Evidence that the corpus luteum of pregnancy contributes to the control of tonic secretion of LH in the ewe

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Summary. Concentrations of LH and FSH were measured in blood samples collected from the jugular vein at 20-min intervals for 7 h (09:00–16:00 h) on Days 60, 80, 100 and 120 of pregnancy in 5 intact ewes and 5 from which the CL had been excised on Day 70. In the 5 intact ewes, plasma LH concentrations remained low and unchanged between Days 60 and 120. During this period, pulsatile release of LH occurred irregularly and infrequently. Removal of the CL resulted in an increase in the basal values of LH and in the frequency and amplitude of LH pulses. Concentrations of FSH were relatively constant in all stages of pregnancy examined and were similar in both groups of ewes. These results show that (1) LH concentrations are low during the second half of pregnancy; and (2) LH, but not FSH, increases after CL excision, presumably by removing some luteal factor inhibitor of LH secretion.

Keywords: ewe; pregnancy; corpus luteum; FSH; LH

Introduction

In 1945, Casida & Warwick reported that ovariectomy performed after Day 55 of pregnancy in the ewe did not always lead to abortion. Ten years later, Denamur & Martinet (1955) showed that ovariectomy can be performed on or after Day 50 of pregnancy without inducing an abortion and suggested that gestation was maintained by an extra-luteal source of progesterone. In fact, it has been demonstrated that the placenta assumes an active role in progesterone secretion during the last two-thirds of pregnancy (Linzell & Heap, 1968; Moore *et al.*, 1972). Therefore, the physiological role, if there is any, of the corpus luteum (CL) after Day 50 of pregnancy in ewes is not known.

A recent study of ovarian follicular populations at Day 140 of pregnancy in the ewe has shown that the ovary bearing a CL contained significantly more small preantral follicles (<0.08 mm in diameter) than did the ovary without a CL (Al-Gubory & Martinet, 1987). These results suggest that the CL has a local stimulatory effect on the recruitment of primordial follicles. This hypothesis is supported by the study of follicular populations at Day 140 of pregnancy in unilaterally ovulating ewes from which the CL was removed at Day 75; there was no difference between the ovary with a CL removed and the contralateral ovary in the number of preantral follicles (Al-Gubory *et al.*, 1987). Furthermore, after surgical removal of the CL, it has been demonstrated that the number of preantral follicles, especially those leaving the reserve of primordial follicles, increased in both kinds of ovary. It is apparent that the CL exerts another effect, thereby limiting the recruitment of primordial follicles during pregnancy by a systemic action.

The fact that the total number of preantral follicles decreased in long-term hypophysectomized ewes (Dufour *et al.*, 1979) and that FSH administration for 4 days in the mouse resulted in an increase in the number of small preantral follicles (De Reviers *et al.*, 1984) could be taken as arguments for a role of FSH in early follicular development.

In view of these observations, it seemed of interest to study the effect of removal of the CL of pregnancy on the secretion of pituitary gonadotrophins.

Materials and Methods

Animals. Ewes of the Préalpes-du-Sud breed, aged 2–3 years, were housed under conditions of natural light and temperature and provided with a diet (hay, straw and concentrates) adequate for gestation needs. Animals had access to water *ad libitum*. At Day 70 of pregnancy, anaesthesia was induced with intravenous injection of 120 mg pentobarbital (Sanofi, Paris, France) and 400 mg thiopental (Abbott, Aubervilliers, France), maintained with halothane, and a midventral laparotomy was performed. In 5 ewes (Group L), selected randomly, CL excision was performed by making an incision on the surface of the ovary and exerting pressure with fingers around the base of the CL. Haemorrhage was arrested by local application of thrombin (Houdé, Paris, France). The other 5 ewes (Group C) were assigned to the sham operation which consisted of exposure of ovaries without removal of the CL. All ewes were injected intramuscularly with penicillin (10^6 U per day) for 3 consecutive days after surgery.

Blood samples. During the breeding season, frequent bleeding (5 ml) consisted of 22 samples taken from the jugular vein into evacuated heparinized tubes every 20 min for 7 h (09:00–16:00 h) on Days 60, 80, 100 and 120 of pregnancy. Blood samples were kept at 4°C before centrifugation (3000 g) for 30 min. Plasma was stored at –20°C until the hormone concentrations were measured.

LH and FSH assay. To avoid inter-assay variation, all plasma samples were run in duplicate in a single assay for each hormone. Concentrations of LH and FSH were measured by the double-antibody RIA according to the procedure described previously (Pelletier *et al.*, 1968; Blanc & Poirier, 1979). The intra-assay coefficient of variation was <12% for LH and <10% for FSH. The minimum detectable concentration of the LH assay was 0.2 ng/ml and that of FSH was 2 ng/ml.

Progesterone assay. Plasma progesterone concentrations were determined for 09:00 h samples in one assay using a previously described double-antibody RIA (Heyman *et al.*, 1984). The intra-assay coefficient of variation and the minimum detectable concentration were <10% and 0.2 ng/ml respectively.

