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TESTOSTERONE REGULATION OF LUTEINIZING HORMONE AND FOLLICLE STIMULATING HORMONE SECRETION IN YOUNG MALE LAMBS¹

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Summary

Polydimethylsiloxane (Silastic) capsules containing crystalline testosterone were implanted subcutaneously in castrate lambs and evaluated for their ability to (1) provide stable, physiological concentrations of serum testosterone and (2) regulate secretion of LH and FSH. Testosterone was nondetectable ($<.2$ ng/ml) between 12 and 24 weeks of age in the serum of castrate lambs implanted with empty capsules but was increased at those ages in lambs implanted with 20 cm ($1.2 \pm .1$ and $.5 \pm .1$ ng/ml), 60 cm ($2.8 \pm .1$ and $.9 \pm .1$ ng/ml) and 180 cm ($6.6 \pm .3$ and $1.9 \pm .2$ ng/ml) of testosterone-filled Silastic tubing. Serum concentrations of LH and FSH were suppressed by exogenous testosterone and did not exceed concentrations in intact control rams as long as serum testosterone concentrations were greater than 1 ng/ml. In conclusion, simultaneous suppression of serum LH and FSH by concentrations of testosterone that cause a dose-dependent stimulatory effect on accessory sex gland weights indicates that testosterone is a physiological regulator of gonadotropin secretion in ram lambs. Although testosterone *per se* may not be directly responsible for negative feedback on LH and FSH secretion, the results

presented herein question the need for a nonsteroidal "inhibin-like" testicular hormone to regulate FSH in lambs.

(Key Words: Lambs, Testosterone Implants, Negative Feedback, Gonadotropin Secretion.)

Introduction

Hypophysectomy of young lambs stops growth of the testis and prevents onset of spermatogenesis, whereas treatment with luteinizing hormone (LH) and follicle stimulating hormone (FSH) overcomes these effects (Courot, 1967). LH stimulates the interstitial cells of Leydig within the testis to produce and secrete steroids, principally testosterone. The effects of FSH on the testis are not as clear-cut; however, FSH apparently interacts with the Sertoli cells within the seminiferous tubules to initiate and maintain spermatogenesis. Thus, the endocrine and exocrine functions of the testes appear to be regulated by these two gonadotropins.

The testis apparently controls the secretion of LH and FSH by negative feedback, because concentrations of both increase in peripheral blood after castration. Testosterone reduces the increased levels of LH and FSH characteristics of castrate rams (Pelletier, 1970; Crim and Geschwind, 1972; Schanbacher and Ford, 1977), but other steroids are also effective (Riggs and Malven, 1974; Karsh and Foster, 1975; Edgerton and Baile, 1977; Schanbacher and Ford, 1977; Parrott and Davies, 1979). Hormones derived from the tubular compartment of the testis are thought to be involved in feedback control of FSH. Indeed, the increase in LH and FSH after selective destruction of the germinal epithelium in rams (Schanbacher and Ford, 1977) appears to require the involvement of hormones other than testosterone. Under normal physiological circumstances, however, testosterone may be

¹The author acknowledges the technical assistance of Ms. Becky Weber and Ms. Donna Taubenheim and the cooperation of the Nebraska Agr. Exp. Sta., Univ. of Nebraska, Lincoln. Portions of these data were presented at the 1979 Annu. Meet. of the ASAS. (Schanbacher, 1979), Univ. of Arizona, Tucson, July 28-31.

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the testicular hormone which regulates LH and FSH secretion in ram lambs.

Single and short-term series injections of testosterone or testosterone propionate neither provide a suitable treatment regimen nor produce stable blood levels of testosterone. The availability of testosterone-filled Silastic capsules that deliver a constant and predictable dose of hormone (Stratton *et al.*, 1973; Ewing *et al.*, 1976) facilitates interpretation of steroidal control of LH and FSH secretion. In this study serum concentrations of LH and FSH were determined during pubertal development of intact, castrate and testosterone-treated castrate ram lambs.

