Control of ovarian follicular growth and maturation by the corpus luteum and the placenta during pregnancy in sheep

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Ovarian follicular growth and maturation and its control throughout pregnancy have not been described fully in sheep. Experiment 1 characterized the size and maturation (steroid production in vitro and aromatase activity) of ovarian follicles obtained at days 20, 50, 80 and 110 of pregnancy compared with those obtained at day 12 of the oestrous cycle. There was no difference in the number of small follicles (< 3 mm in diameter) between cyclic and pregnant ewes, regardless of the stage of pregnancy. There was a marked reduction (P < 0.01) in the number of medium follicles (3–5 mm) starting at day 80 of pregnancy. Large follicles (> 5 mm) were not detected at day 110 of pregnancy. In vitro testosterone output by follicles was constant throughout pregnancy. Oestradiol output remained steady until day 80, but decreased markedly at day 110 of pregnancy. This decrease was associated with a reduction in aromatase activity in follicles obtained at this stage. Experiment 2 examined the effect of administration of high concentrations of progesterone between day 100 and day 120 after mating on resumption of follicular growth in ewes that underwent Caesarean section at day 99 of pregnancy. In ewes that underwent Caesarean section, progesterone supplementation was successful in mimicking the profile found in pregnant ewes, but did not prevent re-initiation of follicular growth, as demonstrated by the presence of large follicles (> 5 mm) at day 120 after mating. Experiment 3 examined the effects of PGF2α-induced regression of the corpus luteum of day 100 of pregnancy on resumption of follicular growth. High concentrations of PGF2α (0.28 mg kg⁻¹ body weight) administrated at day 100 of pregnancy were required to initiate regression of the corpus luteum. At day 120 after mating, the mean (± SEM) diameter of the largest follicle in PGF2α-treated ewes (3.40 ± 0.47 mm) was significantly greater (P < 0.05) than that in control pregnant ewes (2.52 ± 0.34 mm). Experiment 4 examined the effect of removal of the fetus and of the corpus luteum at day 100 of pregnancy on resumption of ovulation. Removal of the corpus luteum by PGF2α treatment at the time of removal of the fetus resulted in earlier occurrence of short luteal phases (27.8 versus 40.6 days, PGF2α-treated versus non-treated) but did not alter the timing of the first normal luteal phases (41 days). In conclusion, the results from these experiments indicate that placental compounds play a major role in inhibiting follicular growth and maturation during late pregnancy in sheep.

Introduction

Knowledge of ovarian follicular growth and maturation throughout pregnancy in sheep is still limited. Macroscopic observations of the follicles present on the ovarian surface showed that 85% of the follicles had a diameter < 3 mm as early as day 50 of pregnancy (Williams et al., 1956) and that the size of the largest follicle decreased progressively as pregnancy advanced (Rüsse, 1971; Rexroad and Casida, 1975). However, atresia, which is the fate of most the follicles, was not documented in these early studies. Microscopic examinations of ovarian follicles of ewes at day 140 of pregnancy showed that the largest follicles did not exceed 1.5 mm in diameter and were atretic (Al-Gubory and Martinet, 1986) in the ovaries bearing a corpus luteum and those not bearing a corpus luteum (Al-Gubory and Martinet, 1987). However, changes in follicular maturation associated with the progression of pregnancy have not been described. It is unclear whether follicles do not grow beyond 2 mm in diameter during late pregnancy because they have insufficient gonadotrophin support or because key maturational...
events such as the acquisition of the ability to aromatize androgens cannot occur. This last possibility is supported by data from Scaramuzzi et al. (1996) who demonstrated high androstenedione and low oestradiol secretion rates in pregnant ewes with one ovary autotransplanted to the neck, although only a single day of pregnancy (day 121) was studied. Therefore, the first aim of the present study was to characterize the number, size and maturation of ovarian follicles of ewes at four stages of pregnancy (days 20, 50, 80 and 110) by comparison with ewes during the luteal phase (day 12) of the oestrous cycle.

