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Changes in the Levels of Progesterone, Corticosteroids, Estrone, Estradiol-17 β, Luteinizing Hormone, and Prolactin in the Peripheral Plasma of the Ewe During Late Pregnancy and at Parturition

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Serial measurements (up to four times per day) of maternal plasma progesterone, corticosteroids, estrone, estradiol-17β, LH, and prolactin have been made in six sheep, during late pregnancy and at parturition (Day 0). The mean progesterone concentration on Day −10 was 15.5 ng/ml. From Day −4 the level began to decrease and by Day 0, the mean progesterone level was 1.0 ng/ml. The mean concentrations of estrone and estradiol-17β on Day −3 (62.4 and 24.0 pg/ml, respectively) began to rise during the 48 hr preceding parturition, reaching peak values of 228.0 and 141.8 pg/ml, respectively. The mean corticosteroid level (17.3 ng/ml) rose during the 18 hr preceding parturition, reaching a peak of 38.6 ng/ml. Estrone, estradiol-17β, and corticosteroids returned to low levels within 12 hr after parturition.

LH levels remained relatively constant (2.0–6.0 ng/ml) from −36 h to +30 h but prolactin levels rose sharply from about Day −2. Possible explanations for the absence of an increase in LH secretion, in response to the preparturient rise in estrogens, are discussed.

The interrelationships between the secretions of the pituitary, ovary, and uterus in the sheep at term are still uncertain because of the lack of detailed information on all available parameters observed in individual animals. For example, changes in peripheral plasma progesterone concentration throughout gestation in the sheep have been reported by Fylling (1970), Obst, Seamark, and McGowan (1971), and Stabenfeldt, Drost, and Franti (1972). Changes in the levels of unconjugated estrogen only have been reported by Challis (1971) and Obst and Seamark (1972a).

No detailed measurements of plasma LH during late pregnancy and after parturition in the sheep have been reported but a rise in plasma prolactin at this time has been reported by Davis, Reichert, and Niswender (1971) and Fell *et al.* (1972).

The published data show that plasma progesterone begins to decline a few days before parturition and this is followed by a sharp rise in unconjugated estrogens on the day before delivery (Challis, 1971). So far, no published information is available on the ability of these steroid changes to elicite a release of LH at this time.

This paper reports a detailed study of the changes in progesterone, corticosteroids, estrone, estradiol-17β, LH, and prolactin in maternal plasma during late pregnancy and at parturition.

MATERIALS AND METHODS

Six ewes from a flock of fine-wool Merino sheep, mated in February, 1972 were used in this study. The animals selected had been mated during a 2-day period, and pregnancy was diagnosed radiologically. Four ewes were carrying single lambs and two carried twins.

Daily blood samples (10 ml) were taken by venipuncture from each animal starting at least 10 days prior to the day of lambing (Day 0). The ewes were moved into an enclosed shed 1 week before the expected day of lambing. They were penned singly and fed oaten hay ad lib. with each ewe receiving a daily intramuscular injection (10^5 units) of Streptopen (Glaxo-Allenbury). On entry into the shed, a silastic cannula (Dow Corning) was inserted into one jugular vein of each animal. Blood samples (20 ml) were then collected from this cannula at intervals of 6 hr for up to 30 hr postpartum.

All blood samples were centrifuged immediately after collection and the plasma stored in separate aliquots at —12C. All lambs were born alive and in each case parturition was not prolonged. The lambs were removed from their dams immediately after birth so that no ewe was suckled. Four days after parturition, laparotomy was performed on each ewe and the gross morphology of the ovaries was noted.

Hormone assays. Plasma corticosteroid concentrations were determined using the protein-binding method of Bassett and Hinks (1969). Progesterone, estrone, and estradiol-17β were measured in duplicate 1.0-ml aliquots of plasma. After extraction of the plasma with 10 ml of 1:1 ether (M & B peroxide-free): petroleum ether (M & B 40–60 C), the steroids in the dried-down extract were separated on celite columns as described by Nagai and Longcope (1971). Estrone was measured by radioimmunoassay (Hasan and Caldwell (1971). Details of the methods used for measurement of progesterone and estradiol-17β have been previously described (Chamley *et al.* 1972). LH and prolactin were measured by solid-phase radioimmunoassay (Goding *et al.*, 1969; Fell *et al.*, 1972).