Materials and Methods

The relationship between exogenous testosterone and serum concentrations of LH and FSH was determined in lambs assigned to a companion study whose objective was to determine the influence of testosterone on the performance of the growing-finishing animal (Schanbacher *et al.*, 1980). Polydimethylsiloxane (Silastic)⁴ capsules containing crystalline testosterone were constructed and then tested in castrate lambs weighing approximately 30 kilograms. Silastic tubing (3.35 mm inside diameter $\times$ 4.65 mm outside diameter) was cut into 10- or 30-cm lengths and sealed at one end with Silastic Adhesive Type A. The tubes were packed with crystalline testosterone⁵ 24 hr later. The open ends were sealed with adhesive, and the capsules were stored in a desiccator until needed. Immediately before use, the Silastic capsules were sterilized by rinsing with absolute ethanol followed by a surgical disinfectant. Capsules were implanted subcutaneously with a trocar just behind the shoulder under local anesthesia.

After the number of Silastic capsules needed to maintain physiological concentrations of serum testosterone was determined, the study was begun. Fifty Suffolk crossbred ram lambs were weaned at approximately 10 weeks of age (15 kg) and used to determine the effect of castration and testosterone replacement therapy on LH and FSH secretion. Ten lambs were left intact to serve as controls, and the remainder were castrated. The wethers were

divided into four groups of 10 and implanted on the day of castration with (1) two 10-cm Silastic capsules to provide a low blood concentration of testosterone (W_L), (2) two 30-cm Silastic capsules to provide an intermediate blood concentration of testosterone (W_I) and (3) six 30-cm Silastic capsules to provide a high blood concentration of testosterone (W_H). Ten castrate controls (W) were implanted with an empty Silastic capsule. Blood samples were collected from each lamb by jugular venipuncture at 12, 16, 20 and 24 weeks of age, and serum was assayed for concentrations of LH, FSH (Schanbacher and Ford, 1977) and testosterone (Schanbacher, 1980a).

Testosterone concentrations were monitored in serum from intact, castrate and testosterone-treated castrate lambs. The seminal vesicles and bulbourethral glands were weighed at the time of slaughter in an effort to estimate the biological potency of the exogenous testosterone. Silastic capsules were retrieved from the carcasses at the time of slaughter.

Split-plot analysis of variance was used to analyze the data.

Results

Preliminary Results with Testosterone-Filled Silastic Capsules. Castrate lambs weighing between 25 and 35 kg had low (.3 ng/ml) to non-detectable (<.2 ng/ml) concentrations of testosterone in serum. Implantation with testosterone-filled Silastic capsules caused serum testosterone to rise in proportion to the dosage (capsule length) administered. Not only were blood concentrations stable within individual lambs, but given the weight of the lamb, the concentrations produced by the implants could be estimated before the capsules were implanted. Figure 1 shows the blood concentrations of testosterone in castrate lambs receiving the three dosages of testosterone via Silastic capsules and compares these patterns with the 24-hr testosterone secretory pattern for a representative intact lamb. Serum testosterone fluctuated in intact rams because of the episodic nature of the LH stimulus to the testes (Schanbacher and Ford, 1976). Testosterone-filled Silastic capsules, on the other hand, maintained essentially constant serum testosterone concentrations in castrate lambs.

Serum Testosterone, LH and FSH in Implanted Lambs. Serum testosterone concentra-

⁴ Dow Corning Corp., Midland, MI.

⁵ Sigma Chemical Co., St. Louis, MO.

