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SEASONAL CHANGES IN SEXUAL ACTIVITY AND SERUM LEVELS OF LH AND TESTOSTERONE IN FINNISH LANDRACE AND SUFFOLK RAMS

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SEASONAL CHANGES IN SEXUAL ACTIVITY AND SERUM LEVELS OF LH AND TESTOSTERONE IN FINNISH LANDRACE AND SUFFOLK RAMS[1]

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SUMMARY

A procedure for assessing sexual activity (libido) in individual rams is described. Mating activity measured by this procedure and serum levels of luteinizing hormone (LH) and testosterone in Finnish Landrace and Suffolk rams was determined at 8-week intervals from October, 1974 through October, 1975. Seasonal changes were observed for serum LH, testosterone and libido index scores. Mating activity in rams was highest for both breeds during the peak breeding season (October) and declined 50% by late spring and summer before it increased the next October.

Serum LH concentrations were lowest in May (.54 ng/ml) and increased abruptly in July (>2 ng/ml) when daily photoperiod began to decrease. Serum testosterone concentrations (>6 ng/ml) and mating activity were highest during the October evaluations. Testosterone decreased gradually through the winter months and reached its lowest levels in late March (2.06 ng/ml in Finn rams and 1.01 ng/ml in Suffolk rams). Thereafter, concentrations gradually increased to levels observed the previous fall. A positive correlation (r = .59) between mean testosterone and mating scores collected across months suggested that seasonal fluctuations in serum testosterone influence the sexual behavior of rams. A temporal relationship was not detected between circulating testosterone and sexual behavior.

(Key Words: Rams, Season, Luteinizing Hormone, Testosterone, Libido.)

INTRODUCTION

Fertility in the domestic ram undergoes continual seasonal changes. Although this species produces semen throughout the year, a period of "summer sterility" or reduced breeding efficiency exists for several months (Dutt, 1960). Testosterone (T), which is involved in several male reproductive processes, fluctuates from one season to the next (Johnson *et al.*, 1973; Sanford *et al.*, 1974a; Gomes and Joyce, 1975). Other investigators have shown marked seasonal variation in sexual activity of rams following their exposure to an estrous female (Pepelko and Clegg, 1965; Lees, 1965). Whether decreased summer activity is a direct result of lowered T or a combination of environmental and physiological factors is unknown. Castration greatly diminishes the breeding activity of rams suggesting that T or other secretory products of the testis are partly responsible for male behavior (Clegg *et al.*, 1969). The following experiment was conducted to evaluate serum luteinizing hormone (LH), serum T and sexual activity in two breeds of rams and assess their possible interrelationships. The data are discussed with regard to ram fertility.

MATERIALS AND METHODS

Animals. Five Finnish Landrace (Finn) and five Suffolk rams from the flock at the U.S. Meat Animal Research Center were used in this study. All rams were 3 or 4 years of age and were maintained in sound breeding condition. Data were collected from these animals for 1 year starting October 7, 1974. At 8-week intervals, blood samples were taken for hormone analyses, and sexual aggressiveness was assessed by the libido test described below. Rams and

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test females were sheared on April 24, 1975, 4 weeks before the May test period.

To measure seasonal changes in LH and T, jugular blood was collected from each ram by venipuncture just prior to a 20-min libido test and again 48 hr later. While the first sample was used to relate temporally the concentrations of T with sexual activity, values obtained from the second sample were averaged with those from the first to better describe seasonal fluctuations.

Radioimmunoassay of LH and T. After blood samples were allowed to clot, serum was separated by centrifugation and stored at −20 C until all samples had been accumulated. Concentrations of LH and T were measured by radioimmunoassay. Luteinizing hormone was determined by the method described by Niswender *et al.* (1969) and the assay procedure validated in our laboratory utilized NIH LH-S18 as reference standard (Schanbacher and Ford, 1976a).

Radioimmunoassay of T used in this study has been previously described (Schanbacher, 1976). Because the correlation between values obtained in the presence and absence of chromatography was high, extracted samples were assayed directly for T content. Briefly, ether extracts were dried and incubated overnight with 30,000 dpm of ^{3}H-T and T-3-BSA antiserum diluted in phosphate buffer so that 50% binding was obtained when unlabeled T was absent. This procedure allowed the quantitation of .1 to 20 ng/ml when 100 μl of serum was assayed.

