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MATING BEHAVIOR AND REPRODUCTIVE HORMONE SECRETION IN RAMS

by

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Dedicated in the Memory of the Late

FRANCIS NAKU GHARTEY

ABSTRACT

Four adult Finnish Landrace rams were used in two experiments to study the interaction between mating behavior and season on circulating levels of LH, FSH, Prolactin (PRL) and Testosterone (T). Jugular blood was collected by venipuncture every 20 min. for 8-h and 36-h periods in Experiment 1 and 2, respectively. In Experiment 1, blood samples were collected in July and October while individual rams were: 1) isolated from, 2) observing, 3) mounting, and 4) mating estrous-induced ewes. Mating activity in July was associated with elevations in mean LH and T levels, basal LH levels and the number of LH peaks; while in October obvious changes in only basal LH levels were noticed. Circulating LH and T during mounting and observation periods were often depressed from control levels in both months. Mean FSH levels remained unaffected by several ejaculations, mounting or observation in both months. Likewise, there was no obvious relationship between sexual activity and mean PRL levels in July; however, all types of sexual activity were associated with higher circulating PRL levels in October.

Experiment 2 studied the effect of repeated mating on the secretion of LH, FSH, PRL and T during non-breeding and breeding seaons and the cause of the decline in circulating LH and T that occurs after several hours of mating. It was done in two parts. In Part 1, conducted in July-August and

in November, blood was collected from rams during 36-h control and mating periods. Part 2, performed in August only was designed as Part 1 with the inclusion of three 10 µg GnRH injections (i.v.) given at 20-min. intervals beginning at h 19. Mating activity produced transient (6 - 12 h) elevations in circulating LH, T and PRL in August, but was associated with consistently lower LH levels and a short-term (6 h) increase in PRL levels in November. In comparison with control periods, treatment with GnRH during the mating period (Part 2) produced smaller elevations (Δ value) in mean LH (83.1 $\pm$ 23.8 for control period vs 45.3 $\pm$ 11.7 ng/ml, $P < .01$, for mating period), FSH (125.1 $\pm$ 23.0 vs 105.1 $\pm$ 39.8 ng/ml) and T (20.0 $\pm$ 2.3 vs 12.5 $\pm$ 1.0 ng/ml, $P < .05$) during either the first or second 2-h interval following the onset of GnRH injections. Normal seasonal endocrine changes were exhibited in both experiments.

Results indicate that: 1) only multiple ejaculations by rams consistently induce short-term increases in LH and T secretion, and then only during the non-breeding season; 2) whereas FSH levels may not be affected by any type of mating behavior, PRL levels may be elevated after a period of several ejaculations, in both seasons, and with all types of sexual activity during the breeding season; 3) the decline in LH secretion following 12 h of mating may be due in part to pituitary refractoriness to endogenous GnRH.

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INTRODUCTION

Traditionally animal scientists have preoccupied themselves with increasing the productivity of livestock species. Methods adopted have included: improvement in animal nutrition, genetic selection for desirable traits, and proper management techniques. These, and other approaches meant to enhance the animals reproductive performance, have also been tried with varying degrees of success, and they still offer a great deal of hope.

With regards to the ovine species, the principal problem to year-round production is that of seasonal breeding. Great strides have been made in the understanding of the seasonality of reproduction in the ewe to such an extent that, it is possible to breed her out of season. The induction of estrus and ovulation by photoperiodic manipulation and hormone therapy, as well as using breeds with extended breeding seasons are alternatives chosen to forestall this problem of seasonal anestrus.

This possibility requires that rams of high fertility be available at all times of the year; but seasonal infertility in the ram is fairly well documented. This problem has been solved in part through the use of hormone therapy. However, complete success in this area requires a clearer understanding of the endocrine control of sexual function in the ram and its interaction with seasonal variables.

Preliminary studies in this laboratory on mating behaviour and reproductive hormone secretion in the ram, have indicated that multiple ejaculations provide a stimulus adequate to trigger increases in the release of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone (T) and that this effect is season dependent. Therefore, it was of interest to investigate further these relationships. Secondly, it had been observed that the rise in hormone levels declined to low levels within a few hours, and this phenomenon was speculated to be due in part to a decrease in the responsiveness of the pituitary to gonadotropin releasing hormone (GnRH). Thus this hypothesis was also investigated. It is hoped that these studies will provide additional insight into the effect of sexual stimuli on the hypothalamic-pituitary-gonadal axis of rams which may in turn suggest ways to improve reproductive efficiency in rams, particularly during the period of summer infertility.

The review of literature focuses on the roles and regulation of secretion of gonadotropins, with emphasis on the latter. The influence of photoperiodic and sexual stimuli in modulating the basic regulatory mechanisms have been considered. Although the discussion has been restricted mainly to the ovine species, information on other species have been included where pertinent.

LITERATURE REVIEW

LH and FSH: Function and Regulation of Secretion

In the male LH and FSH are primarily involved in the maintenance of testicular function. LH stimulates the Leydig cells of the testis to produce androgens which are necessary for the maintenance of spermatogenesis (Steinberger, 1976). FSH binds specifically to Sertoli cells to stimulate the production of androgen binding protein (Means *et al.*, 1976; Steinberger, 1976). Androgen binding protein (ABP) immobilizes T in the vicinity of germ cells to facilitate their maturation (Means *et al.*, 1976). Leydig cells and Sertoli cells were suspected to be the respective target sites for LH and FSH, but it was not until the recent advent of immunohistological and radioautographic techniques that these testicular binding sites were directly demonstrated. Castro *et al.* (1972) using their indirect fluorescent antibody technique on sections of rat testis were able to detect FSH or LH administered *in vivo* after the hormones had formed complexes with their respective rabbit antisera. LH appeared in the interstial and peritubular cells, whereas FSH was localized in Sertoli cells. De Kretser *et al.* (1969) localized binding of ^{125}I-labelled LH to receptor sites on interstitial cells of the testis of immature rats and in the proximal convoluted tubule of the kidney by radioautography. The same group was able to localize receptor sites for LH in the cytoplasm of interstitial cells (De Kretser *et al.*, 1971).

A recent report indicates that FSH may bind to spermatogonia as well; probably to help reduce their rate of degeneration (Orth and Christensen, 1978).

The secretion of LH and FSH from the basophilic cells of the anterior pituitary gland is regulated by a complex mechanism, involving the stimulatory effect of a hypothalamic releasing hormone(s) and the negative feedback action of gonadal steroids (Schally and Kastin, 1970; Fink, 1979) and a non-steroidal factor (Blanc et al., 1978, 1979; Main et al., 1979). There is also the involvement of other hormones, especially prolactin (PRL) and its complex control system (Bartke, 1971; Hafiez et al., 1972; Fink, 1979). While this section of the review will concentrate on the control of LH and FSH secretion, it must be emphasized that the hypothalamic releasing hormone, GnRH, is necessary for their synthesis as well (De Koning et al., 1977; Fraser and Baker, 1978; Liu et al., 1976).

The first demonstration of LH release in rams in response to GnRH injection was by Amos and Guillemin (1969). They injected purified hypothalamic extract of GnRH into the carotid artery and observed a significant surge in LH release 3 min. post-injection, and about 30 min. later, the effect was over. On the contrary, purified thyrotropin releasing hormone (TRH) was without effect. Since then, the propensity of this hormone to elicit pituitary LH and FSH release has been widely documented (Schally and Kastin, 1970; Pelletier, 1976). However, these

and other studies have often involved the use of small numbers of rams of different ages and breeds, and varying dosages and routes of GnRH administration, making interpretation of results difficult. Although, it may suffice to refer to the review by Pelletier (1976) the main tenets could be outlined as follows:

(i) LH responds to GnRH according to the dose administered.

(ii) After GnRH administration LH rises to peak levels within 1 to 2 h.

(iii) FSH release is variable. Increases observed are only discrete.

(iv) The rise of LH and FSH to peak levels occurs after a time lag with FSH requiring more time than LH.

(v) Often, there is a decline from peak values (even with continuous infusion or successive injection) after a period of time, with the drop being more rapid for LH than FSH.

(vi) Very frequently, a biphasic pattern in LH release is observed. This is believed to be due to the presence of two releasable pools of LH in the pituitary; one requiring more time to be released than the other (Bremner et al., 1976; Pelletier, 1976; De Koning et al., 1977).

(vii) Castrate-male LH response differs from entire male response in two ways: first, the magnitude of the peak level is much greater in the former; second, the time to reach the peak level is markedly shorter in castrate males.

Similar results have been observed in rams in more recent studies (Stelmasiak et al., 1977; Wilson and Lapwood, 1978; Lincoln, 1978, 1979).

The differential response of LH and FSH to GnRH and other observations (McCann, 1974; Campbell and Ramaley, 1978) have been interpreted by some to indicate the existence of a separate releasing hormone for FSH. On the contrary, the concurrent increases in the plasma concentrations of both hormones after GnRH administration (Crighton, 1973; Pelletier, 1976; Lincoln, 1978, 1979) points to the control of their release by a single releasing hormone. A strong support for this concept is provided by immunization studies. Fraser et al. (1974) actively immunized male rats against GnRH by injecting GnRH-conjugated to bovine serum albumin. This caused a drastic reduction in the levels of both LH and FSH; and was accompanied by atrophy of the testes and the secondary sex organs, and aspermatogenesis. Also, passive immunization (multiple injection of anti-GnRH for 4 days) of intact or orchidectomized rats against endogenous dramatically suppressed the secretion of LH and FSH (Hauger et al., 1977). The different temporal

patterns of circulating FSH and LH in rams (Bremner et al., 1976; Lincoln, 1978, 1979) may be due to differences in secretion and metabolic clearance rate (Lincoln, 1978, 1979).

The negative feedback effect of gonadal steroids emerges as the next regulatory element of gonadotropin secretion. This is evidenced by both direct and indirect means. Wethers given purified porcine GnRH showed much higher elevated levels of LH than entire males (Reeves et al., 1970; Galloway and Pelletier, 1975) indicating in part, the removal of the inhibitory steroid action at the pituitary level in castrates. Intravenous injection of 2 or 6 mg dihydrotestosterone (DHT) at 4-h intervals for 60 h to long-term castrate rams suppressed LH levels approximately 25%; however, T treatment did not suppress LH levels (Sanford et al., 1976b). In contrast, when Schanbacher and Ford (1976a) administered 25 mg of T or DHT to cryptorchid rams, serum levels of LH were not significantly affected. However, active immunization of rams against steroid conjugates (T and estradiol-17β) resulted in elevated concentrations of both LH and FSH (Schanbacher, 1979).

It is interesting to note that estrogen has been implicated to be a more potent inhibitor of LH and FSH secretion (Schally and Kastin, 1970; Schanbacher and Ford, 1976a). Estradiol-17β is produced by Sertoli cells (Dorrington et al., 1978) and in certain areas of the brain (Reddy et al., 1974; Christensen and

Clemens, 1975). Swift and Crighton (1979) demonstrated that the presence of estradiol-17β in hypothalamic incubation medium greatly enhanced the activity of the peptidase enzyme responsible for the degradation of GnRH. Presumably, the steroid removes an inhibitor from the enzyme or stimulates enzyme activity allosterically. These results do not exclude the probable similar effects of DHT and T. DHT, although present in minute amounts in the peripheral blood of rams (Falvo and Nalbandov, 1974) has been shown to be more androgenic than T (Sanford et al., 1976b). Furthermore, Selmanoff et al. (1977) have located heterogenous neuronal subpopulations in specific regions of the rat brain which may be involved in the conversion of T to estradiol-17β or DHT. Thus, the negative feedback effect of gonadal steroids may be exerted on both the hypothalamus and anterior pituitary.

Control of FSH and probably LH secretion may in part be under the influence of a non-steroidal factor (inhibin) of testicular origin. Blanc et al. (1978) injected 20 ml charcoal-treated rete testis fluid (RTF) into cryptorchid rams and observed a suppression in the secretion of both LH and FSH. Apparently, inhibin voided into RTF and thus into the epididymis does not play a role in the regulation of FSH and LH (Walton et al., 1978; Blanc et al., 1979). When the remaining testes of adult hemi-castrated rams were cannulated in order to remove RTF and to prevent reabsorption by the epididymis, no change in peripheral blood levels of LH and FSH occurred, whereas orchidectomy

induced an increase in the same parameters. Thus regulation of FSH and LH would be under the influence of inhibin absorbed into efferent circulation (lymph and blood). In spite of the fact that the presence of inhibin is virtually irrefutable, its presumed physiological significance as a feedback inhibitor of FSH is yet to be convincingly demonstrated (Review by Main et al., 1979).

Evidence accruing from several authors suggests an additional control mechanism in which an inhibitory impulse is provided by LH and FSH themselves, probably to provide a fine adjustment to their own secretion. For this feedback, the name "short", "internal", "auto" feedback or autoregulation has been proposed. It was demonstrated when LH was implanted into the median eminence with the resultant decrease in LH levels in normal and castrated male and female rats (see review by Schally and Kastin, 1970). Similarly, implants of FSH in the median eminence or its systemic administration, lowered pituitary FSH stores and releasing hormone concentration in normal or castrated adult rats. Clearly, this indicates that at least in the rat the median eminence contains receptors for LH and FSH. When these gonadotropins are present in plasma in high levels they may reduce further LH and FSH secretion by inhibition of the secretion of their releasing hormone. To date, there is no evidence to suggest that this feedback system is present in the ram.

The hypothalamic catecholamines have been shown to influence

anterior pituitary function presumably by affecting either the release of hypothalamic-pituitary regulating hormones or by acting directly on the pituitary (Sawyer, 1975).

Prolactin: Function and Regulation of Secretion

Prolactin has been suggested to participate in the regulation of LH and FSH secretion but experimental results are somewhat contradictory. Hafiez et al. (1972) showed that PRL acts synergistically with LH in controlling the synthesis and secretion of T by the rat testis. In contrast, Ravault et al., (1977) did not observe any change in testis weight or in LH and T secretion when 2-Br-α-ergocryptine (CB 154, a potent dopamine receptor agonist) was injected into rams during pubertal development to selectively block PRL secretion. But they noted a significant decrease in the weight and fructose concentration of seminal vesicles after treatment. Similarly, short-term inhibition of PRL secretion in rams with CB 154 in the non-breeding season did not seem to influence the secretion of LH, FSH and T or the binding of LH and FSH by testicular-gonadotropin receptors (Sanford and Phillips, 1979). However, there is evidence to indicate that PRL is essential for the maintenance of LH receptors, which would undoubtedly help maintain testicular function. Aragona et al., (1977) demonstrated that in the immature male rat inhibition of PRL release by administration of CB 154 resulted in a decrease of testicular LH

receptors. Zipf et al., (1978) indicated that LH receptor concentration in adult hypophysectomized rats depended on the combined effects of PRL, growth hormone (GH) and LH, since a combined administration of these hormones prevented a loss of LH receptors, whereas LH alone decreased its receptor concentration. Interrelationships appear to exist between LH, T and PRL secretion in the ram; this area will be dealt with in the section on seasonal variation in PRL secretion.

Excellent comprehensive reviews on the control of PRL secretion have been published (Meites and Clemens, 1972; Macleod, 1976; Ganong, 1977). In contrast to the other gonadotropins the secretion of PRL in mammals is generally thought to be under tonic inhibition by a substance(s) produced by the hypothalamus. Strong evidence has been obtained which suggests that dopamine is at least one of these agents (Macleod, 1976). In contrast, a prolactin-inhibiting factor (PIF) other than dopamine secreted by the hypothalamus has been hypothesized to exist (Malven, 1975; Macleod, 1976) but has not been identified.

Biogenic amines (cholinergic and adrenergic), and somatostatin have been suggested to alter PRL secretion perhaps by exerting a direct effect on the pituitary or by influencing the secretion of PIF (Macleod, 1976; Lamberts and Macleod, 1978). In addition estrogens and TRH (Macleod, 1976), stressful conditions (Raud et al., 1971) and temperature (Meites and Clemens, 1972; Sanford et al., 1978b) influence PRL secretion.

Similar to LH and FSH, PRL may be under the influence of an autoregulatory mechanism (Meites and Clemens, 1972).

Variation of Reproductive Function with Season

It is now fairly well established that like their female counterparts, rams exhibit seasonal variation in reproductive characteristics. They undergo marked seasonal changes in testicular size (Lincoln, 1976; Land and Sales, 1977; Hanrahan, 1977; Schanbacher and Ford, 1979), spermatogenesis (Johnson et al., 1973; Schanbacher and Ford, 1979), sex drive (Pepelko and Clegg, 1965; Schanbacher and Lunstra, 1976; Mattner, 1977; Sanford et al., 1977) and gonadotropin and gonadal steroid secretion (Katongole et al., 1974; Purvis et al., 1974; Pelletier and Ortavant, 1975a, Lincoln, 1976; Sanford et al., 1974a, 1977). Among the various environmental factors that may promote seasonality in reproductive function (viz. temperature, photoperiod, relative humidity, rainfall), temperature and photoperiod have received considerable attention (Pepelko and Clegg, 1965; Howarth, 1969; Rathore, 1970; Sanford et al., 1974a; Gomes and Joyce, 1975). Photoperiod is emerging as the most important factor affecting these seasonal changes (Pepelko and Clegg, 1965; Lincoln et al., 1977; Sanford et al., 1977, 1978a; Barrell and Lapwood, 1979a, 1979b). Thus it will be the main subject of further consideration.

