Individual Differences in LH and FSH Responses to Orchidectomy and Testosterone Replacement Therapy in Rams

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Profiles of LH and FSH levels and pituitary LH responses to exogenous luteinizing hormone releasing hormone (LHRH) have been characterized in rams, castrated rams (wethers), and wethers implanted with testosterone. Rams were castrated when adult, and, at the time of castration, two groups of wethers were implanted with either four or eight testosterone capsules. Rams showed random pulses of LH and testosterone which were temporally related. The number of LH and testosterone pulses per 24 hours differed among rams, giving rise to large differences in the mean levels of these hormones. Mean FSH levels and pituitary LH responses to LHRH also differed among rams and were positively correlated to differences in LH levels. All three nonimplanted wethers showed a rhythmic pulsatile pattern of LH secretion and had elevated mean LH and FSH levels. There were, however, appreciable differences between wethers with regard to mean LH and FSH levels and pituitary LH responses to LHRH. Both four and eight testosterone capsules were effective in suppressing pulsatile LH secretion and mean LH and FSH levels in two out of three wethers. In a third animal within each of these groups, however, LH and FSH profiles and LH responses to LHRH were characteristic of nonimplanted wethers. These data suggest that individual rams have different inherent capacities to secrete gonadotropins which influence LH and FSH responses to castration and testosterone replacement therapy.

Key Words: rams, wethers, gonadotropin secretion, testosterone, negative feedback, individual differences.

In negative feedback studies involving gonadectomy and steroid replacement therapy, it is generally assumed that between-animal variation in gonadotropin secretion does not contribute appreciably to LH and FSH responses to steroid treatment. However, during the course of feedback studies with male sheep, we have noted that individual animals, both intact and castrated, can differ considerably in their LH and FSH secretory profiles (D'Occhio et al, 1982a,b). This communication presents data which suggest that the LH and FSH responses to castration and testosterone replacement therapy in male sheep may be influenced by an individual animal's inherent capacity to secrete gonadotropin. Parts of these data have been presented briefly elsewhere (D'Occhio et al, 1982c).

Materials and Methods

Animals

Twelve mature (2- to 3-year-old) rams of Finnish Landrace breeding and similar body weight (87.5 ± 2.5 kg, mean ± SE) were used in this study. They were kept under field conditions except during periods of blood collection when they were restrained in metabolism crates under shelter.

Experimental Procedures

The rams were randomly assigned to one of four equal groups. One group was left intact while the other three

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groups were surgically castrated (wether) under xylazine analgesia on October 8. At the time of castration, one group of wethers was implanted with four testosterone capsules and a second group was implanted with eight similar capsules. The capsules consisted of polydimethylsiloxane medical grade tubing (3.35 mm i.d. × 4.65 mm o.d. × 300 mm; Dow Corning, Midland, MI) filled with crystalline testosterone. They were placed subdermally over the ribs using a surgical trochar (Schanbacher, 1980a).

All animals were bled via jugular vein cannulae at 10-minute intervals for 8 hours at two and four weeks after the commencement of treatment and at 10-minute intervals for 24 hours at six weeks. On each occasion, 3 ml of blood were withdrawn every 10 minutes. At the end of each sampling period, the animals were given an intravenous injection of luteinizing hormone releasing hormone (LHRH, 5 ng/kg BW) and additional blood samples were taken every 10 minutes for 1 hour. Blood was allowed to clot and the serum was obtained after centrifugation and stored at −20°C until required for hormone assay. Validated double-antibody radioimmunoassays were used to measure serum LH (Schanbacher and Ford, 1976), FSH (Schanbacher and Ford, 1977), and testosterone (Schanbacher and D'Occhio, 1982). Serum LH concentrations were measured in all samples while FSH and testosterone were assayed only in samples collected at six weeks.

Results

LH, FSH, and Testosterone in Rams

The 24-hour profiles of LH, FSH, and testosterone levels in untreated rams at six weeks are shown in Figure 1. All three rams showed random pulses in serum LH and testosterone which were temporally related. There were large differences between rams in the frequency of hormone secretory episodes (range 5 to 13 per 24 hours) resulting in marked differences in mean LH and testosterone levels. Similar differences were also observed at two and four weeks (data not shown). The pituitary LH responses to exogenous LHRH were related to pre-injection LH levels in individual rams (Figure 1). Serum FSH levels in rams remained relatively constant throughout the 24-hour sampling period with mean levels positively correlated ($r = 0.96$, $P < 0.01$) with mean LH levels.

