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ACUTE AND CHRONIC EFFECTS OF GONADOTROPIN RELEASING HORMONE ON REPRODUCTIVE CHARACTERISTICS OF RAMS DURING THE NONBREEDING SEASON^{1,2}

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SUMMARY

Acute and chronic effects of gonadotropin releasing hormone (GnRH) administration on reproductive characteristics of 32 rams have been assessed during the nonbreeding season. Rams injected intramuscularly with 50 µg of GnRH had elevated ($P < .01$) concentrations of serum testosterone and noticeably higher (60%) mating activities when compared to control animals injected with saline. Daily injections of GnRH resulted not only in higher testosterone concentrations and mating activity, but also in increased testes size ($P < .05$). The apparent change in testicular function may account for the improvement in semen quality which was observed in these animals. Although benefits were only slight for percentages of live sperm, normal sperm and sperm with normal acrosomes, sperm motility was markedly increased ($P < .05$).

These data suggest that reproductive performance in rams is related to testicular androgen secretion and that a spring decline in those characteristics normally associated with high fertility in the male can be lessened by chronic treatment with GnRH.

(Key Words: Rams, Season, GnRH, Testosterone, Reproduction.)

INTRODUCTION

Castration, commonly used to render males infertile, also has been employed to reduce or abolish objectionable sexual behavior in males. Thus, testicular hormones must contribute in some manner towards this reproductive trait. The relationship between testicular hormones and mating behavior appears extremely complex; however, testosterone (T) has been shown to restore mating behavior to the castrate rat (Larsson, 1966), rabbit (Agmo and Kihlstrom, 1974), guinea pig (Alsum and Goy, 1974), hamster (Whalen and DeBold, 1974), boar (Joshi and Raeside, 1973), ram (Clegg *et al.*, 1969) and rhesus monkey (Wilson *et al.*, 1972). Dihydrotestosterone and estradiol-17β, which are known to originate, in part, within the testes and are secreted in response to human chorionic gonadotropin stimulation (Mahoudeau *et al.*, 1975), may play an important role in the regulation of male sexual behavior; but this remains to be investigated.

Sexual aggressiveness in rams is reduced during the "nonbreeding season" or the period during which most females are anestrus (Pepelko and Clegg, 1965, Schanbacher and Lunstra, 1976), and the fact that semen quality is poor at this time (B. D. Schanbacher and D. D. Lunstra, *unpublished data*) suggests that multiple factors contribute to the seasonal reduction in breeding efficiency described by Dutt (1960). The physiological processes involved in maintenance of male sexual behavior and the production of high quality semen are considered to be androgen-dependent. Because several investigators have reported low concentrations of T during the nonbreeding season (Schanbacher and Lunstra, 1976; Schanbacher and Ford, 1976), it was hypothesized that ram fertility or some component thereof could be improved by stimulation of testicular androgen secretion. The following study was conducted during the nonbreeding season to determine the

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⁴Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

acute and chronic effects of GnRH administration on reproductive characteristics of rams.

MATERIALS AND METHODS

Late in February, 16 Finnish Landrace and 16 Suffolk rams, 2 or 3 years of age, were given intramuscular injections of saline or GnRH to determine the subsequent effects on sexual behavior. In preliminary tests, we found that T concentrations were elevated for 3 or 4 hr after a 50 µg dosage of GnRH, but maximum concentrations occurred between 1 and 2 hr after injection. In an experiment designed to monitor the acute response of circulating T and sexual behavior of rams to hormone treatment, blood samples were collected by venipuncture 1 hr after the administration of saline or 50 µg GnRH and serum was prepared for the quantitation of radioimmunoassayable T (Schanbacher, 1976). Immediately following blood collection, individual rams were assessed for their ability to mate ovariectomized estrous-induced ewes (Schanbacher and Lunstra, 1976).

