EXPERIMENTAL STUDY

Absence of regular pulsatile LH secretion during pre- and post-implantation periods in sheep

Kaïs Hussain Al-Gubory
INRA, Unité de Recherches de Physiologie Animale, 78352 Jouy-en-Josas cedex, France
(Correspondence should be addressed to K H Al-Gubory; Email: algubory@jouy.inra.fr)

Abstract
Two experiments were conducted to determine the patterns of LH secretion and to evaluate the LH responses to pulsatile administration of GnRH during early pregnancy in ewes. In experiment 1, pregnant ewes (n=16) were used to determine the concentration of LH in plasma of jugular blood samples collected every 15 min for 6 h before (day 10 post-mating) and after (days 20 and 30 post-mating) implantation. In experiment 2, the pituitary LH responses to exogenous pulsatile administration of GnRH were examined on day 10 post-mating in 4 pregnant ewes. A small dose of GnRH (200 ng/ml saline) was injected (i.v.) every 3 h and jugular blood samples were collected every 15 min for 12 h beginning at the onset of GnRH administration and continuing through the 4th GnRH pulse. During the frequent-sample bleeding at any of the stages of pregnancy examined, LH concentrations oscillated in a pulsatile manner. However, pulsatile LH release occurred irregularly and infrequently. Overall mean LH concentrations, frequency and amplitude of LH pulses were not significantly different between any of the stages of pregnancy examined. Pulsatile administration of GnRH on day 10 post-mating induced regular pulses of LH.

In conclusion, these data demonstrate that: (i) pulsatile LH secretion occurs irregularly during early pregnancy, and (ii) the absence of regular pulsatile LH release during early pregnancy is not attributed to a lack of pituitary responsiveness to GnRH.

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Introduction
In mammalian species, progesterone plays a crucial role in the establishment of a uterine environment suitable for the survival and development of pre-implantation embryos. In the ewe, the corpus luteum (CL) is necessary for the establishment and maintenance of pregnancy during the first 50 days (1–3), because it assumes a predominant role in progesterone secretion (4, 5). It is known that pituitary luteinizing hormone (LH) is required for the formation and development of the CL, and the maintenance of luteal progesterone secretion. Indeed, removal of endogenous LH by hypophysectomy performed before (day 3 or day 10 post-mating) or after (day 30 post-mating) implantation, and treatment of ewes with anti-LH serum between days 10 and 20 of pregnancy, causes luteolysis and abortion (6, 7). In addition, it has been reported that hypophysectomy performed on days 20–23 of pregnancy results in complete degeneration of the CL and resorption of the conceptus, unless LH replacement treatment is carried out (8).

Despite the general acceptance of the importance of LH in the maintenance of luteal structure and progesterone secretion during early pregnancy in the ewe, the secretory patterns of LH before and after implantation have not been well characterized by frequent blood sampling suitable for the determination of pulse parameters. These LH secretory patterns are of the utmost importance to our understanding of the physiological processes that ensure the establishment of pregnancy and subsequent embryonic survival. Therefore, the aims of the present study were to determine (i) the secretory patterns of LH during the pre- and post-implantation periods and (ii) the pituitary LH responses to pulsatile administration of gonadotrophin-releasing hormone (GnRH) during early pregnancy.

Materials and methods

Animals and management
Ewes (aged 2 years) of the Préalpes-du-Sud breed were used in this study. During the breeding season (November), oestrus was synchronized with intravaginal pessaries containing 40 mg fluorogestone acetate (Intervet, Angers, France) which were left in situ for 14 days. After removal of the pessaries, each ewe received an intramuscular injection of 400IU pregnant mare’s serum gonadotrophin (Intervet). At the time of

synchronized oestrus, ewes were mated with a fertile ram. The ewes were housed under conditions of natural day length and temperature, fed straw and hay daily and had free access to mineral licks and water. Since the presence of rams among ewes during early pregnancy affects LH secretion (9), the ewes were isolated from rams throughout the experimental period.

**Experimental design**

**Experiment 1** This experiment was conducted to determine the patterns of LH secretion before and after implantation. Frequent blood samples were collected from the 16 pregnant ewes at 15-min intervals for 6 h (0900–1500 h) on days 10, 20 and 30 post-mating. Pregnancy was ascertained in all the ewes by mid-ventral laparotomy performed on day 35 post-mating.

**Experiment 2** This experiment was conducted to evaluate the responsiveness of the pituitary, in terms of LH secretion, to pulsatile administration of GnRH on day 10 post-mating. On the day of blood sampling, 4 pregnant ewes received i.v. injections of 200 ng GnRH (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂; Sigma, Saint-Quentin-Fallavier, France) in 1 ml saline (0.9% NaCl). The pulse of GnRH was injected every 3 h, beginning at 0700 h, and blood samples were collected at 15-min intervals for 12 h (0700–1900 h) beginning at the onset of GnRH administration and continuing through the 4th GnRH pulse.

**Blood sampling**

Blood samples were taken from the jugular vein using evacuated heparinized tubes. After centrifugation (3000 g, 4 °C) for 30 min, plasma was stored at −20 °C until assayed.

**LH assay**

The concentrations of LH were measured by double-antibody radioimmunoassay (10). To avoid interassay variation, all samples for each experiment were run (in duplicate) in the same assay. The intra-assay coefficient of variation was <10% and the assay sensitivity was 0.2 ng/ml.

**Data analysis**

The Pulsar Algorithmic Program (11) for the study of pulsatile hormone secretion was used to calculate the basal LH concentrations, the frequency and the amplitude of episodic LH release (pulses) in individual profiles of the serial sampling period. The G values, or number of assay coefficients of variation by which a pulse must exceed the base line were: 3.80, 2.60, 1.92, 1.46 and 1.13 for pulses with 1–5 consecutive elevated points. All data were subjected to analysis of variance for repeated measurements using general linear models procedures (12).

