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Original article

The ovarian secretion of androstenedione and oestradiol during late pregnancy and the early postpartum period in sheep with an autotransplanted ovary

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Summary — During late pregnancy in the ewe, ovarian function is suppressed by placental steroids and following parturition ovarian function is restored. This experiment determined the ovarian secretion of oestradiol and androstenedione during late pregnancy and the early postpartum period in ewes. Six ewes with ovarian autotransplants were transplanted with three day 6 embryos and three gave birth on day 147. Ovarian and jugular blood sampled were collected on three different occasions. On each occasion a 4 h period of sampling was followed by a 6 or 8 h period during which the ewes were challenged with 150 ng of gonadotropin-releasing hormone (GnRH). Basal secretion of oestradiol and androstenedione was 0.3 ± 0.1 and 10.5 ± 3.0 ng min^{-1}, respectively, on day 120 of pregnancy. Oestradiol secretion remained low on days 7 and 21 postpartum (0.4 ± 0.3 and 0.3 ± 0.1 ng min^{-1}, respectively). Androstenedione secretion (ng min^{-1}) on days 7 and 21 postpartum was 2.5 ± 0.5 and 4.1 ± 1.8, respectively. The injection of GnRH on day 121 of pregnancy produced luteinizing hormone (LH) release with a peak concentration of 0.6 ± 0.1 ng mL^{-1}, that did not stimulate steroid secretion. On day 8 postpartum GnRH injection induced LH release with a peak concentration of 3.9 ± 1.1 ng mL^{-1} that stimulated secretion of oestradiol (0.2 ± 0.1 to 2.1 ± 0.9 ng min^{-1}; $P < 0.01$) and androstenedione (2.3 ± 0.6 to 17.1 ± 6.9 ng min^{-1}; $P < 0.001$). Similar effects were seen on day 22 postpartum; GnRH injection induced LH release with a peak concentration of 4.7 ± 1.4 ng mL^{-1} that stimulated secretion of oestradiol (0.2 ± 0.1 to 3.7 ± 1.1 ng min^{-1}; $P < 0.001$) and androstenedione (4.2 ± 2.6 to 29.5 ± 9.2 ng min^{-1}; $P < 0.01$). These results suggest that the suppresion of ovarian function during late pregnancy in the ewe is reversed by 7 days postpartum.

oestradiol / androstenedione / LH / pregnancy / ovary

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Résumé — Chez la brebis pendant la gestation, la fonction ovarienne est inhibée par les stéroïdes placentaires. Après la mise bas, la fonction ovarienne est rétablie. *L'expérience suivante a permis de déterminer les sécrétions ovariennes d'œstradiol et d'androstènedione chez la brebis en gestation et post-partum. Six brebis aux ovaires autotransplantés ont été implantées chacune avec trois embryons 6 jours après la fécondation et trois d'entre elles ont mis bas à j147. Des échantillons de sang ovarien et de sang prélevé à la veine jugulaire ont été collectés à trois moments. À chaque fois, une période de prélèvement de quatre heures a été suivie d'une période de six ou huit heures durant laquelle elles ont été soumises à une stimulation par 150 ng de GnRH. Les sécrétions basales d'œstradiol et d'androstènedione ont été respectivement de 0,3 ± 0,1 et de 10,5 ± 3,0 ng min^{-1} à j120 de gestation. La sécrétion d'œstradiol est restée faible aux jours 7 et 21 (respectivement 0,4 ± 0,3 et 0,3 ± 0,1 ng min^{-1}) post-partum. La sécrétion d'androsténedione a été de 2,5 ± 0,5 ng min^{-1} au jour 7 et de 4,1 ± 1,8 ng min^{-1} au jour 21. L'injection de GnRH à j121 de gestation a induit des pics de LH (0,6 ± 0,1 ng mL^{-1}) qui n'a pas stimulé de sécrétion de stéroïdes. À j 8 post-partum, l'injection de GnRH a provoqué des pics de LH (3,9 ± 1,1 ng mL^{-1}) qui ont stimulé la sécrétion d'œstradiol (0,2 ± 0,1 à 2,1 ± 0,9 ng min^{-1} ; P < 0,01) et d'androsténedione (2,3 ± 0,6 à 17,1 ± 6,9 ng min^{-1} ; p < 0,001). Des effets similaires ont été observés à j 22 post-partum ; l'injection de GnRH a provoqué des pics de LH (4,7 ± 1,4 ng mL^{-1}) qui ont stimulé la sécrétion d'œstradiol (0,2 ± 0,1 à 3,7 ± 1,1 ng min^{-1} ; p < 0,01) et d'androsténedione (4,2 ± 2,6 à 29,4 ± 9,2 ng min^{-1} ; p < 0,001). Ces résultats suggèrent que l'inhibition de la fonction ovarienne pendant la gestation est supprimée dès j7 post-partum.*

