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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:

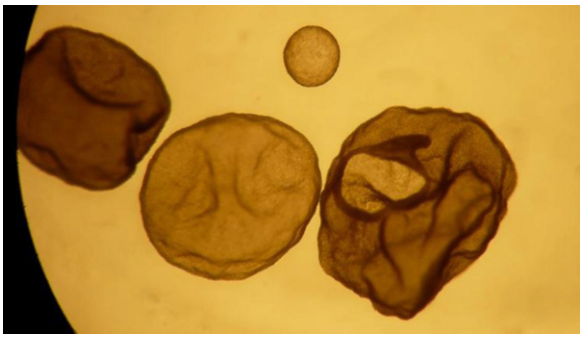

Fig. 1. Day 8 embryos from the same donor showing a Grade 2 embryo (far left), two Grade 1 embryos (middle) and one Grade 4 embryo (far right).

1. The number of corpora lutea as an estimate for ovulation rate.
2. The number of embryos recovered per flush.
3. The recovery rate/percentage recovery (total number of embryos flushed divided by total number of corpora lutea multiplied by one hundred).
4. The quality (grade) of the embryos.
5. The size of the embryos.

2.2. Recipients

Recipient ovarian activity was synchronised to that of each donor using 4 μg buserelin injected intramuscularly between 1 day before and 1 day after the time of donor mating to induce ovulation and formation of a corpus luteum. If the recipient alpaca ovulated 1 day after the donor due to the buserelin being administered one day after donor mating, the degree of synchrony was termed −1 day. Conversely, if the recipient ovulated 1 day before the donor (buserelin was administered the day before donor mating), the degree of synchrony was termed +1 day. The freshly obtained embryos were loaded individually into 0.25 mL straws (IMV, France) and placed into a sheathed, bovine embryo transfer gun (IMV, France) for transfer. Embryos were usually deposited transcervically into the uterus of recipient females within 1 h of collection. The interval from buserelin treatment to day of transfer (in days), the site of embryo placement (left or right uterine horn), depth of embryo placement (shallow, mid, horn tip), ease of transfer (easy, moderate, hard) and the tail score (amount of mucus on underside of tail at level of vulva) was recorded. Recipient females were mostly tested for pregnancy at approximately 60 days of gestation by transrectal or transabdominal ultrasound performed by a local veterinarian. The principal outcome measure was embryo transfer success rate calculated as number of live-born crias divided by number of transferred embryos multiplied by one hundred. Further, the gender of the cria and the length of gestation were recorded.

2.3. Data sampling and statistical analysis

Information was recorded on a wide variety of parameters including year, season, farm and single or multiple ovulations of donors. Age, parity and lactation status were recorded for both donors and recipients. The body condition of donors and recipients was scored using a 1 (thin) to 5 (obese) scale; the desired body condition was considered between 2.5 and 3.5; scores of 2.0 and below and 4.0 and above are considered abnormal (Australian Alpaca Association, 2011; Penn State University, 2011).

The day of flushing after mating, and total number of corpora lutea were recorded in donors. The day of transfer in relation to the buserelin treatment and site of embryo placement (left or right uterine horn) were recorded in recipients. The difference in timing (in days) of donor mating and buserelin treatment of recipients was calculated (ovulation synchrony).

Statistical analysis was performed to determine the factors that influence number and quality of embryos, embryo transfer success and gestation length. Normal distribution was tested using the Kolmogorov–Smirnov test. Data are presented as arithmetic mean and standard deviation. For comparison of the frequencies of outcome measures the Chi-square test was used (Wright, 1981); to calculate associations between parameters Pearson Rank correlation coefficients were calculated. A logistic binary regression model using the maximal likelihood methodology which has been described and used in studies to assess embryo transfer success in cattle (Spell et al., 2001; Peixoto et al., 2007) was used to estimate the effect of several variables on the probability of pregnancy. Pregnancy has to be considered a binomial event (pregnant = 1, non pregnant = 0) and is the dependent variable. The variables: year, season, body condition score (BCS) of donors and recipients, age and lactation status of the donor and recipient, day of flushing, embryo diameter, embryo quality, day of transfer, site of embryo placement, difference in ovarian status synchronisation between donors and recipients were included in the model as independent (explanatory) variables. The software package OpenStat was used for all statistical analyses. Statistical significance level was set at $P < 0.05$.

3. Results and discussion

The logistic regression analysis identified the variables: day of flushing, embryo diameter, embryo quality, day of transfer, and the age of the recipient to have significant impact on the outcome measure pregnancy (Table 1). Embryo quality and development (size) of the embryo have also been found to be associated with embryo transfer success in bovine studies (Spell et al., 2001; Peixoto et al., 2007). The variable 'farm' did not have a significant effect.

