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Relationship Between LH, FSH, and Prolactin Concentration and the Secretion of Androgens and Estrogens by the Preovulatory Follicle in the Ewe

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ABSTRACT

The ovarian secretion of estradiol, androstenedione, testosterone, and progesterone and the concentration of LH, FSH, and prolactin were measured in the periovulatory period of five ewes with ovarian or utero-ovarian autotransplants. Samples of jugular and ovarian venous blood were collected every 1 or 2 h before and for 96 h after induction of luteal regression on Day 10 of the cycle by injection of 100 μg cloprostenol. Progesterone concentration in jugular vein plasma fell to less than 1 ng/ml in all ewes by 24 h, and coincidental to this decline there was a significant increase in the secretion of LH and prolactin and an associated rise in the ovarian venous concentration of estradiol, testosterone, and androstenedione. The rise in LH secretion was associated with a twofold increase in the frequency of episodic pulses each of which stimulated an increase in the secretion of estradiol. In contrast the concentration of FSH declined significantly in the 48 h after luteal regression. Shortly after the onset of estrus (48 ± 2.5 h), there was a marked rise in the concentration of prolactin, FSH, and LH which reached a peak at 61 ± 4 h. At the start of this preovulating LH surge, there was a further substantial stimulation in the secretion of estradiol, testosterone, and androstenedione, followed by a sharp fall within 3, 5, and 7 h, respectively.

In the 24 h after the preovulatory surge, there was a marked decline in the concentration of LH and prolactin as well as all ovarian steroids. However, within 12 h there was a further rise in the concentration of FSH which reached a second peak at 23 h after the LH peak.

These results suggest that the increased secretion of estradiol from the preovulatory follicle is due to stimulation by episodic pulses of LH which occur with increased frequency as the concentration of progesterone falls during luteal regression. The sustained rise in LH which occurs during the preovulatory surge stimulates and then markedly inhibits aromatase activity and eventually all steroid secretion from the follicle. Thus the final stages of development of the preovulatory follicle are determined not by FSH but by the pattern of secretion of LH.

INTRODUCTION

The changes in the concentration of FSH, LH, prolactin, estradiol, and progesterone in jugular venous blood of the sheep in the periovulatory period have been described in a number of publications (Pant et al., 1977; Hauger et al., 1977; Bindon et al., 1979). Following regression of the corpus luteum, the dominant follicle(s) secretes increasing quantities of estradiol in response to a rise in the basal secretion of LH (Baird and Scaramuzzi, 1976a). The rise in estrogen secretion induces estrous behavior and a surge of LH which results in ovulation some 24 h later (Cumming et al. 1971). Important changes occur in the Graafian follicle at this time converting it from a structure which secretes estrogens and androgens to one which secretes mainly progesterone (Bjersing et al., 1972; Moor et al., 1975).

It has been known for some years that the sheep ovary secretes relatively large amounts of androstenedione and testosterone (Baird et al., 1968). These androgens are precursors for estrogen biosynthesis although their role in the regulation of gonadotropins and the factors controlling their secretion are unknown (Baird, 1978a). Active immunization of ewes against androstenedione and testosterone produces profound effects on ovarian function and the secretion of gonadotropins (Martenz et al., 1976; Martenz and Scaramuzzi, 1979; Scaramuzzi et al., 1980; Scaramuzzi et al., 1981).

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The present study was designed to study the secretion of androgens and estrogens from the ovary in the periovulatory period and to relate these findings to the changes in the concentration of FSH, LH, and prolactin measured simultaneously at frequent intervals. In this way it was hoped to gain insight into the factors regulating the marked change in pattern of steroids secreted by the preovulatory follicle.

MATERIALS AND METHODS

Experimental Animals

Four ewes with ovarian (Goding et al., 1967) and one ewe with a utero-ovarian autotransplant (Harrison et al., 1968) were allocated to this experiment in the middle of the breeding season (January). Ewes were either pure Tasmanian Merino (D314 and D410) or crossed with Finnish-Landrace (F × M OU57, 9U6) or Southdown (MX 16). Ewes were housed under natural lighting conditions in a covered pen at Dryden Field Station (Animal Breeding Research Organisation), Roslin, Midlothian, Scotland. The left ovary alone, or together with the oviduct, horn, and body of the uterus, cervix, and anterior vagina, had been autotransplanted to the neck at least 2 years previously. The left ovarian and uterine arteries and utero-ovarian vein were anastomosed to the carotid artery and jugular vein which were exteriorized in separate skin loops (Baird et al., 1976a). The right ovary and uterine horn were removed. The ewe with the utero-ovarian transplant had regular estrous cycles [17.5 ± 1.2 days (mean ± SD), n = 17] in contrast to the ewes with the ovarian transplants which failed to show heat due to persistence of the corpus luteum (Baird et al., 1976b). Heat was tested with a vasectomized ram at least twice per day and on some occasions more frequently (see below).

Because of the absence of cyclical ovarian function in the ewes with ovarian transplants and because of the difficulty of standardizing the timing of collections in relationship to spontaneous luteal regression, estrus, and ovulation in the utero-ovarian transplant, premature luteal regression was induced by i.m. injection (100 μg) of a potent analogue of prostaglandin $F_{2\alpha}$ ICI 80996 (cloprostenol). Luteal regression and estrus (Day 0) occurred within 72 h of this treatment (Baird and Scaramuzzi, 1975).