**Results**

**Experiment 1**

The patterns of LH secretion on days 10, 20 and 30 post-mating for 4 representative pregnant ewes are shown in Fig. 1. During the sampling periods, pulses of LH were either infrequent or absent. Overall mean (±S.E.M.) plasma LH levels were 0.68 ± 0.05, 0.55 ± 0.02 and 0.55 ± 0.03 ng/ml on days 10, 20 and 30 post-mating respectively. These levels were not significantly different (P>0.05). Mean frequency and amplitude of LH pulses did not change as pregnancy advanced (Fig. 2). The mean basal concentration of LH on day 10 post-mating was significantly greater (P<0.01) than that on days 20 and 30 post-mating (Fig. 2).

**Experiment 2**

In the four pregnant ewes treated during early pregnancy with GnRH, spontaneous parturition occurred at term, with live lambs. Exogenous pulsatile administration of GnRH at a dose of 200 ng/pulse on day 10 post-mating induced regular pulses of LH (Fig. 3).

**Discussion**

The present study is the first report describing episodic release of LH during early pregnancy in the ewe. During the frequent-sample bleeding at any of the stages of pregnancy examined (days 10, 20 and 30 post-mating), LH concentrations oscillated in a pulsatile manner. However, pulsatile LH release occurred irregularly and infrequently. The absence of regular pulsatile LH secretion during the implantation period has also been reported in the Rhesus macaque (13). As the frequency and the amplitude of the detected LH pulses were not different between days 10, 20 and 30 post-mating (present study), the LH responses to pulsatile administration of GnRH were only determined on day 10 post-mating. Pulsatile administration of small doses of GnRH induced regular pulses of LH. The dose of GnRH used in the present study (200 ng) appears to be a physiological dose, because the amplitudes of the induced LH pulses are relatively similar to those determined during the sheep oestrous cycle (14, 15). The absence of regular pulses of LH during early pregnancy could be attributed to the refractoriness of the pituitary to the stimulation of GnRH and/or to the decreasing pulsatile GnRH secretion. In vitro studies (16) have demonstrated that the responsiveness of ovine pituitaries in terms of the increase in LH released by GnRH pulses does not decrease during pregnancy. The restoration of regular pulses of LH during early pregnancy by pulsatile
administration of GnRH (present study) provides evidence that the pituitary is not refractory to GnRH stimulation during the pre-implantation period in the ewe, and supports the hypothesis that the absence of regular pulsatile LH release decrease during early pregnancy is probably due to a reduction in the hypothalamic GnRH signal to the pituitary (16).

Several investigations (6, 7) demonstrated the importance of pituitary LH in the formation, secretory activity and maintenance of the sheep CL during early pregnancy. Indeed, the removal of luteotrophic hormones by hypophysectomy performed on days 3, 10 or 30 of pregnancy, and by treating ewes with anti-LH and anti-prolactin sera between days 10 and 20 of pregnancy, caused CL regression and abortion. Thus it must be presumed that the constant and sustained LH secretion during the first 30 days of pregnancy (present study) contributes to maintaining normal luteal function in the ewe.

The secretion of progesterone during the luteal phase of the oestrous cycle in the ewe is independent of LH pulses. Indeed, the abolition of pulsatile LH release by chronic treatment of ewes with a GnRH agonist (17) or by treating ewes with a GnRH antagonist (18) had no effect on luteal function as assessed by changes in plasma concentrations of progesterone. These authors suggested that normal progesterone secretion by the sheep CL of the oestrous cycle can be maintained in the presence of basal concentrations of LH. The establishment and maintenance of pregnancy in the absence of regular LH pulses could be taken as evidence that the sheep CL of pregnancy can be sustained in the presence of basal concentrations of LH.

Experimental evidence indicates that 20% to 30% of the sheep embryos fail to survive the first month of pregnancy (19). These relatively high losses are attributed to genetic, environmental and also endocrine factors which affect animals during the pre- and post-implantation periods. Evidence is available to suggest that elevated secretion of LH may be deleterious to the implantation and embryonic development. Women with elevated LH concentrations showed high rates of pregnancy loss (20, 21). Chronic LH hypersecretion in transgenic mice has been shown to compromise pregnancy (22, 23). An increase in LH pulse frequency induced by the introduction of rams among ewes during

Figure 1 Profiles of LH concentration in plasma of the jugular blood samples collected every 15 min for 6 h on days 10, 20 and 30 post-mating in 4 representative pregnant ewes.
the pre-implantation period severely reduced the incidence of multiple births (9). It is thus possible that the physiological significance of the inhibition of pulsatile LH secretion during early pregnancy in ewes may lie in the establishment of gestation, successful implantation and survival of the developing embryos. The adverse effect, if any, of elevated LH secretion upon the establishment of pregnancy remains to be elucidated.

In conclusion, these data demonstrate that (i) pulsatile LH secretion occurs irregularly and infrequently during early pregnancy and (ii) the absence of regular pulsatile

Figure 2 Characteristics of LH secretion on days 10, 20 and 30 post-mating in 16 pregnant ewes. Values are means ± S.E.M. *P<0.01 compared with the other two days.

Figure 3 Profiles of LH concentration in plasma of the jugular blood samples collected every 15 min for 12 h on day 10 post-mating in 4 pregnant ewes injected (i.v.) with 200 ng GnRH in 1 ml saline (arrows).
LH release during early pregnancy is not attributed to lack of pituitary responsiveness to GnRH.

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