3.1. Donor related factors

The procedure to induce a single ovulation was performed in 822 donor females. The number of detectable corpora lutea was 991, which were equally distributed between both sides (left ovary 500, right ovary 491). The number of corpora lutea is considered as an estimate for ovulation (1.22 ± 0.34 per animal). Out of the 814 females which had ovulated, 680 were single ovulations, 127 double ovulations, 6 triple ovulations and 1 animal had 4 corpora lutea.

Table 1
Results of the logistic regression analysis: β (regression coefficient), standard error, values for X^2 and P (probability).

Variable	β	Standard error	X^2	P
Year	−0.04	0.04	0.71	0.51
Season	0.04	0.03	0.77	0.38
Donor age	0.01	0.08	0.01	0.91
Donor BCS	−0.11	0.10	0.83	0.38
Donor lactational status	−0.12	0.07	0.85	0.36
Day of flushing after mating	0.70	0.18	14.70	<0.001
Embryo diameter	0.64	0.12	5.73	0.02
Embryo quality	−0.38	0.08	20.4	<0.001
Day of transfer after buserelin	0.23	0.14	3.94	0.047
Recipient age	−0.28	0.33	6.42	0.01
Recipient BCS	−0.19	0.09	3.15	0.089
Recipient lactational status	−0.14	0.12	1.58	0.21
Recipient tail mucus score	−0.03	0.063	0.19	0.66
Depth of embryo placement	0.09	0.09	0.01	0.90
Ease of embryo transfer	−0.29	0.15	2.37	0.12
Ovarian synchrony between donor and recipient	0.13	0.10	1.38	0.24

Single-ovulation donor flushing resulted in 667 embryos (259:0 embryos, 450:single embryo, 98:two embryos and 7:three embryos, thus the recovery rate was 66.9 ± 37.1%. The failure of 38% of donors to produce an embryo is slightly higher than rates observed in small-scale llama studies where 7 of 26 (27%) single-ovulation donors (Huanca et al., 2009) and 40 of 123 (33%) single-ovulation donors (Adams and Dominguez, 2007) failed to produce an embryo following natural mating in the presence of a dominant follicle. Reasons for the 259 donors ovulating but failing to produce an embryo on flushing day are multi-factorial. Causes associated with the donor include delayed ovulation, ovulation of an immature or aged oocyte, reproductive tract abnormalities such as segmental aplasia of the oviduct, and uterine infection resulting from recent parturition or copulation interfering with embryo development. Issues associated with the male include failure of fertilisation due to inadequate quality and/or quantity of sperm (male history such as age, current fertility and number of matings per day were not included in the dataset). Embryo-associated issues include early embryonic death arising from chromosomal abnormalities, in-breeding, extreme weather events and other environmental effects such as diet. Lastly, management factors such as imprecise implementation of the protocol, stressful handling, inappropriately timed treatments of anthelmintics/vitamins/minerals, and failure to retrieve the embryo would also contribute to failure of embryo production in donors.

The superovulation regime was applied in 1636 animals. As an estimate of ovulation, a total of 10,796 corpora lutea were detected in donors using transrectal ultrasonographic examination. This reflects 6.59 ± 4.78 ovulations per animal. The ovulations occurred evenly distributed between the ovaries (left ovary 5399 corpora lutea, right ovary 5397 corpora lutea). In total, 4188 embryos were collected during transcervical uterine flushing; the recovery rate was calculated as 41.4 ± 32.2% which is significantly lower than the recovery rate of the animals after single ovulation treatment. The use of superovulatory hormones aims to increase the number of follicles recruited at the start of a new follicle wave. Some of these artificially stimulated follicles may have abnormal oocyte development within, leading to ovulation of oocytes with deficient nuclear and/or cytoplasmic maturation which may in turn affect fertilisation rate and embryo development rate (Sirard et al., 2000). Nevertheless, the embryo recovery rate again compares favourably with findings from small-scale studies in llamas. Correa et al. (1997) collected 10 embryos from 29 ovulations (34.5%) and Ratto et al. (1997) collected 31 embryos from 183 ovulations, (16.9%) using FSH as the superovulatory hormone. Only studies which used equine chorionic gonadotrophin (eCG) in combination with progestagen and oestradiol achieved equally high recovery rates (Huanca et al., 2009; Aller et al., 2010).

