THE UNIVERSITY OF MANITOBA

THE SECRETORY PATTERNS OF LUTEINIZING HORMONE AND TESTOSTERONE IN THE RAM AS INFLUENCED BY SEXUAL ACTIVITY, SEASON, AGE AND BREED

by

LEE MERRITT SANFORD

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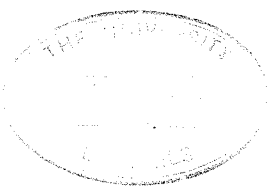

ABSTRACT

The Secretory Patterns of Luteinizing Hormone and Testosterone in the Ram as Influenced by Sexual Activity, Season, Age and Breed

Lee Merritt Sanford

The normal secretory patterns of luteinizing hormone (LH) and testosterone (T) were determined in mature rams. Both hormones were measured in peripheral blood serum using established radioimmunoassay procedures, in this and in subsequent experiments. Four rams were sampled at 20 minute intervals for 24 hour periods. A series of episodic LH releases was observed within each ram. The blood T level was consistently elevated following each release, achieving a peak level within 60 minutes. LH releases occurred at random among rams and at regular intervals within each ram. Results imply cause and effect and temporal relationships between these two hormones.

The effect of sexual activity on blood LH and T in the ram was observed in January. During periods of frequent sampling, seven rams were allowed to singly breed, mount without intromission or observe estrual ewes. These activities were not consistently followed (within 15 minutes) by elevations in the blood levels of LH and T. However, when two rams were allowed to breed for a 24 hour period, pronounced changes in the secretory patterns were observed, when compared to patterns obtained during a comparable non-breeding period. Due primarily to additional LH releases and elevations of T, higher mean levels of both hormones were noted during the first 12 hours. This was followed by marked decreases in LH and T during the second 12 hours.

Two studies were conducted to determine what effect environmental influences characteristic of southern Manitoba had on the blood levels of LH and T. Six ram lambs were sampled bi-monthly for 9 months, April through December. Mean blood T levels were observed to be low until mid-August following which a gradual four fold increase occurred; maximum levels being attained in October. Levels of T decreased sharply in December. LH levels fluctuated considerably prior to the onset of the elevation in T, then decreased gradually and became less variable as T increased. Constant exposure of four rams to an elevated ambient temperature of 32 C and 50 per cent relative humidity for 18 days did not alter their mean levels of LH and T. Therefore, the pronounced increase in blood T which occurs in rams at the onset of the breeding season would seem to be more closely related to changes in photoperiod rather than ambient temperature.

The relationship between reproductive function and blood LH and T was observed from early August through mid-December in eight rams. Frequent sampling at six week intervals revealed gradual changes in the secretory patterns over time. The frequency of LH release increased, although the releases were of smaller magnitude. This resulted in lower mean LH levels. Elevations in blood T became larger and more frequent, raising the mean level substantially. Coincident with the dramatic increase in blood T were improvements in two androgen dependent functions, libido (estimated by observing number of breedings per 8 hours) and semen production (estimated by assessing ejaculate quality). Breeding activity altered the secretory patterns of LH and T in August and September only. The level of T in seminal plasma appeared to remain constant over time in spite of marked increases in blood T.

Possible differences in the mean levels of LH and T

due to age were investigated. In May, 28 rams were bled at 20 minute intervals for 8 hours. Mean levels of both hormones were determined by assaying aliquots of pooled serum. Mean LH levels were highest in the prepuberal rams and lowest in the aged rams. Contrariwise, T levels were lowest in the prepuberal rams and highest in the yearling and aged rams, respectively. Young sexually mature rams with higher mean levels of LH tended to exhibit more releases which were of greater magnitude than did the older rams. Comparatively large LH releases followed closely by small elevations in blood T were observed in the prepuberal rams. The mean level of both hormones was found to be higher in rams of a high prolificacy breed type as compared to those from a low prolificacy breed type.

These results clearly indicate that LH release and T secretion in the ram are episodic in nature and closely related. The pattern of LH and T fluctuation in peripheral blood changes considerably throughout the year primarily due to changes in photoperiod. Partly in response to these seasonal endocrine changes, reproductive function is also modified. Some of the ram to ram variation in the levels of LH and T may also be attributable in part to differences in age, breed and frequency of breeding.

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INTRODUCTION

During the last few years a greater understanding has been achieved of the hypothalamic-pituitary-gonadal relationships in the male. It is now quite evident that a very complex set of hormonal controls governs male reproductive function. It was originally thought that a constant release of luteinizing hormone (LH) from the anterior pituitary prompted a steady secretion of testosterone (T) from the Leydig cells of the testes, which in turn maintained libido and the accessory sex glands, and that similarly, a constant output of follicle stimulating hormone (FSH) maintained spermatogenesis. Recently, however, it has been suggested that a number of different androgens may be active in the various target organs and that the initiation and maintenance of spermatogenesis involves both pituitary and testicular hormones.

Recent reports in the literature also have indicated that in a number of species, LH release from the pituitary in the male is not continuous, but episodic in nature and that at least in the bovine species there is a definite temporal relationship between blood levels of LH and T. Because little is currently known about the pattern of LH release and T secretion in the ram, and the temporal relationship between the two, this area was investigated. In addition, the possible effects of a number of factors thought to alter these secretory patterns such as sexual activity, season, age and breed were studied.

The literature relevant to the above areas of study has been reviewed primarily for the ovine, bovine and human species. Reference is also made to observations for the rat, rabbit and other species where pertinent.

LITERATURE REVIEW

Secretory Patterns of LH and Testosterone

With the recently acquired capability provided by radioimmunoassay to assay small aliquots of serum from frequently, serially collected blood samples, insight has been gained into the patterns of LH release and T secretion, the variation in peripheral blood levels and the temporal relationship between these two hormones. This is particularly true for species with a large body weight and blood volume such as the bovine, ovine and human.

Although some work on the nature of the fluctuations of LH and T in blood has been reported for the rat, the inability to collect appreciable numbers of blood samples at frequent intervals has limited investigation. A technique has been developed for monitoring LH levels in arterial blood of individual rats at 5 minute intervals for up to 4 hours (Gay et al., 1969). However this frequency of collection cannot be maintained when large volumes of blood are required for the measurement of either T or LH and T simultaneously. Blood volume depletion due to sampling may alter the endocrine status of the rat. For example, removal of 5 ml of blood (during a 30 minute period) from 200 to 250 gram female rats results in a significant elevation of LH within 5 minutes (Seyler and Reichlin, 1973).

Serum LH levels in the male rat, based on single determinations, have been observed (Yamamoto et al., 1970) to range from nondetectible (< 0.2 ng/ml) to peak levels of 3.12 ng/ml. It was thought that the occasional high values observed might represent "spikes" in LH release. A diurnal pattern in the fluctuation of both serum and pituitary levels of LH could not be detected.

If LH release in the male rat is spontaneous and episodic in nature, it may possibly result in subsequent elevations of blood T if there is a cause and effect relationship between these two hormones. When LH is injected into either prepuberal (Parlow et al., 1973) or hypophysectomized mature (Hafiez et al., 1972) male rats, it causes a quick, marked elevation in blood T. In addition, tremendous fluctuations in the level of blood T in adult conscious male rats bled every 30 minutes for 2.5 hours have been observed by Bartke et al. (1973). This suggests a pulsatile pattern of T secretion. Peripheral T levels in the rat appear to be consistently elevated at mid-day (Mock and Frankel, 1973) and may fluctuate in response to an inherent rhythm from season to season (Kinson and Liu, 1973), although this is uncertain based on the evidence presented to date.

In the bovine species, LH appears to be released in spontaneous bursts. Katongole et al. (1971) observed from 5 to 10 LH peaks (ranging from 2.0 to 5.0 ng/ml) in the peripheral blood of two bulls sampled for 24 hour periods, the occurence of which appeared to be unrelated to daylight, feeding or sleep. Similarly, Smith et al. (1973) observed a series of from 0 to 8 elevations (>1 ng/ml) of LH during a 24 hour period in the peripheral blood of five bulls. A variable number of LH releases were observed by Bindon et al. (1974) for eight British breed bulls (two Angus, two Guernsey, two Jersey and two Shorthorn x Hereford) sampled at 30 minute intervals for 4 to 6 hours. However, six Zebu crossbred bulls exhibited no significant fluctuations in LH over a 4 hour period.

Results of studies which have investigated the possibility of diurnal variation of the LH level in bulls are not consistent. The mean level of serum LH was noted by

Mongkonpunya et al. (1972) to average 1.81 ± 0.05 ng/ml in five mature dairy bulls when sampled every hour for two 24 hour periods. The mean LH level did not fluctuate significantly throughout the sampling periods. Likewise, a diurnal variation of the mean LH level was not observed by Smith et al. (1973) for five bulls when animals were sampled at hourly intervals for two 24 hour periods (5 days apart). Gombe et al. (1973) found, however, that blood LH levels in four dairy bulls consistently decreased at 18.00 hours, after having been elevated since 07.00 hours. Levels apparently remained low until the time of feeding the following morning.

It has been demonstrated that an elevation in blood LH quickly follows an injection of synthetic gonadotropin releasing hormone (GnRH) in the bull (Golter et al., 1973; Mongkonpunya et al., 1973; Zolman and Convey, 1973). Subsequent increases in the blood T and androstenedione level also occur. LH appears to be the major hormone influencing the synthesis and secretion of androgens in the bull (Seguin et al., 1973). When bulls were injected with comparable doses of LH, FSH, growth hormone (GH) and thyroid stimulating hormone (TSH), only LH produced significant increases in the level of androstenedione and T.

The results of simultaneous estimations of serum LH and T levels in bulls collected at 30 to 60 minute intervals also lends evidence to the contention that LH releases prompt an immediate increase in the secretion of T (Katongole et al., 1971; Smith et al., 1973; Bindon et al., 1974). Spontaneous LH releases are usually followed by elevations of blood T which peak within 60 minutes. It is of interest to note that although this cause and effect relationship seems to hold for both normal and abnormal bulls (those co-twin to freemartin or, infertile and incapable of producing an erection), the magnitude of the T peaks is substantially less in abnormal bulls as opposed to

normal animals (Katongole, 1971).

Early observations by Bolt (1971) and Katongole et al. (1972) suggested that the levels of both LH and T fluctuated greatly in the peripheral blood of rams. This has since been confirmed by a number of people (Falvo et al., 1973; Wettemann and Desjardins, 1973; Purvis et al., 1974). However, it is not known whether a definite temporal or cause and effect relationship exists between these LH and T fluctuations. Falvo et al. (1973) have suggested that the fluctuations of LH and T which occur in the ram do not follow a diurnal rhythm. A consistent rhythm was not detected when six mature rams were sampled at hourly intervals over a 24 hour period.

It is known that a number of hypothalamic-pituitary-gonadal relationships exist in the ram. Recently, Galloway (1973) has shown that LH in intact rams increases to peak levels within 1.5 to 2.5 hours following an intravenous injection of luteinizing hormone releasing hormone (LH-RH) and that doses in excess of 200μg produce significantly greater LH discharges than doses of less than 200μg. Others have also demonstrated that both LH (Falvo et al., 1973; Wettemann and Desjardins, 1973) and T (Falvo et al., 1973) levels increase in the peripheral blood of rams following GnRH administration.

In the normal intact ram, LH levels appear to be depressed due to the negative feedback effect of circulating T. This has been demonstrated by the observation that hemicastration of 18 month old rams results in a transient but significant ($P<0.01$) increase (41%) in the LH level by day 4 (Hochereau-de Reviers and Pelletier, 1971), and that complete castration is associated with a two fold increase in the LH level within 24 hours (Short, 1972). Conversely, 5 mg of T given to rams

as a single intramuscular injection markedly depressed the occurrence of LH releases and the mean level for up to 27 hours (Bolt, 1971). In addition, Pelletier (1970) observed that a pharmacological injection (400 mg injected intramuscualry) of testosterone propionate (TP) significantly ($P < 0.001$) depressed LH levels in castrate (for 1 week) rams for 5 to 7 days by blocking the release of spontaneous bursts of LH into the blood stream. A concomitant decrease in the synthesis of LH-RH was also observed, although synthesis of LH in the pituitary apparently continued uninhibited.

The magnitude of the spontaneous LH releases may be partially related to the levels of circulating T in the blood. The pituitary has been shown to be less responsive to LH-RH in castrated rams when pretreated with TP (Pelletier, 1973b). Rams castrated 3 months previously exhibited decreases in the blood level of LH between hours 6 to 18 and hours 48 to 96 following an intramuscular injection of 600 mg of TP. The amount of LH released when animals were injected with LH-RH at hour 6 and hour 36 was significantly ($P < 0.05$) lower and higher respectively when compared to the amount released in control animals.

A tremendous amount of work has been done in order to investigate the secretory patterns of LH and T in the human male. The first study which demonstrated that repetitive elevations of LH were occurring at frequent intervals (3 to 7 per 12 hours) in peripheral blood of the human male was reported by Nankin and Troen (1971). Males were sampled at 15 minute intervals for a 12 hour period. Since then, a number of workers have noted similar patterns of release for both LH and FSH (Alford et al., 1973a; Boyar et al., 1972; Murray and Corker, 1973; Naftolin et al., 1973; Wieland et al., 1973). There is some evidence which

suggests that a circadian rhythm exists in LH levels in the human male, with levels being higher during the early morning hours. For example, Nankin and Troen (1972) observed from 4 to 7 elevations of LH in the peripheral plasma of men when sampled at 15 minute intervals from 19.00 hours to 07.00 hours. The highest mean LH levels were observed between 03.00 and 07.00 hours. Daily variation in the LH level was also noted by Piro et al. (1973). LH values in the evening were observed to be much lower than those in the morning.

The peripheral T blood level in the human male is also known to fluctuate considerably between baseline and peak values (Alford et al., 1973 b; Elwood et al., 1973; Murray and Corker, 1973; Naftolin et al., 1973; Wieland et al., 1973; Rowe et al., 1974). However, unlike the close relationship that has been found between LH and T fluctuations in the bull (Katongole et al., 1971; Smith et al., 1973), fluctuations of these hormones in the human appear to be occurring at random. Naftolin et al. (1973) reported that only one third of the LH peaks were followed by a rise in serum T. Wieland et al. (1973) reported an unrelated occurrence of fluctuations of LH, FSH and T in normal males. Far more T peaks than LH peaks were noted by Murray and Corker (1973) when males were sampled at 10 minute intervals for an 8 hour period.

An explanation as to why a consistent close relationship cannot be demonstrated between fluctuations of LH and T in the human has been offered by Alford et al. (1973b). He has suggested that there may be a time lag between the occurrence of changes in the level of blood LH and the response of the Leydig cells and (or) that dramatic changes in LH are necessary in order to alter T secretion. This theory is supported by recent work. Tamm and Lindenmeyer (1973) were not able to detect increases in

either T or dihydrotestosterone (DHT) levels when LH-RH (4 or 12μg) was infused over 60 minutes into normal males. However, 150 to 400 per cent increases in the LH level were noted. In a similar study, Kley et al. (1973) noted a marked increase in plasma LH following an injection (25 to 500μg) of LH-RH, although T levels were not altered. Testosterone levels were appreciably elevated only after continuous infusion of LH-RH for 8 hours. In addition, studies by Alford et al. (1973a) and Elwood et al. (1973) have shown that for the human male, changes in plasma T concentration are often significantly positively correlated with the plasma LH level observed during the previous 1 to 3 hours.

There seems to be a considerable difference of opinion as to whether or not a definite diurnal cycle exists for blood T levels in the human male. A number of reports have unquestionably demonstrated that mean plasma T levels are consistently higher during the early morning hours prior to the time of awakening, peaking during 04.00 to 08.00 hours (Faiman and Winter, 1971; Rose et al., 1972; Barberia et al., 1973; de Lacerda et al., 1973; Judd et al., 1974; Rowe et al., 1974). Although concurrent cycles in the LH level could not be detected by Faiman and Winter (1971) and de Lacerda et al. (1973), implying that LH does not cause the T cycle, a close relationship was observed between night time increases in plasma T and preceding LH releases by Judd et al. (1974). Evans and Maclean (1971) have observed an increase in the number of fluctuations of T preceding periods of paradoxical sleep characterized by rapid eye movements during early morning hours. Alford et al. (1973b) however were unable to demonstrate a consistent time-related fluctuation in the blood T level of seven males sampled at 3 to 4 hour intervals. Four exhibited peak levels of T in the morning while the remaining three

exhibited peak levels approximately 12 hours later. Likewise, Boon et al. (1972) observed daily variation in T levels within males but no rhythmicity was seen among subjects.

Sexual Activity and LH and Testosterone

Copulation by the male rat appears to trigger a release of LH within 5 to 10 minutes, since pituitary LH levels are observed to drop while at the same time plasma levels increase (Taleisnik et al., 1966). Copulation also stimulates an increased production of T in the testis, presumably in response to LH stimulation. Herz et al. (1969) have observed a significantly higher level of T in the testes of male rats when housed from 40 to 162 days of age with females (mated males) as opposed to those (unmated males) housed with other males. Plasma T levels are known to be markedly elevated in male rats within 5 to 20 minutes following a single copulation (Purvis and Haynes, 1972a; Purvis and Haynes, 1974). Levels remain high for up to 1 hour. Being placed in close proximity to female rats seems to provide sufficient impetus for the onset of a rise in plasma T in the male (Bliss et al., 1972; Purvis and Haynes, 1974).

Sexual stimulation, which may be provided by a variety of activities, appears to be essential for the maintenance of the male reproductive tract because of the stimulatory effect it appears to have on T secretion. Thomas and Neiman (1968) observed that male rats living in cohabitation with females had heavier reproductive systems as opposed to those living in isolation or in male groups. In addition, either three intromissions or ejaculation every 4 days was sufficient to maintain the weight of the secondary sex glands. However, atrophy of the organs did

occur when males were only allowed to either mount without intromission or come into contact with estrual odors. This is in agreement with Folman and Drori (1966) who also observed that female odors did not enhance development of the reproductive tract in male rats raised in social isolation. Other workers have also observed that compared to sexually inactive males, rats allowed frequent mating exhibit larger weights of seminal vesicles and coagulating glands (Hunt, 1969), had increased fructose levels in the coagulating glands (Drori et al., 1968), and had increased weights of the penis and perineal muscle (Herz et al., 1969). Folman and Drori (1969) reported that hypertrophy of the androgen-sensitive secondary sex organs and the hypophysis, which occurred when male rats were mated, was highly correlated with the number of ejaculations performed. This suggests that T secretion in the male rat is positively related to the frequency of mating and this is mediated via the hypophysis. Male rats previously reared in either sexually segregated groups or in co-habitation with females respond to the presence (close proximity for 4 days without physical contact) of female rats (both castrate and estrual) by exhibiting increases in secondary sex organ weights, pituitary LH content and testicular vein T levels (Purvis and Haynes, 1972b).

Similarly, the male rabbit exhibits a marked increase in plasma T 30 to 45 minutes following copulation with a receptive female (Saginor and Horton, 1968; Haltmeyer and Eik-Nes, 1969). Testosterone was observed to have been increased four fold in a number of rabbits prior to physical contact with the female (Saginor and Horton, 1968). The observation that a human chorionic gonadotropin (HCG) injection will also markedly increase blood T levels in male rabbits within a similar amount of time leads one to speculate that the act of copulation triggers an LH

release which in turn elevates the circulating T level.

Blood levels of LH and T coincident with sexual activity were first reported for the bull by Katongole *et al.* (1971). Teasing (mounting without intromission) prior to ejaculation was shown to be associated with an LH release and a subsequent elevation of blood T in two bulls. Observation of a cow was thought to prompt an LH release via a similar neuroendocrine reflex in one of the bulls. Subsequent investigations, however, have not confirmed that this is a phenomenon which consistently occurs among all bulls.

Convey *et al.* (1971) have shown that only minor nonsignificant changes in serum LH occurred following sexual preparation (three to four false mounts) and ejaculation in both young and mature bulls. Blood samples were collected 30 minutes prior to and 5 and 30 minutes following ejaculation. However, the stimuli associated with ejaculation did cause a marked release of prolactin and a smaller but significant release of GH. Subsequent analysis of the serum samples from these same bulls for T (Smith *et al.*, 1973) revealed that although LH had not increased appreciably 5 minutes following ejaculation (only 13%), the T level had increased an average of 49 per cent, but in only 75 per cent of the bulls. In an additional experiment, bulls were sampled at frequent intervals prior to and following either sexual preparation (three false mounts) or ejaculation on the first mount. Only slight increases of similar magnitude in blood T were observed following either treatment in the four bulls. observed. LH levels were determined in four bulls prior to and following teasing (two incomplete mounts) and ejaculation by Gombe *et al.* (1973). In three of the four bulls, a slight decrease in plasma LH levels was observed

30 minutes following ejaculation. A slight nonsignificant increase occurred in the other animal. Likewise, when Bindon et al. (1974) exposed bulls to estrual heifers for a four hour period, no significant fluctuations in LH occurred.

Comparatively little is known about the effects various types of sexual activity have on blood levels of LH and T in the ram. Purvis et al. (1974) sampled rams at frequent intervals for a 7 hour period during which time they were allowed contact for 1 hour with an estrual ewe. Blood T levels were low throughout the whole period (approximately 1 ng/ml). It was concluded that copulation had no effect on the T level at a time of the year when it was normally low. There is some evidence which suggests that blood T levels in the ram fluctuate throughout the year, decreasing in the spring and increasing again in the fall (Katongole et al., 1974; Purvis et al., 1974). The same cycle has been observed for T levels in all rams, whether they have been engaged in frequent breeding activity or not.

The possible association between sexual activity and elevations in blood T for the human male is still open to question, since conflicting results have been reported. A positive correlation between beard growth and either anticipation of intercourse or intercourse itself has been noted in one male subject (Anon., 1970). Because beard growth is dependent primarily upon androgens, this observed correlation led to the speculation that perhaps T was elevated during these situations. Another study involving only one subject has been reported by Fox et al. (1972). T levels in peripheral plasma were measured prior to, during and following sexual intercourse on a number of

occasions. It was observed that T levels in samples taken either during or immediately after intercourse were significantly higher than those taken under resting conditions. LH levels were apparently unaffected. In the same study, masturbation was observed to have had no measurable effect on the T levels in seven subjects. Stearns et al. (1973) could not detect changes in either LH, GH, prolactin or T in six men following coitus.

An interesting study reported by Rose et al. (1972) demonstrated that male monkeys allowed frequent copulations with receptive females for a 2 week period exhibited two to three fold increases in plasma T levels as compared to levels during 2 week periods (prior to and following the breeding period) when they were caged individually. This suggests that periods of intense sexual activity may have a greater effect on blood T levels than do single copulations.

Variation of Reproductive Function with Season

Semen quality - Environmental factors such as temperature and duration of photoperiod are known to influence the fertilizing capability of the ram. Rams exposed to an elevated ambient temperature of 32 C and relative humidity of 65 per cent for 4 days exhibited increased rectal temperatures (0.9 C) and respiration rates (Howarth, 1969). When allowed to mate during the second and third weeks following treatment they were observed to be incapable of settling ewes. As would be expected, this was associated with marked decreases in semen quality. Epididymal spermatozoa were apparently somewhat resistant to the effects of heat as fertility was unaltered one week following the treatment period. Similarly, Rathore (1970a) noted that the fertility (based on lambing rates) of rams

8 to 16 days following exposure to 40.5 C for either 1 or 3 days (8 hours per day) was significantly lowered. Fertility had returned to normal levels by 25 to 36 days following treatment. The fertility rate (ova fertilized 60 to 70 hours following mating) of superovulated ewes mated to rams previously exposed to 40.5 C for either 1, 2, 3 or 4 days (8 hours per day) was demonstrated to be 65.6, 42.4, 23.1 and 6.4 per cent respectively (Rathore, 1970b), as compared to 93.3 per cent for control rams. Breeding took place 10 to 27 days following heat treatment.

In the above studies, decreases in ram fertility due to elevated temperature was always associated with decreases in semen quality. The morphological changes of ram spermatozoa associated with heat treatment have been investigated by Rathore (1970c). Ejaculates were collected from rams housed at 40.5 C (8 hours per day) for either 2 or 4 days. They were noted to contain progressively greater numbers of pyriform cells during days 9 to 18 following treatment. The percentage of abnormal cells found in ejaculates during this period increased from 4.5-5 per cent (day 9) to 17.5-31.5 per cent (day 18) and was related to the duration of heat exposure. Smith (1971) exposed rams to a temperature of 41 C for either 4, 6, 9 or 13.5 hours. Heat exposure resulted in considerable increases in the percentage of abnormal spermatozoa and decreases in the proportion of motile spermatozoa. These changes were more pronounced with increasing duration of treatment. Results suggest that rams need to be exposed to temperatures of 41 C for at least 9 hours before marked seminal degeneration becomes evident.

The changes in semen quality which occur from season to season appear to be strongly related to photoperiod. Fowler (1965) noted that when Merino rams were subjected to a reversal of the seasonal changes in daylength at a

time of the year when daylength was normally decreasing, they exhibited poor semen quality as compared to the control rams. Semen from treated rams exhibited lower percentages of live, motile and morphologically normal spermatozoa, and when used for artificial insemination, settled fewer ewes as compared to semen from control rams. Recently, Jackson and Williams (1973) exposed Suffolk rams to an artificial light regime consisting of two cycles per year. Photoperiod ranged from 6 to 18 hours of daylight per day, and was changed at the rate of 60 minutes per week. They observed similar 24 week cyclic variations in semen characteristics (fructose level, volume, concentration of spermatozoa and number per ejaculate) that could be related to photoperiod.

Not only are changes in semen quality evident with variation in photoperiod, but in addition, Skinner and van Heerden (1971) have noted alterations in the reproductive tract of the ram. During the course of one year, Merino rams were exposed to natural changes in daylength but were protected from temperature extremes. It was observed that periods of increasing daylength were strongly associated with decreases in the weight of the testes, seminiferous tubule diameter and number of epididymal spermatozoa. The weight of the seminal vesicles and vesicular fructose content also tended to be slightly lower during the same periods.

Libido - Sex drive in the ram is also known to be markedly altered throughout the year when animals are exposed to natural environmental conditions. This has been demonstrated by Pepelko and Clegg (1965). When eight rams were individually exposed to an estrual ewe for 1 hour twice monthly for one year, ejaculations were observed to be most frequent 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).