The mechanisms involved in the progressive arrest of terminal follicular growth and induction of atresia during late pregnancy have not been fully elucidated. In sheep, follicular growth over 2 mm in diameter is known to be FSH dependent (Picton et al., 1990). However, no change in peripheral FSH concentrations during pregnancy have been demonstrated that are able to explain why follicular growth stops during late pregnancy (Al-Gubory et al., 1994a). At least three possible mechanisms could be involved in blocking follicular growth: high concentrations of progesterone, or secretion of inhibiting compounds by the corpus luteum or by the fetoplacental unit. These mechanisms are indicated by the following lines of evidence. Firstly, the high peripheral progesterone concentrations observed during late pregnancy (Bassett et al., 1969) could block the development of large healthy follicles directly (Adams et al., 1992; Rubianes et al., 1996) via inhibition of follicular aromatase activity (Fortune and Vincent, 1983; Manikkam and Rajamaheendran, 1997). Secondly, data collected in vivo (Rexroad and Casida, 1975) and in vitro (Al-Gubory et al., 1994b) indicate that the corpus luteum of late pregnancy may produce compounds that can modulate follicular aromatase activity and growth. Thirdly, the resumption of follicular growth observed after surgical removal of the gravid uterus (Al-Gubory et al., 1992) or of the conceptus (Al-Gubory and Abdennabbi, 1996) indicates that the fetoplacental unit may inhibit follicular growth directly. This proposal is also supported by data in vitro which show that the sheep placenta of late pregnancy contains non-steroidal factors that inhibit aromatase activity of granulosa cells (Al-Gubory et al., 1995). Therefore, the second aim of the present study was to ascertain which of these three possible mechanisms plays a key role in blocking follicular growth during late pregnancy.

Materials and Methods

Animals

All ewes used were housed under conditions of natural daylength and temperature at Jouy-en-Josas (Expts 1, 2 and 3) and at Nouzilly (Expt 4) and fed according to gestation requirements. Before mating, ewes were oestrous synchronized by the intravaginal insertion of sponges impregnated with 40 mg fluorogestone acetate (Intervet, Angers) for 14 days. An i.m. injection of 400 iu eCG (Intervet) was given when the sponge was removed. All ewes were subsequently mated with fertile rams.

Surgery

On the day of surgery, ewes were initially anaesthetized with a mixture of pentobarbital (Sanofi, Paris), thiopentone (Abbott, Aubervilliers) and atropine sulfate (Aguettant, Lyon). After endotracheal intubation, anaesthesia was maintained by constant inhalation of a mixture of oxygen and halothane. Reproductive organs were exposed through a midventral laparotomy. Caesarean section (placental membranes, fetal fluids and fetus were removed) was performed by making an incision of about 10 cm near the uterotubal junction. The fetoplacental unit was removed from the uterine horns after manual separation of the placental cotyledons from the uterine caruncles. Blood clots were removed carefully by washing the uterus and uterine horns with sterile warm saline (0.9% (w/v) NaCl). In Expt 4, surgery was performed as above except that only the fetus was removed. Finally, one tablet (1 g) of chlortetracycline hydrochloride (Auréomycine, Rhône Mérieux, Lyon) was placed in utero. The uterine incision was closed through the myometrium and serosa using 4/0 catgut (Braun Biotrol, Boulogne). The sham-operated ewes underwent laparotomy as above and the reproductive tract was exteriorized and manipulated. All ewes were injected with penicillin (10⁶ iu day⁻¹) for 3 consecutive days after surgery.