An average of 2.57 ± 3.01 embryos was obtained per treatment. Embryos could not be collected from 548 of the 1636 animals, one embryo was collected from 268 animals; two or more embryos (maximal 21) were obtained from 820 animals which indicates that a third (33.5%) of the animals did not respond to the superovulatory treatment producing embryos. Using FSH as the superovulatory hormone, Correa et al. (1997) collected similar numbers of embryos per flush (an average of 2.5 embryos from 4 donors), but Ratto et al. (2003) collected fewer (an average of 1.6 embryos from 20 donors). Huanca et al. (2009) collected more embryos per flush (4.8 ± 2.8 embryos from 26 donors and 3.5 ± 3.0 from 27 donors) using a different superovulatory protocol (eCG ± MPA). Such results may have been achieved due to a more effective superovulatory protocol; however, the cited study was much smaller, and was performed on one farm in one season under controlled conditions. In comparison, our field study was conducted on many farms and therefore under the influence of numerous farm and management factors, potentially affecting the preparation of donors and recipients on each farm. Conversely, Aller et al. (2010) achieved lower numbers of embryos per flush (1.5 ± 0.4 embryos from 18 donors and 1.9 ± 0.4 from 18 donors) using also an eCG-based superovulatory protocol (MPA + eCG ± EB), while Carretero et al. (2010) collected 74 embryos from 40 donors (1.9 embryos per flush) using eCG ± EB ± progesterone.

The association between ovulation (number of corpora lutea) and recovered embryos was characterised by a correlation coefficient of 0.54 ($P < 0.05$), different to findings by Bourke et al. (1995b) who observed a negative correlation

between number of embryos recovered and number of corpora lutea in llamas. More recently, studies indicate a positive correlation between ovulation rate and recovered embryos (Huanca et al., 2009).

No differences were found in parameters describing ovulation and embryo quality among the different years of the study. The variables season (Table 2), lactation (data not shown), and body condition (Table 3) of the donor had no effect on ovulation rate, or embryo number, diameter or quality. Seasonal influences, especially heat stress, have been identified as factors that negatively affect response to superovulation treatment and embryo quality in cattle (Hansen et al., 2001). However, alpacas are relatively well adapted to high environmental temperatures of low humidity (Fowler, 1994), and tend to be farmed in areas of lower humidity in Australia. Lactation was similarly not observed to affect ovulation and embryo recovery rates in a study in llamas (Aller et al., 2010).

The diameter of embryos collected from the single ovulation group (1.29 ± 0.76 mm) was similar to that of the MOET group (1.41 ± 0.75 mm; $P > 0.05$). There was no correlation between size of the embryos and their quality ($r = 0.13$, $P = 0.37$). Embryo diameter significantly influenced the embryo transfer success rate (percentage of crias born alive), with decreased rates following transfer of embryos smaller than 1 mm (Table 4). The day of embryo collection in relation to mating had a significant effect on embryo size and, therefore, indirectly on embryo transfer success rate (Table 5). The embryos collected on Day 7 were significantly smaller than embryos on Day 8. Although there were few embryos collected on Day 9 they were significantly larger; the apparent lower transfer success rate in comparison to Day 8 was not significant. Wright (1981) did not find any differences in pregnancy rates in cattle after transfer of embryos collected on Days 6.5, 7, 7.5 and 8; however, none of the embryos were hatched on any of these days, and significant differences after transferring embryos of different developmental stages were described. The increased recovery rate from Day 7 to Day 9 after mating reported by Taylor et al. (2000) was not observed in the current study.

There was variation in embryo diameter within and between flushes in this study (Fig. 1). Other workers have also observed great variability in embryo diameter between single embryos from the same female in repeated collections and among embryos from different females (Del Campo et al., 2002). Variation in embryo diameter within any day of collection may be attributed to variation in ovulation time and thus developmental time (Trasorras et al., 2010), variation in growth rates of embryos following different superovulatory protocols (Sirard et al., 2000) or other intrinsic factors (Del Campo et al., 2002).

All embryos collected were hatched blastocysts similar to the findings from many camelid workers (Ratto et al., 1997; Taylor et al., 2000; Del Campo et al., 2002; Huanca et al., 2009; Aller et al., 2010). The collected embryos were classified into four groups according their quality (Table 6). Embryo quality classification resulted in similar embryo transfer success rates for each grade of embryo transferred in single ovulation versus MOET groups. Overall, 3386 Grade 1 transferred embryos resulted in 1553 crias (45.9%), 915 Grade 2 embryos resulted in 286 crias (31.2%), 111 Grade 3 embryos resulted in 31 crias (27.9%) and 109 Grade 4 embryos resulted in 17 crias (15.6%). The embryo transfer success rates of Grades 1 and 4 differed significantly to all other groups. Although embryo quality classifications systems differ among studies in domestic livestock, embryo quality is considered a significant factor affecting pregnancy rate in many studies (Wright, 1981; McKinnon et al., 1994; Spell et al., 2001; Peixoto et al., 2007) although not in all (Hanekamp, 1999).