Libido - Sex drive or libido of the ram is markedly altered

throughout the year under conditions of varying photoperiod. Pepelko and Clegg (1965) individually exposed eight rams to an estrual ewe for 1 h twice monthly for 1 yr., and observed frequent ejaculations during late fall and early winter. The highest monthly average (5.9 per ram) occurred in November, and the lowest (4.1 per ram) in March. The average number of mounts per ejaculation significantly increased at a time of the year when breeding activity was low. The greatest number of mounts was observed in April (4.7 mounts per ram). Schanbacher and Lunstra (1976) noticed that mating activity was highest for both Finnish Landrace and Suffolk rams during the peak breeding season (October) and declined 50% by late spring and summer before increasing again the next October. Using two breed types; i.e., Finnish Landrace and Managra Synthetic or Line-M, Sanford et al.,(1974b) noticed seasonal changes in mating activity. Rams averaged 21.5 $\pm$ 1.5 matings in 8 h in November compared to 7.6 $\pm$ 1.6 matings in August. Mating activity remained high in December.

Apparently, there is a marked positive relationship between the seasonal variation in mating behaviour and androgen status. Schanbacher and Lunstra (1976) determined that T levels in peripheral serum for Finnish Landrace (Finn) and Suffolk rams alike, were high (> 6 ng/ml) in October when mating activity was the highest. During the winter, T levels gradually decreased reaching their lowest value in March (2.06 ng/ml for Finns and

1.01 ng/ml for Suffolks). Similarly, Sanford et al., (1977) noticed the highest number of matings per 8 h in November when the level of serum T was maximal. Thus it seems that gonadal hormone levels are maintained below a threshold level necessary to maintain peak mating performance, during those months when libido is relatively low. As the breeding season advances, gonadal hormone levels rise above the threshold to enable more frequent matings to occur.

Alterations in the androgen status per se may not be the only determining factor of sex drive. There is a related variation in the sensitivity of brain mechanisms to androgen feedback, which is believed to be due to the influence of environmental stimuli (e.g., photoperiod). Presumably, environmental stimuli may act on the brain; either directly on the cells of the anterior hypothalamic-preoptic complex which are sensitive to androgen or on extrahypothalamic systems which influence the androgen sensitive cells. It is possible that environmental stimuli could act indirectly by promoting changes in the hypothalamo-pituitary-gonadal axis; this in turn would alter gonadal steroid secretion and subsequently the sensitivity of androgen responsive cells in the hypothalamus (Hutchison, 1978).

LH, FSH and T Secretion - Using citric acid and fructose concentrations as indices of androgenicity, Amir and Volcani (1965) reported that minimum concentrations of citric acid and fructose in the ejaculates of Awassi rams occurred in March through June.

Citric acid levels later rose to a peak in September through November, while peak fructose concentrations occurred in October through November in these rams. Johnson et al., (1973) observed maximal testicular spermatogenic and androgenic activity in Suffolk and Hampshire rams in October; and a gradual decline in subsequent months as ambient temperature and photoperiod increased.

In corroboration with this indirect approach, endocrine data have been presented to indicate that decreasing photoperiod is commensurate with elevated circulating T levels, although contradictory reports exist. Purvis et al., (1974) demonstrated that rams bled at 1/2 to 1-h intervals in November and January exhibited 4 to 8 peaks of T with magnitudes ranging from 8 to 14 ng/ml in serum during a 24-h period. In comparison, T elevations in rams sampled during March and April were comparatively small (< 5 ng/ml) and less frequent. Sanford et al., (1974a) observed a gradual but four-fold increase in mean serum T levels from mid-August through September; serum T remained elevated during October and November but subsequently dropped sharply during December and January. Similarly, Katongole et al., (1974) reported T concentrations for rams of .5 to 10 ng/ml plasma from January to September, whereas from October to December it ranged from 3 to 28 ng/ml. In contrast, Gomes and Joyce (1975) reported that lowest T values occurred in December; then a gradual increase was observed through April with peak levels being

achieved in July after a transient decrease in June. Concentrations decreased again in August and September. Barrell and Lapwood (1979a) performing their study in New Zealand noticed highest T concentrations to occur in January through March while minimum levels occurred between May and November.

Associated with seasonality in T secretion are changes in the levels of LH, which is not surprising owing to the ability of LH to stimulate T production in Leydig cells (Steinberger, 1976). In the short-term, each individual LH discharge can result in a transient stimulation of the testes with a consequent increase in circulating T levels (Katongole et al., 1974; Sanford et al., 1974c; Lincoln, 1976). In spite of these observations, it is not completely understood which components of the fluctuating levels of LH are involved in determining the stimulus to the target organ, and which may be responsible for the seasonal increases in T. Sanford et al. (1977) noticed progressive changes in the profiles of serum LH and T as the breeding season advanced. Peaks in LH became more frequent; and the number and height of the T peaks increased. Another report by the same group revealed that periods of elevated T, brought about by artificially decreasing the photoperiod, were characterized by increases in LH and T peak frequencies and T peak height, while LH peak height decreased (Sanford et al., 1978a) Schanbacher and Ford (1976b) bleeding five mature rams in September and May failed to observe any seasonal difference in

mean LH concentrations, or the number and amplitude of LH peaks however, basal levels of LH were higher in September than in May. Seasonal differences in T levels were dramatic with mean, baseline and peak concentrations elevated during September. The number of T peaks did not vary with season. When Lincoln (1976) subjected Soay rams to contrasting photoperiodic regimes, he observed substantial increases in testicular size during short days in comparison to long days. Accompanying this were more frequent elevations in T of higher amplitudes and longer duration, and higher baseline and mean concentrations of serum T. LH profiles were characterized by more frequent releases with lower amplitudes, but higher baseline values. Indeed, the frequency, amplitude and duration of LH peaks all tended to determine the basal LH level. Thus Lincoln rationalized that basal LH levels may be the most significant in initiating seasonal changes in circulating T. More recently, work by Ponzilius and Sanford seems to reaffirm the claim that the frequency of spontaneous LH release is one of the major factors promoting T secretion in rams (Ponzilius and Sanford, 1980). They attempted to mimic the pattern of serum LH-peak release normally found in the breeding season in June (non-breeding season), by injecting (i.v.) 10 µg LH at 80 min. intervals for 55 h 40 min. By the third day following the start of injection, circulating T level had increased to 50% of the level determined in October.

Two points of significance are conceivable: first, during

periods of decreasing photoperiod the decrease in the amplitude of LH peaks and the associated increase in the amplitude of T peaks, suggest an increase in the sensitivity of the testes at this time (Lincoln, 1976; Lincoln and Peet, 1977). Secondly, the continued increase in the frequency of LH release, even though the amplitude of LH is depressed, suggests that hypothalamic neuronal activity may remain raised in spite of the high T levels. In fact it has been speculated that during short days the frequency of GnRH releases is increased, this perhaps being facilitated at least in part by a lessened negative feedback effect of T (Pelletier and Ortavant, 1975b; Lincoln, 1976, 1978).

Biologically relevant variations that occur in FSH secretion appear to be changes in mean concentration and not any aspect of a secretory profile, since it is not certain if it is secreted episodically (Sanford et al., 1976a; Lincoln, 1976, 1978). Sanford et al. (1976a) reported that FSH levels in rams were significantly lower in January than in August. Lincoln and Peet (1977) observed that both LH and FSH began to increase 6 to 12 days after abrupt exposure to declining photoperiod, and rose progressively until days 33 to 54 before declining. Davies et al. (1977) found an eight-to ten-fold rise in plasma FSH at about the summer solstice; raised levels were maintained until after the autumnal equinox with the lowest level being seen in January and June. Ostensibly, the high plasma levels of FSH in the ram during the initial period of declining photoperiod enhance testicular growth, mediating its effect on the Sertoli

cells and hence spermatogenesis (Courot et al., 1979). Once testicular development becomes maximal, plasma FSH values decline rapidly to a relatively low level (Lincoln and Peet, 1977). Since the negative influence of T on FSH secretion is thought to be minimal or non-existent (see review by Main et al., 1979), it has reasonably been suggested that the putative "inhibin" could, for the most part, account for this decline (Lincoln and Peet, 1977).

Prolactin - It may be generalized that the variations in PRL secretion seem to be in phase with changing photoperiod (Pelletier, 1973; Ravault, 1976; Ravault and Ortavant, 1977; Barrell and Lapwood, 1979a; Sanford and Dickson, 1980). However, the marked effect of ambient temperature on PRL levels deserves mention. Wetteman and Tucker (1974) have shown that a rise or fall in temperature increases or decreases respectively, the secretion of PRL in heifers. Sanford et al. (1978a) found that whereas serum levels of LH and FSH were unaffected, levels of PRL increased roughly two-, three- and four-fold for rams following exposure to elevated temperature for 1, 3 and 6 days, respectively, thus implicating temperature in the modulation of the yearly PRL cycle in the ram. However, experiments in which photoperiod have been altered and temperature held fairly constant (Pelletier, 1973; Alberio and Ravault as cited by Ravault and Ortavant, 1977; Schanbacher and Ford, 1978) indicate that variations in photo-

period might be the major factor regulating the seasonal changes in PRL levels in the ram.

The significance of variations in PRL secretion are difficult to assess owing to the contradictory views that exist. It appears that in the rat PRL synergizes with LH in maintaining spermatogenesis (Bartke, 1971) and testicular androgen secretion (Hafiez et al., 1972). By contrast, in humans it is generally believed that hyperprolactinemia is associated with impaired gonadal function because of either altered hypothalamic-pituitary interactions or a detrimental effect at the gonadal level (Beumont et al., 1974; Child et al., 1975). Nevertheless a short-term hyperprolactinemic condition induced in rams in the summer, only temporarily retarded testicular growth and did not appear to adversely affect T secretion (Sanford and Duffy, 1980). Actually, the springtime increase in circulating PRL in rams seems to provide the proper milieu for normal testicular development. In its absence, the subsequent seasonal increases in testicular growth and circulating T levels are delayed and the daily sperm output reduced (Sanford and Dickson, 1980).

Sexual Activity and Reproductive Hormone Secretion

This area has received much attention in recent times; however, differences in response between species, the influences of non-specific stimuli or the regimen of blood collection have undoubtedly contributed to the somewhat confusing state of the

knowledge at present.

In the male hamster, it appears that the mere exposure to vaginal odour evokes a rapid increase in plasma T levels which compares with increases that occur after pairing with the female (Macrides et al., 1974). Similarly, male house mice which have been paired with females for 1 wk show elevations in plasma T concentration 30 to 60 min. after the resident female is replaced by another female (Macrides et al., 1975). This elevation appears to be a specific response to a strange female as it does not occur if the resident female is replaced by a male. The plasma T elevations following exposure to estrous females seem to be quite marked when basal T levels are low; furthermore, the T levels are maximal at the initiation of mounting and declines during copulation (Batty, 1978). Recently, Coquelin and Bronson (1979) have attempted to isolate and identify cues that modulate hormone secretion by exposing males to estrous females, diestrous females, or pooled female urine. Results obtained therefrom, suggested that two types of cues actively stimulate the release of, at least, LH in mice: (1) a urinary pheromone common to both receptive and non-receptive females; and, (2) a cue, probably tactile, that is specifically associated with intromission or ejaculation; and that both cues yield rapid "all or none" surges inter-spaced by a refractory period. Consistent with these observations are reports with guinea pigs which indicate that changes in T levels do not

depend on copulation *per se* but may be induced by exposure to sight, smell or sound of an estrous female.

In the male rat, it has been inferred that sexual activity has a stimulatory effect on T secretion, since cohabitation appears to be essential for the maintenance of the reproductive tract. Thomas and Neiman (1968) observed that male rats living together with females had heavier reproductive systems as opposed to those living in isolation or in male groups. In addition, either three intromissions or ejaculations every 4 days were sufficient to maintain accessory sex organ weights. However, atrophy of the organs occurred when males were allowed to only mount without intromission or come into contact with estrual odors. Similarly, Folman and Drori (1966) noticed that the reproductive tract of male rats raised in social isolation was not enhanced by exposure to female odors. Other workers have observed that rats allowed frequent mating exhibit larger seminal vesicles and coagulating glands (Hunt, 1969), have increased fructose levels in the coagulating glands (Drori *et al.*, 1968) and have increased weight of the penis and perineal muscle (Herz *et al.*, 1969) in comparison with sexually inactive males.

Direct evidence have also been reported. Herz *et al.* (1969) observed a significantly higher level of T in the testes when male rats were housed from 40 to 162 days of age with females as opposed to those housed with other males. Peripheral plasma

T levels were noticed to rise markedly within 5 min. after the first intromission (Purvis and Haynes, 1974); levels remained high for at least 30 min. As with mice, close proximity of male rats to female rats is adequate to elicit a rise in plasma T (Bliss et al., 1972; Purvis and Haynes, 1974). Even the mere "anticipation of a mating encounter" is enough to stimulate a rise; Kamel et al. (1975) observed a rise in both LH and T secretion in male rats when they were placed alone in an arena used for testing sexual behaviour or with anestrous females.

Although contradictory evidence exists, indications are that the elevations in T levels are accompanied by elevations in the levels of gonadotropins. Taleisnik et al. (1966) reported that copulation by the male rat triggered a release of LH within 5 to 10 mins., since pituitary LH levels were observed to drop while plasma levels increased; FSH levels rose 4 h post-coitum. Kamel et al. (1975) noticed that both sexually experienced and naive male rats had increased plasma levels of LH, PRL and T following mating; but the increases shown by the experienced rats were more pronounced. In a subsequent study, Kamel et al. (1977) reported that the levels of LH and PRL, but not FSH were increased during mating and suggested that the increase in T levels may be due to the prior increase in LH and PRL levels.

In rabbits, the levels of T are seen to increase about 30 to 90 mins. following copulation (Saginor and Horton, 1968;

Haltemeyer and Eik-Nes, 1969; Hilliard et al., 1975; Agmo, 1976), but it is not definite if this rise is preceded by rises of gonadotropins. Hilliard et al. (1975) found that basal serum LH levels remained unaltered after coitus. However, Younglai et al. (1976) observed slight nonsignificant increases in T soon after coitus or exposure to females. Increases in T were usually preceded by rises in LH, but these LH peaks were interpreted to be the occurrence of normal episodes. Furthermore, Agmo (1976) did not find an increase in LH concentration when male rabbits were allowed to ejaculate once or mount once without intromission. Thus they speculated that T may be released from the testes in response to mating without a prior release of LH. The question to ask then, is what triggers T increases in this species. It may well be that the rapidity with which this species copulates makes it difficult to detect early LH peaks.

With regards to the bull, Katongole et al. (1971) were able to show with a limited number of bulls that the sight of a cow, mounting without intromission or ejaculation caused an immediate release of LH which was often followed by an elevation in T when basal T levels were low. Smith et al. (1973) noticed that although LH had not increased appreciably 5 min. following ejaculation, T levels had increased in both mature and young bulls. LH levels were determined in four bulls prior to and following teasing (false mounts) and ejaculation by Gombe et al. (1973). In three of the four bulls, a slight decrease in plasma

LH levels was observed 30 min. following ejaculation; but a slight nonsignificant increase occurred in one bull. Similar to these results was the finding by Bindon et al. (1976) who exposed (single ejaculation or teasing for 4 mins.) bulls of normal libido and low libido to estrous cows. LH levels did not rise after sexual stimulation and T levels actually dropped in both groups.

Ellendorf et al. (1975) determined the pattern of plasma LH and T concentration before and after a single copulation by the male miniature pig by collecting blood samples at 10 min. intervals. Plasma LH was elevated 30 min. after copulation but plasma T levels were not altered. But it is important to mention that in this experiment a sow in estrus, although not visible to the boars, was placed in an adjacent room. Data presented by Wannamaker et al. (1979) suggested that boars in close proximity to or in physical contact with estrual sows exhibited T peaks in serum which may well be normal elevations in response to spontaneous LH pulses. On the other hand, copulation produced more frequent peaks of T. Determination of the levels of 5α-androstenone and T in peripheral plasma of boars before and after copulation, revealed that the response was variable and seemed to depend on the extent to which animals had been sexually stimulated prior to copulation. On the average ($n = 7$) androstenone and T levels 60 to 90 min. post-copulation were elevated 50 and 90%, respectively. Between 24

and 48 h following copulation, levels of both steroids had dropped in most animals. This was speculated to have been due to the stress of handling. Paradoxically, Liptrap and Raeside (1978) noticed that increases in the concentration of plasma T during copulation or periods of aggressive behaviour in the boar were highly correlated with increases in the concentration of corticosteroids. They speculated that the influence of increased corticosteroid secretion on plasma T levels may be biphasic in nature: 1) an initial positive effect of a transient nature due to conversion of corticosteroids to T; and, 2) an inhibitory influence associated with prolonged stressful conditions due to corticosteroids exerting a detrimental effect on T secretion.