Lindsay and Ellsmore (1968) observed that seasonal changes in the libido of rams was breed dependent. They noted that Border Leicester rams were relatively more active (served more estrual ewes) during the autumn and spring as opposed to the summer. Merino and Dorset Horn rams, however, remained quite active during the summer months as well. It was observed that when rams were exposed to adequate and constant sexual stimulation (estrual ewes), animals of the three breeds would mate throughout the year. In a subsequent study, Lindsay (1969) placed four rams of each of three breeds (Merino, Dorset Horn and Border Leicester) into temperature controlled rooms. Animals were exposed to elevated ambient temperatures for 1 month; 26.7 C (first week), 32.2 C (second week), 37.8 C (third week) and 43.3 (fourth week). At the end of each week, rams were given an opportunity to breed every hour for an 8 hour period. Merino rams were observed to remain sexually active throughout each 8 hour period irrespective of the ambient temperature. However, progressive decreases in activity were observed at 37.8 and 43.3 C for the Dorset Horn and Border Leicester rams. Libido was still depressed in the Border Leicester rams after the temperature had been lowered to normal levels.

LH and testosterone secretion - Indirect evidence has been presented which suggests that seasonal variation in androgen secretion from the testis does occur. It is known for example that both semen volume and fructose level are

androgen dependent (Moule et al., 1966; Knight, 1973), and that these two indices of semen quality fluctuate in a yearly cycle (Amir and Volcani, 1965). Libido in the ram also varies with season, as was discussed previously. It has been shown to decrease during the winter months in spite of temperatures which are conducive for breeding (Pepelko and Clegg, 1965). This suggests that temperature is not the only factor contributing to the seasonal changes in sexual behavior. Because the maintenance of sex drive is androgen dependent (Davidson, 1972), it may be that the decreased breeding activity is in part due to a change in androgen secretion.

High ambient temperature has been demonstrated to exert a temporary detrimental influence on T secretion in Hereford bulls (Rhynes and Ewing, 1973). When exposed to 35.5 C and 50 per cent relative humidity for seven weeks, their plasma T levels fell to 43 per cent of control levels by the end of the first two weeks of the experiment. However, levels subsequently rose to near control values during the remaining five weeks. Within 2 days from the onset of the experiment, the rectal temperature had risen an average of 1.6 C and the respiration rate had doubled in the experimental animals.

Wettemann et al. (1973) observed that plasma T levels in Yorkshire boars were not altered significantly when they were maintained at 34 C for 8 hours, and at 31 C for 16 hours during each 24 hour period for 90 days. Levels averaged 2.2 $\pm$ 0.9 ng/ml in treated boars as compared to 2.8 $\pm$ 0.9 ng/ml in control boars. However, increases in both rectal temperature (1.0 C) and respiration rate (three fold) were observed during the first week.

The effect of elevated ambient temperature on T synthesis and secretion in the ram has been investigated by Gomes et al. (1971). Rams were either penned in an open

shed and exposed to Ohio spring conditions, or in an environmental chamber for 14 days where the temperature fluctuated daily from 28 to 32 C. At the conclusion of the experiment, treated animals exhibited significantly lower testis weights (both wet and dry weights) and T levels (μg/grams testis dry weight, μg/testis and μg/100 ml spermatic venous plasma). Testis tissue from treated rams incorporated less labelled precursor (cholesterol and pregnenolone) into T under in vitro conditions than tissue from control rams. Results imply that elevated ambient temperature is detrimental to Leydig cell function in the ram.

Few studies have investigated the relationship between heat treatment and blood LH level. Recently, Madan and Johnson (1973) blood sampled six heifers throughout four estrous cycles. Heifers were housed at 18.2 C for the duration of the first two cycles, and at 33.5 C during the second two cycles. The baseline (day 10 of cycle) and preovulatory peak levels of LH were markedly reduced during the later two cycles.

It is becoming more evident that the blood levels of T and some pituitary hormones fluctuate rhythmically on a yearly cycle, and that these changes are closely related to photoperiod. One of the most dramatic examples is demonstrated by prolactin. There is a strong positive relationship between the hours of daylight and the blood prolactin level in the ram (Pelletier, 1973a), bull (Schams and Reinhardt, 1974) and goat (Buttle, 1974).

Observation of testicular function throughout the year in rams exposed to Oklahoma environmental conditions (Johnson et al., 1973) has revealed that testicular metabolic activity (incorporation of lysine-U-C^{14} into protein, and oxidation of glucose-U-C^{14} to $C^{14}O_2$) is highest in October. This is coincident with a

comparatively higher rate of conversion of acetate-1-^{14}C to T-^{14}C in testicular tissue, and higher levels of plasma T and LH. Gradual declines in both steroidogenic activity and photoperiod took place during the subsequent months.

Two studies, recently reported by British workers, suggest that changes in the secretory patterns of LH and T occur from season to season in the ram. Purvis et al. (1974) demonstrated that rams sampled in November and January exhibited from four to eight marked episodic bursts of T ranging from 8 to 14 ng/ml during a 24 hour period. However, T fluctuations observed in rams sampled in March and April were comparatively small (maximum of 5 ng/ml) and less frequent. The T profile in June was found to be similar to that observed in the rams in November and January.

Katongole et al. (1974) blood sampled three Suffolk rams at weekly intervals for 14 months. LH levels appeared to fluctuate within the same range and at random throughout the year. T levels however, were consistently low from January to September ($<$10 ng/ml). Values were usually well above 10 ng/ml from October to December. Two of the rams sampled hourly for 24 hours in October exhibited more LH peaks and much higher T values than did the one other ram which was sampled in May.

Age and LH and Testosterone

A number of studies investigating the relationship between age and the blood levels of gonadotropins and androgens have been conducted for the domestic livestock species (bull, boar and ram). Particular attention has been given to endocrine changes occurring at the onset of puberty.

It has been suggested (Gallardo and Campbell, 1965;

Campbell and Gallardo, 1965) that in bulls the median eminence contains more LH-RH with increasing age. This was based on the observation that as bulls increased in age from prepuberal (3 to 6 months) to mature (over 24 months) animals, the capability of median eminence extract to induce ovulation in rabbits gradually increased. It is of interest to note that the pituitary in bulls will respond to releasing factor at a very early age. Bull calves 2, 4 and 6 months of age have been shown to respond to GnRH injections by promptly releasing LH (Mongkonpunya et al., 1973), the peak magnitude of which does not appear to vary with age.

Swanson et al. (1971) noted that levels of LH in jugular blood of Hereford bulls increased at the time of puberty from 1.93 ± 0.21 ng/ml (10 months of age) to 3.22 ± 0.24 ng/ml (12 months of age). Plasma LH levels in Angus bulls 7 to 9 months of age were shown to average 3.7 ± 0.3 ng/ml (Gombe et al., 1973). By 12 to 14 months, mean basal values had increased to 4.3 ± 1.1 ng/ml and irregular LH peaks three to four times the basal values had occurred. Values for adult Holstein bulls (over 2 years) ranged from 7 to 10 ng/ml. Convey et al. (1971) however, failed to demonstrate differences in the mean plasma LH level between young (1.5 to 2.5 years) and mature (3 to 6 years) bulls. Recently, linear increases in the mean LH level have been observed in crossbred bulls during the age of 7 to 13 months. Following puberty, LH levels were observed to increase quadratically (Moss and Moody, 1974).

As the immature male calf progresses from 4 to 9 months of age, dramatic changes occur in the levels of testicular androgens (Skinner et al., 1968). Androstenedione levels decrease from 111.0 to 9.4 μg/100 grams testis, whereas T levels only decline slightly from 101.0 to 81.4 μg/100 grams testis. Concomitant increases

in the weight of the testes and diameter of the seminiferous tubules also take place during this period. As androstenedione injections apparently delay these changes, Skinner et al. (1968) have suggested that it normally functions to delay the onset of puberty in the bovine male.

Rawlings et al. (1972) observed that plasma T levels generally remained low in bulls until approximately 5 months of age. Values then rose considerably and remained high for 6 months. This was followed by a subsequent decline at 12 months of age. Plasma androstenedione levels however, were noted to be high in bulls only during 2 to 5 months of age. Swanson et al. (1971) demonstrated that T levels did not change appreciably in bulls during 10 to 12 months of age, although androstenedione levels decreased 50 per cent over the same period. T levels have been shown to increase quadratically in bulls during 7 to 13 months of age (Moss and Moody, 1974). Smith et al. (1973) observed that blood T levels in mature bulls (3 to 6 years) was 116 per cent higher than in younger bulls (1.5 to 2.5 years).

T levels have been determined in spermatic vein blood of boars during puberty. Carlson et al. (1971) observed that plasma T levels were substantially higher in three boars when sampled at 118 to 127 days of age (6.0 to 11.2 μg/100 ml) as compared to levels for the same three animals at 78 to 87 days (1.3 to 2.7 μg/100 ml). High levels of T were found in spermatic vein blood of 3 month old boars (15.9 μg/100 ml) by Gray et al. (1971). Levels steadily increased from 3 months of age and were maintained at peak levels (22.3 to 27.0 μg/100 ml) between 5 and 7 months of age, a time which was coincident with the attainment of sexual maturity.

Testicular levels of T and androstenedione in boars ranging in age from 2 to 20 weeks have been determined by Elsaesser et al. (1972). Relatively high levels of both androgens were found in 2 week old animals (1 μg/g tissue). Levels tended to decline during the following 8 weeks, until the onset of puberty. Values then gradually increased from 10 to 20 weeks of age. The ratio of T to androstenedione was close to unity until boars reached 6 weeks of age, but the ratio shifted to 10:1 during puberty. In a subsequent study, Elsaesser et al. (1973) could not detect changes in the LH level of venous blood in the miniature pig between 1 and 12 weeks of age. T and DHT levels were generally found to be constant up to 5 weeks of age (1.46 ± 0.46 ng T and 0.27 ± 0.09 ng DHT/ ml of plasma). However, a continual, gradual rise in the levels of both androgens could be observed (4.0 to 7.0 ng T and 0.6 to 0.8 ng DHT/ ml of plasma) during the subsequent weeks.

Changes in the pituitary level of LH in rams from birth to puberty have been observed by Skinner et al. (1968). Pituitary LH steadily increased from birth (6 μg/ mg) to 84 days of age (23 μg/mg), following which time levels tended to decline and become quite variable. Similarly, Courot et al. (1972) noted a sharp six or seven fold increase in pituitary LH content between 10 and 80 days of age (from 4 to 26 μg/mg). In 60 day old rams, the pituitary appears to be capable of releasing a greater amount of LH in response to LH-RH (4 μg/kg body weight) than it does in 20 day old rams (Galloway and Pelletier, 1974). This is thought to be due to differences in the LH content of the pituitaries between the two age groups.

Gradual increases in the plasma level of LH in ram lambs during 4 to 11 weeks of age (from 2 to 4 ng/ml) have

been observed by Thimonier and Pelletier (1972). Courot et al. (1972) also noted that plasma LH in spring born lambs initially averaged 1.5 ng/ml and increased up to 70 days of age, peaking at levels averaging 4.5 ng/ml. Elevations in the LH level were observed some time before the onset of puberty. Crim and Geschwind (1972a) demonstrated that plasma LH in groups of ram lambs 30, 60 and 150 days of age was uniformly low, although values tended to be elevated considerably in some lambs at 90 and 120 days of age.

Testicular levels of T and androstenedione have been determined in ram lambs from birth to 168 days of age (Skinner et al., 1968). Although the concentration of T fluctuated at random (4 to 24 μg/100 g) throughout this period, the total amount of T increased steadily concurrently with increases in testicular weight. T was always the predominant androgen, and the relatively small initial androstenedione levels which were observed at birth decreased sharply during the first 56 days. Crim and Geschwind (1972b) reported that T could be found in the spermatic vein plasma of young ram lambs (53 days) and, that there was a significant positive correlation between the secretion rate of T and age (ranging from 47 days to adult) of rams. Androstenedione could not be detected in spermatic vein blood, and if present at all had to be less than 10 ng/ml.

Breed and LH and Testosterone

Part of the animal to animal variation in the levels of pituitary and gonadal hormones may be due to differences in the genetic makeup of the individuals, i.e. strain or breed variation. It has been shown (Karande et al., 1970) that pituitary levels of FSH are different in

two strains of female rats. The pituitaries of intact females from one of the strains (ICRC) always contained more FSH than those of the second strain (C3H). The difference was observed to be greater in the younger age groups. However, following castration the trends were reversed, with females in the C3H strain exhibiting higher levels of pituitary FSH. Similarly, Wolfe (1971) noted that pituitary and plasma levels of gonadotropin differed between two groups of female rats which were genetically different at only a single gene locus.

Gombe et al. (1973) could not detect differences in the mean level of serum LH between Holstein (eleven animals) and Guernsey (seven animals) bulls, aged 2 to 12 years. The LH values for animals of both breeds ranged from 7 to 10 ng/ml. Values from three Jersey bulls also fell within the same range. Although not designed to investigate differences in blood LH between breeds, a study by Bindon et al. (1974) indicated that LH release in Shorthorn x Hereford bulls was considerably different from that of Zebu crossbred bulls. More LH releases of greater magnitude were observed for the Shorthorn x Hereford bulls during a 4 hour period.

Differences in the gonadotropin content of the pituitary have been observed between high and low fertility breeds of sheep. Land and co-workers (1972) noted that pituitary levels of LH on day 10 of the estrous cycle in Finn x Blackface ewes (2.3 lambs produced per ewe) were less than 50 per cent that of Merino x Blackface ewes (1.2 lambs produced per ewe). It was concluded that the Finn x Blackface ewes were releasing a greater amount of LH during that stage of the cycle. This was supported by the observation that LH activity in the urine during

days 9 through 11 of the cycle was three times higher for the Finn x Blackface ewes as compared to the Merino x Blackface ewes. In a subsequent study, Land et al. (1973) found that for ewes of four breeds of sheep, both the mean plasma LH levels during the estrous cycle and the mean ovulation rates could be ranked in the same order.

Thimonier and Pelletier (1972) noted that changes in the plasma level of LH in male lambs between 4 to 11 weeks of age was considerably different between two breeds of sheep. LH in peripheral blood during this period increased at the rate of 0.41 ng per week in crossbred ram lambs and 0.15 ng per week in Prealpe ram lambs. LH levels in the prepuberal lamb also appear to vary with birth type (single, twin or triplet) and fecundity of the ewes. Bindon (1973) demonstrated that LH levels tended to be higher in prepuberal ram lambs born as triplets, as compared to those born as singles or twins. Ram lambs from three different flocks of Merino sheep, each flock exhibiting either a low (0 to 5%), medium (40 to 50%) or high (60 to 65%) incidence of multiple births, were noted to have mean plasma LH levels of 1.73, 2.11 and 2.51 ng/ml respectively at approximately 30 days of age. Results of a subsequent study by Bindon and Turner (1974) on prepuberal lambs of both sexes from these same three flocks have indicated that LH levels above 3 ng/ml occur in a greater proportion of the lambs from the medium (MF) and high (HF) fertility flocks as compared to lambs from the low fertility (LF) flock. There was also a significant difference between lambs from the MF and HF flocks with respect to numbers of lambs exhibiting LH values exceeding 10 to 20 ng/ml. The highest values were recorded in lambs from the HF flock. In addition, frequent sampling (at 09.00, 12.00, 15.00 and 18.00 hours) of lambs from the three flocks at 30 days of age revealed that significant

peaks in plasma LH were occurring in lambs from the MF and HF flocks, the magnitude and frequency of which were greater in the latter group. However, only moderate LH peaks were noted in lambs from the LF flock and these were not observed until 100 days of age.

MATERIALS AND METHODS

Experimental Animals

Experimental animals were obtained from the University of Manitoba breeding flock and were considered to be normal and in good health. A number of the yearling and aged rams had been used routinely for breeding purposes. Animals were housed throughout the year in pens containing 8 to 12 rams, located in an open-front, three-sided barn, and were maintained on a diet of grass-legume hay. When rams were moved to a new location for a period of experimental treatment and (or) bleeding, time was allowed for adaptation to the surroundings before the start of the experiment.

Finnish Landrace rams were used in many of the experiments reported in this study (Experiments 1 through 3 and 5). Rams from different breeds were used in the other experiments. The crossbred rams used in Experiments 4 and 8 were predominantly from crossbreeding Suffolk, Finnish Landrace, Line-M and grade Western sheep. Animals from the Line-M (Managra Synthetic) breed were used in Experiments 6 and 7, in addition to Finnish Landrace and Suffolk rams. This strain was developed from foundation stock and was composed initially of Devon Closewool (43%), Oxford (30%), Southdown (9%), Shropshire (8%), Minnesota 100 (6%) and Suffolk (4%).

Blood Collection and Handling

Prior to blood collection, rams were confined in a relatively small area to minimize possible movement and excitement associated with being restrained and to facilitate blood collection. From 6.0 to 8.0 ml of blood

were collected within 15 to 20 seconds from the jugular vein using either a 7.0 or 15.0 ml vacutainer tube and a 20 gauge needle one and one-half inches long. It was visually observed that neither the frequent handling nor bleeding appeared to stress the animals. The rams were usually not excited by the presence of the people restraining and collecting blood from them, particularly following 1 to 2 hours of frequent collections. However, the ram lambs sampled (2 to 7 months of age) tended to become excited more easily than did the older rams.

Blood samples were collected into chilled tubes and were either stored in crushed ice or refrigerated at 5 C until centrifugation. Samples were centrifuged within 24 to 48 hours following collection and aliquots of serum were stored at -20 C in one dram screw top vials until assayed for LH and T.

Hormone Assay Procedures

LH assay- The LH concentration of serum samples was determined by a modification of the radioimmunoassay described by Niswender *et al.* (1969). Purified ovine LH (LER-1056-C2) was labelled with ^{125}Iodine (Cambridge Nuclear Corporation) by a modification of the method of Greenwood *et al.* (1963).

The iodination procedure initially involved the addition of 25µl of 0.5 M sodium phosphate (NaH_2PO_4) buffer (pH 7.6) and 1 mC ^{125}I to a small glass tube containing 2.5µg of purified ovine LH (in 5µl of distilled water). Next, 10µl Chloramine-T phosphosaline (0.01 M phosphate, 0.15 M sodium chloride, pH 7.8)buffer solution (25 mg/10 ml) was added and the vial agitated. The iodination reaction was allowed to proceed for 2 minutes, and then 25µl sodium metabisulfite ($Na_2S_2O_5$) solution

(25 mg/10 ml phosphosaline) was added to stop the reaction. Lastly, 0.2 ml potassium iodide solution (100 mg/10 ml phosphosaline) was added to increase the volume.

The contents of the tube were then transferred to a Sephadex G-75 column to separate ^{125}I-LH from free ^{125}I. The column was then eluted with phosphosaline and 0.5 ml eluates were collected into tubes containing 0.5 ml of bovine serum albumin (2% in phosphosaline). Aliquots of these eluates were then diluted and counted in order to determine which fraction contained the highest level of activity (usually tube # 5 or 6). The first peak represented LH bound ^{125}I. The eluate associated with this peak was kept for up to 3 weeks (stored at -20 C) for use in the assay.

The Sephadex column was prepared from a disposable 10 ml glass serological pipette. The mouthpiece was cut off and a small glass bead dropped into the tip. The column was then filled to a height of approximately 10 cm with Sephadex G-75, which had been previously equilibrated in phosphosaline at room temperature overnight. Before use, the column was first coated with 1 ml of 2 per cent bovine serum albumin in phosphosaline to reduce nonspecific binding of protein to the glass, and then washed with approximately 30 ml of phosphosaline.

For the LH assay, serum aliquots (0.2 ml), standards (0.0, 0.1, 0.2, 0.4, 0.8, 1.2, 2.0, 4.0 and 6.0 ng NIH-LH-S14) and control pool serum samples (0.2 ml) in duplicate were added to 10 x 75 mm culture tubes and brought to 0.5 ml with diluent. The diluent used was phosphosaline with 0.1 per cent sodium azide and 1.0 per cent dried egg white (pH 7.8). Two tenths of a ml of ovine LH antiserum (GDN-#15) diluted 1:100,000 (phosphosaline and azide with 3% rabbit serum and 0.05 M disodium-ethylenedinitrilo-tetraacetate, pH 7.6) was then added to

all tubes, followed by 0.1 ml ^{125}I-LH (approximately 3000 cpm) in diluent.

After mixing, the tubes were covered with a sheet of Parafilm and incubated for 5 days at 4 C. Two tenths of a ml of 30 per cent goat anti-rabbit gamma globulin (Antibodies Incorporated, Davis, California) in phosphosaline-azide buffer (diluent without egg white) was then added, and the tubes were mixed and reincubated for an additional 24 hours at 4 C. The white precipitates that formed were resuspended by mixing. Then 1.0 ml of phosphate-azide buffer was added to each tube and the tubes were centrifuged for 20 minutes. Following this, the supernatant was decanted from the precipitate and both the supernatant and precipitate were counted for 2 minutes in a well-type automatic gamma counter (Packard Model 3001, Tri-carb scintillation spectrometer). The number of counts in the bound fraction was expressed as a percentage of the total counts. Serum samples with an LH concentration of less than 0.2 ng/ml were considered undetectible and were assigned a concentration of 0.2 ng/ml for statistical purposes.

Samples collected from individual rams were measured in the same assay and when possible samples from equal numbers of rams from each treatment group were assayed together. The inter-assay coefficient of variation for 12 replicate samples from a pooled serum standard with a mean of 1.27 ng/ml was $\pm$ 8.6 per cent.

Testosterone assay - Serum T was determined using a radioimmunoassay procedure developed at the Health Sciences Center, University of Manitoba, by Dr. J. S. D. Winter and co-workers. Antiserum was raised in rabbits immunized with T-3-carboxy-methyloxime conjugated to bovine serum albumin. The extent to which steroids other than T cross-reacted

with this antiserum was estimated by calculating the amount of each steroid detectible when 5 ng (in ethanol) was added to tubes processed for T determination. Using this criterion, the antiserum was shown to cross-react with androstan-17βol-3one (50%) and 1,4-androstediene-17β ol-3one (30%), 4-androstene-3β,17βdiol (5%) and 5∝-androstan-3,17βdiol (2.5%). Cross-reactivity with all other steroids tested was less than one per cent. These cross-reacting androgens are apparently not present in ram's blood at levels which significantly interfere with T determinations. Assay results on extracts of 15 serum samples prior to and following removal of these androgens by thin layer chromatography, as described by Winter and Grant (1971) did not differ significantly when analyzed using a paired t test.

For the T assay, single serum aliquots (0.2 ml), standards (0, 50, 100, 150, 200, 300, 400, 500, 600 and 700 pg) and a control pool sample (0.2 ml) were brought to a 2 ml volume in 20 ml screw top tubes with double distilled (dd) water and extracted (by inverting tubes slowly for 10 minutes) with 12 ml dd methylene chloride. The lower phase was then washed (by inverting tubes slowly for 2 minutes) once with 2 ml of 0.1 N sodium hydroxide and twice with 0.2 ml dd water. In some instances, extracts were then left overnight at 4 C. Duplicate 2 ml aliquots of the extract were transferred to 10 x 75 mm culture tubes and dried in a water bath at 37 C under nitrogen. One tenth of a ml of a solution (1:250) of T antiserum in phosphate buffer (pH 7.4) containing 0.1 per cent gelatin was added to the tubes which were then mixed and incubated at 21 C for 30 minutes. Fifty pg of T-1,2-^{3}H (New England Nuclear) in 0.1 ml of the same buffer was then added and after mixing tubes were incubated at 4 C for 30 minutes. Then 0.1 ml phosphate buffer with 0.5 per cent

gelatin and 1 ml charcoal-dextran solution (0.25 gram Dextran T-80 and 2.5 grams Norit-A in 1000 ml phosphate buffer) was added; the tubes were incubated 5 minutes at 4 C and then centrifuged for 5 minutes. A 0.5 ml aliquot of the supernatant was taken for liquid scintillation counting (Nuclear-Chicago Unilux II).

From a standard curve relating percentage binding to mass of T, the T content of each sample was determined and expressed in ng/ml. The sensitivity of this assay was 15 pg. The coefficient of intra-assay variance was determined to be ± 3.3 per cent. The inter-assay coefficient of variation was ± 5.4 per cent for 13 replicate samples with a mean of 3.15 ng/ml, and ± 8.0 per cent for 12 replicate samples with a mean of 18.96 ng/ml. Whenever possible, all samples collected from individual rams or, samples from equal numbers of rams from each treatment group, were assayed in one assay.

The T concentration was determined in seminal plasma (0.1 ml) samples in accordance with the above procedure.

Semen Collection and Evaluation

Semen Collection - Semen was collected from mature rams with an artificial vagina (AV) warmed by filling with water at 45 to 50 C. Prior to the beginning of the collection period, rams were individually penned with an estrual ewe and were allowed three to five false mounts (without intromission). Ewes had been brought into estrus by receiving an injection (intramuscular) of 1 mg estradiol-17β in 1 ml of ethanol approximately 36 and 12 hours prior to the time of intended use. Although most of the rams had been used and were concurrently being used for natural breeding, they usually ejaculated into the AV within 2 to 10 minutes following the onset of the

collection period. The ambient temperature of the collection area was maintained at 21 to 25 C to minimize the chances of spermatozoa becoming cold shocked.

Semen Evaluation - Immediately following the collection of semen into the 15 ml graduated test tube (protected by a warm water jacket) attached to the AV, a visual estimate of ejaculate volume was made and the tube placed into a water bath at 37 C. Two drops of semen were then pipetted into a 10 x 75 mm culture tube containing 1 ml of Krebs-Ringer buffer (calcium free) which was then covered with Parafilm and gently inverted. Microscopic assessment of percentage of motile spermatozoa was then carried out by two individuals, each estimating the proportion of spermatozoa (to the nearest 5%) exhibiting active progressive movement in three or four different fields.

Within 2 to 4 hours following semen collection, an aliquot (0.1 ml) of the ejaculate was diluted (1:400) in 40 ml of 3 per cent sodium chloride solution. Both chambers of an improved Neubauer (1/10 mm deep) hemacytometer were then filled. Fifteen minutes later, the total number of spermatozoa in five (four corner and the center) 0.2 mm squares were counted in each of the chambers and added together. The number of spermatozoa per ml of semen was determined by multiplying the total number of spermatozoa in the 10 squares by 1×10^7. The counting of spermatozoa on the hemacytometer grid was also done by two individuals.

