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Secretion of LH, FSH and Oestradiol-17β During the Follicular Phase of the Oestrous Cycle in the Ewe

G. B. Thomas,[A,C] *G. B. Martin,*[A] *J. R. Ford,*[A] *P. M. Moore,*[A] *B. K. Campbell*[B] *and D. R. Lindsay*[A]

[A] Animal Science Group, School of Agriculture, University of Western Australia, Nedlands, W.A. 6009.
[B] Division of Animal Production, CSIRO, P.O. Box 239, Blacktown, N.S.W. 2148.
[C] Present address: Medical Research Centre, Prince Henry's Hospital, St Kilda Road, Melbourne, Vic. 3004.

Abstract

Plasma concentrations of LH, FSH and oestradiol-17β were measured in blood samples taken at 15 min intervals for 48 h during the follicular phase of four Merino ewes. The amplitude of pulses of LH and the mean concentration of LH were higher at the beginning of the follicular phase, 36–24 h before the preovulatory surge of LH (amplitude 2·4 ng ml^{-1}, mean concentration 3·9 ng ml^{-1}), than at the end, 24–0 h before the preovulatory surge (amplitude 1·2 ± 0·1 ng ml^{-1}; mean concentration 1·4 ± 0·1 ng ml^{-1}). There was no change in the inter-pulse interval during this time (mean 74 ± 5 min). Over the same period, oestradiol levels increased from 7–8 pg ml^{-1} to a peak of 10–15 pg ml^{-1}. Mean FSH concentrations declined (36–24 h: 3·6 ng ml^{-1} vs 24–0 h: 1·8 ± 0·3 ng ml^{-1}) before rising at the time of the preovulatory surge of LH and again 24 h later. It was concluded that the biphasic response of LH to oestrogen that is seen in ovariectomized ewes may also operate during the follicular phase of the oestrous cycle in entire ewes.

Introduction

Many studies have reported the changes in the plasma concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), progesterone and oestrogen during the oestrous cycle of the ewe (see Baird and McNeilly 1981, for review). Generally, these studies have involved either infrequent blood sampling or intensive collection of blood samples for short periods, and have concentrated primarily on measuring the pulsatile release of LH after prostaglandin-induced luteolysis (e.g. Baird 1978; Baird *et al.* 1981). The collection of frequent blood samples for longer periods would help to define further the precise pattern of endocrine changes during the oestrous cycle. The present study was therefore designed to describe in detail the changes in plasma concentrations of LH, FSH and oestradiol-17β during the follicular phase of the oestrous cycle of the Merino ewe.

Materials and Methods

Animals

The oestrous cycles of eight mature cyclic Merino ewes were synchronized using intramuscular injections of 20 mg of progesterone in oil every second day for 14 days during June (mid-breeding season). After the last injection, four ewes (controls) were placed with a harnessed vasectomized ram to detect the onset of oestrus. The remaining four ewes were placed in individual pens and kept under natural lighting in preparation for blood sampling.

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Blood-sampling Procedure

Five days after the control ewes had been detected in oestrus, the ovaries of all eight ewes were examined by laparoscopy to confirm ovulation. Blood was then collected at 15 min intervals for 48 h from the four experimental ewes during days 15 and 16 after the onset of oestrus in the controls. At night, blood samples were collected with the lights out, using faint illumination from a green lamp. All samples were assayed for LH and a sample every 2 h was measured for FSH and oestradiol-17β.

Radioimmunoassays

Plasma concentrations of LH were determined using the assay described by Martin *et al.* (1983). The reference preparation was CNRS-M3 (1·8 × NIH-LH-S1) and the limit of detection was 0·42 ± 0·08 ng ml^{-1} (mean ± s.e.m.). Pooled samples containing 0·61 ± 0·04 ng ml^{-1}, 2·15 ± 0·12 ng ml^{-1} and 3·28 ± 0·24 ng ml^{-1} were included in each of the eight assays used in this study to estimate the coefficients of variation (c.v.) within (11·6 ± 1·9%; 9·1 ± 1·6%; 6·9 ± 1·0%) and between (11·5%, 8·9%, 7·1%) assays.

Samples were measured for FSH using the NIAMDD assay kit (kindly supplied by Dr A. F. Parlow). The reference preparation was NIAMDD-oFSH-RP-1, which had a biological potency 75 × NIH-FSH-S1. The limit of detection was 0·12 ± 0·01 ng ml^{-1} and a pooled plasma sample containing 7·46 ± 0·62 ng ml^{-1} gave a within-assay c.v. of 8·3%.