RESULTS

Steroid hormones. The changes in the mean concentrations of progesterone and corticosteroids for the six ewes from Day —6 to +1 are shown in Fig. 1. On Day

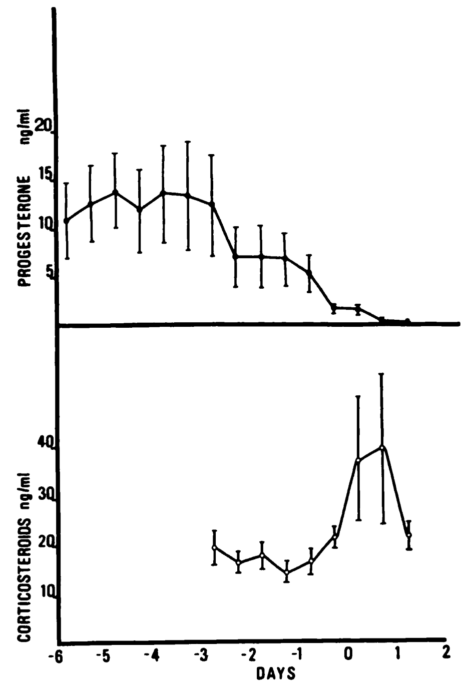

FIG. 1. Changes in concentration of progesterone (●——●) and corticosteroids (○——○) in peripheral plasma (Mean ± SEM) until just before parturition, $n = 12$; in subsequent collections $n = 6$–10. The mean hour of lambing is indicated by the arrow.

—10, plasma progesterone concentrations were in the range 3.8–36.9 ng/ml. The level in individual animals remained relatively constant until Day —5 when progesterone began to decrease. The progesterone concentrations in the last sample taken before parturition were in the range 2.4–3.3 ng/ml for three animals and 0.2–0.5 ng/ml for the remaining three. After parturition the levels in all animals were in the range 0.1–0.6 ng/ml.

Plasma corticosteroids were measured from Day —3 to +30 hr postpartum. The mean corticosteroid levels in blood samples taken on Day —3 were in the range 8.8–25.4 ng/ml. Plasma corticosteroids began to rise in all animals 6–18 hr before birth, reaching peak levels of 14.0–155.0 ng/ml. In five animals, peak values were measured in the last sample taken before parturition, and after this corticosteroids

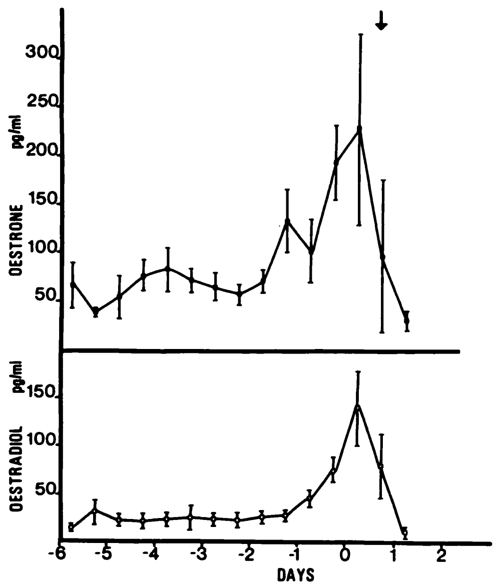

FIG. 2. Changes in concentration of estrone (■——■) and estradiol-17β (□——□) in peripheral plasma (Mean ± SEM) until just before parturition, $n = 12$; in subsequent collections $n = 6$–8. The mean hour of lambing is indicated by the arrow.

declined. At any time before parturition the levels of both progesterone and corticosteroids were generally higher in the twin-bearing ewes than in those ewes which had single lambs.

The changes in the mean concentrations of estrone and estradiol-17β are shown in Fig. 2. On Day −10, levels of estrone were in the range 22.0–129.0 pg/ml while the corresponding levels of estradiol-17β were in the range 7.0–11.0 pg/ml. Estrone began to rise 16–48 hr before parturition while estradiol-17β rose 6–24 hr before this event. The peak concentrations of estrone were in the range 75.0–597.0 pg/ml while those for estradiol-17β were in the range 62.0–320.0 pg/ml. Changes in plasma hormone concentrations in one twin-bearing and one single-bearing ewe are shown in Figs. 3 and 4, respectively.