Reports on the ram appear to be as conflicting as those on other species. While some researchers have not detected an influence of mating activity on T levels in young rams (Illius et al., 1976a, 1976b) and mature rams (Purvis et al., 1974; D'Occhio and Brooks, 1976), others have been able to clearly demonstrate that under certain conditions mating activity does elevate T levels in rams (Illius et al., 1976b; Sanford et al., 1974b; Sanford et al., 1977). It was noticed by Illius et al. (1976b) that rams kept near ewes for 6 mo. had larger testes, higher plasma T levels and greater sexual and aggressive activity. Furthermore, they noticed that when rams were allowed to have physical contact (30-min.) with estrual ewes, there occurred transient increases in the levels of T. Data by

Sanford et al. (1974b) which appears to be consistent with that of Moore et al. (1978) revealed that, when rams were allowed to observe (5 min.), mount without intromission (2 min.) or mate once, with an estrual ewe in January, LH pulses were not triggered. However, when rams were allowed to mate repeatedly during a 24-h period in January, higher baseline and mean serum levels of both LH and T, and a greater number of LH and T peaks were observed during the first 12 h. The serum levels of both hormones then decreased sharply at 13 to 14 h, and remained low for the next 8 to 10 h. Subsequently, a further serum peak of LH and T was observed before the conclusion of the 24-h period. In a subsequent study, rams were allowed to mate during 8-h test periods in August, September, November and December; during the first two mating periods when the frequency of LH peaks was relatively low, additional LH and T peaks were observed (Sanford et al., 1977). Thus the short-term effect of mating on LH release in rams appears to be season dependent. Mating activity has also been shown to significantly increase mean FSH levels especially during the first 12 h (Sanford et al., 1976a).

In an interesting study by Amann et al. (1978) it was demonstrated that there was an increase in testicular blood flow when rams were allowed to mount, intromit or ejaculate. Apparently, this increase in blood flow could result in a local increase in flow through the intertubular capillaries and thereby facilitate steroidogenesis in the Leydig cells which comprise only a small portion of the testis.

MATERIALS AND METHODS

Experimental Animals

Four Finnish Landrace (Finn) rams were selected for this study on the basis of mating capability. Finns were used because they exhibit remarkable sexual aggressiveness during most times of the year in comparison with other breeds of sheep. The experimental animals ranged in age from 2 to 4 years and weighed between 57.3 and 80.9 kg. The animals maintained good health throughout the course of the study, except for one (Ram #3) which developed an abscess on the neck during the latter stages of Experiment 2. Prior to and in between experimental periods of the study, the rams were penned together with other rams of the University flock in a three-sided, open-front barn and had no direct contact with ewes in nearby pens.

Five ovariectomized ewes were used to provide the mating stimulus to the rams. The ewes were brought into estrus by administering intramuscularly, a single progesterone (20 mg in corn oil) injection on days 1, 3, 5 followed by a 17β-estradiol (E_2, 1 mg in corn oil) injection on day 8. Ewes were usually in estrus 18 h after E_2 injection. In Experiment 2, estrus was maintained by half-dosage injections of E_2 (.5 mg) every 8 h.

The animals were fed a legume hay and grain ration and had access to water *ad libitum*.

Collection and Handling of Blood

Although the rams had not been accustomed to blood collection, they quickly adapted to the bleeding routine. However, they showed some signs of physical stress during the first collection period and on the first few samplings of each subsequent collection period. In addition one animal (Ram #3) seemed to abhor being bled over a very long period.

At the time of bleeding about 6 ml of blood were obtained from the jugular vein by venipuncture using 20 gauge, 1 1/2" long needles and 7 ml vacutainer tubes. Blood samples were kept cool on ice and later refrigerated at 4 to 5°C. Samples were centrifuged within 24 to 48 h of collection and the sera decanted into 1 dram vials and stored at -20°C until thawed and assayed for the various hormones.

Hormone Assay Procedures

In order to determine characteristics of LH secretory profiles, aliquots (.2 ml) of all serum samples collected during the bleeding periods in Experiment 1 were assayed for LH. All samples for each ram were included in the same assay. In addition, sera pooled from collections during the 8-h periods in Experiment 1 were assayed for LH, FSH, PRL and T. Aliquots of .2, .2, .025 or .1, and .05 or .1 ml were used for LH, FSH, PRL and T, respectively.

In Experiment 2, sera pooled for each consecutive 2 h of

36-h periods were assayed for the respective hormones. Aliquots (.025, .1 or .2 ml) of pooled sera obtained from all rams were measured for LH in a single assay. The determination of FSH and PRL concentration in aliquots (.1 or .2 ml for FSH; .025 or .1 ml for PRL) of pooled sera were done for two rams at a time. Aliquots (.05 or .1 ml) of pooled sera obtained for each ram were measured for T concentration in two assays.

LH Assay - An established double antibody radioimmunoassay was used to determine LH concentrations. Details of the procedure have been previously described by Niswender et al. (1969) and modified by Howland (1972). Anti-ovine LH serum (GDN #15) supplied by Dr. G. Niswender (Colorado State University) was used in the assay. Labelling of purified ovine LH (LER-1056-C2) with ^{125}I (Cambridge Nuclear Corporation) was by a modification of the method of Greenwood et al. (1963). This modified procedure has been described by Sanford (1974). LH values were expressed as ng/ml of NIH-LH-SI4 standard. The anti-ovine LH serum was used at an initial dilution of 1:100,000 in .5% rabbit serum phosphate-disodium-ethylene dinitrolotetracetate (RS-phosphate-EDTA) buffer. (See Appendix II, Table 23).

The inter-assay and intra-assay (Rodbard, 1971) coefficients of variation for five replicate samples from a pooled serum standard with a mean concentration of 1.31 ng/ml were 13.4% and 10.0%, respectively. The lowest detectable LH level defined as

95% initial binding (B/B_0), ranged from .09 to .13 ng/ml. For statistical purposes, samples yielding values lower than the minimum detectable level in this and other hormone assays were assigned the corresponding minimum detectable value.

Prolactin Assay - Serum PRL levels were determined by a slight modification of the method described by Sanford et al. (1978a), and was very similar to that described for ovine LH. An anti-ovine PRL serum developed in rabbits (Friesen's #73) was used. The assay employed ^{125}I-labelled ovine PRL (LER-860-2) and NIH-PRL-S12 standard. The standard was serially diluted to obtain concentrations in the range of 1 to 80 ng/tube. The anti-ovine PRL serum was intially diluted 1:12,000 in .5% RS-phosphate-EDTA buffer. Anti-rabbit gamma globulin serum was used to separate bound from free hormone.

The inter-assay and intra-assay coefficients of variation for two replicate samples of pooled serum standard were 12.1% and 6.6%, respectively. The mean concentration of the serum standard was 57.5 ng/ml. The sensitivity of the assays ranged from 2.2 to 2.3 ng/ml.

FSH Assay - FSH levels were measured by a procedure developed and described by Dr. Cheng, Health Sciences Centre, University of Manitoba (Cheng et al., 1980). Cheng's rabbit anti-bFSH sera was used at an initial dilution of 1:60,000 in RS-phosphate-EDTA buffer. Purified bFSH labelled with ^{125}I was employed in

this assay system. Anti-rabbit gamma globulin serum was used to separate bound from free hormone. FSH values were expressed as ng/ml of NIH-FSH-S12.

The inter-assay and intra-assay coefficients of variation for two replicate samples from a standard serum pool with a mean concentration of 90.5 ng/ml were 14.9% and 9.2%, respectively. The sensitivity of the assay ranged from .7 to 1 ng/ml.

Testosterone Assay - The assay procedure previously employed in this laboratory (Sanford et al., 1974a; Sanford et al., 1978a) has been extensively modified and is now similar to that described for progesterone and estrogen by Yuthasastrakosol (1975).

Antiserum - The antiserum used was raised in sheep immunized with T-3-carboxy-methyloxime conjugated to bovine serum albumin (Sanford et al., 1978a). The extent of cross reactivity of the antiserum with steroids other than T was determined from the amount of each steroid measured when 5 ng (in ethanol) were added to tubes processed for T determination. The antiserum showed some cross-reactivity with 1, 4-androstediene-17β-ol-3-one (14%), 4-androstene-3 , 17β-diol (10%), androstan-17β-ol-3-one (9%) and 5α-androstan-3α, 17β-diol (5%). However, these cross-reacting steroids are present in ram blood in such minute quantities that they do not interfere with T estimations (Sanford et al., 1974c). The antiserum was used at an initial dilution of 1:2,400.

Labelled Testosterone - Stock solution T-1, 2, 6, 7-H^3 purchased from New England Nuclear was intially diluted with benzene-ethanol (9:1) at a ratio of 1:10 and stored at 5°C. For assay 15 to 20 µl of stock solution were dried under nitrogen gas and reconstituted with 20 ml phosphate buffer solution (PBS; Appendix II, Table 23) containing .1% gelatin to give ^3H-activity ranging from 8,000 to 12,000 cpm/.1 ml. The working trace solution was freshly prepared for each assay.

Testosterone Standards - An aliquot (.1 ml) of stock solution (10 mg % in distilled absolute ethanol) was diluted with 10 ml distilled ethanol to obtain a dilute stock solution. Working standard solutions of 0, 10, 25, 50, 100, 200, 300, 400, 500, 600 and 1,000 pg/ml were prepared by serially diluting the dilute stock solution with PBS. Working standards were usually discarded after a period of 2 weeks.

Extraction Procedure - Single aliquots of unknown serum samples (.05 or .1 ml) and pooled castrate and intact ram sera were pipetted into 20 x 150 mm disposable glass culture tubes and brought up to 1 ml with PBS. Samples were vortexed gently for 30 sec. with 8 ml absolute diethyl ether obtained from a freshly opened can and then allowed to freeze while kept at -20°C for 2 to 3 h. The ethereal layer was then decanted into a second 20 x 150 mm tube and dried under a gentle stream of N_2 gas in a water bath at 37°C (10 - 20 min. required). The

residue was redissolved in 1.2 ml PBS and shaken for 10 min.. Extract (.5 ml) was pipeted into duplicate culture tubes (12 x 75 mm) for radioimmunoassay.

Estimation of procedural losses was done by addition of .1 ml ^{3}H-T, prior to extraction, to a set of three culture tubes containing serum samples chosen at random. Percentage recovery (% R) was calculated based on the equation $\% R = 100 \left(\frac{Ri\text{-}cpm \times 1.2}{Rt\text{-}cpm \times .5}\right)$ from ^{3}H-activity present in .5 ml extract (Ri-cpm) and the total activity (Rt-cpm). Percentage recovery estimations following extraction averaged ($\pm$ S.E.) 83.1 $\pm$ 1.6% (n = 8).

Assay Procedure - A .5 ml volume of each standard solution was added to duplicate culture tubes (12 x 75 mm). Two sets of three culture tubes meant for monitoring total ^{3}H-activity, hereinafter denoted as 'Totals', were also included in each assay; to these tubes were added 1.1 ml of PBS. Except for 'Totals', .1 ml antiserum was added to all assay tubes. Following this, .1 ml of labelled-hormone was added to all tubes including 'Totals'. Then, the tubes were gently vortexed for 5 sec., covered with aluminum foil or parafilm and incubated overnight for approximately 16 h at 4°C.

While maintaining the temperature of constantly stirred dextran-coated charcoal suspension (250 mg activated Norit A charcoal; 25 mg Dextran T-70; 100 ml PBS) at about 4°C, .5 ml of this solution was added to all tubes except 'Totals'; and tubes immediately vortexed for 5 sec. Tubes were then incubated

for 20 min. at 4°C and centrifuged at approximately 3,000 rpm for 10 min.

Each supernatant fraction was decanted and sent for scintillation counting (Nuclear Chicago Unilux II) after addition of 4 ml of scintillation fluid. Vials were shaken vigorously for about 10 sec. and equilibrated in the dark at 4°C for 4 to 16 h before counting.

Calculations - Using the standard curve and relating percentage binding to mass of T, the T content of each unknown sample was determined. Recovery estimates were used to correct for extraction losses. Values were expressed as ng/ml after subtraction of the estimate for the pooled castrate ram serum. Estimates for pooled castrate ram serum were subtracted from calculated values in order to minimize serum effect. The calculation procedure is represented by the equations below:

$$Y = X - BV$$

$$X = \frac{p \times cf_1 \times cf_2}{1{,}000}$$

where Y = corrected concentration of T (ng/ml) in serum sample

X = unknown concentration of T (ng/ml) in serum sample

BV = unknown concentration of T (ng/ml) in blank (castrate serum)

p = picogram T determined from standard curve using % binding

cf_1 = correction factor due to extraction losses $(\frac{1}{\%\ R})$

cf_2 = dilution factor (e.g., $\frac{1.2}{.5} \times 10$).

$$\text{N.B.} \quad BV = \frac{p \times cf_1 \times cf_2}{1{,}000} .$$

Estimated values for castrate ram serum pool ranged from 1.87 to 3.31 ng/ml. From the eight duplicate determinations on the intact ram serum pool, the inter- and intra-assay coefficients of variation were calculated to be 13.7% and 4.3%, respectively. T concentration averaged 8.43 ng/ml. The sensitivity of the assay ranged from .42 to .60 ng/ml.

Definitions of the LH-Profile Characteristics

MEAN BASELINE. The mean of the lowest single value(s) between peaks; i.e., usually those which immediately preceded an elevation (Sanford et al., 1977).

PEAK. A measurable rise followed by a fall in concentration. The rise was considered measurable when the difference between consecutive low and high values were higher than three standard errors of the overall sampling period mean (Blanc et al., 1978).

PEAK HEIGHT. The highest value associated with the peak.

DELTA (Δ) VALUE. The difference between peak height and baseline value.

Statistical Procedures

As mentioned previously, the intra-assay coefficient of variation was calculated according to the method of Rodbard (1971). Estimation of inter-assay coefficient of variation was by standard statistical procedure (Snedecor and Cochran, 1976).

In Experiment 1, data obtained for various treatments were subjected to analysis of variance, and differences between means tested by the Student-Newman-Keuls (SNK) test; seasonal differences in hormone concentration were subjected to a paired t-test (Snedecor and Cochran, 1976).

Data for Experiment 2 were analyzed as a 2 x 4 x 6 factorial (i.e., mating and control periods for four rams with six time intervals). The BMDP Biomedical Computer Program (P-series 2V) developed by R. Jennrich and P. Sampson (1977) for the analysis of variance and covariance was used. This program takes into account the fact that data were collected from the same rams on many occasions.

EXPERIMENTAL

Experiment 1. Variation in Serum Levels of LH, FSH, Prolactin and Testosterone in Rams Engaged in Various Types of Sexual Activity During the Ovine Non-Breeding and Breeding Seasons

Seasonal changes in peripheral blood levels of LH, FSH and T (Sanford et al., 1976a; Lincoln et al., 1977; Sanford et al., 1977; Sanford et al., 1978a) and in libido (Pepelko and Clegg, 1965; Sanford et al., 1974b; Schanbacher and Lunstra, 1976) and semen production (Cupps et al., 1960; Jackson and Williams, 1973) have been demonstrated to occur in the ram; the changes being more pronounced in the fall breeding season.

The direction of these seasonal-endocrine changes remained unaffected in rams allowed to copulate (Katongole et al., 1974; Purvis et al., 1974; Sanford et al., 1977), although close proximity of rams to estrual ewes has been found to increase mating activity and T levels during the breeding season (Illius et al., 1976b). By contrast, short periods of exposure to estrous-induced ewes could not be associated with alterations in LH and T secretion in the ram (Sanford et al., 1974b). However, extended mating periods (8-12 h) evoked transient increases in the levels of LH, FSH and T secretion which appeared to be season dependent; it was evident only in the early and latter parts of the breeding season (Sanford et al., 1974b; Sanford et al., 1976a; Sanford et al., 1977).

It is important to determine the type of sexual activity or specific stimuli that initiates the changes in the levels of LH, FSH and T, and the significance of these changes. Preliminary investigation (Sanford et al., - unpublished) indicated that elevations in hormone secretion in the nonbreeding season may be initiated by the act of ejaculation. Thus a major objective of this experiment was to investigate this possibility, as well as the influence of various sexual activities on hormone secretion during the breeding season. Prolactin was included due to its importance in the regulation of sexual function (see Review).

Experimental Plan

The study was designed as a 4 x 4 Latin Square. Four mature Finnish Landrace rams were bled at 20-min. intervals for 8 h when allowed to: serve as controls, observe, mount without intromission, or mate ovariectomized estrous-induced ewes. Rams were prevented from intromitting by covering their undersides completely with burlap aprons. When a ram was serving as a control he was bled while penned away from the auditory, visual and olfactory influences of the other rams and ewes involved in sexual activity. Each of the three remaining rams exposed to ewes were placed individually in three adjacent pens (1.8 x 2.8 m) in an enclosed area of the barn. The animals

that mated or mounted were placed in the end pens.

Placement of rams in their respective pens was done at approximately 0800 h on the day of blood collection, and rams were left undisturbed for 1 h to become accustomed to the different surroundings. A single blood sample was taken from each ram at approximately 0900 h before the introduction of the estrual ewes. Just prior to the second bleeding, an estrual ewe was placed with the rams designated to mount or mate. The ewes were changed after 4 h to provide additional incentive to rams to continue mating and mounting.

The sexual behavior of all rams was closely observed during the specified time periods. The number of mounts and mounts culminating in ejaculation during each 20-min. interval were recorded for each ram when mating. Ejaculation was considered to have occurred when a mount was succeeded by a marked pelvic thrust, followed by a period of complete sexual inactivity. Similarly, the number of mounts were recorded for aproned rams. Only minimal physical contact was allowed between the observing ram and estrual ewes in adjacent pens.

This experiment was performed in July (nonbreeding season) and repeated in October (breeding season). Blood collection periods in July occurred on day 5, 7, 18 and 21 of the month. In October blood was collected on day 11, 15, 20 and 25.

