Effects of pregnancy on pulsatile secretion of LH and gonadotrophin-releasing hormone-induced LH release in sheep: a longitudinal study

K. H. Al-Gubory¹, J. Hervieu² and P. A. Fowler³

¹Unité de Recherches de Physiologie Animale, 78352 Jouy-en-Josas Cedex, France; ²Domaine Expérimental de Brouëssy, 78114 Magny-les-Hameaux, Institut National de la Recherche Agronomique, France; and ³Department of Obstetrics and Gynaecology, University of Aberdeen, Aberdeen AB25 2ZD, UK

Pulsatile LH secretion and its control throughout pregnancy have not been fully determined in sheep. Expt 1 determined the patterns of LH secretion in five ewes on days 10, 20, 60 and 120 of pregnancy and on day 10 postpartum, compared with those on day 10 of the oestrous cycle. Mean ($\pm$ SEM) concentrations of LH declined steadily throughout pregnancy (ANOVA, $P < 0.01$) and were lower ($P < 0.01$) on day 60 (0.19 ± 0.3 ng ml⁻¹) and on day 120 (0.18 ± 0.4 ng ml⁻¹) of pregnancy than on day 10 of the oestrous cycle (0.55 ± 0.04 ng ml⁻¹). This decrease was due to a significant reduction in the number and the amplitude of LH pulses. Only on day 120 of pregnancy were progesterone concentrations higher ($P < 0.01$) than on day 10 of the oestrous cycle. Although concentrations of progesterone on day 10 postpartum were barely detectable, mean LH concentration (0.45 ± 0.09 ng ml⁻¹) was not different from that on day 10 of the oestrous cycle. Expt 2 examined the LH responses in a separate group of four ewes to a physiological dose of GnRH (0.2 μ g) on days 10, 20, 60 and 120 of pregnancy and on day 10 postpartum, compared with those on

day 10 of the oestrous cycle. The area under the LH response curve and the maximum LH concentrations induced by GnRH declined steadily throughout pregnancy (ANOVA, $P < 0.01$) and were lower ($P < 0.01$) on days 60 and 120 of pregnancy than on day 10 of the oestrous cycle, but these parameters were not different between day 10 postpartum and day 10 of the oestrous cycle. Expt 3 examined the LH responses in a separate group of four ewes to a potent GnRH agonist, buserelin (0.5 μ g), on days 10, 60 and 120 of pregnancy. The area under the LH response curve and the maximum LH concentrations induced by GnRH were lower ($P < 0.01$) on days 60 and 120 than on day 10 of pregnancy, but were not different between days 60 and 120. This longitudinal study demonstrates that the pulsatile LH release and pituitary responsiveness to GnRH decreases progressively as pregnancy advances, but does not support the hypothesis that high concentrations of progesterone are solely responsible for the inhibition of pulsatile LH secretion and GnRH-induced LH release during pregnancy in sheep.

Introduction

LH is released by the pituitary gland in a pulsatile manner, resulting in peripheral blood LH patterns characterized by peaks. Pulsatile LH secretion in sheep is driven by the episodic discharges of the hypothalamic peptide, GnRH (Caraty *et al.*, 1982; Clarke and Cummins, 1982; Levine *et al.*, 1982). Variations in pulsatile LH release occur during different stages of the oestrous cycle in sheep (Hauger *et al.*, 1977) and reflect changes in pulsatile GnRH release (Clarke *et al.*, 1987). A great deal of information is available regarding pituitary LH responsiveness to GnRH (Chamley *et al.*, 1974; Jenkin and Heap, 1974; Jenkin *et al.*, 1977; Wright *et al.*, 1981),

pituitary content of LH (Chamley *et al.*, 1976; Jenkin *et al.*, 1977; Crowder *et al.*, 1982), the amount of mRNA encoding pituitary LH α and β subunits (Wise *et al.*, 1985; Fowler and McNeilly, 1997) and GnRH receptor (Fowler and McNeilly, 1997), and the quantity of pituitary GnRH receptors and the stores of GnRH in the median eminence, the preoptic area and the hypothalamus (Crowder *et al.*, 1982) in pregnant ewes. Despite these investigations, the pulsatile secretion of LH and its control throughout pregnancy have not been elucidated. Previous efforts to characterize the pattern of LH secretion by intensive blood sampling have failed to show changes in pulsatile LH release in advancing pregnancy in ewes (Al-Gubory *et al.*, 1994). In this investigation, the study of pulsatile LH release was limited because blood sampling was not performed during the oestrous