After aliquots of the ejaculate had been taken for determination of concentration, the remainder of the sample was centrifuged for 20 to 30 minutes and the seminal plasma was removed and stored at -20 C in one dram screw top vials until assayed for T.

Statistical Procedures

The data obtained from many of the experiments (Experiments 1 through 5) conducted during the course of this study could not be meaningfully analyzed by any test of significance. Either the nature of the experiment or the lack of numbers of animals precluded meaningful statistical comparisons. Some of the data in Experiment 6 however, was analyzed using a mixed factorial design analysis of variance computer program (Computer Center, Health Sciences Center). Other statistical procedures used such as the paired t test (comparing serum T levels prior to and following chromatography of extracts), the simple one-way analysis of variance (Experiment 7), Duncan's new multiple range test (Experiment 7) and the t test (Experiment 8) were done in accordance with the methods prescribed by Steel and Torrie (1960).

EXPERIMENTAL

Experiment 1. Profile of LH and Testosterone Secretion in the Mature Ram

It is known that both LH and T are secreted in episodic bursts in the ram (Bolt, 1971; Falvo et al., 1973; Wettemann and Desjardins, 1973; Katongole et al., 1974; Purvis et al., 1974). Evidence to date however tends to suggest that the variations in blood levels of LH and T occur independently (Falvo et al., 1973; Wettemann and Desjardins, 1973) and that there is no definite temporal relationship between the appearance of peak levels of these two hormones. The data collected in these studies however, have been obtained from hourly or less frequent blood samplings. In addition, the half-life of LH in the peripheral blood of sheep has been determined to be approximately 30 minutes (Geschwind and Dewey, 1968). Therefore, in order to more accurately determine the profile of LH and T secretion and the possible temporal relationship between these two hormones in the ram, the following experiment was conducted.

Experimental Plan

In May, two mature crossbred rams 2 years of age (rams #1 and 2) were confined together in a portion of a larger pen which they had previously occupied with 10 to 15 other rams. This area was located in an open-front barn. From each of these two rams blood samples were collected at 20 minute intervals for a 24 hour period. During the sampling period it was observed that the rams periodically became sexually stimulated and attempted to mount each other.

Because it was thought that periods of mild sexual

stimulation such as periodic mounting might be altering the normal secretory patterns of LH and T, two mature Finnish Landrace rams 2 and 3 years of age (rams #3 and 4) were blood sampled in August and in January. Again, sampling was at 20 minute intervals for a 24 hour period, but the rams were penned in an enclosed area of the barn in isolation from each other and from the rest of the flock. However, they could still hear each other and the other sheep. Two weeks prior to the January sampling period, the rams were placed in pens inside the barn in a heated area (21 to 24 C). Each of the three sampling periods began at 09.00 hours.

Results

The results of frequent serial observations of serum levels of LH and T for the rams sampled in May, August or January are displayed in Figures 1, 2 and 3 respectively. A summary of the characteristics of the secretory patterns for LH and T for each of the 24 hour periods is given in Table 1. Mean baseline levels of LH and T reported in Table 1 were determined by averaging all values which were not part of the profiles of obvious peaks. The single values of greatest magnitude associated with well defined peaks were averaged to determine mean peak levels.

The secretory patterns for LH and T for each of the four rams sampled in May or August were observed to be similar. However, the secretory patterns observed in January for rams #3 and 4 were considerably different from the patterns for these two rams observed in August. Although LH releases occurred more frequently in January, mean serum LH levels were found to be substantially lower because of the smaller magnitude of the peaks. Mean T levels were considerably higher in January since baseline

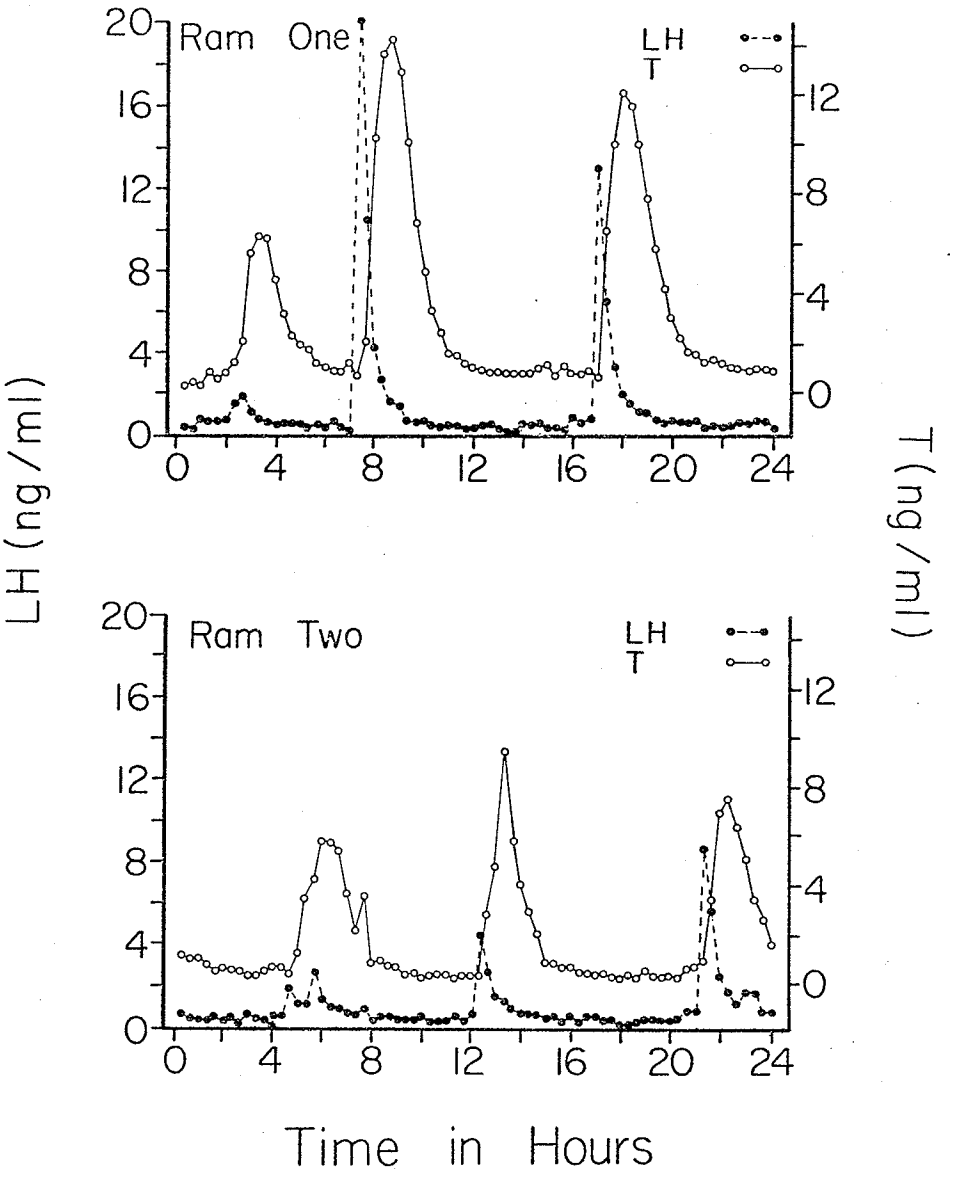

FIGURE 1. Secretory patterns of serum LH and T in two crossbred rams, bled by jugular venipuncture every 20 minutes for 24 hours in May.

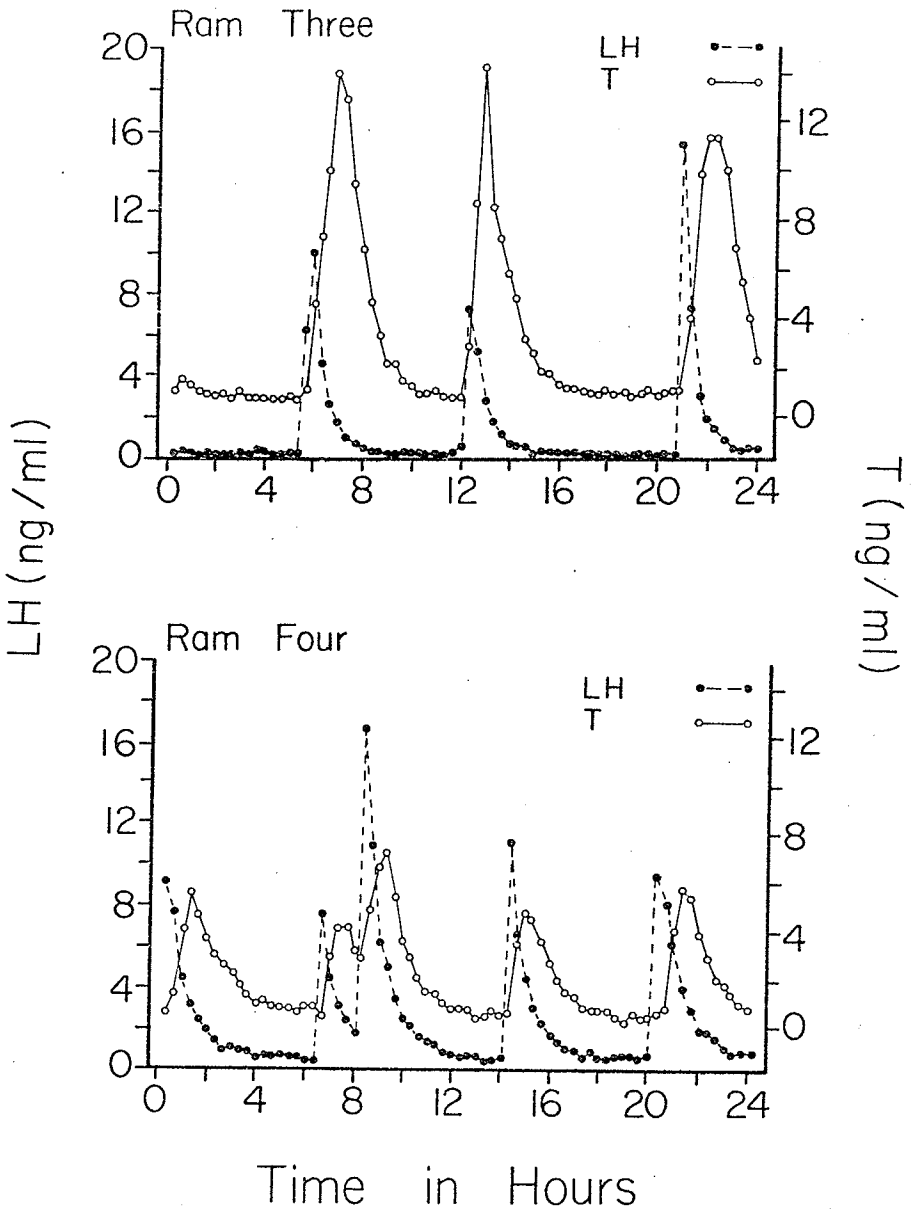

FIGURE 2. Secretory patterns of serum LH and T in two Finnish Landrace rams, bled by jugular venipuncture every 20 minutes for 24 hours in August.

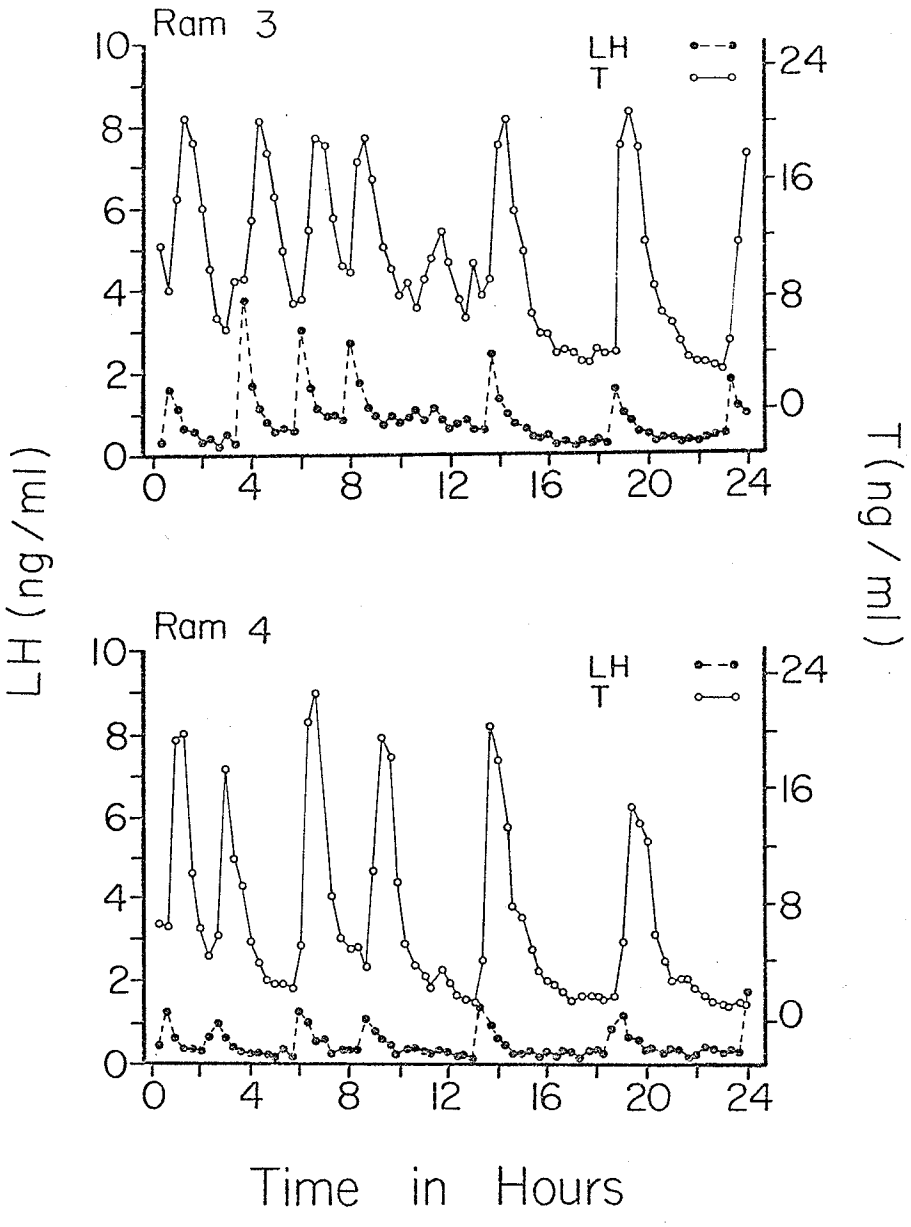

FIGURE 3. Secretory patterns of serum LH and T in two Finnish Landrace rams, bled by jugular venipuncture every 20 minutes for 24 hours in January.

TABLE 1.

Characteristics of the 24 Hour Secretory Patterns of Serum LH and T in Four Mature Rams at Various Times of the Year

	Month		
	May[a]	August[b]	January[b]
LH Level			
mean (ng/ml)	1.15 ± 0.24	1.94 ± 0.72	0.64 ± 0.16
baseline (ng/ml)	0.46 ± 0.03	0.49 ± 0.21	0.40 ± 0.08
LH Peaks			
number	3	3-5	7
magnitude (ng/ml)	8.37 ± 3.25	10.82 ± 0.01	1.80 ± 0.55
freguency (h)	7.50 ± 0.33	6.25 ± 1.25	3.85 ± 0.05
T Level			
mean (ng/ml)	2.54 ± 0.72	2.79 ± 0.58	8.58 ± 1.81
baseline (ng/ml)	0.69 ± 0.21	0.86 ± 0.13	4.26 ± 1.58
T Peaks			
number	3	3-5	6
magnitude (ng/ml)	9.26 ± 1.74	8.77 ± 3.31	19.66 ± 0.42
occurrence post LH peak (min)	56.7 ± 3.4	56.0 ± 4.0	35.0 ± 5.0

[a] values represent the mean ± SE for rams #1 and 2

[b] values represent the mean ± SE for rams #3 and 4

and peak levels and number of peaks per 24 hours had increased.

Among rams, LH releases appeared to occur at random and could not consistently be associated with feeding, light intensity or physical activity. For each ram, LH releases did however seem to occur at regular intervals. The interval between releases was shorter in rams #3 and 4 when sampled in January as opposed to August and releases seemed to occur more frequently during the daylight hours. Also in January, rams #3 and 4 exhibited slightly higher mean levels of both LH (0.75 ± 0.26 ng/ml vs 0.52 ± 0.07 ng/ml) and T (10.48 ± 1.86 ng/ml vs 6.69 ± 1.74 ng/ml) for the first 12 hours of the sampling period as compared to the mean levels observed during the second 12 hours.

In every instance, a release of LH was quickly followed by a definite increase in blood T. Blood T levels were usually elevated within 20 minutes following an LH surge. In the four rams sampled in May or August, peak T levels were reached within 60 minutes while peak T levels were achieved within 40 minutes in rams #3 and 4 when sampled in January.

Although the magnitude of the T peaks appeared to be related to the magnitude of the preceding LH peaks in ram #1 sampled in May, this relationship did not consistently apply within all rams. The comparatively small LH releases which occurred in rams #3 and 4 in January were apparently sufficient to maintain blood T at substantially higher levels than were the larger LH releases which occurred in these same two rams in August.

Discussion

The pattern and magnitude of fluctuations in serum levels of LH and T determined for rams in this study are

in good agreement with previous findings (Bolt, 1971; Falvo et al., 1973; Wettemann et al., 1973; Katongole et al., 1974; Purvis et al., 1974). A consistent, close relationship was observed between the occurrence of LH releases and increases in blood T for each ram irrespective of time of sampling. Peak T levels were observed to occur within 40 to 60 minutes following the LH peak. The temporal relationship which was observed had not previously been shown to occur in the ram. It had been noted however, that rams with elevated T levels also tended to exhibit higher LH levels (Falvo et al., 1973) and that fluctuations of serum LH and T were occasionally associated (Wettemann et al., 1973).

A similar relationship between LH and T fluctuations had been reported for the bull. Katongole et al. (1971) sampling two bulls every hour for two 24 hour periods observed that LH peaks preceded T peaks by about 30 minutes in most instances. In a similar study, Smith et al. (1973) collected blood hourly from five bulls for two 24 hour periods. Within 1 hour, 64 per cent of the LH peaks were accompanied or followed by elevations in serum T. In contrast to the relationship which exists in the ram and bull, individual fluctuations in the levels of LH and T in the human male appear to be unrelated (Murray and Corker, 1973; Wieland et al., 1973). Changes in plasma T may be correlated with the mean plasma LH level during the previous 1 to 3 hours however (Alford et al., 1973a; Elwood et al., 1973).

These results suggest that LH releases in the ram exert a strong positive influence on the Leydig cells, prompting a quick, marked increase in the secretion of T. This is consistent with the observations that injections of LH-RH promptly elevate blood levels of LH in the ram (Falvo et al., 1973; Galloway, 1973; Wettemann and

Desjardins, 1973) and T (Falvo et al., 1973). It seems likely that the release of LH spurts at regular intervals could be controlled primarily by some inherent central rhythm, as was suggested by Katongole et al. (1971) to explain the onset of LH releases in the bull.

It is questionable whether the LH-induced high levels of T in turn exert an influence on the LH level via a negative feedback effect. LH spurts were released quickly, usually reaching peak levels within 20 minutes. Also, it was observed that LH levels had often declined considerably from peak values before T levels had been elevated appreciably. This is particularly evident in rams sampled in May and August. Other workers suggest that in a general way, circulating levels of T exert an influence on the pattern of LH release in the ram. LH levels are known to rise following castration (Hochereau-de Reviers and Pelletier, 1971; Short, 1972) and to be depressed following T injections (Pelletier, 1970; Bolt, 1971). In addition, T levels may influence the frequency and (or) magnitude of the LH releases (Pelletier, 1973b). However, our data suggest that negative feedback is not involved in lowering LH levels following the release. LH release in human males does not appear to be consistently related to preceding declines or subsequent elevations in T levels (Wieland et al., 1973) which suggests that they are not the result of negative feedback control.

Although the numbers of animals are too few for meaningful statistical analysis, there appears to be a marked seasonal difference in the secretory patterns, with LH peaks in January being more frequent but of lesser magnitude than in May or August. While mean levels of LH in January were lower than in May or August, mean T levels were observed to be higher. This may be further evidence of a seasonal difference in pituitary-testicular function

in the ram (Johnson et al., 1973; Katongole et al., 1974; Purvis et al., 1974).

Summary

Jugular blood was collected at 20 minute intervals for 24 hours from each of two mature rams in May, August and January. Episodic increases in blood levels of LH and T were observed throughout the 24 hour periods. LH peaks always prompted an immediate elevation in blood T, which reached peak levels within 40 to 60 minutes. The magnitude of the T peaks did not appear to be consistently related to the magnitude of the preceding LH releases. LH levels following the release often appeared to decline markedly (within 20 minutes) prior to appreciable increases in the T level. Characteristics of the secretory patterns of LH and T tended to be considerably different in January as compared to May and August. Mean and baseline levels of T and the number and magnitude of T peaks tended to be greater in January, whereas, mean levels of LH and the magnitude of LH peaks tended to be lower.

Experiments 2 and 3. Influence of Sexual Activity on LH and Testosterone Secretion in the Ram

Recently, there has been a considerable amount of interest in the effect of various types of sexual activity on blood levels of LH and T in the male, particularly for the bovine (Convey et al., 1971; Katongole et al., 1971; Gombe et al., 1973; Smith et al., 1973), the human (Fox et al., 1972; Stearns et al., 1973) and the rat (Thomas and Neiman, 1968; Herz et al., 1969; Bliss et al., 1972; Purvis and Haynes, 1972; Purvis and Haynes, 1974). Limited data on the ram suggests that blood T levels are not altered by either copulation for 1 hour with estrual ewes (Purvis et al., 1974) or the collection of semen twice a week when sampled at a time of year when T levels are normally low (Katongole et al., 1974; Purvis et al., 1974).

The objective of the present two experiments was to determine if various types (observation of ewes, frequent mounting without intromission, and single breeding) and intensities (single breeding vs breeding at will over 24 hours) of sexual activity produced either a temporary change in the blood levels of LH and T or, more marked alterations in the secretory patterns of these two hormones in the ram. The experiments were conducted in early January, a time of the year when T levels are thought to be comparatively high in Finnish Landrace rams in southern Manitoba (Experiment 1).

Experimental Plan

Experiment 2.

Seven Finnish Landrace rams (two aged - 48 months, two yearling - 22 and 23 months, and three ram lambs - 11

months) were taken from an outside pen and placed in individual pens inside the barn. They were left undisturbed for 2 hours to become accustomed to the different surroundings. Blood samples were taken from the jugular vein at 20 minute intervals for approximately 4 hours (starting at 13.00 hours). Following 2 hours of sampling, rams were individually taken to two estrual ewes housed in a separate nearby area. Each ram was allowed to breed once and then returned to his pen where blood sampling was continued for another 2 hours.

The next day (day 2) blood samples were again collected from each ram at 20 minute intervals for 2 hours (starting at 09.40 hours). Rams were then taken individually to the ewes and permitted numerous mounts without intromission over a 2 minute period. Following this, they were returned to their pens and sampled for a further 2 hours. At the conclusion of this 2 hour period, the ewes were brought to the rams and walked in front of them for 5 minutes. Blood samples were collected from the rams 10 and 30 minutes following this observation period.

Experiment 3.

Two 4 year old Finnish Landrace rams (the same two used in Experiment 2) were placed in individual pens and after 2 weeks of adaptation to the surroundings, were sampled from the jugular every 20 minutes for two separate 24 hour periods (starting at 09.00 hours) which were spaced one week apart. Rams did not have visual or physical contact with each other or the other sheep during the first (control) 24 hour period. An estrual ewe was put in with each ram during the second (breeding) period at the beginning of hour 2 and, a record of mountings and breedings was kept. Ewes were switched between rams three

times during the 24 hours to provide further stimulation to the rams.

Results

Experiment 2.

Each of the seven Finnish Landrace rams bred one of the two estrual ewes within a 2 minute period, usually following from one to four mounts. On day 2, all rams exhibited a great deal of interest in the ewes and persisted in mounting for the full 2 minute period. Rams also became extremely excited when the ewes were walked in front of them for 5 minutes. Therefore, it was concluded that each type of activity had definitely sexually stimulated all of the rams.