Statistical analysis. The presence, frequency and amplitude of LH and FSH pulses in individual profiles of the 22 blood samples collected from each ewe were determined by the PULSAR program (Merriam & Wachter, 1982). The G values used in this program were $G(1) = 3.8$, $G(2) = 2.0$, $G(3) = 1.5$, $G(4) = 1.2$ and $G(5) = 1.0$. These values specify the number of assay coefficients of variation over a smoothed baseline that 1, 2, 3, 4 or 5 consecutive elevated points must be to qualify as a pulse. The overall mean concentrations of FSH and LH for the 5 ewes per group were calculated as the average of the 22 samples collected from each ewe during period of sampling. All data were subjected to analysis of variance for repeated measurements using general linear models procedures (SAS, 1987) followed by Duncan's multiple range test. A logarithmic transformation was applied to the data, if necessary, to equalize variances.

Results

No significant differences were found between ewes in Groups C and L in the mean progesterone concentrations for any of the four stages of pregnancy examined (Fig. 1). However, 3 of the 5 Group L ewes and 1 of the 5 Group C ewes had 2 lambs while the other ewes had only 1 lamb and this might explain the variation between ewes in Group L in the concentration of progesterone. The analysis of variance revealed an interaction ($P < 0.05$) between groups by time. This could be explained by the difference between ewes in Groups C and L in the evolution of peripheral concentrations of progesterone after Day 60 of pregnancy due to a small, but nevertheless statistically not significant, decrease in mean progesterone concentration 10 days after CL excision (4.5 and 3.2 ng/ml on Days 60 and 80). In both groups of ewes, plasma concentrations of progesterone increased markedly after Day 100.

Plasma concentrations of FSH were relatively constant in all stages of pregnancy examined and were similar in the two groups of ewes (Fig. 2). No episodic release of FSH was detected in any of the ewes.

Plasma concentrations of LH in Group C ewes were low and remained unchanged (about 0.3 ng/ml) between Days 60 and 120, but in Group L ewes increased ($P < 0.01$, Duncan's multiple range test) from 0.4 ng/ml at Day 60 (10 days before CL excision) to 0.7 and 0.6 ng/ml respectively at Days 80 and 100 (10 and 30 days after CL excision) (Fig. 2).

Fig. 1. Mean ($\pm$ s.e.m.) plasma concentrations of progesterone for 5 intact (Group C) pregnant ewes and 5 ewes from which the CL was removed at Day 70 (Group L).

In intact ewes, occasional LH pulses of low amplitude (<0.64 ng/ml) were detected in some stages of pregnancy (Fig. 3). The mean frequency (pulses/7 h) and amplitude (ng/ml) of LH pulses were relatively stable between Days 60 and 120 (Table 1).

Before CL excision (at Day 60), pulsatile release of LH was infrequent (Fig. 3) and the amplitude of LH pulses detected never exceed 0.64 ng/ml. After CL excision, episodic releases of LH occurred frequently (Fig. 3). Compared to Day 60, LH pulse frequency increased ($P < 0.05$, Duncan's multiple range test) at Day 80 (10 days after CL excision). The amplitude of LH pulses increased ($P < 0.05$) 10 days after CL excision: 69% of LH pulses had an amplitude greater than 0.64 ng/ml (χ^2 test).

Table 1. Characteristics of LH secretion during the second half of pregnancy for 5 intact ewes (Group C) and 5 ewes from which the CL was removed at Day 70 (Group L)

Group		Day of pregnancy			
		60	80	100	120
C	No. of LH pulses/7 h for the 5 ewes	3	4	2	2
	Pulse amplitude (ng/ml)	0.48 ± 0.08	0.29 ± 0.04	0.47 ± 0.18	0.32 ± 0.02
	Basal conc. (ng/ml)	0.28 ± 0.02	0.30 ± 0.03	0.34 ± 0.02	0.32 ± 0.02
L	No. of LH pulses/7 h for the 5 ewes	5	13	8	3
	Pulse amplitude (ng/ml)	0.50 ± 0.05	0.88 ± 0.11	0.73 ± 0.14	0.89 ± 0.45
	Basal conc. (ng/ml)	0.38 ± 0.02	$0.61 \pm 0.05^*$	$0.53 \pm 0.05^*$	0.42 ± 0.02

Values are mean $\pm$ s.e.m.

* $P < 0.01$ (Duncan's multiple range test) compared to value at Day 60.

Fig. 2. Mean ($\pm$ s.e.m.) plasma concentrations of FSH and LH for 5 intact (Group C) pregnant ewes and 5 ewes from which the CL was removed at Day 70 (Group L). *Significantly different from Day 60 ($P < 0.01$, Duncan's multiple range test).

The interaction ($P < 0.04$ after square root +0.5 transformation) between groups by time for LH pulse frequency might be explained by the fact that this parameter remained stable between Day 60 and 120 in Group C ewes but increased in Group L ewes (Table 1).

Basal concentrations of LH in Group C ewes were low and remained stable between Days 60 and 120 (Table 1). Basal values of LH at Days 80 and 100 (10 and 30 days after CL excision) were significantly greater ($P < 0.01$, Duncan's multiple range test) than at Day 60 (10 days before CL excision) (Table 1).