Eight of these rams were used in a continuing experiment to evaluate the effects of chronic GnRH administration on serum T concentration, sexual behavior, scrotal circumference and semen characteristics. Four animals were chosen randomly from both the saline- and GnRH-treated groups (acute study) to receive twice daily intramuscular injections of saline or 50 µg of GnRH for the duration of the study. At the end of the first, third and seventh week of treatment, sexual behavior data were recorded for the subsequent calculation of

mating index scores (Schanbacher and Lunstra, 1976). Blood samples were collected from these animals at the seventh week evaluation period and 2 days later, semen was collected by an artificial vagina that was retained within the vagina of an estrous-induced ewe. The artificial vagina, made of latex liner and fitted with a 10 × 75 mm test tube, was used very effectively for the collection of rams not trained to serve a regular artificial vagina. As soon as the ram had ejaculated, the estrous ewe was restrained, the artificial vagina was removed and the semen was expressed into the test tube and kept in a water bath at 37 C while semen quantity and quality were measured. Normal acrosomes were assessed by the criteria of Saacke and White (1972).

In addition to the evaluation of behavior and semen characteristics of chronically treated rams, scrotal circumference was measured at the beginning and end of this study to determine whether hormonal stimulation resulted in increased testes size. Changes in the scrotal circumference of saline- and GnRH-treated rams were evaluated by paired t analysis; whereas the Student's t test was used for other statistical comparisons except mating activity of chronically treated rams. Mating activity was analyzed by procedures outlined by Steel and Torrie (1960) for a completely random split-plot design.

RESULTS

Rams treated acutely with a single intramuscular injection of 50 µg of GnRH had

TABLE 1. ACUTE EFFECTS OF SALINE OR GONADOTROPIN RELEASING HORMONE (GnRH) ON SERUM TESTOSTERONE CONCENTRATIONS AND MATING ACTIVITY OF RAMS^a

Treatment ^b	Serum testosterone (ng/ml)	Mating index	Correlation coefficient (r)
Saline	4.08 ± .54	23.5 (1.4) ±5.5	.36
GnRH	7.97** ±1.25	35.9 (2.3) ±6.4	.05

^aMeans ± SEM for 16 animals. Values in parentheses represent mean no. of completed matings per 20-min test period.

^bAssessment 1 hr following a single intramuscular injection of saline or 50 µg of GnRH.

**P<.01.

TABLE 2. CHRONIC EFFECTS OF SALINE OR GONADOTROPIN RELEASING HORMONE (GnRH) ON MATING ACTIVITY OF RAMS^a

Treatment ^b	Mating index scores for various treatment periods			
	Initial	1 week	3 weeks	7 weeks
Saline	35.7 (2.0) ±12.3	50.9 (3.0) ±10.9	58.9 (3.5) ±16.1	47.7 (2.7) ± 4.9
GnRH	55.7 (3.5) ±13.2	56.1 (3.2) ±15.5	87.4 (5.3) ± 7.4	59.5 (3.5) ±19.4

^aMeans ± SEM for four animals. Values in parentheses represent mean no. of completed matings per 20-min test period.

^bTwice daily intramuscular injections of saline or 50 µg of GnRH.

higher ($P < .01$) concentrations of serum T than those of control animals injected with saline (table 1). Similarly, treated animals appeared to be sexually more active because their mating index scores and the number of completed matings during a 20-min test period averaged 60% higher than those observed for control animals. Although increased serum T coincided with the increased mating activity of GnRH-treated rams, the simple correlation between these two characteristics was small ($r = .05$). The correlation coefficient between these two characteristics in control animals was higher ($r = .36$) but not significant.

Chronic administration of GnRH did not significantly increase mating activity of rams above those responses elicited by a single intramuscular injection of releasing hormone

(table 2). Although the mating index scores tended to increase at each successive test period for both saline- and GnRH-treated rams, the GnRH group of rams had consistently higher scores although they were not significant. Serum T concentrations in rams treated daily for 7 weeks (8.47 ± 3.60 ng/ml) were similar to those concentrations observed after an acute exposure to GnRH (7.97 ± 1.25 ng/ml) and were higher ($P < .01$) than those observed in control rams ($2.00 \pm .29$ ng/ml).

Although scrotal circumference among rams varied considerably, the variation that could be measured within each ram was small. Seven weeks of continual GnRH stimulation increased ($P < .05$) the size of ram testicles which is reflected by the scrotal circumference of these animals (table 3).

The characteristics of semen collected from rams that received saline or GnRH for a 7-week period are shown in table 4. Although sperm concentration was higher in treated rams than in control rams, total sperm numbers were not significantly different. The quality attributes of semen samples from GnRH-treated animals appeared to be improved over those of control samples. Percentage of motile sperm was markedly increased ($P < .05$) by treatment; whereas, the percentages of live sperm, normal sperm and sperm with normal acrosomes were increased only slightly.