Pituitary hormones. LH was measured in five ewes from about −36 h to +30 h. Prolactin concentrations were measured from Day −3 up to the time of lambing in two ewes and up to +30 hr in the other four. No major change was observed in

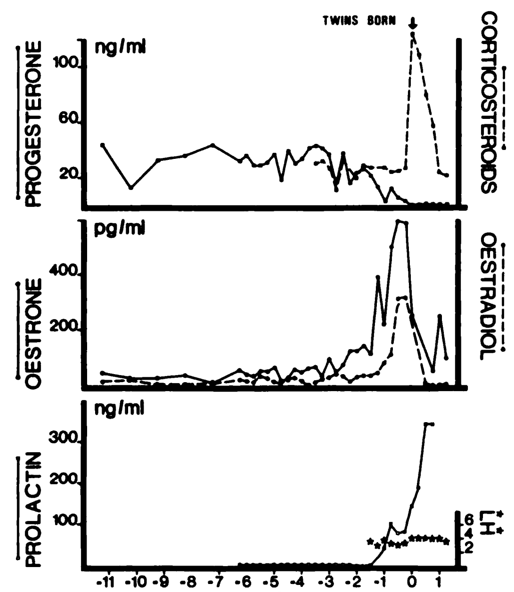

FIG. 3. Changes in the peripheral plasma concentration of progesterone, corticosteroids, estrone, estradiol-17β, LH, and prolactin in one ewe which delivered twin lambs.

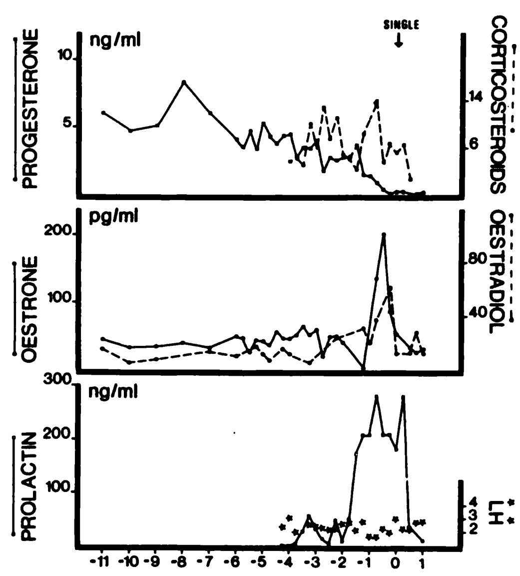

FIG. 4. Changes in the peripheral plasma concentration of progesterone, corticosteroids, estrone, estradiol-17β, LH, and prolactin in one ewe which delivered a single lamb.

plasma LH concentrations which remained in the range 2.0–6.0 ng/ml.

In all animals plasma prolactin levels began to rise sharply at about 2 days before parturition. In two animals minor fluctuations in prolactin concentration preceded this sharp rise. The highest concentrations of prolactin measured in samples taken on Day −3 were in the range 2.0–29.0 ng/ml, while the highest concentrations of prolactin measured before parturition were in the range 100.0–640.0 ng/ml. In three ewes prolactin fell to low levels after lambing, but continued to rise in a fourth.

At laparotomy, no follicles >2.0 mm diameter or recent ovulations were observed in the ovaries of any ewe.

DISCUSSION

The decline in peripheral progesterone levels which preceded parturition is similar to the pattern described by other workers. Bassett *et al.* (1969) reported peak values of 7.6 ± 0.8 and 14.2 ± 1.9 ng/ml (mean $\pm$ SEM) for groups of single- and twin-bearing ewes, respectively, within 10 days prior to lambing. In similar studies, Stabenfeldt, Drost, and Franti (1972) measured peak concentrations of 10.0 ± 1.5 and 16.0 ± 3.4 ng/ml (mean $\pm$ SEM) in similar groups of ewes. In both of these studies maternal plasma progesterone levels began to fall 6–10 days before parturition.