Discussion

By using frequent bleeding, we have shown that peripheral concentrations of LH in the ewe during periods when the CL is not necessary for maintenance of pregnancy (i.e. after Day 50) were maintained at low levels with an overall mean of 0.3 ng/ml. This finding is in agreement with a previous study (Alexander *et al.*, 1973) in which only one blood sample was collected at each stage of pregnancy.

There have been two reports in the literature dealing with the episodic release of LH during pregnancy in ruminants (cow: Little *et al.*, 1982; goat: Buttler & Morant, 1986). These authors demonstrated that the pattern of LH secretion is pulsatile and Little *et al.* (1982) also showed that LH pulses frequency and amplitude decrease during the second half of gestation. No information is

Fig. 3. LH profiles from jugular vein blood samples collected at 20-min intervals for 7 h during the second half of pregnancy in 5 intact ewes (Group C) and in 5 ewes from which the CL was removed at Day 70 (Group L). The arrows indicate LH pulses.

available on the tonic secretion of LH during pregnancy in the ewe. In the present study, we showed by using frequent bleeding that plasma concentrations of LH are not static during the second half of gestation in the ewe. However, pulsatile release occurred irregularly and infrequently and may reflect reduced pituitary sensitivity to LHRH (Chamley *et al.*, 1974), pituitary content of LH (Chamley *et al.*, 1976) or episodic release of LHRH.

The fact that LH concentrations and pulse frequencies are higher during the follicular phase after luteolysis, when peripheral concentrations of progesterone declined dramatically from about 3 to 0.1 ng/ml, than during the luteal phase of the oestrous cycle in the ewe (Hauger *et al.*, 1977), provide evidence for the role of progesterone in the control of pulsatile discharge of LH. This hypothesis was strongly supported by the finding that treatment of ovariectomized ewes during the breeding season with implants which provided high peripheral progesterone concentrations (3–5 ng/ml) decreased LH pulse frequency (Goodman & Karsch, 1980; Goodman *et al.*, 1981). These authors showed also that oestradiol administered alone reduced LH pulse amplitude. Goodman *et al.* (1981) and Martin *et al.* (1983) found that implants which provided lower progesterone concentrations (1.3 and 1.6 ng/ml respectively) in ovariectomized ewes did not affect LH pulse frequency in the absence of exogenous oestradiol and suggested that oestradiol and progesterone interact synergistically to inhibit the tonic secretion of LH. During the anoestrous season, Goodman *et al.* (1982) and Tamanini *et al.* (1986) reported that oestradiol administered alone decreases both frequency and amplitude of LH pulses. During the second half of pregnancy in the ewe, the increasing secretion of oestradiol (Terqui, 1974) and progesterone (Moore *et al.*, 1972; Ricketts & Flint, 1980) by the placenta may play a major role in suppression of LH secretion to basal levels.

The present study is the first report concerning the effect of removal of the CL on the secretion of pituitary gonadotrophins during pregnancy in the ewe.

The fact that peripheral concentrations of FSH were not affected by CL excision provides a clear demonstration that the bilateral increase in the recruitment of the primordial follicles in ewes with excised CL (Al-Gubory *et al.*, 1987) could not be attributed to increasing secretion of FSH. This finding is consistent with the hypothesis that the passage of follicles from the reserve of primordial follicles to the early growth phase is FSH-independent (Cahill & Mauléon, 1981).

After surgical removal of the CL at Day 70 of pregnancy, basal concentrations of LH, frequency and amplitude of LH pulses increased considerably. Bilateral ovariectomy performed at Day 75 (Beal *et al.*, 1986) and CL excision at Day 70 of pregnancy in the present study, results in a small transitory decrease in peripheral concentrations of progesterone. In Group L ewes of the present experiment, the concentration of progesterone at Day 80 (10 days after CL excision) was similar to that during the mid-luteal phase of the oestrous cycle (about 3 ng/ml), and thereafter increased, reaching 5.2 ng/ml at Day 100. Nevertheless, LH values on Days 80 and 100 (10 and 30 days after CL excision) were significantly greater than those before CL excision at Day 60. In ewes, oestradiol is secreted exclusively from the large non-atretic antral follicles (Baird & Scaramuzi, 1976). Since CL excision performed at Day 75 of pregnancy had no subsequent effect on antral follicle population and growth (Al-Gubory *et al.*, 1987) and oestradiol is mainly secreted by the placenta after Day 50, we suspect that surgical removal of the CL of pregnancy in the present study induced a decrease in peripheral oestradiol concentration. Taken together, the results of the present study suggest that some luteal factor, presumably not progesterone, may contribute to the inhibition of LH secretion during the second half of pregnancy in the ewe. The decrease of LH secretion after treatment of ovariectomized ewes with steroid-free extract of ovine corpora lutea (unpublished data) strongly supports our hypothesis. In ewes with excised CL, the decrease of LH secretion after Day 100 of pregnancy suggests the involvement of extra-ovarian factors from the fetoplacental unit in LH regulation.

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