Oestradiol-17β was measured in three assays using a double antibody radioimmunoassay recently developed in our laboratory. The guidelines of Barnard *et al.* (1975) and Chard (1981) were followed for optimization of sensitivity. Oestradiol-17β-6-CMO-HSA was used to produce the antiserum (R27-2). It was used at a final dilution of 1 : 175 000 and cross-reacted significantly with only oestrone (4·9%) and oestriol (1·2%). Samples of plasma (2 ml) were extracted in duplicate with three volumes of diethyl ether and the dried residues were chromatographed on Sephadex LH-20 columns using a system derived from methods described by Carr *et al.* (1971) and Pearson-Murphy and Diez D'Aux (1975). The mean percentage recovery after extraction and chromatography was 73·9 ± 1·3%. Standards and samples were incubated in assay buffer for 60 min at room temperature and then with the antiserum for 24 h at 4°C before the addition of 6000 dpm [2,4,6,7,16,17-H^3] oestradiol-17β (90–130 Ci mmol^{-1}; New England Nuclear). After a further 24 h at 4°C the bound and free hormone were separated using donkey anti-rabbit serum. One ml of assay buffer containing 0·4% Al_2O_4 was added to the tubes before centrifugation. The precipitate was dissolved in 0·05 M HCl and counted. Sample values were not corrected for assay blanks or procedural losses. The limit of detection of the standard curve was 0·55 ± 0·19 pg ml^{-1}. Fifty per cent displacement of labelled oestradiol was produced by 21·3 ± 2·0 pg oestradiol (n = 3 assays). Values for the solvent blanks were 1·13 ± 0·57 pg ml^{-1}. Concentrations below the assay blank were defined as undetectable. The concentration of oestradiol measured in 2 ml ovine plasma was highly correlated with the mass of exogenous oestradiol added (1·56 to 50 pg) to these samples (r = 0·997, n = 3) and with the volume of plasma assayed (r = 0·995, n = 3). The addition of 2 ml of ovine plasma resulted in a displacement curve that was parallel with the standard curve (data not shown). The within-assay c.v. for plasma pools containing 8·8 ± 0·7 or 11·8 ± 0·3 pg ml^{-1} oestradiol were 9·4 ± 3·6% and 4·9 ± 2·9% respectively. The between-assay c.v. for the same pools were 4·8% and 2·3%.

Statistical Analysis

Pulses of LH were identified using the definition of Martin *et al.* (1983). Pulse amplitude was calculated by subtracting the concentration at the onset of an LH pulse (nadir) from the maximum concentration, and the intervals between pulses of LH were measured as the time between consecutive pulses. The onset of the LH surge was arbitrarily defined as the time at which LH concentrations increased above 10 ng ml^{-1}.

Results

Due to variation in the time of onset of the LH surge, it was only possible to study the changes in pulsatile LH secretion during the entire 36–0 h before the preovulatory surge of LH in two ewes (Table 1). Pulse amplitude was highest 36–24 h before the onset of the surge but then decreased during the next 24 h (Table 1, Fig. 1). There was no change in pulse interval during this time (Table 1). The nadir and mean concentrations of LH followed

a similar trend to pulse amplitude, both decreasing from the early- to late-follicular phase.

During the 24 h preceding the surge, the concentration of FSH was lower than that 36–24 h before the surge (Table 1, Fig. 1). At the same time as the preovulatory surge of LH, there was a five-fold increase in the concentration of FSH, from $1{\cdot}4 \pm 0{\cdot}1$ ng ml^{-1} to a peak of $7{\cdot}1 \pm 3{\cdot}0$ ng ml^{-1} (Fig. 1), followed by a decline back to basal levels. In two ewes the surge began within 24 h of the start of sampling and a second increase in FSH was observed 8–12 h later, with peak values of around 3 ng ml^{-1} approximately 24 h after the initial LH/FSH surge.

On day 15 of the cycle, 36–24 h before the LH surge, the concentration of oestradiol-17β was 7–8 pg ml^{-1}. During the 24–0 h before the LH surge, oestradiol levels increased to a peak of 10–15 pg ml^{-1} around the time of the onset of the surge (Table 1, Fig. 1). Within 2 h of the start of the LH surge the concentration of oestradiol began to decrease and by the end of the surge the level of oestradiol was below the limit of detection of the assay.

Table 1. The secretion of LH, FSH and oestradiol-17β during the follicular phase of the Merino ewe
Values are mean ± s.e.m. (when $n = 4$) or individual values for each ewe (when $n = 2$)

Time from LH surge (h)	n	LH pulse Interval (min)	LH pulse amplitude (ng ml^{-1})	LH pulse nadir (ng ml^{-1})	Mean LH concn (ng ml^{-1})	Mean FSH concn (ng ml^{-1})	Mean oestradiol-17β concn (pg ml^{-1})
36–24	2	55, 73	2·1, 2·6	1·7, 1·9	3·1, 4·8	3·0, 4·1	6·8, 8·1
24–12	2	84, 93	1·1, 1·3	0·7, 0·7	1·5, 1·6	2·5, 2·6	6·8, 8·6
12–0	4	71 ± 5	1·2 ± 0·1	0·7 ± 0·1	1·4 ± 0·1	1·5 ± 0·4	10·7 ± 2·1
36–0 (pooled)	4	74 ± 5	1·4 ± 0·4	1·1 ± 0·3	2·4 ± 0·8	2·5 ± 0·5	9·2 ± 1·9

Discussion

The biphasic effect of oestrogen on LH in ovariectomized ewes is well documented (Radford *et al.* 1969; Scaramuzzi *et al.* 1971), but to our knowledge this is the first report that LH concentrations decrease during the late-follicular phase in the non-ovariectomized ewe. We only observed this phenomenon in two sheep, but similar profiles have been reported by others (McLeod *et al.* 1983). Furthermore, LH pulse amplitude falls during the period leading up to the surge in anoestrous ewes induced to ovulate by the 'ram effect' (Martin *et al.* 1985). The present study suggests that the decline in LH during the late-follicular phase of the spontaneously ovulating ewe is due to a similar reduction in LH pulse amplitude.

Goodman and colleagues have shown that oestradiol implants that provide circulating levels of oestradiol similar to those found in the follicular phase will reduce LH pulse amplitude in ovariectomized ewes in the breeding season (Goodman and Karsch 1980; Goodman *et al.* 1981). In more recent studies the short-term negative feedback following oestrogen administration has been found to be exerted directly on the pituitary gland, with no effect on the release of gonadotrophin-releasing hormone from the hypothalamus (Clarke and Cummins 1985*a*). LH pulse amplitude is also dependent on the frequency with which the pulses are released (Clarke *et al.* 1984) because this frequency regulates the size of the releasable pool of LH in the pituitary gland (Clarke and Cummins 1985*b*). Therefore, the decrease in pulse amplitude observed in this study was probably due to a combination of the increases in the secretion of oestradiol and in the frequency of LH pulses. Although we did see a continual rise in plasma oestradiol concentrations during the late-follicular phase, our experimental design did not allow us to sample frequently enough to determine if there

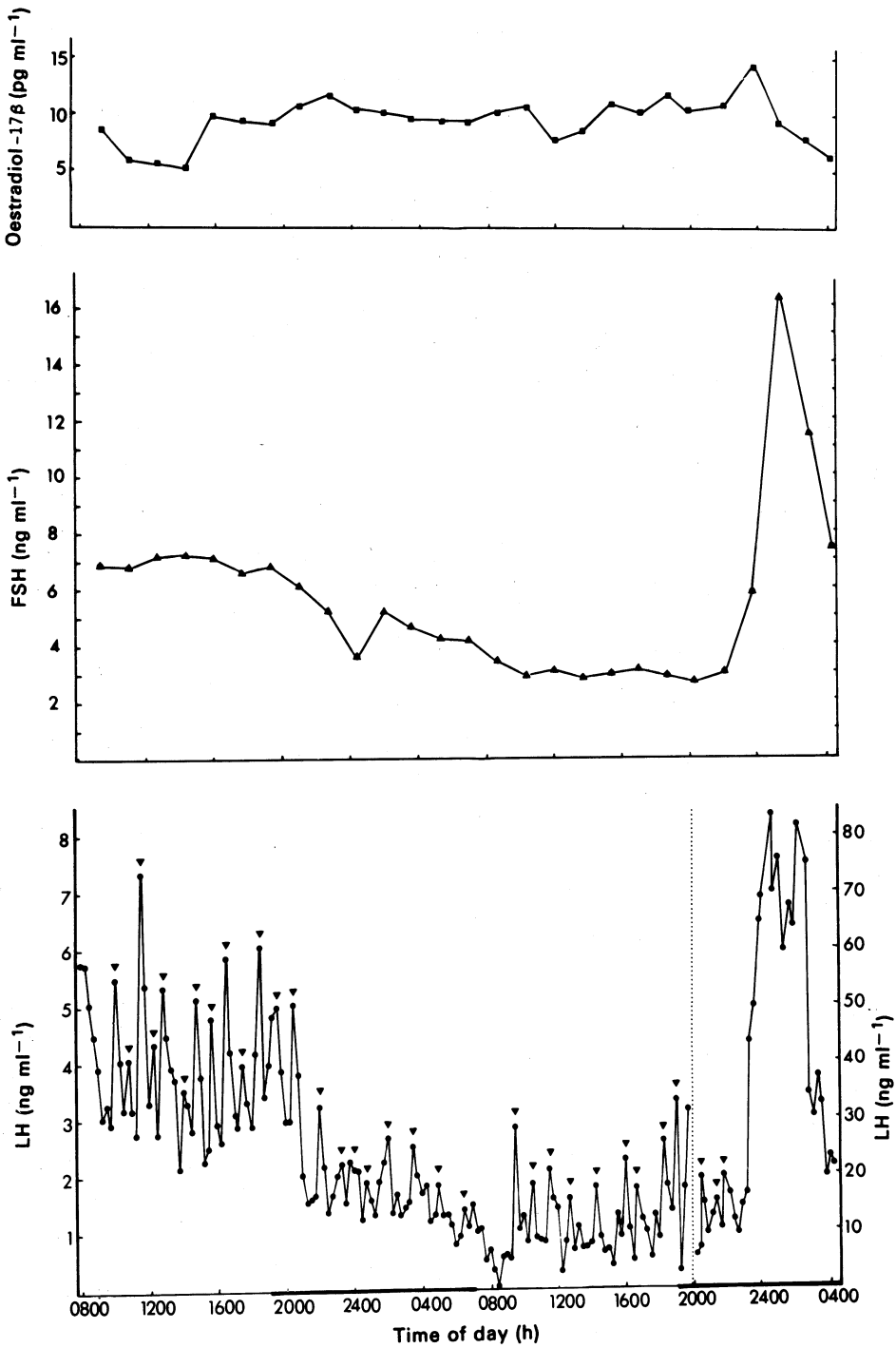

Fig. 1. Changes in the concentration of LH, FSH and oestradiol-17β during days 15 and 16 of the oestrous cycle. The timing of the day and night periods are shown on the horizontal axis. Note the change in scale on the right vertical axis for LH during the preovulatory surge. Pulses of LH indicated by ▼.

is an increase in LH pulse frequency from the early- to late-follicular phase of the oestrous cycle. Further studies are required to answer this question.

Samples collected during the late-follicular phase also included the preovulatory LH surge. These samples were not collected frequently enough for the detection of LH pulses during the surge, but there were occasional large fluctuations in the concentration of LH (Fig. 1). In a subsequent study, samples were collected at 5 min intervals during the preovulatory surge and it was confirmed that LH release is pulsatile during this stage of the oestrous cycle (Martin *et al.* 1987).

Apart from the preovulatory surges, the mean plasma concentration of FSH was relatively constant and did not exhibit the major fluctuations that have been reported in other studies (Salamonsen *et al.* 1973; Miller *et al.* 1981). However, the concentration of FSH did progressively decrease prior to the first preovulatory surge. Large Graafian follicles, which are the major source of oestradiol (Moore *et al.* 1969) and inhibin (Tsonis *et al.* 1983), are developing as the follicular phase progresses, so the fall in FSH levels was probably due to increasing negative feedback.

The inhibition of gonadotrophin secretion at a time when it is necessary to stimulate ovarian activity is interesting. On the one hand, it allows the emergence of a dominant follicle, one that is independent of FSH yet very responsive to the smallest of LH pulses (Baird 1978; Baird and McNeilly 1981), and on the other hand it may also provide a means for the anterior pituitary gland to build up its stores of gonadotrophins in readiness for the preovulatory surge (see Martin 1984, p. 29).

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