Email: algubory@jouy.inra.fr

cycle and during the very early stages of pregnancy. On the basis of the proposed negative feedback action of progesterone on pulsatile secretion of LH in cyclic ewes (Hauger *et al.*, 1977) and ovariectomized non-pregnant ewes (Karsch *et al.*, 1977; Goodman and Karsch, 1980; Goodman *et al.*, 1981), the present study was designed to determine the longitudinal patterns of LH secretion (Expt 1) and pituitary LH responses to exogenous administration of GnRH (Expt 2), as follows: (1) at the active luteal phase of the oestrous cycle (day 10), which is characterized by a steady state secretion of progesterone; (2) at critical stages of early pregnancy (days 10 and 20), which are comparable with day 10 of the oestrous cycle in terms of progesterone secretion and are considered very important for the recognition and maintenance of early pregnancy in the ewe; (3) at the time of luteo-placental shift (day 60), which is characterized by a transitional period of progesterone secretion; (4) during late pregnancy (day 120), when high concentrations of progesterone are secreted by the placenta; and (5) during the early postpartum period (day 10) when peripheral concentrations of progesterone are very low or undetectable. Expt 3 was designed to achieve maximum LH responses at three specific stages of pregnancy (days 10, 60 and 120) by the exogenous administration of a large dose of a highly potent GnRH agonist, buserelin, to determine whether changes in pulsatile LH release throughout pregnancy are associated with changes in the content of pituitary LH (Chamley *et al.*, 1976).

Materials and Methods

Animals and management

Three experiments were performed during the breeding season in 13 mature ewes of the PréAlpes-du-Sud breed. Oestrus was synchronized using a 14 day treatment of intravaginal pessaries containing 40 mg fluorogestone acetate (Intervet, Angers). After removal of the pessaries, the ewes were given 400 iu of equine chorionic gonadotrophin (Intervet) and were mated with fertile rams. The ewes were housed under conditions of natural daylength and temperature, fed straw, hay and concentrate adequate for gestation needs, and were given free access to mineral licks and water. Ewes were isolated from rams throughout the experimental period as the presence of rams among ewes during early pregnancy affects pituitary LH secretion (Al-Gubory, 1998). All procedures relating to the care and use of animals were approved by the French Ministry of Agriculture according to French regulations for animal experimentation.

Experimental design

Experiment 1. This experiment was designed to determine the patterns of LH secretion during the luteal

phase of the oestrous cycle, throughout pregnancy and during early postpartum in a group of five ewes (mean $\pm$ SEM body weight, 57.6 ± 2.4 kg). Blood samples were collected from the jugular vein at 15 min intervals for 6 h beginning at 09:00 h on day 10 of the oestrous cycle. This blood collection regimen was repeated on four selected periods of pregnancy (days 10, 20, 60 and 120) and during the early postpartum period (day 10).

Experiment 2. This experiment was designed to determine pituitary LH responsiveness to a small dose of GnRH on day 10 of the oestrous cycle, days 10, 20, 60 and 120 of pregnancy and day 10 postpartum in a separate group of four ewes (mean $\pm$ SEM body weight, 59.7 ± 3.7 kg). Ewes were given a single i.v. injection of 0.2 μ g GnRH (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂; Sigma, Saint-Quentin-Fallavier) in 1 ml saline. A dose of 0.2 μ g was chosen because it was thought to be physiological in terms of LH release (A. Caraty, personal communication). Blood samples were collected from the jugular vein at 15 min intervals for 1 h before GnRH injection and 3 h after GnRH injection.