Experimental Design

On Day 9 of the subsequent estrous cycle, both jugular veins were cannulated as described previously (Collett et al., 1973). On the side of the utero-ovarian anastomosis, a Silastic cannula (2.00 mm i.d., 3.2 mm o.d.; Dow Corning Corp., Medical Products, MI) was advanced so that the tip lay opposite the entrance of the utero-ovarian vein. Following cannulation the ewes were kept in metabolism cages in a heated room (about 15°C) until the end of the experiment.

On the morning of Day 10, 5 ml samples of jugular venous blood were collected every hour for 6 h prior to and for 60 h after a second i.m. injection of 100 μg cloprostenol. From 60–96 h the sampling frequency was reduced to every 2 h.

Samples (10 ml) of ovarian venous blood were collected every 2 h from −6 to +96 h. Samples of jugular and ovarian venous blood were collected more frequently every 10 min for two periods of 4 h during the luteal and follicular phases of the cycle, i.e., −4 to 0 h and 24 to 28 h after injection of cloprostenol. During the periods of frequent sampling, timed samples (15 ml) of ovarian blood were collected every hour and the plasma flow then calculated from the hemacrit reading. Blood was collected into sterile heparinized containers, centrifuged immediately at 4°C, the plasma aspirated, and stored at −20°C until analysis. During the 10 min sampling period the red cells were returned to the ewe via the cannula in the right jugular vein.

Commencing 36 h after the injection of cloprostenol, the ewes were tested for heat every 4 h through estrus until two successive tests were negative. The ewes were removed from the metabolism crates after the collection of the blood sample and observed in a pen for 10 min with a vasectomized ram.

Analytical Methods

Progesterone, estradiol, androstenedione, and testosterone were measured in duplicate in ovarian venous plasma by methods which have been described in detail in publications from this laboratory (Scaramuzzi et al., 1975; Baird et al., 1976; Baird et al., 1974; Corker and Davidson, 1978). The precision and sensitivity of the methods are listed in Table 1.

Prolactin, LH, and FSH were measured in duplicate by radioimmunoassays described in detail by McNeilly and Andrews (1974), McNeilly et al. (1976), and Martenz et al. (1976). The sensitivities of the assays were 0.1 ng prolactin (NIH P-S6)/ml, 0.1 ng LH (NIH-LH-S14)/ml, and 16 ng FSH (NIH-FSH-S10)/ml. All samples were analyzed in one assay with coefficients of variations (%) of three quality control sera (at 27, 50, and 75% B/Bo) of 8, 9, and 7% for prolactin, LH, and FSH, respectively. High values were reassayed in dilution in assays with intraassay coefficients of variation of 7%, 8%, and 8% and interassay variations of 10%, 9%, and 12% for prolactin, LH, and FSH, respectively.

Statistical Analysis

The differences between groups were analyzed statistically by two-tailed or paired Student's t test and the pulse frequency by χ^2 test.

RESULTS

Estrous Behavior

All the ewes showed estrus 43–56 h after the injection of the cloprostenol (47.6 ± 2.5; mean ± SEM). As expected the duration of estrus in the F × M crosses (38 and 40 h) was longer than that in the Merino ewes (20, 25, and 32 h).

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TABLE 1. Precision and sensitivity of methods used for hormone assay.

	Antiserum	Interassay precision (%)	Limit of sensitivity (pg/ml)
Estradiol	R3	9.5	10
Androstenedione	Shopman	15.0	50
Testosterone	E 0 1	11.5	10
Progesterone	91920/9	13.0	100

Changes in Steroids and Gonadotropins

Because of the dilution of ovarian with uterine venous blood, the concentrations of ovarian steroids in the ewe with a utero-ovarian transplant were considerably less than in the ovarian blood of those ewes with ovarian transplants, although the pattern of hormone changes was similar. The values from this ewe were excluded from the mean of the values from the four ewes with ovarian transplants which were grouped around the injection of cloprostenol and the peak concentration of LH. The LH peak occurred 60 h after the cloprostenol injection (52–68 h) so that the mean results illustrated in Figs. 1 and 2 are an accurate representation of the time scale in spite of the break in the ordinate. The results in ewe 9U6 are illustrated separately in Figs. 3 and 4 to demonstrate the exact temporal relationships between the changes in gonadotropins and steroids in an individual ewe.

There was a marked decline in the concentration of progesterone in ovarian and jugular venous blood within 6 h of the injection of cloprostenol, and functional luteolysis (progesterone concentration in jugular venous plasma <1 ng/ml) was complete in all ewes by 24 h (Figs. 1, 3). Within 6 h of the decline in the secretion of progesterone, there was a threefold rise in the concentration of LH from 0.40 ± 0.11 to 1.45 ± 0.20 ng/ml ($P<0.05$). In contrast, as the secretion of estradiol increased, the concentration of FSH fell so that by 48 h after the cloprostenol injection it was significantly lower than the preinjection value (56 ± 19 vs 92 ± 10 ng/ml; $P<0.05$, paired t test).

Six to 12 h after the cloprostenol injection there was a rise in the secretion of estradiol, androstenedione, and testosterone (Figs. 2, 4). Thereafter, there was a further marked increase in the secretion of estradiol reaching a peak of 3190 ± 1272 pg/ml at ∿54 h (44–66 h). In contrast there was no significant change in the secretion of androstenedione and testosterone so that a marked increase was found in the ratio of estradiol to both testosterone and androstenedione as the follicle reached maturity (Fig. 5).