Experimental designs

Experiment 1. The aim of Expt 1 was to characterize number, size and maturation of ovarian follicles during pregnancy. A total of 37 ewes were used and ovaries were obtained at specific stages of pregnancy (day 20, n = 8; day 50, n = 8; day 80, n = 8; day 110, n = 6). A group of seven non-mated ewes acted as controls; ovaries were obtained from the controls at day 12 of the oestrous cycle. The ovaries were collected when the animals were killed and immediately transported to the laboratory. All follicles visible on the ovarian surface were counted and the diameters was estimated by comparison with callipers of known size. The follicles were divided into three size classes: small (< 3 mm), medium (3–5 mm) and large (> 5 mm). After dissection of the largest follicles and at least one to four smaller follicles, the diameters were determined using a stereomicroscope fitted with an ocular graticule. All follicles were cultured intact for 1 h as described for sheep follicles by Webb et al. (1989). Follicles were placed in 1 ml Egle’s minimum essential medium (MEM, Sigma, Saint Quentin Fallavier) and incubated at 37°C in an incubator containing 5% CO₂ in air. At the end of culture, the media were recovered and stored frozen until assayed for oestradiol and testosterone. Follicular fluid was aspirated immediately and the follicle shell was used to assess its aromatase activity as described by Driancourt et al. (1996). The test system used was the conversion of [¹²⁵I]testosterone (DuPont NEN, Les Ulis) to [³H]O₂ during aromatization. Details on the validation of this procedure for sheep follicles were provided by Driancourt et al. (1996). Briefly, at the initiation of culture, 35 ng [¹H]testosterone was added to 1 ml MEM containing each follicular wall (one follicular wall per well). Culture was
for 3 h in an incubator containing 5% CO₂ in air. At the end of culture, each follicular wall was carefully weighed and the amounts of ³H₂O produced were measured using SepPak C18 columns (Waters, Milford). Aromatase was expressed as the ratio (expressed per mg of tissue) between the d.p.m. present in the aqueous phase and the total label provided. Blanks were included in all cultures.

**Experiment 2.** The aim of Expt 2 was to assess the effect of administration of high progesterone concentrations (mimicking those occurring during late pregnancy) on resumption of follicular growth in ewes undergoing Caesarean section. Eight ewes were used, which were randomly assigned to two groups (n = 4 ewes per group) at day 99 after mating: sham-operated pregnant ewes (group 1) and progesterone-treated ewes that underwent Caesarean section (group 2). The ewes were treated twice a day (at 08:00 h and 16:00 h) by a s.c. injection of 1 ml ethanol (group 1) and 50 mg progesterone ml⁻¹ ethanol (group 2). Treatment (ethanol or progesterone in ethanol) started 2 days before surgery and continued for 20 days until the animals were killed at day 120 after mating. In a preliminary trial involving ovarioctomized ewes, this progesterone supplementation regimen produced peripheral concentrations of progesterone similar to those found during late pregnancy in ewes undergoing Caesarean section. At day 120 after mating, the ovaries were collected when the animals were killed and immediately transported to the laboratory. The largest and second largest follicles were dissected and measured under a stereomicroscope.

**Experiment 3.** The aim of Expt 3 was to assess the effects of PGF₂α-induced regression of the corpus luteum of pregnancy on resumption of follicular growth. A total of 28 ewes were randomly assigned to one of three groups at day 100 after mating: pregnant ewes (n = 12) acted as controls, whereas the other two groups received a single i.m. injection of PGF₂α (Lutalyse, Upjohn Co., Paris) at a dose of 0.14 mg kg⁻¹ body weight (n = 10) or 0.28 mg kg⁻¹ body weight (n = 6). The doses of PGF₂α were selected according to Campbell et al. (1994) who reported that the minimum effective dose of PGF₂α causing regression of the sheep corpus luteum of days 90–100 of pregnancy was 0.14 mg kg⁻¹ body weight. Blood samples were collected before injection of PGF₂α and once a day for at least 5 days after treatment. At day 120 after mating, the ovaries were collected when the animals were killed and immediately transported to the laboratory. The status of the corpus luteum (regressed or not regressed) was recorded. The largest and second largest follicles were dissected and measured under a stereomicroscope.