Experiment 3. Experiments 1 and 2 demonstrated that the pulsatile secretion of LH and the pituitary responsiveness to a physiological dose of GnRH decreased progressively as pregnancy advanced, and that the period at about day 60 was a transition phase in pituitary LH release. In addition, with advancing pregnancy in ewes there is a progressive decline in pituitary LH stores (Chalmey *et al.*, 1976). Thus, this experiment was designed to achieve maximum LH responses at three specific stages of pregnancy (days 10, 60 and 120) by exogenous administration of a large dose of a highly potent GnRH agonist in a separate group of four ewes (mean $\pm$ SEM body weight, 57.3 ± 1.7 kg). Ewes received a single i.v. injection of 0.5 μ g of the GnRH agonist buserelin (Hoechst Laboratories, Paris) in 1 ml saline on days 10, 60 and 120 of pregnancy. Blood samples were collected from the jugular vein at 30 min intervals for 1 h before GnRH injection and 10 h after GnRH injection.

Hormone assay

All plasma samples from the same experiment were run in duplicate in a single assay to avoid interassay variation. Plasma concentrations of LH were measured by a double-antibody radioimmunoassay (Montgomery *et al.*, 1985). Plasma progesterone concentrations were determined in the samples taken at 09:00 h using a double-antibody radioimmunoassay (Heyman *et al.*, 1984). The intra-assay coefficient of variation was $<10\%$ for LH and progesterone. The assay sensitivity was 0.1 ng ml⁻¹ for LH and 0.2 ng ml⁻¹ for progesterone.

Data analysis

The pulsar Algorithmic Program (Biomedical Computing Technology Information Center, USA) for the study

Fig. 1. Representative patterns of LH secretion in jugular blood samples for two ewes sampled every 15 min for 6 h on (a) day 10 of the oestrous cycle, on days (b) 10, (c) 20, (d) 60 and (e) 120 of pregnancy, and on (f) day 10 postpartum.

of pulsatile hormone secretion (Merriam and Wachter, 1982) was used to calculate basal LH concentrations and the frequency and amplitude of LH pulses in individual profiles of the serial sampling period. The pituitary LH responses to GnRH were assessed by measuring the maximum plasma concentration of LH after GnRH injection (peak concentration minus the value before GnRH injection) and the area under the curve of LH release which represented the total response to GnRH over the test period. Data were subjected to ANOVA with repeated measures, followed by Dunnett's *post hoc* tests. The pulse frequencies required a special approach because they were discrete variables and some ewes presented zero pulses in 6 h. Therefore, LH pulse frequencies were transformed by adding 0.5 to the number of pulses observed in each sampling

Fig. 2. Mean ($\pm$ SEM, $n=5$) concentrations of (a) LH and (b) progesterone in jugular blood samples collected from ewes on day 10 of the oestrous cycle (C10), on days 10 (P10), 20 (P20), 60 (P60) and 120 (P120) of pregnancy, and on day 10 postpartum (PP10). The overall mean concentrations of LH for the five ewes were calculated as the average of the 24 samples collected every 15 min for 6 h from each ewe. Concentrations of progesterone were determined in the samples at 09:00 h at various reproductive stages. * $P < 0.01$ compared with values on day 10 of the oestrous cycle.

period for each ewe, and were subjected to square root transformation before analysis.

Results

Experiment 1: effect of pregnancy on pulsatile secretion of LH

Representative patterns of LH secretion in jugular blood collected every 15 min for 6 h at the various reproductive stages are shown (Fig. 1). Mean ($\pm$ SEM) concentrations of LH declined steadily throughout pregnancy (ANOVA, $P < 0.01$), decreasing ($P < 0.01$) from 0.55 ± 0.04 ng ml⁻¹ on day 10 of the oestrous cycle to 0.19 ± 0.3 and 0.18 ± 0.4 ng ml⁻¹ on days 60 and 120 of pregnancy, respectively (Fig. 2). Mean ($\pm$ SEM) LH concentration on day 10 postpartum (0.45 ± 0.09 ng ml⁻¹) was not different from that on day 10 of the oestrous cycle (Fig. 2). Concentrations of progesterone on days 10,

Table 1. The characteristics of pulsatile LH secretion on day 10 of the oestrous cycle (C10), on days 10 (P10), 20 (P20), 60 (P60) and 120 (P120) of pregnancy, and on day 10 postpartum (PP10) in five ewes