Six hours before the peak value, the basal concentration of LH increased above 5 ng/ml. Coincidental to this rise there was a marked increase in the secretion of estradiol, androstenedione, and testosterone (Fig. 5). However, within 3 h of the start of the LH surge the secretion of estradiol fell markedly, although the secretion of testosterone and androstenedione did not reach its maximum value until some 2–3 h later. The secretion of all steroids then declined rapidly so that by 12 h after the LH peak the secretion of ovarian steroids was lower than at any other time of the estrous cycle.

Coincidental to the LH surge (61 ± 4 h) there was a fourfold rise in the concentration of FSH (Figs. 1, 3, 5) which rose from 56 ± 19 ng/ml at −12 h to a peak of 248 ± 42 ng/ml ($P<0.02$) before returning to basal values (56 ± 18 ng/ml) 6–12 h after the LH peak. Subsequently there was a rise in the concentration of FSH in all five ewes, the peak value (218 ± 70 ng/ml) occurring 16–28 h (mean 22.5 h) after the LH peak. This second FSH peak, which lasted between 18 and 32 h (mean 23 ± 4 h), was not associated with any significant change in concentration of LH (mean 0.92 ± 0.24 ng/ml). Because of slight differences in timing, the magnitude of these peaks of FSH is underestimated by the mean data illustrated in Figs. 1 and 5 in which the data are grouped relative to the LH peak.

Pulsatile Release of Gonadotropins

The secretion of estradiol, LH, FSH, and

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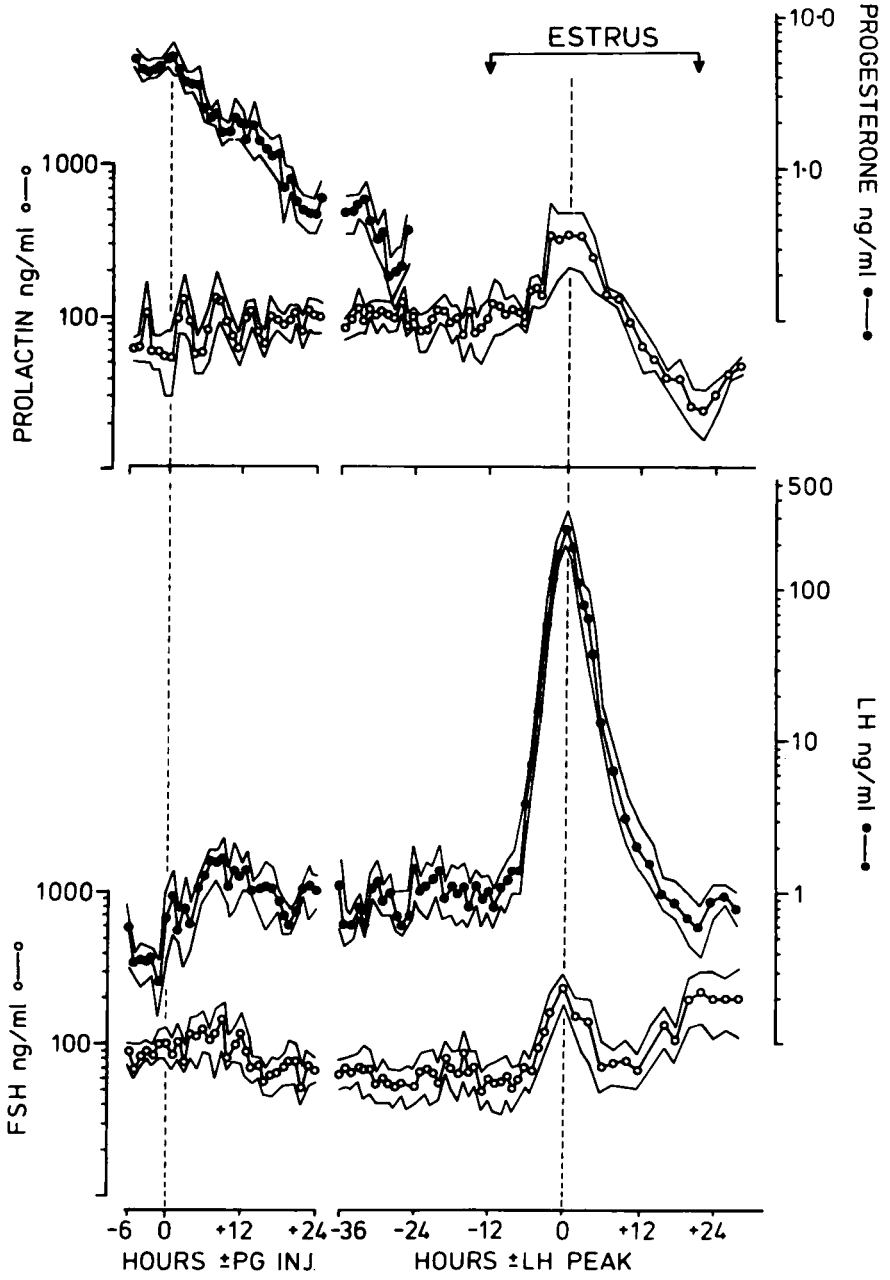

FIG. 1. Mean concentration (± SEM) of progesterone, prolactin, LH, and FSH in jugular venous plasma of four ewes with ovarian autotransplants. Results have been grouped around the injection of 100 μg cloprostenol (PG Inj.) and the peak LH value.