**Experiment 4.** The aim of Expt 4 was to assess the effect of removal of the fetus and of the corpus luteum on resumption of ovulation. Fetuses were removed from 13 ewes at day 100 after mating. In addition, seven of these ewes were given an i.m. injection of 0.14 mg PGF₂α kg⁻¹ body weight at surgery to induce regression of the corpus luteum. Resumption of ovulation was followed by monitoring progesterone concentrations in jugular blood samples collected twice a week according to Terqui and Thimonier (1974). A short luteal phase was defined by a brief period (one or two samples) of progesterone production not exceeding 1 ng ml⁻¹. A normal luteal phase was defined by a sequence of samples (generally three or four) containing > 1 ng progesterone ml⁻¹.

**Steroid assays**

Oestradiol and testosterone concentrations in culture media were quantified by radioimmunoassay (Terqui, 1978; Hochereau-de Reviers et al., 1990). The intra-assay coefficients of variation were 7.7% and 11.5% for oestradiol and testosterone, respectively. The minimum detectable values for oestradiol and testosterone were 20 pg ml⁻¹ and 0.2 ng ml⁻¹. Plasma progesterone concentrations were determined by a double-antibody radioimmunoassay (Heyman et al., 1984). The intra-assay coefficient of variation was < 10%. The minimum detectable value was 0.1 ng ml⁻¹.

**Statistical analysis**

Steroid concentrations in medium and aromatase activity were analysed by ANOVA using the general linear models procedures (SAS, 1987). For all parameters, the analysis included the group effect, the animal within group effect, and the size by group interaction. Differences in follicle size were tested by t tests or ANOVA on raw or transformed data (unequal variances). Comparison of progesterone concentrations in ewes in Expt 3 was achieved by ANOVA for repeated measurements.

**Results**

**Experiment 1: characterization of antral follicular development and maturation during pregnancy**

There was no difference in the number of small follicles between cyclic ewes and pregnant ewes, regardless of the stage of pregnancy (Table 1). There was a marked reduction (P < 0.01) in the number of medium follicles from two to three in the early pregnant groups to about 0.5 starting at day 80 of pregnancy (Table 1). There were few large follicles and individual variations precluded visualization of the statistical differences (Table 1). However, no large follicles were observed at day 110 of pregnancy. The changes in the diameter of the largest follicle reflected the changes in the number of medium and large follicles. A significant decrease (P < 0.01) occurred in the diameter of the largest follicle as pregnancy advanced. The mean (± SEM) diameter of the largest follicle at day 80 and day 110 of pregnancy was significantly smaller (P < 0.05) than that at day 12 of the oestrous cycle and day 20 of pregnancy (Table 1).

Follicular maturation was compared between stages using two parameters, namely steroid production in vitro during
short-term incubation and estimation of aromatase activity during a 3 h incubation. The model used tested the effect of the stage of pregnancy, of ewe within stage of pregnancy and the size by stage of pregnancy interaction. Least square means for oestradiol and testosterone present in culture medium and aromatase activity are presented (Table 2). For oestradiol production, no significant stage of pregnancy effect or ewe within stage of pregnancy effect was detected. In contrast, the size by stage of pregnancy interaction was significant \((P < 0.01)\). This effect was related to the low oestradiol output of follicles obtained at day 110 of pregnancy. For testosterone concentration, none of the main effects (stage of pregnancy, size by stage of pregnancy interaction) reached significance. The only effect that was significant was the ewe within stage of pregnancy effect \((P < 0.01)\). There was a marked reduction in oestradiol output by follicles at day 110 of pregnancy, but the testosterone output by these follicles was relatively similar to that of follicles obtained during early pregnancy. When aromatase activity, measured by the release of tritiated water, was compared between groups, a significant stage of pregnancy effect \((P < 0.01)\) was detected, whereas the ewe within stage of pregnancy effect and the size by stage of pregnancy interactions were not significant (Table 2). A separate analysis was carried out on all follicles 3–4 mm in diameter to confirm that the changes in aromatase activity were not related to the changes in follicle size described above (Table 2). A significant \((P < 0.01)\) stage of pregnancy effect was again identified; aromatase activity was significantly \((P < 0.01)\) depressed in follicles from day 110 of pregnancy compared with that in follicles from day 80.