LH, FSH, and Testosterone in Nonimplanted Wethers

Characteristics of the LH profiles of nonimplanted wethers at two, four, and six weeks after castration are presented in Table 1. A rhythmic pulsatile pattern of LH secretion had developed in all three wethers by two weeks after castration (inter-pulse interval: 50 to 55 minutes). However, at two weeks there were appreciable differences between wethers in basal LH and LH peak amplitude resulting in 2- to 3-fold differences in mean LH levels. By six weeks, LH peak amplitudes were similar while differences in basal LH levels persisted despite increases in basal LH in all three animals between the second and sixth week of study. The 24-hour profiles of LH, FSH, and testosterone at six weeks are shown in Figure 2. Testosterone levels in nonimplanted wethers (0.1 to 0.2 ng/ml) were at the lower limit of assay sensitivity. The 24-hour LH profiles were characteristic of the individual profiles observed during the 8-hour bleeds at two and four weeks. Differences in mean LH levels at six weeks were the result of differences in basal LH levels, rather than variations in the number or amplitude of LH peaks. The areas under the LH response curves after LHRH injection were related to pre-injection LH levels (Fig. 2). Serum FSH levels were stable over 24 hours in nonimplanted wethers and mean levels for individual animals were positively correlated with LH levels ($r = 0.98$, $P < 0.01$).

LH, FSH, and Testosterone in Implanted Wethers

The 24-hour hormone profiles at six weeks in wethers implanted with either four or eight testosterone capsules are shown in Figures 3 and 4, respectively. The capsules provided stable testosterone levels which ranged from 1.4 to 2.0 ng/ml and 3.3 to 4.1 ng/ml for individual animals within the four- and eight-implant groups, respectively. In both groups, the respective levels of testosterone were effective in suppressing the rhythmic pattern of LH secretion in two wethers. These wethers also had low FSH levels and showed dampened LH responses to LHRH. However, a third animal within each group showed LH and FSH profiles, and LH responses to LHRH, that were characteristic of nonimplanted wethers. The lack of effect of testosterone capsules in these animals was also observed at two and four weeks (data not shown). Mean serum LH and FSH levels were positively correlated at six weeks ($r = 0.91$, $P < 0.01$; pooled data for four- and eight-implant groups).

