130,D128,C116) ewes showed an apparent short luteal phase followed by a normal luteal phase. Apparently none of the ovulations were

96

accompanied by estrus; no crayon marks of intensity 1 or 2 were observed. One D ewe (F381) did show an intensity 3 crayon mark just prior to the normal luteal phase. Thus, about one half of the ewes in this category had what appeared to be an ovulation without estrus (silent ovulation) followed by a short (approximately 7 d) luteal phase, and a second silent ovulation with a normal (14 to 16 d) luteal phase. The other 6 ewes showed one silent ovulation followed by a normal luteal phase. After all ewes in this group completed the normal luteal phase, they returned to baseline P_4 levels apparently signalling a return to anestrus after one or two ovulations in response to the ram introduction. Two ewes in this group (G175,D128) showed luteal phases of normal length but a dual peak in P_4 during this phase such that the middle bleeding had lower P_4 levels than the previous and subsequent breedings. No explanation for this observation can be given.

The third profile type is 'two peaks' and encompassed 12% of the D and 10% of the H ewes. All eleven ewes in this category were in group A. As a general pattern, these ewes were not cycling at ram introduction, apparently ovulated silently about 4 d after the study began, apparently ovulated a second time, now with estrus (and usually with mating) and then returned to anestrus at about the time the rams were removed. Both luteal phases for all ewes in this category were of normal length. Of the 6 D ewes, all showed ovulation

within 4 d of ram exposure and all but one ewe mated at the time of their second ovulation. Four of the 5 H ewes mated at their second ovulation. The only obvious difference between the breeds was that 4 of the 5 H ewes did not first ovulate until 8 to 11 d after ram introduction. The other H ewe, like the D ewes, ovulated within 4 d. These ewes apparently all responded to ram introduction and at least 9 of the ewes exhibited behavioral estrus. They could be considered successfully 'teased' by the rams but the design did not allow us to know whether these would have continued to cycle had the rams remained with them. They did not continue to ovulate when the ram was removed.

The two ewes which fell under the 'start cycling' category tended to be ewes with indeterminate P_4 patterns. Interestingly enough, however, these two ewes had remarkably similar P_4 profiles. Each ewe (one H and one D) came from group B and were initially at baseline P_4 levels (E359) or at baseline with sporadic low peaks of .4 ng/ml (E117). Then on d 18 to 22, the ewes had an apparent ovulation (whether estrus was shown is not known since these ewes were isolated from rams at that time). At about the time the rams were introduced the ewes had had baseline P_4 levels for two or three consecutive bleedings. At 3 d after ram introduction both ewes had a silent ovulation and normal luteal phase followed by an ovulation accompanied by estrus. Both ewes

mated at this time. If one were to look at the P_4 profiles of these ewes during the second half of the study (day 36 to 68 when rams were present) it would appear to be a classic example of the ram effect: ram introduction, silent ovulation, normal luteal phase, normal ovulation, mating and a second normal luteal phase. However, during the first half of the profile both ewes showed a distinct peak P_4 elevation that lasted the normal 14 d during the first 36 d of the study. These ewes appear to have had erratic ovarian activity at this point characterized by an isolated cycle surrounded by mostly baseline P_4 values. Hence, possibly some ewes do not have clearly anestrus and cycling states. Perhaps there are times during the transition from one to the other where the negative feedback effect of estradiol on LH secretion is changing so that sensitivity to estradiol may be phasing in and out and allowing for isolated intermittent ovulations when the ewe really is not truly cyclic.

The 'late induction' category contained 7 D and 14 H ewes, all from group B. Since they were from group B, these ewes were not bled after ram removal so ewes in this group could actually have had the same response to rams as some of the ewes in the 'one' and 'two peak' categories that first ovulated at least 7 d after ram exposure. All ewes in the 'late induction' group were baseline throughout the first 46 d of the experiment. Some ewes showed baseline P_4 until d

60 of the experiment (14 d after ram exposure). With the classic ram effect, ewes are expected to respond to ram introduction within 3 to 4 d by ovulating. None of the ewes in this group ovulated before their 10th day with the rams. Five of the seven D ewes first ovulated so late in the study that it could not be determined if the second ovulation was to be accompanied by estrus (all D ewes' first ovulation was silent). The two D ewes who ovulated a second time during the study were marked (intensity = 1), indicating that they showed estrus (A333,G6). Three of the D ewes (G11,F11,H33) showed an apparent short luteal phase prior to the first normal phase. With the H ewes, the same patterns did not follow. Nine of the 14 H ewes first ovulated so late that the start of the second cycle was not within the bleeding period. However, 2 of these ewes apparently mated before their first ovulation (intensity = 2; F102,G180). These two ewes marked at least 3 to 4 d before the expected time of estrus (elucidated from the P_4 profile) so it cannot be concluded that these ewes showed behavioral estrus at their first ovulation. A third ewe (F117) apparently ovulated silently with a short luteal phase and then ovulated with estrus (mark intensity = 1). The remaining 5 H ewes first ovulated early enough so that the first cycle was completed during the bleeding period. Two of these 5 ewes started second cycles; one marked (intensity = 1; B127) and one did

not (G123). The other 3 H ewes completed their first cycle
on the last day of the study and one of these ewes marked
(intensity = 1) on that day (day 68; E113). Seven of the 14
H ewes showed a short luteal phase prior to a normal length
luteal phase (F117,C154,D107,B154,A124,G132, F120). Overall,
this group of 21 ewes was either delayed in responding to the
ram effect or naturally entered the breeding season at this
time independent of the ram effect. The results could easily
represent a combination of the two possibilities. Possibly
in the first 1/2 to 3/4 of the study the ewes were in deep
anestrus and could not respond to the ram. As the onset of
the natural breeding season approached, these ewes may have
become able to respond to the ram and ovulate. Hence the rams
may still have hastened the onset of the breeding season.
This conclusion must be guarded, however, because the end of
the study did not allow further ovarian activity of these
ewes to be monitored after ram removal. A proportion may
have returned back to anestrus after one or two cycles in-
stead of cycling continuously thereafter.

