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Factors influencing embryo transfer success in alpacas—A retrospective study☆

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ABSTRACT

Embryo transfer offers great advantages to South American camelid farmers to reach their breeding goals but the technology still plays a relatively minor role in comparison to other domestic farm animals like cattle. The aim of the present study was to analyse a data set of 5547 single or multiple ovulation embryo transfers performed in commercial alpaca farms in Australia to determine the factors that influence number and quality of embryos produced, embryo transfer success (percentage of crias born) and gestation length following transfer. Logistic binary regression identified the variables day of flushing after mating, embryo diameter, embryo quality, day of transfer after GnRH, and the age of the recipient to have significant impact on the outcome measure embryo transfer success. Transfer of smaller embryos or lower quality embryos resulted in decreased transfer success rates. Optimal days for obtaining embryos from donors were Days 8 and 9 after mating, optimal days for transfer into recipients were Days 7 and 8 after GnRH treatment. Age (>15 years) and body condition of recipients <2 also lowered transfer success rates, while the summer heat had no adverse impact. However, season did influence gestation length, while cria gender did not. In conclusion, results from the analysis of this very large dataset can underpin new recommendations to improve embryo transfer success in alpacas.

1. Introduction

The international interest in breeding alpacas and other South American camelids for their fine fleece, has increased over the last two decades. This development has been accompanied by an increasing demand for assisted reproductive technologies like artificial insemination and embryo transfer to improve fleece quality more rapidly. In comparison to other domestic livestock, South American camelids show some unique reproductive characteristics. They are induced ovulators but follicular growth occurs in waves (Vaughan et al., 2004). The gestation period of approximately 340 days is relatively long and can vary considerably between years, seasons and individual animals despite sex of the cria and age of the dam not appearing to influence gestation length (Knight et al., 1995; Davis et al., 1997). Ovulation occurs equally on both ovaries, however the embryo implants into the left uterine horn 95–98% of the time (Fernandez-Baca et al., 1973; Bravo and Varela, 1993). The migration of the embryo from the right to the left uterine horn has been considered a possible reason for exceptionally high rates of embryonic mortality during the first month of pregnancy (Fernandez-Baca et al., 1979).

Although non-surgical embryo transfer has been described for many years (Wilson Wiepz and Chapman, 1985; Fernandez-Baca, 1993) the technology still plays a

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minor role in South American camelids in comparison to other domestic farm animals like sheep and cattle. The synchronisation of ovarian activity of South American camelid donors and recipients, superovulation, embryo recovery and transfer is also more challenging due to their specific reproductive physiology (Bourke et al., 1995a,b). This may account for the very variable but overall low reported embryo transfer success rates (65 transferred embryos resulted in 12 pregnancies and 7 live born crias; Del Campo et al., 1995). However, in recent years progress has been made in reproductive biotechnologies and embryo transfer has become a routine technique in some herds with very high genetic merit (Vaughan et al., 2002), also showing improved success rates (18 out of 49 transfers in llamas, Taylor et al., 2000). South American camelid embryos are commonly transferred immediately after recovery from donors because cryopreservation of camelid embryos is difficult due to their stage of development (hatched blastocyst) and their relatively large size (Aller et al., 2002; Taylor et al., 2005).