Assessment of Sexual Aggressiveness (Libido). A libido test was developed to evaluate sexual aggressiveness in rams. Results were satisfactory when a single ram was allowed to mate estrus-induced ewes. Three days before the test, experienced rams were placed individually with five pregnant ewes in an isolated pen measuring approximately 3.3 × 6 meters. This permitted the ram to adjust to the testing environment before the actual test period. Between 0800 and 1200 hr on the fourth day, each ram was evaluated 20 min for sexual activity following the introduction of five estrus-induced females. The 20 min, arbitrarily chosen in this study, was found to be sufficient time for the expression of sexual aggressiveness.

Ovariectomized ewes were used as test females. Eleven days before libido evaluation, each ewe was treated with an intravaginal, progestogen passary (Searle Synchro-mate; 20 mg flurogestone acetate). Pessaries were removed 48 hr before the day of testing and daily intramuscular injections of .5 mg 17β-estradiol were begun. These ovariectomized, estrus-induced ewes were sexually receptive and no favoritism was noted by any group of rams.

Behavioral events used to assess ram libido included: a) sniffing the vulva of an estrous ewe (S); b) abortive mating without intromission or ejaculation (A); and c) completed mating with intromission and ejaculation (C). These particular events were recorded separately to the nearest 5 sec for each of the five estrus-induced females. A libido index was mathematically derived for each ram so highest scores represent highest sexual activity. Points were arbitrarily set at one for a single sniff, three for an abortive mount and 10 for a completed mating. Elapsed time (ET) was incorporated, and each ram's libido index with a single ewe (LI_E) was calculated by the formula:

$$LI_E = \sum_S [1(2\text{-}ET_S)] + \sum_A [3(2\text{-}ET_A)] + \sum_C [10(2\text{-}ET_C)]$$

where elapsed times were expressed as a fraction of the total test period. The libido index for an individual ram (LI_R) was obtained when libido scores calculated for each ewe were summed.

$$LI_R = \sum_E [LI_E]$$

A mating index (MI_R) involving only completed matings was calculated by the following formula and results compared with the libido index.

$$MI_R = \sum_E [\sum_C (10(2\text{-}ET_C))]$$

Data were analyzed by analyses of variance techniques and group means tested by least significant differences (Steel and Torrie, 1960).

Hours of daylight (photoperiod) and temperature ranges are plotted in figure 1. These monthly averages were taken from the National Oceanic and Atmospheric Administration Report, Clay Center Station, for the experimental period October, 1974 through October, 1975.

RESULTS AND DISCUSSION

Seasonal Variation in Ram LH and T. Seasonal changes in serum LH and T concentrations for the two breeds of rams, Finn and

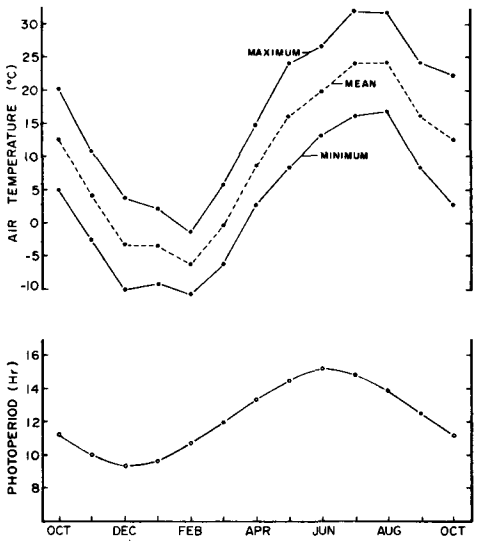

Figure 1. Minimum, maximum and mean air temperature (●) and mean photoperiod (○) at monthly intervals for the period October, 1974 through October, 1975.

