Effect of exogenous estradiol on maternal recognition of pregnancy after embryo transfer in llamas

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Abstract

The objective of this study was to evaluate the effect of exogenous estradiol during maternal recognition of pregnancy (MRP) on extending the life span of the corpus luteum (CL) in embryo transfer (ET) recipient females. Eleven females were used as embryo donors and 23 females as recipients. Recipients were randomly divided into two groups: control (n=10) and treatment (n=13). In both groups follicular activity was monitored by transrectal ultrasonography and when a dominant follicle was observed in the left ovary a single intravascular (IV) dose of 8 µg of an homologous of GnRH agonist (buserelin) was administered (Day 0). Ovulation was confirmed by transrectal ultrasonography, and transcervical ET was carried out on Day 6 after buserelin administration. Embryos were recovered by flushing the uterus on Day 8 after the first mating of the donor. Once the embryos were washed and evaluated, those competent for transfer were transferred to the left uterine horn of the recipient females. On days 8 and 9 after buserelin administration the treatment group received 0.2 mg intramuscular injection (IM) of estradiol benzoate (EB). Fisher's exact test was used for statistical analysis. Embryo size varied within a range of 0.3-1.2 mm in diameter. Pregnancy rate was 50% (5/10) vs. 30.7% (4/13) for control and treatment groups respectively. No significant differences were observed between groups (P = 0.4173) with the dose of 0.2 mg of EB. Further research is needed to determine if a higher dose of EB could increase the luteal phase in ET recipients and if it helps the embryo when inducing MRP in llamas.

Keywords: Lama glama, estradiol benzoate, embryo transfer, maternal recognition of pregnancy.

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Introduction

There has been a great increase in the use of assisted reproductive techniques in South American Camelids (SAC) (Miragaya et al., 2006). Nevertheless, certain basic reproductive physiology remains unknown. Embryo transfer (ET) has spread widely as a practical reproduction method in various domestic animals (e.g. equine and bovine) with its purpose being to accelerate genetic improvement and reproductive efficiency. It has not yet been possible to develop an ET protocol in llamas that has comparable results to those obtained in other species. The corpus luteum (CL) has a half life of 8-9 days (Aba et al., 1995) thus limiting the period necessary for transferring embryos to the uterus and for maternal recognition of pregnancy (MRP) to maintain the CL viable. Although the events leading to MRP in SAC are not fully understood, it is possible that the process depends on the location of the embryo in the uterus, on the embryo's mobility or on both factors simultaneously. In a previous study we observed that maximum pregnancy rates were obtained when transferring the embryo to the left uterine horn in the presence of an ipsilateral CL; these results decreased when the embryo had to migrate to carry out MRP (Trasorras et al., 20010). The exogenous administration of estradiol can maintain and extend luteal production of progesterone (Palomino et al., 2006; Powell et al., 2007) indicating that the estradiol produced might play a role in MRP and in early support of the CL. Furthermore, prior to implantation, the blastocyst produces estradiol and the increase coincides with the period of MRP

(Powell et al., 2007). Hence, this hormone produced by the llama blastocyst may be involved in migration by causing a localised increase in myometrial contractility and propelling the blastocyst to the contralateral side. So, estradiol is a potential candidate for the blastocyst signal responsible for MRP in llama.

The objective of this study was to evaluate the effect of exogenous estradiol during the period of MRP on extending the life span of the CL in ET recipient llama females.

Materials and methods

Experimental animals

Non-pregnant, non-lactating *Lama glama* females (n=34) ranging in ages between 4 and 8 years and with an average body weight of 120 ± 22 kg were used as embryo donors and recipients. Females were kept separate from males during the experiment and fed bales of hay and water *ad libitum*. The study was carried out at the Veterinary School of the University of Buenos Aires, Buenos Aires, Argentina, situated 34º 36' S and 58º 26' W, at sea level.

Treatment of donor llamas

Ovarian follicular activity of all donor females (n=11) was examined by linear-array ultrasonography using a 5 MHz transrectal electronic transducer (Berger LC 2010). The absence of follicles bigger than 5 mm was confirmed before beginning the superstimulation treatment. A single IM dose (1000 IU) of PMSG (Novormon 5000®, Syntex, Argentina) was administered (Trasorras et al., 2009). Daily ultrasonographic examinations of the ovaries were continued until two or more dominant follicles (≥ 7mm in diameter) were observed and at that moment donor females were mated twice, 24 h apart. After the first mating, ovulation was induced in all donor llamas with a single IV dose of 8 µg of buserelin (Receptal®, Intervet, Argentina).