œstradiol / androsténedione / LH / gestation / ovaire

INTRODUCTION

There is a paucity of information concerning follicular development and function during pregnancy of the sheep. During late pregnancy (day 140) in the ewe virtually all follicles of 1 mm or greater are atretic (Al-Gubory and Martinet, 1986). Other data from cattle and pigs (Rexroad and Casida, 1975) suggest that although some follicular development occurs early in pregnancy, by the final third of pregnancy the ovaries are almost totally devoid of large antral follicles. The same appears to be true for humans (Govan, 1968, 1970) but not for rodents (Greenwald, 1966, 1967; Greenwald and Choudary, 1969; Pedersen and Peters, 1971) or rabbits (Adams, 1968).

The functional state of these follicles has been difficult to assess in vivo because of the production of steroids by placental tissue and by the relative inaccessibility of the ovary with advancing pregnancy. Several in vitro studies with cultured ovarian follicular cells from pregnant rats (Richards and Kersey, 1979; Bogovich et al, 1981; Carson et al, 1981) suggest that follicular steroidogenesis is impaired in late pregnancy; in particular, there is a thecal deficiency of the 17α-hydroxylase and C17-20 lyase enzymes (Bogovich and Richards, 1982). Following parturition follicular function is restored although the rate at which it is restored shows considerable intra- and interspecies variation. In some species a postpartum ovulation occurs normally, within 1 or 2 days of birth (rat; Connor and Davis, 1980) while in others the first postpartum ovulation can be normally delayed for several months (cow; Peters and Lamming, 1990; Short et al, 1990). In the ewe postpartum ovulation can occur within a week (Mauléon and Dauzier, 1965) or it can be delayed by several months depending on the time of the year (Abecia et al, 1992) and on nutrition (Shevah et al, 1975; Loudon, 1987). The time of the first postpartum ovulation can be delayed by lactation and the intensity of the suckling stimulus (Mauléon and Dauzier, 1965; Schirar et al, 1989).

The physiology of the ovary during the inactive state of late pregnancy and over the period of transition to an active state in the postpartum period is not clear. The low

level of gonadotrophic support may be sufficient to explain ovarian inactivity in late pregnancy. Alternatively, the ovary may be inactive because of the direct effects of placental hormones on ovarian function.

Using the technique of superovulation and embryo transfer (Ward et al, 1986; Murray et al, 1989), pregnancies were established in three ewes with an ovarian autotransplant and ovarian function was studied prepartum and twice at postpartum. We are able to report the pattern of endogenous and gonadotropin-releasing hormone (GnRH)-induced secretion of follicular steroids from the ovary of the conscious pregnant ewe. A preliminary account of this experiment has been published as an abstract (Scaramuzzi et al, 1989).

MATERIALS AND METHODS

Embryo transfer

Donor animals

Twenty Border Leicester X Merino cross ewes were used as donor animals.

Recipient animals and diet

Six Border Leicester X Merino cross ewes with a mean body weight of 58.0 ± 0.6 kg had the left ovary and vascular pedicle autotransplanted (Goding et al, 1967). The animals were studied in the normal breeding season and housed in a group pen with ad libitum access to a pelleted ration and water.

Oestrous synchronization: embryo production and embryo transfer

The procedure employed has been described elsewhere in detail (Ward et al, 1986; Murray et al, 1989). The embryo transfers were carried out in the nonbreeding (anoestrous) season so that the ewes would give birth and lactate during the natural breeding season. Briefly, the oestrous cycles of both donor and recipient ewes were synchronized using progestagen (medroxy-progesterone actate) sponges (Repromap; Upjohn Pty Ltd, Rydalmere, NSW, Australia). Donor and recipient ewes were injected intramusculary with PMSG (Pregnecol; Intervet, Artarmon, NSW, Australia) 24 h before sponge removal. Donor ewes received 1 000 IU while recipient ewes received 750 IU. The donor ewes were inseminated with 100 x 10^6 sperm 24 h after sponge removal. The embryos were recovered from donor ewes at the early blastocyst stage 100 to 120 h postinsemination and assessed morphologically for normality (Ward et al, 1986; Murray et al, 1989). Three morphological normal blastocysts were transfered to each of six recipient ewes. The successful establishment of pregnancy was confirmed by ultrasound carried out 30 to 35 days post-transfer, in three of the six ewes.