Suffolk are shown in figure 2. Although only five rams (10 serum samples) represent a breed at each test period, marked changes in serum T

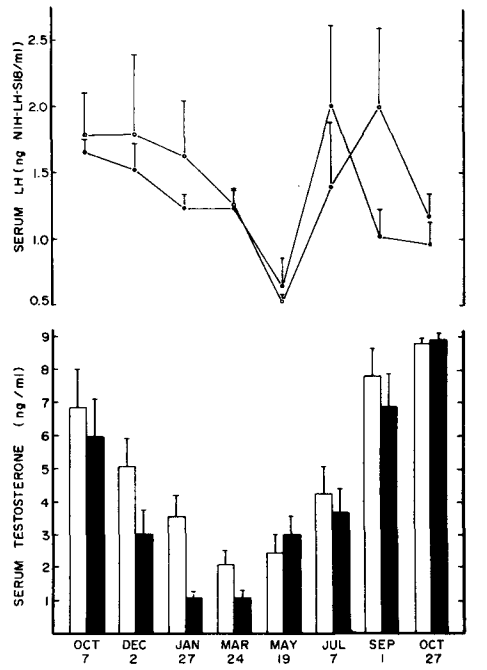

Figure 2. Serum LH and testosterone concentrations for eight evaluation periods from October, 1974 through October, 1975. Means ± SEM are given for Finn (open bars and circles) and Suffolk (closed bars and circles) rams.

were observed.

Considerable variation existed for concentrations of serum LH. Nonetheless, levels tended to decrease during the winter and spring months until May when levels for Finn (.54 ng/ml) and Suffolk rams (.69 ng/ml) were lowest ($P<.05$). Thereafter, levels rose sharply but were not sustained. Luteinizing hormone trends in the present study closely parallel results collected by Pelletier and Ortavant (1975a,b) and suggest that the major factor controlling LH release in the ram is photoperiod length; i.e., when photoperiod increased in spring, LH decreased; and when photoperiod decreased in late June, LH release was stimulated and serum levels were increased.

Other investigators have indicated the existence of seasonal variation in ram plasma (Katongole *et al.*, 1974) and serum LH (Sanford *et al.*, 1974a,b). In addition to seasonal sources of variation, individual as well as time-related (episodic) variation exist. Results by Katongole *et al.* (1974) and Schanbacher and Ford (1976b) suggest that the relatively large variation in serum LH found during the fall breeding season may be the result of increased frequency of LH release observed at that time.

Changes in T concentrations varied significantly between test periods (table 1). Testosterone was decreased as early as December in this study; however, levels were not lowest until early spring (March). Gradual increases through the summer months resulted in elevated T at the fall breeding season (October).

Except for two test periods, Finn rams had consistently higher T concentrations than Suffolk rams when maintained and bled under identical conditions. This breed difference was significant ($P<.01$) during the January test period. To our knowledge, breed differences in T levels of the ram have not been previously shown; however, small animal numbers are generally used, and other breeds may not be as dissimilar as those chosen for this study.

Males of species with restricted breeding seasons are known to have marked variation in plasma T concentration (Neaves 1973; Whitehead and McEwan, 1973). Although a similar phenomenon exists in sheep, the seasonal fluctuations are not as evident. Investigations with Suffolk (Katongole *et al.*, 1974), Southdown (Johnson *et al.*, 1973), Hampshire (Purvis *et al.*, 1974) and crossbred rams (Sanford *et al.*, 1974a) have reported highest concentrations of

