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Embryo transfer technique: Factors affecting the viability of the corpus luteum in llamas

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ABSTRACT

The aim of the present study was to evaluate the effect of the embryo transfer (ET) maneuvers on plasma progesterone concentrations in recipient *Lama glama* females and the relationship between the site the embryo was transferred to and corpus luteum (CL) localization. Experiment I (effect of transcervical threading): adult non-pregnant, non-lactating llama females were randomly assigned into two groups: control group (without cervical threading, $n = 10$) and group A (with cervical threading, $n = 10$). In both groups, CL activity was evaluated through measurement of progesterone plasma concentrations. In group A, on Day 6 after inducing ovulation with buserelin, the cervix was threaded to evaluate the effect of the maneuver on CL viability. No significant differences were observed in mean progesterone concentrations between groups ($P > 0.05$). Experiment II (effect of depositing PBS): females ($n = 66$) were randomly assigned into six groups ($n = 10$ per group and control group: $n = 6$) to evaluate the effect of depositing PBS in different sites in the uterus in relation to the localization of the CL: group 'Left-Ipsilateral': transcervical placing of PBS in the left uterine horn (CL in left ovary); group 'Left-Contralateral': transcervical placing of PBS in the left uterine horn (CL in right ovary); group 'Right-Ipsilateral': transcervical placing of PBS in the right uterine horn (CL in right ovary); group 'Body-Left': transcervical placing of PBS in the uterine body (CL in left ovary); group 'Body-Right': transcervical placing of PBS in the uterine body (CL in right ovary) and control group. Corpus luteum activity was evaluated in all groups by measuring plasma progesterone concentrations. On Day 6 post-buserelin, the corresponding maneuver was carried out according to the group. No significant differences were found for the mean plasma progesterone concentrations between groups ($P > 0.05$). Experiment III (effect of ET on CL viability): females ($n = 22$) were used as embryo donors and 50 females as recipients, in order to evaluate if placing the embryo in different areas of the uterus influences CL viability. Recipients were randomly divided into five groups, according to the place in the uterus where the ET was conducted with respect to the ovary where ovulation occurred: group 'Left-Ipsilateral':

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ET in the left uterine horn (CL in left ovary); group 'Left-Contralateral': ET in the left uterine horn (CL in right ovary); group 'Right-Ipsilateral': ET in the right uterine horn (CL in right ovary); group 'Body-Left': ET in the uterine body (CL in left ovary) and group 'Body-Right': ET in the uterine body (CL in right ovary). Corpus luteum activity was evaluated in all groups by measuring plasma progesterone concentrations. Embryos were recovered by flushing the uterus on Day 8 after the first mating of the donor and transcervical ET was carried out in recipients 6 days after buserelin administration. Pregnancy rates were: group 'Left-Ipsilateral': 50%; group 'Left-Contralateral': 20%; group 'Right-Ipsilateral': 30%; group 'Body-Left' and 'Body-Right': 10%. No significant differences ($P = 0.4728$) were detected between the pregnancy rates in the five groups. Threading the cervix and transcervical placing of PBS either in the uterine horns or the body did not affect plasma progesterone concentrations in the llama, indicating that the different embryo transfer maneuvers do not interfere with CL viability. To improve pregnancy rates it could be suggested that ET in the left uterine horn with an ipsilateral CL, is the most desirable option.

1. Introduction

There have been minimal amounts of reproduction-focused research with South American Camelids (SAC). Complex reproductive characteristics of these species contribute to the lack of research: induced ovulation, short half-life of the CL, different luteolytic activity between the two uterine horns, maternal recognition of pregnancy (MRP) beginning before Day 10 after mating, pregnancies only in left uterine horn, highly viscous semen with low sperm concentration, etc. Nevertheless, some assisted reproductive techniques (such as synchronization of time of ovarian follicular development, superstimulation and embryo transfer) indicate a greater increase in knowledge while others show less advances (artificial insemination, *in vitro* fertilization and intracytoplasmic sperm injection) (Miragaya et al., 2006; Tibary et al., 2005) and certain basic reproductive physiology remains unclear such as MRP signaling.

In their natural habitat (Andean regions) SAC demonstrate a seasonal reproductive behavior, with mating and parturitions occurring predominantly in the rainy season when more food is available (November–March). In areas at sea level (Buenos Aires, Entre Ríos, etc.) characterized by a higher quality food supply, llamas are considered non-seasonal because of follicular growth throughout the year. Nevertheless in these areas during the same months, with greater ambient temperatures, fertilizing capacity in males is less (Giuliano et al., 2000, 2008). This short reproductive period added to the long gestation of approximately 11 months (Johnson, 1989; Leon et al., 1990), are partly responsible for the poor reproductive efficiency observed in camelids. Application of reproductive biotechnologies such as embryo transfer (ET) would allow an increase in the number of offspring from selected males and females and consequently increase the proportion of genetically superior animals. This technique has spread widely as a practical production method in domestic animals (horses: Allen, 2005; cattle: Hasler, 2003). In llamas there has been little success (Aller et al., 2002; Bourke et al., 1992; Taylor et al., 2000). The luteolytic release of $PGF_{2\alpha}$ starting on Day 7 or 8 post-mating and completed by Day 9 or 10 after mating, shortens the half-life of the CL to 8 or 9 days (Aba et al., 1995, 2000) limiting the period necessary for transferring embryos to the uterus and for MRP to maintain the CL viable in llamas.

Del Campo et al. (1996) found luteolytic action of the right uterine horn is solely local, while the luteolytic action of the left uterine horn is on the CL located on the left and right ovaries. This difference in luteolytic activity between uterine horns can be explained by the different oviduct, ovarian and uterine vascular anatomy in the camelid female. The main vein originating from the left uterine horn crosses the midline and ends as a branch of the right uterine horn vein. Therefore, vascular anatomy indicates that much of the blood-flow of the left uterine horn vein crosses over to the right side, thus being able to exert a luteolytic control of the CL in the right ovary (Del Campo et al., 1996). Although ovulations occur with equal frequency from both ovaries, the majority of pregnancies are localized in the left uterine horn (alpaca: 97.5 and 99.3% with a CL in the right and left ovaries respectively, Fernández-Baca et al., 1973, 1979; llama: 100%, Sumar, 1988). When ovulation occurs in the right ovary, the embryo must probably migrate from the right uterine horn to the left to be able to conduct MRP (Sumar and Leyva, 1979). These observations allow us to hypothesize that the success of ET could depend on the site where the embryo is deposited in the uterus.

To date, the poor pregnancy rates obtained using transcervical ET are attributed to a high release of prostaglandin because of cervix manipulation, a weak signal from the embryo or a deficient embryo migration. In mares, the cervical threading involved in ET produces an increase in endometrial $PGF_{2\alpha}$ release, thus compromising CL viability (Kask et al., 1997) but this has not yet been studied in SAC.

Therefore, the aim of the present study was to evaluate the effect of uterine handling during ET on plasma progesterone concentrations in recipient *Lama glama* females and the relationship between the place the embryo is deposited and CL localization, to improve the pregnancy rates using ET.

2. Materials and methods

2.1. Animals

Non-pregnant, non-lactating female llamas ($n = 70$) ranging between 4 and 8 years of age and with an average