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M. J. D'Occhio
University of Nebraska-Lincoln

B. D. Schanbacher
USDA-ARS

J. E. Kinder
University of Nebraska-Lincoln, kinder.15@osu.edu

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Testosterone feedback on FSH secretion in male sheep*

M. J. D'Occhio, B. D. Schanbacher† and J. E. Kinder

Department of Animal Science, University of Nebraska, Lincoln, Nebraska 68583, and †Roman L. Hruska U.S. Meat Animal Research Center, Agricultural Research Service, U.S. Department of Agriculture, Clay Center, Nebraska 68933, U.S.A.

Summary. Testosterone-filled Silastic capsules were implanted into mature rams at the time of castration (wethers). After 6 weeks, a tonic pattern of FSH secretion was observed in rams, wethers and wethers implanted with testosterone. Castration caused serum concentrations of FSH to increase 4–5-fold. Relatively low serum concentrations of testosterone (25–50% of intact ram values) did not significantly affect FSH secretion in wethers, but wethers exposed to concentrations of testosterone equivalent to those of intact rams had serum concentrations of FSH similar to those of intact rams. We suggest that testosterone feedback can account for the gross differences in FSH observed between intact and castrated male sheep.

Introduction

It is generally accepted that one hypothalamic releasing hormone, gonadotrophic hormone releasing hormone, stimulates the release of both LH and FSH in the ram (Lincoln, 1979). However, feedback mechanisms which modulate the secretion of LH and FSH may differ. Feedback on LH, for example, is thought to be mediated exclusively by gonadal steroids (Crim & Geschwind, 1972; Schanbacher & Ford, 1977; Parrott & Davies, 1979). FSH secretion, on the other hand, is influenced by both gonadal steroids (Schanbacher & Ford, 1977; Parrott & Davies, 1979; Schanbacher, 1979; 1980) and a non-steroidal factor referred to as inhibin (Setchell & Jacks, 1974; Baker *et al.*, 1976; Blanc & Dacheux, 1976; Keogh *et al.*, 1976). The specific increase in FSH in rams after hemicastration provides the classical type of evidence for the existence of inhibin (Walton, Evins & Waites, 1978; Walton, Evins, Hillard & Waites, 1980).

The purpose of the present study was to investigate the finer control of FSH secretion by testosterone in rams and at the same time examine further the inhibin hypothesis.

Materials and Methods

Experimental procedure. The testosterone implants were prepared from Silastic medical grade tubing (3·35 mm i.d. × 4·65 mm o.d.: Dow Corning, Midland, Michigan, U.S.A.) as described by Schanbacher (1980). The implants were 15 cm or 30 cm in length and were placed subdermally over the ribs at the time of castration (Schanbacher, 1980).

Fifteen mature Finnish Landrace rams (84·6 ± 2·4 (s.e.m.) kg body weight) were randomly assigned to 1 of 5 equal groups during the spring of 1981. One group of rams served as intact controls. The remaining animals were surgically castrated under xylazine analgesia and were

* Reprint requests to Dr B. D. Schanbacher.

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treated with no implants (control wethers), one 15-cm implant; two 30-cm implants, or four 30-cm implants. The implants were left in place for 6 weeks since previous studies in the ewe had indicated that gonadotrophin secretion stabilizes within this time after gonadectomy (Reeves, O'Donnell & Denorscia, 1972).

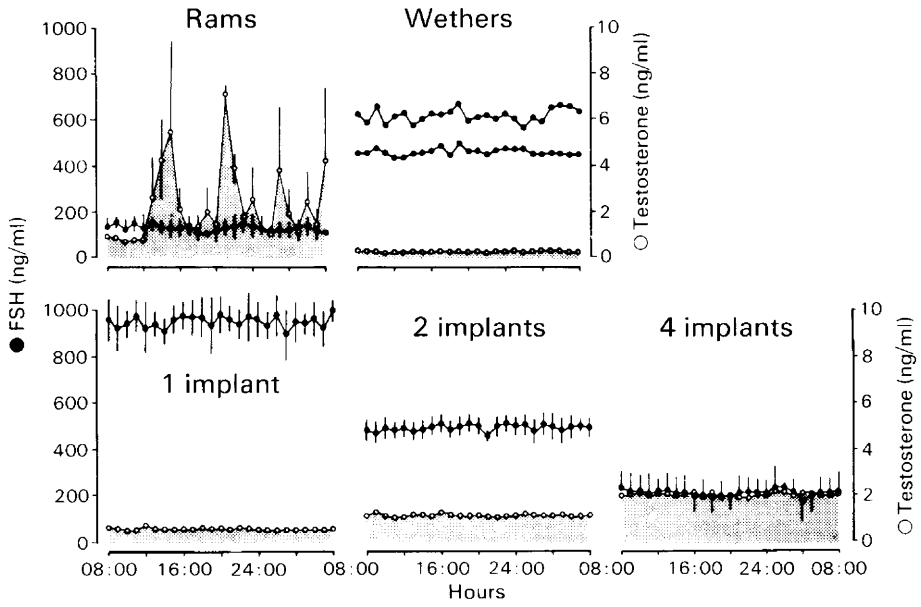
After 41 days of treatment, a jugular cannula was inserted into each animal which was bled at hourly intervals from 08:00 h the next day for 24 h. Blood was allowed to clot and the serum obtained after centrifugation was stored at −20°C until assayed for testosterone and FSH.