TABLE 1. EFFECT OF SEASON ON SEXUAL ACTIVITY AND SERUM LEVELS OF LH AND TESTOSTERONE (T) IN FINN AND SUFFOLK RAMS

Date of evaluation	Breed	Serum hormones		Sexual activity	
		LH	T	Libido index	Mating index
October 7	Finn	1.78 ± .31[a]	6.86 ± 1.18[ab]	150 ± 17[a]	119 ± 19[a] (7.4)[f]
	Suffolk	1.66 ± .14[ab]	6.00 ± 1.25[bc]	118 ± 13[a]	93 ± 12[a] (5.6)
December 2	Finn	1.80 ± .59[a]	5.07 ± .86[bc]	122 ± 17[ab]	90 ± 16[ab] (5.4)
	Suffolk	1.54 ± .17[ab]	3.01 ± .74[de]	90 ± 8[abc]	57 ± 9[bc] (3.4)
January 27	Finn	1.67 ± .39[a]	3.56 ± .65[cd]	169 ± 32[a]	65 ± 12[bc] (4.2)
	Suffolk	1.22 ± .11[ab]	1.01 ± .27[e]	81 ± 5[bcd]	44 ± 10[c] (2.6)
March 24	Finn	1.23 ± .18[ab]	2.06 ± .43[d]	161 ± 34[a]	66 ± 14[bc] (4.0)
	Suffolk	1.22 ± .12[ab]	1.01 ± .31[e]	70 ± 15[cd]	39 ± 9[c] (2.4)
May 29	Finn	.54 ± .05[b]	2.39 ± .61[d]	85 ± 8[b]	51 ± 6[c] (3.0)
	Suffolk	.69 ± .16[b]	2.97 ± .58[de]	69 ± 11[cd]	41 ± 5[c] (2.4)
July 7	Finn	1.41 ± .47[ab]	4.25 ± .81[cd]	116 ± 5[ab]	84 ± 3[abc] (3.8)
	Suffolk	2.01 ± .65[a]	3.61 ± .76[cd]	58 ± 7[d]	37 ± 6[c] (2.2)
September 1	Finn	1.98 ± .66[a]	7.80 ± .81[a]	84 ± 3[b]	67 ± 1[bc] (3.6)
	Suffolk	1.00 ± .21[ab]	6.82 ± 1.02[ab]	56 ± 5[d]	45 ± 3[c] (2.6)
October 27	Finn	1.17 ± .15[ab]	8.75 ± 1.36[a]	122 ± 16[ab]	96 ± 14[ab] (5.8)
	Suffolk	.95 ± .18[ab]	8.87 ± 1.54[a]	98 ± 7[ab]	70 ± 8[ab] (4.4)

[a,b,c,d,e]Within breed, means ± SEM with no superscript in common differ significantly ($P<.05$) using the LSD test.

[f]Values in parentheses represent mean no. of completed matings per 20-min test period.

T during the fall months and lowest levels occurring during the spring. Results presented in this paper agree with those in previous studies. Although T levels appear maximum during late September to early November, the minimum levels are more variable. Perhaps a combination of breed and geographical (environmental) effects is responsible for the noted differences.

In contrast to results from the studies mentioned above, recent results from Ohio by Gomes and Joyce (1975) are somewhat contradictory. Using seven rams of mixed breeds, these investigators observed highest T concentrations during the months of May and July with the nadir occurring in December. Although environmental conditions in Ohio may partly account for these results, these data are difficult to explain when they are compared with results of the present and previously mentioned studies.

Seasonal Variation in Ram Libido. Seasonal variation in sexual activity of Finn and Suffolk rams was indicated by the significant fluctuations in index scores (table 1). As suggested by the mating index or simply the mean number of completed matings in table 1, Finn rams tended to be more active than Suffolk rams. Furthermore, the fact that certain individuals within each breed group consistently scored high suggests that, regardless of hormone levels, sexual aggressiveness is an inherent trait for which genetic selection may be useful.

Behavior is much more complicated than we have depicted in figure 3. Libido and mating indices are only two ways to describe this behavioral trait. Although mating index scores were used preferentially in this study, the events used to calculate a libido index may yield additional information. For instance, the high libido scores observed for Finn rams during the months of January and March were due to an excessive number of abortive mounts by two rams. Why this was noted in only two Finn rams and at the months indicated is unknown. In this regard, we might note that Pepelko and Clegg (1965) recorded the greatest number of mounts per ejaculation during the

