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Development of corpus luteum susceptibility to an analog of prostaglandin $F_{2\alpha}$, throughout the luteal phase in llamas (*Lama glama*)

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ABSTRACT

The aim of the present study was to evaluate the susceptibility of the corpus luteum to d-cloprostenol (synthetic analog of $PGF_{2\alpha}$) throughout the luteal phase in llamas. Female llamas ($n = 43$) were induced to ovulate by GnRH injection in the presence of an ovulatory follicle and randomly assigned into one of six groups: control and treated with an injection of d-cloprostenol on Day 3, 4, 5, 6 or 8 post GnRH. Blood samples were collected to determine plasma progesterone concentrations. There was no effect of treatment on animals injected on Day 3 or 4 post-GnRH. In animals treated on Day 5, different responses were observed. No effect of treatment was recorded in 27% of the animals whereas 55% of the llamas showed a transitory decrease followed by a recovery in plasma progesterone concentrations after d-cloprostenol injection, indicative of a resurgence of the corpus luteum, extending the luteal phase a day more than in control animals. In the remaining 18% of the animals injected on Day 5, (corresponding to those exhibiting the greatest plasma progesterone concentrations at the day of injection), complete luteolysis was observed. Plasma progesterone concentrations decreased to below 1 ng ml^{-1} 24 h after d-cloprostenol in llamas injected on Day 6 or 8 post-GnRH. In conclusion, the corpus luteum of llamas is completely refractory to $PGF_{2\alpha}$ until Day 4 after induction of ovulation, being partially sensitive by Day 5 and fully responsive to $PGF_{2\alpha}$, by Day 6 after induction of ovulation.

1. Introduction

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has been recognized as the most important luteolysin in most domestic animals (McCraken et al., 1999). Luteolytic pulses of $PGF_{2\alpha}$ are released from the endometrium at the end of the luteal phase to induce luteolysis. Nevertheless, the corpus luteum is sensitive to $PGF_{2\alpha}$ earlier during the luteal phase although the time when a corpus luteum becomes susceptible to $PGF_{2\alpha}$ varies between species (Cows: Louis et al., 1975; mares: Allen and Cooper, 1975; sheep: Rubianes et al., 2003; gilts: Estill et al., 1993).

In cows, the corpus luteum becomes sensitive to $PGF_{2\alpha}$ on Day 4 following ovulation. Administration of $PGF_{2\alpha}$ by either intrauterine or intramuscular route induces a decrease in the diameter of the corpus luteum and a decrease in plasma progesterone concentrations while the female returns to estrus 3–4 days later (Tervit et al., 1973; Louis et al., 1974). The corpus luteum of the ewe is susceptible to $PGF_{2\alpha}$ at Day 3 post ovulation (Rubianes et al., 2003), which is earlier than had been previously known (Acritopoulou and Haresing, 1980). The corpus luteum of mares fully responds to $PGF_{2\alpha}$ when it is administered by intrauterine, intramuscular or subcutaneous route after Day 4 post ovulation, inducing complete luteolysis (Allen and Cooper, 1975; Douglas and Ginther, 1975). In this species, when $PGF_{2\alpha}$ is injected on Day 3 after ovulation an immediate functional and structural regression occurs followed by a later resurgence of the corpus luteum (Bergfelt et al., 2006). The period of luteal sensitivity seems to be shorter in pigs in comparison to other domestic species. $PGF_{2\alpha}$ is effective to induce luteolysis around Day

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12 after ovulation (Guthrie and Polge, 1976; Hallford et al., 1975). However, multiple injections between Day 5 and 10 are effective to cause premature luteolysis (Estill et al., 1993).

Llamas are induced ovulators, requiring copulation at the time when an ovulatory follicle is present to initiate the ovulatory process (San-Martín et al., 1968; Aba et al., 1995). The interval between mating and ovulation is approximately 30 h (range 24–48 h) (Adams et al., 1989; Vaughan and Tibary, 2006). Thereafter, plasma concentrations of progesterone increase on Day 4 after induction of ovulation, peak on Day 8 and, in non pregnant llamas, decrease by Days 10–11 in response to the release of $PGF_{2\alpha}$ from the endometrium, attaining basal concentrations by Day 12 post mating (Aba et al., 1995). Small peaks of $PGF_{2\alpha}$ are observed as early as Day 7 after mating, being the luteolytic pulses recorded between Days 8 and 13 when luteolysis occurs (Aba et al., 1995, 2000).

