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Effects of leptin administration on development, vascularization and function of *Corpus luteum* in alpacas submitted to pre-ovulatory fasting

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ABSTRACT

The objective of this study was to determine the effect of leptin administration on the development, vascularization and function of *Corpus luteum* (CL) in alpacas submitted to pre-ovulatory fasting. Fourteen alpacas were kept in fasting conditions for 72 h and received five doses of o-leptin (2 μg/kg e.v.; Leptin group) or saline (Control group) every 12 h. Ovulation was induced with a GnRH dose (Day 0). The ovaries were examined every other day by trans-rectal ultrasonography (7.5 MHz; mode B and power Doppler) from Day 0 to 13 to determine the pre-ovulatory follicle diameter and ovulation, and then to monitor CL diameter and vascularization until the regression phase. Serial blood samples were taken after GnRH treatment to determine plasma LH concentration; and every other day from Days 1 to 13 to determine plasma progesterone and leptin concentrations. The pre-ovulatory follicle and CL diameter, LH, progesterone and leptin plasma concentrations were not affected by treatment ($P > 0.05$). The vascularization area of the CL was, nevertheless, affected by the treatment ($P < 0.01$) with significant differences between groups at Days 3, 7 and 9 ($P < 0.05$). The Leptin group had a larger maximum vascularization area (0.67 ± 0.1 compared with 0.35 ± 0.1 cm^2; $P < 0.05$). In addition, there was a positive correlation between CL vascularization, CL diameter and plasma progesterone. The exogenous administration of leptin during pre-ovulatory fasting increased the vascularization of the CL in alpacas *in vivo*.

1. Introduction

Leptin is a protein hormone (16 kDa) produced mainly by adipocytes, although also by the digestive and reproductive systems, among others (Chehab, 2014; Friedman, 2014). Its role as a molecule linking nutrition and reproduction has been studied since the discovery that hypoleptinemic rodents were obese and infertile and that both conditions were reversed following administration of leptin (Barash et al., 1996; Farooqi et al., 1999).

In ruminants it has been documented that the relative abundance of adipose tissue leptin mRNA and leptin plasma concentration is markedly decreased by nutritional restriction, and increases after feeding is resumed (Chilliard et al., 2005; Delavaud et al., 2007).

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These authors suggested that the reduction of leptin may function as an acute signal to stimulate feeding behavior, reduce energy expenditure and inhibit reproduction.

Several studies have determined that leptin administration reverses the negative effect caused by fasting on the secretion of LH in cows and rodents (Nagatani et al., 2000; Zieba et al., 2003). It has also been shown that the hypothalamus, hypophysis, ovarian follicles and *Corpus luteum* (CL) have leptin receptors in different species (Di Yorio et al., 2008; Sarkar et al., 2010). Interestingly, it has been determined that the greatest leptin plasma concentration or relative abundances of leptin and leptin receptor mRNA are observed in the middle and late luteal stages and decrease coincident with luteal regression in llamas (Norambuena et al., 2013), cattle (Sarkar et al., 2010) and buffalo (Kumar et al., 2012). There is also a positive correlation between the amount of steroidogenic acute regulatory (StAR) protein, side-chain cleavage cytochrome P450 (P450scc) and 3beta-hydroxysteroid dehydrogenase/isomerase (3beta-HSD) factors and ObR receptor activity, suggesting a role of leptin in progesterone and other steroid production (Kumar et al., 2012).

Only a few *in vivo* studies have been conducted to understand the role of leptin in luteal function. Kendall et al. (2004) reported that ovarian leptin infusion during the early follicular phase resulted in an increase in progesterone production during the subsequent luteal phase in sheep. Roman et al. (2005), however, observed that chronic leptin administration reversed the negative effect on progesterone plasma concentration induced by severe food restriction in rodents.

Wiles et al. (2014) provided *in vitro* evidence that leptin may be involved in the luteal angiogenic process because it stimulated Ang1, FGF2 and VEGF gene expression in luteal cell cultures. The aim of the present study was to determine the effect of leptin administration on the development, vascularization and function of the CL in alpacas submitted to pre-ovulatory fasting.