Figure 1. Serum testosterone concentrations in an intact ram lamb (●) and three castrate lambs implanted with testosterone-filled Silastic capsules to provide low (X), intermediate (Δ) and high (◻) blood concentrations of testosterone. Note the episodic secretory pattern in the intact ram and the constant release provided by the Silastic implants. Nondetectable (—).

tions remained nondetectable throughout the experiment in castrate lambs implanted with an empty Silastic capsule (W), whereas serum testosterone in intact lambs increased gradually from low values at 12 weeks of age (0.6 ± 0.3 ng/ml) to relative high values at 24 weeks of age (3.1 ± 0.7 ng/ml). This pubertal increase in serum testosterone paralleled growth of the testes. Serum testosterone in implanted lambs was dictated by the length and number of capsules administered as well as the animal's body weight. Testosterone decreased gradually and consistently between 12 and 24 weeks of age in serum of wethers implanted with two 10-cm Silastic capsules (W_L ; 1.2 ± 0.1 to 0.5 ± 0.1 ng/ml), wethers implanted with two 30-cm Silastic capsules (W_I ; 2.8 ± 0.1 to 0.9 ± 0.1 ng/ml) and wethers implanted with six 30-cm Silastic capsules (W_H ; 6.6 ± 0.3 to 1.9 ± 0.2 ng/ml); This decrease in serum testosterone was not a result of capsule depletion, because the capsules were used to maintain expected serum testosterone concentrations in a subsequent implant experiment.

Castration resulted in increased serum concentrations of LH and FSH; however, maximum concentrations were not reached until lambs were 20 weeks of age (figures 2 and 3). LH and FSH concentrations in intact lambs did not change during the course of the study

Figure 2. Serum LH concentrations in intact rams (R), castrate lambs (W) and castrate lambs treated with low (W_L), intermediate (W_I) and high (W_H) dosages of exogenous testosterone. Means are plotted at 12, 16, 20 and 24 weeks of age for each group ($n=10$). Assay sensitivity, .5 ng/ml.

in spite of increasing concentrations of testosterone. In castrates, concentrations of LH and FSH were suppressed by all three dosages of testosterone at 16 weeks of age. Interestingly, serum LH in implanted lambs was reduced to concentrations below those seen in intact lambs (figure 2), whereas serum FSH concentrations were similar to those in intact lambs (figure 3). The increase in serum LH and FSH appeared as serum testosterone fell below 1 ng/ml. Furthermore, these results suggest that the decrease in feedback inhibition of LH and FSH occurs simultaneously. At 24 weeks, serum LH and FSH had reached concentration characteristics of castrates in lambs treated with low levels of testosterone (W_L ; 0.5 ± 0.1 ng/ml), but both gonadotropins remained low in lambs given high levels of testosterone (W_H ; 1.9 ± 0.2 ng/ml).

Figure 3. Serum FSH concentrations in intact rams (R), castrate lambs (W) and castrate lambs treated with low (W_L), intermediate (W_I) and high (W_H) dosages of exogenous testosterone. Means are plotted at 12, 16, 20 and 24 weeks of age for each group ($n=10$). Assay sensitivity, 30 ng/ml.

Accessory Sex Glands in Implanted Lambs. Weights of the seminal vesicles and bulbourethral glands of intact, castrate control and testosterone-treated castrate lambs are presented in table 1. Both glands were regressed in the castrate lamb; however, weights of these glands appeared to be responsive to exogenously administered testosterone in a dose-dependent manner.

Discussion

The testes of domestic rams, like the testes of other mammals, produce and secrete the principal androgen, testosterone (Schanbacher, 1976; Schanbacher and Ford, 1977). Both testosterone (Garnier *et al.*, 1977; Schanbacher and Ford, 1977) and testosterone propionate (Pelletier, 1970; Crim and Geschwind, 1972; Garnier *et al.*, 1977; Parrott and Davies, 1979) have been given by injection to decrease the high concentrations of LH characteristic of castrates. In general, such studies do

TABLE 1. WEIGHTS OF SEMINAL VESICLES AND BULBOURETHRAL GLANDS AT THE TIME OF SLAUGHTER FOR INTACT (R), CASTRATE (W) AND CASTRATE LAMBS TREATED WITH LOW (W_L), INTERMEDIATE (W_I) OR HIGH (W_H) LEVELS OF TESTOSTERONE VIA SILASTIC CAPSULES^a

