J. Reprod. Fert. (1978) **53**, 67–70

Effects of sexual stimulation on plasma levels of LH and testosterone in rams from high- and low-fertility flocks

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Summary. Plasma LH and testosterone levels did not differ significantly between high and low fertility rams before or after sexual stimulation (ejaculation or teasing). Repeated stimulation caused significant elevation of mean plasma LH and an almost significant rise in testosterone concentration. Plasma testosterone peaks followed those of LH after 30–60 min. A single sexual stimulation did not always cause an LH peak or increase the mean LH level.

Introduction

Several groups of workers (Thimonier, Pelletier & Land, 1972; Bindon & Turner, 1974; Carr & Land, 1975) have found differences in the plasma LH concentrations of genetically distinct groups of ram lambs to be positively correlated with differences in the prolificacy of female relatives, but to our knowledge there are no comparable data on adult rams. Finnish Landrace rams had higher serum concentrations of LH and testosterone during a 24-h session in which they were allowed to mate freely with an oestrous ewe than when penned alone (Sanford, Palmer & Howland, 1974a), and differences between adult rams may therefore be easier to detect during periods of sexual stimulation. The present study was an examination of plasma levels of LH and testosterone in high- and low-fertility Romney rams before and during sexual stimulation.

Materials and Methods

The experiments were carried out during the spring (October) because Sanford, Winter, Palmer & Howland (1974b) found that plasma LH peaks in rams occurred less frequently in the spring than in the winter months, and hence a more stable baseline level of LH secretion was expected against which the effects of copulation could most effectively be measured.

The 6 high-fertility (H) Romney rams, 2–3 years old, were obtained from a flock selected primarily for the the number of lambs weaned (Hight, Gibson, Wilson & Guy, 1975) and were compared with 6 low-fertility (L) 2-year-old Romney rams from a separately run randomly bred flock. In the 4 years before this investigation the percentages of lambs born to the ewes lambing in the two groups was 164 and 114 respectively. The rams were penned singly indoors and fed pellets containing 50% maize meal and 50% lucerne. They had been trained for semen collection with an artificial vagina.

In Exp. 1 each of the rams was bled 12 times at 30-min intervals, the last 6 blood samples from each ram being preceded by sexual stimulation which began 15 min before the samples were taken. Sexual stimulation consisted of an attempted collection of semen into the artificial vagina while using an oestrous ewe as a teaser. Unsuccessful attempts were terminated after 5 min.

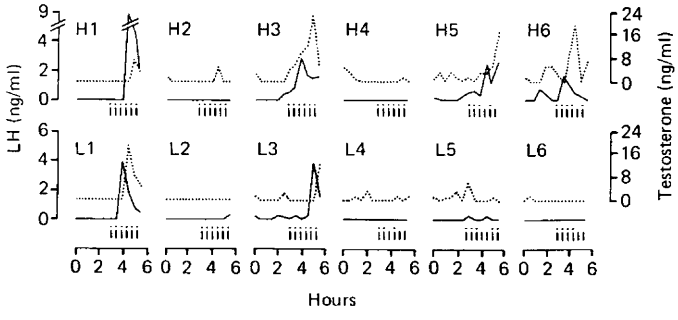
Four of the high-fertility rams (H1–H4) and 3 of the low-fertility rams (L1–L3) were compared in Exp. 2, each ram being given two forms of sexual stimulation on 2 different days. In the first (mating) the ram was allowed to mate an oestrous ewe with a single ejaculation, and the second (teasing) consisted of 2½ min of contact with an oestrous ewe during which mounting took place but intromission and ejaculation were prevented. Starting 2 h before the sexual stimulation began, blood samples were taken every 30 min for 1½ h, every 5 min for the next 60 min, and then every 30 min for a further 3 h.

The blood samples (15 ml) were collected from an indwelling jugular cannula into heparinized test tubes, which were immediately placed in a refrigerator at 4°C. After centrifugation at 4°C aliquots of plasma were stored at −15°C until assayed. LH concentrations were determined in all the plasma samples but testosterone was measured only in the samples collected in Exp. 1.

The plasma LH concentrations were determined by the double-antibody radioimmunoassay method of Niswender, Reichert, Midgley & Nalbandov (1969) with the minor modifications described by Barrell (1976). Plasma samples and standards were measured in triplicate, with all samples from a particular ram included in the same assay. Results were analysed by the method of Berger, Lee & Rennie (1972). The sensitivity of the assay ranged from 0·04 to 0·11 ng/ml and within-assay coefficients of variation were 4·6 and 7·5% for two samples with mean LH concentrations of 4·17 and 0·55 ng/ml respectively. The corresponding between-assay coefficients of variation were 13·1 and 12·7%. Plasma levels of testosterone were measured by a modification of the method of Smith & Hafs (1973) as described by Barrell (1976). This method had a sensitivity ranging from 0·06 to 0·13 ng/ml. All testosterone estimations were performed in a single assay and the data were computed as for LH. The within-assay coefficients of variation for two samples with mean testosterone concentrations of 0·19 and 7·05 ng/ml were 5·8 and 16·6% respectively.

Results and Discussion

Seven of the 12 rams in Exp. 1 successfully ejaculated into the artificial vagina on each of the 6 occasions when the stimulus was offered. Of the remainder, 1 H ram failed to ejaculate on one occasion, 1 H and 2 L rams failed on two occasions, and 1 L ram failed on three occasions. For these animals the stimulus was regarded as one of teasing.



Text-fig. 1. The effect of repeated sexual stimulation, as ejaculation (i) or teasing (ı), on plasma LH (——) and testosterone (····) concentrations in high-fertility (H) and low-fertility (L) rams.