Experimental protocol

From day 100 of pregnancy until 28 days after parturition the three pregnant ewes were bled three times a week by jugular venepuncture. On day 111 of pregnancy the ewes were placed in metabolism cages (Till and Downes, 1963) in a temperature controlled (20 °C) room and allowed to acclimatize for 1 week. While in the metabolism cages they were provided ad libitum with a pelleted ration and fresh water. The ewes were placed in a single metabolism cage and following parturition with a second cage placed alongside and the lower rails removed from both cages. This provided exclusive space for the lambs and allowed them to suckle the ewes during the experimental period, including the periods of intensive blood sampling.

Experimental design

Over the course of the experiment the ewes were subjected to three periods of intensive blood sampling: days 119 and 120 of pregnancy, days 7 and 8 postpartum and days 21 and 22 postpartum. On the first day of each period of intensive sampling the ewes were sampled at 15 min intervals for a total period of 6 h. The next day the sampling was repeated for 5 h on day 120 of pregnancy and for 9 h on days 8 and 22 postpartum. On the second day of sampling each ewe

was injected intravenously with 2 mL of saline containing 150 ng of GnRH (Sigma Chemical Company, MO, USA) at 2 h (day 121 of pregnancy) and at 1 and 5 h (days 8 and 22 postpartum) of the intensive sampling period. Each sampling consisted of a timed ovarian venous blood (5 mL) followed immediately by a sample of jugular venous blood (3 mL). The blood was centrifuged at 4 °C, the plasma harvested and stored at –20 °C.

Cannulations

On the day prior to the start of the intensive blood sampling all ewes had a polyvinyl cannula inserted into the jugular vein distal to the ovarian and jugular vein anastomosis, and a second polyvinyl cannula inserted into the contralateral jugular vein (Downing, 1994).

Pharmaceutical treatments

Following cannulation and daily thereafter, all ewes were given a 4 mL intramuscular injection of antibiotic (Hydropen; Bomac Laboratories, Castle Hill, NSW, Australia). Over the intensive blood sampling period all ewes were given a rapid intravenous injection of 5 000 IU heparin (Boots Australia Pty Ltd, North Rocks, NSW, Australia) at the start of sampling and then every 3 h. Between blood samples the cannulae were filled with heparinized saline (50 IU mL^{-1}).

Hormone analysis

The concentrations of luteinizing hormone (LH), progesterone (Downing et al, 1995), oestradiol and androstenedione (Dowing, 1994) were determined using established radioimmunoassays. Details of the antibodies and their specificities, the sensitivity and the inter- and intra-assay variability for these assays carried out in the same laboratory have been published in detail elsewhere (Downing, 1994; Downing et al, 1995).

Progesterone

The concentration of progesterone was determined in jugular venous blood collected three times weekly.

Luteinizing hormone

LH concentrations were determined in jugular venous plasma samples collected during the periods of intensive sampling.

Oestradiol

The concentration of oestradiol was determined in ovarian venous samples collected during the periods of intensive sampling. The time taken to collect each ovarian venous sample was recorded and the blood haematocrit determined hourly. These values were then used to determine the secretion rate of oestradiol (Collett et al, 1973).

Androstenedione

The concentration of androstenedione was determined in ovarian venous plasma collected during the periods of intensive sampling. The time taken to collect each ovarian venous sample was recorded and the blood haematocrit determined hourly. These values were then used to determine the secretion rate of androstenedione (Collett et al, 1973).

Statistical analysis

Differences in basal hormone concentrations and secretion rates were compared using a repeated measures analysis of variance (ANOVA, CRL; Clear Lake Inc, Houston, TX, USA). The maximum responses of LH, oestradiol and androstenedione to GnRH injection were compared using Student's unpaired *t*-test.

RESULTS

Embryo transfer

Pregnancy was successfully established in three of the six recipient ewes with ovarian autotransplants. All three ewes carried to term and after an uneventful pregnancy delivered normally and unassisted on day

147 of gestation. One ewe delivered a single lamb, one a set of twins and the third a set of triplets. Lactation was established and lambs suckled normally.

Progesterone

Progesterone concentrations were high over the last 40 days of pregnancy but fell rapidly at parturition (fig 1). Two ewes appeared to have ovulated within a few days of parturition and progesterone concentrations rose indicative of luteal activity (fig 1).

Endogenous pulses of LH

No LH pulses were detected over the 6 h sampling period in any ewe on day 120 of pregnancy. On day 7 postpartum one ewe had a single LH pulse (amplitude 4.5 ng mL^{-1}); no LH pulses were detected in the other two ewes. On day 21 postpartum one ewe (the same one) had a single LH pulse (amplitude 2.8 ng mL^{-1}); no LH pulses were detected in the other two ewes. The mean concentrations of LH were low and not significantly different from each other at all three sampling periods (table I) and this is reflected in low endogenous secretion rates of oestradiol (table I). The secretion rate of androstenedione was elevated on day 120 of pregnancy when compared to the secretion rates on days 7 and 21 postpartum (table I); however, the differences were only significant on day 7 postpartum ($P = 0.05$).