2. Material and methods

2.1. Animal management

Eighteen adult non-lactating alpacas (*Vicugna pacos*), property of the Universidad Católica de Temuco, Chile (38° 46′ S latitude, 72° 38′ longitude, and 200 m above sea level) were used in this study. All procedures were reviewed and approved by the Universidad Católica de Temuco Bioethics Committee and were performed in accordance with the animal care protocols established by the University. Alpacas were kept indoors at night and during the afternoon (from 12:00 to 17:00) the animals grazed in natural pastures of *Ballica* sp. The animals were also fed daily *Ballica* sp. hay (6.2% crude protein [CP], 4.4 Mcal/kg, metabolic energy [ME], 4.7% total ash [TA)] – dry matter basis), water and mineral salts (Usablock®, Sweetlix, Mankato, Minnesota, USA) *ad libitum*; plus 200 g of a commercial concentrate (16.0% CP, 5.4% TA, and 3.0 Mcal/kg ME – dry matter basis; Cosetán®, Iansagro S.A., Osorno, Chile).

2.2. Experimental design

Stage of follicle wave development was synchronized using intra-vaginal progesterone devices for 7 days (CIDR®, 0.3 g, Pfizer Chile S.A., Chile) as described in previous studies (Chavez et al., 2002; Cavilla et al., 2016). After removal of the devices, the alpacas were examined by trans-rectal ultrasonography (7.5 MHz linear-array transducer; Sonovet3, Samsung Medison, South Korea) every other day for 10 days to evaluate follicle diameter and retrospectively, the emergence of the new wave of follicular development. Four alpacas which had follicles > 5 mm after the removal of the progesterone devices were not used for the study. Eight days after the removal of the devices, the remaining alpacas were fasted for 72 h with water *ad libitum* and randomly assigned to the Leptin or Control group (n = 7 alpacas/group). Alpacas in the Leptin group received five intravenous doses (at 12, 24, 36, 48 and 60 h) of o-Leptin (2 μg/kg; 0.1 mg/mL; e.v.), while those in the Control group received a similar number of saline administrations (1 mL; e.v.). Six hours before the conclusion of the fasting period, ovulation was induced with an intramuscular dose of GnRH (Day 0 = GnRH treatment; 50 μg; gonadorelin acetate; Gonasyn GDR, Syntex, Buenos Aires, Argentina). The ovaries were subsequently examined by trans-rectal ultrasonography to determine the pre-ovulatory follicle diameter at Day 0 and to detect ovulation at Day 3. Ovulation was defined as the disappearance of a large follicle (≥7 mm) that had been detected in the previous examination. The ovaries were examined every other day thereafter until Day 13 to monitor CL diameter and vascularization. During each examination, power Doppler mode was activated after initial B-mode evaluation to examine CL vascularization. Fixed, preinstalled Doppler system controls were used, to exclude variations in recording. Cineloops (10 s in length) of the CL were recorded during power Doppler imaging and downloaded onto a VLC media player (www.videolan.org, Version 2.0, Boston, MA, USA). The cineloops were examined frame by frame to select three images that represented the maximum vascular signal near the maximum cross-sectional area of the CL. Images were saved in JPG format with minimal compression, and analyzed by ImageJ software (National Institute of Health, Bethesda, MD, USA). The degree of vascularization was estimated by measuring the area (cm^2) of the vascular flow signals (power Doppler mode) overlaying the B-mode image of the CL. The average of the three images was taken as the value for a given animal on a given day. The maximum CL diameter, the maximum vascularization area and the CL lifespan were determined. The lifespan of the CL was calculated using the interval in days between the 5 mm stage at the growing phase to the 5 mm stage during the regression phase. The body live weight (BLW) and body condition score (BCS; 1–5 scale according to Van Saun, 2009) were determined before and after fasting, and every 4 days from Day 1 to 13 (Day 0 = day of ovulation induction). Blood samples for measuring the plasma concentration of triglycerides (GPO-PAD enzymatic method), cholesterol (CHOD-PAD enzymatic method) were collected from the jugular vein in heparinized tubes before and after fasting. Metabolic variables were analyzed at the Universidad Católica de Temuco using Metrolab 2300® plus Random Access Clinical Analyzer.