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Theriogenology 77 (2012) 1802–1810

Theriogenology

www.theriojournal.com

GnRH dose reduction decreases pituitary LH release and ovulatory response but does not affect corpus luteum (CL) development and function in llamas

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Received 29 June 2011; received in revised form 25 November 2011; accepted 24 December 2011

Abstract

Gonadotrophin releasing hormone (GnRH) is commonly used in llamas to induce ovulation; however, the consequence of reduced doses of GnRH on luteinizing hormone (LH) release, ovulatory response, and subsequent corpus luteum (CL) development and function have apparently not been investigated. Hence, we examined the effect of gradual reduction of gonadorelin acetate (GnRH) dosage on pituitary LH release, ovulatory response, CL development, and plasma progesterone concentrations in llamas. Non-pregnant, non-lactating adult llamas were examined once daily by transrectal ultrasonography, and those with a follicle ≥8 mm in diameter that had grown for three consecutive days were randomly assigned to receive 50 (GnRH50, n = 23), 25 (GnRH25, n = 29), 12.5 (GnRH12.5, n = 29), or 6.25 μg (GnRH6.25, n = 29) of GnRH, or 0.5 mL of PBS (Control group, n = 16) im. In a subset (7 or 8 animals/group), intense blood sampling was done to measure LH concentrations. All females were examined by ultrasonography every 12 h from treatment (Day 0) to Day 2 to determinate ovulation, and thereafter on alternate days until Day 16 to evaluate CL development (9–13 animals/group). Also, blood samples for progesterone determination were taken (9 or 10 animals/group) on alternate days from Days 0–16. Ovulatory response (%) was highest ($P < 0.05$) in the GnRH50 (82.6), intermediate in the GnRH25 (72.3) and GnRH12.5 (75.9) groups, and lowest in the GnRH6.25 group (48.3). No ovulations were detected in the Control group. Mean peak LH concentrations (ng/mL) were highest ($P < 0.05$) for GnRH50 (6.2), intermediate for GnRH25 (4.4) and GnRH12.5 (2.9), and lowest for GnRH6.25 (2.2) groups. In addition, based on regression analysis, llamas with an LH peak <4 ng/mL were less likely to ovulate. Llamas given 50 μg of GnRH released more ($P < 0.05$) pituitary LH and had an LH surge of longer duration than those given 25, 12.5, or 6.25 μg. However, in those that ovulated, neither GnRH treatment nor treatment by time interaction affected ($P > 0.05$) CL diameter or plasma progesterone concentrations. In summary, reducing the dose of GnRH gradually decreased the magnitude of the preovulatory LH surge and ovulatory response; however, subsequent CL development and plasma progesterone concentrations were not affected.

Keywords: Llamas; GnRH; Corpus luteum; Ovulation

1. Introduction

Synthetic GnRH molecules have been used to manipulate ovarian function for controlled breeding purposes and development of artificial reproductive technologies (ART) in various species. An acute release of

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doi:10.1016/j.theriogenology.2011.12.024

LH occurs after exogenous administration of GnRH agonists in several species. In llamas, an LH surge similar to that triggered by copulation was reported after administration of a GnRH agonist, gonadorelin acetate [1,2].

Llamas and alpacas are induced ovulators, since copulatory stimulation is required to elicit ovulation [3,4]; therefore, the application of ARTs in these species usually requires induction of ovulation by hormonal treatments. In this regard, various doses of GnRH have been used to induce a luteal phase in llamas and alpacas [1,2,5,6], as well as to synchronize ovulation for embryo transfer in llamas [7–9] or AI in alpacas [10,11]. However, results have been variable and comparisons among studies are difficult because of differences in agonists and doses used.

Luteinizing hormone is the most relevant luteotropic agent in several animal species [12,13]. Interestingly, several studies reported a correlation between the magnitude and duration of the preovulatory LH surge and the subsequent degree of luteogenesis in several laboratory animals [14–18]. Although other studies reported increased LH release in response to increased doses of GnRH in cattle [19–21] and ewes [22], the consequent effect on luteal function remains equivocal.

In South American camelids, there is a lack of information regarding luteal function in response to different hormonal stimuli. Adams, et al. [23] suggested that the difference in plasma progesterone concentration between llamas treated with either llama seminal plasma or GnRH was due to the variation in LH secretion pattern after treatment. In a recent study [6], 50 μg of gonadorelin acetate induced an ovulatory response and corpus luteum (CL) development in llamas that was comparable to that after mating. However, the effect of reduced doses of GnRH on pituitary response and subsequent CL development and function have apparently not been investigated in this species. The acquisition of this new knowledge would be valuable for improvement of AI schemes, female recipient synchronization protocols, and development of fixed-time AI programs in llamas.

The objective of the present study was to examine the effects of 50, 25, 12.5, or 6.25 μg of gonadorelin acetate (GnRH) on pituitary LH release, ovulatory response, CL development, and plasma progesterone concentrations in llamas. We hypothesized that reduced doses of GnRH would result in: a) decreased LH secretion, b) reduced ovulatory response, and c) impaired CL development and function.

2. Materials and methods

The present study was conducted during May to September 2010 at the Universidad Austral de Chile, Valdivia, Chile (39°38′ S - 73°5′ W and 19 m above sea level). All procedures were reviewed and approved by the Universidad Austral de Chile Bioethics Committee and were performed in accordance with the animal care protocols established by the Universidad Austral de Chile.

2.1. Animals

Non-pregnant, non-lactating adult llamas (n = 126), with a mean age of 7 yrs (range 5–9), weighing 112 ± 22 kg, with a mean body condition score of 3.0 ± 0.5 and a mean parity of 3 ± 1 were maintained on pasture supplemented with hay and water *ad libitum*. Llamas were housed indoors at night and offered 200 g/animal of a commercial diet supplement containing 140 g/kg crude protein and 150 g/kg crude fiber (Vaca14, Cisternas Nutrición Animal, Paine, Chile).

2.2. Experimental design

Llamas were examined once daily by transrectal ultrasonography using a B-mode scanner with a 7.5 MHz linear-array transducer (Aloka SSD 500, Aloka Co., Tokyo, Japan). Females with a follicle ≥8 mm in diameter that had grown for three consecutive days were randomly assigned to receive 50 (GnRH50, n = 23), 25 (GnRH25, n = 29), 12.5 (GnRH12.5, n = 29), or 6.25 μg (GnRH6.25, n = 29) of gonadorelin acetate (Ovalyse, Pfizer, Chile SA, Santiago, Chile), or 0.5 mL of PBS (Control group, n = 16) intramuscularly (day of treatment was designated day 0). More females were allocated to the GnRH25, GnRH12.5 and GnRH6.25 groups, since the expected ovulatory response was unknown; in contrast, 50 μg of gonadorelin consistently induced ovulation in more than 90% of llamas, whereas no ovulations were reported in llamas treated with PBS (Adams, et al., 2005; Ratto, et al., 2005, Silva, et al., 2011). Intramuscular injections were given in the semimembranosus muscle using a 21-gauge, 40 mm-long needle.

2.3. Ultrasonographic examinations

After treatment, ovaries were examined by ultrasonography every 12 h for 2 days to determine ovulation. Ovulation was defined as the sudden disappearance of a large follicle ≥8 mm that was detected during the previous examination and was confirmed by subsequent CL formation on Day 8. The interval from GnRH