During the two sampling periods (9 hours total) LH fluctuated among rams from baseline levels averaging 0.96 $\pm$ 0.22 ng/ml to peaks (four to eight) averaging 3.67 $\pm$ 1.09 ng/ml. Peaks were considered to be the obvious elevations of LH which prompted a subsequent rise in the blood T level. Approximately 40 minutes after the occurrence of the LH peaks, T had increased from baseline levels averaging 7.00 $\pm$ 1.32 ng/ml to peaks averaging 17.89 $\pm$ 2.24 ng/ml. If serum LH was observed to have been elevated at the time the first blood sample was taken following either breeding, mounting or observation of the ewes (5 to 15 minutes later) it was thought that this increase may have been attributed to the stimulation. In one of the ram lambs (#5) an elevation in serum LH appeared after all three types of sexual activity (Figure 4). Increases were also observed following breeding and continued mounting in one of the yearling rams (#4), following breeding in a second of the ram lambs (#6) and following observation of the ewes in an

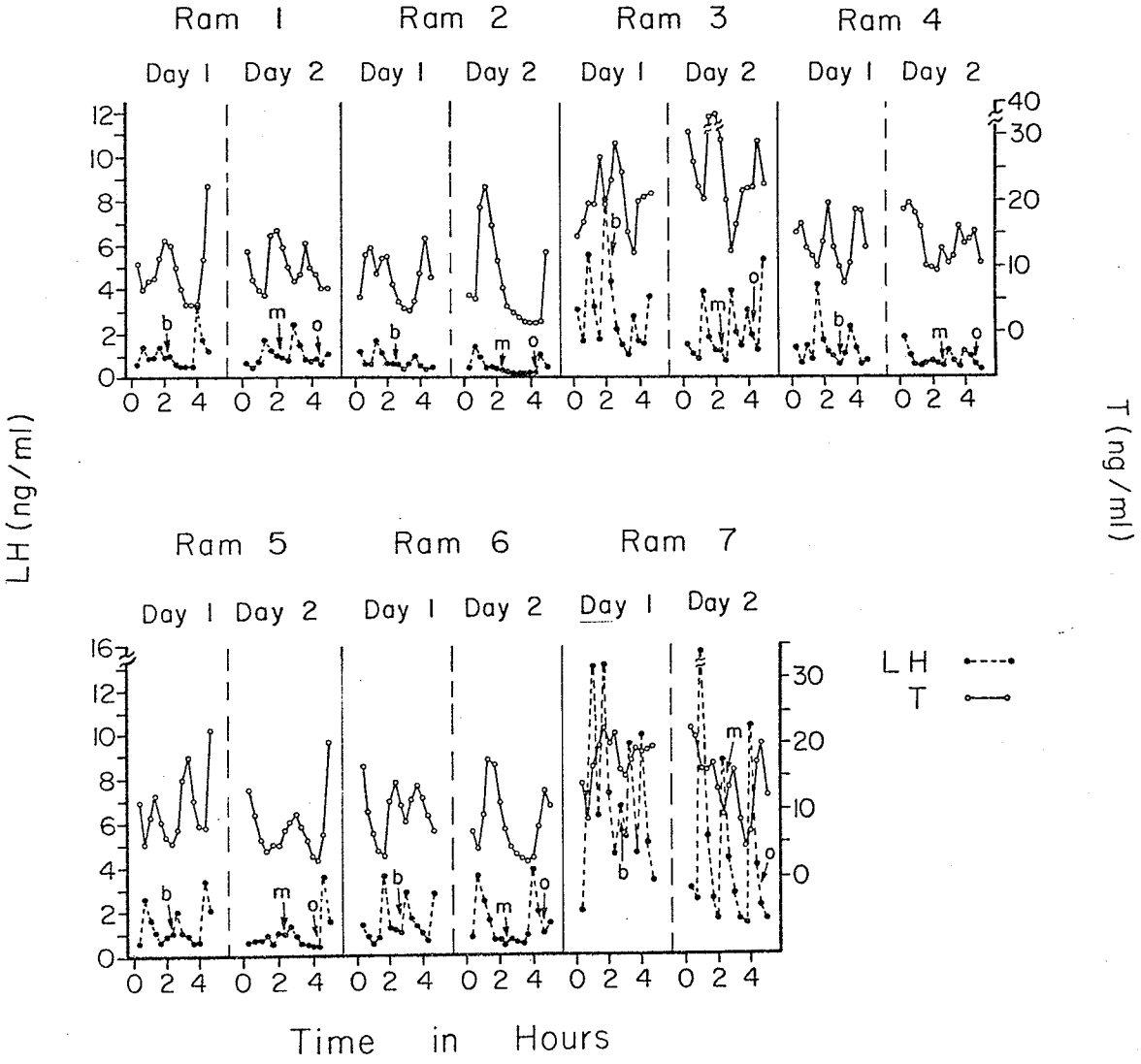

FIGURE 4. Secretory patterns of serum LH and T in two aged (#1 and 2), two yearling (#3 and 4) and three Finnish Landrace ram lambs (#5-7) concurrent with a single breeding (b), 2 minutes of mounting (m) and 5 minutes of observation (o) of estrual ewes when bled by jugular venipuncture every 20 minutes.

aged ram (#2). There was however, no association between increases in serum LH and any of the forms of sexual activity in three of the rams; one aged (#1), one yearling (#3) and one lamb (#7). The magnitude of the LH peak which appeared to be associated with sexual activity was on the average similar to or less than that of other peaks occurring spontaneously within the same ram (1.94 ± 0.31 ng/ml vs 2.66 ± 0.51 ng/ml).

Experiment 3.

The results of frequent serial observations of serum levels of LH and T for the two rams sampled during the 24 hour control and breeding periods are displayed in Figures 5 and 6, respectively. The observations in Figure 5 have been presented previously (Experiment 1, Figure 3). In the present experiment these observations served as a control for purposes of comparison with the patterns obtained during the 24 hour breeding period.

During the breeding period, ram #167 was observed to have bred the ewe 44 times while ram #130 bred 24 times. Most of the breeding activity (74%) took place during the first 12 hours and appeared to be associated with a marked alteration of the secretory patterns of LH and T. Mean levels of LH and T were slightly higher in both rams during the first 12 hours of breeding as compared to the comparable control period (Table 2). These differences appeared to be due to the greater number of LH releases (nine vs four) and resultant elevations of serum T (seven and eight vs four) as well as the slightly higher baseline levels of LH (0.83 ± 0.16 ng/ml vs 0.47 ± 0.14 ng/ml) and T (9.09 ± 1.28 ng/ml vs 5.99 ± 2.09 ng/ml) which occurred during this period. LH and T levels began decreasing in both rams during hour 13 and 14 and remained at low levels

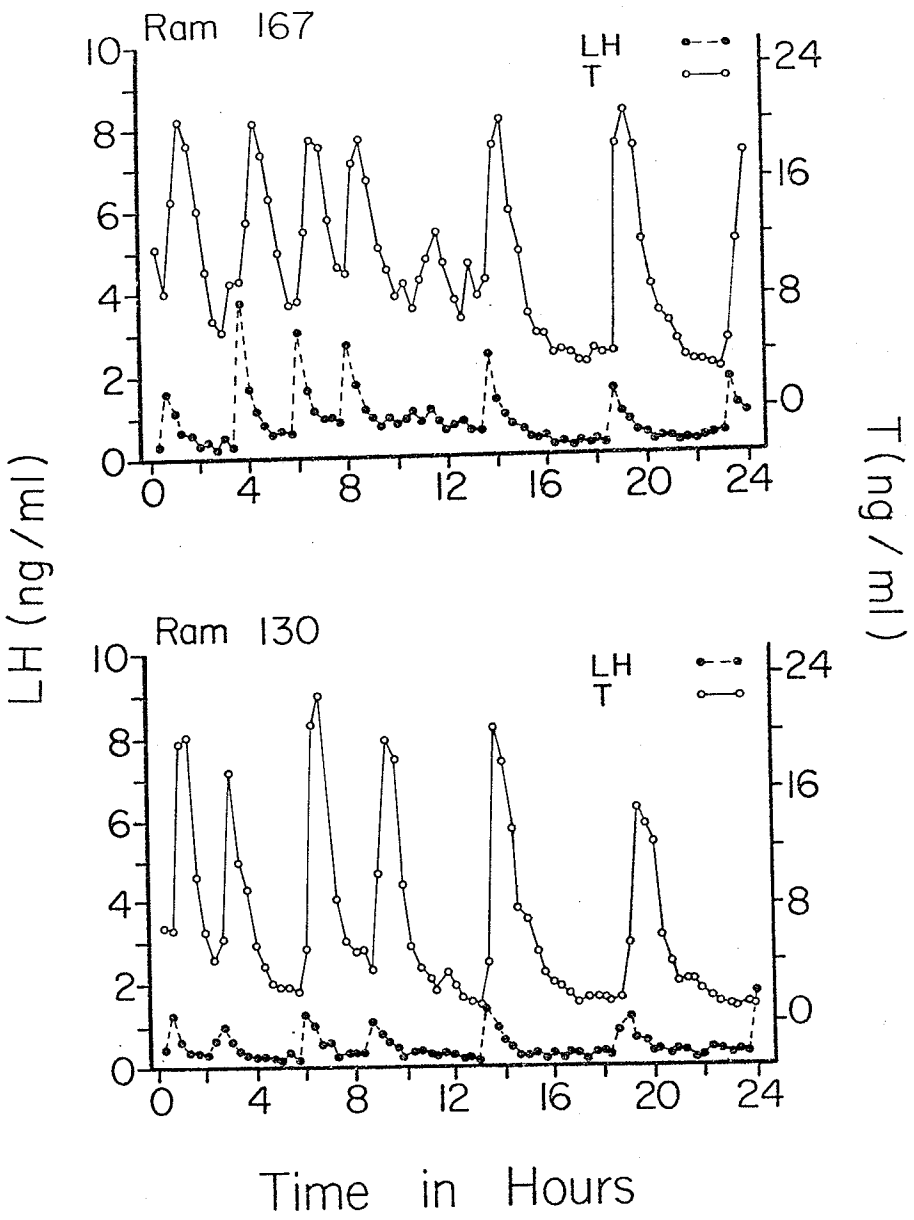

FIGURE 5. Secretory patterns of serum LH and T in two aged Finnish Landrace rams when each was penned alone and bled by jugular venipuncture every 20 minutes for 24 hours. Hours 0 through 8 represent daylight hours.

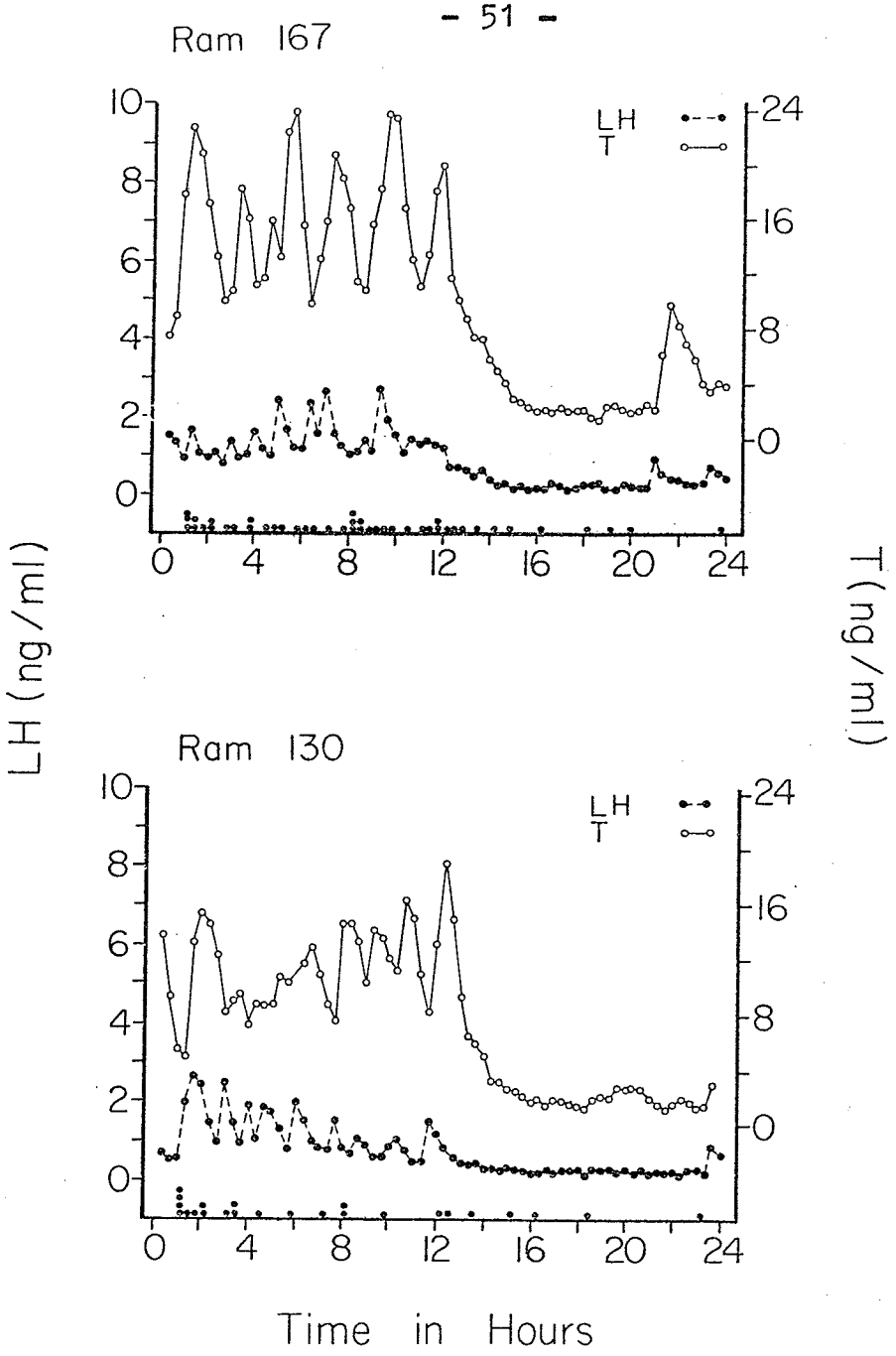

FIGURE 6. Secretory patterns of serum LH and T in two aged Finnish Landrace rams when each was penned with an estrual ewe for 24 hours and allowed to breed (•) at will, and bled by jugular venipuncture every 20 minutes. Hours 0 through 8 represent daylight hours.

TABLE 2.

Mean Serum LH and T Levels (ng/ml) in Two Aged Finnish Landrace Rams During the Control and Breeding Periods

	Ram 167		Ram 130	
Period	LH	T	LH	T
Control				
h 1-12	1.01[a]	12.34	0.49	8.62
h 13-24	0.59	8.43	0.44	4.94
Breeding				
h 1-12	1.36	15.86	1.16	11.03
h 13-24	0.29	4.28	0.27	3.68

[a] each value represents the mean of 36 observations

for 8 to 10 hours. Mean levels during the second 12 hours tended to be lower than the mean levels observed during the comparable control period (Table 2). Although breeding occurred periodically during this time, further LH releases were not observed until hour 21 and 23 in the two rams, respectively.

Discussion

The stimuli which promote sexual arousal and behavior in the male appear to bring about a quick release of LH in some species. Releases occur within 5 to 10 minutes following copulation in the rat (Taleisnik et al., 1966) and immediately or within minutes after teasing or ejaculation in the bull, according to one report (Katongole et al., 1971). Although all seven rams became strongly sexually stimulated when engaged in either a single breeding, false mounting or observing estrual ewes, none of these activities appeared to be consistently associated with the appearance of elevations of blood LH and T. In only one ram (#5) was each period of stimulation coincident with an LH release. It is also possible that the other observed associations between sexual activity and LH release may have been due to the occurrence of coincident spontaneous releases. These results together with the recent observation by Purvis et al. (1974) that T levels in four rams were not appreciably influenced by breeding activity when rams were penned with estrual ewes for 1 hour suggests that these types of sexual activity probably do not affect to any great extent the release of LH and secretion of T.

The apparent inability of certain types of sexual activity (single breeding, mounting and observation of ewes) to trigger an immediate release of LH in the ram is

similar to results reported for the bull and human male. Some bulls (Katongole et al., 1971) have been shown to respond to the sight of a cow, false mounting or ejaculation by exhibiting an immediate pronounced release of LH and rise of blood T. Other studies have shown however that false mounting and ejaculation may or may not be followed by slight non-significant increases in LH (Convey et al., 1971; Gombe et al., 1973) and moderate elevations of T (Smith et al., 1973). Coitus has recently been shown to have had no effect on blood LH and T levels in the human male (Stearns et al., 1973) although an earlier study with only one subject (Fox et al., 1972) reported that significant increases in T did occur during and immediately following coitus.

When the two aged rams were each penned with an estrual ewe and allowed to mate at will for 24 hours a dramatic change in the secretory pattern of LH and T occurred within each of the rams, as compared to the non-breeding pattern. The secretory patterns were probably altered in response to the continued intense stimuli associated with the numerous breedings which took place, particularly during the first 12 hours. These same two rams were used in Experiment 2 and in only one instance in one of the rams (#2, Figure 4) were any of the three forms of sexual activity associated with an LH release. A greater number of LH releases and elevations of T were observed during the first 12 hours of the breeding period as compared to the non-breeding period. This resulted in an elevation of the mean levels of these hormones as compared to the respective non-breeding values. Likewise, plasma T levels have been shown to increase dramatically in male monkeys when they were placed with receptive females and allowed to engage in frequent copulations (Rose et al., 1972).

During hours 12 through 14 of the breeding period the

blood levels of LH and T decreased dramatically in both rams to lower than normal mean levels for the remainder of the 24 hours. This was associated with a marked decrease in breeding activity which also occurred at this time. Despite some breeding activity during hours 12 through 20, LH releases, spontaneous or otherwise, were not evident again until hour 21. This suggests that for a period of time, the hypothalamus had become refractory to incoming neural stimuli and (or) that the pituitary had temporarily become refractory to LH-RH. There is some evidence to suggest that the human pituitary exhibits partial refractoriness when two LH-RH injections are given within a short time interval (Schneider and Dahlen, 1973). However, Gay et al. (1970) did not observe refractoriness of the pituitary when three injections of hypothalamic extract were given to anestrual ewes at 2 hour intervals. Similarly, Kinder et al. (1974) observed no pituitary refractoriness when LH-RH injections (150 μg) were given to heifers at four hour intervals for 96 hours during days 16 through 19 of the estrous cycle. When either anestrual ewes (Sandow et al., 1973; Chakraborty and Reeves, 1973) or rams (Wettemann and Desjardins, 1973) were continuously infused with LH-RH, serum LH levels decreased markedly within 3 to 4 hours, despite continuous infusion, to baseline levels or lower. Perhaps intensive breeding activity results in a more or less continuous release of LH-RH which in turn causes the pituitary to become insensitive to further stimulation after 12 to 13 hours.

The decrease in the level of serum LH during hours 12 and 13 may be attributed in part to possible increases in the level of corticosteroids in response to the emotional and physical stresses associated with intensive breeding. Although cortisol levels were not raised by coitus in the human male (Fox et al., 1970), plasma corticosterone levels

were significantly elevated above resting levels following a single copulation in the rat (Szechtman et al., 1974). Increases however, were probably not related to copulation per se but to slower habituation of some males to the novelty of being introduced to a receptive female. Increased corticosteroid production in the boar due to adrenocorticotrophic hormone (ACTH) stimulation is thought to be able to lower the plasma levels of LH and hence T secretion by the Leydig cells (Liptrap and Raeside, 1968). Likewise, Beitins et al. (1973) have demonstrated that when human males were given injections of ACTH (20 USP units per m^2 of body surface area) every 12 or 6 hours, plasma T and DHT levels had decreased significantly within 24 hours, although changes in the LH level were not detectible.

Although this study was conducted at a time of the year when most sheep in this latitude may be entering anestrus, it is believed that the alteration in the secretory patterns of LH and T obtained with intense breeding activity probably represents a true breeding effect and is not the result of seasonal influence. The two sampling periods were only one week apart. In addition, the type of secretory patterns thought to be exhibited in the ram with the advancement of the non-breeding season are not similar to the patterns we observed during the second 24 hour period (Katongole et al., 1974; Purvis et al., 1974). It is possible however, that the alterations observed are only characteristic of Finnish Landrace rams when actively engaged in continuous breeding activity in mid-winter.

Summary

Observations were made on the levels of serum LH and T in seven Finnish Landrace rams when sampled at 20 minute intervals prior to and following a single breeding,

continuous mounting without intromission (2 minutes) and observation of estrual ewes (5 minutes). Elevations in blood LH and T levels were evident in only two or three rams following these types of sexual activity and may have occurred spontaneously. The secretory patterns of LH and T for two mature Finnish Landrace rams sampled at 20 minute intervals for 24 hours while penned alone (control period) and with estrual ewes (breeding period) were markedly different. The intense breeding activity which took place during the first 12 hours appeared to be associated with temporary increases in the mean and baseline levels and number of elevations of both LH and T. Levels of both hormones markedly decreased during hours 12 to 14 and remained low throughout the next 8 to 10 hours in spite of limited breeding activity.

Experiment 4. Seasonal Variation of Blood LH and Testosterone in the Ram Lamb

Reproductive function in the ram is known to vary throughout the year. The alterations in libido and semen quality which occur from season to season appear to be closely related to changes in photoperiod and ambient temperature (as described earlier). Modification of reproductive function may be a reflection of alterations in the levels of various hormones, particularly those which are known to be directly involved in the maintenance of libido and semen production. Recent reports suggest that blood levels of LH, T and prolactin follow a yearly cycle in the ram (Johnson et al., 1973; Pelletier, 1973a;Katongole et al., 1974; Purvis et al., 1974). This experiment was conducted to determine mean serum levels of LH and T in ram lambs when exposed over a 9 month period to the yearly extremes of natural daylength and environmental temperature characteristic of southern Manitoba.

Experimental Plan

Six crossbred ram lambs (initially 3 to 4 months of age) were penned together and starting on April 26, 1972, a blood sample was collected from each ram at 2 to 3 week intervals until January 10, 1973. Rams were housed in a well ventillated barn until mid-August and then moved to an outside pen. Although ewes were kept in the same barn, rams did not come into physical contact with them at any time. Information on daily ambient temperature and daylength (daylight hours per 24 hours) was obtained from the Winnipeg International Airport Surface Weather Station (lat. 49°54' N, long. 97°15' W) which was located approximately 15 miles away.

Results

Mean serum levels of LH and T observed throughout the experimental period for the six rams are given in Figure 7. During May through July, the LH level appeared to be considerably more variable among rams, with some exhibiting comparatively high fluctuations (6.35 to 17.65 ng/ml). Coincident with this was an increase in age of the younger rams from 90 to 180 days. From mid-August through September, serum T gradually increased approximately four fold from a mean of 2.72 $\pm$ 0.44 ng/ml and remained at this elevated level (12.56 $\pm$ 0.90 ng/ml) during October and November. The mean LH level however, appeared to be fairly constant throughout this period. Concurrent with gradual increases in the T level and its maintenance for a 2 month period was a continual decrease in both environmental temperature and daylength (Figure 8). Daylength decreased from approximately 12 to 8.5 hours during the period of highest T levels. Both the T level and to a lesser extent the LH level were observed to decrease sharply during December and early January. This coincided with slight increases in the ambient temperature and daylength.

Discussion

Results of this study demonstrate that the mean level of blood T in young rams (7 to 8 months of age) increases dramatically and remains elevated at a time of the year which coincides with the normal breeding season. Others have also observed elevated levels of T during the fall and early winter months in peripheral blood of rams (Johnson *et al.*, 1973; Katongole *et al.*, 1974; Purvis *et al.*, 1974).

Under the experimental conditions of this preliminary study, changes in the mean level of LH coincident with

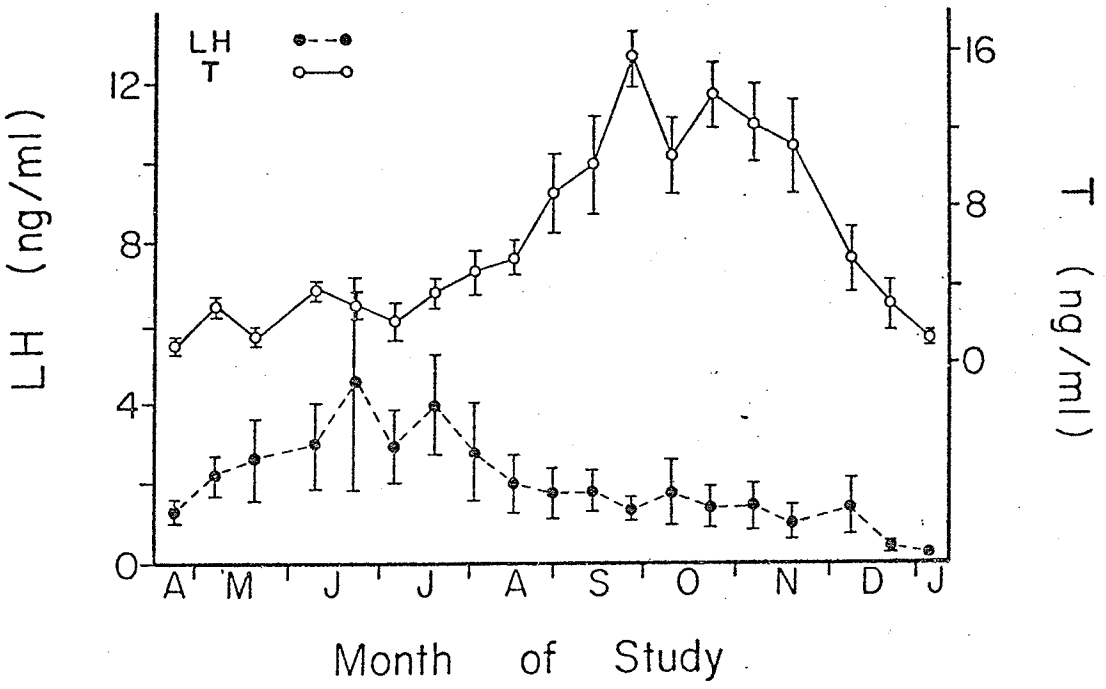

FIGURE 7. Mean (± SE) serum LH and T levels in six crossbred ram lambs bled at 2 to 3 week intervals from April, 1972, to January, 1973.

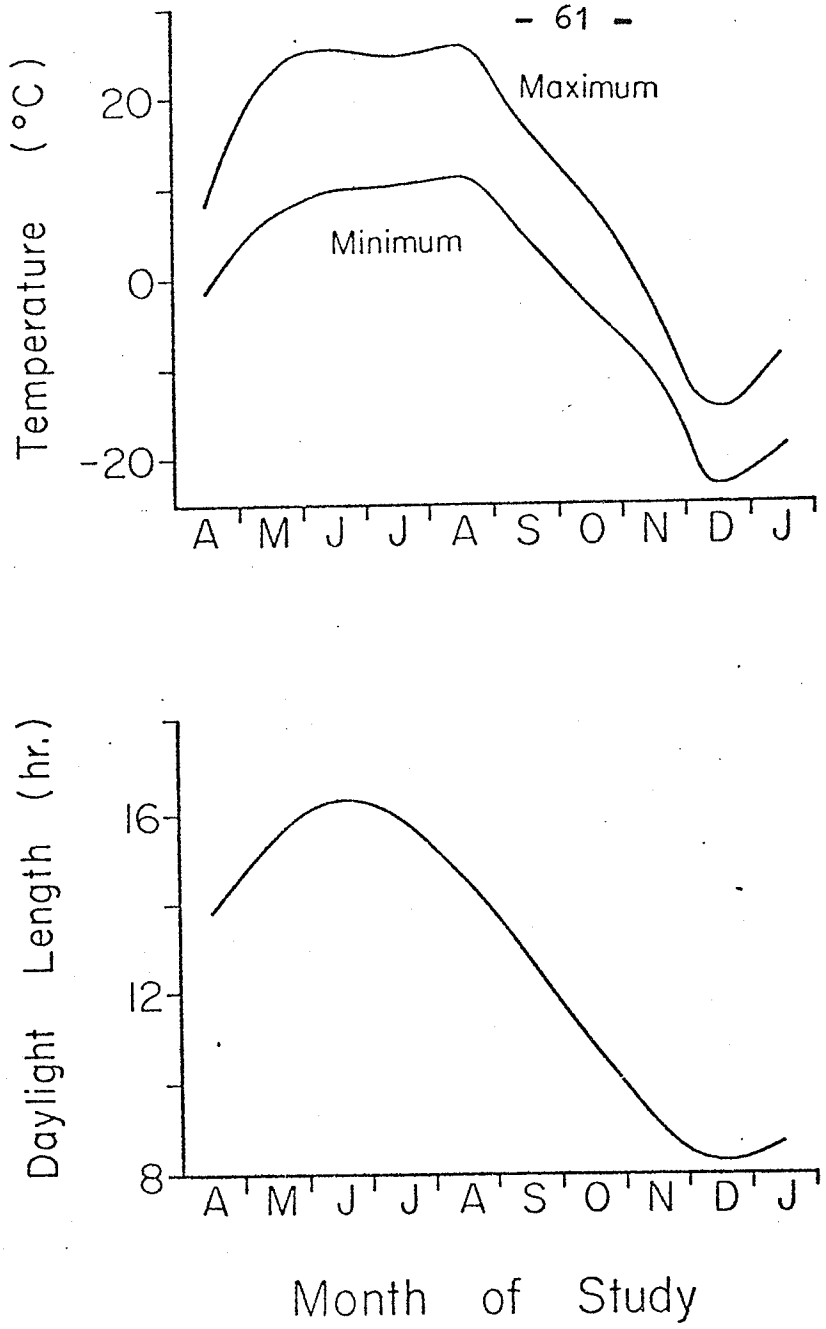

FIGURE 8. Mean monthly maximum and minimum temperature and daylength for Winnipeg, Manitoba (lat. 49°54' N, long. 97° 15' W).

increases in T could not be detected. It has been shown however, that increases in the mean LH level (Johnson et al., 1973) and number of LH releases (Katongole et al., 1974) accompany the high levels of T observed in the ram during the breeding season.