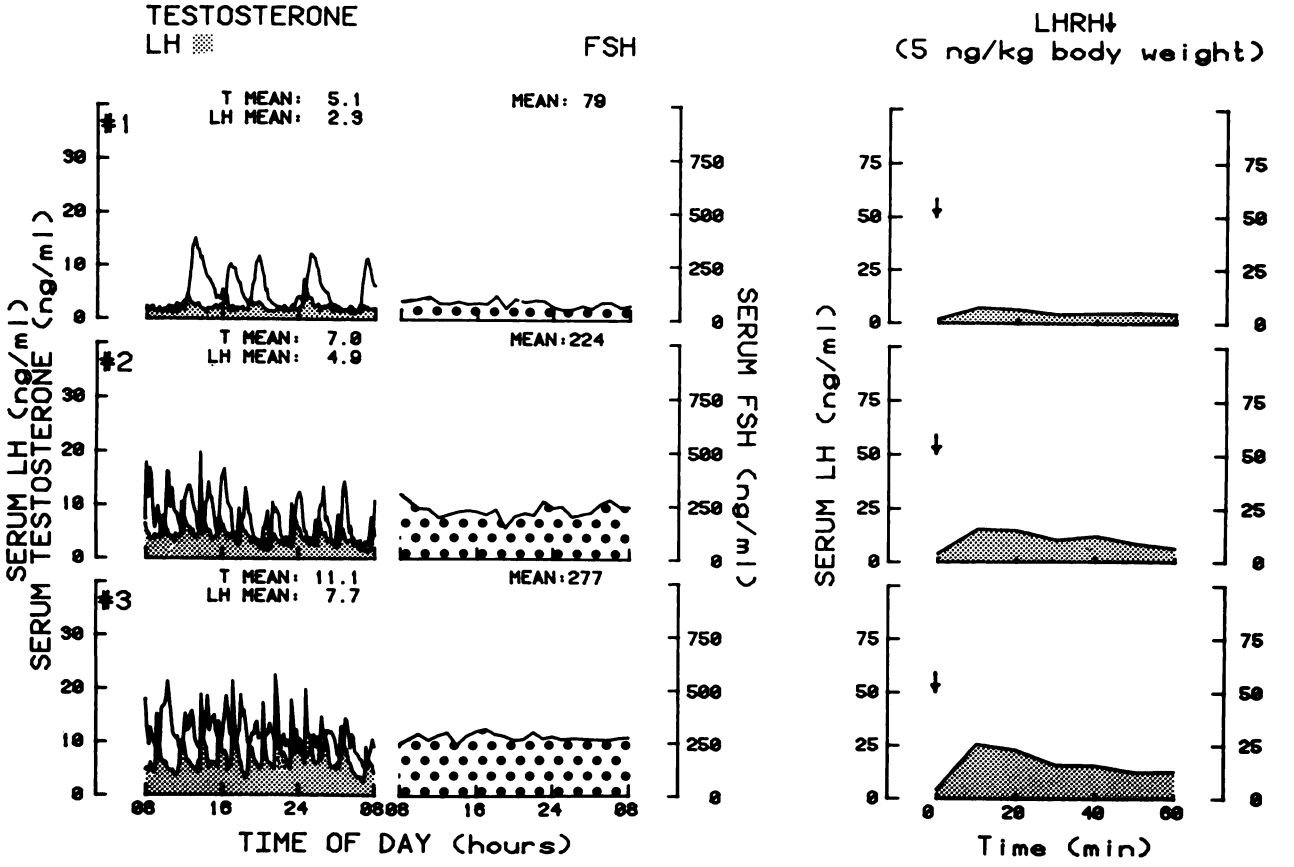

Fig. 1. Twenty-four hour profiles of LH, FSH, and testosterone levels in three intact rams. Also shown are the LH responses to an intravenous injection of LHRH given at the end of the 24-hour bleed (0800 hours).

TABLE 1. Characteristics of the Serum LH Profiles of Nonimplanted Wethers 2, 4, and 6 Weeks after Castration*

	Wether	Week 2	Week 4	Week 6
Mean LH (ng/ml)	1	18.7	21.6	24.6
	2	12.3	18.9	19.8
	3	6.4	10.1	15.9
Basal LH† (ng/ml)	1	12.0	16.7	19.8
	2	7.5	11.9	15.8
	3	4.7	6.8	11.8
Number of LH peaks per 8 hours	1	9	10	11
	2	9	11	11
	3	10	9	10
LH peak‡ amplitude (ng/ml)	1	15.7	11.7	10.2
	2	8.7	14.3	9.8
	3	3.9	7.7	9.6

* LH secretory profiles were determined by bleeding wethers at 10-minute intervals for eight hours starting at 0800 hours.

† Calculated from points not associated with either the ascending or descending portions of LH peaks.

‡ Represents LH peak height minus basal LH and is presented as the average for each wether.

Discussion

Large differences among rams in the profiles of gonadotropin secretion similar to those observed in the present study have been reported previously (Wilson and Lapwood, 1978; D'Occhio et al, 1982a,b). Although the physiologic basis for these differences has not been determined, the present findings suggest that the inherent pattern of gonadotropin release in individual rams may have a bearing on LH and FSH responses to castration and testosterone replacement therapy. Castration led to the development of a rhythmic pulsatile pattern of LH secretion in nonimplanted wethers (Riggs and Malven, 1974; D'Occhio et al, 1982a). Even though all three nonimplanted wethers showed this pattern of LH release, there were consistent individual differences in mean LH concentrations across the study. Corresponding differences were also observed in mean FSH levels at six weeks. It is