Reproductive state	LH pulse frequency (pulses per 6 h per ewe)	LH pulse amplitude (ng ml ⁻¹)	Basal LH (ng ml ⁻¹)
C10	1.6 ± 0.4 (5)	1.65 ± 0.50	0.38 ± 0.03
P10	1.6 ± 0.2 (5)	2.02 ± 0.58	0.31 ± 0.04
P20	1.2 ± 0.4 (4)	1.14 ± 0.15	0.20 ± 0.03*
P60	0.8 ± 0.4 (3)	0.69 ± 0.07*	0.15 ± 0.01*
P120	0.6 ± 0.6 (1)	0.45 ± 0.04*	0.17 ± 0.03*
PP10	1.2 ± 0.4 (4)	2.05 ± 0.47	0.30 ± 0.07

Values are mean ± SEM.

The number of ewes showing pulses of LH is indicated in parentheses.

* $P < 0.01$ compared with values on day 10 of the oestrous cycle.

20 and 60 of pregnancy were not different from those on day 10 of the oestrous cycle and increased markedly ($P < 0.01$) on day 120 of pregnancy (Fig. 2). Concentrations of progesterone were barely detectable on day 10 postpartum (Fig. 2). The characteristics of pulsatile LH secretion are shown in Table 1. Although there was a tendency for the frequency of LH pulses to decrease as pregnancy advanced, the difference was not significant. However, the number of ewes that exhibited one or more, but never three, LH pulses decreased markedly during pregnancy. The amplitude of LH pulses on days 60 and 120 of pregnancy was significantly different from that on day 10 of the oestrous cycle. Basal LH concentrations on days 20, 60 and 120 of pregnancy were significantly different from those on day 10 of the oestrous cycle.

Experiment 2: effect of pregnancy on pituitary LH response to a physiological dose of GnRH

Pituitary LH responses to a single i.v. injection of 0.2 µg GnRH on day 10 of the oestrous cycle, days 10, 20, 60 and 120 of pregnancy and day 10 postpartum are shown (Fig. 3). The GnRH-induced LH release decreased progressively as pregnancy advanced. The area under the LH response curve (Fig. 4a) and the maximum LH concentration (Fig. 4b) induced by GnRH declined steadily throughout pregnancy (ANOVA, $P < 0.01$) and all these parameters were significantly lower ($P < 0.01$) on days 60 and 120 of pregnancy than on day 10 of the oestrous cycle. Concentrations of progesterone on days 10, 20 and 60 of pregnancy were not different from those on day 10 of the oestrous cycle and increased markedly ($P < 0.01$) on day 120 of pregnancy (Fig. 4c).

Experiment 3: effect of pregnancy on pituitary LH response to a highly potent GnRH agonist

Pituitary LH responses to a single i.v. injection of 0.5 µg of the GnRH agonist buserelin on days 10, 60 and 120 of pregnancy are shown (Fig. 5). The magnitude of

GnRH agonist-induced LH release was markedly lower on days 60 and 120 than on day 10 of pregnancy. The area under the LH response curve (Fig. 6a) and the maximum LH concentration (Fig. 6b) induced by buserelin administration were lower ($P < 0.01$) on days 60 and 120 than on day 10 of pregnancy. These parameters were not different between days 60 and 120 of pregnancy.

Discussion

To the authors' knowledge, the present study is the first longitudinal study characterizing the pattern of LH release before, during and after pregnancy in ewes. Frequent sequential blood sampling revealed that LH is released in a pulsatile fashion throughout pregnancy. However, pulsatile secretion of LH occurred irregularly, as has been reported during early pregnancy in rhesus macaques (Monfort *et al.*, 1989) and ewes (Al-Gubory, 1999). The present study demonstrates that pulsatile secretion of LH decreases progressively with advancing pregnancy. An *in vitro* study of pituitaries from pregnant ewes determined that basal LH secretion falls from day 45 of pregnancy onwards (Fowler and McNeilly, 1997). In the present study, decreased basal LH concentration was the most prominent change in LH secretion observed on and after day 20 of pregnancy. By day 60 of pregnancy, there was a clear-cut decrease in all the parameters of pulsatile LH secretion compared with those on day 10 of the oestrous cycle. Pulses of LH were either absent or infrequent at this time. This marked inhibition of pulsatile LH secretion occurred at a time when circulating concentrations of progesterone were comparable with those measured on day 10 of the oestrous cycle and on days 10 and 20 of pregnancy. Although concentrations of progesterone showed a threefold increase on day 120 compared with those measured on day 60 of pregnancy, no further changes in LH secretion parameters occurred. These results indicate that the marked increase in the peripheral concentration of progesterone during the last trimester of pregnancy cannot be the sole cause of the

Fig. 3. Mean ($\pm$ SEM, $n = 4$) concentrations of LH in jugular blood samples collected from ewes on (a) day 10 of the oestrous cycle, on days (b) 10, (c) 20, (d) 60 and (e) 120 of pregnancy, and on (f) day 10 postpartum before and after a single i.v. injection (arrow) of 0.2 μ g GnRH. Blood samples were collected every 15 min for 1 h before and 3 h after GnRH injection.

inhibition of pulsatile LH secretion at this time. Treatment of ovariectomized non-pregnant ewes with oestradiol at concentrations that mimic circulating concentrations of oestradiol (24 pg ml^{-1}) present during the last few days before parturition (Tsang, 1978) suppresses serum and hypophyseal concentrations of LH (Moss *et al.*, 1981). Such observations have led to the conclusion that the high concentrations of oestradiol in the circulation at the week before parturition inhibit synthesis and secretion of LH in ewes. If oestradiol is an important inhibitor of LH pulse amplitude and frequency on days 60 and 120 of pregnancy (present study), then circulating concentrations of this steroid should be noticeably increased after midpregnancy. This does not appear to be the case as concentrations of circulating oestradiol remain low (less than 8 pg ml^{-1}) throughout pregnancy

Fig. 4. (a) Mean ($\pm$ SEM, $n = 4$) area under the LH response curve and (b) maximum LH concentrations induced by a single i.v. injection of 0.2 μ g GnRH; and (c) concentrations of progesterone on day 10 of the oestrous cycle (C10), on days 10 (P10), 20 (P20), 60 (P60) and 120 (P120) of pregnancy, and on day 10 postpartum (PP10) in sheep. Concentrations of progesterone were determined in the samples collected just before GnRH injection. * $P < 0.01$ compared with values on day 10 of the oestrous cycle.

and increase to 11–27 pg ml^{-1} only in the last few days of pregnancy (Tsang, 1978). Thus, it is reasonable to hypothesize that the inhibition of pulsatile LH secretion observed on days 60 and 120 are probably not related to oestradiol concentration at those times.

The observation that exogenous administration of progesterone to ovariectomized ewes during the breeding season (which produced luteal phase concentrations of progesterone) reduced the frequency of LH pulses (Goodman and Karsch, 1980), combined with the finding that the frequency of LH and GnRH pulses increase during the follicular phase of the ovarian cycle in sheep (Clarke *et al.*, 1987), have led to the hypothesis that the rise in LH pulse frequency during the follicular phase

Fig. 5. Mean ($\pm$ SEM, $n = 4$) concentrations of LH in jugular blood samples collected from ewes on days (a) 10, (b) 60 and (c) 120 of pregnancy before and after a single i.v. injection (arrow) of 0.5 µg of the GnRH agonist buserelin. Blood samples were collected every 30 min for 1 h before and 10 h after GnRH injection.

is due to the withdrawal of progesterone at luteolysis. This is clearly not the case during the early postpartum period as the present study has demonstrated that the peripheral concentrations of progesterone are very low or undetectable on day 10 postpartum, whereas the frequency and amplitude of LH pulses at this time remain comparable with those observed during the active luteal phase of the oestrous cycle. This finding indicates that acute withdrawal of high concentrations of progesterone immediately after parturition is insufficient to allow the generation of pulsatile LH and probably GnRH release, characteristic of the follicular phase. Evidence that non-steroidal factors from the ovary may play a role in the inhibition of LH secretion have emerged from several studies in pregnant and postpartum ewes. In the course of a study to elucidate the role of the ovary in the control of LH secretion in the early puerperium in sheep, it

Fig. 6. (a) Mean ($\pm$ SEM, $n = 4$) area under the LH response curve and (b) maximum LH concentrations induced by a single i.v. injection of 0.5 µg of the GnRH agonist buserelin on days 10 (P10), 60 (P60) and 120 (P120) of pregnancy in sheep. * $P < 0.01$ compared with values on day 10 of pregnancy.

was observed that pituitary and serum LH concentrations remain low during the last third of pregnancy and that the rate of increase of LH after parturition is less in intact ewes than in ewes from which the ovaries have been removed at day 28 and pregnancy maintained using progesterone implants (O'Reilly and Dziuk, 1973). Removal of the sheep corpus luteum at midpregnancy has no subsequent effect on peripheral concentrations of progesterone, but results in an increase in the frequency and amplitude of LH pulses (Al-Gubory *et al.*, 1989a). In addition, the frequency and amplitude of LH pulses increases more rapidly during the early postpartum period in ewes from which the corpora lutea have been removed at midpregnancy than in intact ewes (Al-Gubory *et al.*, 1989b). From these studies, it was hypothesized that non-steroidal factors from the corpus luteum could play a role in the inhibition of pulsatile release of LH during pregnancy in ewes. These findings were confirmed when it was shown that addition of charcoal-treated aqueous extract of sheep corpus luteum of pregnancy resulted in inhibition of pulsatile LH in ovariectomized ewes (Al-Gubory *et al.*, 1992). The putative factor was shown to be a protein and it was designated an LH-release inhibiting factor, LH-RIF (Al-Gubory *et al.*, 1992). A candidate protein with a potent LH-RIF bioactivity that stains as

a single band in SDS-PAGE with an apparent molecular mass of 16 kDa has been isolated from sheep corpus luteum of pregnancy (Al-Gubory *et al.*, 2002). Therefore, the results of the present study, together with the evidence currently available, indicate that ovarian non-steroidal factors probably constitute part of the feedback systems reducing LH secretion during pregnancy in the ewe.

The present longitudinal study is the first to link pulsatile LH secretion with the pituitary LH responsiveness to GnRH throughout pregnancy in ewes, in the presence of low or high concentrations of endogenous progesterone. The inhibition of pulsatile LH secretion on day 60 of pregnancy coincides with the reduction of LH release induced at this time by exogenous administration of a physiological dose of GnRH or a large dose of the highly potent GnRH agonist, buserelin. During the last two trimesters of pregnancy, when the corpus luteum is no longer the main source of progesterone (Neher and Zarrow, 1954), peripheral concentrations of progesterone increase markedly (Bassett *et al.*, 1969), predominantly as a result of the increased synthetic capacity of the placenta (Ricketts and Flint, 1980). The marked increase in the concentrations of progesterone during late pregnancy may reduce both hypothalamic GnRH output and pituitary LH content, as sustained high concentrations of progesterone inhibit pulsatile LH (Karsch *et al.*, 1977; Goodman and Karsch, 1980) and GnRH (Karsch *et al.*, 1987; Skinner *et al.*, 1999) secretion in ovariectomized ewes, and because of the close relationship between GnRH and LH during the oestrous cycle in sheep (Clarke *et al.*, 1987). This would compromise the ability of the pituitary to secrete LH in response to GnRH stimulation. However, in the present study pituitary LH responses to exogenous GnRH on day 120 of pregnancy, when circulating concentrations of maternal progesterone reach maximal values, were no lower than those measured on day 60 of pregnancy. In addition, the pituitary responses to GnRH during the early postpartum period, when peripheral concentrations of progesterone are barely detectable, remain comparable with those observed during the luteal phase of the oestrous cycle. This finding indicates that acute withdrawal of high concentrations of progesterone immediately after parturition is insufficient to promote a large release of LH. Previous studies have shown that GnRH injected into ewes at postpartum anoestrus fails to promote a large release of LH until day 40 after parturition, similar to that induced during the oestrous cycle and seasonal anoestrus (Jenkin and Heap, 1974). When amounts of exogenous progesterone mimicking circulating progesterone concentrations occurring during the luteal phase of the oestrous cycle are injected into ovariectomized ewes, pituitary LH response to exogenous GnRH (Goodman and Karsch, 1980) is not reduced, indicating that pituitary insensitivity to GnRH during the last two trimesters of pregnancy and the early postpartum period is not associated with an acute