Results

Rams quickly exhibited the desired sexual behavior upon introduction of estrous-induced ewes into their pens. When allowed to mate, they mated more frequently during the first 40-min. and their frequency of mating became less in subsequent 20-min. intervals. Nevertheless, overall mating was considerable (Table 1) with no significant ($P > .05$) difference between the seasons. Rams allowed to mount only, mounted often and fairly consistently during the 8-h period (Table 2); and again there was no significant difference between the seasons. Rams which observed, were obviously excited judging from their attempted contacts with estrous-induced ewes in adjacent pens. This activity was maintained throughout the 8-h period.

Mean hormone levels and the characteristics of LH profiles determined for rams performing different sexual activities during the non-breeding season are shown in Tables 3 and 4, respectively. In addition the LH profiles for control and sexually active periods of each ram in the non-breeding season are depicted in Figure 1. Mating triggered significant ($P < .05$) increases in mean LH levels which were associated with nonsignificant increases in basal LH and the number of LH peaks. LH peaks during the mating periods seemed to occur at less regular intervals and exhibited substantial variation in their magnitudes. The other two forms of sexual activity

TABLE 1

Mean (± S.E.) Number of Mates and Mounts Recorded For Rams During the 8-h Mating Periods

Season	Activity	$\bar{x}$ ± S.E.
Non-breeding (July)		
	Mounts	30.0 ± 3.6
	Mates	24.5 ± 4.7
	Mounts/Mate	1.28 ± 0.10
Breeding (October)		
	Mounts	26.3 ± 4.0
	Mates	23.0 ± 3.6
	Mounts/Mate	1.15 ± 0.07

Each value represents the mean of four rams

TABLE 2

Mean (± S.E.) Number of Mounts Recorded for Rams During the 8-h Mounting Periods

Season	$\bar{x}$ ± S. E.
Non-breeding (July)	187.3 ± 26.2
Breeding (October)	177.5 ± 6.7

Each value represents the mean of four rams

TABLE 3

Mean (± S.E.) levels (ng/ml) of LH, FSH, Prolactin (PRL) and Testosterone (T) in Peripheral Blood Serum of Rams Bled at 20-minute Intervals During 8-h Control or Sexually Active Periods in the Non-Breeding Season (July)

	TREATMENT				
		Interaction With Estrual Ewes			
Hormone	Control	Observed	Mounted	Mated	Pooled S. E.
LH	.6[a]	.5[a]	.4[a]	1.2[b]	.15
FSH	42.3[a]	48.4[a]	45.6[a]	52.4[a]	3.9
PRL	251.2[a]	125.9[b]	284.4[a]	150.7[b]	26.7
T	2.5[a]	3.1[a]	2.4[a]	4.3[a]	.5

Each value represents the mean of four rams. Horizontal means followed by the same superscript are not significantly ($P > .05$) different

TABLE 4

Characteristics of Serum LH Profiles for Rams Bled at 20-minute Intervals During 8-h Control and Sexually-active Periods in the Non-breeding Season (July)

	TREATMENT				
		Interaction With Estrual Ewes			
	Control	Observed	Mounted	Mated	Pooled S. E.
Baseline level (ng/ml)	.39	.24	.24	.68	.15
Peak frequency (per 8h)	1.0	1.5	1.4	3.0	.5
Peak height (ng/ml)	7.3	5.0	5.7	6.3	2.0
Δ value (ng/ml)	6.9	4.8	5.5	5.6	2.0

Values represent the mean of four rams

No significant ($P > .05$) differences between horizontal means

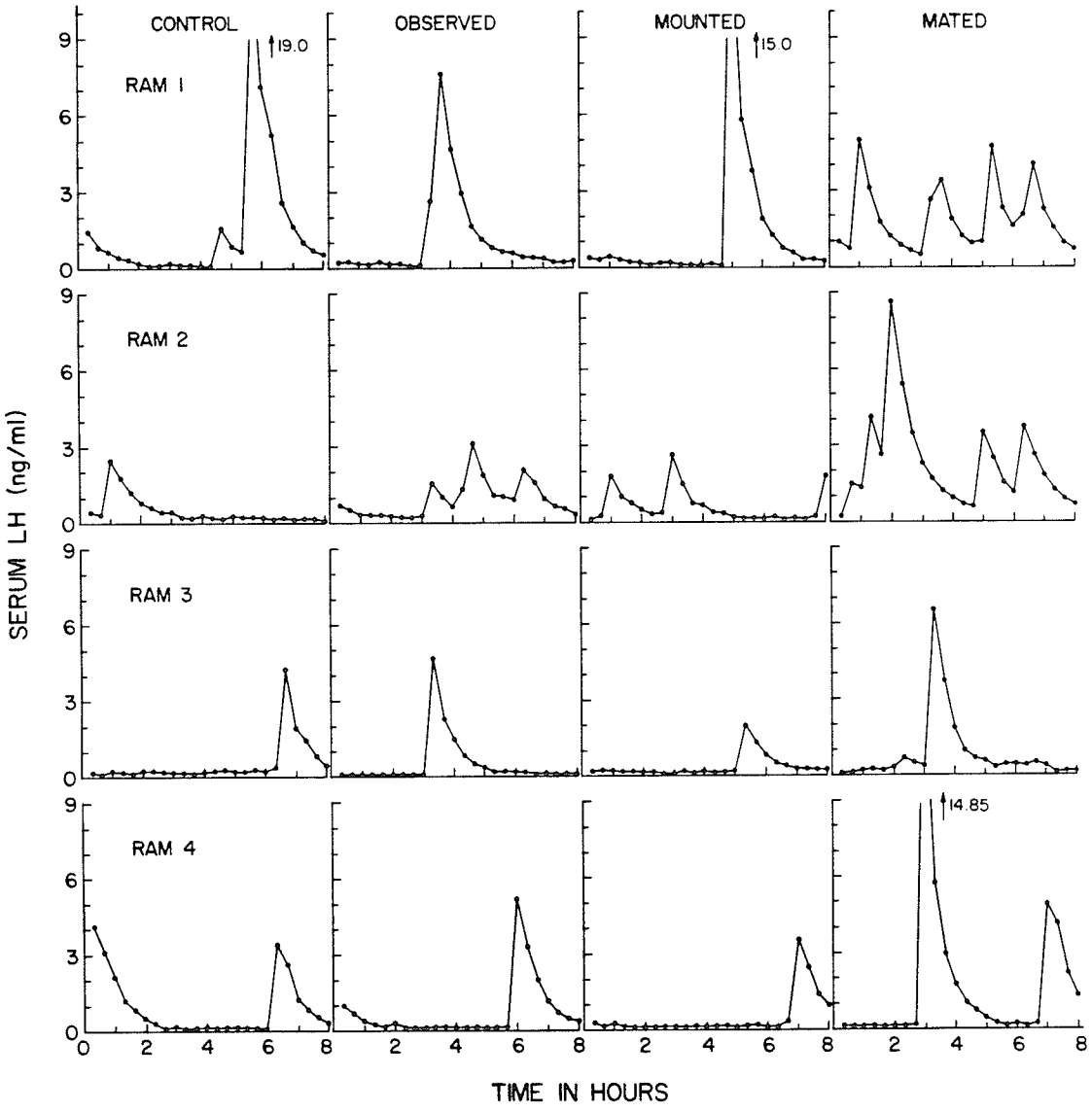

Figure 1. Profile of LH fluctuations in peripheral blood serum of rams bled at 20-min. intervals during 8-h control and sexually-active periods in the non-breeding (July) season. Bleeding started at approx. 0900 h.

resulted in only minor depressions of mean and basal levels of LH. Although, sexual activities other than mating generally did not seem to affect LH-peak frequency, in one ram (Ram #2) both observation and mounting of ewes evoked an increase in the number of LH peaks but not to the same extent as mating did (Figure 1). None of the sexual activities affected mean FSH levels. Mating estrual ewes resulted in a substantial but nonsignificant elevation in mean T levels. Mean PRL levels were relatively low and comparable when rams were either observing or mating, and were significantly ($P < .05$) higher when they were either mounting or serving as controls. However, this pattern was not consistent among rams (Appendix I, Table 4).

The breeding season hormonal responses are presented in Tables 5 and 6. The LH profiles of control and sexually-active periods for each ram during the same season are displayed in Figure 2. Mating activity resulted in a significant ($P < .05$) increase in basal LH, but only a slight increase in mean LH and in the frequency of LH peaks. Serum LH-profile changes were not associated with a significant increase in mean T. The mean levels of LH and particularly mean T ($P < .05$) were depressed from control levels during mounting periods. Mounting also resulted in a slight decrease in LH-peak frequency and a significant ($P < .05$) decrease in LH-peak height and Δ values. Similarly, observation resulted in a decrease in the frequency of LH-peaks, basal and mean LH levels, and especially mean T level ($P < .05$). FSH did not vary among treatments. Mean PRL

TABLE 5

Mean (± S.E.) Levels (ng/ml) of LH, FSH, Prolactin (PRL) and Testosterone (T) in Peripheral Blood Serum of Rams Bled at 20-minute Intervals During 8-h Control or Sexually Active Periods in The Breeding Season (October)

	TREATMENT				
		Interaction With Estrual Ewes			
Hormone	Control	Observed	Mounted	Mated	Pooled S. E.
LH	1.0^{ab}	.7^{a}	.7^{a}	1.2^{b}	.1
FSH	107.6^{a}	114.5^{a}	148.6^{a}	124.8^{a}	10.7
PRL	4.7^{a}	32.0^{a}	148.6^{b}	55.1^{a}	25.3
T	17.9^{b}	13.7^{a}	13.8^{a}	18.8^{b}	.8

Each value represents the mean of four rams
Horizontal means followed by the same superscript are not significantly ($P > .05$) different

TABLE 6

Characteristics of Serum LH Profiles for Rams Bled at 20-minute Intervals During 8-h Control and Sexually-Active Periods in the Breeding Season (October)

	TREATMENT				
	Interaction With Estrual Ewes				
	Control	Observed	Mounted	Mated	Pooled S. E.
Baseline level (ng/ml)	.61[a]	.40[a]	.43[a]	.83[b]	.06
Peak frequency (per 8h)	3.6[ab]	2.5[a]	2.6[a]	4.3[b]	.3
Peak height (ng/ml)	3.2[b]	3.1[b]	1.9[a]	3.1[b]	.2
Δ value (ng/ml)	2.6[b]	2.6[b]	1.4[a]	2.2[b]	.2

Horizontal means followed by the same superscript are not significantly ($P > .05$) different

Values represent the mean of four rams

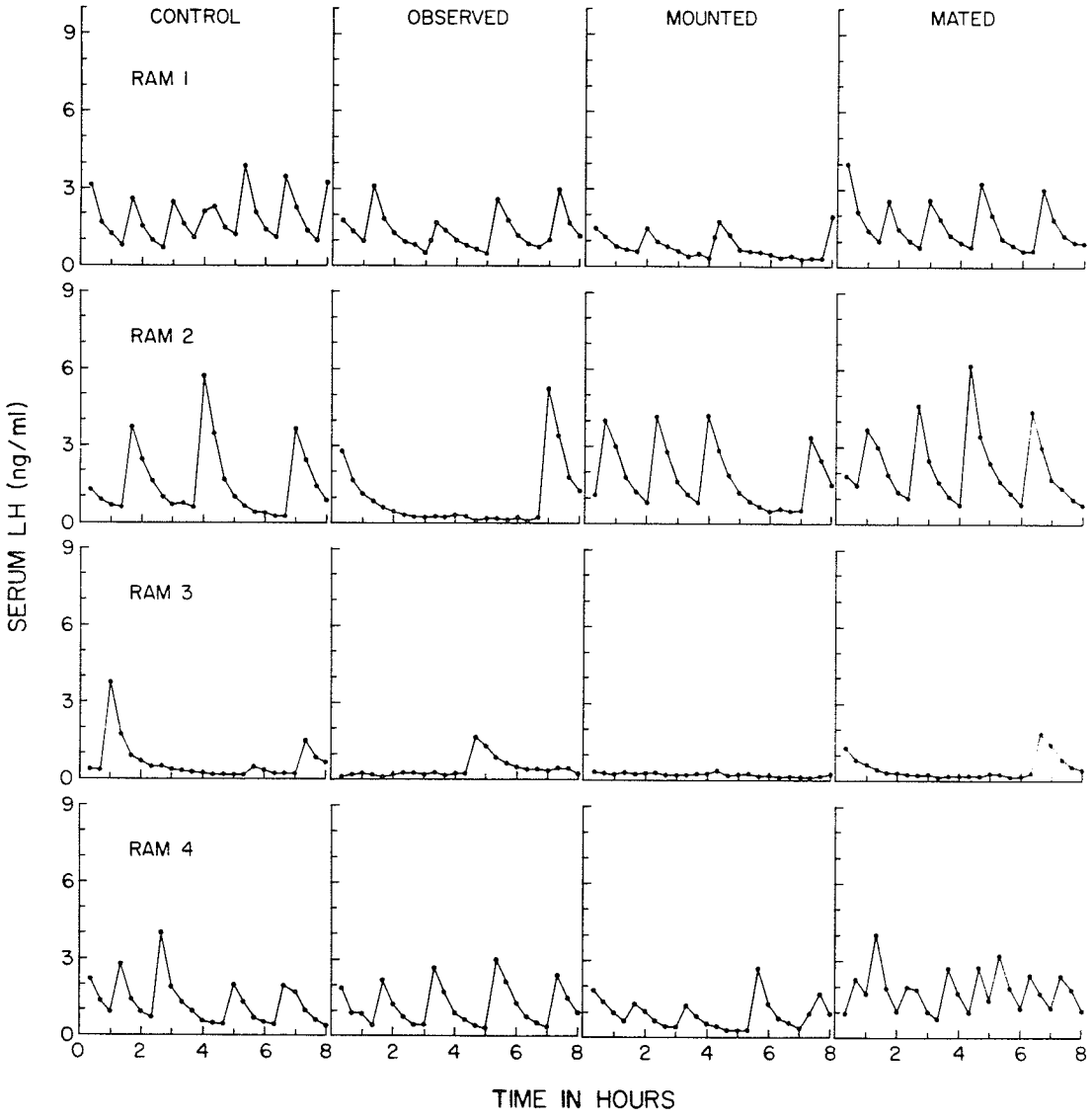

Figure 2. Profile of LH fluctuations in peripheral blood serum of rams bled at 20-min. intervals during 8-h control and sexually-active periods in the breeding (November) season. Bleeding started at approx. 0900 h.

levels for the 8-h control periods averaged 4.7 ± 1.6 ng/ml. The incidence of sexual activities triggered substantial elevations in PRL concentration above control levels, with levels for mounting being remarkably higher (148.6 ± 41.9 ng/ml, $P < .05$).

In July there were not substantial variations in mean hormone levels or LH-profile characteristics from one bleeding period to the next; an exception was the very high PRL level noticed for the first bleeding period: 396 ± 83.9 ng/ml ($P < .01$). PRL values for the second, third and fourth bleeding periods were 118.8 ± 27.8, 154.7 ± 33.8 and 142.7 ± 27.5 ng/ml, respectively. Similarly, in October, hormone levels of the rams generally remained unchanged from one period to the next except for significant ($P < .01$) differences in basal LH level and the number of LH peaks.

The normal and expected seasonal-endocrine changes were seen in the rams (Tables 7 and 8). The frequency of LH release, and mean LH, FSH and T levels were higher in October compared to July. In contrast, LH peak height and mean PRL levels were low in October but high in July.

Discussion

It has been reported that the mating performance of a subordinate ram may decline when viewed by a dominant ram (Lindsay *et al.*, 1976). Also, mating performance of rams is known to decrease after a single mate or a period of several copulations (Thiery and Signoret, 1978). These detrimental

TABLE 7

Mean (± S.E.) Levels (ng/ml) of LH, FSH, Prolactin (PRL) and Testosterone (T) in Peripheral Blood Serum of Rams Bled at 20-minute Intervals During an 8-h Control Period in the 'Non-breeding (July) and Breeding (October) Seasons

Hormone	Non-Breeding Season	Breeding Season
LH	.6 ± .2	1.0 ± .2*
FSH	42.3 ± 4.5	107.8 ± 26.3*
PRL	251.2 ± 90.1	4.7 ± 1.4*
T	2.51 ± .3	17.9 ± 3.5**

Season differences significant at $P < .10$* or $P < .05$**

Each value represents the mean (± S.E.) of four rams

TABLE 8

Characteristics of Serum LH Profiles for Rams Bled at 20-minute Intervals During an 8-h Control Period in the Non-Breeding (July) and Breeding (October) Seasons

Characteristic	Non-Breeding Season	Breeding Season
Baseline level (ng/ml)	.4 ± .1	.6 ± .1
Peak frequency (per 8-h)	1.0 ± 0.0	3.6 ± .7**
Peak height (ng/ml)	7.3 ± 3.4	3.2 ± .4
Δ value (ng/ml)	6.9 ± 3.3	2.6 ± .4

Season difference significant at $P < .05$**

Values represent the mean (± S.E.) of four rams

influences to mating behavior may have been corrected in part by changing the teaser ewes. However, it is difficult to assess if the change after 4 h was adequate to compensate for the presumptive decline in mating behavior. In any case, stimulation could be regarded to be substantial since rams performed their various activities promptly and fairly consistently over the 8-h test periods.