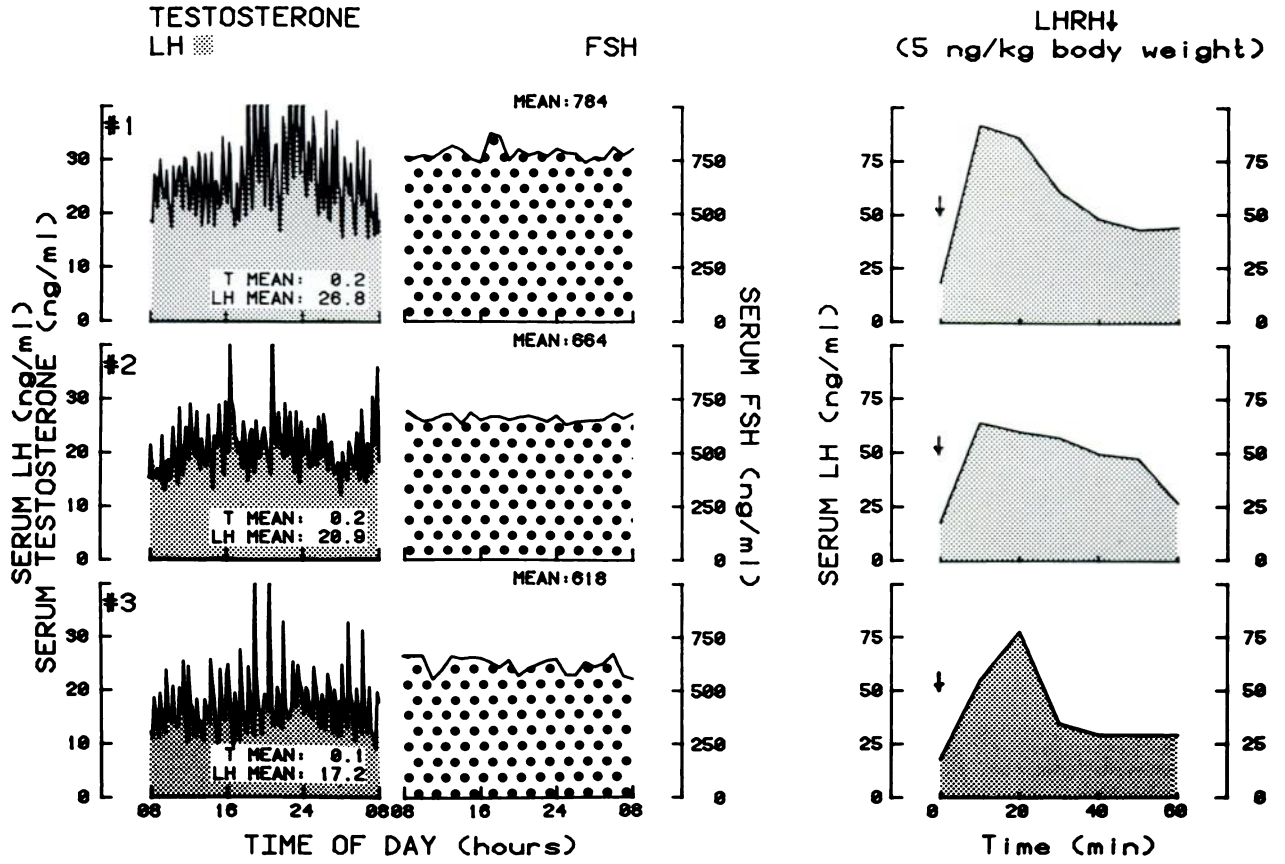

Fig. 2. Serum LH, FSH, and testosterone profiles and LH responses to LHRH in nonimplanted wethers six weeks after castration. The LHRH was administered intravenously at the end of the 24-hour bleed (0800 hours).

proposed that the differences in LH and FSH levels observed in nonimplanted wethers were due, in part, to inherent differences in gonadotropin secretion prior to castration. Such an explanation might also account for the failure of testosterone to suppress gonadotropin levels in two wethers that may have had relatively high serum LH and testosterone levels at the time of castration (eg, levels similar to those observed in ram number 3). It is acknowledged that the above conclusions are based on data from the limited number of animals that were available for this study. The present findings, therefore, need to be substantiated by characterizing gonadotropin profiles in individual animals both before and after castration.

Sensitivity to steroid feedback in male sheep is influenced by photoperiod (Parrott and Davies, 1979; Schanbacher, 1980b). The present study was performed at a time of the year (autumn) when the hypothalamic-pituitary axis is thought to be least sensitive to negative feedback. This also corresponds to the time of year when differences among rams in gonadotropin secretion are most accentuated (Wilson and Lapwood, 1978). In previous studies with rams conducted during the spring (a time when rams are particularly sensitive to steroid feedback), gonadotropin responses to testosterone therapy after castration were similar (D'Occhio et al, 1982a,b). Combined, these studies suggest that differential responses to testosterone replacement therapy may be more evident at certain times of the year in seasonal breeding species.