**Experiment 2: resumption of follicular growth in progesterone-treated ewes that underwent Caesarean section**

In ewes that underwent Caesarean section, progesterone supplementation was successful in mimicking the profile observed in pregnant ewes. In treated ewes, peripheral concentrations of 10–20 ng progesterone ml\(^{-1}\) were achieved, which are similar to those measured in pregnant ewes (Fig. 1). The high concentrations of progesterone did not prevent re-initiation of follicular growth as the mean \((6 \pm SEM)\) diameters of the largest and the second largest follicle measured at day 120 after mating were significantly \((P < 0.01)\) increased in the progesterone-treated ewes that underwent Caesarean section compared with sham-operated pregnant ewes (Fig. 2).

**Experiment 3: resumption of follicular growth in prostaglandin-treated pregnant ewes**

The ability of the two doses of PGF\(_{2\alpha}\) to initiate regression of the corpus luteum was assessed using two parameters. Firstly, when the morphology of the corpus luteum was assessed when the animals were killed, the corpus luteum of all pregnant untreated ewes appeared functionally active,
the corpus luteum was functionally active in half of the ewes (5/10) treated with 0.14 mg PGF$_2$ kg$^{-1}$ body weight, whereas in all ewes treated with 0.28 mg PGF$_2$ kg$^{-1}$ body weight, the corpus luteum appeared to be regressed. Secondly, when the concentrations of progesterone (Fig. 3) were compared by ANOVA among the three groups of ewes (excluding the five ewes treated with 0.14 mg PGF$_2$ kg$^{-1}$ body weight that failed to show luteolysis), a significant group effect ($P < 0.01$), time effect ($P < 0.01$) and group by time interaction ($P < 0.01$) was detected. The interaction was related to a larger decrease in progesterone concentrations 1 day after PGF$_2$ treatment in the 0.28 mg kg$^{-1}$ body weight group (50%) compared with the 0.14 mg kg$^{-1}$ body weight group (33%).

Analysis of the follicle size data by ANOVA (excluding the five ewes treated with 0.14 mg PGF$_2$ kg$^{-1}$ body weight that failed to show luteolysis) demonstrated a significant ($P < 0.05$, diameter of the largest follicle) and highly significant ($P < 0.01$, diameter of the second largest follicle) treatment effect. In ewes treated with 0.28 mg PGF$_2$ kg$^{-1}$ body weight, the diameter of the largest follicle (3.40 $\pm$ 0.47 mm, $n = 6$) was significantly larger ($P < 0.05$) than that in ewes treated with 0.14 mg PGF$_2$ kg$^{-1}$ body weight (2.02 $\pm$ 0.08 mm, $n = 5$) and in control ewes (2.52 $\pm$ 0.34 mm, $n = 10$). The diameter of the second largest follicle was also significantly larger ($P < 0.01$) in ewes treated with 0.28 mg PGF$_2$ kg$^{-1}$ body weight (2.80 $\pm$ 0.11 mm) than that in the two other groups (0.14 mg PGF$_2$ kg$^{-1}$ body weight: 1.86 $\pm$ 0.05 mm; control: 1.95 $\pm$ 0.11 mm).

**Experiment 4: resumption of ovulation after removal of the fetus and removal of the corpus luteum**

Five of seven ewes treated with a dose of 0.14 mg PGF$_2$ kg$^{-1}$ body weight at the time of removal of the fetus underwent immediate luteolysis. In the remaining two ewes that failed to undergo immediate luteolysis, the corpus luteum remained functional for only 1 week. Since the duration of corpus luteum function was limited and since these two ewes behaved as the other five ewes, the timing to ovulation for these ewes was included in the analysis. Resumption of ovulation was estimated from the time after mating.
between surgery and the first sample containing concentrations of progesterone in excess of 1 ng ml⁻¹. Samples containing an excess were obtained significantly earlier (P < 0.05) when PGF₂α was injected (27.8 ± 4.3 days, mean ± SEM) than in untreated ewes (40.6 ± 2.6 days). When the time interval to the first normal luteal phase was analysed, no differences between groups were detected (41.0 ± 2.5 days and 44.5 ± 3.3 days for ewes that did not or did receive PGF₂α at surgery, respectively).