The considerable variation in the LH level observed among rams during May through July could have been due to an effect of aging. The younger rams increased in age during this period from 90 to 180 days. A similar observation has been reported by Crim and Geschwind (1972a) for 90 to 120 day old rams. Although the effects of age and season are confounded in this experiment, the observations that the highest T levels occurred at a time later than the expected attainment of puberty and that T levels dropped in December and January (the oldest age studied) suggests that differences in T were not a result of differing ages.

Summary

Six crossbred ram lambs (initially 3 to 4 months of age) were sampled at 2 to 3 week intervals from late-April, 1972, to mid-January, 1973. A number of comparatively large fluctuations of serum LH were observed in the rams from May through July. Whereas mean LH levels were observed to be comparatively low and fairly constant throughout the breeding season, serum T levels increased four fold from mid-August through September and remained elevated for 2 months. Throughout December and January, both the T level and to a lesser extent the LH level decreased markedly.

Experiment 5. Effect of Elevated Ambient Temperature on Blood LH and Testosterone Levels in the Yearling Ram

Rams exposed to the natural environmental conditions characteristic of southern Manitoba appear to exhibit pronounced alterations in the secretory patterns (Experiment 1) and mean levels (Experiment 4) of blood LH and T. Both photoperiod (Johnson et al., 1973) and ambient temperature (Gomes et al., 1971) have been implicated as modifiers of Leydig cell function. The present experiment was designed to determine the effect of continuous exposure to elevated ambient temperature on mean blood levels of LH and T in the ram. In southern Manitoba, the daytime ambient temperature seldom rises above 32 C during the summer and the relative humidity (RH) is usually less than 50 per cent. Therefore, rams were continuously exposed to an ambient temperature of 32 C and 50 per cent RH for 18 days, during which time blood levels of LH and T were monitored.

Experimental Plan

Four Finnish Landrace rams (yearlings) were housed in an environmental chamber (Cold Stream, Flemming and Pedlar Ltd., approximately 6 feet wide x 10 feet long x 9 feet high) for about one month. Rams were placed in the chamber in mid-May, 2 weeks following being shorn. The length of the photoperiod was 12 hours of light (between 08.30 and 20.30 hours) per day for the duration of the experimental period. The RH was kept constant at 50 per cent. Rams were allowed one week to become adapted to the surroundings and following this blood samples were collected twice daily (09.00 and 17.00 hours) for 27 consecutive days. The temperature was kept constant at 21.1 C for the first nine days and was then elevated to 32.2 C for days 10 through 27.

While in the chamber, rams were periodically (days 9, 13 20 and 27) introduced to an estrual ewe, at which time an approximate estimate of libido was attempted. This was done by recording the time interval from introduction of the ewe until mating had occurred.

Results

Because of the episodic nature of LH release and T secretion in the ram, it was felt that determination of LH and T in only two blood samplings per day probably would not have accurately reflected the mean levels of these hormones in individual animals. Therefore, the determinations of the individual samples were averaged for each of three 9 day periods (control and two treated) for each ram. This was done in order to more accurately assess the changes in mean levels for the rams.

Mean levels ($\pm$ SE) of serum LH (1.33 $\pm$ 0.32 ng/ml) and T (2.56 $\pm$ 0.63 ng/ml) observed during the control period did not appear to be altered appreciably due to prolonged exposure of the rams to 32.2 C (Table 3). Although quantitative measurements were not taken, the elevated temperatures did appear to increase the respiration rate and greatly decrease the amount of physical activity (standing, walking and fighting). Appetite, however, seemed to be unaffected. Regardless of the number of days housed at 32.2 C (4, 11 or 18 days), rams were observed to have bred the estrual ewe when given the opportunity within a 1 minute period following from three to five mounts. This was similar to their performance when allowed to breed at the end of the control period (day 9). In spite of constant mean levels of LH and T throughout the experimental period, one of the rams showed no interest in the ewe during the first two breeding periods.

TABLE 3.

Mean Serum LH and T Levels (ng/ml) in Yearling Finnish Landrace Rams when Continuously Exposed to Ambient Temperatures of either 21.1 C or 32.2 C and 50% Relative Humidity

	Period		
	Days 1-9 21.1 C	Days 10-18 32.2 C	Days 19-27 32.2 C
LH	1.33 ± 0.32[a]	1.73 ± 0.61	1.69 ± 0.32
T	2.56 ± 0.63	2.12 ± 0.18	2.75 ± 0.22

[a] each value represents the mean ± SE for the 4 rams.
individual ram values equal the mean of 18 observations (2 per day).

Discussion

It was rather surprising to find that T levels were not depressed in the rams after 18 days of constant exposure to 32.2 C. On the contrary, Gomes et al. (1971) observed that T levels in spermatic venous plasma of rams previously housed at 28 to 32 C for 14 days were only 25 per cent that of control animals which had been exposed to Ohio spring environmental conditions. Perhaps differences of breed, fleece length, or percentage of RH the animals were exposed to could account in part for the discrepancy between our results and these previous findings.

Although heat treatment markedly reduced the amount of physical activity and seemed to increase the respiration rate slightly, it did not noticeably alter blood levels of LH and T, sexual behavior or appetite. This tends to suggest that the rams were probably not excessively heat stressed. The length of fleece on the rams was initially less than one-fourth of an inch and, in addition, the RH was kept at a low level (50%). These two factors may have tended to alleviate the effects of the high temperature. Lindsay (1969) observed that the rectal temperature of Dorset Horn, Merino and Border Leicester rams was not elevated when animals were exposed to an ambient temperature of 32.2 C for one week. He also observed that libido was not adversely affected.

Results of this study would seem to suggest that, at least for the recently shorn Finnish Landrace ram, fluctuation of the ambient temperature during the summer months within the normal range for southern Manitoba does not adversely effect T secretion. Therefore, the dramatic increase in the blood T level which occurs in rams of this region at the onset of the normal breeding season (mid-August through September), as indicated by Experiments 4

and 6, is most likely prompted by decreases in the photoperiod rather than because of decreases in the ambient temperature.

Summary

Four yearling Finnish Landrace rams were exposed to an ambient temperature of 21.1 C for 9 days and 32.2 C for an additional 18 days while housed in an environmental chamber. During this time, the relative humidity was kept at 50 per cent and the photoperiod was 12 hours of light per day. Mean blood levels of LH and T for the rams during each of the three 9 day periods were observed to be similar. Likewise, the time required for rams to breed an estrual ewe did not appear to change, regardless of the number of days (0, 4, 11 or 18) previously exposed to the elevated temperatures.

Experiment 6. Seasonal Variation of the Secretory Patterns of LH and Testosterone, Libido and Semen Quality in the Mature Ram

Results of previous experiments strongly suggest that levels of LH and T in peripheral blood of rams fluctuates rhythmically on a yearly cycle (Experiments 1 and 4). This is in agreement with other findings (Katongole et al., 1974; Purvis et al., 1974). However, the precise nature of the changes in the secretory patterns of these two hormones, which may take place at the onset of the breeding season, has not been investigated. Intense breeding activity appears to result in temporary increases in blood levels of both LH and T (Experiment 3). It may be however, that this observed endocrine response to breeding is dependent upon the status of LH and T secretion in the ram immediately prior to treatment. Libido (Pepelko and Clegg, 1965; Lindsay and Ellsmore, 1968) and semen quality (Amir and Volcani, 1965; Fowler, 1965; Skinner and van Heerden, 1971; Jackson and Williams, 1973), the maintenance of which is androgen dependent (Moule et al., 1966; Davidson, 1972; Knight, 1973), changes considerably throughout the year. It seems, therefore, that seasonal modifications in reproductive function in the ram are associated with concomitant changes in the blood levels of LH and T.

In the following investigation, the secretory patterns of LH and T were determined at regular intervals for rams of high (Finnish Landrace) and low (Line-M) prolificacy breed types, prior to and during the normal breeding season. In the University flock, Finnish Landrace ewes had been averaging 2.5 to 3 lambs per ewe, whereas the Line-M ewes were averaging approximately 1.5 lambs per ewe. In addition, changes in; 1) libido, 2) semen quality, and 3)

LH and T levels with breeding were observed throughout the same period.

Experimental Plan

Seasonal changes in reproductive function were observed for four Finnish Landrace and four Line-M rams (two yearlings and two aged animals from each breed). The secretory patterns of LH and T were determined for the rams in early-August, mid-September, early-November and mid-December, 1973. Blood samples were collected at 20 minute intervals for an 8 hour period (starting at 09.00 h) while the rams were penned in groups of two in an enclosed area of the barn. One week following each of these non-breeding (control) periods, rams were individually penned with an estrual ewe for an 8 hour period. Blood samples were again collected at 20 minute intervals (starting at 09.00 h). Rams were allowed to breed at will during the 8 hours and a record of number of mountings and breedings was kept.

Single ejaculates were collected from either three or four of the Finnish Landrace rams and from either two or three of the Line-M rams one week following each of the above breeding periods. An assessment of ejaculate volume (ml) and spermatozoan motility (percentage exhibiting active progressive movement) were made within 2 to 4 minutes following collection. Estimation of concentration of spermatozoa (billions per ml) was made 2 to 3 hours later, upon arrival at the laboratory. Aliquots of seminal plasma (0.1 ml) were also assayed for T content.

Results

Serum LH Levels

The mean serum LH level for rams of each breed during

control and breeding periods are given in Table 4. Analysis of variance revealed a significant ($P<0.05$) period x treatment x breed interaction. Although mean LH levels during the control periods tended to decrease with time, reaching a low in November, levels first increased slightly in September in the Finnish Landrace rams. Breeding activity appeared to elevate the mean LH level above control values only in the Finnish Landrace rams in August. During all other breeding periods, and for both breeds, breeding was associated with a slight decrease in the mean level of LH.

The number of LH releases which occurred per 8 hours appeared to increase with time. The greatest frequency was observed in November and December (Table 5). Breeding appeared to trigger additional LH releases in August and September, although this effect was not demonstrated in November and December (significant period x treatment interaction, $P<0.01$). The magnitude of the LH peaks tended to steadily decrease from August through November (Table 6). In September, however, the peaks which occurred during the control period were considerably higher in the Finnish Landrace rams as compared to the Line-M rams. In general, the magnitude of the LH releases which occurred during the breeding periods tended to be lower than those observed during the control periods. In August and December, however, releases were slightly higher during the breeding periods in the Finnish Landrace and Line-M rams, respectively. A significant period x treatment x breed interaction ($P<0.01$) was observed.

Serum T Levels

The mean level of serum T was observed to have increased dramatically from August on, reaching its highest point in November (Table 7). The breeding periods were

TABLE 4.

Mean Serum LH Level (ng/ml) in the Rams During the Control and Breeding Periods

Breed	Treatment	Period Aug	Sept	Nov	Dec
Finn	control	2.37[a]	3.05	1.70	2.04
	breeding	5.22	2.45	1.12	1.70
Line-M	control	3.78	2.12	1.33	1.61
	breeding	3.48	1.74	1.04	1.57

significant period x treatment x breed interaction ($P < 0.05$)

[a] each value represents the mean for 4 rams (pooled SE $\pm$ 0.44)
individual ram estimates are the mean of 24 observations

TABLE 5.

Mean Number of LH Releases Occurring within Rams During the Control and Breeding Periods

Treatment	Period			
	Aug	Sept	Nov	Dec
Control	2.1[a]	3.1	5.3	5.4
Breeding	3.6	4.3	4.9	4.6

significant period x treatment interaction ($P < 0.01$)

[a] each value represents the mean for the 8 rams (pooled SE $\pm$ 0.3)

TABLE 6.

Mean Magnitude (ng/ml) of LH Peaks Occurring within Rams During the Control and Breeding Periods

Breed	Treatment	Period Aug	Sept	Nov	Dec
Finn	control	14.30[a]	12.22	3.27	4.47
	breeding	15.54	8.16	2.40	3.92
Line-M	control	13.72	5.77	2.39	2.99
	breeding	10.18	3.57	1.92	3.44

significant period x treatment x breed interaction ($P<0.01$)

[a] each value represents the mean for 4 rams (pooled SE $\pm$ 0.51)

TABLE 7.

Mean Serum T Level (ng/ml) in the Rams During the Control and Breeding Periods

Treatment	Period			
	Aug	Sept	Nov	Dec
Control	3.53[a]	8.52	17.35	13.68
Breeding	5.93	12.28	16.72	12.52

significant period x treatment interaction ($P < 0.01$)

[a] each value represents the mean for the 8 rams (pooled SE $\pm$ 0.73)
individual ram estimates are the mean of 24 observations

associated with substantial increases in the mean level of T in August and September, but slight decreases occurred in November and December (significant period x treatment interaction, $P<0.01$). During each control and breeding period, increases in blood T were consistently observed to follow LH releases within 40 to 60 minutes. Throughout the course of this experiment, T peaks were observed to be significantly higher ($P<0.05$) in the Finnish Landrace rams (21.64 ± 1.69 ng/ml) as compared to the Line-M rams (14.79 ± 1.69 ng/ml). The magnitude of the T peaks steadily increased from August to November, when maximum levels were observed (Table 8). In September, breeding was associated with T peaks which were of substantially greater magnitude than were those for the comparable control periods, although this did not occur during the other sampling periods (significant period x treatment interaction, $P<0.01$).

The secretory patterns of LH and T observed during the 8 hour control and breeding periods in August and November are presented for one of the Finnish Landrace rams (#1) in Figures 9 and 10, respectively. The alterations in the secretory patterns that occurred in this animal were also observed in the other seven rams.

Libido

Based on the number of breedings per 8 hours, libido increased dramatically throughout the experimental period (250%). Breeding performance reached its peak in November (21.5 ± 1.5 breedings per 8 hours) and remained high during December. There appeared to be a close association between the mean level of serum T and libido (Figure 11), although this does not necessarily imply that a cause and effect relationship existed. Breeding performance for the two breeds was similar throughout the duration of this study.

TABLE 8.

Mean Magnitude (ng/ml) of T Peaks Occurring within Rams During the Control and Breeding Periods

Treatment	Period			
	Aug	Sept	Nov	Dec
Control	9.35[a]	15.47	25.08	21.03
Breeding	10.04	19.97	25.08	19.74

significant period x treatment interaction ($P<0.01$)

[a] each value represents the mean for the 8 rams (pooled SE $\pm$ 0.77)

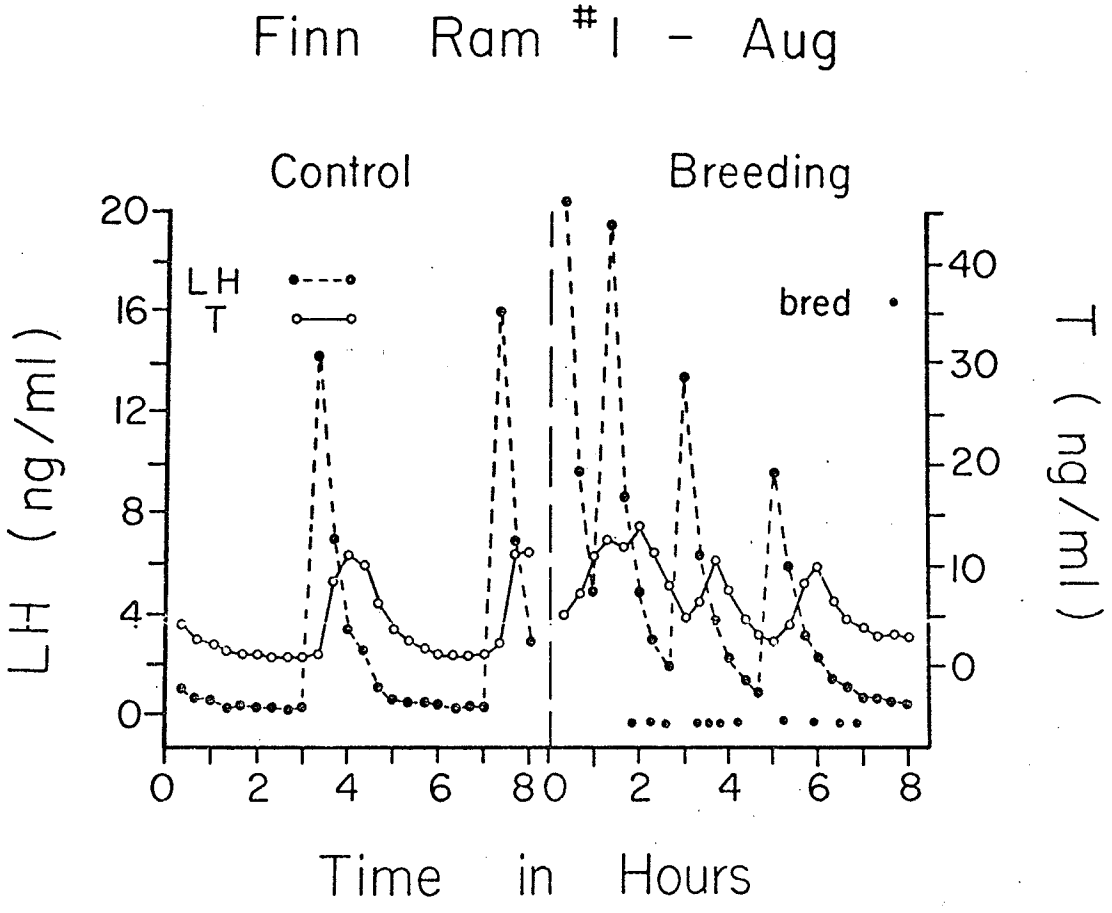

FIGURE 9. Secretory patterns of serum LH and T in Finnish Landrace ram #1, bled in August by jugular venipuncture every 20 minutes for an 8 hour control (penned alone) and breeding (penned with an estrual ewe) period. (•) represents a breeding.

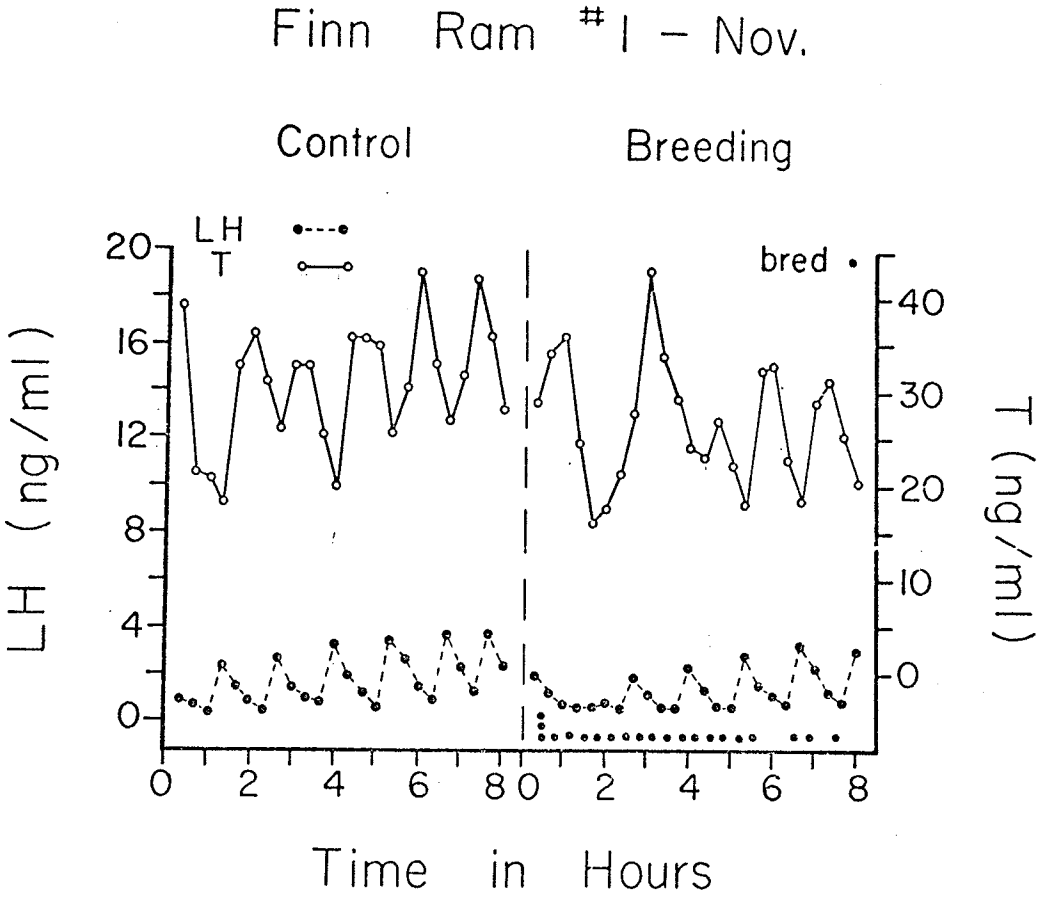

FIGURE 10. Secretory patterns of serum LH and T in Finnish Landrace ram #1, bled in November by jugular venipuncture every 20 minutes for an 8 hour control (penned alone) and breeding (penned with an estrual ewe) period. (●) represents a breeding.

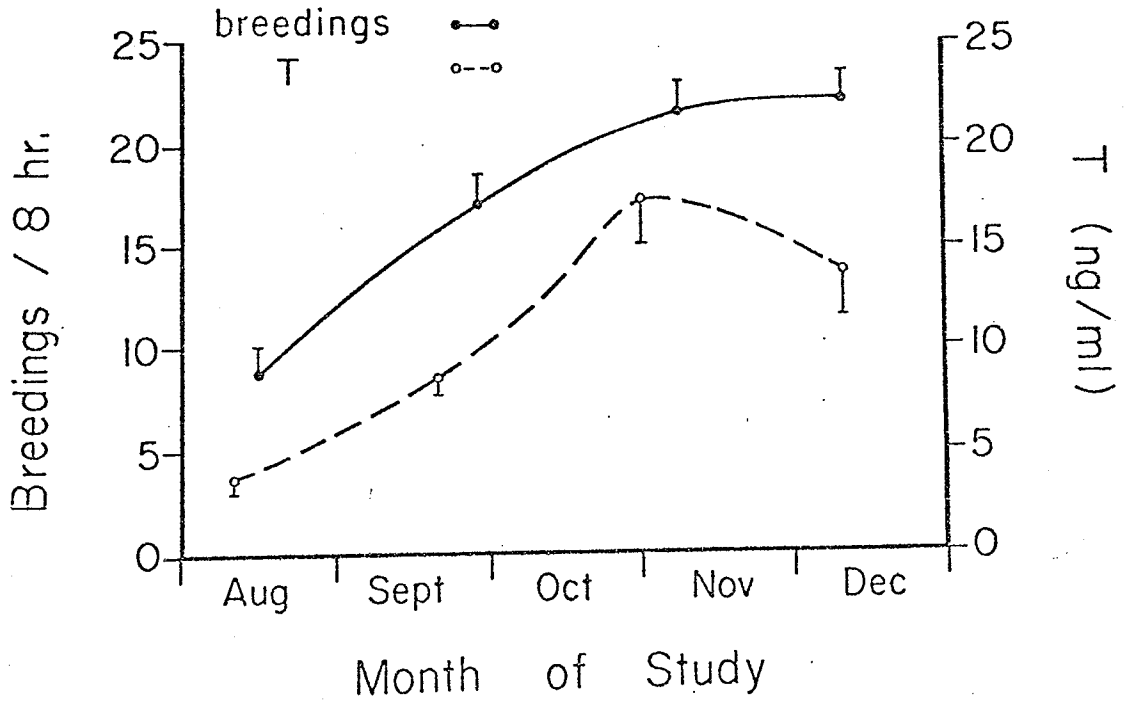

FIGURE 11. Mean level of serum T observed during the control periods and number of breedings per 8 hours observed 1 week later during the breeding periods for the eight rams. The vertical bars represent the standard error of the mean.

One of the Line-M rams, however, did not mount or breed during the first two breeding periods.

Semen Quality

There was a gradual improvement in all measures of semen quality throughout the experimental period (Table 9). The mean ejaculate volume tended to be higher during September through December than in August, although the greatest volumes were observed in November for the Finnish Landrace rams (0.8 ± 0.1 ml) and in December for the Line-M rams (1.7 ± 0.3 ml). Throughout the duration of this experiment, ejaculate volume averaged 40 per cent less for the Finnish Landrace rams as compared to the Line-M rams. The concentration of spermatozoa per ml of ejaculate was similar for both breeds throughout the experimental period and was substantially higher in ejaculates collected in December. The percentage of motile spermatozoa in the ejaculate was estimated to be the highest in November and December. Percentages tended to be higher for Finnish Landrace ejaculates in November (72 ± 6%) and for Line-M ejaculates in December (85 ± 2%). Other measures of semen quality, such as the total number of spermatozoa per ejaculate and the total number of motile spermatozoa per ejaculate tended to give optimum values in December. Due to the larger ejaculate volume and greater proportion of motile spermatozoa (up to 25%) in the ejaculate, both of these measurements were appreciably higher for the Line-M rams in November and December.

