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Effect of GnRH analogue administration on Day 7 after natural mating on formation accessory corpus luteum, progesterone concentration and conception rate in llamas (*Lama glama*)

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ABSTRACT

The objectives of the present study were to determine the effects of exogenous GnRH administered 7 days after breeding on the formation of an accessory corpus luteum (ACL), plasma progesterone (P_4) concentrations and pregnancy rates. Adult females ($n = 71$) having a follicle ≥ 7 mm in diameter in the ovary were naturally mated (Day 0). On Day 7, ultrasonic examination was performed to confirm the occurrence of ovulation as evidenced by presence of an induced corpus luteum (ICL). Females with an ICL plus a dominant follicle ≥ 7 mm ($n = 56$) were treated with saline solution (SS, $n = 29$) or GnRH analogue ($n = 27$). On Day 14, the formation of an ACL was observed by ultrasonography. Blood samples were collected on Days 7 and 14 to quantify plasma P_4 concentrations. On Day 14, 21 of 27 (77.8%) females in the GnRH group developed an ACL, whereas females in the SS group did not. Progesterone concentrations on Day 7 and 14 in those llamas diagnosed as pregnant on Day 30 were not different ($P > 0.05$) between groups. In addition, P_4 concentration was similar for GnRH-treated females having two CL to those with a single CL. Pregnancy rates were similar ($P > 0.05$) between SS and GnRH groups (55.2% compared with 74.1% respectively) and the pregnancy rate for the GnRH group was not affected ($P > 0.05$) by the number of CL observed at Day 14 (66.6% and 75.6% for females with one and two CL respectively). In conclusion, GnRH administration on Day 7 after breeding leads to ACL formation; however, neither the plasma P_4 concentration nor pregnancy rate was affected by having an ACL.

1. Introduction

South American camelids belong to two genera. One genus, *Lama* includes two species: *Lama glama* (llama) and *Lama guanicoe* (guanaco) and the other genus, *Vicugna* includes *Vicugna vicugna* (vicuna) and *Vicugna pacos* (alpaca). Llamas, alpacas y vicuñas are limited to the puna (i.e., dry Andean highlands of the Peru, Bolivia, Chile and Argentina; between 3500 and 5000 m above sea level) and it is a cold, dry and often windy environment.

South American camelids are considered to be non-seasonal breeders and females have continuous ovarian activity and may breed, conceive and give birth at any time of the year (Smith et al., 1994). Ovulation is induced after mating by the β-NGF (β-nerve growth factor) that was identified as the main ovulation-inducing factor in the seminal plasma (Ratto et al., 2011; Adams and Ratto,

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2013).

The herd productivity is considered to be very low and reproductive failure is one reason for the low productivity (Alarcon et al., 1990). Embryo mortality can reach 10% to 15% in the first 60 days of pregnancy (Bravo et al., 1995) and may reach up to 80% in the first 90 days of pregnancy with some conditions (Alarcon et al., 1990). Luteal insufficiency is often suspected as a cause of pregnancy loss (Vaughan and Tibary, 2006).

The ovulatory response to copulation was dependent upon follicular size and 7 mm in diameter is the minimum size of follicles from which ovulation occurs in llamas and alpacas (Bravo et al., 1991). The mean follicle diameter at Day 6 of the first wave of ovarian follicular development after mating was 6–7 mm (Adams et al., 1990); therefore, if GnRH, hCG or LH are administered in this timeframe, there is a high probability of ovulation and formation of a new corpus luteum (CL).

It was hypothesized that GnRH administered at Day 7 after natural mating produces an ACL, increases the plasma P_4 concentration and pregnancy rate. The objectives of this experiment were to determine the effects of the exogenous GnRH agonist when administered 7 days after natural mating on the formation of an ACL, plasma P_4 concentration and conception rate in non-lactating adult llamas.

2. Materials and methods

2.1. Research conditions

This research was conducted at the Abra Pampa Experimental Station of Altitude of the National Institute of Agricultural Technology (22°49'S, 65°47'W) that is located in the High Andean plateau of northwest Argentina in the Jujuy province (3484 m above sea level), termed the "dry puna", during summer season (January to March 2017). Summer is the wet season in the puna, with most of the annual rainfall occurring between December and March (250–350 mm). In the dry winter season the temperature can be-20 °C; however, in the summer season the maximum temperature can be 25 °C.

2.2. Animals and treatments

The llamas at the experimental station number about 400 tagged llamas, maintained in four enclosed areas of approximately 200 ha each. About 100 non-lactating adult females, 4–8 years old (105 ± 8 kg body weight) were selected by clinical examination and *trans*-rectal ultrasonography (Honda HS 101 V, 5 MHz linear-array transducer, Japan) of the reproductive tract to detect any gross abnormalities or pregnancy before natural mating and only those with a healthy reproductive status were included in the study. The animals were kept on natural pasture (*Festuca scirpifolia*) and water was provided ad libitum.

Trans-rectal ultrasonography was performed on Day 0 and only 71 females with a follicle ≥ 7 mm were naturally mated (individual controlled breeding) with males of proven fertility. Each female was mated only once and 25 males were used (three matings total per male; one mating every 72 h).

On Day 7, ultrasonic examinations of the ovaries were performed to confirm the occurrence of ovulation as evidenced by presence of an induced corpus luteum (ICL). Ovaries were assessed and images were recorded on a diagram of the ovary by sketching the relative location of dominant follicle (DF) and visible ICL. The size of largest follicle was determined by averaging the largest and smallest diameter. Females with an ICL + DF ≥ 7 mm ($n = 56$) were randomly assigned to receive either 2 mL of 0.85% NaCl (saline solution – SS; $n = 29$) or GnRH analogue (100 μg of gonadorelin, Gonasyn GDR®, Zoetis, Argentina; $n = 27$).

On Day 14, *trans*-rectal ultrasonography was performed to confirm the formation of an ACL. Pregnancy was confirmed on Day 30–32 post-natural mating by ultrasonic examination and again the pregnancy diagnosis was performed by rectal palpation after 60–62 days following breeding.

2.3. Blood collection and hormone assays

Blood samples were collected on Day 7 and 14 in GnRH and SS groups. Each sample was collected by jugular venipuncture into evacuated tube containing sodium heparin and immediately placed on ice for up to 2 h until centrifugation at 1500*g* for 20 min. Plasma was then stored at −20 °C until hormone analyses. Concentrations of P_4 in plasma samples were determined by a radioimmunoassay commercial kit (IM1188 Beckmman Coulter, USA) for use in cattle and validated for llamas (Bianchi et al., 2010) only in females that were pregnant at Day 30 of gestation. The intra-assay coefficient of variation was < 4% for concentrations between 0.15 and 55.0 ng/mL. The estimated sensitivity of this method was 0.10 ng/mL and all samples were assayed in duplicate.

2.4. Statistical analyses

Data analyses were performed using SAS Institute Inc. (1989). The dominant follicle diameter on Days 0 and 7 in the females that had ovulations (SS and GnRH groups) was analyzed by one-way ANOVA. Student́s *t* test was employed to compare plasma P_4 concentrations between SS and GnRH groups on Days 7 and 14 as well as to analyze the difference between ICL and ICL + ACL plasma P_4 concentrations within the GnRH-treated group.

Proportional data were compared between groups by Fischer Exact Test. Probability of $P < 0.05$ was considered to be significant. Pregnancy rate was defined as the number of females diagnosed pregnant in a treatment group divided by the number of females with an ICL present at Day 7 after natural mating.

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