Discussion

The data reported in the present study extend the knowledge of the mechanisms controlling follicular growth during pregnancy in sheep. The data show that the absence of large healthy follicles in ovaries of late pregnant sheep is the consequence of two sequential blocks, affecting both follicular growth and follicular maturation, and clarify, at least in part, the origin of the compounds involved in these locks.

The results of the present study confirm other reports that follicular growth is inhibited as pregnancy progresses (Rüsse, 1971; Rexroad and Casida, 1975). In this respect, ovaries from pregnant sheep are similar to those from cattle (Ginther et al., 1996). The present study extends the findings of Rüsse (1971) and Rexroad and Casida (1975) by showing that after the block in follicular growth occurs between days 50 and 80 of pregnancy, an additional block, which occurs at a key step in follicular maturation (that is the development of active aromatase activity in granulosa cells), is set up between days 80 and 110 of pregnancy. At least three lines of evidence support this proposal. Firstly, oestradiol was not detected in most samples of incubation medium of follicles dissected from ovaries of day 110 of pregnancy, whereas the testosterone output of these follicles was similar to that observed at earlier stages. Secondly, aromatase activity in follicles dissected at day 110 of pregnancy, as determined by the release of tritiated water, was similar to blank values. Thirdly, the ANOVA run on a subgroup of follicles of a similar size (2–4 mm) demonstrated a marked decrease in aromatase activity between days 80 and 110 of pregnancy. These observations are in agreement with those made in pregnant sheep with an ovarian autotransplant (Saccomuzzi et al., 1996). The model used by Saccomuzzi et al. (1996) demonstrated that ovaries of late pregnant ewes showed a high androstenedione secretion rate associated with a minimum oestradiol secretion rate. As an FSH induced increase in aromatase activity is one of the key triggers of terminal follicular growth (Tsonis et al., 1984a; Picton et al., 1990; Driancourt et al., 1996), the marked reduction in aromatase activity in follicles obtained between days 80 and 110 of pregnancy may reduce further the ability of follicles to grow and may reinforce the initial block in follicular growth that appears earlier at days 50–80. The result of the combination of these two suppressive mechanisms is the absence of follicles > 2 mm in ovaries during late pregnancy. In cattle, repression of follicular growth has been reported at about the fifth month of pregnancy and almost total disappearance of follicular waves during the last month of pregnancy (Ginther et al., 1996). Whether a defect in aromatase activity in the follicles present at this stage of pregnancy is also involved in repression of follicular growth in cattle remains to be established.

In the present study, the possible mechanisms involved in the block in follicular growth, developing between days 50 and 80 and expanding between days 80 and 110 of pregnancy, were explored. Gonadotrophins (FSH and LH) were not considered relevant candidates because concentrations are not markedly affected as pregnancy progresses (Al-Gubory et al., 1994a). Three other candidates (that is high concentrations of circulating progesterone, inhibitory action of compounds produced by the corpus luteum of pregnancy or by the fetoplacental unit) were tested for potential inhibitory effects on follicular growth.

In Exp 2, the ability of progesterone to inhibit follicular growth was tested. Evidence supporting inhibitory effects of exogenous progesterone on follicular development have been obtained in a number of species, including sheep (Rubianes et al., 1996). In addition, progesterone may suppress follicular development by inhibiting aromatase activity of granulosa cells, as reported in studies conducted in vitro (Fortune and Vincent, 1983) and in vivo (Manikkam and Rajamahendran, 1997). In the present study, injection of concentrations of exogenous progesterone that mimicked circulating progesterone concentrations occurring in late pregnant ewes (Bassett et al., 1969) failed to prevent the resumption of follicular growth after Caesarean section performed at day 99 after mating. This finding indicates that the sheep placenta secretes factors other than progesterone that contribute to the block in terminal follicular growth during late pregnancy.