Responses to the GnRH challenge

On day 121 of pregnancy the injection of GnRH induced significant LH release (fig 2) but, the maximum LH concentration was greatly reduced compared to the corresponding release induced by the same dose of GnRH on days 8 ($P < 0.05$) and 22 ($P < 0.05$) postpartum (table II). There was no detectable increase in the secretion of either oestradiol or androstenedione around the time of the induced LH release (fig 2) although the basal secretion of androstenedione was elevated.

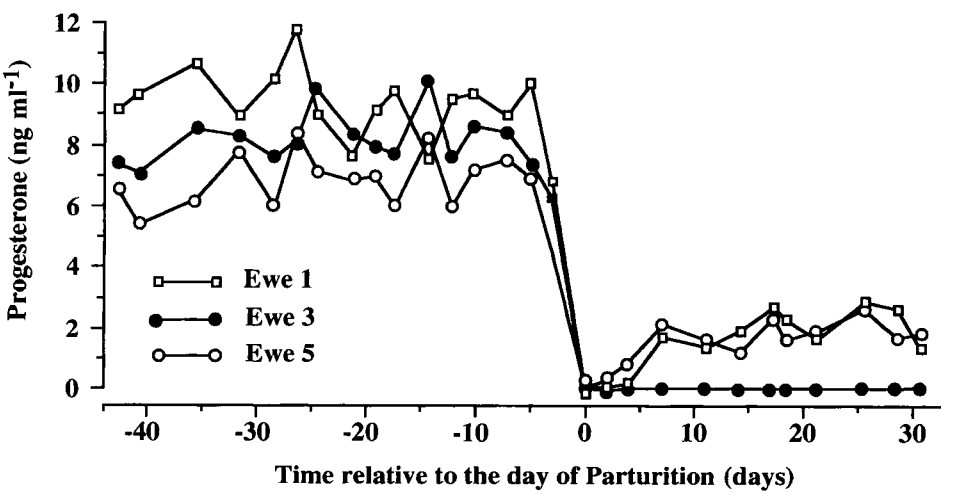


Fig 1. The plasma concentrations of progesterone in three ewes with an ovarian autotransplant from day 100 of pregnancy until day 28 postpartum.