Hormone assays. Serum testosterone concentrations were determined by a direct double-antibody radioimmunoassay which has a sensitivity of 0·1 ng/ml (Schanbacher & D'Occhio, 1982). FSH concentrations were measured using a double-antibody radioimmunoassay (Schanbacher & Ford, 1977). This assay has a sensitivity of 6 ng/ml. For each hormone, all samples were assayed together and the intra-assay coefficients of variation of duplicate samples were 8·3% for FSH and 4·2% for testosterone.

Statistical analysis. Regression analysis was applied to the data to ascertain the relationships between the implant treatments and serum testosterone and FSH concentrations. The responses in each group were compared with the hormone values in intact rams by a t test for populations with unequal variances (Dixon & Massey, 1969). The significance level for all analyses was set at $P < 0{\cdot}05$.

Results

The 24-h serum testosterone concentrations are shown in Text-fig. 1. Serum testosterone in intact rams (2·25 ± 0·49 ng/ml; mean ± s.e.m. of individual 24-h means) showed episodic pulses which were asynchronous between animals. The number of testosterone pulses per 24 h (1–5) varied



Text-fig. 1. Serum testosterone and FSH profiles in rams, control wethers, and wethers bearing one 15-cm implant, two 30-cm implants or four 30-cm implants which had been implanted at the time of castration 6 weeks earlier. Results are presented as the mean of 3 animals (except for control wethers, for which individual FSH profiles are plotted) with the s.e.m. indicated by the vertical bars. Where no s.e.m. is indicated, the variation was smaller than the symbol.

between rams, giving rise to appreciable differences in 24-h mean testosterone concentrations (range 1·39–3·07 ng/ml). There was a linear relationship ($y = 0{\cdot}28 + 0{\cdot}43x$; $r^2 = 0{\cdot}98$) between the number of testosterone implants and serum testosterone concentration in wethers. The stable testosterone concentrations in animals with implants indicated a constant release of hormone from the implants over the 6-week period. Testosterone levels in control wethers (0·21 and 0·23 ng/ml; N = 2 since one animal died) and wethers with the 15-cm (0·54 ± 0·01 ng/ml) or two 30-cm (1·11 ± 0·08 ng/ml) implants were significantly lower than those in rams. Wethers with four 30-cm implants had testosterone levels (2·01 ± 0·09 ng/ml) that were similar to those of rams.

The FSH profiles showed only minor fluctuations within animal for rams and wethers (Text-fig. 1). By 6 weeks after castration, control wethers had significantly higher FSH levels (610 and 457 ng/ml) than did rams (127 ± 35 ng/ml). The dose-response relationship between the number of testosterone implants and serum FSH concentration was best described by a quadratic regression ($y = 747{\cdot}9 - 31{\cdot}4x - 27{\cdot}4x^2$; $r^2 = 0{\cdot}63$). Wethers with a 15-cm or two 30-cm implants had serum FSH levels of 943 ± 72 and 480 ± 31 ng/ml, respectively. FSH levels in wethers with four 30-cm implants (205 ± 71 ng/ml) did not differ from those in rams.

Discussion

The biological importance of inhibin in rams has been questioned in recent studies since it has been shown that gonadal steroids suppress both LH and FSH in cryptorchid rams and wethers (Schanbacher & Ford, 1977; Parrott & Davies, 1979, Schanbacher, 1979, 1980). In the present study, wethers treated with 4 testosterone implants had normal concentrations of serum testosterone and showed a reduction in serum FSH from values typical of castrates to those of intact animals. These results therefore confirm that testosterone feedback can account for the gross differences in serum FSH observed between wethers and rams. Similar findings have also been reported for the rat (Gay & Kerlan, 1978). From studies of male rats, it has been suggested that constant physiological concentrations of testosterone provide a stronger feedback signal to gonadotrophin secretion than do similar mean concentrations resulting from endogenous fluctuations in testosterone secretion (Moger, 1976; Damassa, Kobashigawa, Smith & Davidson, 1976). If this is true of sheep, FSH concentrations in wethers with four testosterone implants should perhaps have been lower than those in rams. Since this was not the case, it may be that testosterone normally synergizes with inhibin to regulate FSH concentrations in rams.

Serum FSH concentrations in wethers were not suppressed by circulating levels of testosterone that ranged from 25 to 50% of the values in rams. FSH in wethers with one 15-cm implant appeared to be elevated above levels normal for castrates but this was not borne out by the regression analysis and additional treatments with intermediate testosterone implant doses are required to characterize the FSH response within this range. Also, appreciable differences in FSH between wethers are normal (Schanbacher, 1979). Small numbers of animals were used in the present study and the results therefore do not justify the conclusion that FSH secretion is enhanced in wethers treated with low doses of testosterone. Nevertheless, serum FSH was increased in male rats receiving relatively small doses of 5α-dihydrotestosterone, the principal androgenic metabolite of testosterone (Mittler, Ertel & Ourednik, 1981). Although two testosterone implants had no effect on FSH in the present study, the same treatment altered the pattern of LH secretion in wethers (D'Occhio, Schanbacher & Kinder, 1982). This apparent differential effect of testosterone on LH and FSH may further indicate an inhibin component in the control of FSH secretion in rams.

In summary, this study has demonstrated that the gross differences in serum FSH between rams and wethers can be explained by testosterone feedback. However, the results do not exclude the possibility that the finer control of FSH secretion in rams may involve the combined actions of testicular androgens and inhibin.

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