Donor age did not influence ovulation parameters and the quality of the collected embryos during single ovulation or MOET embryo collection (Table 7). The decrease in size and embryo transfer success rate of embryos out of donors older than 15 years was not significant possibly due to the small number of animals in this age group. In a study in cattle (Breuel et al., 1991), embryo number and quality were reduced by the age of the donor. Farmers aim to flush younger female alpacas to access their superior genetics, thus only a small number of older donors were present in the study. Despite the lack of significance it seems reasonable to suggest that older alpaca donors may also produce fewer embryos with decreased quality.

3.2. Recipient related factors

Overall 4516 embryos were transferred which resulted in 1952 pregnancies (43.2%) and finally in 1892 crias born alive (41.9%). The average gestation length of pregnancies with crias born alive was 344 ± 11.6 days with a minimal length of 319 and a maximal length of 387 days. For 1887 crias information on their gender was recorded; 977 male and 910 female crias were born giving a sex ratio of 1.07/1. The gestation length did not differ between male (344 ± 11.2 days) and female (343 ± 11.6 days) offspring nor was it different between dams of different age, which corroborates the findings of Knight et al. (1995) and Davis et al. (1997).

It was not possible to transfer all embryos, due to insufficient recipient availability on some farms and poor quality of embryos. It was not possible to retrieve birthing data in some instances. From the 667 obtained embryos using the single ovulation protocol 553 embryos (82.9%) were transferred resulting in 243 (44.8%) pregnancies detected by ultrasound and 235 (42.4%) crias born alive (Table 5). Of the 4188 embryos collected from MOET 3963 (94.6%) were transferred. The embryo transfer resulted in 1709 pregnancies and 1657 live crias; the transfer success rates were 43.1% and 41.8%, respectively. The transfer success rates did not differ between single ovulation and MOET protocols; there were also no differences between the years of the study period. The embryo transfer success rate reported in the present study seems to be higher in comparison to the reported pregnancy rates in studies on embryo transfer in South American camelids (Del Campo et al., 1995; Taylor et al., 2000; Trasorras et al., 2010). However comparison is difficult or impossible as the results of previous studies were based on very low numbers of transfers. Generally, success rates higher than 40% can be considered as satisfactory, although they appear lower compared to cattle, possibly due to different developmental stage of the

Table 2
Seasonal effects on ovulation and embryo collection, diameter and quality in donor alpacas which have been treated to induce a single ovulation or multiple ovulation embryo transfer (MOET) and on embryo transfer (ET) success rate (percentage of crias born) in recipient alpacas; different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation ET

Season	Spring	Summer	Autumn	Winter
Donors flushed	236	167	253	166
Ovulations	254	194	335	208
Ovulations per animal	1.08 ± 0.30	1.16 ± 0.39	1.32 ± 0.89	1.25 ± 0.44
Collected embryos	162	134	219	145
Recovery rate (%)	63.8 ± 50.0	69.0 ± 46.2	65.4 ± 44.0	69.7 ± 0.65
Embryo diameter (mm)	1.19 ± 0.62	1.28 ± 0.88	1.39 ± 0.89	1.26 ± 0.64
Embryo quality	1.38 ± 0.77	1.37 ± 0.78	1.35 ± 0.79	1.38 ± 0.72
Transferred embryos	146	115	179	113
Crias born	56	59	76	44
ET success rate (%)	38.3[a]	51.3[b]	42.4[a,b]	38.9[a]

II. MOET

Season	Spring	Summer	Autumn	Winter
Donors flushed	402	391	500	334
Ovulations	2531	2615	3361	2289
Ovulations per animal	6.30 ± 4.35	6.69 ± 4.62	6.72 ± 4.88	6.85 ± 4.87
Collected embryos	1006	1030	1326	826
Recovery rate (%)	39.7 ± 40.0	39.4 ± 37.3	39.4 ± 39.1	36.1 ± 38.9
Embryo diameter (mm)	1.44 ± 0.72	1.47 ± 0.78	1.43 ± 0.79	1.28 ± 0.70
Embryo quality	1.33 ± 0.64	1.35 ± 0.72	1.36 ± 0.64	1.30 ± 0.62
Transferred embryos	933	968	1273	788
Crias born	389	390	549	329
ET success rate (%)	41.6	40.3	43.1	41.7

embryo being more vulnerable (Wright, 1981), embryo defects, chromosomal abnormalities and gamete ageing (Trasorras et al., 2010). The corpus luteum produced in response to exogenous GnRH, as in the case of recipients used in this study, has been shown to have a similar form and function to those produced by natural mating in llamas (Ratto et al., 2006) and should not have had a deleterious effect on pregnancy rates and numbers of crias born.