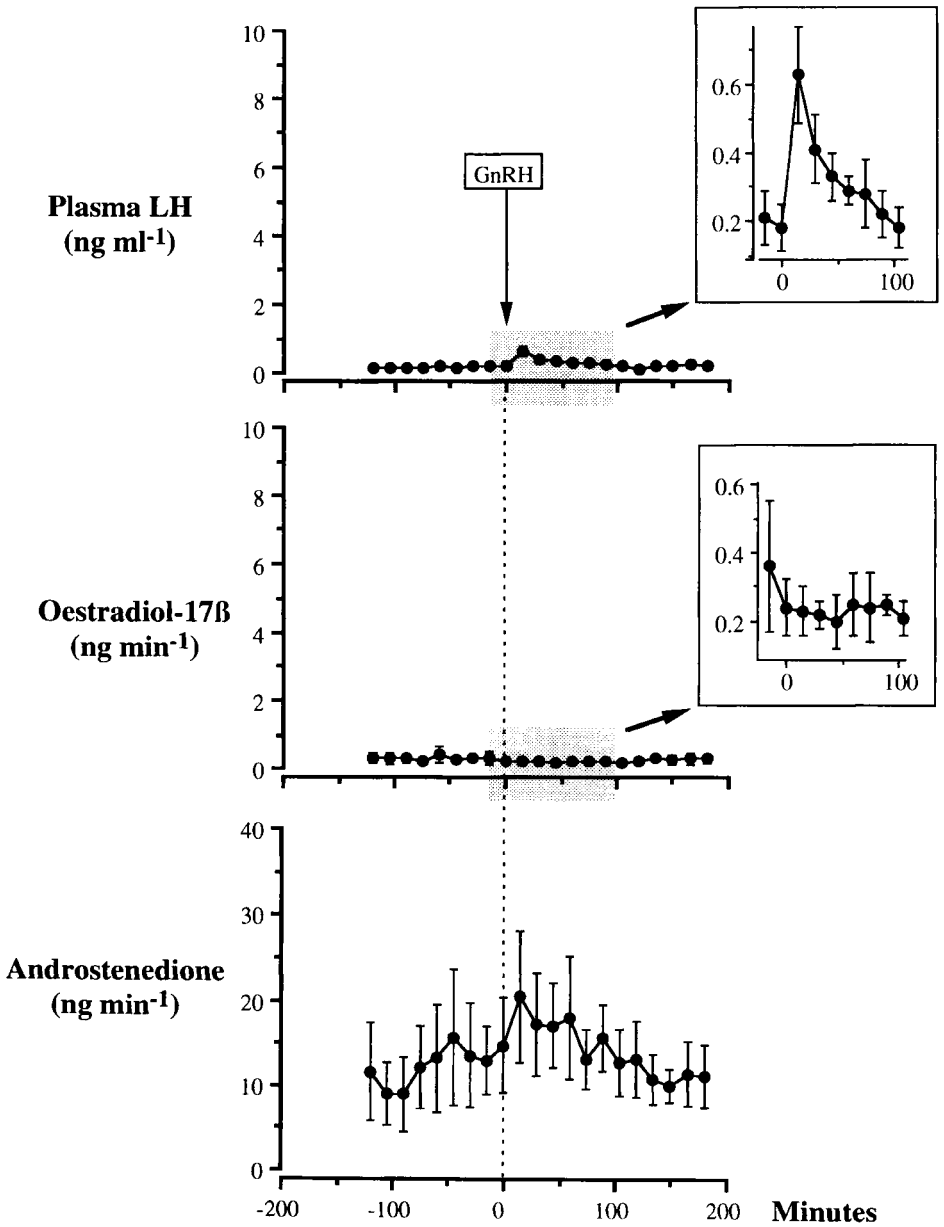


Fig 2. The mean (± SEM) concentration of luteinizing hormone (LH) and the mean (± SEM) ovarian secretion rate of androstenedione and oestradiol in three ewes with an ovarian autotransplant on day 121 of pregnancy. The arrow indicates the time of a rapid intravenous injection of 2 mL of saline containing 150 ng of gonadotropin-releasing hormone (GnRH).

On day 8 postpartum both injections of GnRH induced significant LH release. There was no difference in the maximum LH concentration between the two injections (table II, fig 3). The secretion of both oestradiol ($P < 0.001$) and androstenedione ($P < 0.001$) increased rapidly to a peak 75 min after GnRH and then declined significantly to basal secretion by 4 h after GnRH. The second injection of GnRH also increased the secretion rates of oestradiol ($P < 0.01$) and androstenedione ($P < 0.01$) and maximum secretion was observed 60 and 45 min after GnRH (fig 3).

Table I. The mean (± SEM) concentration of luteinizing hormone (LH) and the mean (± SEM) secretion rate of androstenedione and oestradiol in three ewes with an ovarian autotransplant on day 120 of pregnancy and on days 7 and 21 postpartum.

	Day 120 pregnant	*Day 7 postpartum*	*Day 21 postpartum*
LH (ng mL^{-1})	0.18 ± 0.07	0.42 ± 0.23	0.30 ± 0.15
Oestradiol (ng min^{-1})	0.34 ± 0.09	0.33 ± 0.27	0.29 ± 0.10
Androstenedione (ng min^{-1})	10.54 ± 3.02 [a]	2.49 ± 0.52 [b]	4.11 ± 1.77 [ab]

[ab] Within rows values with the different superscripts differ at $P < 0.05$.

Table II. The mean (± SEM) maximum concentration of luteinizing hormone (LH) and the mean (± SEM) maximum secretion rates of oestradiol and androstenedione in three ewes with an autotransplanted ovary and challenged with a rapid intravenous injection of 150 ng of gonadotropin-releasing hormone (GnRH) on day 121 of pregnancy and on days 8 and 22 postpartum.

	Day 121 pregnant	*Day 8 postpartum*	*Day 22 postpartum*
LH pulse height (ng mL^{-1})	0.63 ± 0.14 [a]	3.89 ± 1.69 [b]	4.67 ± 1.39 [b]
Maximum oestradiol secretion rate (ng min^{-1})	0.24 ± 0.03 [a]	2.09 ± 0.89 [b]	3.66 ± 1.14 [b]
Maximum androstenedione secretion rate (ng min^{-1})	20.43 ± 7.66	17.11 ± 6.95	29.45 ± 9.23

The maximum secretion rates of oestradiol and androstenedione are the mean maximum secretion rate observed following GnRH and it occurred between 30 and 75 min after GnRH injection. [ab] Within rows values with different superscripts differ at $P < 0.05$.

On day 22 postpartum both injections induced significant LH release. There was no difference in the maximum LH concentration between the two injections (table II, fig 4). The secretion of both oestradiol ($P < 0.001$) and androstenedione ($P < 0.001$) increased rapidly to a peak 75 and 60 min after GnRH and then declined to basal secretion by 4 h after GnRH. The second injection of GnRH also increased the secretion rates of oestradiol ($P < 0.001$) and androstenedione ($P < 0.01$) and maximum secretion was observed 30 min after GnRH (fig 4).