prolactin was measured during a period of intensive sampling at 10 min intervals for 4 h on Day 10 of the cycle (luteal) and again during the follicular phase 24–28 h after the injection of cloprostenol. The results were analyzed for each phase of the cycle in terms of the number and frequency of pulses of each hormone, a pulse being defined as occurring when two consecutive samples were higher than the two preceding samples (basal) and when the value of the highest sample (peak) exceeded the mean basal sample by at least four times the coef-

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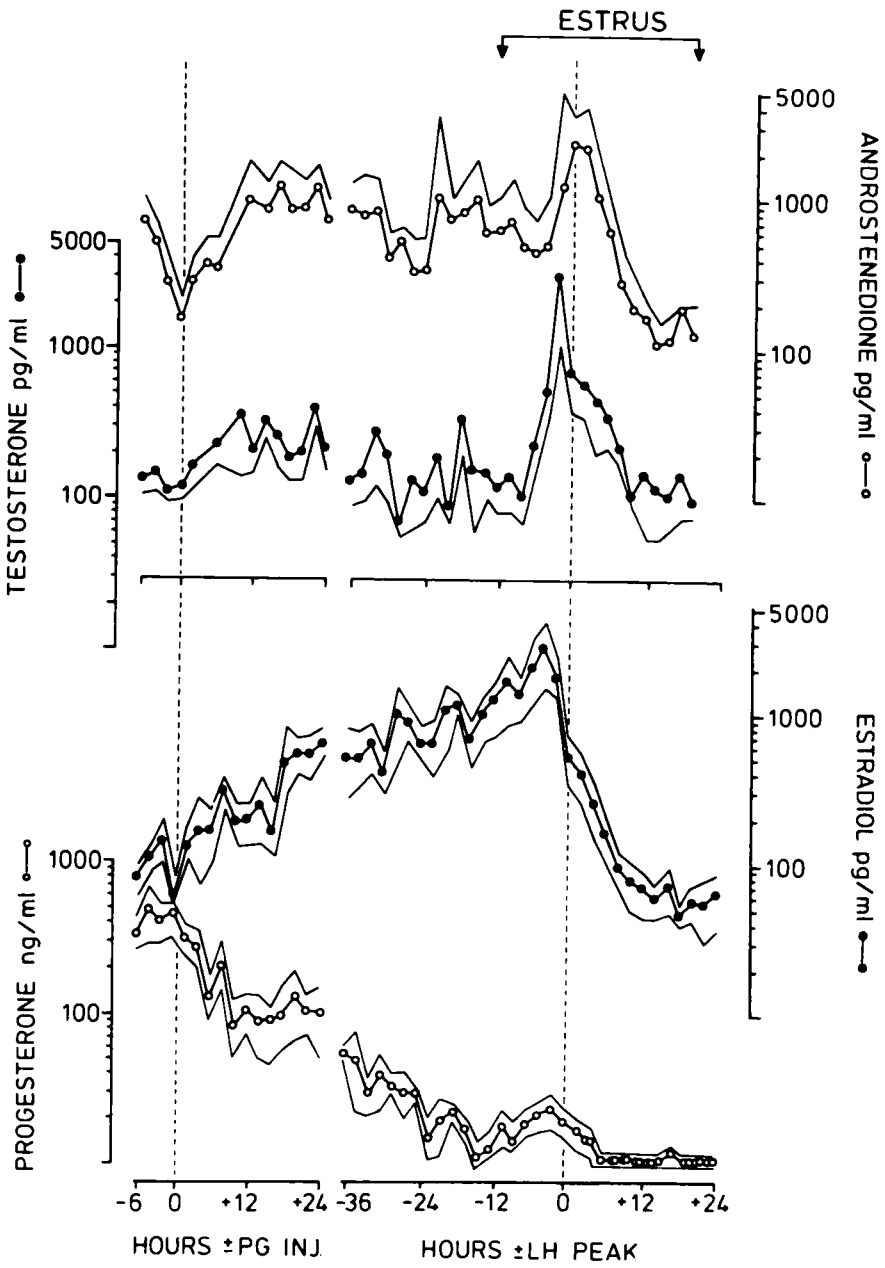

FIG. 2. Mean concentration (± SEM) of progesterone, estradiol, androstenedione, and testosterone in ovarian venous plasma of four ewes with ovarian autotransplants. Results have been grouped around the injection of 100 μg cloprostenol (PG Inj.) and the peak LH value.

ficient of variation of the assay of the hormone concerned (Table 2).

Although the secretion of all hormones showed marked fluctuations, the most obvious episodic pulses were recorded for LH and estradiol (Fig. 6). Each LH pluse was followed within a few minutes by a rise in the secretion of estradiol. The number of LH and estradiol pulses in the 20 h of observation increased from 8 and 6 pulses, respectively, during the luteal phase to 18 and 20 pulses, respectively, during the follicular phase of the cycle (χ^2 = 3.87 and