In a large study by Fernandez-Baca et al. (1973), 472 corpora lutea in the right ovary were associated with 12 pregnancies in the right uterine horn and 460 pregnancies in the left horn, while 440 corpora lutea in the left ovary were associated with 3 pregnancies in the right horn and 437 pregnancies in the left horn. Thus, approximately 50% of left horn pregnancies had a corpus luteum on the right ovary. Since embryo implantation occurs predominately in the left uterine horn the majority of embryos were placed there in our study (4200). Fewer embryos were transferred into the right uterine horn (244). The placement of the embryo in the left horn resulted in 1782 crias (transfer success rate 42.4%), the placement in the right horn in 108 crias (44.3%); there was no difference between the two sides. Although the movement and implantation of the embryo was not followed up, our data suggest that in contrast to the assumption of Fernandez-Baca et al. (1979) the movement of the embryo from the right into the left uterine horn is not likely to be a major reason for embryo mortality in alpacas. However, if hatched embryos move around the uterine horns generally, regardless of where embryos are placed during transfer (for example, as a means of sending the signal of maternal recognition to the recipient reproductive tract), this intrauterine movement may account for the lower pregnancy rates seen in camelids generally, compared to other domestic livestock.

The comparison between embryo placements in the left or right horn has previously been performed in llamas (Trasorras et al., 2010), also resulting in pregnancy rates that were not significantly different from each other. However, in the current study embryo transfer rate was significantly higher in recipients in which the embryo was transferred into the left uterine horn when the corpus luteum was on the ipsilateral (left) ovary (2241 transferred embryos, 963 crias born, 43.0%) in comparison to recipients that had the corpus luteum on the contralateral (right) ovary (1976 transferred embryos, 795 crias born, 40.2%). This finding corroborates the suggestion of Trasorras et al. (2010) that to improve pregnancy rates embryos should be placed in the left uterine horn with an ipsilateral corpus luteum, however, it might have little practical impact, as the number of recipients available on camelid farms is limited and the 3% difference found does not justify transferring embryos only when the corpus luteum is on the left ovary. No differences could be found in percentage of crias born when embryos were transferred into the right uterine horn nor in recipients that had corpora lutea on both ovaries due to the relatively low number of embryo transfers in these females.

Ease of embryo transfer into the recipient uterine horn and depth of embryo placement by the operator did not significantly affect the percentage of crias born (Table 1). Our results support similar findings, where ease of transfer did not significantly affect pregnancy rate in cattle (Wright, 1981) and depth of embryo transfer was not significant in llamas (Trasorras et al., 2010). It seems that it is not

Table 3
Effect of body condition score (BCS) of the donor on ovulation, embryo collection, diameter and quality in alpacas treated to induce a single ovulation or multiple ovulation embryo transfer (MOET); effect of BCS of the recipient on the embryo transfer (ET) success rate (percentage of crias born); different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation ET – Donors

BCS	1 ≤ BCS ≤ 2	2 < BCS < 4	4 ≤ BCS ≤ 5
Donors flushed	178	630	14
Ovulations	228	749	14
Ovulations per animal	1.28 ± 0.57	1.19 ± 0.48	1.00 ± 0.00
Collected embryos	162	496	5
Recovery rate (%)	71.9 ± 41.8	66.2 ± 47.9	35.7 ± 45.5
Embryo diameter (mm)	1.40 ± 0.85	1.25 ± 0.73	0.84 ± 0.36
Embryo quality	1.27 ± 0.63	1.41 ± 0.81	1.20 ± 0.45

I. Single ovulation ET – Recipients

BCS	1 ≤ BCS ≤ 2	2 < BCS < 4	4 ≤ BCS ≤ 5
Recipients	104	440	6
Crias born	39	194	2
ET success rate (%)	37.5[a]	44.1[b]	33.3[a,b]

II. MOET – Donors

BCS	1 ≤ BCS ≤ 2	2 < BCS < 4	4 ≤ BCS ≤ 5
Donors flushed	349	1260	18
Ovulations	2368	8310	118
Ovulations per animal	6.78 ± 5.00	6.59 ± 4.61	6.56 ± 5.38
Collected embryos	863	3292	33
Recovery rate (%)	36.4 ± 41.0	39.6 ± 36.3	28.0 ± 32.9
Embryo diameter (mm)	1.50 ± 0.71	1.39 ± 0.76	1.33 ± 0.79
Embryo quality	1.30 ± 0.59	1.34 ± 0.67	1.62 ± 0.79

II. MOET – Recipients

BCS	1 ≤ BCS ≤ 2	2 < BCS < 4	4 ≤ BCS ≤ 5
Recipients	852	3073	38
Crias born	324	1321	12
ET success rate (%)	38.0[a]	43.0[b]	31.6[a,b]

important where the embryo is deposited during transfer as it will move to the appropriate location and send the signal of maternal recognition regardless of site of placement, but selection of recipients with a corpus luteum on the left ovary should optimise embryo transfer success rate. It was not possible to determine whether grading the amount of mucus deposited on the underside of the recipient's tail at the level of the vulva predicted her ability to carry a cria to full term (Table 1).