$PGF_{2\alpha}$ and its synthetic analogs have been widely used in different species. $PGF_{2\alpha}$ is considered the most potent luteolytic substance and it has been broadly used in different estrous synchronization protocols in cows (Hiers et al., 2003; Stevenson and Phatak, 2010), sheep (Menchaca et al., 2004) and mares (Loy et al., 1980) to ensure corpus luteum regression. Due to the capacity of $PGF_{2\alpha}$ to induce myometrial contractions, it has also been used to treat uterine infections (Hirsbrunner et al., 2003; Kasimanickam et al., 2005) and to prevent post mating endometritis in mares (Combs et al., 1996). In llamas, cloprostenol, an analog of $PGF_{2\alpha}$, has been used as part of ovarian follicular superstimulation treatments (Bourke et al., 1995) and as abortifacient (Smith et al., 2000).

Information regarding the precise moment when the corpus luteum attains the capacity to respond to $PGF_{2\alpha}$ in many domestic species has been known for many years, however, no reports are available regarding when the corpus luteum of llamas becomes susceptible to $PGF_{2\alpha}$. Thus, the aim of the present study was to investigate the susceptibility of the llama corpus luteum to an injection of d-cloprostenol (synthetic analog of $PGF_{2\alpha}$) on different days of the luteal phase.

2. Materials and methods

Field studies were performed in compliance with animal welfare regulations set by the Faculty of Veterinary Sciences, UNCPBA where activities were conducted. Facilities are located in Tandil, Argentina, at 37° S, 60° W. Animals were kept in pens isolated from males and fed pasture hay and water *ad libitum*. Female llamas ($n = 43$) were examined daily by transrectal ultrasonography to assess ovarian status (Mindray, DP 6600 Vet, with 5.0/7.5 variable traducer probe). When a follicle with a diameter ≥8 mm, considered ovulatory in this species (Bravo et al., 1991), was observed, ovulation was induced with a single injection of GnRH (16.8 μg Receptal®, Intervet, Argentina). Occurrence of ovulation was assessed based on ultrasonographic visualization of the corpus luteum and further confirmed by the progesterone profiles. Thereafter, animals were randomly divided into six different groups: Control ($n = 10$; untreated) and G3 ($n = 4$); G4 ($n = 7$); G5 ($n = 11$); G6 ($n = 5$) and G8 ($n = 6$) which were treated with an injection of d-cloprostenol (112.5 μg, Baker, Tecnofarm®, Argentina) into the hind leg on Days 3, 4, 5, 6 or 8 post-GnRH, respectively.

Blood samples for progesterone determinations were collected every second day until the day of d-cloprostenol injection and thereafter samples were obtained daily until the end of the study (Day 11 or 12 post-GnRH). Samples were centrifuged and plasma was stored at −20 °C until hormone assays were performed. Progesterone was measured using an RIA kit (COAT-A-COUNT®, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) previously validated for use with llama plasma (Bianchi et al., 2007). The sensitivity of the assay was 0.1 ng ml^{-1} and the intra and inter-assay coefficient of variation were below 14% for concentrations between 0.1 and 40 ng ml^{-1}.

Data were analyzed using an ANOVA test to detect differences in the diameter of the largest follicle at the time of induction of ovulation between the different groups. Serial data were compared using the mixed model procedure of SAS (Statistical Analysis System Institute Inc., Cary, NC, USA) to determine main effect of treatment, days and their interactions. The compound symmetry model with a covariance structure was used. When the interactions were significant, the effect of days within treatment was compared by least squares means (adjustment Tukey–Kramer). Data are presented as mean ± S.E.M., and differences were considered to be significant when $P < 0.05$.

3. Results

Mean diameters of the largest follicle at the time when GnRH was injected were similar in all groups ($P = 0.59$) and averaged 9.6 ± 0.42 mm.

Plasma concentrations of progesterone were similar for llamas injected on Days 3 or 4 to controls and increased above 1 ng ml^{-1} on Day 5, had peaked on Day 8, started to decrease by Days 9 to 10 and had attained basal concentrations between Days 11 and 12 post induction of ovulation (Fig. 1A–C). In animals injected with d-cloprostenol on Day 5, diverse responses were observed. In 27% (3/11) animals, d-cloprostenol injection did not cause any effect, the pattern of progesterone concentrations were similar to that reported in the control group. A transient decrease (below 1 ng ml^{-1}) in plasma progesterone concentrations was recorded 24 h after treatment in 55% (6/11) of the animals. One day later, circulating progesterone concentrations had increased and were maintained above 1 ng ml^{-1} until Day 11, one day longer than in animals from the control group. By Day 12, an important decrease was observed (0.17 ± 0.07 ng ml^{-1}). Meanwhile, in 18% (2/11) of the animals in which plasma progesterone concentrations were above 3 ng ml^{-1} on Day 5, d-cloprostenol injection induced a complete luteolysis (Fig. 1D). In animals treated on Day 6 or 8 post GnRH, plasma progesterone concentrations had decreased to less than 1 ng ml^{-1} by 24 h after d-cloprostenol injection while in the control group plasma progesterone concentrations remained greater than 2 ng ml^{-1} on those days ($P \leq 0.0001$; Fig. 1E and F).