TABLE 3. CHRONIC EFFECTS OF SALINE OR GONADOTROPIN RELEASING HORMONE (GnRH) ON SCROTAL CIRCUMFERENCE OF RAMS^a

Treatment ^b	Initial circumference (cm)	Final circumference (cm)
Saline	31.60 ± .99	31.65 ± .77
GnRH	31.27 ±1.50	34.32* ±1.96

^aMeans ± SEM for four animals.

^bTwice daily intramuscular injections of saline or 50 µg of GnRH for 7 weeks.

* $P < .05$.

Discussion

Seasonal changes in the reproductive characteristics of rams have been documented by several investigators (Dutt, 1960; Emmens and Robinson, 1962; Schanbacher and Lunstra, 1976); however, few attempts have been made

TABLE 4. CHRONIC EFFECTS OF SALINE OR GONADOTROPIN RELEASING HORMONE (GnRH) ON SEMEN CHARACTERISTICS OF RAMS^a

Treatment ^b	Volume (ml)	Sperm concentration (10 ⁹ /ml)	Total sperm (10 ⁹)	pH (1/log[H ⁺])	Progressive motility (%)	Live sperm (%)	Normal acrosome (%)	Normal sperm ^c (%)
Saline	1.18 ±.06	1.74 ±.61	2.00 ±.68	7.21 ±.13	47.5 ±9.4	63.8 ±1.7	49.8 ±12.6	83.3 ±5.5
GnRH	.82 ±.14	3.30 ±.36	2.82 ±.62	7.06 ±.08	75.8 ±4.1	68.3 ±1.0	60.3 ± 6.5	91.0 ±2.1
Significance	NS	†	NS	NS	*	†	NS	NS

^aMeans ± SEM for four animals.

^bTwice daily intramuscular injections of saline or 50 µg of GnRH for 7 weeks.

^cNormal sperm, morphologically, excluding acrosome status.

†P<.10, *P<.05, NS = P>.10.

to prevent the spring decline in breeding performance of these animals.

Data presented herein indicate that reproductive performance could be improved after the administration of GnRH to rams that are to be used in a spring breeding program; and although actual fertility data were not collected, the increased sexual behavior and improved semen quality of these animals should increase the number of ewes mated and result in higher conception rates. This becomes especially important when the number of rams is limiting or the estrous cycles of a large group of ewes have been synchronized.

Increased size of the testes and improvement in semen quality suggest that GnRH has a beneficial effect on spermatogenesis in rams. Because no attempt was made to quantitate daily sperm output in these animals and testicular tissue was not taken for histological evaluation, we do not know the specific effects of GnRH on testicular function. However, directly or indirectly, increased steroidogenic activity of the interstitial cells may account for the increase in testes size, as well as change the ram's sexual behavior.

The lack of a significant correlation between serum T concentrations and mating activity as observed during the spring implies that a temporal relationship does not exist between these two reproductive characteristics; yet mean sexual behavior was elevated nearly 50% when T secretion was stimulated by GnRH. Perhaps only a threshold level of T is required for the full expression of mating performance. The threshold concept might explain the lower correlation coefficient between serum T concentrations and mating activity of GnRH-treated rams ($r = .05$) compared with control rams ($r = .36$). The increased mating index scores in control animals, as well as in those chronically treated with GnRH, suggests that the rams were adjusting to the test procedures. Nevertheless, the consistent difference between the two groups of rams indicates a response to hormone therapy.

The hypothesis that reproductive capacity of rams is androgen-dependent appears to have merit because the GnRH-elevated concentrations of serum T in this study were associated with improvement of the characteristics indicative of high fertility in the male. On the other hand, T *per se* probably is not the only hormone regulating sexual behavior and seminal characteristics of rams. For example, estradiol-

17 β restores most aspects of sexual behavior in the castrate rat (Sodersten, 1973) and red deer (Fletcher and Short, 1974), whereas dihydrotestosterone is believed to play an active role in the function of the epididymides (Vreeburg, 1975; Djoseland, 1976) and accessory sex glands (Liao and Fang, 1969; Wilson and Gloyna, 1970). Our limited understanding of the effect of the various hormones on reproductive performance of males and the potential benefits of hormone therapy (such as those depicted in this study with GnRH) indicate a serious need for additional research on the interaction of hormones with all facets of male reproduction.

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