Season did not influence embryo transfer success rate when considering the complete dataset for embryos produced from single and multiple ovulation embryo transfer (Table 1). However, there was a significantly higher embryo transfer success rate in summer compared to winter and spring in the single ovulation group (Table 2). Similarly, Knight et al. (1992) showed conception rates following natural matings in spring (10/20, 50%) were lower than those in autumn (20/25, 80%) but the difference was not

Table 4
Effect of embryo diameter on embryo transfer (ET) success rate (percent of crias born) after treatment to induce a single ovulation or multiple ovulation embryo transfer (MOET); different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation ET

Embryo size (mm)	≤1	>1 and ≤2	>2 and ≤3	>3
Transferred embryos	237	278	32	6
Crias born	88	131	13	3
ET success rate (%)	37.1[a]	47.1[b]	40.6[a,b]	50.0[a,b]

II. MOET

Embryo size (mm)	≤1	>1 and ≤2	>2 and ≤3	>3
Transferred embryos	1451	2068	382	61
Crias born	418	1015	191	31
ET success rate (%)	28.8[a]	49.1[b]	50.0[b]	50.8[b]

Table 5
Effect of the interval between mating the donor alpaca and embryo collection on ovulation and embryo collection, diameter and quality in donor alpacas treated to induce a single ovulation or multiple ovulation embryo transfer (MOET) and on embryo transfer (ET) success rate (percentage of crias born); different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation

Mating to embryo collection interval	Day 6	Day 7	Day 8	Day 9
Donors flushed	1	256	564	1
Ovulations	1	298	691	1
Ovulations per animal	1.00	1.16 ± 0.37	1.22 ± 0.59	1.00
Collected embryos	0	197	465	1
Recovery rate (%)	0	66.1 ± 47.7	67.3 ± 47.3	100
Embryo diameter (mm)		0.77 ± 0.41[a]	1.51 ± 0.49[b]	2.0
Embryo quality		1.31 ± 0.67	1.40 ± 0.8	1
Transferred embryos		165	387	1
Crias born		57	178	0
ET success rate (%)		34.5[a]	46.0[b]	0

II. MOET

Mating to embryo collection interval	Day 6	Day 7	Day 8	Day 9
Donors flushed	0	356	1273	5
Ovulations		2438	8327	31
Ovulations per animal		6.8 ± 4.3	6.54 ± 4.7	6.2 ± 2.4
Collected embryos		809	3361	18
Recovery rate (%)		33.2 ± 38.8	40.4 ± 38.9	58.1 ±
Embryo diameter (mm)		0.63 ± 0.26[a]	1.58 ± 0.66[b]	4.69 ± 1.26[c]
Embryo quality		1.37 ± 0.56	1.28 ± 0.63	1.22 ± 0.73
Transferred embryos		789	3156	18
Crias born		144	1505	8
ET success rate (%)		18.2[a]	47.7[b]	44.4[b]

significant. It has been shown in cattle that season may influence pregnancy rate, particularly warmer seasons having a negative impact (Peixoto et al., 2007). The adaptation of camelids to high environmental temperatures in the presence of low humidity (Fowler, 1994) is reflected in the observation that the percentage of crias born was not significantly reduced in summer transfers.

Season did have an effect on gestation length. Animals that gave birth in autumn (March, April, May) and winter (June, July, August) had shorter gestation lengths (340.0 ± 9.1 days and 340.4 ± 9.1 days) in comparison to those that gave birth in spring (September, October, November; 348.4 ± 11.4 days) and summer (December, January, February; 348.0 ± 10.5 days). Similarly, in a New Zealand study, 102 autumn-mated females averaged 336.4 ± 1.2 days gestation while 60 spring-mated alpacas averaged 348.9 ± 1.4 days gestation (Davis et al., 1997). Davis et al. (1997) suggested that a shorter gestation in autumn could be an evolutionary adaptation that makes it more likely for alpaca females to deliver and raise a cria every year. Alpacas are native to the South American highlands where rainfall is concentrated between January and March. Alpacas that are therefore mated in autumn (March–May) of any year give birth approximately a month earlier the following year, during/soon after the next wet season, thus optimising their nutrition during late gestation and early lactation.