It is known in farm animals such as cattle and sheep that a number of factors such as species, breed, age, health and body condition, metabolism and energy balance, treatment protocols, lactational status of donors and recipients, time of embryo recovery after insemination, site of embryo placement in the recipient uterus, embryo size, quality and stage of development influence implantation and overall embryo transfer success rate (among others: Wright, 1981; Donaldson, 1984; Misra et al., 1999; Spell et al., 2001; Bari et al., 2003; Hidalgo et al., 2004; Raz et al., 2011). In contrast, little is known about factors affecting embryo transfer success in South American camelids. Lactation reduces dominant follicle size in llamas, however, the size of the corpora lutea do not differ (Adams et al., 1990, 1991; Ratto et al., 2003), and thus the significance of lactation for ovulation, and early embryonic development in camelids is not known. There is limited information about the optimum day of embryo collection. It is known that embryos reach the uterine cavity on Day 6 after ovulation and such embryos have already hatched (Del Campo et al., 1995). This would indicate that embryo collection should be performed later than 6 days after ovulation or 7 days after mating. Indeed, flushing of embryos from the donor uterus has been performed 7 days (Smith et al., 1994) and 8–8.5 days after mating (Aller et al., 2002), with a small scale study reporting an increasing percentage of embryo recovery from Day 7 (55%, 27 embryos out of 49 collections) to Day 8 (79%, 37 embryos out of 47 collections) and to Day 9 (100%, 3 embryos out of 3 collections) after mating (Taylor et al., 2000).

From the studies cited above it becomes obvious that information about factors that influence the outcome of embryo transfer in alpacas is scarce and often based on very low numbers of animals. Therefore, the aim of the present study was to review a very large data set collected from commercial embryo transfer undertaken in alpacas in recent years. Numerous factors were studied to investigate their influence on embryo numbers, size, quality, transfer success rate and birth rate. Results from these retrospective analyses are aimed at optimising embryo transfer protocols in alpacas.

2. Materials and methods

Single-embryo and multiple-ovulation embryo transfers (MOET) were performed in alpacas throughout Australia between February 2004 and December 2008 on 53 different farms. A dataset containing information on 5547 embryo transfers was considered for this analysis.

2.1. Donors

The donors were treated to either induce a single ovulation ($n = 822$) or multiple ovulations ($n = 1636$). To induce a single ovulation 4 μg buserelin (Receptal®, Intervet Australia Pty Ltd), a GnRH analogue, was injected intramuscularly to induce ovulation of the existing dominant follicle of unknown age and generate a new follicular wave. These animals were injected with 200 μg cloprostenol (Juramate®, Jurox Pty Ltd) intramuscularly nine to ten days later and then mated 24 h later. For multiple ovulation embryo transfer (MOET) the donors were treated as above, and then 24 h after cloprostenol treatment were injected with 4 μg buserelin intramuscularly, followed 2 days later by twice daily, diminishing doses of FSH for 4 days (Folltropin V®, Bioniche Animal Health Australasia; Vaughan and Hopkins, unpublished). Donors were injected intramuscularly with 200 μg cloprostenol 24 h after the last FSH treatment, then mated with fertile males approximately 48 h after the last FSH treatment.

On the day of flushing, a transrectal ultrasound was performed on donors to visualise the ovaries and count corpora lutea as an estimate for number of ovulations using an Aloka SSD-500 ultrasound machine (Aloka Co, Japan) equipped with a 7.5 MHz linear array transducer. There was no prior evaluation of ovarian status of donors using ultrasound before commencing hormonal treatments as all programmes were performed on a commercial basis and it was only possible to visit farms and ultrasound females on the day of flushing. Donor females that had no copora lutea on their ovaries were deemed to have failed to respond to hormonal treatment. They were therefore not flushed nor included in the dataset.

Each donor uterus was flushed non-surgically to obtain embryos using a commercial flushing solution (Complete Ultra Embryo Flushing Solution®, ICPbio Reproduction) via a 14 or 16 gauge Foley catheter on Day 6, 7, 8 or 9 after mating as described by Correa et al., 1992, 1997. Donors were treated with a single dose of 200 μg cloprostenol intramuscularly immediately after flushing to induce lysis of corpora lutea. The recovered fluid was examined for embryos under a stereomicroscope at ×20 magnification. Hatched blastocysts were measured and classified according to the visual appearance of the surrounding trophectoderm cells (Grade 1 – excellent, Grade 2 – moderate to good, Grade 3 – poor, Grade 4 – holed; Fig. 1). Grade 5 embryos were not transferred for economic reasons and thus discarded. In the absence of sufficient recipients left-over embryos were also discarded.

To evaluate embryo production by donors the following outcome measures were used: