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Short communication

Leptin infusion during the early luteal phase in ewes does not affect progesterone production

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Abstract

Infusion of leptin during the ovine follicular phase has been shown to increase progesterone secretion during the subsequent luteal phase. In this study, we have assessed the effects of infusing leptin during the early luteal phase. Infusion of leptin (2.5 μg/h) into the ovarian artery of ewes with ovarian autotransplants ($n = 5$) on day 3 of the luteal phase for 12 h did not affect progesterone estradiol or LH concentrations compared to control ewes ($n = 5$). These results suggest no direct effect of leptin on ovarian function at this stage of the estrous cycle.
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1. Introduction

Numerous studies have reported effects of leptin on steroidogenesis in a number of in vitro culture systems, establishing the hypothesis that leptin may have a direct role in controlling ovarian function, as well as its central action on the hypothalamo–piuitary axis

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[1]. More recent studies have demonstrated an effect on ovarian steroidogenesis of infusing physiological quantities of either recombinant ovine [2] or recombinant human [3] leptin. In one study [2], infusion of leptin during the follicular phase caused an inhibition of estradiol during the infusion period followed by an increase in progesterone during the subsequent luteal phase. Thus, leptin-induced effects on the developing ovulatory follicle can influence subsequent luteal function. Furthermore, leptin receptors have been detected on the bovine corpus luteum [4].

The aim of the present study was to determine whether leptin can act directly on the developing corpus luteum to stimulate progesterone secretion.

2. Materials and methods

The study was undertaken in 12 mature Finn-Merino ewes with ovarian autotransplants [5] during the breeding season. Initiation and synchronisation estrous cycles was achieved with two injections of the $PGF_{2\alpha}$ analogue cloprostenol (125 μg i.m. Estrumate; Schering-Plough Animal Health, Harefield, UK), given 17 days apart.

Ewes were randomly assigned to receive a continuous intravenous infusion of either saline (3 ml/h; control group, $n = 6$) or recombinant human leptin (supplied by Professor J.W. Goding, Monash University Medical School, Australia) (2.5 μg/h; treatment group; $n = 6$). This dose was selected, based on results from a previous study [2], to generate concentrations of leptin within the physiological range. Animals were infused on day 3 of the estrous cycle, directly into the carotid ovarian artery for 12 h.

Jugular blood samples were taken twice daily until day 12 of the estrous cycle with additional samples collected at 10 min intervals from hour 4 to hour 8 of infusion for LH analysis. During the infusion period ovarian venous blood samples were collected at hourly intervals. All samples were centrifuged at $3000 \times g$ for 20 min at 4 °C and plasma stored at −20 °C. Following the infusion period, animals were scanned daily (real time Aloka 500 ultrasound scanner with a linear 7.5 MHz transducer probe, Dynamic Imaging, Livingston, UK) to confirm the presence of a corpus luteum.

Progesterone concentrations were measured by radioimmunoassay [6] with a sensitivity of 0.11 ng/ml and intra- and inter-assay coefficients of variation of 8.8 and 9.2%. Leptin concentrations were determined in both ovarian and jugular plasma samples by radioimmunoassay [7] (antibody supplied by Dr. D Blache, University of Perth, Australia) with a sensitivity of 0.37 ng/ml and intra- and inter-assay coefficients of variation of 7.4 and 8.4%. LH concentrations were determined by radioimmunoassay [8] with a sensitivity of 0.13 ng/ml and the intra- and inter-assay coefficients of variation of 6.0 and 7.5%. Plasma samples collected from ovarian venous blood were assayed for estradiol by double antibody radioimmunoassay [9] with a sensitivity of 12.1 pg/ml and the intra- and inter-assay coefficients of variation of 12.9 and 13.1%.

Progesterone, estradiol and leptin concentrations were analysed by repeated sample analysis of variance on data that had been log-transformed using the general linear model (SPSS 11.0 software) with data partitioned on the basis of treatment and time. The characteristics of pulsatile LH secretion were determined using the Munro Pulse analysis programme (Zaristow software, Haddington, UK). An LH pulse was defined as a value that was greater

than the mean LH concentration over the intensive sampling period plus two standard deviations and was calculated for each individual animal. LH pulse characteristics, time of LH surge and ovulation rate were analysed by Student's *t*-test.

3. Results

One control ewe with low progesterone prior to induction of luteolysis and one treated ewe that failed to ovulate were removed from further analysis.

Infusion of leptin did not cause any measurable increase in circulating concentrations of leptin in jugular or ovarian venous blood, which did not differ between control and treated ewes at any time point.

The LH surge occcurred 66 ± 3 h post-prostaglandin treatment, and did not differ between groups. Furthermore, there was no significant effect of infusion on LH pulse frequency or amplitude on day 3 (Table 1).

Prior to and during infusion peripheral progesterone concentrations were similar between the two groups. Following infusion there was a significant effect of time on progesterone concentrations ($p < 0.01$) associated with the development of corpora lutea. However, there was no effect of leptin infusion on progesterone concentrations (Fig. 1). Furthermore, progesterone concentration in ovarian venous blood during infusion were similar in control and treated ewes (Fig. 2a).

Table 1
LH characteristics in ewes during infusion of saline (control; $n = 5$) or 2.5 μg/h human recombinant leptin (treated; $n = 5$) for 12 h

	Control ($n = 5$)	Treated ($n = 5$)
Baseline concentration (ng/ml)	1.00 ± 0.13	0.78 ± 0.23
Pulse frequency (per 4 h)	2.8 ± 0.4	2.6 ± 0.3
Pulse amplitude (ng/ml)	1.07 ± 0.841	0.84 ± 0.38

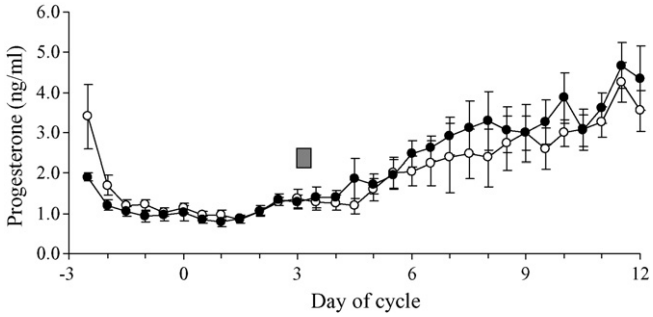

Fig. 1. Mean ± S.E.M. concentrations of progesterone in jugular venous blood throughout the luteal phase (LH surge, day 0) in ewes receiving a 12 h arterial infusion on day 3 of either leptin (treated; $n = 5$; ●) or saline (control; $n = 5$; ○).

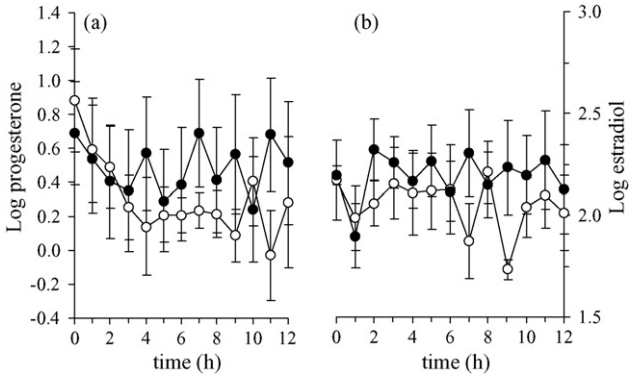

Fig. 2. Mean ± S.E.M. log concentrations of: (a) progesterone and (b) estradiol in ovarian venous blood during a 12 h arterial infusion on day 3 of the luteal phase of either leptin (treated; $n = 5$; ●) or saline (control; $n = 5$; ○).

The concentration of estradiol in ovarian venous blood was also similar between the two groups (Fig. 2b). There was no significant difference in ovulation rate between control (1.7 ± 0.2) and treated (1.4 ± 0.3) animals.

4. Discussion

The results demonstrate that, unlike the increase in progesterone seen following a follicular phase infusion of leptin [2], infusion of leptin on day 3 of the luteal phase, during the period of rapid corpus luteum development, did not influence progesterone secretion. This is in keeping with in vitro results in which leptin did not influence basal progesterone production by cultured bovine luteal cells collected during early luteal development [4]. Thus, any influence of leptin on luteal function appears to result from an indirect effect on the developing preovulatory follicle.

The lack of effect of leptin infusion of estradiol secretion is in contrast to another study [2] that demonstrated a reduction in estradiol concentrations in the ovarian autotransplant ewe following infusion of a physiological dose of leptin during the early follicular phase and is in contrast to in vitro studies that have shown an inhibitory effect of leptin on estradiol production [10].

The fact that leptin infusion did not affect LH pulse frequency, amplitude or baseline concentration is in agreement with previous studies [2,11,12] and suggests little involvement of leptin in the short term regulation of LH.

The failure to measure changes in plasma leptin concentration was not unexpected as an earlier study [2], though demonstrating increase plasma leptin following supra-physiological infusion, found a similar dose of leptin, infused during the follicular phase also failed to increase plasma leptin levels, despite showing clear biological activity. In this study, we used recombinant human rather than ovine leptin. However, this recombinant human leptin had previously been shown to exhibit biological activity in sheep [3].

In summary, the results of this experiment showed that direct ovarian exposure to physiological concentrations of leptin on day 3 of the luteal phase had no effect on the local or peripheral endocrine status of the animals.

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