The 'ram induction' profile type consisted of six D and
six H ewes. All 12 of these ewes were from group B. These
ewes were exposed to rams on day 36 of the experiment and all
responded with a silent ovulation by day 43. All the
Hampshire ewes except one (G158) mated at their second
ovulation, indicating a classic ram induction response. This

ewe did show a mark of intensity = 3 but this is only a very weak indication that the ewe stood for the ram. One ewe (F132) may have had a short luteal phase starting on day 39. Progesterone levels rose slightly to approximately .3 ng/ml for two bleedings and then showed the elevation characteristic of a normal luteal phase. Possible a drop in P_4 occurred after the short phase but was missed due to the bleeding regimen. Four of the 6 D ewes showed a silent ovulation by day 43 and then mated at the second ovulation. One ewe (F23) marked (intensity = 2) prior to her first ovulation but the ram may have mounted the ewe while she was confined during bleeding procedures. Except for this peculiar mark, the ewe followed the pattern of the others under this profile type by mating at her second ovulation. A second ewe (G7) also probably had a short luteal phase but declining P_4 levels were not detected prior to when they peaked during the first normal luteal phase. This ewe had a slight mark (intensity = 3) at her first ovulation after the apparent luteal phase, then remarked (intensity = 1) at her first ovulation after the initial normal luteal phase. Because these were all group B ewes, bleeding was stopped before a third cycle could occur. This does not allow us to know whether or not these ewes would have continued cycling. Generally, the ewes in this group could have shown the same response to ram induction as did some of the ewes in the 'two

peaks' group (especially F124, G121, F116, E21, W79, H45, D51, C30).

Three D and 2 H ewes fell under the category of 'cycling then stopped'. All the D and one of the H ewes were from group A. Generally, these ewes had at least one estrous cycle (ovulation or mating) at the beginning of the experiment and then stopped cycling and had baseline P_4 values for the remainder of the study. The one ewe from group B (H ewe E167) showed one cycle and fell to baseline on d 15. She did not mate. Levels remained at baseline for the remainder of the study. The remaining four ewes showed three cycles before falling to baseline. Hence, it appears the ewes started and continued cycling in the presence of rams and then became anestrus when the rams were removed. Two ewes (D ewe F24, H ewe F161) cycled with estrus until the rams were removed, whereas one ewe (H14) showed three ovulations in the presence of rams, but only mated once (at the second ovulation). The final ewe of this pattern (F387) ovulated twice in the presence of the rams but never mated. It is difficult to determine if these ewes' breeding states were affected by the presence of the rams. The breeding period apparently ended upon the removal of the rams. Apparently the rams started the cycling but this activity could not be maintained once the rams were removed.

Another profile pattern that was unexpected is the 'time off' pattern. Twenty-two percent of the D ewes fell under this category: four from group A and seven from group B. No H ewes fit this category. Generally ewes of this profile type showed some ovulation activity early in the study, followed by baseline P_4 levels for about four blood sampling periods, with a subsequent return to cyclicity. Within this group, ewes showed several different sub-patterns. Six of the D ewes fell into a fairly explainable sub-pattern. All six were in group B and showed one period of elevated P_4 coming into the study. After completion of the one cycle, P_4 levels fell to baseline for 6 to 9 blood sampling periods. Then upon the introduction of rams, ewes responded by ovulating within 3 to 7 d, presumably in association with the ram effect. Two of the ewes (E388,F100) mated at their first ovulation, while the other four (E45, H32, E344, F373) had a silent ovulation followed by an ovulation with estrus. At this time all 4 ewes mated (intensity = 1). Possibly, the two ewes which mated at their first ovulation had a short enough time from the last luteal phase to exclude the need for progestational priming before showing behavioral estrus. Three of these ewes (E388, E344, F373) showed possible short luteal phases before the first ovulation and normal luteal phase. One ewe (E344) actually appears to have shown a short luteal phase prior to ram introduction. This indicates that

possibly the 20+ d of baseline values may be an actual anestrus period and the ewe spontaneously resumed cyclic activity after this time. The other ewes which responded after ram introduction may have been close enough to resuming their breeding season that ram introduction hastened the onset of breeding. The remaining 5 ewes did not fall into such a well-defined profile sub-type. The remaining ewe from group B (H22) cycled sporadically during the entire first half of the study then went baseline for six consecutive bleeding periods. On day 53 she had a silent ovulation with a short luteal phase followed by a second silent ovulation and a normal luteal phase. This ewe again may only have an anestrus period of about 17 d in the presence of rams. The final 4 ewes from this profile type (all group A) were quite variable in specific patterns, but generally these ewes cycled with heat during the first 36 d, then fell to baseline a few days after ram removal, and finally spontaneously resumed cycling before the blood sampling ended. Overall, it appears some of the ewes show a very short anestrus period that really may only involve not cycling for one 17 d period. Again, these conclusions can only be speculative due to small numbers and lack of data on other hormones.

The final profile type is 'continuous cycles' and encompasses those ewes that cycled continuously throughout the entire study. Twenty-four percent of the D ewes but no H ewes

fell into this category. Nine of the ewes were from group A while 3 were from group B. All ewes in this profile type mated when ovulation occurred and rams were present. The 68 d of the experiment did not fall during the anestrus period of the ewes and the data does not allow one to tell if the anestrus was prior to or after the experiment. Some of these ewes may not have exhibited an anestrus period.

A few observations and conclusions are apparent after looking at all 100 P_4 profiles for the ewes. There were clear breed differences in ovarian activity throughout. There were 24 (48%) D ewes in the two most active ovarian profile types ('time off' and 'continuous cycles') compared with no H ewes. By contrast 44% of the H ewes and only 8% of the D ewes in the two profile types indicative of low ovarian activity ('baseline' and 'one peak'). Generally, this was expected. Dorset ewes have been shown to have the ability to cycle and mate in spring while the H ewes have been shown to be in a 'deep' anestrus at this time of year. The unexpected result was the high frequency of H ewes which responded to ram introduction. Thirty-four percent of H ewes were in either the 'one peak' or 'two peak' group compared with 16% of the D ewes. Also 28% of the H ewes were in the 'late induction' group compared with 14% D and 12% of each breed fell under the classic 'ram induction' profile heading. The impressive numbers of H ewes responding to rams compared with D ewes is

deceiving, however. Almost a quarter of the D ewes cycled continuously and hence were not available to respond to the rams. Overall, even though many more D ewes cycled spontaneously in the study, H ewes were at least able to respond to rams by ovulating during this period.