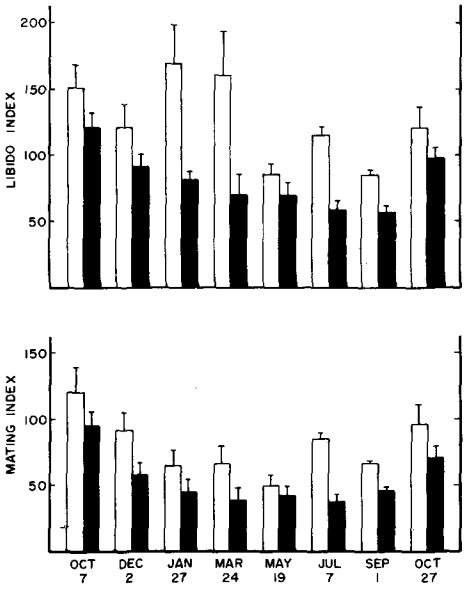

Figure 3. Libido and mating index scores (defined in text) for eight evaluation periods from October, 1974 through October, 1975. Means ± SEM are given for Finn (open bars) and Suffolk (closed bars) rams.

TABLE 2. CORRELATION COEFFICIENTS BETWEEN SERUM TESTOSTERONE (T) LEVELS AND MATING INDEX SCORES WITHIN BREED FOR EACH EVALUATION PERIOD

Date of evaluation	Finn[a]	Suffolk[a]	Finn[b]	Suffolk[b]
October 7	-.49	.69	-.86	.29
December 2	.57	-.18	.84	.62
January 27	-.29	.91*	-.29	.87
March 24	.02	.01	.71	-.24
May 29	.60	.32	.82	.23
July 7	.94*	.35	.98**	.61
September 1	.14	.10	.92*	.40
October 27	-.17	.25	.09	.63

[a]Correlations calculated with T levels at the time of libido evaluation.

[b]Correlations calculated with mean T levels for each ram.

*P<.05, **P<.01.

TABLE 3. CORRELATION COEFFICIENTS (r) BETWEEN SERUM TESTOSTERONE (T) LEVELS AND MATING INDEX SCORES IN INDIVIDUAL RAMS FROM OCTOBER, 1974 THROUGH OCTOBER, 1975

	Finn ram no.					Suffolk ram no.				
	1	2	3	4	5	6	7	8	9	10
r^a	.67	-.33	.77*	.50	.38	.53	.64	.28	.34	.28
r^b	.54	-.12	.54	.41	.33	.71*	.66	.36	.42	.49

[a]Correlations calculated with T levels at the time of libido evaluation.

[b]Correlations calculated with mean T levels for each ram.

*$P<.05$.

winter.

Scores from both the libido and mating indices show seasonal trends which suggest that ram behavior may be influenced by serum T concentrations. Mean T levels and mating index scores were correlated (r = .59) across months, while correlations for all rams within months (table 2) and within individual rams across months (table 3) were highly variable (range −.86 to +.98). Correlations for individual rams were not improved by using T values from serum taken immediately before the libido test as compared to using mean T values calculated from two independent samples for each ram (table 3). Since it is possible that two samples do not accurately reflect "average" serum T, we hesitate to conclude that a temporal relationship does not exist between circulating T and sexual activity in normal functioning rams.

Data accumulated for castrated rams (Clegg *et al.*, 1969) and laboratory animals (Grunt and Young, 1952; Champlin *et al.*, 1963; Agmo and Kihlstrom, 1974) indicate that "high" doses of T, as compared with physiological doses, do not increase mating activity above precastrational levels. Possibly, for an individual ram at a given point in time, sufficient T is present to yield maximum behavioral responses. However, when T was elevated as it was during the fall, libido and mating scores were relatively high, and when T was low as seen during the non-breeding season (anestrous period), index scores were lowest. The fact that sexual activity did not increase with T during July and September is interesting; however, two explanations are plausible. First, the high summer temperatures that generally exist until mid-September in Nebraska may have suppressed mating activity without affecting normal endocrine activity; in fact, decreasing photoperiod in July appears to have stimulated gonadotropin (LH) secretion. The second possibility remains that endocrine-induced changes in behavior may require several months before maximum activity resumes.

In conclusion, sexual activity in the ram changes with corresponding seasonal changes in serum T levels. Whether a cause and effect relationship exists is unknown. To evaluate sexual activity in breeding rams, the mating index may be the preferred method, particularly when the ultimate goal is to obtain the maximum possible number of ewes inseminated by a specific sire.

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