Results of the present study indicated no significant differences between seasons in the various types of sexual activity. However, several investigators (Pepelko and Clegg, 1965; Schanbacher and Lunstra, 1976; Sanford et al., 1977) have noticed an increase in mating activity during the breeding season. Probably the wide variation that existed between rams in terms of their mating performance, coupled with the fact that only four rams were used precludes valid statistical inference. But, it may be possible to explain the absence of a seasonal increase in mating behavior in the presence of an increase in mean serum T based on results obtained by Schanbacher and Lunstra (1976) and Sanford et al. (1977). They found a high and positive correlation between seasonal changes in T levels and mating activity of rams. Therefore, it is probable that, although levels of mean T in rams in the present study during the non-breeding season were low in comparison to those of the breeding season, the levels for these rams at this time were above or close to threshold levels required to maintain maximal

behavioral responses. Also, the fact that Finnish Landrace rams are extremely sexually active, in comparison to other breed types, may preclude extensive seasonal variations in mating behavior.

The hour-to-hour changes that occur in the levels of LH and T in peripheral blood are not regularly influenced by brief involvement in various kinds of sexual activity. Neither the observation nor mounting of estrual ewes for 2 to 5 min. is consistently followed by elevations in the levels of these hormones (Sanford et al., 1974b). Furthermore, rams allowed to ejaculate once or several times during a period of 1 h generally do not exhibit increases in blood LH and T (Purvis et al., 1974; Sanford et al., 1974b). Nevertheless, when rams are allowed to mate for prolonged periods of time (8-12 h) during the latter part of the breeding season, the levels of FSH (Sanford et al., 1976a), LH and T (Sanford et al, 1974b; Sanford et al., 1977) are temporarily elevated. Similarly, mating activity early in the breeding season (August and September) is associated with an increase in the frequency of LH pulses and mean T levels, but no change in mean FSH levels (Sanford et al., 1977). Clearly, these results together, indicate that repeated ejaculation exerts a positive short-term influence on the secretion of LH and T and perhaps FSH, in rams during periods of the year when levels are relatively low. The results of the present study support these observations. During

the non-breeding season when control values of LH and T were low, mating triggered increases in mean and basal LH levels, the number of LH peaks and mean T levels in the rams. Although mating activity during the breeding season was associated with significant ($P < .05$) increases in basal LH levels and a slight increase in LH-peak frequency, these changes in the pattern of release could not stimulate substantial elevations in circulating T levels. The reason for the lack of a significant T response may have been due in part to the high levels of T already present at that time of the year. It is reported that during all seasons the episodic releases of LH induce a maximum response from the testes in terms of T secretion, which varies according to the synthetic state of the gonad, and that supra-physiological levels of LH do not induce short-term rises in circulating T outside the normal range (Katongole et al., 1971; Lincoln, 1976). Thus one should expect T levels to rise with the occurrence of repeated mating, only during those months of the year when both LH-peak frequency and T levels are relatively low.

It is of interest to know what component of the rams sexual behavior is actually responsible for the transitory increases in hormone levels, but as yet, published data in this area is lacking. Preliminary investigation in this laboratory suggested that it is the stimuli associated with the act of repeated ejaculation which triggers increases in hormone levels, since the act of repeated mounting or observing

an estrual ewe was associated with gradual depressions in LH and T levels (Sanford et al. - unpublished data). Data obtained in this study are in agreement with the earlier findings. Similarly, the act of repeated mounting and observation produced declines in LH and T levels except that, in one ram these acts led to an increase in the number of LH episodes during the non-breeding season. Although the occurrence of these episodes was possibly normal (i.e., spontaneous and unrelated to the presence of ewes), it is still likely that in this particular ram, and may be in other rams, the act of observation or mounting during the non-breeding season provides adequate stimulation for the secretion of additional LH. In rats, it has been observed that, even though copulation elicits an increase in the levels of LH and T, their mere anticipation of a mating encounter is enough to trigger a rise in the levels of these hormones (Kamel et al., 1975). Similarly, boars kept in close proximity to or allowed physical contact with estrual sows exhibited increased numbers of T peaks in serum but not to the same extent as that produced by copulation (Wannamaker et al., 1979). Some bulls have been shown to respond to the sight of a cow, false mounting or ejaculation by exhibiting an immediate and pronounced release of LH and T in blood (Katongole et al., 1971). Other studies have shown however, that false mounting and ejaculation may or may not be followed by slight nonsignificant increases in LH (Convey et al., 1971; Gombe et al., 1973) and moderate elevations in T (Smith et al., 1973).

This effect of observation or mounting on additional LH secretion may be especially true following long-term exposure since it has been observed that keeping rams adjacent to ewes brought into estrus periodically, increased rams' circulating T levels and testis sizes (Illius et al., 1975; Illius et al., 1976b; Sanford and Yarney, 1980).

In the present study, the levels of FSH were unaffected by mating in both the non-breeding and breeding seasons. Similarly, Sanford et al. (1977) could not detect increases in mean FSH when rams were allowed to mate for 8-h periods in August through December. In contrast, when two rams were allowed to mate estrual ewes during a period of 24-h in January, mean FSH levels were found to have increased during the first 12 h. Mounting or observation during the non-breeding or breeding season could not be associated with increases in FSH levels and this conforms to the results of a subsequent study performed in January (Sanford et al. - unpublished data).

The pattern of response of PRL levels during the non-breeding season does not indicate any apparent relationship with sexual activity. Nevertheless, during the breeding season, all types of sexual activity appeared to have stimulated substantial elevations in PRL concentrations. Yet, it may be argued that PRL levels were elevated in sexually-active rams because they were penned in the enclosed area of the barn where the ambient temperature was higher than the outside

temperatures. During this time of the year the outside temperatures ranged from a daily average of 3.8 to 12°C, whereas the temperature in the enclosed area of the barn was approximately 23°C. Sanford et al. (1978b) reported that when rams were moved from an outside environment (temperature ranging from -10 to -25°C) to an inside environment (temperature ranging from 24 to 30°C), PRL levels were raised about two-fold within one day. Therefore, there is ample reason to believe that the elevated PRL levels encountered in this experiment were due in part to the elevated temperatures. Alternatively, the stress effect associated with sexual activity (mounting in particular) was probably a major cause of the elevated PRL levels.

Alterations in hypothalamic-hypophyseal function brought about primarily by changes in photoperiod are thought to account for seasonal variation in the pattern of LH release. Although, direct measurement of GnRH secretion during changes in photoperiod has not been made in the ram, it has been demonstrated that the hypothalamic content of GnRH is influenced by photoperiod, and that rams exposed to natural daylength have increased GnRH activity in their hypothalami before the autumn mating season at the time when the testes are increasing in size and function (Pelletier, 1971). Presumably, the increased GnRH activity is due to a decrease in the negative-feedback effect of gonadal steroids (T) at the level of the hypothalamus

(Pelletier and Ortavant, 1975b). Since, it is probable that the episodes of LH release which produce transitory peaks in serum LH concentration are the result of episodes of GnRH (Lincoln, 1976; Lincoln, 1978), LH profiles may be used as an indirect indicator of some aspects of hypothalamic activity. Additionally, the pituitary gland does not appear to possess an intrinsic capacity to cause episodic gonadotropin release (Bremner et al., 1976). In this regard, a change from long to short days would stimulate episodic GnRH release and consequently episodic LH release, as was the case in this study. Likewise, the additional LH peaks that were observed in some rams during the period(s) of sexual activity may have been due to an increase in the frequency of pulsatile releases of GnRH.

It is also purported that the responsiveness of the pituitary to a standard dose of GnRH increases as the breeding season advances (Lincoln, 1976). This increase in responsiveness could account for the rise in LH levels. These changes could be brought about by a decrease in the negative-feedback effect of gonadal steriods directly on the pituitary, although it is curious that the greatest pituitary response to exogenous GnRH is seen during months of rising T levels. Mean T concentrations in rams used in this study had increased about seven-fold by October. This seasonal increase is in general agreement with previous reports (Katongole et al., 1974; Sanford et al; 1974b; Schanbacher and Lunstra, 1976) and is

undoubtedly due in part to the increase in the number of spontaneous LH releases. An increase in the responsiveness of the testes to LH stimulation (Lincoln, 1976; Sanford et al., 1977) is likely another factor that accounts for higher circulating levels of T during the breeding season.

It is not clear which component of the fluctuating levels of LH are involved in determining the major stimulus to the target organ (i.e., Leydig cells) and which may promote the seasonal elevation in T level. Some studies (Sanford et al., 1977; Ponzilius and Sanford, 1980) seem to suggest that LH-peak frequency is an important factor. This is indicated by the fact that the magnitude of LH-peaks decreases in the breeding season, while the smaller pulses, although greater in number, stimulate increasingly larger T elevations. In other studies basal LH levels have been observed to increase considerably either alone (Schanbacher and Ford, 1976b) or in association with increases in LH-peak frequency (Lincoln, 1976); perhaps changes in basal LH levels is an important factor as well. In the present study, in spite of the fact that each LH-profile characteristic increased or decreased in the normal and expected direction, the changes were generally slight and nonsignificant. Only LH-peak frequency increased significantly ($P < .05$) and therefore could be considered of prime importance in elevating T secretion.

The significant seasonal increases in mean FSH are in agreement with other reports (Lincoln et al., 1977; Sanford et al., 1977, 1978a). Likewise, the seasonal decrease in PRL level agrees with the results of other investigators (Pelletier, 1973; Ravault and Ortavant, 1977; Sanford et al., 1978a; Sanford and Dickson, 1980).

Experiment 2. Effect of Repeated Mating on Reproductive Hormone Secretion and Pituitary Responsiveness to Gonadotropin Releasing Hormone

Whereas single ejaculations are not consistently associated with variations in LH and T secretion in the ram (Sanford et al., 1974b), it is apparent that during certain months of the year several matings will induce increases in the circulating levels of LH, FSH, and T (Sanford et al., 1974b; Sanford et al., 1976a; Sanford et al., 1977). During the non-breeding season serum concentrations of LH and T in particular are elevated for about 12 h; this is followed by a decline and inhibition of pulses for about 9 h (Sanford et al., 1974b; Sanford et al., - unpublished). Thus it was hypothesized that the temporary discontinuance of LH releases may have been due to: (i) refractoriness of the pituitary to hypothalamic inputs of GnRH; and/or (ii) suppression of GnRH release; and that both phenomena may be influenced by elevated T levels as suggested by Pelletier (1976). The object of this experiment was to investigate the first possibility. Additionally, the effect of repeated mating on circulating levels of LH, FSH, PRL and T during both the ovine non-breeding and breeding seasons was examined.

Experimental Plan

This experiment was carried out in two parts and used the same four rams of Experiment 1. In Part 1, the four rams were

bled by jugular venipuncture at 20-min. intervals during 36-h control and mating periods. This part was performed on July 27 and August 3 for the control and mating periods, respectively; and was repeated on November 10 and 17, respectively. Part 2, was similar to Part 1 with the inclusion of three 10 μg GnRH (NIH-NICHD-72-2722CPR; Lot 26-306 AL) injections (iv) given at 20-min. intervals beginning at h 19 of the 36-h control and mating periods; Part 2 was carried out on August 10 and 16 for control and mating periods, respectively. Blood collections in all periods started at approximately 0830 h. Rams were allowed 5 days of rest before the start of the next blood collection.

Rams were penned individually in three adjacent and one nearby pen in an enclosed area of the barn during experimental periods. Since it was noticed in Experiment 1 that, rams were able to anticipate a mating encounter when brought into the mating area, they were penned alone for about 17 h prior to blood collection. During the mating periods, an ovariectomized estrous-induced ewe was introduced into each of the pens after the first blood sample had been taken. After every 4 h, estrual ewes were sequentially switched from ram to ram to provide additional mating incentive.

Number of mounts and mounts ending in ejaculation were recorded for each 20-min. interval. Ejaculation was considered to have occurred as indicated in Experiment 1.

Results

Records of mating behavior of rams during the 36-h mating periods in the non-breeding and breeding seasons are summarized in Tables 9 and 10, respectively. Hormone concentrations determined in sera pooled from collections within each consecutive 2 h were averaged to obtain 6-h means. Values obtained for both Parts 1 and 2 of the experiment are presented for LH, FSH, PRL and T in Tables 11, 12, 13 and 14, respectively.

In both parts of the experiment, all rams mated frequently throughout the 36-h periods, but marked differences existed between rams in the frequency with which they mated. The highest number of mates were recorded during the first 6 h of each mating period but considerable mating occurred during subsequent 6-h intervals. Rams often exhibited renewed interest in mating whenever estrual ewes were changed. During the non-breeding season rams mated significantly ($P < .05$) more frequently during the first mating period (65.3 ± 12.3) than in the mating period with GnRH injection (42.5 ± 8.8). The overall number of mounts were also greater for the first mating period compared to the second (108.8 ± 20.8 vs 65.5 ± 12.4, $P < .05$). However, there was no significant ($P > .10$) difference between the two mating periods in the non-breeding season in the number of mounts per mate. Comparison of sexual activities between seasons (Part 1), showed no significant ($P > .10$, paried t-test) differences in

TABLE 9

Mean (± S.E.) Number of Mounts and Mates Recorded For Rams During The 36-h Mating Periods in The Non-breeding (July-August) Season

Treatment	Activity	Time Interval (h) 1 - 6	7 - 12	13 - 18	19 - 24	25 - 30	31 - 36	Total
Mating	Mounts (Mo)	22.3 ± 4.8	15.8 ± 5.2	28.8 ± 9.7	13.3 ± 2.5	12.3 ± 2.7	14.0 ± 5.4	108.0 ± 24.1
	Mates (Ma)	17.8 ± 3.4	10.5 ± 3.3	12.3 ± 3.0	8.5 ± 2.3	5.8 ± 1.8	8.0 ± 2.4	65.3 ± 14.2
	Mo/Ma index	1.26 ± .05	1.46 ± .16	2.77 ± 1.07	1.65 ± .15	2.42 ± .54	1.72 ± .35	1.72 ± .28
Mating + GnRH	Mounts (Mo)	15.0 ± 4.1	18.0 ± 4.3	10.0 ± 2.2	8.8 ± 1.7	5.5 ± 1.8	8.3 ± 1.4	65.5 ± 14.4
	Mates (Ma)	11.5 ± 4.1	10.8 ± 1.9	6.3 ± 1.5	6.5 ± .9	3.0 ± .7	4.5 ± 1.8	42.5 ± 10.2
	Mo/Ma index	1.59 ± .35	1.65 ± .39	1.75 ± .39	1.32 ± .10	2.10 ± .97	2.13 ± .96	1.57 ± .15

Each value represents mean of four rams

TABLE 10

Mean (± S.E.) number of Mounts and Mates Recorded For Rams During the 36-h Mating Period in the Breeding (November) Season

Treatment	Activity	Time Interval (h) 1 - 6	7 - 12	13 - 18	19 - 24	25 - 30	31 - 36	Total
Mating	Mounts (Mo)	34.0 ± 7.2	23.5 ± 11.9	26.5 ± 8.5	17.5 ± 2.5	16.0 ± 5.8	13.0 ± 3.8	135.5 ± 33.0
	Mates (Ma)	16.3 ± 4.4	7.5 ± 2.1	11.5 ± 2.5	8.8 ± .6	8.0 ± 2.7	8.0 ± 2.1	60.0 ± 10.5
	Mo/Ma index	2.21 ± .17	3.79 ± 2.04	2.10 ± .45	2.02 ± .27	3.03 ± 1.06	1.63 ± .34	2.20 ± .24

Each value represents mean of four rams

TABLE 11

Six-hour Mean (± S.E.) Levels (ng/ml) of LH in Peripheral Blood Serum of Rams Bled at 20-minute Intervals for 36-h During Control and Mating Periods in the Non-breeding (July-August) and Breeding (November) Seasons

Season	Treatment	Time Interval (h) 1 - 6	7 - 12	13 - 18	19 - 24	25 - 30	31 - 36
Non-breeding[a]	Control	.4 ± .2	.6 ± .2	.4 ± .2	.3 ± .1	.7 ± .2	.7 ± .2
	Mating	1.2 ± .3	1.1 ± .3	.8 ± .2	.5 ± .1	.8 ± .3	.8 ± .3
Non-breeding[b]	Control + GnRH*	.6 ± .2	.6 ± .2	.8 ± .3	45.9 ± 12.8	1.2 ± .3	.6 ± .1
	Mating + GnRH*	1.5 ± .2	1.2 ± .2	1.1 ± .2	29.9 ± 7.6	1.1 ± .2	.6 ± .1
Breeding[c]	Control	1.0 ± .2	1.2 ± .2	.7 ± .1	.5 ± .1	.6 ± .1	.8 ± .1
	Mating	.8 ± .1	.7 ± .1	.5 ± .1	.3 ± .1	.4 ± .1	.5 ± .1

Each value represents the mean of four rams

*Three 10 µg GnRH injections were given intravenously at 20-minute intervals beginning at hour 19

[a]Treatment, time, time x treatment, significant ($P < .05$ or $P < .01$)

[b]Treatment, time, time x treatment, significant ($P < .01$)

[c]Treatment, time, significant ($P < .01$)

TABLE 12

Six-hour Mean (± S.E.) Levels (ng/ml) of FSH in Peripheral Blood Serum of Rams Bled at 20-minute Intervals for 36-h During Control and Mating Periods in the Non-breeding (July-August) and Breeding (November) Seasons