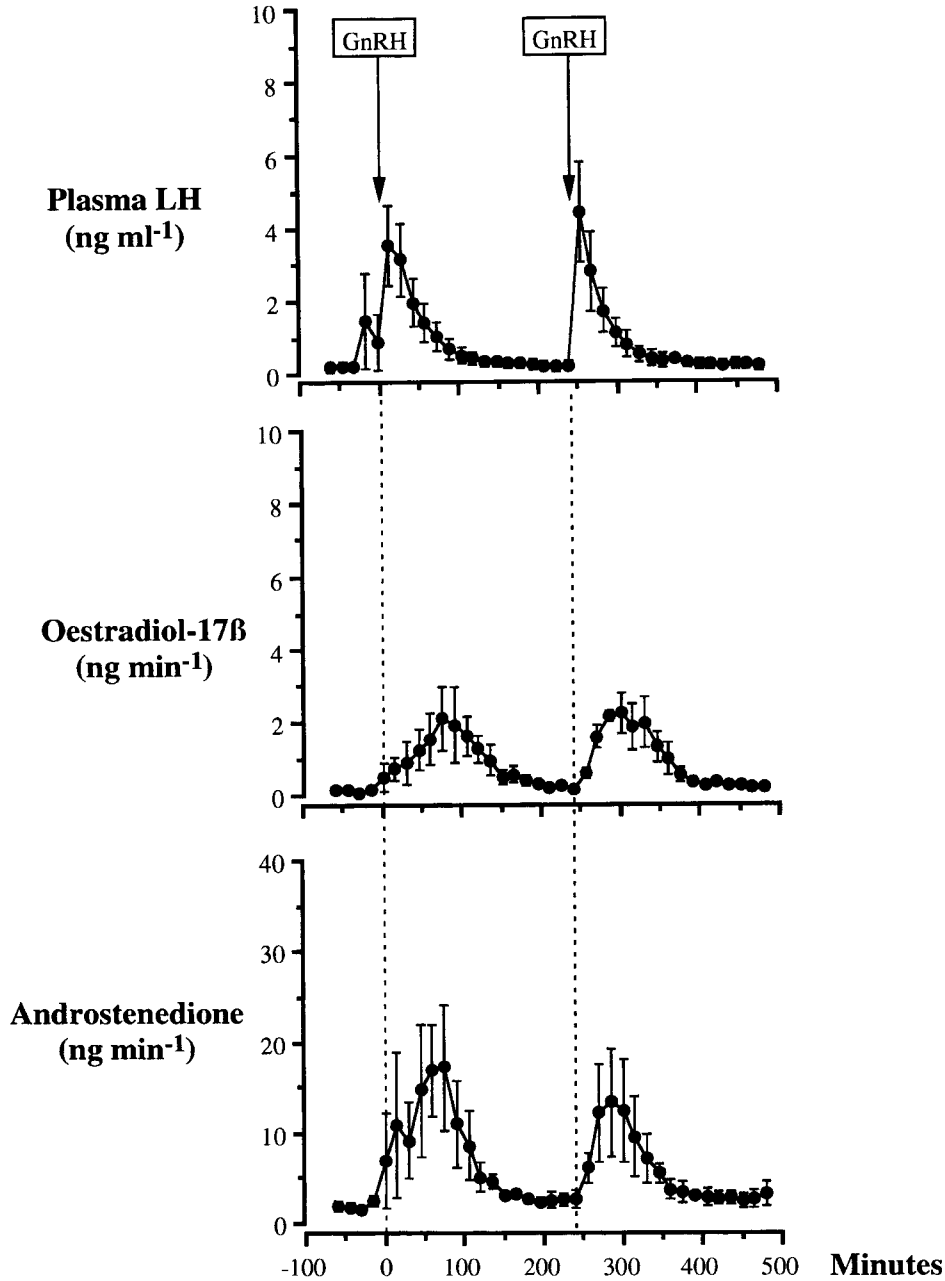
DISCUSSION

This experiment reports the first account of the establishment of pregnancy in ewes with an autotransplanted ovary. Although only three of the six ewes became pregnant, the resulting data derived from these three animals is unique and offers further insights into the control of ovarian activity during the final trimester of pregnancy in the ewe. Clearly removal of the ovary and its relocation to a superficial site under the skin in the neck region (Goding et al, 1967) has not compromised the ability of the ovary to maintain pregnancy until such time as the developing placenta takes over this function. Parturition on day 147 of pregnancy was normal and progesterone concentrations fell sharply immediately prior to birth, confirming that luteolysis at the end of pregnancy does not require an intact utero-ovarian vasculature (Moor et al, 1970). Spontaneous luteolysis at the end of the oestrous cycle does not occur in nonpregnant ewes with an ovarian autotransplant and the corpora lutea persist beyond their normal life span of 14 days. The corpora lutea in ewes with an ovarian autotransplant regress spontaneously sometime between 100 and 150 days (Wheeler, 1973) and this is similar to the pattern of spontaneous regression of corpora lutea seen in the hysterectomized ewe (Moor et al, 1970). The corpora lutea of pregnancy appear to behave in a similar fashion because it is apparent that the corpora lutea had regressed before the time of the normal fall in placental progesterone that takes place just before parturition (Bassett et al, 1969).