Long-term castrated male sheep develop an insensitivity to testosterone feedback as a result of the prolonged absence of gonadal steroids (Edgerton and Baile, 1977; Parrott and Davies, 1979). It is unlikely that similar changes account for the failure of testosterone to suppress LH in

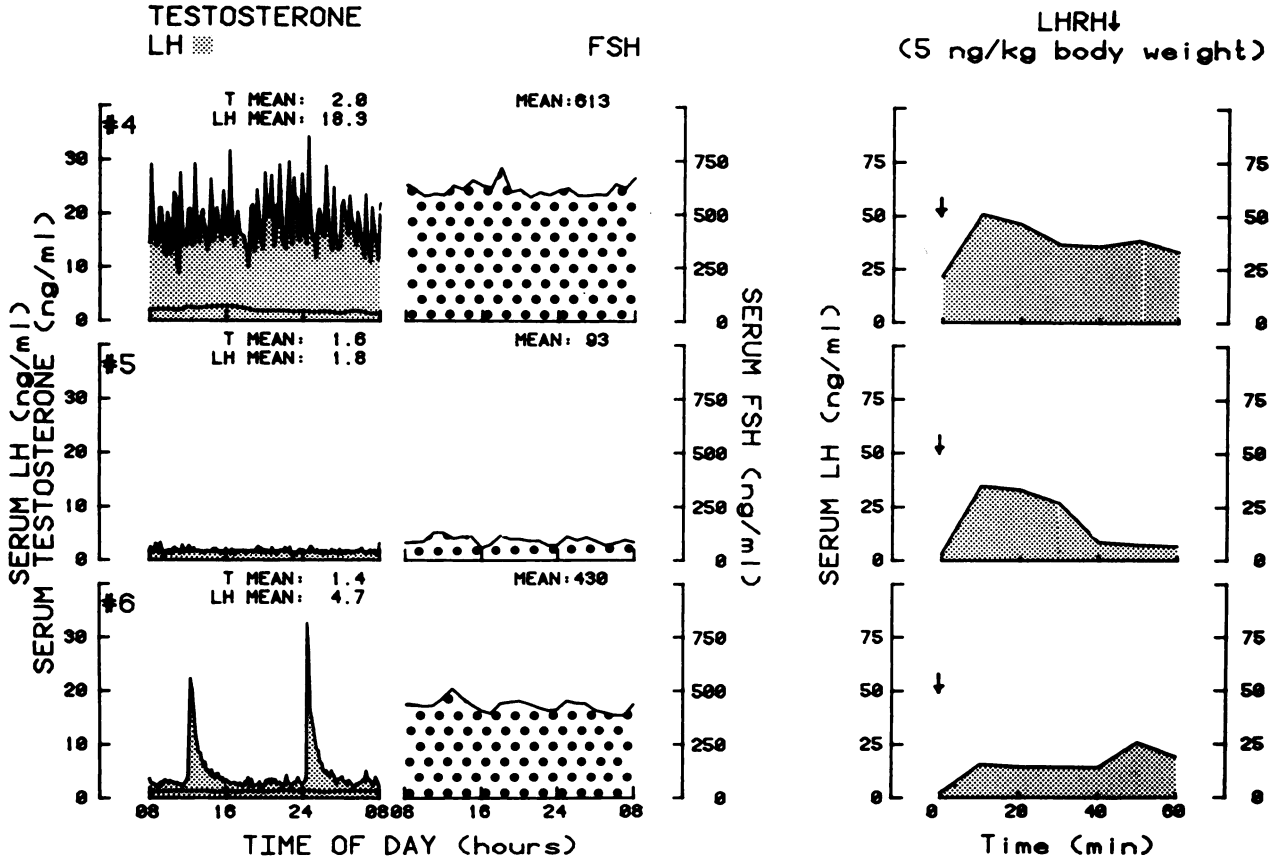

Fig. 3. Serum LH, FSH, and testosterone profiles and LH responses to LHRH in wethers implanted with four testosterone capsules. Wethers were implanted at the time of castration and bled six weeks later at 10-minute intervals for 24 hours. The LHRH was administered intravenously at the end of the 24-hour bleed (0800 hours).