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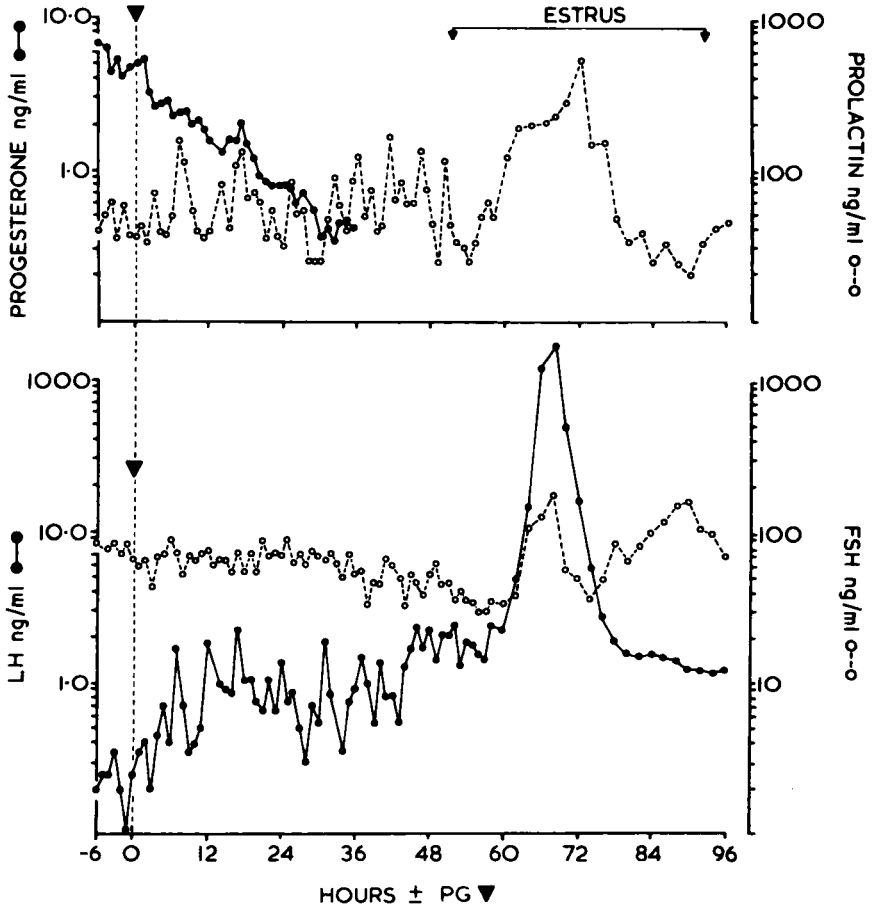

FIG. 3. Concentration of progesterone, FSH, LH, and prolactin in jugular venous plasma in ewe 9U6 with ovarian autotransplant. Values have been grouped around the injection of 100 μg cloprostenol (▼) given on Day 10 of the cycle.

5.76, $P<0.05$). There was a marked increase in the basal and peak secretion of estradiol although there was no significant rise in basal LH concentration or the pulse amplitude (Table 2). The overall mean LH concentration increased significantly due mainly to the increased number of episodic pulses. The pulses of prolactin and FSH did not always coincide with those of LH (Figs. 6, 7), and there was no significant change in the frequency of the pulses of these two hormones in the two phases of the cycle. Compared with the luteal phase, the concentration of FSH during the follicular phase was significantly lower (64 ± 9 vs 93 ± 11 ng/ml).

DISCUSSION

The changes in LH, progesterone, and estradiol secretion in this study confirm and extend those previously reported following spontaneous (Pant et al., 1977; Hauger et al., 1977) and prostaglandin-induced luteal regression (Chamley et al., 1972; Baird and Scaramuzzi, 1976). We have reported (Baird et al., 1968; Baird et al., 1976) that secretion of androstenedione (and testosterone) correlated with that of estradiol and was stimulated by endogenous (Baird et al., 1976) and exogenous LH (McCracken et al., 1969). In the present study the rise in LH secretion occurring during luteal regression was associated initially with an increased secretion of androgens as well as estrogens (Figs. 2, 4). However, in the 36 h before the preovulatory LH surge there was no further rise in the secretion of androstenedione and testosterone in spite of a tenfold increase in

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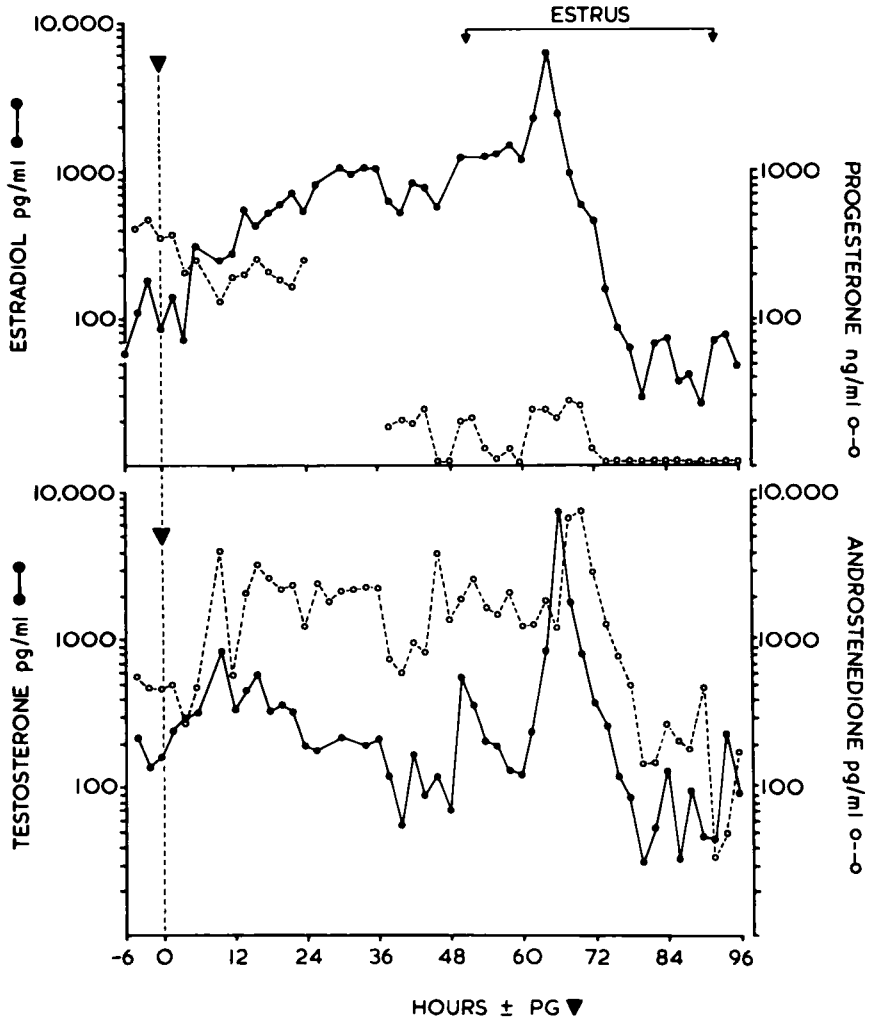