The mean levels of T found in seminal plasma samples are given in Figure 12. It appeared that on the average, the levels of T in the ejaculates remained fairly constant throughout the experimental period, in spite of definite increases in the mean level of serum T. However, there was a great deal of variation among and within rams. Values ranged from 1.44 to 10.20 ng T/ml of seminal plasma.

TABLE 9.

Quality of Semen Collected from Rams One Week Following the Breeding Periods

	Periods			
	Aug	Sept	Nov	Dec
Number of Rams	5	6	6	5
Ejaculate volume (ml)	0.7 ± 0.1[a]	0.9 ± 0.2	1.0 ± 0.1	1.0 ± 0.3
Sperm conc./ml (x 10^9)	3.53 ± 0.40	3.26 ± 0.36	3.74 ± 0.41	6.15 ± 0.46
Sperm/ejaculate (x 10^9)	2.49 ± 0.36	3.10 ± 0.88	3.88 ± 0.56	6.20 ± 2.13
Motile sperm (%)	64.0 ± 2.9	69.2 ± 2.4	74.2 ± 3.5	74.0 ± 5.1
Motile sperm/ejaculate (x 10^9)	1.58 ± 0.22	2.19 ± 0.64	2.86 ± 0.42	4.94 ± 1.91

[a] each value represents the mean ± SE

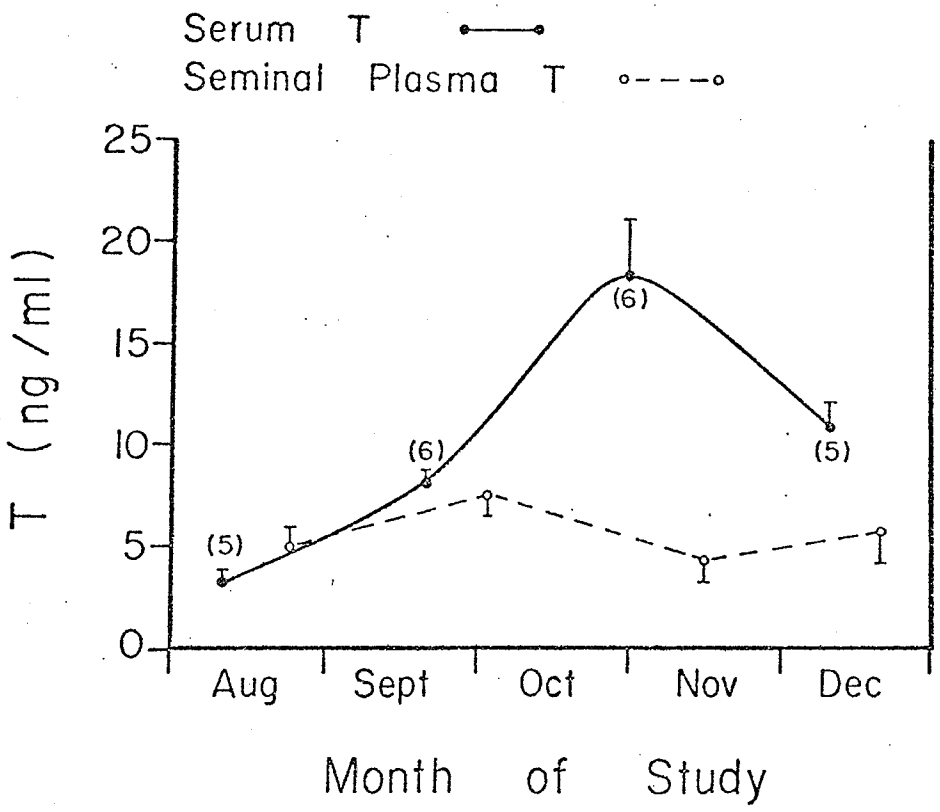

FIGURE 12. Mean level of T in the serum during the control periods and in seminal plasma 2 weeks later for either five or six of the rams. The vertical bars represent the standard error of each mean.

Discussion

Pronounced seasonal alterations in the release of LH and secretion of T were observed in the rams. The pattern of LH release changed noticeably as the breeding season progressed; fluctuations of comparatively lower magnitude tended to occur with increased frequency. As a consequence, mean blood levels of LH tended to decline, reaching their lowest levels in November. Similar changes in the pattern of LH release with season have been observed in two rams during earlier studies (Experiment 1). A number of other workers have reported different findings. Johnson et al. (1973) observed that mean serum LH levels in mature rams were at their highest in October, and then gradually decreased during the subsequent months, reaching a low in May. Katongole et al. (1974) noted that LH levels were extremely variable, but remained within the same range, in three Suffolk rams sampled at weekly intervals throughout the year. However, LH releases were observed to occur more frequently in the one ram sampled (hourly) in October as compared to the other two rams sampled in May. Peak levels of LH within the three animals were observed to be of similar magnitude. The discrepancy between our results and those of others could be due in part to differences in sampling frequency and numbers of rams observed. A more accurate assessment of the pattern of LH release is possible with a 20 minute sampling interval, as opposed to the hourly, daily, weekly or bi-monthly samplings employed in the above studies.

Mean levels of blood T in the rams rose very quickly from August on, the highest mean T levels being observed in November. This trend is in agreement with the findings of Johnson et al. (1973). They noted that T synthesis and secretion in rams exposed to natural environmental

conditions appeared to be at its maximum in October. Similarly, Katongole et al. (1974) reported that mean T levels in rams increased from August through November. In the present study, it was noted that the frequency and magnitude of the T fluctuations increased steadily during the fall and early winter months. Purvis et al. (1974) observed decreases in both the frequency and magnitude of T peaks in rams sampled in March and May, as compared to January.

Johnson et al. (1973) observed yearly rhythms in the mean levels of LH and T in the ram. Because increases and decreases in the mean levels of these hormones occurred concurrently, it may be concluded that the T cycle was primarily regulated by the LH cycle. Katongole et al. (1974) noted that LH levels tended to be constant throughout the year, even during periods when T levels were comparatively high. This led to the suggestion that the sensitivity of the hypothalamus to steroid feedback may be changing during the year, the sensitivity to T inhibition increasing during the summer and decreasing during the winter. However, in the present study, blood levels of LH were observed to gradually decrease as the T levels markedly increased during August to November. This suggests that T may be acting via a negative feedback effect to modify the pattern of LH release, the end result of which is a lowering of the mean level. This contention is supported by the observations that in the intact ram, LH release is normally partially suppressed by circulating T (Hochereau-de Reviers and Pelletier, 1971; Short, 1972), and that injections of T (Bolt, 1971) or TP (Pelletier, 1970) are capable of suppressing LH release for up to a number of days. In addition, the pituitary has been shown (Pelletier, 1973b) to be less responsive to LH-RH when castrate male sheep were pretreated with TP.

Intensive breeding activity appeared to prompt additional LH releases which in turn elevated the mean blood LH and (or) T levels slightly above control levels at the onset of the breeding season (August and September), but not during the subsequent months. Breeding activity was not associated with an increase in the frequency of LH releases at a time of the year when the number of spontaneous releases was at its maximum, perhaps suggesting that an upper limit of frequency of releases had already been achieved. It is of interest to note that intensive breeding activity in early-January was also associated with temporary (for approximately 12 hours) increases in the blood levels of LH and T (Experiment 3). However, neither observation of estrual ewes, mounting without intromission and single breedings (Experiment 2), nor breeding for 1 hour (Purvis et al., 1974) appears to alter the levels of LH and T in the ram.

The rams exhibited a marked improvement in libido throughout the first 3 months of the experimental period, which was associated with considerable increases in the blood levels of T. Although it would be tempting to attribute the improved breeding performance to the higher levels of T, the effect of ambient temperature cannot be excluded. Lindsay (1969) has shown that exposure of rams to elevated ambient temperature (1 week at 37 C) reduces breeding activity. The ambient temperature decreased considerably during the course of this study and therefore may have been an influencing factor. In addition, rams may have become more accustomed to the breeding area and to our presence with time, and therefore may have become less reluctant to breed during each of the subsequent breeding periods.

Testosterone has been demonstrated to be the most effective androgen for inducing sexual behavior in castrate

male rats (Beyer et al., 1973; Luttge and Hall, 1973). The daily dose of T required to maintain normal copulatory behavior in castrates has been demonstrated to be only 50 per cent of that estimated to be the physiological replacement dose, i.e. that which will maintain the accessory sex glands (Davidson, 1972). This implies that a wide safety margin exists in the hormonal control of reproductive behavior in the male rat. Similarly, reproductive behavior in the ram may not be affected by a marked decrease in blood T, providing a minimum level is always maintained. It is known that TP injections will not increase the libido of sexually active rams (Knight, 1973) or bulls (Macmillan, 1971). In view of these observations, it is difficult to determine what affect the level of blood T had on the breeding performance of the rams at any given time in the present study. The observation made by Pepelko and Clegg (1965) that libido of rams decreases during the winter months in spite of temperatures which are conducive for breeding implies that the decreases in blood T which are probably occurring at this time are adversely affecting libido. The apparent close association observed between blood T levels and breeding performance for rams in the present study also tends to suggest that low levels of libido in the ram during the non-breeding season are in part attributable to low T levels.

One of the Line-M rams would not breed or even exhibit interest in the estrual ewes during the first two breeding periods. During these periods, the blood T level in this animal was similar to that of the other rams which were sexually active. Sexually inhibited rams, i.e. those that refuse to mate estrual ewes after repeated, short-interval exposure (Hulet et al., 1964), have been shown to exhibit concentrations of seminal fructose (Knight, 1973) and semen quality, when collected by electroejaculation (Hulet et al.,

1964), which is comparable to that of sexually active rams. These observations suggest that low T levels are probably not the cause of sexual inactivity in sexually inhibited rams.

The quality of semen collected from the rams showed a gradual improvement over the experimental period and was most favorable during November and December, a period when blood T levels were also relatively high. During August and September, gradual increases were observed in the volume of the ejaculate and the number of spermatozoa per ejaculate, both of which are thought to be positively influenced by androgen (Moule et al., 1966; Steinberger, 1971) and by periods of decreasing daylength (Cupps et al., 1960; Fowler, 1965; Skinner and van Heerden, 1971; Jackson and Williams, 1973). A 10 per cent increase in the proportion of motile spermatozoa was observed by November and was probably attributable to the rams being exposed to more favorable environmental temperatures, as temperatures above 32 C during a 2 week period prior to the onset of this study were not uncommon. Exposure of rams to ambient temperatures above 32 C for a few hours has been shown to adversely affect spermatozoan motility (Howarth, 1969; Rathore, 1970a; Smith, 1971), although exposure to increased photoperiod (long days) is also known to be detrimental (Fowler, 1965).

Biological fluids within the male reproductive tract are known to contain appreciable amounts of T and other androgens. Rete testis fluid in particular contains very high levels of T (Ganjam and Amann, 1973; Harris, 1973; Setchell, 1974), levels which are known to be as high as those in the internal spermatic vein (Waites and Setchell, 1969) and which have been shown to be 6 to 8 fold higher than T levels in peripheral blood. Testosterone-binding protein, also found in rete testis fluid (French and Ritzen,

1973a; French and Ritzen 1973b) is thought to facilitate the entry of T into the fluid and its transport within the tract. Testosterone is taken up quickly by the seminiferous tubules (Setchell, 1974) and therefore may aid in maintaining spermatogenesis (Steinberger, 1971). The metabolism of testicular spermatozoa is known to be influenced by T under *in vitro* conditions (Voglmayr *et al.*, 1970; White *et al.*, 1971). Both epididymal function (Waites and Setchell, 1969) and the maintenance of the accessory sex glands (Skinner and Rowson, 1968) are thought to be partially dependent upon the presence of T in the fluid which passes through the male reproductive tract. Testosterone has been reported to be present in human (Oertel and Treiber, 1968; White and Hudson, 1968), bull (Ganjam and Amann, 1973; White and Hudson, 1968) and ram (White and Hudson, 1968) semen, although at levels substantially lower than in rete testis or epididymal fluid.

In the present study, levels of T in the seminal plasma were observed to be relatively constant throughout the experimental period (5.70 ± 0.60 ng/ml). Similar levels of T have been reported in the seminal plasma of the bull (4.60 ± 0.50 ng/ml) by White and Hudson (1968). In spite of marked increases in blood T in the rams during August through November, only a slight rise in seminal plasma T (from 4.91 ± 0.99 ng/ml to 7.43 ± 0.97 ng/ml) was observed in September, followed by a return to lower levels (4.72 ± 0.91 ng/ml and 5.74 ± 1.48 ng/ml) during November and December. These results would at first appear to suggest that the levels of T within the male reproductive tract remain fairly constant throughout the year. However, it is known that the T level in rete testis fluid normally decreases as it passes through the epididymis (White and Hudson, 1968; Ganjam and Amann, 1973), perhaps due to absorption and (or) utilization (French and Ritzen, 1973b).

It may be that the rate of disappearance of T in the epididymis changes with season. Considerable variation in the seminal plasma T levels was also noted among rams. For example, values ranged from 3.84 to 10.20 ng/ml for the six rams collected in September. This may suggest that T levels in semen vary considerably over time and that estimates of T in aliquots of seminal plasma from a number of ejaculates are necessary in order to accurately determine the mean level at a given time. White and Hudson (1968) have shown that the T level in semen collected from bulls was approximately 40 per cent lower in the second ejaculate as compared to the first.

The higher level of natural fertility which is characteristic of the Finnish Landrace breed seems to be attributable to increased fecundity of the ewe. Land (1970a) demonstrated that Finnish Landrace ewes had longer estrual periods and a greater number of lambs than British breeds. Ewes of the Finnish Landrace breed were observed by Bradford et al. (1971) to ovulate more than twice as many ova per ewe as compared to ewes of other breeds in response to a superovulatory dose of pregnant mare serum gonadotropin. Finnish Landrace rams do not appear to have been capable of influencing the reproductive performance of ewes of British hill breeds to which they were mated (Barker and Land, 1970). When compared with Border Leicester rams, the range of litter sizes, mean litter size and percentage of ewes lambing were similar for rams of both breeds.

It was noted in this experiment that during each of the breeding periods, the Line-M rams on the average, bred the estrual ewes as frequently as did the Finnish Landrace rams. Similarly, Land (1970b) observed that over a 19 month period it was not possible to differentiate between the ability of Finnish Landrace and Scottish Blackface rams

(initially 9 months of age) to mate ewes presented to them once they had become sexually active, although at the onset of the experiment Finnish Landrace ram lambs mated ewes more frequently than the Blackface ram lambs. In general, the quality of the ejaculates (ejaculate volume, percentage of motile spermatozoa, spermatozoa per ejaculate) collected from the Finnish Landrace rams appeared to be less favorable than those from the Line-M rams. Neither differences in motility, density nor proportion of live spermatozoa could be found between ejaculates collected from Scottish Blackface and Finnish Landrace rams by Land (1970b). Results of this study further suggest that the high level of prolificacy observed for the Finnish Landrace breed as compared to other breeds is not due to improved reproductive function in the male.

Summary

A number of measures of reproductive function were observed for eight mature rams (four Finnish Landrace and four Line-M) on four occasions (early-August, mid-September, early-November and mid-December). On each occasion, blood samples were collected from the rams at 20 minute intervals for two 8 hour periods spaced one week apart, one while penned without (control) and one while penned with (breeding) an estrual ewe. One week following the breeding periods, a semen sample was collected from each of five or six of the rams.

Numerous alterations were noted in the secretory patterns of LH and T from August to November. Both the mean level of blood LH and the magnitude of the LH releases decreased, whereas the frequency of LH releases gradually increased. Contrariwise, the mean level of serum T and the magnitude of the T peaks increased during this period, and

the greater number of LH releases resulted in the occurence of additional T fluctuations. Breeding activity tended to elevate blood LH above control levels in August, whereas, it resulted in increases in the mean level of blood T in August and September.

Libido increased markedly from August through November and remained at a high level during December. Likewise, the quality of the ejaculates collected in November and December was superior to those collected in August and September in terms of volume, concentration of spermatozoa and percentage of motile cells. The level of T in the seminal plasma did not appear to fluctuate with season, as did the blood T levels, although considerable variation among and between rams was observed.

Experiments 7 and 8. Effect of Age and Breed on Blood LH and Testosterone Levels in the Ram

Marked increases in pituitary LH are known to occur in the ram prior to puberty (Skinner et al., 1968; Courot et al., 1972). Similarly, levels of plasma LH have been observed to steadily increase in ram lambs from birth to 3 to 4 months of age, at which time large fluctuations may be observed (Crim and Geschwind, 1972a; Thimonier and Pelletier, 1972). T levels in testicular tissue (Skinner et al., 1968) and the secretion rate of T (Crim and Geschwind, 1972b) appear to rise substantially throughout the first year of life in the ram. Nothing is known, however, about the secretory patterns of LH and T in the prepuberal ram and how it compares with that for the yearling or aged ram. Recently, differences in pituitary and blood levels of LH have been noted between prepuberal ram lambs from breeds or flocks of varying fertility (Land et al., 1972; Thimonier and Pelletier, 1972; Bindon, 1973; Land et al., 1973; Bindon and Turner, 1974), which suggests that there may be differences in the secretory patterns of LH and T in these animals. Two experiments were conducted to further investigate these areas.

Experimental Plan

Experiment 7

In mid-May, a total of 28 rams were blood sampled at 20 minute intervals for an 8 hour period (starting at 09.00 h). From eight to ten rams were sampled per day. Sampling took place on three consecutive days. Finnish Landrace (F), Line-M (L) and Suffolk (S) rams were used in this experiment. The ram population was arbitrarily broken down into three age groups; eight lambs 2 to 7 months of

age (one F, two L and three S), twelve yearlings 14 to 15 months of age (three F, six L and three S) and eight aged 25 to 62 months of age (five F, two L and one S). An estimate of the mean serum level of LH and T was made for each ram by pooling the 24 samples collected and assaying an aliquot. However, individual samples were assayed for each of the three yearling and five aged Finnish Landrace rams.

Experiment 8

In late-October, twelve prepuberal ram lambs (six Finnish Landrace and six crossbred) ranging in age from 74 to 92 days were weighed and then penned in two groups. Blood samples were collected over an 8 hour period (starting at 10.00 h) at 20 minute intervals from eight of the rams, and at hourly intervals from the remaining four rams. Serum pools were made from the samples collected at hourly intervals for all rams for purposes of estimating mean levels of LH and T. The individual samples collected from eight of the rams at 20 minute intervals were also assayed for LH and T.

Results

Experiment 7

The mean levels of LH and T for the rams in each of the three age groups are given in Table 10. The highest levels of LH were observed in the ram lambs, which were assumed to be either prepuberal or nearing puberty. LH levels tended to be lower in the yearling rams. Aged rams exhibited levels which were significantly ($P < 0.05$) lower than those of either the lambs or yearlings. The highest mean T levels were observed in the yearlings, although levels in the aged rams tended to be only slightly lower.

TABLE 10.

Mean Serum LH and T Levels (ng/ml) in Various Age Groups of Rams

Age Range (mo)	Number of Rams	LH	T
2-7	8	2.06 ± 0.38^{a}	1.31 ± 0.18^{a}
14-15	12	1.14 ± 0.17^{a}	2.75 ± 0.41^{b}
25-60	8	0.48 ± 0.13^{b}	2.38 ± 0.25^{ab}

means $\pm$ SE not followed by the same letter are significantly different ($P<0.05$)

The T level was significantly lower ($P < 0.05$) in the lambs than in the yearlings. The difference between levels in the aged rams and lambs closely approached significance ($P < 0.05$).

Although the number of animals in each age group was small, the secretory patterns of LH and T appeared to be markedly different between the yearling and aged Finnish Landrace rams (Table 11). The yearling rams exhibited both higher mean levels of serum LH and a greater number of comparatively larger LH releases. As a consequence, the number of elevations of T was greater in these rams. Baseline levels of LH and T however, were similar in all rams.

Experiment 8

The crossbred and Finnish Landrace rams sampled in this experiment were of comparable age and weight (Table 12). Both the mean levels of LH ($P < 0.10$) and T ($P < 0.05$) were observed to be significantly lower in the crossbred rams as compared to the Finnish Landrace rams. The secretory patterns of LH and T for an 8 hour period are displayed in Figure 13 for four prepuberal (two Finnish Landrace and two crossbred) rams which were 3 months of age or less. Within each of the four rams, LH releases (one to three) were observed during the 8 hour period and ranged in magnitude from 6.25 to 12.00 ng/ml. In most instances, LH releases were followed within 60 minutes by obvious, well defined T peaks. The magnitude of these peaks varied a great deal from ram to ram. Of the eight rams frequently sampled, one of the Finnish Landrace and two of the crossbreds showed no LH releases during the 8 hour period.

TABLE 11.

Characteristics of the Secretory Patterns for Serum LH and T in Finnish Landrace Rams of Various Ages in May

	Age Groups		
	14 mo	26-34 mo	54 mo
Number of rams	3	3	2
LH Level			
mean (ng/ml)	1.37 ± 0.63[a]	0.72 ± 0.25	0.37 ± 0.01
baseline (ng/ml)	0.44 ± 0.09	0.56 ± 0.14	0.37 ± 0.01
LH Peaks			
rams exhibiting	2	2	0
number/8 h	2.5	1	-
magnitude (ng/ml)	6.14 ± 0.69	3.73 ± 1.22	-
T Level			
mean (ng/ml)	3.03 ± 1.07	2.72 ± 0.82	2.57 ± 0.48
baseline (ng/ml)	0.96 ± 0.19	1.50 ± 0.32	1.13 ± 0.28
T Peaks			
rams exhibiting	2	2	1
number/8 h	2.5	1	1
magnitude (ng/ml)	8.98 ± 0.34	8.79 ± 2.70	6.75

[a] mean ± SE

TABLE 12.

Mean Age, Weight and Serum LH and T Levels in Prepuberal Crossbred and Finnish Landrace Rams in October

	Breed	
	Cross	Finn
Number of rams	6	6
Mean age (day)	84.3 ± 1.8[a]	86.7 ± 2.5
Mean weight (kg)	17.4 ± 2.8	20.7 ± 2.7
LH level (ng/ml)[b]	0.43 ± 0.22	1.02 ± 0.19
T level (ng/ml)[c]	0.37 ± 0.14	2.82 ± 0.86

[a] each value represents the mean ± SE

[b] significant LH level ($P<0.10$)

[c] significant T level ($P<0.05$)

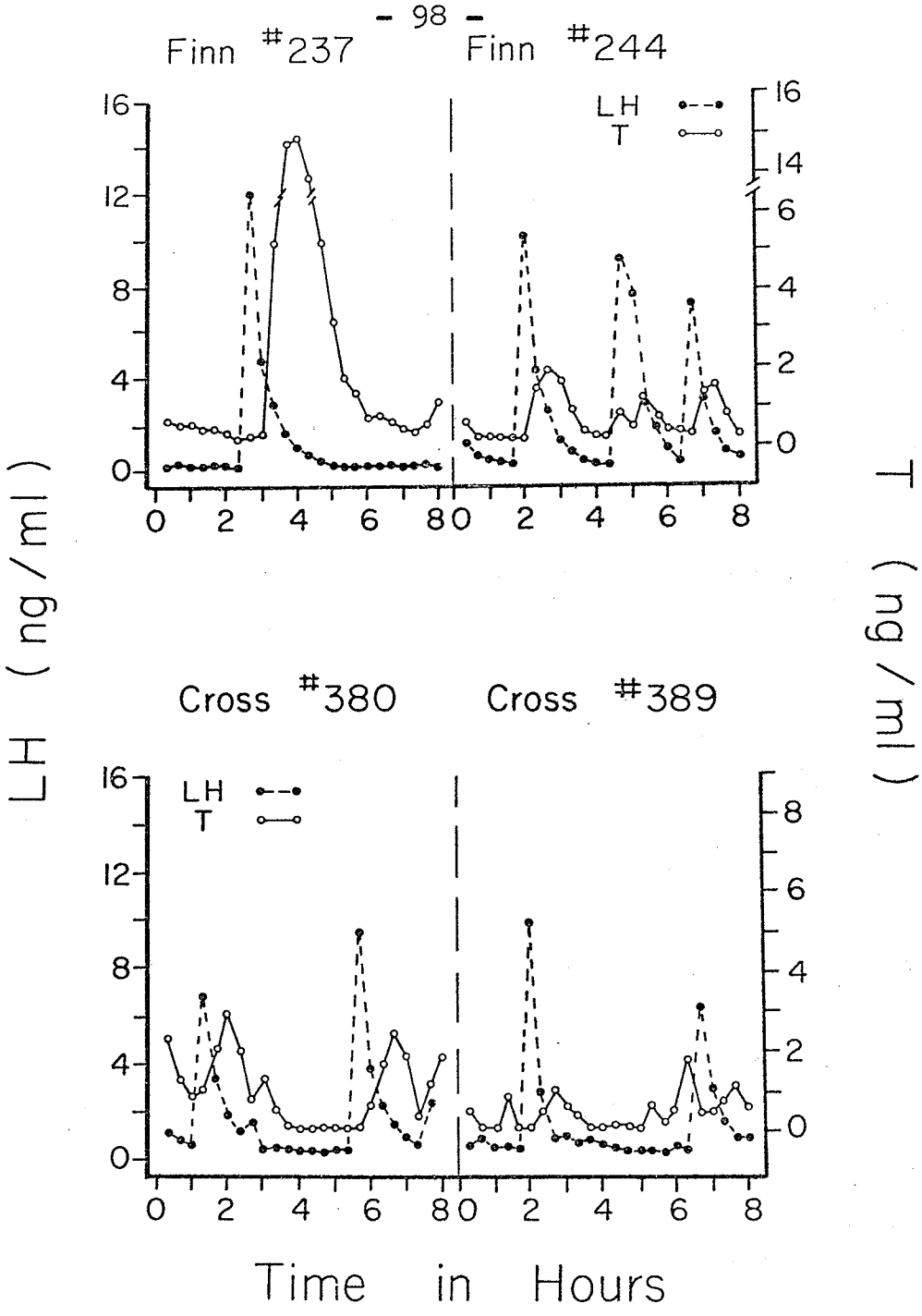

FIGURE 13. Secretory patterns of serum LH and T in four 3 month old rams, bled by jugular venipuncture every 20 minutes for 8 hours in October.