Season	Treatment	Time Interval (h) 1 - 6	7 - 12	13 - 18	19 - 24	25 - 30	31 - 36
Non-breeding[a]	Control	66.5 ± 8.6	72.1 ± 8.5	66.7 ± 7.7	63.5 ± 7.5	65.2 ± 9.4	61.5 ± 7.9
	Mating	82.9 ± 9.0	89.8 ± 9.3	88.4 ± 10.1	89.0 ± 9.4	86.8 ± 9.0	88.5 ± 9.8
Non-breeding[b]	Control + GnRH*	119.4 ± 12.2	128.9 ± 13.5	124.7 ± 11.1	189.2 ± 17.2	102.8 ± 9.0	113.7 ± 8.3
	Mating + GnRH*	171.1 ± 22.0	163.3 ± 14.9	173.0 ± 17.5	223.8 ± 24.7	128.8 ± 15.9	139.5 ± 17.0
Breeding	Control	175.2 ± 26.7	158.7 ± 14.5	156.8 ± 24.4	147.3 ± 14.7	148.8 ± 13.0	177.7 ± 22.1
	Mating	166.4 ± 17.5	164.5 ± 16.0	175.1 ± 27.2	155.6 ± 12.5	177.8 ± 22.5	159.2 ± 17.8

Each value represents the mean of four rams

*Three 10 µg GnRH injections were given intravenously at 20-minute intervals beginning at hour 19

[a]Treatment, significant ($P < .01$)

[b]Treatment, time, significant ($P < .01$)

TABLE 13

Six-hour Mean (± S.E.) levels (ng/ml) of Prolactin in Peripheral Blood Serum of Rams Bled at 20-minute Intervals for 36-h During Control and Mating Periods in the Non-breeding (July-August) and Breeding (November) Seasons

		Time Interval (h)					
Season	Treatment	1 - 6	7 - 12	13 - 18	19 - 24	25 - 30	31 - 36
Non-breeding[a]	Control	63.7 ± 8.8	81.5 ± 11.9	130.5 ± 18.8	106.3 ± 12.3	158.2 ± 8.4	123.8 ± 15.0
	Mating	144.0 ± 25.7	151.5 ± 21.7	160.9 ± 16.2	100.9 ± 15.3	154.8 ± 16.2	134.2 ± 18.4
Non-breeding[b]	Control + GnRH*	68.3 ± 15.5	118.3 ± 20.5	87.9 ± 10.2	81.8 ± 11.3	149.0 ± 20.3	223.8 ± 24.4
	Mating + GnRH*	134.6 ± 16.2	180.5 ± 31.5	165.0 ± 12.1	101.0 ± 12.4	197.2 ± 25.1	150.3 ± 19.6
Breeding[c]	Control	34.0 ± 10.5	22.5 ± 4.8	21.5 ± 4.7	12.4 ± 3.3	15.8 ± 5.3	31.3 ± 7.2
	Mating	51.4 ± 17.1	10.5 ± 3.3	14.1 ± 2.2	14.1 ± 1.4	11.6 ± 4.5	5.6 ± 1.3

Each value represents the mean of four rams

*Three 10 µg GnRH injections were given intravenously at 20-minute intervals beginning at hour 19

[a]Treatment, time, significant ($P < .01$)

[b]Treatment, time, time x treatment, significant ($P < .01$)

[c]Treatment, time, time x treatment, significant ($P < .05$ or $P < .01$)

TABLE 14

Six-hour Mean (± S.E.) Levels (ng/ml) of Testosterone in Peripheral Blood Serum of Rams Bled at 20-minute Intervals for 36-h During Control and Mating Periods in the Non-Breeding (July-August) and Breeding (November) Seasons

Season	Treatment	Time Interval (h) 1 - 6	7 - 12	13 - 18	19 - 24	25 - 30	31 - 36
Non-breeding	Control	2.3 ± .8	4.6 ± 1.1	2.6 ± 1.0	3.4 ± 1.2	3.5 ± 1.0	5.3 ± 1.9
	Mating	4.9 ± .9	4.4 ± .8	3.3 ± .9	2.6 ± .6	3.0 ± 1.0	4.1 ± 1.0
Non-breeding[a]	Control + GnRH*	2.8 ± 1.1	2.6 ± .9	2.8 ± 1.2	14.2 ± 1.9	.8 ± .3	.3 ± .1
	Mating + GnRH*	5.1 ± .5	4.5 ± .5	3.7 ± .7	12.7 ± 1.7	1.1 ± .2	.5 ± .2
Breeding[b]	Control	18.6 ± 2.6	20.6 ± 2.8	17.8 ± 2.5	16.1 ± 2.6	15.8 ± 2.3	17.9 ± 2.0
	Mating	20.9 ± 2.9	23.8 ± 4.1	17.9 ± 3.5	14.3 ± 3.1	14.4 ± 2.9	15.0 ± 2.3

Each value represents the mean of four rams

*Three 10 µg GnRH injections were given intravenously at 20-minute intervals beginning at hour 19

[a]Time, time x treatment, significant ($P < .05$ or $P < .01$)

[b]Time, significant ($P < .01$)

the total number of mates. However, the number of mounts were significantly ($P < .10$) greater in the breeding season (135 $\pm$ 33.0) than the non-breeding season (108.0 $\pm$ 24.1). Thus, it is not surprising that the number of mounts per mate were significantly ($P < .05$) greater in the breeding season (2.2 $\pm$ .2) than the non-breeding season (1.7 $\pm$ .3).

In Part 1, mating activity during the non-breeding season resulted in a prolonged elevation of mean LH (approximately 12 h) which later declined to normal levels (treatment x time, significant, $P < .01$). Mean T level rose by about two-fold but this increase was maintained for only 6 h. Mean FSH levels were consistently and significantly ($P < .01$) elevated above control levels during the entire 36-h period. Similarly, the levels of PRL were significantly ($P < .01$) raised but this was especially prevalent during the initial 18 h.

During the breeding season (Part 1) mating apparently caused a significant ($P < .01$) depression in mean LH levels, which was consistent across all time intervals. But this did not seem to affect mean T levels since they were consistently comparable to control values. Variations in mean FSH levels could not be detected in response to mating. PRL levels for rams when mating were found to be above their control values only during the first 6 h. In later time intervals the levels decreased to below control values, with the extent of the decrease differing among time intervals (treatment x time, significant, $P < .01$).

Endocrine changes due to mating activity observed in the non-breeding season of Part 1 were confirmed in Part 2 of the study. Mating activity triggered increases in mean levels of LH, PRL and T in the 18 h prior to GnRH injection. Treatment with GnRH during the control and mating periods produced marked elevations above preinjection levels (h 13-18) in mean LH (45.1 $\pm$ 10.9 vs 28.8 $\pm$ 7.6 ng/ml, $P < .05$), FSH (64.4 $\pm$ 12.6 vs 50.8 $\pm$ 21.2 ng/ml) and T (11.4 $\pm$ 2.0 vs 9.0 $\pm$ .6 ng/ml) during the subsequent 6-h period (h 19-24).

In order to determine the response to GnRH in more detail, concentrations of the hormones in the 2-h pools were examined. It was noticed that, although elevations in LH and T levels were evident during the first 2 h (h 19-20) following the onset of GnRH injections, peak levels were generally reached during h 3 and 4 (h 21-22) in all rams except Ram #2 which exhibited peak LH levels during the first 2 h. Mean FSH levels did not consistently peak in either h 1 and 2 or h 3 and 4. However, levels of FSH as well as LH and T had declined to relatively low levels within 4 h following peak levels. Pre-injection levels determined from 2-h pools for control and mating periods were .2 $\pm$ .04 vs 1.4 $\pm$ .2 ng/ml ($P < .01$) for LH, 116.4 $\pm$ 12.2 vs 168.4 $\pm$ 25.6 ng/ml for FSH and .6 $\pm$ .1 vs 5.4 $\pm$ .4 ng/ml ($P < .01$) for T, respectively. The GnRH injections produced marked elevations (Δ value) in mean LH (83.1 $\pm$ 23.8 vs 45.3 $\pm$ 11.7 ng/ml, $P < .01$), FSH (125.1 $\pm$ 23.0 vs 105.1 $\pm$ 39.8 ng/ml)

and T (20.0 $\pm$ 2.3 vs 12.5 $\pm$ 1.03 ng/ml, $P < .05$) during either the first or second 2-h interval following onset of injection.

In Part 2, PRL levels during almost the entire mating period were clearly well above control levels ($P < .01$) indicating a mating effect. During the control period, levels changed considerably between 6-h intervals prior to GnRH injection. Levels were not influenced by the injection of GnRH but did eventually increase, and this occurred in all rams. However, there was a marked drop in PRL during the 6 h following GnRH treatment preceding this increase.

Comparison between hormone levels obtained from rams when they were bled during control periods in either the non-breeding or breeding season (Part 1), revealed normal seasonal changes. Mean LH, FSH and T levels were significantly ($P < .01$) higher in the breeding season than the non-breeding season. In contrast, PRL levels were significantly ($P < .01$) greater in the non-breeding season than in the breeding season.

Discussion

As in Experiment 1, rams mated consistently over the 36-h test period and this elicited the characteristic changes in reproductive hormone secretion. It is obvious from the data accrued for the non-breeding season that the mating performance of rams had declined considerably during the mating period with GnRH injection. Since these two mating periods were separated by a period of about 2 weeks (August 3 to August 16) it is

doubtful that the decline in performance would have been due solely to the previous exposure to ewes. It cannot be attributed to the effect of GnRH injection because mating activity had decreased considerably prior to GnRH injection, when compared to the identical time period of the first mating session. It is also doubtful that the higher ambient temperature during the second mating period could have resulted in the decrease in mating activity. During the first mating period temperature ranged between 5.6 to 27.8°C; the temperature range for the second mating period was 11.7 to 28.3°C. Mating performance may have declined because of strains imposed by previous experimental manipulations since the rams had also been used for Experiment 1 at various times, from July 5 through July 27.

Comparison between the sexual activities exhibited during the two mating periods of Part 1 of the study, revealed no seasonal difference in the total number of mates. However, the number of mounts were significantly ($P < .10$) greater in the breeding season than the non-breeding season, and consequently the number of mounts per mate ($P < .05$). This is in disagreement with the results of Pepelko and Clegg (1965) who observed more frequent and efficient ejaculation during the fall after exposing rams to estrual ewes for 1 h twice monthly for 1 year; the greatest number of mounts per ejaculation was observed in April. Schanbacher and Lunstra (1976) also noticed Finn rams to be more sexually aggressive (judged from a libido index)

in October. Thus, as indicated in Experiment 1, the circulating T levels needed to maintain maximum mating performance in these rams in the non-breeding season may have been close to or above the required threshold level.

Undoubtedly, in this study frequent ejaculation in the non-breeding season stimulated temporary increases in the levels of LH and T, whereas LH levels were decreased during the breeding season. This is in concert with the results of Experiment 1 and those of other studies (Sanford et al., 1974b; Sanford et al., 1977). The depressed levels of LH during the breeding season were not associated with a concurrent decrease in T levels. Perhaps LH-peak frequency and height were not reduced enough to have an effect.

Mean levels of LH and T declined to normal after the temporary 6- to 12-h increase. A similar incident was reported by Sanford et al.(1974b). If the increase in mean levels of LH is due to an increase in GnRH release from the hypothalamus, then the subsequent decline in mean levels during the remainder of the mating periods may have been the result of the pituitary becoming refractory to GnRH. Indeed, prolonged infusion of GnRH is known to inhibit LH release in intact rams (Bremner et al., 1976). However, in this study when mating rams were injected with GnRH (three 10 μg injections at 20-min. intervals) at h 19, a time when LH was known to have decreased to normal, there were marked elevations in mean LH and T levels. This

suggests that the pituitary had not become totally refractory due to previous GnRH stimulation. However, the lower response of mating rams to GnRH (in terms of lower mean LH and T values post-injection), does suggest a partial loss of responsiveness of the pituitary to GnRH, perhaps attributed in part to the higher mean T levels resulting from mating activity preceding the injection. In corroboration with this is the fact that T or its metabolites have been shown to exert inhibitory influences on LH release at the level of both the hypothalamus and the pituitary (Reeves et al., 1970; Galloway and Pelletier, 1975; Sanford et al., 1976b; Schanbacher, 1979).

The refractory state of the pituitary may also have been due in part to depletion of pituitary LH stores. Rippell et al. (1974) demonstrated a refractory state of the pituitary up to 96 h following a single 50 µg GnRH injection. Chakraborty et al. (1974) also demonstrated a four-fold decrease in pituitary LH content following 24 h of GnRH infusion at 2.3 µg/h. But, the fact that Bremner et al. (1976) illustrated refractoriness of the pituitary even at very low doses of GnRH (.05 µg/min.) infusion, suggests that pituitary LH depletion may not be a valid explanation. LH itself when at high levels may exert "short-loop" inhibitory feedback effect on the pituitary (Motta et al., 1969). Also high levels of GnRH may feedback on its own receptors to result in a depression in responsiveness. These possible alternative explanations for the decline in LH, observed in the present study, remain to be tested.

Furthermore, it is impossible to ignore the possibility that the decline in LH may have been due to a suppression of GnRH release by the high circulating T levels or that the hypothalamus had become refractory to incoming neural stimuli associated with mating.

It is interesting to consider the benefit of these transitory increases in LH and T with the occurrence of mating, to the rams reproductive processes. Illius et al. (1975) penned one of two groups of rams in close proximity to ewes induced into estrus every 18 days from May through March in the following year. They observed that the older heterosocial group had larger testes as compared to the homosocial group suggesting a long-term effect on the endocrine system of these animals. Likewise, when rams were allowed to mate or were penned adjacent to ewes brought into estrus every 10 days for a period of 18 mo. (May 1978 - October 1979) their mean T levels and scrotal circumference were found to have increased earlier at the onset of the second breeding season (Sanford and Yarney, 1980). Thus, it may not be far reaching to proclaim that testicular function of rams can be enhanced early in the breeding season or perhaps out of season, by constantly keeping rams in close proximity to estrual ewes.

In this study, mean FSH levels were consistently elevated above control levels during the entire 36-h mating period in the non-breeding season but there was no apparent increase in the breeding season. One should exercise caution

in attributing the increase in mean FSH to the effect of mating since levels had progressively increased from one bleeding period to the next, an indication of photoperiodic effect. Initial months of diminishing photoperiod (July through September) have been associated with increases in FSH levels (Sanford et al., 1977; Sanford et al., 1978a) and in fact in the present study daylength decreased from approximately 15.3 h to 14.3 h from the start to finish of the experiment in the non-breeding season. Additionally, because increases in circulating FSH were not detected in Experiment 1, it would seem appropriate not to associate FSH increases in this experiment with the effect of mating, taking cognizance of the fact that others have been able to demonstrate a relationship in rats (Taleisnik et al., 1966) and rams (Sanford et al, 1976a).

During the non-breeding season PRL levels during the two control periods were comparable; and mating induced elevations in mean PRL levels above the respective control values. This observation is in accordance with those of Kamel et al. (1977); they exposed male rats to estrual females and observed increases in serum LH, T and PRL levels but not FSH levels. Whether the high levels of PRL were due to the direct action of neural stimuli on pituitary cells via the hypothalamus, increased LH and T secretion, or otherwise, is a question to be resolved by further experimentation. Treatment of wethers with testosterone propionate (100 mg/day for 3 weeks) significantly ($P < .05$) increased mean amplitude of PRL secretory spikes

and overall PRL concentration but not baseline PRL concentration (Davis et al., 1978). Similarly, Herbert (1978) demonstrated an increase in serum PRL, pituitary PRL and the number of PRL cells following one week of administering several testosterone propionate injections to juvenile male rhesus monkeys. While these findings may suggest an effect of T on PRL secretion, it only applies to the long-term situation. Increases in PRL secretion resulting from mating may be of significance if one considers that PRL is believed to synergize with LH in the maintenance of testicular LH receptors (Zipf et al., 1978) and spermatogenesis (Bartke, 1971; Hafiez et al., 1972); it also synergizes with T to maintain accessory sex gland function (Ravault et al., 1977).

Seasonal trends in LH, FSH, PRL on T secretion observed were comparable to that observed in Experiment 1 and other investigators (Schanbacher and Lunstra, 1976; Lincoln et al., 1977; Ravault and Ortavant, 1977; Sanford et al., 1977, 1978a).

SUMMARY AND CONCLUSIONS

Four adult Finnish Landrace rams were used in two experiments to study the interaction between mating behavior and season on circulating levels of LH, FSH, PRL and T. Experiment 1, was designed to determine the influence of different components of the rams mating behavior on secretion of LH, FSH, PRL and T in the ovine non-breeding and breeding seasons. Blood was collected by jugular venipuncture at 20 min. intervals during 8-h periods in July and October while individual rams were: 1) isolated from, 2) observing, 3) mounting and 4) mating estrous-induced ewes. Mating activity in July was associated with elevations in mean LH (.6 $\pm$ .2, control vs 1.2 $\pm$ .3 ng/ml, mating, $P < .05$) and T levels (2.5 $\pm$ .3 vs 4.3 $\pm$ 1.0 ng/ml), basal LH levels (.39 $\pm$ .10 vs .68 $\pm$ .21 ng/ml) and the number of LH peaks (1.0 vs 3.0 $\pm$ .9 per 8h); while in October obvious changes in basal LH levels (.6 $\pm$ .1 vs .9 $\pm$ .2 ng/ml, $P < .05$) were noticed. Circulating LH and T during mounting and observation periods were often depressed from control levels in both months. Mean FSH levels remained unaffected by several ejaculations, mounting or observation in both months. Likewise, there was no obvious relationship between sexual activity and mean PRL levels in July; however, the sexual activities which involved the most physical exertion tended to be associated with much higher circulating

PRL levels in October.