FIG. 4. Concentration of estradiol, progesterone, testosterone, and androstenedione in ovarian venous plasma of ewe 9U6 with an ovarian autotransplant. Values have been grouped as in Fig. 3.

the secretion of estradiol. As an increasing proportion of androgen produced by the preovulatory follicle is converted to estradiol, it seems likely that the quantity of estradiol secreted is limited not solely by the aromatase activity but also by the amount of androgen available as precursor. In the sheep virtually all the estradiol secreted into the ovarian vein as derived from the dominant follicle(s) (Bjersing et al., 1972; Moor, 1973; Baird and Scaramuzzi, 1976b). Although atretic follicles and stroma can synthesize androstenedione and testosterone (Moor et al., 1975), the dominant follicle is the source of over 90% of androgen secreted into the ovarian vein (Baird and Scaramuzzi, 1976b; Scaramuzzi et al., 1980; Scaramuzzi et al., 1981; McNatty et al., 1981). Thus, although in the present study the source of steroids was not studied directly, it can be assumed that the ovarian secretion of estradiol, androstenedione, and testosterone reflects predominantly the activity of the preovulatory follicle.

Although the cellular origin of estrogens in the follicle remains a controversial issue, it is generally accepted that androgens arise from the theca (Moor, 1977). As receptors for LH are present on the cells of the theca (Carson et al., 1979), it is hardly surprising that androgen secretion is so respon-

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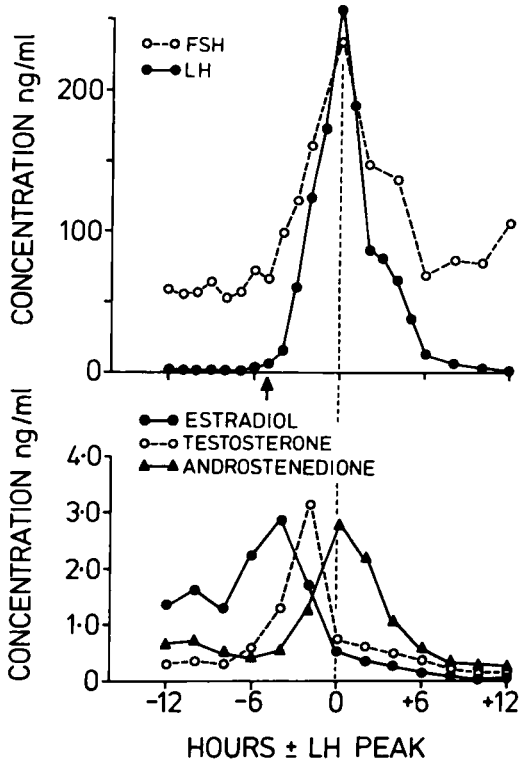

FIG. 5. Mean concentration of estradiol, testosterone, and androstenedione in ovarian venous plasma and LH and FSH in jugular venous plasma of four ewes with ovarian autotransplants. Samples have been grouped around the LH peak. Start of the LH surge, defined as a concentration >5 ng/ml, is indicated by ↑.

sive to each episodic pulse of LH (Baird et al., 1976). The rise in frequency of LH pulses occurring during luteal regression is probably related to the fall in progesterone concentration although a direct stimulatory effect of estradiol cannot be excluded (Legan and Karsch, 1979; Baird and McNeilly, 1981). In seasonal anestrus the final stages of follicular development and ovulation can be induced by infusion or repeated pulses of LH alone without any addition of FSH (Ryan and Foster, 1980; Goodman and Karsch, 1980; McNeilly et al., 1980). Thus the increased frequency of episodic pulses of LH that occurs at luteal regression stimulates secretion of androgen precursor, and is the most important factor in determining the preovulatory increase in estradiol secretion (Baird, 1978b).

In contrast to LH, there was no rise in the frequency of episodic pulses of FSH at the time of luteal regression. In fact the overall concentration of FSH was significantly suppressed due to a decrease in the amplitude of the pulses (Table 2). As LHRH is thought to be the sole hypothalamic hormone responsible for stimulating the release of both gonadotropins, the divergent secretion of FSH and LH during the follicular phase is probably due to a differential effect of estradiol or some other ovarian factor (e.g., inhibin) on the sensitivity of the anterior pituitary to LHRH. Estradiol in physiological

TABLE 2. Concentration of LH, FSH, and prolactin in jugular venous plasma and the ovarian secretion of estradiol in the follicular and luteal phase of the estrous cycle of five ewes (mean ± SEM).