Treatment of recipient llamas

The ovaries and uterus of recipient females (n=23) were examined by transrectal ultrasonography. Two days after donor's buserelin injection and when a dominant follicle was observed in the left ovary of the recipient females, a single IV dose of 8 µg of buserelin was administered (Day 0) to induce ovulation. Ovulation was confirmed by transrectal ultrasonography on Day 2. Recipients were randomly divided into two groups: control (n=10) and treatment group (n=13). Transcervical ET was carried out on Day 6 after buserelin administration and the treatment group then received 0.2 mg IM of estradiol benzoate (EB; Estradiol 10®, Lab. Río de Janeiro, Argentina) on days 8 and 9. Pregnancy diagnosis was performed 13 days after the transfer by transrectal ultrasonography visualisation of the embryo vesicle and three days later by a normal heartbeat confirming viability.

Embryo recovery and transfer

The uterus of the 11 mated donor llamas were flushed non-surgically eight days after the first mating by transcervical uterine flushing. Aggressive females received 0.2 mg/kg IV of xylazine (Rompun®, Bayer) before flushing. Collection was carried out using a Foley catheter (12 or 16 Fr, according to female size) and a stylet was inserted into the catheter to keep it from bending during recto-vaginal manipulation. Uterine flushing was done by placing the catheter cuff in the uterine body, 1 cm cranial to the internal cervical orifice and inflating it with 5 or 10 ml of air (according to catheter gauge). The uterus was flushed four to five times with D-PBS (Gibco, Grand Island, NY, USA) supplemented with 1% fetal calf serum (FCS), with a total volume of 500 ml. Once the flushing was finished, donor females received a single IM dose of 250 µg of cloprostenol (Estrumate®, Schering-Plough, Germany) to cause the lyses of all the CLs in both ovaries.

Embryos recovered were graded according to Tibary and Anouassi (1997) after being washed four times in D-PBS supplemented with 20% FCS. Grade 1 and 2 embryos recovered were aspirated individually into 0.25 ml straws (IMV® ET Straws, France), which were loaded into a sheathed bovine/equine embryo transfer pipette (IMV® ET Sheath, 21'', France). Embryos were then transcervically transferred into the left uterine horn of the recipient llamas belonging to either control or treatment groups.

Statistical analysis

Pregnancy rate was compared in treatment group versus the control group using Fisher's exact test. Statistical significance was set at $P \leq 0.05$.

Results

A total of 34 embryos were recovered from the 11 llamas flushed (recovery from each female 3.09 ± 1.86 embryos, mean ± SD). Embryo size varied within a range of 0.3-1.2 mm in diameter and all the embryos were in the hatched blastocyst stage. Pregnancy rate was 50% (5/10) and 30.7% (4/13) for control and treatment group respectively. No significant differences were observed between groups (P = 0.4173) with the dose of 0.2 mg of EB.

Discussion

In all domestic farm animal species, the developing embryo must release a biochemical message to the maternal organism to prevent the normal release of $PGF_{2\alpha}$ from the endometrium, thereby effectively prolonging the lifespan and secretory functions of the CL. In SAC, this signal must be secreted before Day 7 after mating if it is to prevent luteolysis occurring, which is much earlier than in other species. In ruminants such as cattle and sheep that have a cotyledonary placenta, this MRP signal has been identified as interferon Tau (IFN-τ; Bazer et al., 1996) whereas in pigs and horses, both of which are non-ruminants and have a diffuse, non-invasive epitheliochorial placenta, interferon-like molecules of embryonic origin are not secreted (porcine: La Bonnardière et al., 1991; La Bonnardière, 1993; equine: Baker et al., 1991). However, the embryonic tissues of both these species possess high aromatase activity, and they can synthesise large amounts of estrogens *in vitro* from as early as Day 10 of gestation (porcine: Bazer and Thatcher, 1977; Bazer, 1992; equine: Ginther, 1984).

Camelids have a diffuse epitheliochorial placenta (Steven et al., 1980; Fowler and Olander, 1990; Smith et al., 1994) similar to that of pigs and horses. Correspondingly, llamas do not possess the IFN-τ gene (Leaman and Roberts, 1992) but the blastocyst produces estradiol prior to implantation, coinciding with the MRP period (Powell et al., 2007). Exogenous administration of this hormone was able to maintain and extend luteal production of progesterone in non-pregnant females (Powell et al., 2007) and increased the percentage of pregnancy from 58% (control group) to 75% in females that received 0.2 mg EB on days 8 and 9 after mating (Palomino et al., 2006). Our results were less satisfactory. Using the same experimental design, but on recipient females in an ET program, only 30.7% (4/13) became pregnant, compared with 50% of the control group. Although no significant differences were observed between groups, 0.2 mg of EB did not favour an increase in pregnancy percentage. However, Powell et al. (2007) used 10 mg EB daily during seven days after inducing ovulation in non-pregnant females and were able to maintain progesterone levels high during days 14 to 17. Thus, administration of higher doses of estradiol seems to prolong the luteal phase and increase progesterone secretion over the period in which luteolysis should occur. Consequently, more research is needed to determine if this dose increases

the luteal phase in ET recipients and if it helps the embryo when inducing MRP in llamas.

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