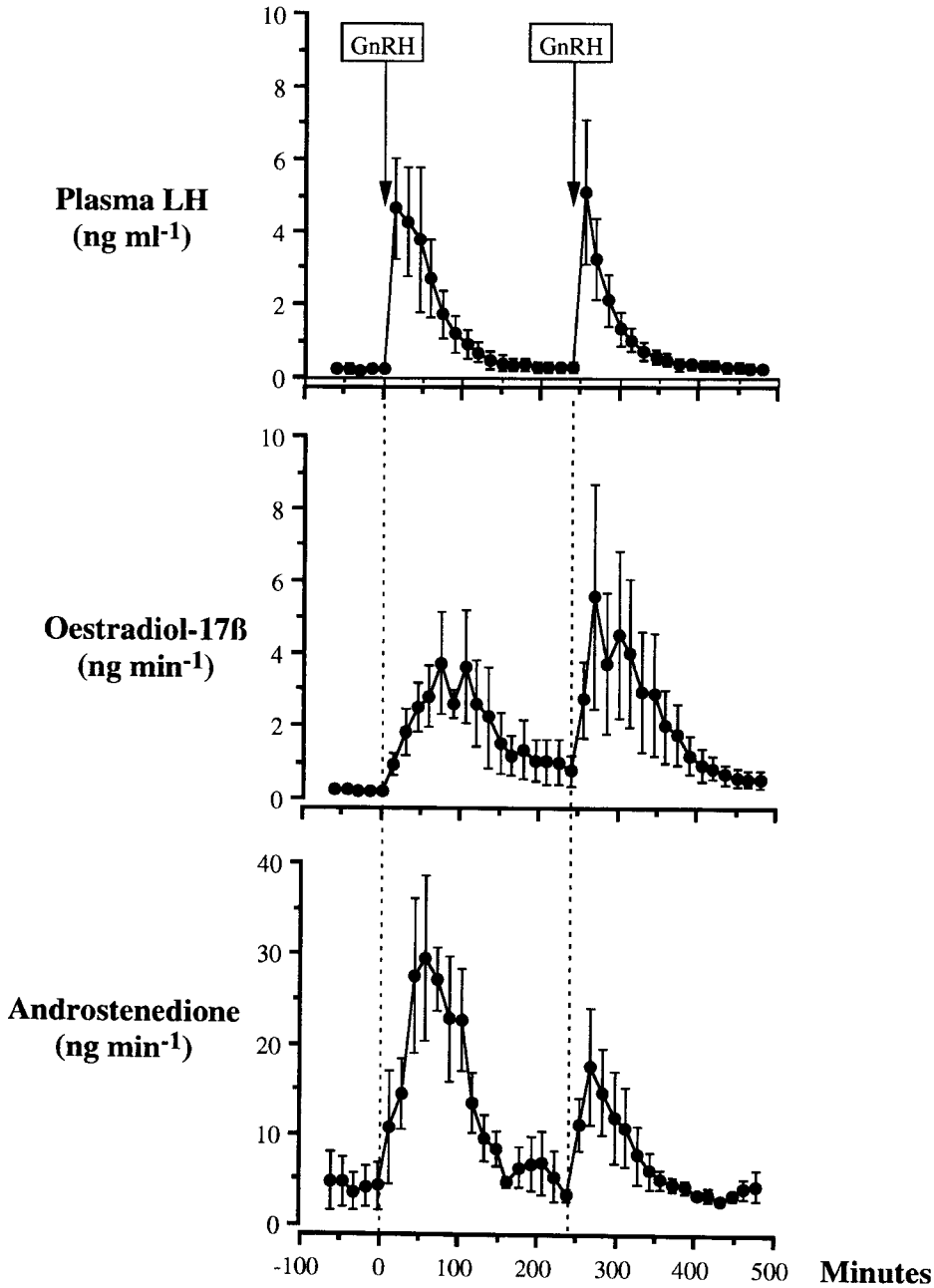
There was a complete inhibition of LH pulse secretion on day 120 of pregnancy and this is consistent with other reports (Al-Gubory et al, 1989; 1994a) and is undoubtedly associated with the high rate of secretion of placental steroids, especially progesterone. Exogenous GnRH was able to induce pulses of LH on day 121 of pregnancy, but the amplitude of the resulting pulses was reduced. These data suggest that both the GnRH neuronal system and pituitary sensitivity of GnRH stimulation are strongly inhibited during pregnancy in the ewe. These inhibitory effects both appear to be rapidly reversed in ewes after parturition so that by day 7 postpartum, pulsatile LH secretion was restored and LH pulse amplitude was normal; this is consistent with other reports (Al-Gubory et al, 1989, 1994a). The influence of pregnancy on ovarian follicular development is not uniform across species and this presumably reflects species differences in placental function, particularly placental steroid secretion. Thus, in the rodent LH pulses are maintained and follicular development to the antral stage continues more or less throughout pregnancy (Greenwald and Roy, 1994). On the other