Besides these breed differences, there was a difference within breeds among those ewes in groups A and B. Some were automatic differences due to the way the profile types were categorized (i.e., 'ram induction' had all group B while 'two peaks' had all group A ewes) while others may have been real differences (i.e., the 'late induction' profile type had all group B ewes possible because only these ewes were exposed to rams late enough in their anestrus season to respond by cycling earlier than normal).

There was also considerable variation in detected peak heights within one animal. A ewe that had 2 to 4 cycles sometimes showed a 1 to 2.5 ng/ml increase of one peak over another. This probably is a result of the bi-weekly sampling. Daily sampling may have shown a more consistent peak height in consecutive cycles. Regardless, this makes interpretation of peak height variation across groups, breeds, and profile types difficult. Actual P_4 concentrations really were dependent on the day of the cycle when bleeding was done and since ewes were not synchronized, this problem was unavoidable. This also made identification of short luteal

phases difficult. With biweekly bleeding a rapid return of P_4 to baseline levels may go undetected. Bleeding occurred every 3 or 4 d and a short luteal phase will have elevated P_4 for only approximately 4 d.

Finally, while breed differences in ovarian activity and response to ram introduction were expected, the amount of variation within each breed was quite surprising. There was at least one D ewe in each of the nine profile types and at least one H ewe in seven of the nine types. Apparently individual differences in timing and duration of the anestrus period accounted for these differences. Depth, length, timing and differences within each breed for anestrus period are indicated from the data.

Conclusions

Generally, both D and H ewes can apparently be stimulated to ovulate in May and June as shown by the P_4 profiles. However, many H ewes had silent ovulations and subsequently returned to anestrus. While ewes from both breeds will ovulate in response to ram introduction, a larger proportion of D ewes will actually show behavioral estrus and mate.

In addition to the D ewes' ability to be stimulated by the ram, many ewes of this breed were apparently cycling spontaneously in May in the absence of rams. Also, many D ewes which responded to ram introduction in May continued to cycle in June after ram removal. Hence H ewes responded to the ram effect but generally the breed is anestrus in the late spring. Of H ewes which ovulated, more showed an accompanying behavioral estrus in June. The H ewes were beginning to terminate their anestrus toward the beginning of July.

While both breeds did respond favorably, the D ewes would appear to be a greater source of genetic material for out-of-season breeding. Those ewes which were not cycling spontaneously were able to be stimulated by rams and subsequently continued to cycle. The conclusions above are a generalization made from observing trends seen in the results. Much variation was present within each breed in re-

sponse to ram introduction and the subsequent P_4 profile for each ewe.

Lambing date affected spring estrus behavior in H but not D ewes. The H ewes which lambed earlier were more likely to mate. This effect was not significant for D ewes but the same trend was apparent.

Evaluation of actual P_4 profiles showed that many response types were present within each breed. Generally, peak height values were affected by external environment as well as individual ewe variation while baseline levels of P_4 were affected only by individual ewe variation. Some of the external factors affecting peak height were ewe group (month of ram exposure), presence or absence of ram, lambing date and ewe weight.

EFFECTS OF RAM BREED AND RAM PRE-EXPOSURE ON

FERTILITY OF EWES IN SUMMER BREEDING

Materials and Methods

Animals. Eighty-eight and 78 Suffolk x Rambouillet ewes were used in 1984 and 1985, respectively. Ewes were purchased in 1977 and were approximately 8 yr old in 1984. All ewes in the 1985 experiment were also used in 1984. The ewes had last lambed in spring prior to initiation of the 1984 treatments. All sheep were held on open pasture at the Shenandoah Valley Research Station in Steeles Tavern, VA. Ewes were given water ad libitum. No supplemental feed was provided. Weights and condition scores (as previously described) were recorded prior to and just after the experiment period in 1985 only.

Experimental Design. This study used the same design in both years. Ewes were first randomly split into three groups and one of three treatments was applied. Ewes were either pre-exposed to three intact yearling Dorset (D) or Suffolk (S) rams or isolated from rams by at least 1 km. Pre-exposure consisted of keeping ewes on pastures during the day and then penning them in a three-sided roofed shelter during the night. Ewes also had access to the shelters during the day. Shelters were approximately 10 m by 50 m. Within the shelter a smaller open pen was constructed (approximately 3 x 3 m). According to pre-exposure group, ei-

ther S or D rams were held in the smaller pens. For the third pre-exposure group, no rams were held with the ewes. The objective of the 2 wk pre-exposure period was to allow the rams to have visual, olfactory, and tactile contact with ewes without allowing mating. Ewes were confined to the shelters during the night to ensure a reasonable level of contact with rams. Presumably these rams could elicit the "ram effect" in some ewes so that they would show behavioral estrus about 17 d after first being pre-exposed. Any breed differences in ability to elicit the ram effect could be detected by determining which ewes mated earliest during subsequent breeding.

After the pre-exposure period, ewes from each of the pre-exposure groups were rerandomized to one of two breeding groups. The first group was placed with the D rams. The second group was placed with the S rams. All rams were equipped with marking harnesses. Marking crayon color was changed on June 27. The rams used for breeding were the same as for pre-exposure within each year but different yearling rams were used across the 2 yr. Hence, after being pre-exposed the ewes were placed with fertile rams and given the opportunity to mate for 27 d. After this time harnesses were removed but rams stayed with the ewes until late July.

In 1984 the exact schedule was as follows: ewes had their lambs weaned on May 28 and were immediately randomized

into the three pre-exposure groups. The D and S groups consisted of 30 ewes each while the control group (N) contained 28 ewes. On June 8 blood samples were collected from the jugular vein of all ewes, and on June 11 ewes were rerandomized into the two breeding groups. On June 15, 22, 29 and July 6, all ewes were bled by jugular venipuncture and crayon marks on the rumps of the ewes were recorded. Marks were checked twice weekly and ewes were bled once weekly. The color of the marking crayon was changed on June 27. Marking harnesses were removed and bleeding was terminated on July 6, but rams remained with the ewes until July 24.

In 1985 the same basic schedule was followed except that ewes were bled twice weekly so that a more complete picture of each ewe's ovarian state could be determined. Lambs were again weaned on May 28. On May 30 the ewes were divided into three groups. At this time all ewes were weighed and condition-scored as previously described. Blood samples were also taken on this day and on every subsequent Monday and Thursday until July 8, at which time marking harnesses were removed from the rams. A total of 12 blood samples were drawn from each ewe. As before, crayon marks were recorded at every bleeding after ewes were rerandomized into the two breeding groups on June 13. The color of the marking crayon was changed on June 27. Rams were removed on July 25.