In Exp 3, the effect of removal of the corpus luteum of pregnancy on follicular growth was monitored. The sheep corpus luteum is not necessary for the maintenance of pregnancy after day 60 as the placenta produces and secretes sufficient progesterone to maintain gestation until term (Al-Gubory et al., in press). Therefore, in the present study PGF₂α induced regression of the corpus luteum at day 100 of pregnancy was used to provide evidence for the possible role of this structure in ovarian follicular development. A possible inhibitory role of the corpus luteum in follicular development was indicated by data obtained in cattle (Saiuddin et al., 1967; Foote and Peterson, 1968; Bellin et al., 1984; Nation et al., 1999) showing that, after parturition, the first large non-atretic follicle or resumption of ovulation preferably occur on the ovary opposite to the corpus luteum of pregnancy. Furthermore, in postpartum ewes, the total number of follicles and the maximum follicle diameter of all follicles > 2 mm are reduced in ovaries bearing the past corpus luteum of pregnancy (Bartlewski et al., 1995). Finally, evidence collected in vitro (Al-Gubory et al., 1994b) indicates that the sheep corpus luteum of late pregnancy contains non-steroidal compounds inimical to follicular maturation. In the present study, ablation of the corpus luteum of pregnancy was achieved via injection of PGF₂α. High concentrations of PGF₂α were required to induce luteolysis efficiently. At 20 days after injection of PGF₂α a 1 mm increase was noted in the diameter of the largest and second largest follicle. Although this finding indicates that the corpus luteum of...
pregnancy produces compounds that affect follicular growth, the limited magnitude of the size increase indicates that it is not the main organ involved in blocking follicular growth during late pregnancy. Alternatively, the placenta may mask an apparent inhibitory effect of the corpus luteum on ovarian follicular development during late pregnancy in sheep.

In Expt 4, the effect of removal of the fetus or of the corpus luteum on resumption of ovulation was tested. Studies in vivo in early pregnancy of cattle (Thatcher et al., 1991) and sheep (Al-Gubory et al., 1992; Al-Gubory and Abdennebi, 1996) have indicated that the fetoplacental unit may inhibit follicular growth. A study in vitro showed that the sheep placenta of late pregnancy contains a non-steroidal factor that inhibits aromatase activity of granulosa cells (Al-Gubory et al., 1995). Resumption of ovarian activity, as observed in cattle induced to abort during pregnancy (Wright and Kiracofe, 1988), was progressive, and short luteal phases preceded the occurrence of normal luteal phases. In the present study, removal of the corpus luteum at the time of removal of the fetus resulted in an earlier occurrence of short luteal phases, but did not alter the time of the first normal luteal phase. In contrast, the long delay between removal of the fetus (with or without removal of the corpus luteum) and ovulation (27–40 days) is in contrast to the time required for a 2 mm follicle to reach ovulation (2–3 days) as reported by Tsonis et al. (1984b) and Driancourt and Cahill (1984). This finding indicates that placental compounds play a major role in inhibiting follicular growth and maturation during late pregnancy in sheep.

The placenta of ruminant species, including sheep and cattle, produces and secretes proteins such as the pregnancy-specific protein B (PSPB) (Butler et al., 1982), pregnancy-associated glycoproteins (PAG) (Zoli et al., 1991), and the pregnancy serum protein 60 (PSP60) (Mialon et al., 1993), which are present in the peripheral circulation in increasing amounts as pregnancy progresses (Sasser et al., 1986; Willard et al., 1987; Mialon et al., 1993) and decrease slowly during the early postpartum period (Sasser et al., 1986; Mialon et al., 1993; Ranilla et al., 1994). The delay in resumption of ovulation when circulating PSPB is high during the early postpartum period in cattle (Kiracofe et al., 1993) and the inhibition of aromatase activity of sheep ovarian follicles in vitro by PSP60 (Al-Gubory et al., 1997) indicate that these secreted placental proteins are involved directly in the block in follicular development during late pregnancy.

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