effect of progesterone. Therefore, the inhibition of GnRH-induced LH release observed in the present study on days 60 and 120 of pregnancy may have been attributable to factors other than progesterone which exert a direct effect at the pituitary to inhibit the secretion of LH in response to GnRH and pituitary LH content. In recent years, gonadotrophin surge-inhibiting factor or attenuating factor (GnSIF/AF) has emerged as a novel gonadal non-steroidal hormone playing an important role in the control of LH secretion in mammals, through its putative inhibitory effect on GnRH-induced LH release (Danforth *et al.*, 1987; Messinis and Templeton, 1990). The human placenta appears to be a source of GnSAF bioactivity, because addition of charcoal-treated, inhibin-depleted serum from women in the third trimester of pregnancy suppresses GnRH-induced LH secretion from perfused sheep pituitaries (Fowler *et al.*, 1995) and rat pituitary cell monolayers (Fowler and Templeton, 1996). The inhibition of GnRH-induced LH release on and after day 60 of pregnancy (present study), and the fact that charcoal-treated, inhibin-depleted extract from sheep corpus luteum of midpregnancy is able to reduce GnRH-induced LH release from cultured rat pituitary cells (Fowler *et al.*, 1997) indicates that GnSAF, probably from the corpus luteum and the placenta, may be a contributory factor to reduce the pituitary function during the last two-thirds of pregnancy in ewes. *In vitro* studies are required to explore this possibility.

In vitro studies using tissue culture of rat pituitaries have shown that GnRH stimulates LH synthesis (Redding *et al.*, 1972; Liu *et al.*, 1976). In a similar way, *in vivo* studies using disconnection of the pituitary from the hypothalamus by surgical approach in ovariectomized ewes have provided evidence for a role of a hypothalamic signal, presumably GnRH, in the synthesis and secretion of LH (Clarke *et al.*, 1983; Hamernik *et al.*, 1986). The finding that the administration of high frequency GnRH pulses (1 per h), compared with low frequency GnRH pulses (1 per 4 h), stimulates the pituitary LH subunit mRNA expression and LH release has been demonstrated in ovariectomized ewes (Leung *et al.*, 1987). Furthermore, episodic discharges of GnRH pulses induce concurrent pulses of LH in both cyclic (Clarke *et al.*, 1987) and anoestrous postpartum (Wise, 1990) ewes. A large dose of a highly potent GnRH agonist, buserelin, was used to achieve maximum LH responses on days 10, 60 and 120 of pregnancy. However, buserelin treatment failed to promote a large release of LH on days 60 and 120 of pregnancy, like that induced on day 10 of pregnancy, and this can be causally related to the pituitary LH content at that time. Taken together, these observations support the hypothesis that decreasing pulsatile GnRH release is probably the limiting factor reducing pituitary LH subunit mRNA expression (Wise *et al.*, 1985; Fowler and McNeilly, 1997), pituitary content of LH (Chamley *et al.*, 1976; Jenkin *et al.*, 1977; Crowder *et al.*, 1982), the pituitary LH responsiveness to

GnRH (Chamley *et al.*, 1974; Jenkin and Heap, 1974; Jenkin *et al.*, 1977; Wright *et al.*, 1981; present study), and the frequency and amplitude of LH pulses (present study) during the last two trimesters of pregnancy in ewes.

In summary, this longitudinal study demonstrates that the release of LH into the peripheral circulation occurs in a pulsatile fashion throughout pregnancy, and that this pattern of release decreases progressively as pregnancy advances. The inhibition of pulsatile LH release observed during the last two trimesters of pregnancy coincides with decreases in pituitary responsiveness to GnRH at this time. This study does not support the hypothesis that sustained high concentrations of progesterone are solely responsible for the inhibition of pulsatile LH secretion and GnRH-induced LH release during pregnancy in sheep.

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