Overall, ewes were pre-exposed for 14 d and then turned in to breeding pastures for 44 d. Blood collection, handling, and radio- immunoassay procedures were described previously. Progesterone profile interpretation was handled as previously described except that ewes could become pregnant during the course of the study. Also, the fact that ewes were only bled once per week in 1984 prevented accurate assessment of P_4 profiles. Short-lived CL's and other transient changes in P_4 concentrations could not be detected by this bleeding regimen. Ewes with clearly elevated P_4 concentrations over four consecutive bleeding periods in 1985 were deemed pregnant. The same could be said of 1984 ewes with three consecutive raised P_4 concentrations. These interpretations were confirmed by subsequent lambing dates.

<u>Statistical Analysis</u>. Comparisons among years in traits such as number of ewes mated per total exposed were done with t-tests as previously described in the statistical analysis section of the Backsburg ewe breed experiment.

Analysis of variance for lambing date used the following model:

$$Y_{ijlm} = K_i + P_j + B_l + KP_{ij} + KB_{il} + PB_{jl} + KPB_{ijl} + e_{ijlm}$$

where:

Y_{ijlm} = lambing date;

K_i = year: i = 1 = 1984; i = 2 = 1985;

P_j = preexposure treatment: j = 1 = Dorset; j = 2 = Suffolk; j = 3 = none;

B_l = breeding treatment: k = 1 = Dorset; l = 2 = Suffolk;

$K_i, P_j, and B_l$ were assumed to represent fixed effects.

Analysis of variance for 1985 lambing dates only was also run using the model:

$$Y_{ijlmn} = K_i + P_j + B_l + PB_{jl} + B_1 W_{ijlm} + B_2 C_{ijlm} + e_{ijlm}$$

where:

Y_{ijklm}, K_i, P_j, K_l are defined above;

W_{ijlm} = mean ewe weight;

C_{ijlm} = mean ewe condition score;

$B_1 and B_2$ are regression coefficients reating weight and condition score, respectively to lambing date.

e_{ijlm} = random error.

Results

Analysis of Mating and Lambing

If ewes were stimulated to cycle during the pre-exposure period, previous ram effect research indicates that an ovulation accompanied by estrus should occur at 17 to 24 d after the beginning of the experiment. If ewes did not respond to the pre-exposure but were stimulated to ovulate by actual ram contact during the breeding period, ewes would not mate until 17 to 24 d after the beginning of breeding. Hence, the number of ewes mating within the first 14 d of the breeding period should be indicative of either the number of ewes which responded to ram pre-exposure or were spontaneously cycling at the beginning of breeding.

Seventeen percent of the ewes mated within the first 2 wk in each of the years (tables 11 and 12). In 1984 an additional 37 ewes mated after the first 2 wk but before bleeding of ewes was discontinued and a total of 55 ewes lambed. (table 11). Of the 52 total ewes that mated, 10 did not lamb. This means 13 additional ewes mated and conceived after blood sampling was stopped and harnesses were removed from the rams. Overall for 1984, 59% of the ewes mated during the first 25 d and 62.5% lambed in the fall.

In 1985, 32 ewes mated first after two or more weeks in the breeding pasture but before marking harnesses were removed and a total of 51 ewes lambed (table 12). Nine of the

116

TABLE 11.
1984 LAMBING AND MATING DATA
ON STEELES TAVERN EWES[a]

Number ewes mated by 6/25 (d 14)	15 (17%)
Number ewes mated by 7/6 (d 25)	52 (59%)
Number ewes lambing by 11/20[b]	11 (13%)
Number ewes lambing total	55 (63%)
Number ewes mated by 6/25 but not lambing	3
Number ewes mated by 7/6 but not lambing	10
Total number ewes	88

[a]Mating began on 6/11.

[b]Within the first 2 wk of lambing.

TABLE 12.
1985 LAMBING AND MATING DATA
ON STEELES TAVERN EWES[a]

Number ewes mated by 6/27 (d 14)	13 (17%)
Number ewes mated by 7/8 (d 25)	45 (58%)
Number ewes lambing by 11/22[b]	13 (17%)
Number ewes lambing total	51 (65%)
Number ewes mated by 6/25 but not lambing	2
Number ewes mated by 7/8 but not lambing	9
Total number ewes	78

[a]Mating began on 6/13.
[b]Within the first 2 wk of lambing.

45 ewes which mated in the first 25 d did not lamb. One of these ewes developed severe mastitis and was removed from the study. Similarly to 1984, 58% of the ewes mated before marking harnesses were removed and 65% of the total exposed lambed in the fall.

Analysis of Exposure and Pre-exposure Ram Breeds

There was no significant difference in overall lambing rate (number of ewes lambing per total number exposed) among pre-exposure treatments during the 2 yr. Ewes pre-exposed to D rams had a slightly higher rate than ewes pre-exposed to S rams or isolated ewes in 1984 but not 1985 (tables 13 and 14). Similarly, year had no significant effect on over-all lambing rate. However, ewes bred to D rams had higher overall lambing rates than ewes bred to S rams in 1984 but not in 1985 (tables 13 and 14).

Early lambing rate did not differ between years. Ewes pre-exposed to S rams and isolated ewes had similar early lambing rates in each year whereas ewes pre-exposed to D rams had higher early lambing rates in 1985.

A preliminary analysis of variance with lambing date as the dependent variable and with effects of year, pre-exposure treatment, breeding treatment, and all 2- and 3-way inter-actions in the model indicated that interactions involving year were not significant. Subsequent analysis of lambing dates for each year individually indicated that mean ewe weight (average of beginning and end weights) and mean con-dition scores over the experiment period did not effect lambing dates in 1985.

The final analysis of variance for 1984 lambing dates is shown in table 15. As was seen with lambing rate for 1984,

TABLE 13.
1984 OVERALL AND EARLY LAMBING RATES

Effect		Overall lambing rate	Early lambing rate[a]
Pre-exposure	D	70%	17%
	S	57%	10%
	N	61%	11%
Breeding	D	77%[b]	
	S	48%[c]	

[a]Ewes lambing by 11/20.