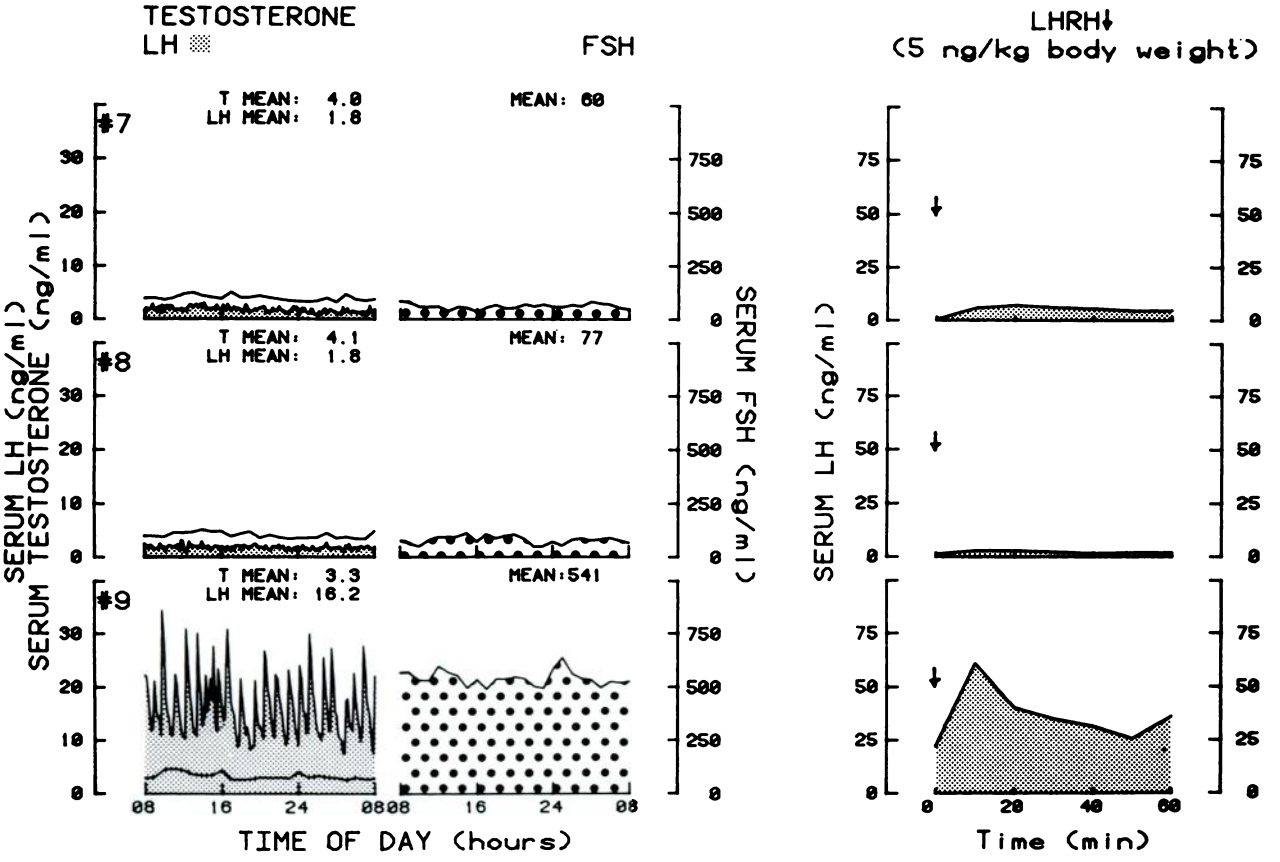

Fig. 4. Serum LH, FSH, and testosterone profiles and LH responses to LHRH in wethers implanted with eight testosterone capsules. Wethers were implanted at the time of castration and bled six weeks later at 10-minute intervals for 24 hours. The LHRH was administered intravenously at the end of the 24-hour bleed (0800 hours).

two wethers in the present study since testosterone was provided concurrently with castration. However, it is noteworthy that serum testosterone levels in implanted wethers were less than the mean levels observed in intact rams. The possibility remains, therefore, that gonadotropin secretion might have been effectively inhibited in all animals had higher levels of testosterone been provided. Nevertheless, differential responses of wethers to a given dose of testosterone, together with large differences in gonadotropin secretion among nonimplanted wethers, is viewed as evidence that individual rams have different inherent capacities to secrete gonadotropin. This conclusion is further supported by the positive relationship between serum LH levels and pituitary LH responses to exogenous LHRH in both rams and wethers.

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