In a study of four pregnant ewes, Bassett and Thorburn (1969) reported 3- to 6-fold increases in fetal plasma corticosteroids around parturition, but did not find any substantial changes in maternal plasma corticosteroid levels. This is in contrast to the findings reported in this paper where substantial increases in maternal corticosteroid levels occurred before parturition in five out of six ewes. In view of the brief interval during which these levels are elevated (12–30 hr) it is likely that the reason why this sharp rise was not reported in the study of Bassett and Thorburn was that blood samples were taken only once daily. An increase in maternal plasma corticosteroid levels at parturition was also observed by Obst and Seamark (1972b) in ewes grazing grass or Yarloop clover.

Had high levels of estrone appeared consistently before the rise in estradiol, it would have been tempting to assume that the estrone was being converted to estradiol in the dam. However, the patterns of estrogen changes reported in this paper show that rises of estrone and estradiol-17β occur at different times in the different animals. The explanation for this phenomenon is, therefore, uncertain. Increases in both estrone and estradiol-17β in one ewe were reported by Bedford *et al.* (1972). In this animal, uterine vein plasma estrone and estradiol-17β levels rose from 173.9 and 24.0 pg/ml at 8.5 hr before parturition to values of 697.1 and 98.1 pg/ml, respectively, 17 min before parturition. However, their values may well be open to question as estrone and estradiol-17β, in virtually every case, was more than double the reported "total" estrogen. Their explanation was that this was due to competitive interaction between the estrogens concerned. However, such a conclusion seems unacceptable without detailed validation. Thorburn *et al.* (1972) found that maternal estradiol-17β rose to 880 pg/ml before parturition. No individual values for estrone were given, and these workers reported that the mean estrone:estradiol-17β ratio was 1.9 ± 0.3 (SEM $n = 7$). The value for peak estrone concentration would appear excessively high and it seems unlikely that the estrone:estradiol-17β ratio is constant near term.

Fell *et al.* (1972) have shown that a release of prolactin occurs in association with the surges of LH resulting from infusion or intramuscular injection of estradiol-17β. On the basis of this evidence it is tempting to suggest that elevation in circulating estrogen levels may in part be responsible for the observed release of prolactin at parturition.

An interesting finding in this present

study is the absence of a release of LH at parturition. In the nonpregnant ewe, a rise in estradiol-17β secretion, in the presence of low levels of progesterone, triggers the release of LH and FSH (Goding *et al.*, 1969; Radford, Wheatley, and Wallace, 1969; Jonas *et al.*, 1973).

Although different in terms of absolute values, the changes in progesterone and estrogens before parturition follow a pattern similar to that during the cycle. However, progesterone can block the release of LH by estrogen (Cumming *et al.* 1971; Scaramuzzi *et al.*, 1971). Raised plasma progesterone levels would not explain the absence of LH release in three of these ewes as in these animals, peripheral progesterone levels had fallen before parturition to values comparable to those measured at the end of a normal cycle (Thorburn, Bassett, and Smith, 1969). Jaume and Shelton (personal communication) have measured LH by solid-phase radioimmunoassay (Goding *et al.*, 1969) and FSH by heterologous radioimmunoassay (Salamonsen *et al.*, 1973) in the pituitaries taken from groups of parous ewes. These animals were slaughtered on the day of parturition and on Day 3 and 14 of the third estrous cycle after parturition. LH levels in the three groups were 77.8 ± 34.7 S.D. ($n = 18$); 478.8 ± 191.3 ($n = 18$); 1239.7 ± 667.0 ($n = 17$) μg/gland, while FSH levels were 228.9 ± 113.1; 478.3 ± 203.4; 1085.4 ± 315.4 μg/gland, respectively. It, therefore, seems probable that the failure of the fall in progesterone and the rise of estradiol-17β to evoke a preovulatory surge of LH is due to a low pituitary content of LH at the time of parturition.

In summary, the sequence of hormonal events at parturition include a fall in progesterone, a subsequent rise in estrogens and corticosteroids, and a rise in prolactin with no change in LH. There is also a terminal rise in secretion of prostaglandin $F_{2\alpha}$ (Liggins and Grieves, 1971) and oxytocin (Chard, 1972).

Although this paper provides further detailed information concerning the time course of secretion of some of these hormones, the underlying mechanism leading to the initiation of parturition is as yet unexplained.

On the other hand, the present study does give some insight into the cause of the preparturient rise of prolactin secretion and the failure of ovulation to occur at that time.

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