[b,c]Values in same column and effect class with different superscripts differ (P < .01).

TABLE 14.
1985 OVERALL AND EARLY LAMBING RATES

Effect		Overall lambing rate	Early lambing rate[a]
Pre-exposure	D	65%	31%[b]
	S	65%	8%[c]
	N	65%	8%[c]
Breeding	D	72%	
	S	59%	

[a]Ewes lambing by 11/22.

[b,c]Values in same column and effect class with different superscripts differ ($P < .01$).

TABLE 15.
ANALYSIS OF VARIANCE FOR 1984 LAMBING DATES

Source	df	MS	P-value
Pre-exposure breed (P)	2	45.341	0.6202
Exposure breed (B)	1	425.280	0.0385
P x B	2	38.764	0.6643
Error	49	93.989	-

the breed of ram to which the ewes were mated had a significant effect on lambing date. Pre-exposure to a ram resulted in slightly (but non-significantly) earlier lambing dates. Lambs sired by D rams were born an average of 6 d earlier than those sired by S rams (table 16). Analysis of P_4 profiles suggest that D rams may have been able to stimulate more ewes to cycle during the actual breeding treatment than did the S rams.

The second year of the experiment yielded different results (tables 17 and 18). Pre-exposure treatment had a significant effect on lambing date. Pre-exposure to D rams resulted in the earliest lambings and no pre-exposure resulted in earlier lambings than did pre-exposure to S rams. Breed of ram for mating was not significant in 1985. Across both years, ewes bred to S rams lambed on average on day 332, whereas ewes bred to D rams had an average lambing date of day 330 (table 19). Generally, over the 2 yr the S rams appeared less able to stimulate ewes to cycle. This result was expected because the blackface S rams should be more reproductively seasonal than the D rams.

Progesterone Profile Analysis

As with the Blacksburg ewes, patterns of serum P_4 concentrations during the experiment for each ewe were grouped into one of several fairly distinct categories for 1985 ewes (sample profiles of each category are in the appendix).

TABLE 16.
LEAST SQUARES MEANS AND STANDARD ERRORS
FOR JULIAN LAMBING DATE IN 1984

<u>Pre-exposure (P)</u>

Dorset		329.72 ± 2.14
Suffolk		328.41 ± 2.39
None		331.85 ± 2.58

<u>Breed (B)</u>

Dorset		327.08 ± 1.67
Suffolk		332.91 ± 2.18

<u>P</u>	<u>B</u>	
D	D	325.33 ± 2.80
D	S	334.11 ± 3.23
S	D	325.40 ± 3.07
S	S	331.43 ± 3.66
N	D	330.50 ± 2.80
N	S	333.20 ± 4.34

TABLE 17.
ANALYSIS OF VARIANCE FOR 1985 LAMBING DATES

Source	df	MS	P-value
Pre-exposure breed (P)	2	435.313	0.0226
Exposure breed (B)	1	18.826	0.6746
P x B	2	30.052	0.7533
Error	45	105.411	-

TABLE 18.
LEAST SQUARES MEANS AND STANDARD ERRORS
FOR JULIAN LAMBING DATE IN 1985

Pre-exposure (P)

Dorset		327.18 ± 2.49
Suffolk		337.17 ± 2.42
None		332.50 ± 2.65

Breed (B)

Dorset		331.67 ± 1.94
Suffolk		332.90 ± 2.17

P	B	
D	D	328.11 ± 3.42
D	S	326.25 ± 3.63
S	D	335.89 ± 3.42
S	S	338.44 ± 3.42
N	D	331.00 ± 3.25
N	S	334.00 ± 4.19

TABLE 19.

MEAN LAMBING DATE [a]

1984	329.40 ± 1.33
1985	332.27 ± 1.49

[a]Julian date.

TABLE 20.

STEELES TAVERN PROGESTERONE PROFILE TYPES 1985

Group	Number of ewes/treatment*						Total
	11	12	21	22	31	32	
Early pregnancy	7	6	5	3	8	2	31
Mated pregnancy	1	0	3	0	0	0	4
Baseline	0	4	0	4	1	4	13
Elevated	1	0	1	2	0	1	5
Mated - open	2	1	2	1	2	1	9
Late pregnancy	1	1	1	3	2	3	11
Slight activity	1	1	1	0	0	2	5

*First number is pre-exposure treatment: 1 = D, 2 = S, 3 = none; second number is ram breed exposed to: 1 = D, 2 = S.

Weekly bleedings in 1984 did not allow a comparable discrimination. Seven profile groups were defined (table 20).

The 'early pregnancy' group are those ewes which mated and became pregnant before day 40 (the last day blood samples were taken). Of these 31 ewes, 19 had no apparent cyclic activity until at least day 15 (when fertile rams were turned in with the ewes). These ewes seemed unaffected by pre-exposure to rams (5-D, 5-S, and 9-N). The other twelve ewes showed some cyclic activity in the presence of the restrained teaser rams. Of the 31 which became pregnant in this group, 20 were bred to D rams and 11 to S rams.

The second group listed is the 'mated-pregnancy' ewes. These ewes mated during the study period but apparently became pregnant after blood sampling was terminated. Hence, these ewes were cycling with estrus before day 40. All four of these ewes were bred to D rams. Three were pre-exposed to S and one to D rams.

'Baseline' category ewes had constant serum P_4 concentrations that were below .3 ng/ml throughout the study. All but one of 13 ewes were bred to S rams. Eight of the 13 did not mate during the 40 d sampling period and did not lamb in the fall. Three other ewes were not marked by the ram but lambed in mid-December. These ewes probably initiated estrous activity late in the breeding period after blood sampling had been terminated. Interestingly, two ewes

(51,111) marked on day 40 and lambed in the fall without showing any large rise in serum P_4. These ewes apparently showed behavioral estrus at the first ovulation of their breeding season.

'Elevated' ewes did not mate with rams before day 40 and did not lamb. Three ewes had consistently high serum P_4 levels throughout the bleeding period while the other 2 started with baseline levels and showed increasing P_4 throughout the next 40 d. No conclusive explanation can be given for the ovarian activity of these two ewes except a possible cystic luteal structure.