Experiment 2 was designed to investigate the effect of repeated mating on the secretion of LH, FSH, PRL and T during the non-breeding and breeding seasons, and the cause of the decline in circulating LH and T that occurs after several hours of mating. The experiment was done in two parts and employed the same rams used in Experiment 1. In Part 1, conducted in July-August and in November, the rams were bled at 20-min intervals during 36-h control and mating periods. Part 2, performed in August only, was designed as Part 1 with the inclusion of three 10 μg GnRH injections (i.v.) given at 20-min intervals beginning at h 19. Mating activity produced transient (6-12 h) elevations in circulating LH, T and PRL in August, but was associated with consistently lower LH levels and a short-term (6 h) increase in PRL levels, in November. Treatment with GnRH during control and mating periods (Part 2) produced marked elevations (Δ value) in mean LH (83.1 $\pm$ 23.8 vs 45.3 $\pm$ 11.7 ng/ml, $P < .01$), FSH (125.1 $\pm$ 23.0 vs 105.1 $\pm$ 39.8 ng/ml) and T (20.0 $\pm$ 2.3 vs 12.5 $\pm$ 1.0 ng/ml, $P < .05$) during either the first or second 2-h interval following the onset of injection (h 19-23).

Seasonal variations in the secretion of hormones occurred in both experiments. LH secretion (especially the number of LH-peaks), mean FSH and T levels were higher in the breeding season as opposed to the non-breeding season. In contrast,

PRL levels were greater in the non-breeding season than the breeding season.

These results indicate that during the non-breeding season when levels of LH and T are relatively low only multiple ejaculations by rams induce short-term increases in the secretion of these hormones, and that all forms of sexual activity when exhibited in the breeding season may lead to disruptions in the secretion of LH and T. While circulating FSH levels may remain unaffected by any type of ram's mating behavior, PRL levels may be elevated after a period of several ejaculations, in both seasons. Finally, the decline in LH secretion following 12 h of mating may be due in part to pituitary refractoriness to endogenous GnRH but the effect of other possible factors needs to be examined. It is likely, that these endocrine changes could be exploited to enhance ram testicular function early in the ovine breeding season or out of season, by keeping rams as sexually active as possible throughout the year.

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APPENDIX I

Data for Experiment 1

TABLE 1

Experimental Design
- 4 x 4 Latin Square

Non-breeding Season (July):

	R1	R2	R3	R4
P1	C	Mo	O	Ma
P2	Mo	O	Ma	C
P3	Ma	C	Mo	O
P4	O	Ma	C	Mo

Breeding Season (October):

	R1	R2	R3	R4
P5	Ma	C	Mo	O
P6	Mo	O	Ma	C
P7	O	Ma	C	Mo
P8	C	Mo	O	Ma

P: Bleeding period

R: Ram

C: Control

O: Observing

Mo: Mounting

Ma: Mating

TABLE 2

Mean Serum LH Levels (ng/ml) in Rams Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July and October

Month	Period	Ram 1	Ram 2	Ram 3	Ram 4
July	Control	1.14	.33	.19	.81
	Observed	.61	.57	.33	.57
	Mounted	.59	.35	.25	.32
	Mated	1.50	1.56	.44	1.30
October	Control	1.19	1.11	.44	1.15
	Observed	.87	.60	.15	1.05
	Mounted	.54	1.33	.09	.84
	Mated	1.09	1.72	.29	1.70

TABLE 3

Mean Serum FSH Levels (ng/ml) in Rams Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July and October

Month	Period	Ram 1	Ram 2	Ram 3	Ram 4
July	Control	55.0	31.0	37.0	46.0
	Observed	84.0	30.5	30.0	49.0
	Mounted	61.0	29.0	40.0	52.5
	Mated	81.0	30.0	40.0	58.5
October	Control	195.0	84.0	55.0	97.0
	Observed	239.0	85.0	30.5	103.5
	Mounted	322.5	96.5	81.5	94.0
	Mated	248.0	91.0	70.0	90.0

TABLE 4

Mean Serum PRL Levels (ng/ml) in Rams Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July and October

Month	Period	Ram 1	Ram 2	Ram 3	Ram 4
July	Control	540.0	262.0	75.2	127.6
	Observed	132.8	110.0	184.8	76.0
	Mounted	196.8	580.0	132.0	228.8
	Mated	148.8	134.0	40.8	279.2
October	Control	< 2.2	5.5	9.0	< 2.2
	Observed	16.0	56.5	18.7	36.7
	Mounted	255.0	57.5	115.0	167.0
	Mated	51.8	56.3	23.9	88.2

TABLE 5

Mean Serum T Levels (ng/ml) in Rams Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July and October

Month	Period	Ram 1	Ram 2	Ram 3	Ram 4
July	Control	3.19	2.65	1.85	2.35
	Observed	2.89	4.14	3.63	1.88
	Mounted	2.23	3.75	2.17	1.46
	Mated	5.24	6.46	3.19	2.23
October	Control	24.26	17.77	6.61	23.01
	Observed	20.68	12.71	3.57	18.01
	Mounted	16.93	17.29	1.70	19.37
	Mated	20.15	22.23	8.48	24.37

TABLE 6

Serum LH (ng/ml) Values for Ram 1 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July

Control	Observed	Mounted	Mated
1.47	.23	.39	1.02
.84	.23	.33	.75
.70	.17	.42	5.00
.44	.17	.33	3.10
.33	.23	.23	1.79
.21	.14	.21	1.25
.14	.18	.14	.84
.16	.14	.20	.62
.24	.14	.24	.50
.16	2.65	.14	2.65
.16	7.60	.14	3.40
.17	4.70	.14	1.89
.14	2.95	.17	1.21
1.59	1.65	.14	.91
.88	1.16	15.10	.94
.69	.81	5.80	4.65
19.00	.67	3.80	2.35
7.15	.61	1.91	1.60
5.25	.42	1.28	2.00
2.60	.42	.77	4.00
1.66	.39	.62	2.25
1.06	.27	.34	1.55
.76	.27	.35	.94
.57	.31	.27	.67
$\bar{x}$ 1.93	1.10	1.39	1.91
S.E .81	.36	.65	.26

TABLE 7

Serum LH (ng/ml) Values for Ram 1 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in October

Control	Observed	Mounted	Mated
3.15	1.78	1.50	4.00
1.68	1.38	1.17	2.15
1.24	.99	.80	1.46
.82	3.10	.64	1.06
2.60	1.87	.61	2.60
1.57	1.30	1.54	1.50
1.09	.94	.98	1.09
.72	.83	.81	.79
2.50	.54	.62	2.60
1.64	1.69	.40	1.87
1.13	1.41	.52	1.22
2.10	1.01	.37	.97
2.30	.82	1.77	.80
1.51	.72	1.26	3.25
1.24	.50	.68	2.05
3.90	2.60	.62	1.15
2.10	1.80	.59	.89
1.44	1.21	.52	.64
1.15	.89	.37	.63
3.50	.77	.46	3.00
2.30	1.05	.33	1.85
1.40	3.00	.36	1.22
1.00	1.73	.34	.96
3.25	1.19	1.19	.94
$\bar{x}$ 1.89	1.38	.80	1.61
S.E .18	.15	.10	.19

TABLE 8

Serum LH (ng/ml) Values for Ram 2 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July

Control	Observed	Mounted	Mated
.45	.70	.12	.23
.34	.54	.23	1.53
2.50	.36	1.81	1.38
1.79	.34	1.03	4.10
1.21	.35	.73	2.65
.81	.27	.50	8.65
.65	.23	.34	5.40
.46	.21	.39	3.50
.45	.28	2.65	2.30
.26	1.59	1.55	1.69
.19	1.06	.74	1.20
.29	.65	.69	.90
.23	1.38	.39	.69
.19	3.15	.36	.60
.28	1.90	.23	3.50
.25	1.08	.15	2.50
.26	1.07	.15	1.56
.25	.71	.14	1.17
.17	2.10	.20	3.75
.21	1.61	.13	2.60
.16	.94	.17	1.80
.20	.66	.12	1.25
.16	.58	.23	.88
.12	.39	1.81	.71
$\bar{x}$ .50	.92	.61	2.27
S.E .12	.14	.14	.38

TABLE 9
Serum LH (ng/ml) Values for Ram 2 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in October

Control	Observed	Mounted	Mated
1.34	2.80	1.13	1.89
.89	1.65	4.00	1.53
.70	1.17	3.05	3.70
.63	.84	1.80	3.00
3.75	.60	1.27	1.95
2.50	.47	.84	1.28
1.64	.35	4.20	1.09
1.02	.26	2.80	4.60
.74	.28	1.65	2.50
.75	.26	1.15	1.65
.61	.24	.84	1.11
5.75	.34	4.25	.80
3.50	.29	2.90	6.20
1.72	.14	1.91	3.45
1.02	.22	1.26	2.40
.66	.21	.91	1.69
.45	.15	.71	1.26
.40	.23	.55	.79
.30	.14	.60	4.40
.31	.23	.53	3.05
3.65	5.25	.56	1.79
2.45	3.40	3.40	1.45
1.45	1.81	2.45	1.02
.88	1.29	1.55	.78
$\bar{x}$ 1.55	.94	1.85	2.22
S.E .28	.26	.25	.29

TABLE 10

Serum LH (ng/ml) Values for Ram 3 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July

Control	Observed	Mounted	Mated
.20	.13	.19	.13
.15	.13	.22	.13
.23	.13	.24	.22
.20	.13	.18	.25
.16	.13	.20	.20
.22	.13	.17	.30
.27	.13	.16	.68
.20	.13	.13	.50
.19	.13	.13	.40
.17	4.70	.18	6.55
.15	2.30	.14	3.75
.20	1.52	.19	1.85
.24	.86	.15	.94
.28	.58	.18	.70
.25	.40	.20	.59
.23	.21	2.00	.35
.30	.23	1.32	.22
.23	.20	.84	.23
.39	.17	.54	.20
4.25	.13	.42	.30
1.95	.14	.30	.20
1.42	.13	.30	.13
.82	.13	.26	.18
.50	.13	.22	.18
$\bar{x}$ .55	.54	.37	.80
S.E .18	.21	.09	.30

TABLE 11

Serum LH (ng/ml) Values for Ram 3 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in October

Control	Observed	Mounted	Mated
.40	< .13	.35	1.29
.39	.20	.30	.82
3.75	.24	.26	.67
1.75	.20	.32	.42
.92	.13	.27	.34
.75	.19	.30	.35
.51	.27	.33	.27
.54	.26	.23	.23
.40	.20	.24	.25
.37	.29	.23	.16
.28	.19	.28	.20
.25	.25	.28	.20
.22	.26	.39	.21
.23	1.65	.20	.15
.19	1.35	.23	.30
.19	.89	.26	.25
.51	.66	.18	.18
.40	.54	.20	.20
.25	.42	.16	.31
.27	.43	.17	1.85
.21	.37	.17	1.42
1.52	.44	.13	.81
.89	.43	.20	.54
.71	.27	.26	.43
$\bar{x}$ .66	.43	.25	.49
S.E .16	.08	.01	.09

TABLE 12

Serum LH (ng/ml) Values for Ram 4 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in July

Control	Observed	Mounted	Mated
4.15	1.00	.25	.13
3.10	.69	.15	.13
2.15	.40	.25	.13
1.17	.25	.16	.13
.82	.17	.14	.13
.50	.28	.13	.13
.32	.13	.13	.13
.13	.13	.13	.13
.17	.13	.13	14.85
.13	.13	.13	5.75
.13	.13	.16	2.95
.13	.13	.13	1.75
.13	.13	.13	1.06
.13	.13	.13	.68
.13	.13	.14	.42
.13	.13	.13	.23
.13	.13	.19	.13
.13	5.20	.13	.19
3.45	3.30	.13	.13
2.65	2.05	.32	.20
1.25	1.17	3.55	4.90
.82	.69	2.45	4.10
.56	.50	1.39	2.20
.32	.38	.94	1.29
$\bar{x}$ .94	.72	.48	1.74
S.E .25	.24	.17	.66

TABLE 13

Serum LH (ng/ml) Values for Ram 4 Bled at 20-minute Intervals During 8-h Control or Sexually-active Periods in October

Control	Observed	Mounted	Mated
2.20	1.89	1.84	.95
1.38	.89	1.40	2.30
.90	.90	.98	1.72
2.80	.41	.64	4.00
1.41	2.15	1.29	1.95
.92	1.24	1.04	1.00
.72	.78	.63	1.95
4.00	.45	.46	1.90
1.90	.43	.42	1.08
1.27	2.70	1.25	.75
.98	1.75	.83	2.70
.58	.90	.54	1.75
.48	.65	.47	1.02
.44	.45	.30	2.75
2.00	.36	.31	1.44
1.30	3.00	.29	3.20
.70	2.15	2.70	1.93
.57	1.29	1.28	1.13
.42	.79	.72	2.45
1.95	.55	.57	1.75
1.74	.40	.38	1.17
.99	2.40	.96	2.40
.62	1.54	1.69	1.92
.41	.94	.93	1.09
$\bar{x}$ 1.28	1.20	.91	1.85
S.E .18	.16	.12	.16

TABLE 14

Number of Mates and Mounts Recorded for Rams During The 8-h Mating Periods

Season	Activity	Ram 1	Ram 2	Ram 3	Ram 4
Non-breeding (July)	Mounts	37	22	35	26
	Mates	34	15	31	18
	Mounts/Mate	1.09	1.47	1.13	1.44
Breeding (October)	Mounts	33	15	31	26
	Mates	26	14	31	21
	Mounts/Mate	1.27	1.07	1.00	1.24

TABLE 15

Number of Mounts Recorded for Rams During The 8-h Mounting Periods

Season	Ram 1	Ram 2	Ram 3	Ram 4
Non-breeding (July)	207	128	165	249
Breeding (October)	231	103	159	217

TABLE 16

Mean LH Levels (Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	.522	5.93*
Period	3	.022	.25
Ram	3	.302	3.43
Error	6	.088	
Total	15		

*P < .05

TABLE 17

Mean FSH Levels (Non-breeding Season)

Analyis of Variance

Source	df	ms	F
Treatment	3	73.52	1.20
Period	3	63.18	1.03
Ram	3	1267.39	20.66**
Error	6	61.35	
Total	15		

**P < .01

TABLE 18

Mean PRL Levels (Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	23505.2	8.23*
Period	3	67076.8	23.45**
Ram	3	22629.1	7.91*
Error	6	2850.0	
Total	15		

*P < .05

**P < .01

TABLE 19

Mean T Levels (Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	2.969	2.84
Period	3	.055	.05
Ram	3	3.747	3.58
Error	6	1.047	
Total	15		

TABLE 20

Mean LH Levels (Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	.251	8.10*
Period	3	.147	4.74
Ram	3	.796	25.68**
Error	6	.031	
Total	15		

*P < .05

**P < .01

TABLE 21

Mean FSH Levels (Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	1281.64	2.82
Period	3	1162.31	2.56
Ram	3	29795.43	65.65**
Error	6	453.83	
Total	15		

*P < .01

TABLE 22

Mean PRL Levels (Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	15626.1	6.11*
Period	3	1638.4	.64
Ram	3	1328.5	.52
Error	6	2559.2	
Total	15		

*P < .05

TABLE 23

Mean T Levels (Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	28.510	10.85**
Period	3	8.545	3.25
Ram	3	224.65	85.50**
Error	6	2.627	
Total	15		

**P < .01

TABLE 24

Baseline Values for LH Secretory Profiles
(Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	.201	2.21
Period	3	.356	.36
Ram	3	.147	1.63
Error	6	.091	
Total	15		

TABLE 25

Baseline Values for LH Secretory Profiles
(Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	.182	13.69**
Period	3	.169	12.69**
Ram	3	.207	15.56**
Error	6	.013	
Total	15		

**P < .05

TABLE 26

Peak Frequency Values for LH Secretory Profiles
(Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	3.099	3.18
Period	3	.182	.19
Ram	3	2.766	2.84
Error	6	.974	
Total	15		

TABLE 27

Peak Frequency Values for LH Secretory Profiles
(Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	2.792	7.44**
Period	3	4.625	12.33**
Ram	3	11.667	31.11**
Error	6	.375	
Total	15		

**P < .01

TABLE 28

Peak Height Values for LH Secretory Profiles
(Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	4.01	.25
Period	3	22.21	1.35
Ram	3	57.65	3.51*
Error	6	16.40	
Total	15		

*P < .05

TABLE 29

Peak Height Values for LH Secretory Profiles
(Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	1.494	7.93**
Period	3	.177	.94
Ram	3	6.658	35.35**
Error	6	.188	
Total	15		

**P < .01

TABLE 30

Delta Values for LH Secretory Profiles
(Non-breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	3.305	.20
Period	3	23.548	1.42
Ram	3	57.528	3.47
Error	6	16.564	
Total	15		

TABLE 31

Delta Values for LH Secretory Profiles
(Breeding Season)

Analysis of Variance

Source	df	ms	F
Treatment	3	1.229	5.98**
Period	3	.196	.95
Ram	3	5.474	26.62**
Error	6	.206	
Total	15		