Discussion

The highest mean levels of LH were found in the ram lambs. Of the 28 rams sampled, the highest values were observed in the six youngest rams (2 to 3 months of age), mean levels ranging from 1.65 to 3.75 ng/ml. Similar blood LH values have been reported by Thimonier and Pelletier (1972) and Courot et al. (1972) for ram lambs of this age. Mean LH levels tended to decrease progressively with increasing age. This may have been due to a reduction in the frequency and (or) magnitude of LH releases. The observation that LH releases tended to be of greater frequency and magnitude in yearling as compared to aged Finnish Landrace rams supports this contention.

It would appear that in prepuberal rams the comparatively greater mean levels and number of releases of LH are only transient, occurring prior to and during the early stages of puberty. This was suggested by the observation in the present study that ram lambs 6 to 7 months of age exhibited much lower mean LH levels than did 2 to 3 month old rams. Similarly, Crim and Geschwind (1972a) found that LH values tended to be elevated in some ram lambs at 90 and 120 days of age, but were consistently low in lambs 30, 60 and 150 days of age. Courot et al. (1972) observed high levels of LH in the peripheral blood of rams prior to the onset of puberty and rapid testicular growth. The total content of LH in the pituitary of rams has been shown to steadily increase from birth to 3 months of age, following which there is a marked decline (implying increased release) which is coincident with increases in the testicular growth rate (Skinner et al., 1968). Pituitary LH content was then found to rise again sharply between 4 and 5 months of age.

The magnitude of LH releases in response to LH-RH

seems to be age dependent. Up to 60 days of age, the pituitary in the ram appears to be less responsive to LH-RH (Galloway and Pelletier, 1974). Galloway and Pelletier (1974) suggested that the increased magnitude of the LH release observed in response to LH-RH after 60 days of age may be due to the increased pituitary LH content observed by Skinner et al. (1968) for rams 2 months of age and older. It may be that the pattern of LH release which occurs in ram lambs between approximately 2 and 6 months of age is modified during subsequent months due to the presence of increasing levels of circulating T. It has been suggested that the magnitude and frequency of LH releases from the pituitary are related to the level of T circulating in the blood (Bolt, 1971; Pelletier, 1970; Pelletier, 1973b; Debeljuk et al., 1974). As expected, blood T levels in the prepuberal lambs were comparatively lower than those in the yearling and aged rams. Because this study was conducted at a time of the year (May) when blood T levels are normally low (Experiment 1), the relative differences between the age groups were not very pronounced.

Crim and Geschwind (1972b) reported a highly significant correlation between the secretion rate of T and the age of the ram. A less obvious relationship was observed between plasma T concentration and age. In the present study, blood T levels in May were observed to be significantly ($P < 0.05$) higher in the yearling rams as compared to the ram lambs. Testosterone levels in the aged rams were on the average slightly lower than in the yearlings, perhaps due to the occurrence of fewer LH releases and subsequent elevations of T, and not significantly different from T levels in the lambs. Mean T levels for the twelve prepuberal rams (74 to 92 days of age) sampled in October were observed to be 1.59 ± 0.54 ng/ml. When sampled at approximately the same time of the

year, mean levels of T were shown to average 12.03 ± 1.64 ng/ml in six ram lambs 8 to 9 months of age (Experiment 4), and 17.35 ± 2.26 ng/ml in eight yearling and aged rams (Experiment 6). In addition, no differences in the T level were detectible between four aged rams (28 to 30 months of age) and four yearling rams (16 to 18 months of age) when sampled frequently from early-August to mid-December (Experiment 6). These observations imply that differences in the blood T level do exist, and that the T levels gradually increase from prepuberal to adult levels within the first 12 to 18 months of life.

Ewes from high fertility breeds have been shown to have lower pituitary (day 10 of the estrous cycle) and higher plasma levels of LH, as compared to ewes from low fertility breeds (Land et al., 1972; Land et al., 1973). Similarly, prepuberal ram lambs (30 days of age) from either high fertility breeds (Thimonier and Pelletier, 1972) or flocks (Bindon, 1973; Bindon and Turner, 1974) tend to exhibit higher levels of blood LH as compared to animals from low fertility breeds and flocks. Rams born as triplets or twins also demonstrate higher LH levels than do singles. Results of the present study are in agreement with these observations. Finnish Landrace rams on the average exhibited significantly higher levels of blood LH and T at approximately 3 months of age, when compared to crossbred rams from ewes of considerably lower fertility. The mean levels of both hormones tended to be higher in the Finnish Landrace group, perhaps in part due to a larger proportion of the animals exhibiting LH releases and elevations of T. Similarly, Bindon and Turner (1974) found that a higher percentage of 30 day old rams from a high fertility flock exhibited elevations of blood LH as compared to rams from a low fertility flock.

Of interest in the present study was the observation

that the secretory patterns of LH and T in the prepuberal rams were strikingly similar to those in mature rams. LH releases were noted to occur at regular intervals within animals and to prompt elevations in blood T within 60 minutes, although the elevations were comparatively small in most rams.

Summary

A number of aged (eight), yearling (twelve) and ram lambs (eight) were blood sampled in May for an 8 hour period at 20 minute intervals. Finnish Landrace, Suffolk and Line-M rams were represented in the three age groups. Lambs were observed to have had the highest mean serum LH level, whereas the yearling and aged rams showed progressively lower levels. Serum T levels were highest in the yearling and aged rams. Observation of the secretory patterns of LH and T for the yearling and aged Finnish Landrace rams revealed that the younger rams exhibited higher mean levels of LH, more frequent and larger LH releases, and more elevations of T per 8 hours.

Prepuberal Finnish Landrace (six) and crossbred (six) rams of comparable age and weight were blood sampled hourly for an 8 hour period in October. The mean serum levels of both LH and T were significantly lower in the crossbred animals. Secretory patterns of LH and T were also observed in a number of the rams which had been sampled at 20 minute intervals. A series of large LH releases followed by comparatively small elevations of T were noted for most of the rams sampled. Apparent temporal and cause and effect relationships between these two hormones were found to exist in these rams.

CONCLUSIONS

(1) LH releases from the pituitary in the ram appear to occur quickly as peak levels are achieved within 20 minutes. They also occur spontaneously and in an episodic fashion at regular intervals within each animal.

(2) Elevations in blood LH stimulate the synthesis and (or) secretion of T from the Leydig cells of the testis, as evidenced by the rapid increase in blood T, reaching peak levels within 60 minutes, following an LH release.

(3) The cause and effect, and temporal relationships which exist between LH and T consistently occur irrespective of the frequency of discharges of LH from the pituitary.

(4) The magnitude of the LH release does not appear to consistently relate to the magnitude of the subsequent T elevation.

(5) Exposure of rams to mild forms of sexual stimulation such as single breedings, mounts without intromission, and observation of estrual ewes does not consistently elevate the levels of blood LH and T.

(6) Intense breeding activity at the onset (August and September) and conclusion (January) of the normal breeding season appears to be associated with modifications of the secretory patterns of LH and T. Temporary rises in the mean levels of LH and T occur during these periods due to increases in the frequency and magnitude of LH releases and number of elevations of T.

(7) Mean levels of LH and T fluctuate dramatically in spring born ram lambs during their first year of life. Large fluctuations in the LH level during the summer months (between 3 to 6 months of age) subside as the breeding season approaches, a time which is coincident with the onset of marked elevations in blood T and decreasing photoperiod and ambient temperature.

(8) The ambient temperatures and relative humidity which are characteristic of southern Manitoba during the summer months do not appear to adversely affect the mean levels of blood T in the ram, suggesting that seasonal changes are due mainly to alterations in the photoperiod (either periods of decreasing daylength and/or long days).

(9) During the late summer to early winter months (August to December), a number of gradual changes in reproductive function occur within the ram.

(a) There are progressive modifications in the pattern of LH release. The frequency of release increases while both the mean and peak levels of LH in peripheral blood decrease, perhaps in response to rising mean levels of blood T which were due to the occurence of more frequent and larger elevations.

(b) Marked increases in libido and semen production occur concurrently with rapidly rising levels of blood T, lending further evidence to the contention that these functions are androgen dependent.

(c) Although marked increases in blood T occurred, levels of T in the seminal plasma appeared to remain constant.

(10) Mean blood LH levels are higher in prepuberal rams between 2 and 3 months of age as compared to either yearling or aged rams, while the reverse is true for T levels. This observation supports the hypothesis that elevations in blood T and testicular growth noticeable at the time of puberty are not associated with early high levels of LH in the prepuberal ram.

(11) A greater proportion of yearling rams, as

compared to aged rams, exhibited a greater number of comparatively larger LH releases over time, which may explain the higher mean levels of LH observed within this age group.

(12) Levels of LH and T characteristic of the adult ram seemed to be achieved by 12 to 18 months of age.

(13) The secretory patterns of LH and T in prepuberal rams were similar to those of mature rams. A series of comparatively large LH releases followed closely by comparatively smaller elevations in blood T occured.

(14) Between breeds of varying fertility, differences in the mean level of LH and T were observed in prepuberal rams, and differences in the magnitude of the T peaks were observed in mature rams.

(15) In order to accurately assess the secretory patterns of LH and T in the ram, sampling intervals of 20 minutes or less must be employed.

APPENDIX

Experiment 1A. LH Release in the Castrate Male Sheep

The pattern of LH release from the pituitary following castration of male (Foster et al., 1972; Osland et al., 1972; Riggs and Malven, 1972; Riggs and Malven, 1974) and female (Reeves et al., 1972; Diekman and Malven, 1973) sheep has been shown to be considerably different from that of intact animals. The LH level in the peripheral blood of rams increases approximately two fold within 24 hours following castration (Short, 1972). A characteristic pattern of LH release (frequent, spontaneous releases which occur at regular intervals) is established. In the following experiment, the pattern of LH release was observed during a 24 hour period in two groups of castrate male sheep, those which had been castrated either 3 weeks or 5 months prior to the sampling period.

Experimental Plan

Four castrate male sheep (crossbreds) were bled at 20 minute intervals for a 24 hour period in June, starting at 09.00 hours. Animals were penned together and given 48 hours to become accustomed to the new surroundings before sampling began. Two of the castrates (#2327D and #2334D) were 6 months of age at the time of the experiment and had been castrated shortly after birth (long-term). The other two castrates (#1399C and #1400C) were 17 months of age. They had been castrated 3 weeks prior to the sampling period (short-term). Serum samples were assayed for LH as in the previous experiments with one exception. Anti-ovine LH serum #573 was used in the assay and at an initial dilution of 1:100,000.

Results

The characterization of LH release during the 24 hour period in the two long-term castrates is presented in Figure 1A, and in the two short-term castrates in Figure 2A. A series of frequent, spontaneous LH releases were observed throughout the 24 hour period in all animals. Based on visual observation of the plotted data, LH releases were thought to occur at the frequency of about one per hour. However, when the data was analyzed for possible rhythmic periodicity using the MINCOS computer program (designed to find the best fitting cosine function for the data), it became apparent that the releases did not occur in a consistent rhythmic fashion at intervals of from 50 minutes to 4 hours. The mean level of LH was found to be 30 per cent higher in the long-term castrates (14.70 ng/ml) as compared to the short-term castrates (11.27 ng/ml). There also appeared to be slightly more variability associated with the mean level of LH in the preceding group. The coefficient of variation averaged $\pm$ 18.5 per cent for the long-term castrates and $\pm$ 14.2 per cent for the short-term castrates.

Discussion

The fluctuating pattern in the level of LH which was observed in peripheral blood of castrate male sheep was similar to that reported by Osland *et al.* (1972) and Riggs and Malven (1974). The mean level of LH and the pattern of release, particularly for the short-term castrates, appeared to be very similar to that of ewes 50 days post ovariectomy when sampled for 24 hours at 15 minute intervals (Reeves *et al.*, 1972). Osland *et al.* (1972) noted the occurrence of hourly (circhoral) oscillations in the

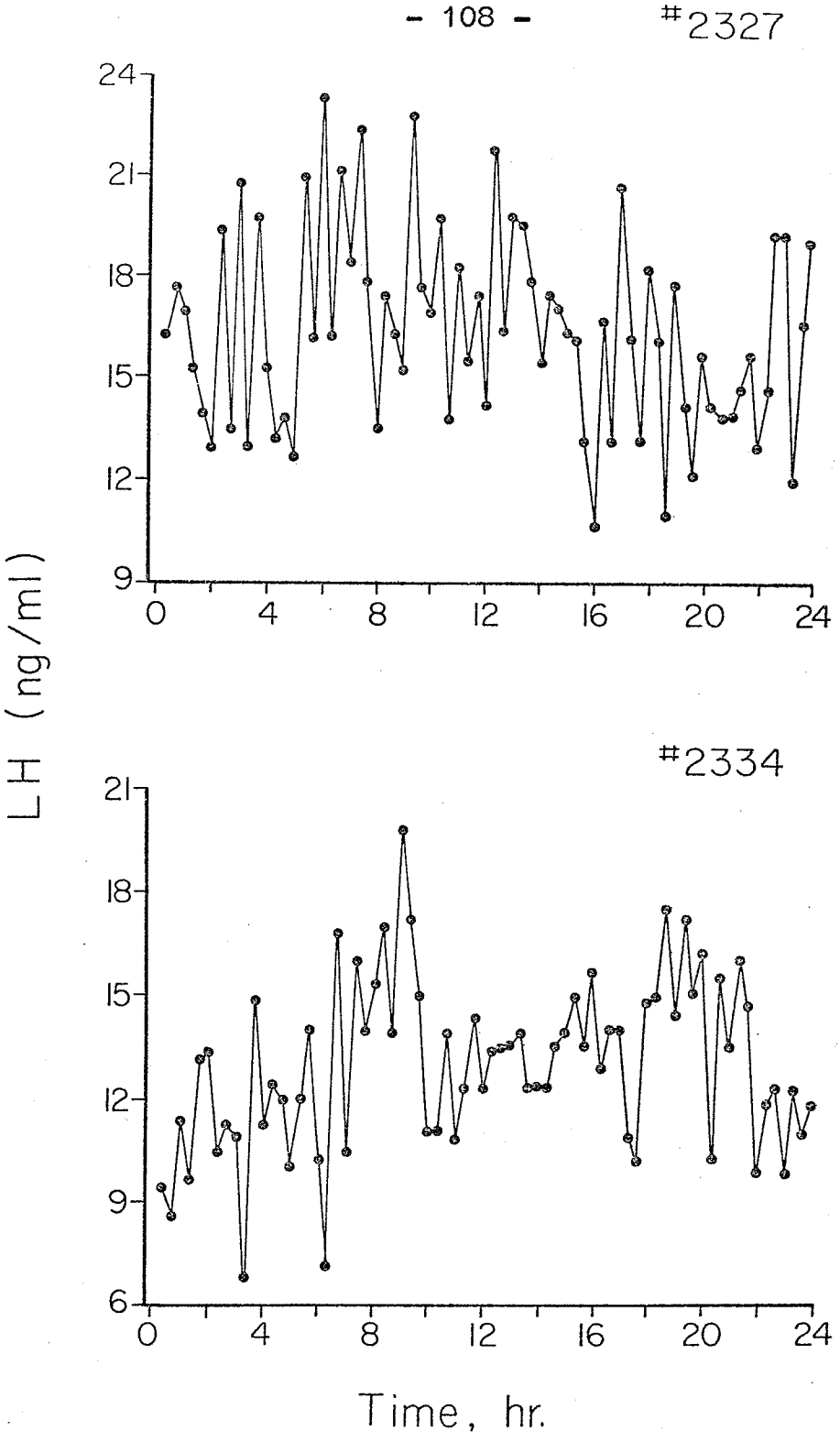

FIGURE 1A. Characterization of LH release in two 6 month old castrate (at birth) male sheep, bled by jugular venipuncture every 20 minutes for 24 hours in June.

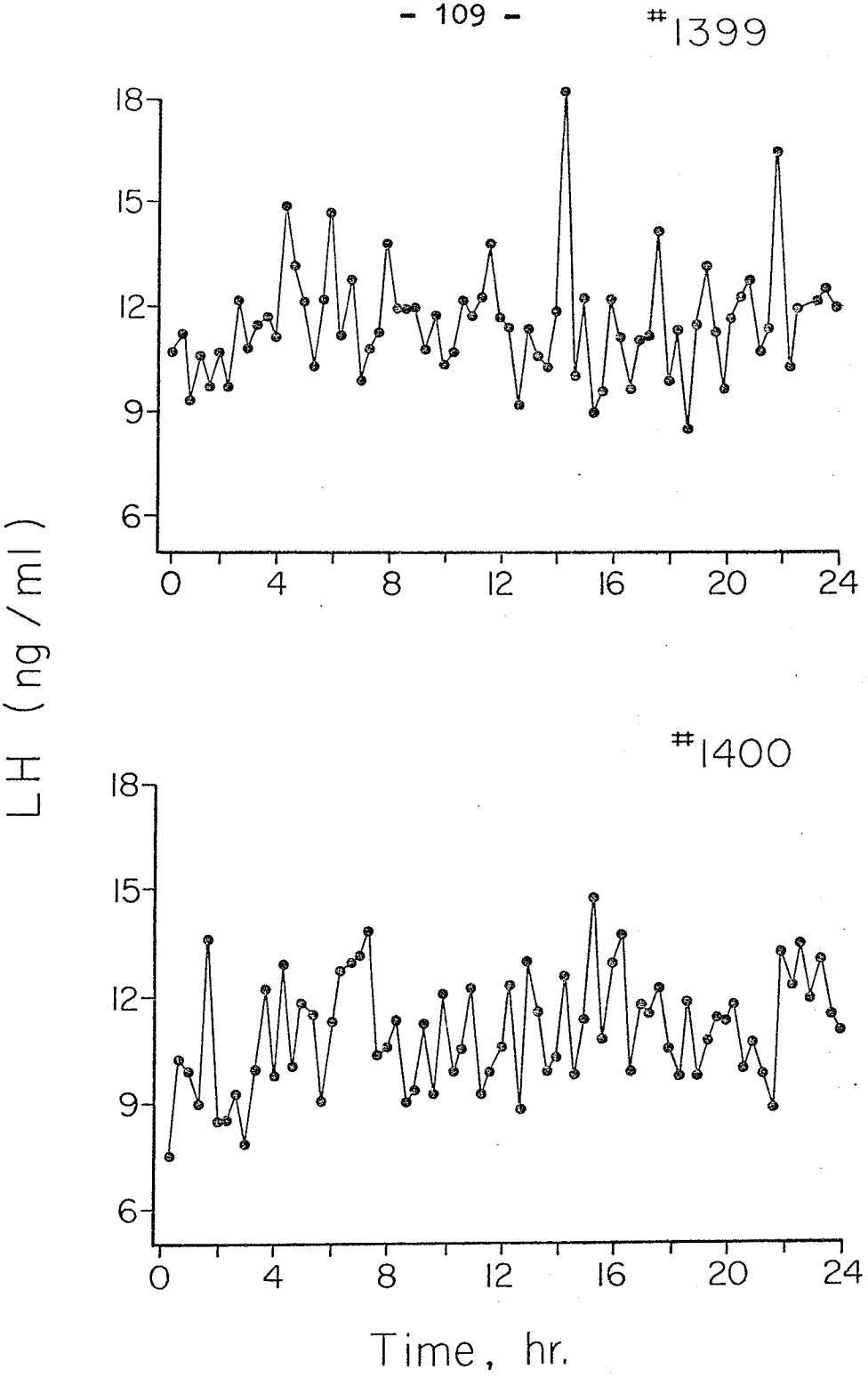

FIGURE 2A. Characterization of LH release in two 16 month old castrate (at 15 months) male sheep, bled by jugular venipuncture every 20 minutes for 24 hours in June.

blood level of LH in mature castrate male sheep sampled at 20 minute intervals for 2 hour periods, but noticeable daily (circadian) oscillations were not observed. Although the data obtained in the present study were not analyzed to detect possible circadian rhythms, visual observation revealed that obvious rhythmical circadian fluctuations were not present. The occurrence of successive rhythmic peaks at approximately 30 minute intervals (an estimated 39 pulsatile discharges per 24 hours) were observed by Riggs and Malven (1974) in young castrate male sheep sampled at 10 minute intervals for 4 to 6 hours. When the data was subjected to computer analysis, a rhythmical pattern of LH release was detected. Failure to detect a similar rhythm following computer analysis of our data was probably due to the comparatively longer sampling interval (20 minutes) employed in this study. It would appear that the mean level of LH and the greater variability associated with the mean level was greater in the long-term castrates as compared to the short-term castrates. These differences may have been due to either differences in the length of time animals were exposed to high levels of blood T and (or) the age of the animals.

Summary

Two long-term (castrated for 5 months) and two short-term (castrated for 3 weeks) male sheep were blood sampled at 20 minute intervals for 24 hours in June. Spontaneous LH releases occurred within each animal at a frequency of about 1 per hour on the average. However, the presence of a consistent rhythmic release pattern (of 50 minutes to 4 hours) could not be established following computer analysis. LH levels in the long-term castrates were 30 per cent higher and considerably more variable than in the short-term castrates.

Experiment 2A. Effect of Testosterone and Dihydrotestosterone Injection on LH Release in the Castrate Male Sheep

The maintenance of reproductive function in the male seems to be dependent upon the action of many different biologically active androgens. This has been implicated by a number of recent studies. Sexual behavior in the castrate male rat has been shown to be more effectively reinstated by T as compared to either DHT or androstenedione (McDonald et al., 1970; Luttge and Hall, 1973; Beyer et al., 1973). Testosterone has also been observed to be more effective than DHT in maintaining the weight of the seminal vesicles in castrate rats (McDonald et al., 1970; Naftolin and Feder, 1973; Zanisi et al., 1973a), although both of these hormones appear to maintain the weight of the prostate to the same extent (McDonald et al., 1970; Swerdloff et al., 1972; Naftolin and Feder, 1973). Zanisi et al. (1973b) has demonstrated that 5α-androstane-3α,17β-diol (a reduction product of DHT) has a greater influence on the growth of the accessory sex glands in immature castrated male rats than does DHT. LH release from the pituitary appears to be inhibited equally as well (Naftolin and Feder, 1973) or to a greater extent (Swerdloff et al., 1972; Zanisi et al., 1973a; Zanisi et al., 1973b) following administration of DHT as compared to T. The objective of the present investigation was to determine the effectiveness of DHT and T in lowering the level of serum LH in castrate male sheep.

Experimental Plan

Eight 21 month old castrate (for 6 months) male sheep were randomly assigned to four groups of two in October. Each of the two animals were then given injections of

either 2 or 6 mg of T or DHT (in 1.0 ml ethanol) directly into the jugular vein at 4 hour intervals for 60 hours (total of 16 injections per animal). Blood was collected from all animals at 20 minute intervals for 1 hour (four collections) prior to the first, fourth, tenth and sixteenth injections, and, for 2 hours (six collections) following the fourth, tenth and sixteenth injections. Sampling took place during hours 1, 13 through 15, 37 through 39 and 61 through 63 of the experimental period.

All samples obtained from the animals were assayed for LH as in the previous experiments, with the exception that anti-ovine LH serum #573 was used and at an initial dilution of 1:100,000. Aliquots of serum samples from the T treated animals were assayed for T. DHT levels were determined in the samples collected from animals given DHT injections using the procedure employed for determining T, with the exception that DHT standards (0 to 500 pg) were assayed with the unknowns instead of T standards.

Results

Following T injections, peak levels in the blood were observed within 20 minutes and averaged ($\pm$ SE) 26.29 $\pm$ 5.04 ng/ml in castrates injected with 2 mg, and greater than 36.00 ng/ml in castrates injected with 6 mg. Similarly, peak levels of DHT were observed within 20 minutes following the 2 mg (4.95 $\pm$ 0.40 ng/ml) and 6 mg (12.20 $\pm$ 0.94 ng/ml) injections, and were noted to be of considerably smaller magnitude than were the T peaks. Irrespective of the previous number of injections, levels of both T (following the 2 mg dose) and DHT (following the 2 and 6 mg doses) had usually returned to baseline levels ($<$1.00 ng/ml) within 2 hours. However, T levels were comparatively higher (2.83 $\pm$ 0.86 ng/ml) at the end of 2

TABLE 1A.