Information about age of the recipients was available for 4213 recipients (504 in the single ovulation protocol and 3709 recipients in the MOET protocol). In contrast to donor age, recipient age had a significant effect on the percentage of crias born after embryo transfer. Recipients greater than 15 years of age had a significantly decreased embryo transfer rate in comparison to younger animals (Table 7). We are

Table 6
Effect of embryos with different quality on embryo transfer (ET) success rate (percentage of crias born) after treatment to induce a single ovulation or multiple ovulation embryo transfer (MOET); different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation ET

Embryo quality	Grade 1	Grade 2	Grade 3	Grade 4
Transferred embryos	423	77	32	21
Crias born	191	29	10	5
ET success rate (%)	45.2[a]	37.7[b]	31.2[b]	23.8[c]

II. MOET

Embryo quality	Grade 1	Grade 2	Grade 3	Grade 4
Transferred embryos	2963	838	79	88
Crias born	1362	257	21	12
ET success rate (%)	46.0[a]	30.7[b]	26.5[b]	13.6[c]

Table 7
Effect of donor age on ovulation, embryo collection, diameter, quality and embryo transfer (ET) success rate (percentage of crias born) after treatment to induce single ovulations or multiple ovulation embryo transfer (MOET), and effect of recipient age on ET success rate; different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation ET – Donors

Age (years)	≤5	>5 and ≤10	>10 and ≤15	>15
Donors flushed	349	357	97	8
Ovulations	416	435	119	8
Ovulations per animal	1.20 ± 0.44	1.22 ± 0.68	1.23 ± 0.53	1.00 ± 0.00
Collected embryos	309	267	73	3
Recovery rate (%)	74.3 ± 45.6	61.4 ± 48.0	61.3 ± 46.1	37.5 ± 51.8
Embryo diameter (mm)	1.30 ± 0.67	1.26 ± 0.63	1.33 ± 0.67	1.03 ± 0.82
Embryo quality	1.38 ± 0.79	1.33 ± 0.70	1.50 ± 0.72	1.66 ± 1.15
ET success rate (%)	41.5	42.5	47.8	33.7

I. Single ovulation ET – Recipients

Age (years)	≤5	>5 and ≤10	>10 and ≤15	>15
Recipients	150	278	70	6
Crias born	62	132	29	2
ET success rate (%)	41.3[a]	47.5[a]	41.4[a]	33.3[b]

II. MOET – Donors

Age (years)	≤5	>5 and ≤10	>10 and ≤15	>15
Donors flushed	709	720	178	9
Ovulations	4558	5026	1066	57
Ovulations per animal	6.43 ± 4.62	6.98 ± 4.76	5.99 ± 4.60	6.33 ± 14.55
Collected embryos	1985	1797	384	3
Recovery rate (%)	43.6 ± 31.0	35.8 ± 38.9	36.0 ± 39.0	5.3 ± 3.5
Embryo diameter (mm)	1.47 ± 0.81	1.37 ± 0.70	1.28 ± 0.61	0.52 ± 0.11
Embryo quality	1.32 ± 0.65	1.29 ± 0.59	1.34 ± 0.63	1.33 ± 0.47
ET success rate (%)	41.9	42.1	40.0	0

II. MOET – Recipients

Age (years)	≤5	>5 and ≤10	>10 and ≤15	>15
Recipients	1221	1946	505	37
Crias born	528	829	213	10
ET success rate (%)	43.2[a]	42.6[a]	42.2[a]	27.0[b]

not aware of studies evaluating effects of recipient age on embryo transfer in other farm animals, possibly because cattle and sheep are slaughtered at a relatively young age. Information on recipient parity (primiparous versus pluriparous) was also available for 2421 animals. There was no difference in embryo transfer success rate between 245 primiparous females and 2176 females that had given birth at least one time before (39.8 and 42.2% respectively).

Thin animals (BCS ≤ 2) had a significantly lower transfer success rate in comparison to animals with optimal body condition (BCS 2.5–3.5; Table 3). The apparent reduction in embryo transfer success rate in obese alpacas (BCS ≥ 4) was not significant because of low numbers in the group. Overall, however, recipient BCS at the time of transfer did not have a significant effect on the transfer success rate according to the regression analysis which is most likely the result of a low number of animals with abnormally high or low BCS (Table 1).