The patterns of plasma LH and testosterone concentrations for the individual rams are shown in Text-fig. 1. Only one obvious LH peak was recorded during the period before stimulation (Ram H6) and this may be attributed to the random pattern of spontaneous LH discharge shown to occur in rams by Sanford *et al.* (1974b) and Wilson, Barrell, Carter & Lapwood (1975). This peak was followed by a transient rise in plasma testosterone concentration. During the period of repeated sexual stimulation, 4 of the 6 H rams and 2 of the 6 L rams showed an elevation of plasma LH concentrations to peak levels of 1·7 to 8·8 ng/ml. Each of these LH peaks was followed by a rise in the concentration of testosterone with maximum recorded levels ranging from 8·1 to 23·3 ng/ml. The interval between contiguous peak levels of plasma LH and testosterone varied between 30 and 60 min in this study. A similar temporal relationship between LH and testosterone has been recorded by other workers for the Romney ram, both in the absence of stimulation (Barrell, 1976) and following the administration of gonadotrophin-releasing hormone (Wilson, 1976). A similar interval has been found in Finnish Landrace rams (Sanford *et al.*, 1974b).

Mean LH and testosterone levels before and during the period of stimulation are shown in Table 1. The hormone concentrations have been transformed into logarithms, such that log hormone concentration = $100 \times \log_{10}(x + 1{\cdot}1)$ where x = plasma hormone concentration in ng/ml (Barrell, 1976). This transformation takes account of the positive correlation between the mean and standard error for different subgroups of hormone data and eliminates zeros. However, statistical analysis of individual LH levels in Exp. 1 was invalidated because of the large number of zeros in the unstimulated period which, even when transformed, yielded an almost invariant statistic. Nevertheless, calculation of the 5% confidence limits on the mean difference between the concentrations, during the 'stimulated' and 'unstimulated' periods showed that the mean LH concentration during stimulation was greater ($P < 0{\cdot}05$) than in the unstimulated period. A positive LH response to copulation has been reported previously for the ram by Sanford *et al.* (1974a).

Table 1. Effect of sexual stimulation through multiple ejaculations on mean (± s.e.m.) plasma LH and testosterone levels in rams of high and low fertility (Exp. 1)

Fertility group	No. of rams	LH conc.*		Testosterone conc.*	
		Unstimulated	Stimulated	Unstimulated	Stimulated
High	6	6·2 ± 1·1	27·3 ± 8·7	30·3 ± 7·2	59·8 ± 15·1
Low	6	4·7 ± 0·7	14·3 ± 4·2	15·7 ± 5·7	32·8 ± 12·3
All rams	12	5·4 ± 0·7	20·8 ± 5·4	23·8 ± 4·8	46·3 ± 10·1

* Values are $100 \times \log_{10}(x + 1{\cdot}1)$ where x is hormone concentration in ng/ml.

Table 2. Comparison of the effect of mating and teasing on mean (± s.e.m.) plasma LH levels* in rams of high and low fertility (Exp. 2)

Fertility group	No. of rams	Mating		Teasing	
		Before	After	Before	After
High	4	12·5 ± 4·5	10·0 ± 3·6	10·8 ± 2·6	7·3 ± 0·8
Low	3	7·3 ± 1·2	12·3 ± 5·4	8·3 ± 1·9	14·7 ± 8·2
All rams	7	10·3 ± 2·7	11·0 ± 2·8	9·7 ± 1·6	10·4 ± 3·5

* Values are $100 \times \log_{10}(x + 1{\cdot}1)$ where x is hormone concentration in ng/ml.

An analysis of variance revealed that the mean levels of testosterone did not differ significantly between the high- and low-fertility rams, although the difference between testosterone levels in the 'stimulated' and the 'unstimulated' periods approached significance at the 5% level. Sanford *et al.* (1974a) reported a similar increase in testosterone levels in two mature Finnish Landrace rams during a period of natural mating, whereas Purvis, Illius & Haynes (1974) reported that testosterone secretion was not affected by copulation. However, Illius, Haynes & Lamming (1976) subsequently found increases in plasma testosterone after copulation in some rams which had previously been kept in close proximity to ewes, but D'Occhio & Brooks (1976) reported that the plasma testosterone level of rams was not influenced by contact with oestrous ewes and was not correlated with the ram's performance in a standard libido test. The 'high versus low fertility ram × stimulated versus unstimulated period' interaction was not significant, which suggests that the period of sexual stimulation did not improve the ability to detect differences in hormone concentration between high- and low-fertility rams.

In Exp. 2, marked elevations in LH concentration occurred in 3 of the rams, with maximal recorded levels ranging from 1·4 to 8·1 ng/ml: Rams L1 and H3 showed an increase in LH levels at the end of the monitoring period, 3½ h after mating, while Ram L3 showed an LH peak 1½ h after teasing and a second elevation after a further 2 h. No obvious elevations above mean prestimulation LH levels were evident in the remaining 4 rams. The transformed values for mean plasma LH levels in the high- and low-fertility rams before and after mating and teasing are shown in Table 2. An analysis of variance revealed no significant differences between the high- and low-fertility rams, the mating and teasing

treatments, or the prestimulation and post-stimulation periods, and no significant interactions were evident. This experiment shows that a single sexual stimulation, either teasing or ejaculation, will not necessarily result in a rise in plasma LH, and confirms the finding of Sanford *et al.* (1974a) with mature Finnish Landrace rams.

We thank Mrs Susan Rowe, Mrs Hilary Carter and Mr Murray Peat for valuable technical assistance. Rabbit anti-ovine LH was generously supplied by Dr G. D. Niswender, and Mr K. E. Jury advised on statistical methods.

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Received 22 August 1977