Mean Serum LH Expressed as Percentage of Control Level in Castrate Male Sheep During Various Time Intervals Following the Onset of Continuous T or DHT Injections[a] at 4 Hour Intervals

Time Interval	Injection Number	% of Control[b] T	DHT
h 1	pre-1	100.0	100.0
h 13	pre-4	88.7 ± 1.7	92.7 ± 6.2
h 14-15	post-4	96.2 ± 3.6	85.9 ± 6.1
h 36	pre-10	118.5 ± 8.8	86.0 ± 1.8
h 37-38	post-10	115.6 ± 5.5	78.5 ± 2.9
h 60	pre-16	108.6 ± 8.3	88.3 ± 7.6
h 61-62	post-16	102.7 ± 4.5	74.9 ± 3.6

[a] either 2 mg or 6 mg per injection in 1 ml ethanol

[b] significant hormone x period interaction ($P<0.01$)

[c] each value represents the mean ± SE for 4 animals

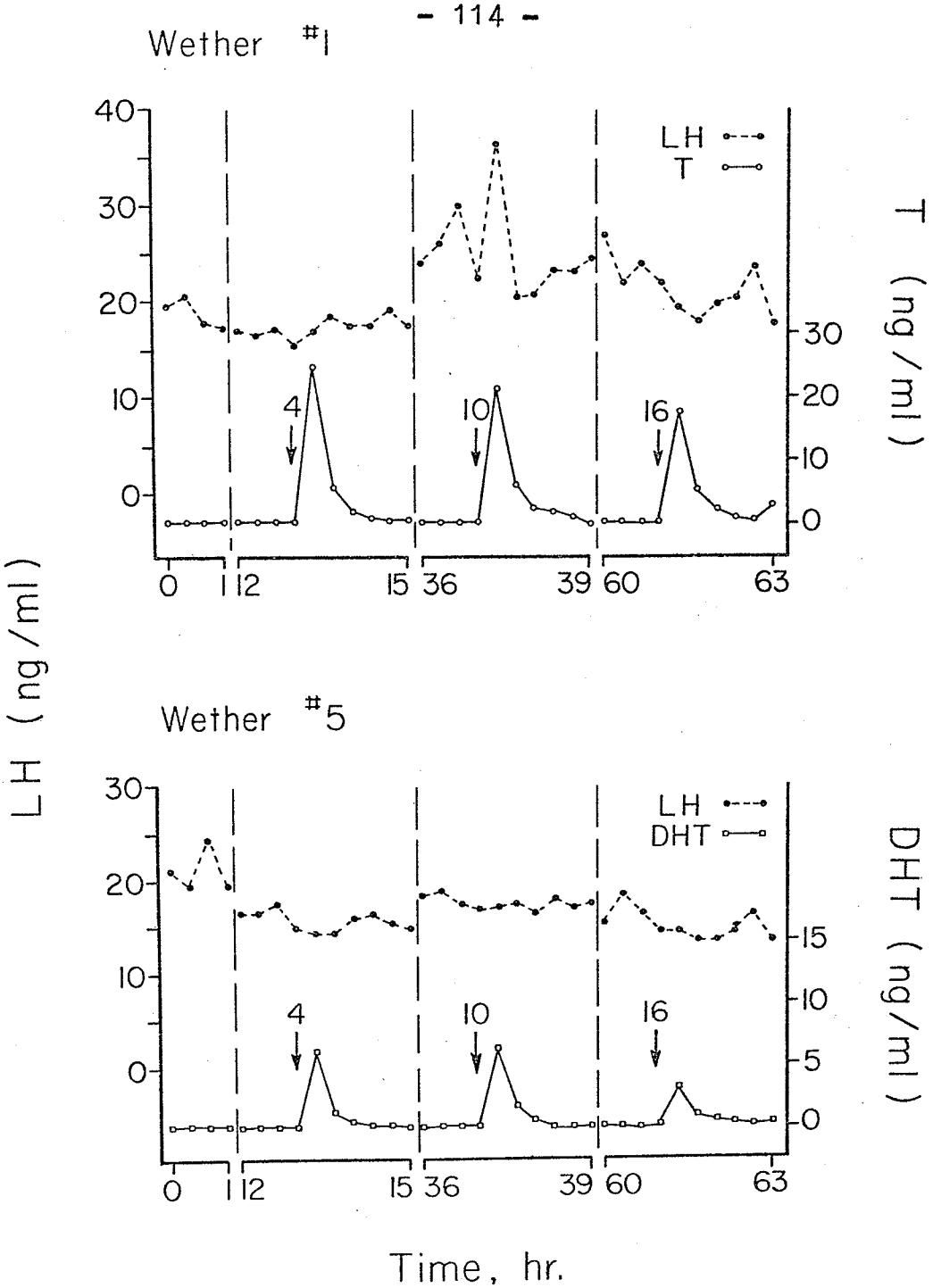

FIGURE 3A. Characterization of LH release and serum T and DHT levels in two 21 month old castrate (at 15 months) male sheep when given IV injections of either 2 mg T or DHT every 4 hours for 60 hours, and bled by jugular venipuncture at 20 minute intervals for 1 hour prior to injections #1,4,10 and 16, and for 2 hours following injections #4, 10 and 16.

hours in animals injected with 6 mg.

For each animal, mean levels of LH were estimated for the pre- and post- injection periods by averaging the individual observations. Because a similar response was observed for all animals treated with the same androgen, regardless of amount injected, data for the four animals in each treatment were combined. Fluctuations from the initial level of serum LH observed during hour 1 (22.1 ± 6.3 ng/ml for the T treated animals, and 17.3 ± 1.9 ng/ml for the DHT treated animals) were expressed as a percentage of the initial level.

The response of the pituitary to T and DHT appeared to be quite different (Table 1A). Although slight depressions in the LH level were observed in both groups by hour 13, animals treated with T exhibited marked elevations in the mean LH level between hours 36 and 38. Concurrently, LH levels continued decreasing in the DHT treated animals. By the end of the experimental period, DHT treatment had depressed LH levels by approximately 25 per cent, whereas, LH levels in the T treated group were observed to be similar to initial values. Analysis of the data revealed a significant ($P < 0.01$) hormone x period interaction.

Individual observations of serum LH, T and DHT are plotted for one representative animal from each treatment group (Figure 3A). Testosterone treatment was associated with the occurrence of LH releases during hours 36 through 39 in animal #1. In animal #5 however, comparable LH releases were not obvious during the same time period. The LH level generally tended to decline throughout the experimental period.

Discussion

Exogenous doses of T are known to suppress the blood

level of LH in the intact ram. Bolt (1971) observed that a 5 mg intramuscular injection suppressed the release of LH in rams for up to 27 hours. Similarly, Pelletier (1970) demonstrated that a pharmacological dose of TP (400 mg injected intramuscularly) significantly depressed LH levels in the castrate (for 1 week) ram for 5 to 7 days. In the present study, the objective was to treat castrate males with physiological doses of two androgens in a manner which would simulate the normal pattern of T secretion in the intact ram. The secretory pattern of T observed in the mature ram in January (Experiment 1) showed the occurrence of six T peaks over a 24 hour period with an average magnitude of approximately 20.00 ng/ml. Peaks as high as 35.00 to 38.00 ng/ml have been observed in some rams in November (Experiment 6). Therefore, 2 and 6 mg doses of androgen and an intravenous route of administration were chosen. This method of treatment elevated blood T to peak levels which were in the physiological range, although the subsequent decreases from peak values were slightly faster and the baseline levels between peaks were substantially lower than normal.

The observation that levels of blood LH were increased considerably in the T treated animals on day 2 of the experiment (hours 36 through 39) is in agreement with the results of Osland et al. (1972). They noted that when mature castrate male sheep were given single injections of either 1 or 10 mg T on each of three consecutive days, periods of significant increases in the LH level were observed by day 2. Likewise, low doses of T have been shown to temporarily increase blood LH levels in castrate male rats (Swerdloff and Walsh, 1973; Bloch et al., 1974). These observations suggest that under certain conditions T exerts a positive feedback influence on the pituitary. However, DHT seems to have the opposite effect as the mean serum LH

levels were observed to be gradually depressed throughout the experimental period in the DHT treated animals. Dihydrotestosterone has also been shown to be more potent than T in suppressing LH levels in the castrate male rat (Swerdloff et al., 1972; Naftolin and Feder, 1973; Zanisi et al., 1973a; Zanisi et al., 1973b).

A number of studies have clearly demonstrated that in the intact male rat circulating T is quickly converted by 5∝-reductase to DHT in a number of androgen-responsive tissues (Williams-Ashman and Reddi, 1972) and that it is the principle steroid retained in the cell nuclei. The conversion of T to DHT is known to take place in the hypothalamus and pituitary (Zanisi et al., 1973b). These observations imply that T may normally be partially suppressing blood LH in the intact male following its conversion to DHT. The concentration of 5∝-reductase in the ventral prostate of the rat is known to decrease following castration (Williams-Ashman and Reddi, 1972). However, Kniewald and Milkovic (1973) have reported that the amount of conversion of T to DHT in the rat pituitary increases following gonadectomy. More work needs to be done in order to determine if the inability of low doses of T to lower serum LH in male sheep and rats following castration is related to changes in either the hypothalamic or pituitary level of 5∝-reductase, and subsequently DHT formation.

Summary

Eight 21 month old castrate (for 6 months) male sheep were treated with either 2 or 6 mg of T (four animals) or DHT (four animals) at 4 hour intervals for 60 hours (16 injections per animal). Regardless of the dose given, all animals treated with a particular androgen exhibited

similar changes in serum LH. In the DHT treated animals, mean levels of serum LH were observed to have gradually declined 25 per cent by the end of the experimental period. However, LH levels were observed to be elevated (17 to 19%) in the T treated animals during hours 36 through 38 of the experimental period. By the conclusion of the experiment, serum LH had decreased to levels similar to those observed prior to treatment.

Experiment 3A. Influence of Triiodothyronine on Blood LH and Testosterone Levels in Ram Lambs

Considerable attention has been devoted to attempting to improve reproductive function in the male by treatment with thyroid hormones. Farris and Colton (1958) demonstrated that a number of men who were subfertile due to poor semen quality became fertile when treated with thyroxine (T_4). Improvements in ejaculate volume and, percentage and number of motile spermatozoa per ejaculate were noted. Jackobovits (1970) claimed to have increased the libido of impotent men by treating them with methyltestosterone and thyroid extract. The 5 month nonreturn rate for dairy cows inseminated with extended bull semen treated with T_4 was observed by Schultze and Davis (1949) to have improved considerably over the rate obtained with untreated semen. Both T_4 and triiodothyronine (T_3) are known to stimulate oxygen uptake (Schultze and Davis, 1949; Gassner and Hopwood, 1955), fructolysis (Casillas and Hoskins, 1970) and cyclic adenosine monophosphate production (Casillas and Hoskins, 1970, 1971) in spermatozoa incubated under <u>in vitro</u> conditions. Libido and spermatogenic function in the ram are thought to be improved during periods of elevated ambient temperature when animals are fed thyroprotein (Reece, 1968). The improvements in reproductive function which seem to occur following T_3 or T_4 treatment may be due to increases in the circulating levels of LH and T. This possibility was investigated by observing blood levels of these hormones in ram lambs treated for 10 days with T_3.

Experimental Plan

Eight Finnish Landrace ram lambs (10 to 11 months of

age) were taken from an outside pen and placed in groups of two in pens inside the barn (kept at 21 to 24 C) in December. Rams were allowed one week to become acquainted with the surroundings before the experimental period began. Blood samples were collected from each ram twice a day (09.00 and 20.00 hours) for 20 consecutive days. Animals were weighed on days 5 and 16. Treatment with T_3 began on day 6 and was concluded on day 15. Each ram received 125μg T_3 on day 6, 250μg on day 7 and 500μg on days 8 through 15. Following each bleeding, one-half of the daily dose (in 0.5 ml alkaline saline) was injected subcutaneously.

All serum samples were assayed for LH and glucose (Technicon System Auto Analyzer). The mean levels of LH and glucose were determined for each ram during four separate 5 day periods (pre-treatment, two treatment and a post-treatment period) by averaging all of the observations within each period. T levels were determined on aliquots of serum pools made for each ram within each period (ten samples per pool, two per day for 5 days).

Results

Treatment with T_3 appeared to induce a mild hyperthyroid condition in the rams. This was suggested by the observation that rams exhibited a slight reduction in weight over the 10 day treatment period. Initial weights averaged 38.7 $\pm$ 1.6 kg, whereas, final weights averaged 36.8 $\pm$ 1.5 kg. Blood glucose levels were observed to have increased significantly ($P<0.05$) during the first 5 days, and again during the second 5 days following the onset of treatment (Table 2A). In addition, animals appeared to be extremely nervous and excitable from the third day of T_3 treatment to the second day of the post-treatment period.

Triiodothyronine treatment appeared to have no effect

TABLE 2A.

Mean Serum LH, T and Glucose Levels in Ram Lambs when Subjected to T_3 Treatment

	Control	Period T_3 Treatment[1]		Post-T_3
	days 1-5	days 6-10	days 11-15	days 16-20
LH Level (ng/ml)	0.63 ± 0.10[2]	0.70 ± 0.13	0.69 ± 0.12	0.74 ± 0.16
T Level (ng/ml)	6.10 ± 2.24[a]	4.61 ± 1.35[ab]	4.06 ± 1.51[ab]	2.69 ± 0.67[b]
Glucose Level (mg/100 ml)	65.9 ± 1.9[a]	75.6 ± 1.9[b]	86.3 ± 1.8[c]	75.3 ± 1.6[b]

[1] 125μg on day 6, 250μg on day 7 and 500μg on days 8-15

[2] each value represents the mean ± SE for the 8 rams

[a-c] means not followed by the same letter are significantly different ($P < 0.05$)

on the mean level of LH (Table 2A). Mean levels were observed to have been similar throughout the pre-treatment and treatment periods. However, there was considerable ram to ram variation in the magnitude of the fluctuations of LH observed during the experimental periods. In ram #1, larger fluctuations of LH appeared to occur during the treatment period (Figure 4A) and the level was depressed during the post-treatment period. A much different trend was observed in ram #8 (Figure 5A) as comparatively large LH fluctuations were observed during the last one-half of the treatment period, and during the post-treatment period.

When compared to the levels observed during the pre-treatment period, the mean T level was noted to be slightly depressed during the T_3 treatment periods, and further depressed ($P<0.05$) during the post-treatment period. Although quantitative measurements were not taken, the level of libido appeared to increase after the onset of T_3 treatment, particularly during days 11 through 15. Rams were more frequently engaged in mounting activity during this period.

Discussion

LH levels in peripheral blood throughout the menstrual cycle have been shown by Akande and Hockaday (1972) to be significantly elevated in women with thyrotoxicosis, a severe hyperthyroid condition. Ruder *et al.* (1971) demonstrated that induced hyperthyroidism in human males (300μg T_3 for 18 to 20 days) resulted in increased LH levels which were apparently caused by a decrease in the level of unbound T brought about by an increased synthesis of testosterone binding globulin (TBG). Indirect evidence suggesting that slight transient increases in the level of serum LH occur in male rats with T_3 treatment has been

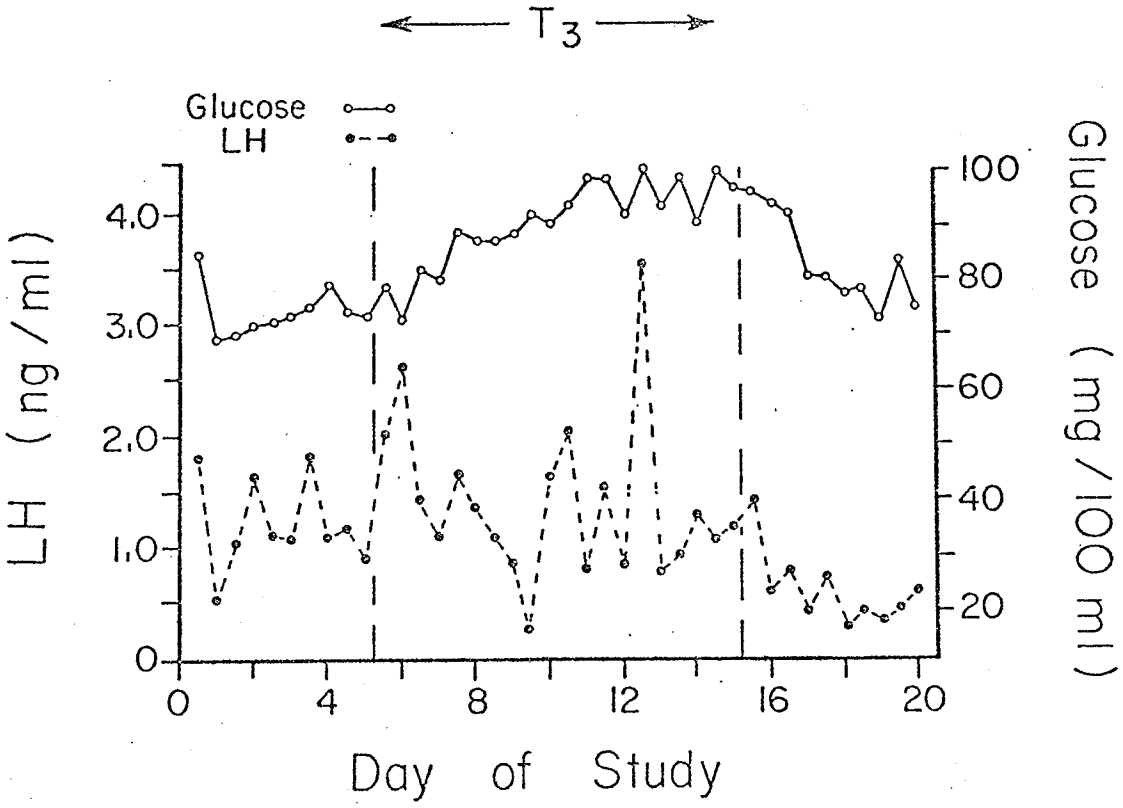

FIGURE 4A. Serum glucose and LH levels in ram lamb #1 when bled by jugular venipuncture twice daily (09.00 hours and 18.00 hours) prior to (days 1-5), during (days 6-15) and following (days 16-20) SC injections of T_3 (125μg on day 6, 250μg on day 7 and 500μg on days 8-15).

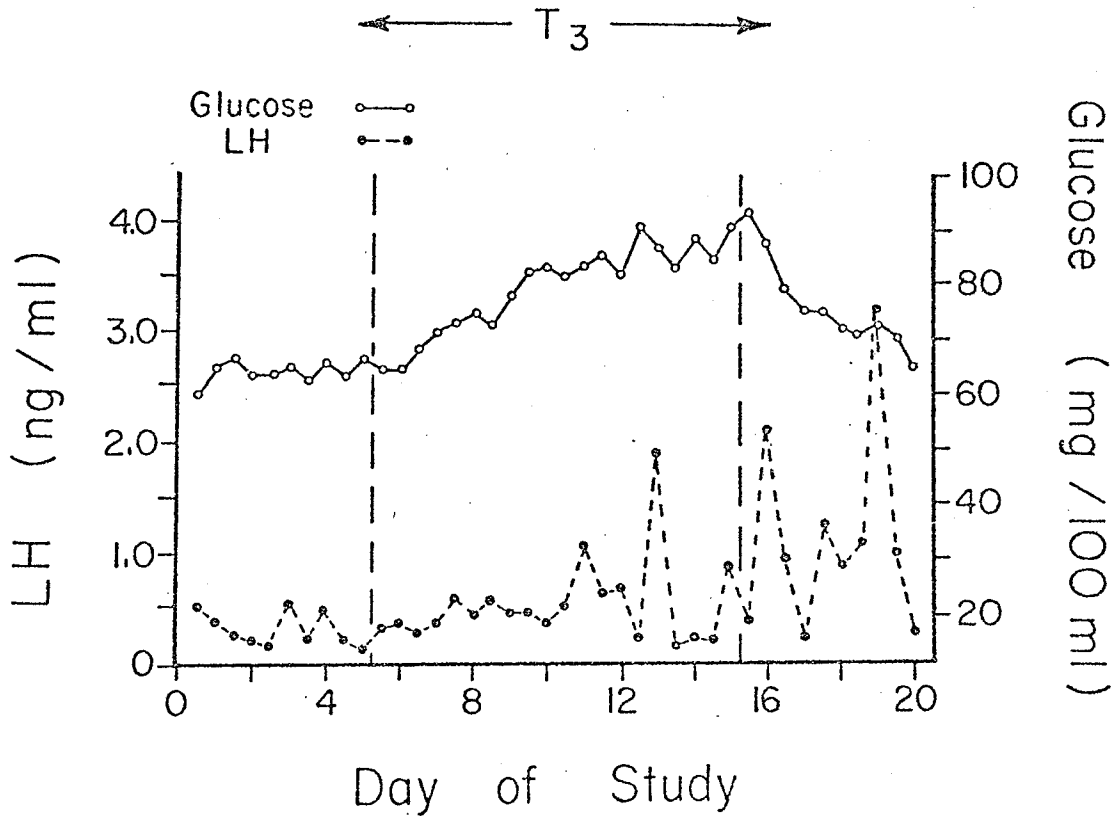

FIGURE 5A. Serum glucose and LH levels in ram lamb #8 when bled by jugular venipuncture twice daily (09.00 hours and 18.00 hours) prior to (days 1-5), during (days 6-15) and following (days 16-20) SC injections of T_3 (125μg on day 6, 250μg on day 7 and 500μg on days 8-15).

presented by Howland and Ibrahim (1973, 1974). In the present study, mean LH levels were observed to be unchanged throughout the experimental period. However, noticeable elevations in the LH level were found in five of the eight rams during either the treatment and (or) post-treatment periods. These may have been in part the result of less negative feedback on the pituitary as T levels were significantly ($P<0.05$) depressed during these periods. The marked increase in the frequency of mounting activity, which was particularly prevalent throughout the treatment period, may have prompted additional LH releases.

Triiodothyronine treatment of the ram lambs was shown to adversely affect the blood T level. Serum T was significantly lower throughout the treatment period and was further depressed following withdrawal of T_3 injections. Induced hyperthyroidism in male rats has been shown by Massie et al. (1969) to slightly reduce the utilization of oxygen by testicular tissue and to produce obvious histological degeneration of the Leydig cells. Although glucose is known to be the primary energy source utilized by the testis (Waites and Setchell, 1969) abnormally high levels in the blood stream may be detrimental to Leydig cell function. Wall et al. (1973) demonstrated that T levels in normal human males decreased an average of 34 per cent within 60 to 90 minutes following the oral administration of 50 grams of liquid glucose. Levels returned to pretreatment values by 150 minutes. It was suggested that glucose may have depressed blood T by suppressing LH release, affecting T synthesis and secretion, or altering the metabolic clearance rate (MCR) of T. The further depression in the level of blood T which we observed in the rams following withdrawal of T_3 treatment is in disagreement with the work of Gordon et al. (1969) and Olivo et al. (1970). They noted that plasma T in the human

male returned to pre-treatment levels following the discontinuance of T_3 doses.

Our results suggest that induced acute hyperthyroidism in the ram is detrimental to Leydig cell function. The observance of unaltered LH levels throughout the experimental period implies that T_3 does not suppress serum T by inhibiting LH release, but that it may have a direct effect on the Leydig cells. This situation is unlike that which occurs in the human male when made hyperthyroid. It is known that T_3 treatment is associated with a two to three fold increase in the plasma T level, which is probably due to the seven to eight fold increase in the concentration of testosterone binding globulin and the subsequent increases in both the percentage and total amount of T bound (Gordon et al., 1969; Olivo et al., 1970; Ruder et al., 1971).

Summary

Eight Finnish Landrace ram lambs (10 to 11 months of age) were treated with T_3 for a 10 day period in December. Rams were bled twice a day prior to (days 1 through 5), during (days 6 through 15) and following (days 16 through 20) T_3 treatment. During the 10 day treatment period blood glucose levels were observed to increase significantly ($P<0.05$). In addition, rams became nervous and excitable, and weight losses (0 to 2.8 kg) were noted. Mean serum levels of LH did not change appreciably in the rams as a result of treatment, although within some of the rams large fluctuations in the LH level were observed during the treatment and (or) post-treatment period. Mean T levels were depressed during the period of T_3 administration, and further depressed ($P<0.05$) during the post-treatment period. Libido (based on the frequency of mounting behavior) appeared to increase during the treatment period.

TABLE 3A.

Experiment 6 - Mean LH Levels
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Breed	1	2.21	0.18
Error 1	6	12.00	
Period	3	17.69	7.03**
B x P	3	0.37	0.15
Error 2	18	2.52	
Treatment[a]	1	0.02	0.07
B x T	1	1.39	3.71
Error 3	6	0.37	
P x T	3	2.78	3.65*
B x P x T	3	2.93	3.85*
Error 4	18	0.76	
Total	63		

[a] breeding vs non-breeding

** $P<0.01$

* $P<0.05$

TABLE 4A.

Experiment 6 - LH Peaks per 8 Hours
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Breed	1	16.00	1.85
Error 1	6	8.66	
Period	3	18.10	9.43**
B x P	3	0.54	0.28
Error 2	18	1.92	
Treatment[a]	1	2.25	1.76
B x T	1	0.06	0.05
Error 3	6	1.28	
P x T	3	4.88	5.73**
B x P x T	3	0.69	0.81
Error 4	18	0.85	
Total	63		

[a] breeding vs non-breeding

** $P < 0.01$

TABLE 5A.

Experiment 6 - LH Peak Magnitude
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Breed	1	103.02	0.88
Error 1	6	116.49	
Period	3	386.95	14.27**
B x P	3	19.99	0.74
Error 2	18	27.12	
Treatment[a]	1	24.97	31.25**
B x T	1	0.56	0.72
Error 3	6	0.80	
P x T	3	7.09	6.72**
B x P x T	3	8.93	8.45**
Error 4	18	1.05	
Total	63		

[a] breeding vs non-breeding

** $P<0.01$

TABLE 6A.

Experiment 6 - Mean T Levels
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Breed	1	162.02	2.27
Error 1	6	71.39	
Period	3	472.34	26.38**
B x P	3	25.64	1.58
Error 2	18	16.19	
Treatment[a]	1	19.06	3.37
B x T	1	5.67	1.00
Error 3	6	5.66	
P x T	3	22.55	5.29**
B x P x T	3	4.04	0.95
Error 4	18	4.26	
Total	63		

[a] breeding vs non-breeding

** $P<0.01$

TABLE 7A.

Experiment 6 - T Peak Magnitude
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Breed	1	748.77	8.18*
Error 1	6	91.56	
Period	3	664.86	57.10**
B x P	3	40.51	3.48
Error 2	18	11.64	
Treatment[a]	1	15.36	3.46
B x T	1	4.77	1.07
Error 3	6	4.44	
P x T	3	24.77	5.28**
B x P x T	3	21.18	4.52*
Error 4	18	4.69	
Total	63		

[a] breeding vs non-breeding

** $P<0.01$

* $P<0.05$

TABLE 8A.

Experiment 6 - Breedings per 8 Hours
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Breed	1	30.03	0.42
Error 1	6	70.78	
Period	3	368.11	27.83**
B x P	3	7.61	0.58
Error 2	18	13.23	
Total	31		

** $P<0.01$

TABLE 9A.

Experiment 7 - Mean LH and T Levels
Analysis of Variance

Source of Variation	d.f.	LH M.S.	LH F	T M.S.	T F
Age	2	5.06	9.55**	5.08	4.64*
Error	25	0.53		1.09	
Total	27				

** $P < 0.01$

* $P < 0.05$

TABLE 10A.

Experiment 2A - Mean LH Levels[a]
Analysis of Variance

Source of Variation	d.f.	M.S.	F
Hormone	1	5129.46	15.31**
Error 1	6	335.06	
Period	2	321.32	2.58
H x P	2	1035.33	8.31**
Error 2	12	124.59	
Treatment[b]	1	283.24	3.92
H x T	1	231.44	3.20
Error 3	6	72.33	
P x T	2	98.96	2.35
H x P x T	2	24.86	0.59
Error 4	12	42.08	
Total	47		

[a] percentage of control value

[b] pre-injection vs post-injection

** $P<0.01$

TABLE 11A.

Experiment 3A - Mean T and Glucose Levels
Analysis of Variance

Source of Variation	d.f.	T M.S.	T F	Glucose M.S.	Glucose F
Rams	7	63.64	14.70**	0.91	18.20**
Period	3	15.95	3.68*	5.52	110.40**
Error	21	4.33		0.05	
Total	31				

** $P<0.01$

* $P<0.05$

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