**P < .01

APPENDIX II

Data for Experiment 2

TABLE 1

Mean LH Levels (ng/ml) During 6-h Intervals

Season	Treatment	Hours	Ram 1	Ram 2	Ram 3	Ram 4
Non-breeding (July-Aug.)	Control	1-6	.17	.47	.09	.85
		7-12	1.13	.23	.46	.72
		13-18	.36	.43	.09	.77
		19-24	.11	.14	.32	.58
		25-30	.62	.42	.59	1.00
		31-36	.71	.46	.31	1.28
	Mating	1-6	1.48	1.93	.33	1.02
		7-12	1.93	.89	.66	.78
		13-18	1.26	.76	.36	.64
		19-24	.65	.41	.09	.79
		25-30	.84	.98	.42	.81
		31-36	1.57	.36	.53	.90
	Control + GnRH	1-6	.98	.79	.30	.49
		7-12	.41	.62	.40	.78
		13-18	1.67	.41	.11	.86
		19-24	79.38	31.85	20.15	52.13
		25-30	2.02	.88	.61	1.31
		31-36	.82	.58	.31	.75
	Mating + GnRH	1-6	1.24	2.08	.96	1.59
		7-12	1.79	1.32	.52	1.16
		13-18	1.64	1.04	.39	1.22
		19-24	55.57	20.53	14.55	28.92
		25-30	1.55	1.05	.50	1.16
		31-36	.70	.39	.35	.79
Breeding (November)	Control	1-6	.97	1.81	.24	1.05
		7-12	1.11	1.87	.44	1.23
		13-18	.84	.73	.32	.70
		19-24	.55	.58	.24	.50
		25-30	.71	.74	.16	.79
		31-36	1.01	.94	.37	.78
	Mating	1-6	1.10	.54	.33	1.29
		7-12	1.01	.41	.17	1.25
		13-18	.44	.30	.31	.84
		19-24	.29	.21	.16	.56
		25-30	.61	.29	.10	.68
		31-36	.35	.44	.28	.77

TABLE 2

Mean FSH Levels (ng/ml) During 6-h Intervals

Season	Treatment	Hours	Ram 1	Ram 2	Ram 3	Ram 4
Non-breeding (July-Aug.)	Control	1-6	76.2	34.2	54.5	101.2
		7-12	79.5	29.2	78.0	101.5
		13-18	61.3	32.5	72.5	100.5
		19-24	61.7	30.2	68.2	94.2
		25-30	49.7	30.8	68.7	111.7
		31-36	58.2	29.0	59.8	99.2
	Mating	1-6	73.2	41.7	99.5	117.2
		7-12	94.3	41.7	101.3	121.7
		13-18	99.3	37.7	94.2	122.5
		19-24	98.2	37.8	100.5	119.5
		25-30	103.8	44.2	83.0	116.3
		31-36	95.8	41.2	86.0	131.0
	Control + GnRH	1-6	136.3	51.3	139.5	150.3
		7-12	119.7	63.0	169.2	163.7
		13-18	123.5	73.7	135.7	166.0
		19-24	197.3	155.3	156.7	247.3
		25-30	129.7	60.7	103.7	117.3
		31-36	138.3	84.7	104.3	127.3
	Mating + GnRH	1-6	282.2	95.3	144.0	163.0
		7-12	224.5	100.7	187.2	140.7
		13-18	241.3	99.5	202.3	148.7
		19-24	322.7	137.0	190.0	245.3
		25-30	209.0	75.0	99.3	131.7
		31-36	222.7	66.7	139.0	129.7
Breeding (November)	Control	1-6	310.8	115.8	105.3	168.8
		7-12	228.0	135.0	111.0	160.8
		13-18	280.5	100.8	99.7	146.0
		19-24	216.2	101.7	108.3	163.0
		25-30	203.8	108.3	107.0	175.8
		31-36	279.7	142.8	118.3	170.0
	Mating	1-6	252.2	108.5	159.8	145.2
		7-12	245.7	105.7	141.7	165.2
		13-18	311.8	104.5	146.2	138.0
		19-24	221.2	108.3	153.8	139.0
		25-30	298.3	108.5	149.3	155.0
		31-36	259.2	111.5	128.3	137.7

TABLE 3

Mean PRL Levels (ng/ml) During 6-h Intervals

Season	Treatment	Hours	Ram 1	Ram 2	Ram 3	Ram 4
Non-breeding (July-Aug.)	Control	1-6	73.1	56.5	78.4	46.7
		7-12	44.4	88.1	70.4	123.1
		13-18	109.5	92.0	90.8	229.7
		19-24	88.9	93.9	96.3	146.1
		25-30	130.0	141.5	145.2	216.0
		31-36	117.1	138.4	93.3	146.3
	Mating	1-6	203.6	108.5	25.6	148.3
		7-12	214.5	139.6	107.7	144.3
		13-18	196.0	155.1	105.1	187.3
		19-24	136.7	114.1	23.3	129.6
		25-30	203.3	122.3	140.0	153.5
		31-36	190.7	93.2	87.1	165.9
	Control + GnRH	1-6	44.9	44.7	92.8	90.9
		7-12	77.9	104.1	114.4	176.9
		13-18	80.0	95.7	54.3	121.5
		19-24	83.1	75.2	66.7	102.1
		25-30	122.0	127.6	174.5	171.9
		31-36	240.4	231.3	211.1	232.3
	Mating + GnRH	1-6	156.1	145.7	61.2	175.2
		7-12	209.6	236.3	91.7	184.3
		13-18	161.2	204.7	120.5	173.6
		19-24	111.7	115.5	55.9	120.8
		25-30	202.3	181.6	149.1	255.9
		31-36	120.0	119.6	127.1	234.0
Breeding (November)	Control	1-6	3.2	74.2	19.9	38.6
		7-12	2.4	22.8	20.2	44.5
		13-18	2.8	26.6	14.2	42.7
		19-24	2.2	14.3	6.8	26.4
		25-30	5.9	16.9	7.1	33.5
		31-36	10.2	48.9	10.1	55.9
	Mating	1-6	19.2	94.2	21.5	70.6
		7-12	7.5	19.5	9.2	5.8
		13-18	8.0	16.8	11.2	20.5
		19-24	2.2	2.9	3.9	7.6
		25-30	2.3	12.5	5.5	26.0
		31-36	2.4	6.8	3.1	9.9

TABLE 4

Mean T Levels (ng/ml) During 6-h Intervals

Season	Treatment	Hours	Ram 1	Ram 2	Ram 3	Ram 4
Non-breeding (July-Aug.)	Control	1-6	.21	3.56	3.17	2.30
		7-12	4.11	5.98	6.25	2.05
		13-18	1.81	4.60	1.63	2.47
		19-24	.41	2.47	8.30	2.53
		25-30	2.62	3.67	6.25	1.80
		31-36	1.96	4.32	11.85	3.03
	Mating	1-6	3.09	7.50	5.51	3.41
		7-12	3.75	5.51	6.31	2.12
		13-18	2.39	4.32	4.93	1.63
		19-24	2.42	4.09	1.65	2.40
		25-30	1.30	3.52	4.93	2.20
		31-36	3.74	3.70	6.38	2.43
	Control + GnRH	1-6	1.18	3.58	3.19	3.07
		7-12	3.15	.88	3.68	2.54
		13-18	4.57	3.43	.32	2.96
		19-24	14.60	9.04	15.67	17.54
		25-30	.55	.53	1.35	.73
		31-36	.14	.16	.67	.40
	Mating + GnRH	1-6	4.37	5.76	5.14	5.12
		7-12	4.31	5.52	3.72	4.49
		13-18	3.93	4.61	2.26	3.88
		19-24	12.98	12.43	10.39	14.78
		25-30	.71	1.88	1.07	.59
		31-36	.76	.61	.61	-
Breeding (November)	Control	1-6	25.13	18.83	5.36	25.14
		7-12	27.68	24.26	5.54	24.80
		13-18	20.79	20.12	5.76	24.47
		19-24	22.66	17.60	4.33	19.98
		25-30	20.20	17.54	5.01	20.47
		31-36	19.91	21.50	7.04	23.04
	Mating	1-6	28.35	15.73	8.55	30.75
		7-12	31.04	18.05	5.92	40.22
		12-18	21.88	11.42	6.38	32.08
		19-24	15.75	9.87	5.39	26.11
		25-30	19.67	11.18	2.10	24.62
		31-36	11.23	14.91	9.03	24.86

TABLE 5

Number of Mates Recorded for Rams During the 36-h Mating Periods in the Non-breeding and breeding seasons

Season	Treatment	Ram	Time Interval (h) 1-6	7-12	13-18	19-24	25-30	31-36	Total
Non-breeding	Mating	1	25	20	20	15	11	13	104
		2	13	7	6	5	3	3	37
		3	22	10	10	8	4	11	65
		4	11	5	13	6	5	5	55
Non-breeding	Mating + GnRH	1	20	15	10	8	5	10	68
		2	3	10	3	5	2	3	26
		3	17	12	7	8	3	3	50
		4	6	6	5	5	2	2	26
Breeding	Mating	1	16	13	17	9	16	8	79
		2	7	3	5	7	5	5	32
		3	28	6	11	9	5	14	73
		4	14	8	13	10	6	5	56

TABLE 6

Number of Mounts Recorded for Rams During the 36-h Mating Periods in the Non-breeding and Breeding Seasons

Season	Treatment	Ram	Time Interval (h) 1-6	7-12	13-18	19-24	25-30	31-36	Total
Non-breeding	Mating	1	30	29	26	20	20	17	142
		2	15	9	18	10	12	6	70
		3	31	19	57	14	9	28	158
		4	13	6	14	9	8	5	65
Non-breeding	Mating + GnRH	1	23	30	12	13	7	12	97
		2	8	15	8	6	10	4	51
		3	21	17	15	10	3	15	81
		4	8	10	5	6	2	2	33
Breeding	Mating	1	31	15	45	25	31	21	168
		2	18	8	5	14	11	7	63
		3	53	59	33	16	31	18	210
		4	34	12	23	15	11	6	101

TABLE 7

Six-hour Mean LH Levels for Control and Mating Periods During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	1.360	32.38**
Ram (R)	3	.744	17.71**
Time (P)	5	.232	5.52**
P x T	5	.121	3.12*
P x R	15	.127	3.02*
R x T	3	.433	10.31**
Error (T x R x P)	15	.042	

*P < .05
**P < .01

TABLE 8

Six-hour Mean LH Levels for Control and Mating Periods with GnRH During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	69.12	10.18**
Ram (R)	5	1822.81	268.59**
Time (P)	3	184.18	27.14**
P x T	5	88.87	13.10**
P x R	15	155.64	22.93**
R x T	3	6.91	1.02
Error (T x R x P)	15	6.79	

**P < .01

TABLE 9

Six-hour Mean LH Levels for Control and Mating Periods During the Breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	.738	15.38**
Ram (R)	3	.782	18.17**
Time (P)	5	.402	8.38**
P x T	5	.026	.54
P x R	15	.039	.81
R x T	3	.373	7.77**
Error (T x R x P)	15	.048	

**P < .01

TABLE 10

Six-hour Mean FSH Levels for Control and Mating Periods During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	5657.19	61.50**
Ram (R)	3	11568.53	125.76**
Time (P)	5	44.70	.49
P x T	5	33.00	.36
P x R	15	34.07	.37
R x T	3	242.87	2.64
Error (T x R x P)	15	91.99	

**P < .01

TABLE 11

Six-hour Mean FSH Levels for Control and Mating Periods with GnRH During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	16250.9	54.97**
Ram (R)	5	7877.6	26.65**
Time (P)	3	23863.9	80.72**
P x T	5	241.9	.82
P x R	15	685.4	2.32
R x T	3	7451.5	25.20**
Error (T x R x P)	15	295.6	

**P < .01

TABLE 12

Six-hour Mean FSH Levels for Control and Mating Periods During the Breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	393.88	1.09
Ram (R)	3	52288.62	145.2**
Time (P)	5	371.88	1.03
P x T	5	604.04	1.68
P x R	15	551.05	1.53
R x T	3	1858.30	5.16*
Error (T x R x P)	15	360.08	

*P < .05
**P < .01

TABLE 13

Six-hour Mean PRL Levels for Control and Mating Periods During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	8533.33	10.20**
Ram (R)	3	10319.75	12.33**
Time (P)	5	4812.69	5.75**
P x T	5	2021.98	2.42
P x R	15	572.17	.68
R x T	3	7192.76	8.59**
Error (T x R x P)	15	837.01	

**P < .01

TABLE 14

Six-hour Mean PRL Levels for Control and Mating Period with GnRH During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	14155.63	12.73**
Ram (R)	3	7426.75	10.78**
Time (P)	5	11986.11	12.73**
P x T	5	6445.86	5.80**
P x R	15	510.56	.46
R x T	3	3829.18	3.44*
Error (T x R x P)	15	1111.56	

*P < .05
**P < .01

TABLE 15

Six-hour Mean PRL Levels for Control and Mating Periods During the Breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	540.02	6.88*
Ram (R)	3	2073.39	26.42**
Time (P)	5	1134.93	14.46**
P x T	5	391.78	4.99**
P x R	15	261.60	3.33*
R x T	3	198.50	2.53
Error (T x R x P)	15	78.48	

*P < .05
**P < .01

TABLE 16

Six-hour Mean T Levels for Control and Mating Periods During the Non-breeding Season

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	.074	.03
Ram (R)	3	31.340	12.50**
Time (P)	5	4.419	1.76
P x T	5	3.818	1.52
P x R	15	2.571	1.02
R x T	3	2.955	1.17
Error (T x R x P)	15	2.508	

**P < .01

TABLE 17
Six-hour Mean T Levels for Control and Mating Periods with GnRH During the Non-breeding Season
Analysis of Variance

Source	df	ms	F
Treatment (T)	1	5.33	4.27
Ram (R)	3	1.15	.92
Time (P)	5	178.56	142.94**
P x T	5	3.96	3.17*
P x R	15	2.84	2.27
R x T	3	3.44	2.75
Error (T x R x P)	15	1.25	

*P < .05
**P < .01

TABLE 18
Six-hour Mean T Levels for Control and Mating Periods During the Breeding Season
Analysis of Variance

Source	df	ms	F
Treatment (T)	1	.089	.02
Ram (R)	3	939.634	170.14**
Time (P)	5	62.121	11.25**
P x T	5	11.642	2.11
P x R	15	13.388	2.42*
R x T	3	90.159	16.33**
Error (T x R x P)	15	5.523	

*P < .05
**P < .01

TABLE 19

Six-hour Mean LH Levels for Control Periods Without GnRH During the Non-breeding and breeding Seasons

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	.945	15.49**
Ram (R)	3	.685	12.55**
Time (P)	5	.257	4.72**
P x T	5	.137	2.50
P x R	15	.054	.90
R x T	3	.408	7.47**
Error (T x R x P)	15	.055	

**P < .01

TABLE 20

Six-hour Mean FSH Levels for Control Periods Without GnRH During the Non-breeding and Breeding Seasons

Analysis of Variance

Source	df	ms	F
Treatment (T)	1	107806.5	460.6**
Ram (R)	3	18545.8	79.24**
Time (P)	5	329.1	1.41
P x T	5	394.95	1.69
P x R	15	370.17	1.58
R x T	3	12785.97	54.63**
Error (T x R x P)	15	234.06	

**P < .01

TABLE 21

Six-hour Mean PRL Levels for Control Periods
Without GnRH During the Non-breeding and Breeding Seasons
Analysis of Variance

Source	df	ms	F
Treatment (T)	1	92330.6	218.60**
Ram (R)	3	5230.9	12.38**
Time (P)	5	1928.3	4.57**
P x T	5	3073.2	7.28**
P x R	15	527.5	1.25
R x T	3	972.2	2.30
Error (T x R x P)	15	422.4	

**P < .01

TABLE 22

Six-hour Mean T Levels for Control Periods
Without GnRH During the Non-breeding and Breeding Seasons
Analysis of Variance

Source	df	ms	F
Treatment (T)	1	2405.92	931.96**
Ram (R)	3	125.99	48.80**
Time (P)	5	10.35	4.01*
P x T	5	6.88	2.67
P x R	15	4.76	1.84
R x T	3	311.64	120.72**
Error (T x R x P)	15	2.58	

*P < .05
**P < .01

TABLE 23
Composition of Buffer Solutions

Gonadotropin assays

1. Phosphate-Azide buffer

$NaH_2PO_4.H_2O$	.19 gm
Na_2HPO_4	2.64 gm
Nacl	17.53 gm
Na-Azide	4.00 gm

Dissolve in 2000 ml distilled water in a volumetric flask. Final pH should range from 7.6 to 7.8.

2. Phosphate-EDTA buffer

Put 18.6 gm EDTA in 1000 ml volumetric flask and add 800 ml PO_4-Azide buffer. Warm and mix solution. Bring up volume to 1000 ml with distilled H_2O. Adjust pH to 7.6 with 5N NaOH.

Testosterone assay

Phosphate buffer solution (PBS)

$NaH_2PO_4.H_2O$	10.76 gm
Na_2HPO_4 (anhyd)	17.32 gm
Nacl	18.00 gm
Na-Azide	2.00 gm
Knox gelatin	2.00 gm

Dissolve first four components in approximately 1500 ml distilled H_2O in 2000 ml volumetric flask. Add 2 gm gelatin to buffer, warm slightly and stir until dissolved. Bring volume up to 2000 ml with distilled H_2O. Adjust pH to 7.4 with 5N NaOH.