The nine ewes in the 'mated-open' groups mated but did not lamb. One of these ewes (20) had severe mastitis and was removed from the experiment after day 22. Except for this ewe and one other (69), all ewes in this group had baseline P_4 levels at least until day 19. One ewe (20) appeared to be cycling from the start and another (69) had a silent ovulation in the presence of the confined teaser rams. Six of the nine ewes mated with D rams.

The 'late pregnancy' ewes did not mate with rams before day 40 but did lamb in the fall. Generally, these ewes showed limited cyclic activity prior to being exposed to fertile rams. Three ewes (40, 55, 98) did ovulate once during the pre-exposure period but then showed baseline P_4 levels for

at least three consecutive bleeding periods. Seven of the 11 ewes bred to S rams.

The final group, 'slight' ewes did not mate during the experiment and did not lamb in the fall. These ewes generally had baseline P_4 concentrations with one silent ovulation.

Overall, there was again much variation in the patterns of P_4, even within the seven profile groups. Response to pre-exposure appeared to be low but ewes were probably in their 'deepest' anestrous state at this time. Fifty-one of 78 ewes exposed eventually lambed. Hence, by the end of the breeding period, most ewes were cycling.

As stated, 1984 ewes were not bled as frequently, therefore, P_4 profile interpretation was difficult. Forty ewes mated and became pregnant before day 40. Thirty of these ewes were bred by D rams. In 1985, 20 of the 31 ewes pregnant before day 40 were bred by D rams.

Nine ewes in 1984 showed baseline levels throughout the bleeding period. Between the two years, 21 of 22 baseline ewes were bred to S rams. It appears that the S rams were less likely to stimulate the ewes to cycle once they were actually turned in to breeding pastures. The more seasonal nature of the Suffolk breed probably is the cause of this occurrence.

Conclusions

Generally, results collected from each of the 2 yr were similar. The percentage of ewes mating in the first 2 wk was the same and the percentage lambing in the fall of each year was similar. Accordingly, year had no significant effect on lambing rate. There was also no significant effect in over-all lambing rate among pre-exposure treatments. Ewes pre-exposed to S rams or isolated from rams had similar early lambing rates in each of the 2 yr while ewes pre-exposed to D rams had higher early lambing rates.

When the years were analysed seperately, pre-exposure breed significantly affected lambing date in one year (1985), while in the other year breed of ram used for mating had a significant effect. Over the entire study, S rams appeared less able to stimulate ovarian activity in anestrus ewes. As seen with the Blacksburg study, many different P_4 profile types were observed and categorized.

The two studies described within this thesis indicate that the ram effect could be a useful tool to sheep producers in Virginia. Caution must be used, however, since a response to ram intrduction occurs in only a percentage of the ewes exposed. Varying responses may be seen from year to year and from breed to breed.

The endocrinology of the Dorset ewe needs to be studied as extensively as that of the Suffolk . Many conclusions are

drawn about all ewes from the work that has been done at the University of Michigan with Suffolk ewes. It would be interesting to see the timing of the changes in estradiol negative feedback sensitivity in Dorsets and whether they parallel those seen in the Suffolk. The Dorset also needs to be monitored year round for ovarian activity. A clear cut breeding season does not seem as apparent for the Dorset as it does for some other breeds. Some Dorset ewes may cycle contiuously throughout the year. The heritability and repeatability of the number of days cycling per year could be estimated.

Another area which needs more investigation is the identification of the putative pheromone. The presence and then the source of the compound needs to be further verified before any isolation and biochemical identification can take place. If a compound does indeed exist, its production on a commercial basis could provide the producer with another method of stimulating anestrus ewes to cycle and show a relatively synchronized estrus. The pheromone in a concentrated form may be able to stimulate a larger percentage of ewes to ovulate than the introduction of rams.

It has been suggested that a larger proportion of anestrus ewes will respond to the ram effect if they are run with some ewes which are already cycling. This phenomenom has not been well documented. Studies in this area may in-

dicate whether anestrus ewes can receive cues from cycling ewes or some type of stimulation that eases their transition from anestrus to a cycling state.

Overall, research dealing with the ram effect has documented its existance and some of its endocrinological effects. It is still unclear whether the normal pathway of termination of negative feedback of estradiol is bypassed or not. Research into possible opiod modulation may answer some of these questions.

The commercial use of the ram effect is being realized now especially in countries other than the United States. The research possibilities are apparent and need to be undertaken to completely understand the mode of action and full potential of this phenomenom.