Group	No.	Seminal vesicles, g	Bulbourethral glands, g
R	9	6.42 ± .66 ^b	2.50 ± .27 ^b
W	10	.42 ± .05 ^c	.51 ± .04 ^c
W_L	8	1.67 ± .08 ^d	1.39 ± .09 ^d
W_I	9	5.19 ± .42 ^b	2.13 ± .14 ^b
W_H	9	9.57 ± .49 ^e	3.24 ± .23 ^e

^aValues are mean ± SEM. Means not followed by the same superscript are different ($P<.05$).

not allow one to determine whether physiological concentrations of testosterone can regulate serum gonadotropin concentrations, because single and short-term series injections of testosterone in long-term castrate animals simply do not reflect endogenous testosterone secretory patterns.

Decreases in LH and FSH in testosterone-treated castrate rams to concentrations equal to or below those found in intact rams suggest that both gonadotropins are regulated by testicular steroids. Castration of young rams resulted in increased serum LH and FSH; however, it took 6 to 10 weeks for concentrations to reach those characteristic of the mature castrate adult. The gradual change in serum gonadotropins after castration may reflect maturational changes in the central nervous system as well as simple postcastration effects. Maintenance of serum LH and FSH in the presence of increasing serum concentrations of testosterone in developing ram lambs supports the contention that a change in sensitivity of the hypothalamus and pituitary takes place during puberty. Similar changes in the concentrations of LH and testosterone were observed by Lee *et al.* (1976a) in the developing ram lamb. Initiation of pulsatile LH secretion (Foster *et al.*, 1978) and heightened pituitary sensitivity to LH-RH stimulation (Lee *et al.*, 1976b) in the young lamb are thought to be centrally important for the initiation of puberty. Foster and Ryan (1979) have shown recently that puberty in the ewe lamb coincides with a decrease in the inhibitory feedback of estradiol on tonic LH secretion.

Inhibition of the postcastration increase in LH by testosterone replacement therapy in this experiment is supportive of results from similar studies with male rats (Damassa *et al.*, 1976; Moger, 1976), dogs (Vincent *et al.*, 1979) and rhesus monkeys (Plant *et al.*, 1978). Simultaneous, dose-dependent suppression of LH and FSH by testosterone is somewhat surprising in view of the suggestion that FSH secretion is largely regulated by a nonsteroidal factor that is thought to originate within the Sertoli cells of the seminiferous tubule (DeJong and Sharpe, 1977; Setchell *et al.*, 1977). Because other investigators have shown that testosterone can regulate serum FSH concentrations (Crim and Geschwind, 1972; Moger, 1976; Plant *et al.*, 1978), we must conclude that physiological concentrations of testosterone are important in the control or modulation of FSH secretion. In this study, LH and FSH escaped from the negative influence exerted by testosterone, but only when testosterone concentrations fell below 1 ng/ml. It has been suggested that the decrease in testosterone in young implanted animals is related to dilution effects caused by the animal's growth (Moger, 1976) and not to capsule failure or altered steroid metabolism by the animal. Implantation of mature wethers with testosterone to maintain serum levels near 4 ng/ml also reduces serum LH concentrations (Schanbacher, 1980b).

The results presented herein indicate that implantation of Silastic capsules containing testosterone can maintain serum testosterone concentrations in castrate rams at concentrations similar to those observed for intact rams. On the basis of suppression of serum gonadotropins and the dose-dependent stimulatory effects on accessory sex gland weights, the need for episodic testosterone secretion is not readily apparent. However, the difference between constant steroid release via Silastic capsules and normal episodic release by the testes should be kept in mind, particularly since Resko *et al.* (1977) were unable to suppress LH and FSH in the castrate male monkey with testosterone capsules alone, whereas Plant *et al.* (1978) were successful when they supplemented capsules with pulsatile (injected) testosterone. In the castrate ram, however, administration of testosterone by Silastic capsules appears to be a suitable method of replacement therapy.

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