	No. of pulses in 20 h	Concentration			
		Overall (n = 5)	Basal	Peak	Amplitude
LH (ng/ml)					
Follicular	18*	1.2 ± 0.4**	0.8 ± 0.2	1.6 ± 0.3	0.7 ± 0.1
Luteal	8	0.9 ± 0.4	0.5 ± 0.2	1.4 ± 0.4	0.9 ± 0.3
FSH (ng/ml)					
Follicular	12	64 ± 9*	58 ± 5	86 ± 8***	30 ± 5*
Luteal	11	93 ± 11	66 ± 11	125 ± 9	51 ± 7
Prolactin (ng/ml)					
Follicular	7	90 ± 14	68 ± 9*	104 ± 17*	35 ± 8*
Luteal	6	55 ± 11	39 ± 5	55 ± 6	16 ± 1
Estradiol secretion (ng/min)					
Follicular	20*	8.19 ± 2.31*	5.69 ± 0.72***	12.39 ± 2.05***	6.60 ± 1.53***
Luteal	6	2.21 ± 0.32	1.44 ± 0.28	4.26 ± 0.98	2.81 ± 0.76

*P<0.05; **P<0.01; ***P<0.001; significantly different from luteal phase (paired Student's t test or χ^2 test).

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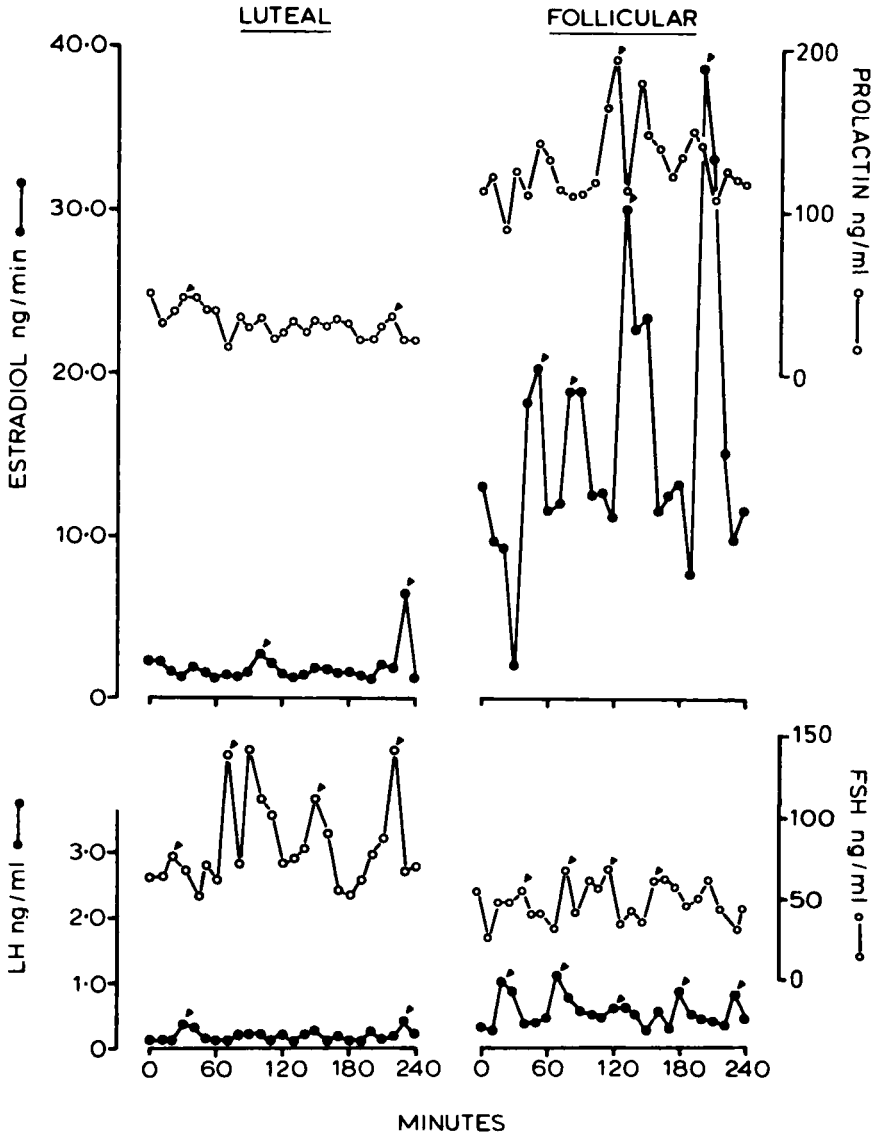

FIG. 6. Secretion of estradiol and concentration of LH, FSH, and prolactin in ewe OU57. Samples were collected between 1000 and 1400 h on Day 10 (luteal) and 24 h after injection of 100 μg cloprostenol (follicular). An episodic pulse is indicated by the arrow ▼ (see text for definition).

concentrations (10^{-11} to 10^{-9} M) suppresses the output of FSH from isolated ovine anterior pituitary cells cultured in vitro (Miller et al., 1977), and release of FSH from rat pituitary glands in response to LHRH is inhibited at a lower concentration of estradiol than of LH (Schally et al., 1973).

Although FSH is essential for folliculogenesis and is thought to play a role in estrogen synthesis by stimulating aromatization of androgens to estrogens by granulosa cells (Dorrington et al., 1975), the final rise in estradiol secretion by the dominant follicle in women (Ross et al., 1970) occurs in the face of a falling secretion of FSH (Fig. 1). It is possible that the relatively high concentration of FSH in follicular fluid protects the dominant follicle (McNatty et al., 1981) from the deleterious effect of low levels of FSH in blood.