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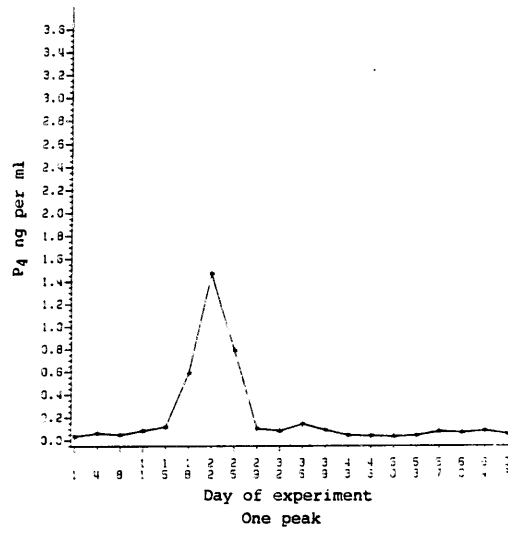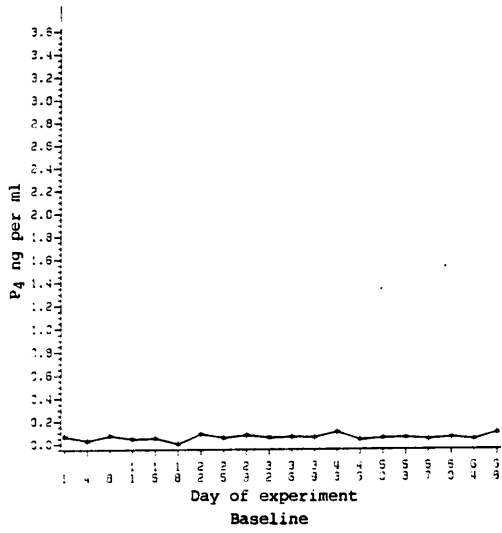
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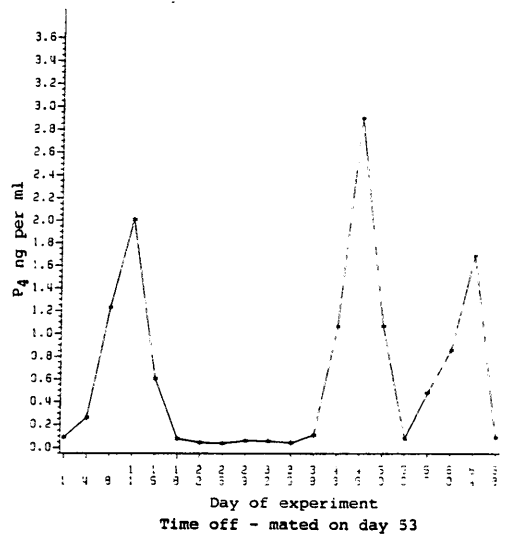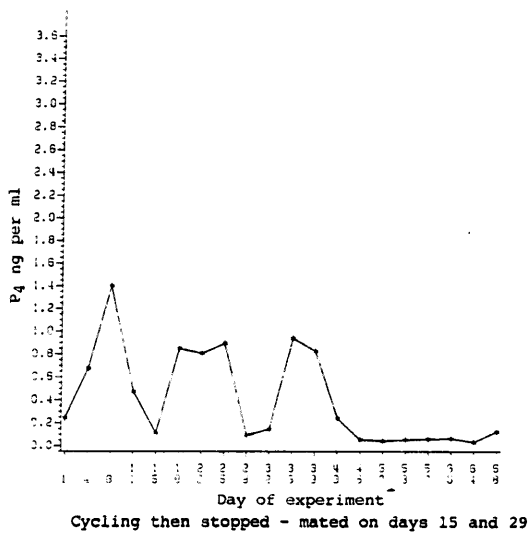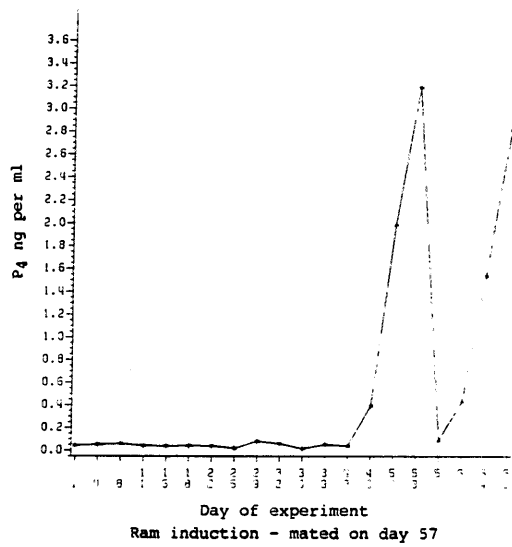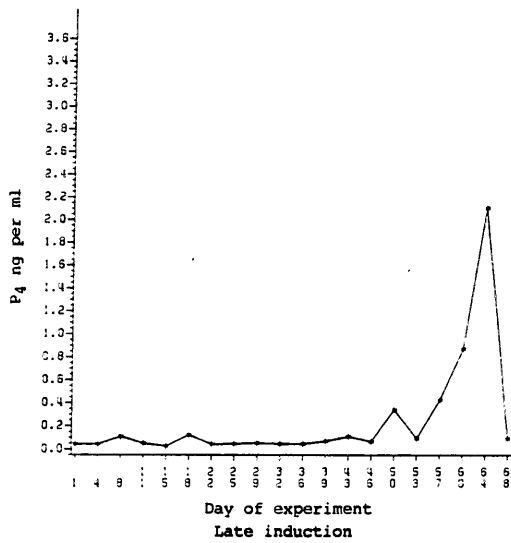
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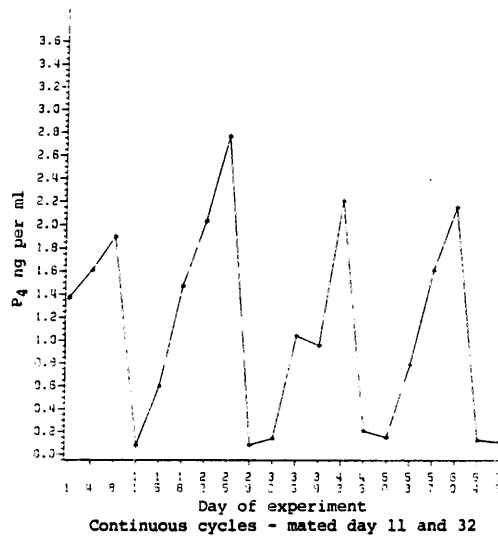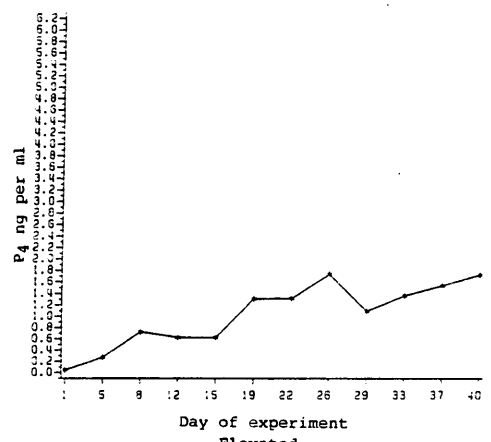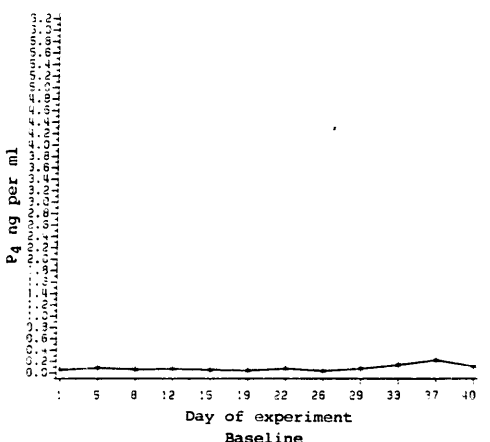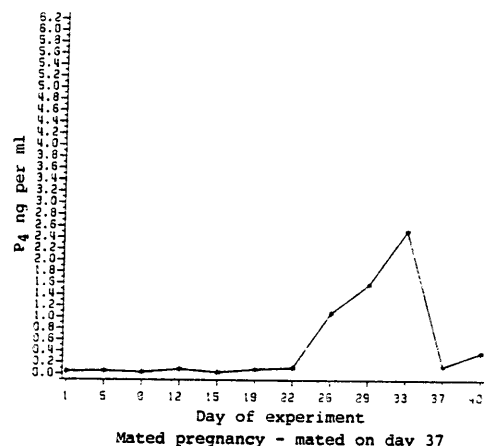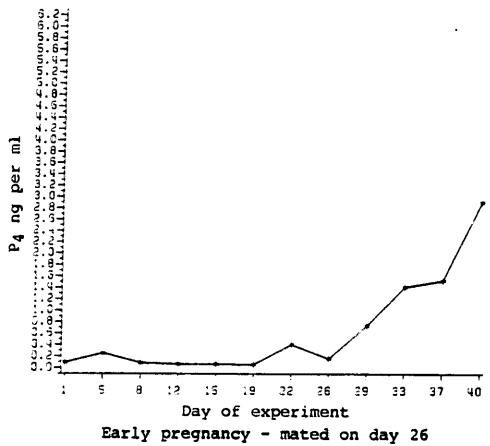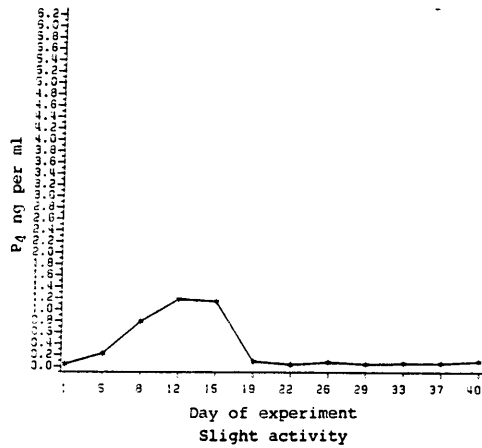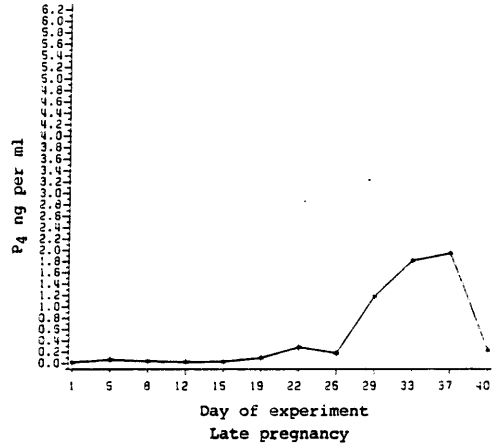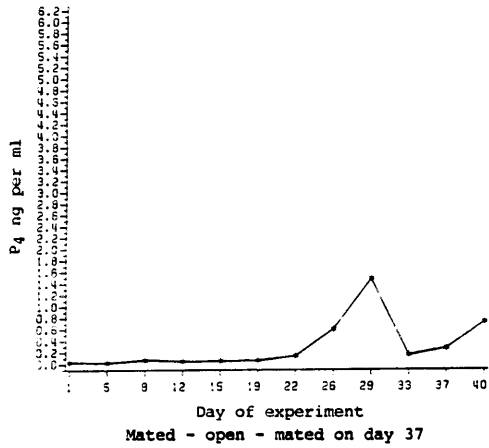
APPENDIX