Effect of lactational status was investigated in 4356 recipient alpacas. Females that were lactating at the time of embryo transfer (1832) had similar pregnancy rates (43.0%) in comparison to non-lactating animals (2524, 42.3%). A number of studies describe the negative influence of milk yield, poor nutrition and negative metabolic status on pregnancy rate in dairy cattle (Hidalgo et al., 2004; Vasconcelos et al., 2006; Jones and Lamb, 2008). In comparison, studies in beef cattle did not show an effect of lactational status on pregnancy rate after embryo transfer (Wright, 1981), likely as a result of the lower milk yield of beef cattle without the risk of negative energy balance. In the present study, the milk yield of alpacas, and hence its impact on metabolism, was unknown. However, it seems reasonable to propose that the metabolic stress caused by lactation is much higher in a high yielding dairy cow than in an alpaca.

The day of embryo transfer into each recipient had a significant effect on embryo transfer success rate (Tables 1 and 8). The window of opportunity for transferring embryos into alpaca recipients appears to be 7 or 8 days after induction of ovulation despite the observation that the luteolytic release of PGF2α already starts 7 or 8 days after mating (Aba et al., 2000). Animals in which embryos were transferred 5 or 6 days after buserelin treatment had lower success rates (16.9%) in comparison to recipients in which the transfer was performed on Day 7 or 8 (47.1% and 45.1% respectively). The apparent decrease in success rate (26.3%) on Day 9 was not significant due to the low number of transfers (19) in that group. While the

Table 8
Effect of the interval between buserelin treatment of the recipient and transfer on embryo transfer (ET) success rate (percentage of crias born); different superscripts within rows indicate significant differences ($P < 0.05$).

I. Single ovulation ET				
Transfer after buserelin	Day 6	Day 7	Day 8	Day 9
Transferred embryos	77	461	9	1
Crias born	14	214	7	0
ET success rate (%)	18.2[a]	46.4[b]	77.8[a,b]	0.0
II. MOET				
Transfer after buserelin	Day 6	Day 7	Day 8	Day 9
Transferred embryos	663	2965	317	18
Crias born	111	1401	140	5
ET success rate (%)	16.7[a]	47.2[b]	44.2[b]	27.7[a,b]

corpus luteum produced in response to exogenous GnRH treatment is similar to that of a corpus luteum induced by natural mating, the corpus luteum does not reach maximal diameter until after Day 7 after mating (Ratto et al., 2006). Plasma progesterone levels in alpacas are likely to be at their highest 7 or 8 days after induction of ovulation (Ratto et al., 2006; Trasorras et al., 2010) and may contribute to higher success rates of embryo transfer in this study on Days 7 and 8. Significantly lower pregnancy rates following embryo transfer in the early stages of the recipient ovarian cycle (Days 5, 5.5 and 6) have also been shown in cattle (Wright, 1981) and sheep (Bari et al., 2003).

The interval in days between ovarian synchrony of donor and recipient females was +1 day in 21 donor-recipient pairs (6 crias born, ET success rate 28.6%), 0 days in 501 pairs (214 crias born, ET success rate 42.7%), −1 day in 3904 pairs (1665 crias born, ET success rate 42.6%) and −2 days in 76 pairs (6 crias born, ET success rate 7.9%). Although there were significant differences between both Day −1 and Day 0 versus Day −2 in embryo transfer success rates the regression analysis did not identify ovarian synchrony as a significant factor (Table 1) most likely caused by the low number of pairs with a difference greater than 24 h. Synchrony of donor and recipient ovarian activity has been described as an important factor in cattle (Peixoto et al., 2007), buffalo (Misra et al., 1999) and camel embryo transfer success (McKinnon et al., 1994), although other studies in cattle showed no or little effect (Wright, 1981; Spell et al., 2001). The synchrony of ovarian activity seems to be less important in alpacas as long as the asynchrony does not exceed 24 h in either direction.

4. Conclusions

Embryo transfer in alpacas can be successfully performed as single ovulation embryo transfer or MOET in the field. The variables of day of flushing, embryo diameter, embryo quality, day of transfer, and the age of the recipient had a significant impact on the outcome measure of embryo transfer success rate (percentage of crias born). Embryo transfer in alpacas is equally successful in all seasons; the heat during summer does not have a negative impact. Both the transfer of embryos of lower quality and the transfer of smaller embryos result in reduced transfer success rates. Optimal days for obtaining embryos from donors are Days 8 and 9 after mating, optimal days for transfer into recipients are Days 7 and 8 after buserelin treatment. Older recipients (>15 years of age) are less likely to become pregnant, and it seems that recipients in poor body condition will have decreased success rates.

Conflict of interest

Jane Vaughan, Monika Mihm-Carmichael and Thomas Wittek do declare that there is no financial or other conflict of interest which could have influenced the results, interpretation or writing of the manuscript.

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