As in previous studies there was a very rapid decline in estradiol secretion within hours of the preovulatory LH surge (Chamley et al.,

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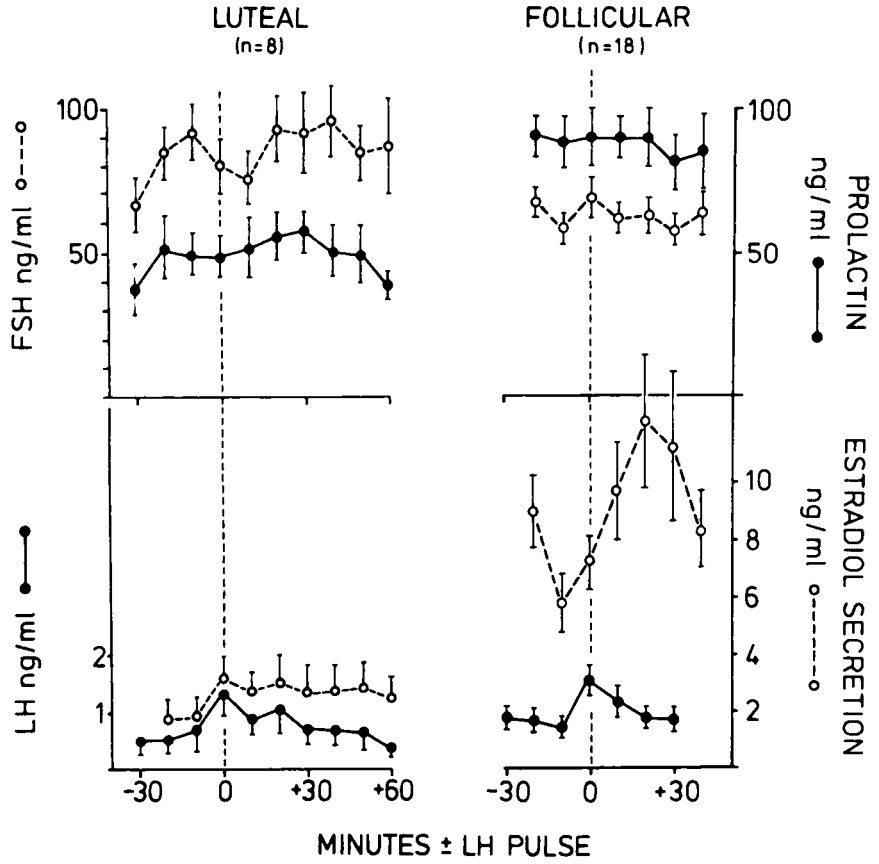

FIG. 7. Concentration of LH, FSH, and prolactin in jugular venous plasma and ovarian secretion of estradiol in five ewes with ovarian or utero-ovarian autotransplants. Results have been grouped around the peak concentration of LH. Each point represents the mean ± SEM of 8 or 18 observations in the luteal (Day 10) or follicular (24 h after cloprostenol) phase, respectively.

1972; Baird and Scaramuzzi, 1976). In the present study the sampling was sufficiently frequent to define the precise relationship between the changes in the secretion of ovarian steroids and pituitary gonadotropins. It was apparent that following a short-lived stimulation of estradiol, androstenedione, and testosterone, there was a very rapid decline of all ovarian steroids (Chamley et al., 1972; Baird and Scaramuzzi, 1976a). That the secretion of estradiol was inhibited before that of testosterone and androstenedione is compatible with the suggestion that LH in large amounts inhibits aromatase activity (Rado et al., 1970; Moor, 1974). Certainly there is no evidence of lack of androgen precursor, and the concentration of FSH, which is known to stimulate aromatase activity in granulosa cells, increases markedly at this time. Testosterone and its 5α-reduced metabolites, e.g., dihydrotestosterone, have been demonstrated to inhibit the conversion of androgens to estrogens by granulosa cells in vitro (Hillier et al., 1980). It is possible, therefore, that the preovulatory surge of LH stimulates a large increase in the secretion of thecal androgens which then accumulate within the follicle and inhibit aromatase. The total inhibition of androgens and estrogens by the follicle after the LH surge is probably due to desensitization and loss of LH receptors on the theca cells (Harwood et al., 1978).

The cause or function of the second peak of FSH which occurs about 22 h after the preovulatory LH peak is unknown (Salamonsen et al., 1973; Pant et al., 1977; Bindon et al., 1979). In other species, e.g., rat, the second peak of FSH determines the number of follicles available for ovulation at the next estrus

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(Sheela-Rani and Moudgal, 1977) although in the sheep the ovulation rate is unaltered by unilateral ovariectomy as late as Day 14 (Land, 1973; Findlay and Cumming, 1977). There is no associated release of LH or prolactin, and the secretion of ovarian steroids is lower than at any other phase of the estrous cycle. It would seem likely that a change in the pituitary sensitivity of LHRH is responsible for the selective release of FSH, as antiserum to LHRH will inhibit the first but not the second FSH peak (Narayana and Dobson, 1979). The second FSH peak occurs at a time when the secretion of ovarian steroids and hence negative feedback is minimal. However, the factors regulating the secretion of FSH are complex and in both the rat (Schwartz and Channing, 1977) and the sheep (Goodman et al., 1980) steroids and/or factors in addition to estradiol are involved.

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