Procedures for the radioimmunoassay were as follows:

(1) extract P_4 from 200 μ l of serum with 3 ml petroleum ether;

(2) add tritium-labelled P_4 and antiserum to bovine serum albumen-conjugated P_4 (Beal et al., 1980);

(3) incubate at 4 C for approximately 18 h;

(4) add dextran-coated charcoal to separate free and bound P_4;

(5) centrifuge and then remove bound P_4;

(6) add scintillation cocktail;

(7) transfer bound P_4 and scintillation cocktail to Beckman LS 1800 scintillation counter;

(8) transform scintillation counts to actual P_4 concentration for each serum sample using RIA computer program.

Baseline

One peak

Two peaks - mated on day 22

Start cycling - mated on day 57

150

Day of experiment
Late induction

Day of experiment
Ram induction – mated on day 57

Day of experiment
Cycling then stopped – mated on days 15 and 29

Day of experiment
Time off – mated on day 53

Day of experiment
Continuous cycles - mated day 11 and 32

Early pregnancy - mated on day 26

Mated pregnancy - mated on day 37

Baseline

Elevated

153

P$_4$ ng per ml

Day of experiment
Mated – open – mated on day 37

P$_4$ ng per ml

Day of experiment
Late pregnancy

P$_4$ ng per ml

Day of experiment
Slight activity

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EFFECTS OF BREED AND RAM EXPOSURE ON SPRING ESTROUS BEHAVIOR AND SUMMER FERTILITY IN DOMESTIC EWES

by

Russell A. Nugent III

(ABSTRACT)

The present studies were conducted to check the effects of acute ram introduction into a flock of anestrus ewes in Virginia. Ewes were bled via jugular venipuncture twice weekly and serum samples were radioimmunoassayed for progesterone (P_4) content as an indicator of estrous activity. All rams were fitted with crayon equipped marking harnesses for use as an indicator of mating behavior in ewes.

The first study tested the effects of introduction of vasectomized rams into a flock of 50 Dorset (D) and 50 Hampshire (H) purebred ewes in either May or June. More D ewes ovulated (96% vs 72% for H ewes) and mated (80% vs 20% for H ewes) in May. Of ewes which mated in May 65% D but no H ewes continued to cycle in June after removal of rams. Of ewes exposed to rams in June no difference among breeds was observed in percentage of ewes ovulating but more D ewes (72%) mated than H ewes (44%). Twenty-four percent of D but no H ewes cycled continuously throughout the 68 d of the

study. Lambing date significantly affected mating behavior in H but not D ewes.

The second study tested the effects of ram breed on incidence of mating and subsequent lambing in Rambouillet x Suffollk ewes In June and July of 1984 and 1985. Ewes were pre-exposed to either confined Suffolk (S) or Dorset (D) yearling rams or no (N) ram for 2 wk prior to breeding by either S or D rams. Lambing date was significantly affected by breeding treatment in 1984 and by pre-exposure treatment in 1985. Sixty-three percent of the ewes lambed in 1984 while 65% lambed in 1985.