Fig 3. The mean (± SEM) concentration of luteinizing hormone (LH) and the mean (± SEM) ovarian secretion rate of androstenedione and oestradiol in three ewes with an ovarian autotransplant on day 8 postpartum. The arrows indicate the time of a rapid intravenous injection of 2 mL of saline containing 150 ng of gonadotropin-releasing hormone (GnRH).

Day 8 Post-partum



Day 22 Post-partum



hand, pulsatile LH secretion is greatly inhibited during pregnancy in the sheep (Al-Gubory et al, 1989, 1994a) and the cow (Schallenberger et al, 1985) and the numbers of antral follicles are greatly reduced.

Some aspects of ovarian function were also suppressed in pregnancy. Basal secretion of oestradiol was low, suggesting an absence of large oestrogenic follicles at this stage of pregnancy and a low frequency of pulsatile LH release. Unlike the cow, the peripheral concentration of oestradiol in the pregnant ewe remains low until a day or so before parturition (Challis and Patrick, 1981). The secretion of androstenedione was markedly elevated on days 121 and 122 of pregnancy (table I, fig 2) and the peripheral concentration of androstenedione is elevated in maternal plasma during pregnancy in the ewe (Yu et al, 1983). These observations suggest that both the foetus and the ovary contribute to the elevated peripheral androgen concentrations seen in the pregnant ewe. The source of ovarian androstenedione is most likely small non-oestrogenic follicles (Al-Gubory and Martinet, 1986), although the pattern of secretion is atypical in that there appears to be a high rate of constitutive secretion (table I, fig 2). The high secretion of ovarian androstenedione leads to the novel suggestion that sheep ovary may be responding to placental hormones.

Since the amplitude of the GnRH-induced LH release was low in the pregnant ewes it is not possible to conclude definitively from these observations that ovarian responsiveness to LH is diminished during late pregnancy. There is little published information on the responsiveness of the ovine ovary to gonadotrophin stimulation during pregnancy and the question of ovarian responsiveness to gonadotrophins remains open.

Following parturition ovarian function was spontaneously and rapidly reestablished so that by day 7 two of the ewes had already ovulated before the first sampling period and before the first GnRH test on day 8. In all three ewes the ovarian responses to GnRH-induced LH release were normal and the secretion of both oestradiol and androstenedione responded with a pulse of secretion similar to that observed in cycling ewes (Campbell et al, 1994). However, the frequency of spontaneous LH pulses was low suggesting that re-activation of the GnRH neuronal system from the inhibition of pregnancy is an important component of postpartum recovery (Jolly et al, 1995).

The present study clearly shows that the secretion rate of androstenedione was elevated on days 120 and 121 of pregnancy and that its basal secretion was greater than that observed on days 7 and 21 postpartum (table I). Since androgens are normal precursors for oestrogen synthesis it appears that aromatization may be inhibited in late pregnancy. The inhibition of follicular oestradiol production during late pregnancy, even in the presence of high amounts of androstenedione, may result from low follicle-stimulating hormone (FSH) concentrations in the blood. After parturition the rapid increase in follicular growth (Al-Gubory and Martinet, 1986) coincides with increased secretion of FSH (Al-Gubory et al, 1989). Unfortunately, we did not measure jugular plasma concentrations of FSH in this experiment. There is also evidence that the corpus luteum (Al-Gubory et al, 1994b) and the placenta (Al-Gubory et al,

Fig 4. The mean (± SEM) concentration of luteinizing hormone (LH) and the mean (± SEM) ovarian secretion rate of androstenedione and oestradiol in three ewes with an ovarian autotransplant on day 22 postpartum. The arrows indicate the time of a rapid intravenous injection of 2 mL of saline containing 150 ng of gonadotropin-releasing hormone (GnRH).

1995) contain nonsteroidal factors that inhibit granulosa cell aromatase activity.

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