

Embryo transfer in domestic South American camelids[☆]

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ARTICLE INFO

Article history:

Available online 1 November 2012

Keywords:

Alpaca
Llama
Embryo transfer

ABSTRACT

Intraspecific and interspecific embryo transfer in domestic South American camelids is developing into a well-established technique. Reports reveal many benefits of using reproductive biotechnologies to allow rapid propagation of alpacas and llamas of high genetic merit (e.g., high fiber quality, preserve color variation). The objective of this review is to provide up-to-date information about embryo transfer in domestic South American camelids. Specific information is provided on criteria for male selection, donor and recipient synchronization, the practice of single- vs. super-ovulation protocols, embryo recovery and transfer techniques, advances in cryopreservation of embryos, results of intra- and inter-specific transfer, and the future of the embryo transfer in domestic South American camelids.

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1. Introduction

The embryo transfer technique in South American camelids involves embryo collection from a female donor by uterine lavage 7–8 days after breeding, and transfer to a recipient female that has been synchronized with the donor. Embryo transfer in South American camelids offers the potential to take advantage of the reproductive capacity of donors with high genetic merit in domestic herds, and to facilitate preservation and repopulation of endangered wild camelid species. Embryo transfer is also a valuable tool for identifying and eliminating genetic flaws or hereditary defects because it involves rapid proliferation of known genetics and a detailed data-base of parentage.

Through embryo transfer, a donor can produce eight or ten offspring per year instead of just one. Taylor et al. (2000) reported a 66% pregnancy rate in a commercial embryo transfer program in llamas in the United States, and Sumar (2008) reported a pregnancy rate of 40% in an embryo transfer program in alpacas in Peru. Many studies have

been done and studies are on-going to develop of a super-ovulation protocol (Bourke et al., 1992; Correa et al., 1997; Gomez et al., 2002; Vaughan and Tibary, 2006; Huanca et al., 2009). Using a superovulation protocol, Vaughan (2012) reported an average of 2.5–3 embryos per uterine flush; hence a single donor can then produce up to 21 embryos per year.

Finally, the embryo transfer technique enables the preservation of endangered species such as vicuna, guanaco and some breeds of alpacas and llamas with specific characteristics (Del Campo et al., 1995). One interesting method used to preserve valuable species or breeds of llamas and alpacas is to perform interspecies embryo transfer (Taylor et al., 2001; Von Baer et al., 2003; Sumar, 2008). Another alternative to achieve preservation of endangered species of South American camelids is embryo freezing, a technique that is actively being studied (Aller et al., 2002; Lattanzi et al., 2002; Palasz and Adams, 2000; Vasquez et al., 2007, 2011).

2. History of embryo transfer in domestic South American camelids

The first surgical embryo collection and transfer in alpacas was reported 42 years ago in Peru. Novoa and Sumar (1968) reported in alpacas the first collection of

[☆] This paper is part of the special issue entitled: International Conference on Camelid Genetics and Reproductive Biotechnologies, Guest Edited by Gregg P. Adams and Ahmed Tibary.

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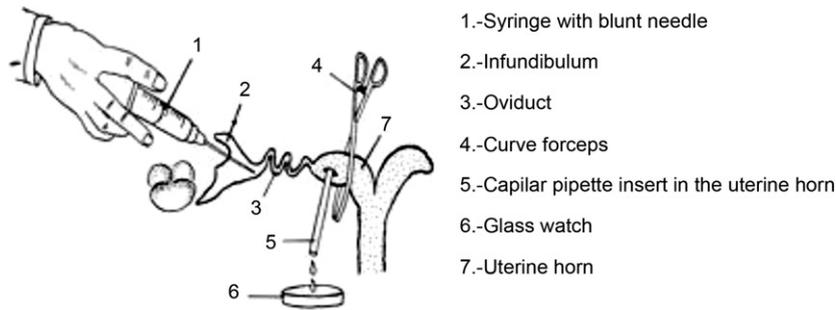


Fig. 1. Method of oviductal flushing at laparotomy for embryo collection in alpacas (Novoa and Sumar, 1968).

zygotes from the oviduct after fertile mating. At Day 3 (Day 1 = day of mating), the embryos were flushed with Ringer solution from the fimbriated end of the oviduct to the uterus (ante grade surgical flush), similar to the procedure used by Smith and Murphy (1987) in ewes. A tight sphincter at the uterotubal junction makes it impossible to flush liquids from the uterus to the oviducts in camelids, embryos were recovered from 80% of single-ovulating females by placing a pipette through an incision in the uterine wall, and clamping the uterine bifurcation with forceps (Fig. 1). Embryos at the 2, 4, 8-cells and morula stages were recovered. In another study (Sumar and Franco, 1974), superovulation was induced in 8 donor alpacas using 750 IU of eCG followed by 1000 U of hCG. Fifty six embryos were collected from the oviducts by laparotomy 3 days after mating and 44 morulae were transferred into the oviducts of 44 recipients by laparotomy, resulting in 4 pregnancies and one live-born cria.

The first llama born by non-surgical collection and transfer technique was reported by Wiepz and Chapman (1985) in the United States. In this study, synchronization and ovulation of the recipient was induced by GnRH, and collection and transfer, of hatched blastocysts was done 7 days after GnRH treatment. One cria was born after transfer of two embryos to two synchronized females. Bourke et al. (1991) reported that, in llamas, superovulation can be achieved using eCG, and embryos can be collected non-surgically. Gatica et al. (1994) reported a successful pregnancy through embryo transfer in a llama in Chile. Excellent reviews about reproductive technologies in domestic South American camelids, have been published previously (Pugh and Montes, 1994; Del Campo et al., 1995; Tibary et al., 2005; Miragaya et al., 2006; Vaughan and Tibary, 2006).

3. Selection of males, donors, and recipients

Breeders should emphasize the use of high ranking sires, free of lesions of the external and internal genitalia, as detected by palpation and ultrasonography. The cost of embryo transfer is high and will not pay dividends on offspring unless they are from the meritorious females and males of the breed. Females selected as donors for the embryo transfer should be well above the breed and herd average in genetic value. They should be of good conformation and free of heritable defects. Females with a history of reproductive problems do not make good donor animals;

- 1.-Syringe with blunt needle
- 2.-Infundibulum
- 3.-Oviduct
- 4.-Curve forceps
- 5.-Capilar pipette insert in the uterine horn
- 6.-Glass watch
- 7.-Uterine horn

therefore, females with a history of producing live crias are preferable. Donors are further evaluated by transrectal palpation, ultrasonography and vaginoscopy to identify potential congenital or acquired abnormalities of the internal genitalia.

Two to three recipients for each donor animal are generally required in single embryo collection programs (i.e., without superovulation). The number of recipients required for multiple ovulation embryo transfer programs has not yet been established. Recipients should be well-grown, between 2 and 8 years of age, and have no history of reproductive failure. The same clinical approach used for donors should be implemented for recipients. Other than systemic health, recipients may be selected irrespective of undesirable heritable or congenital defects (e.g., blue eyes, multicolor or coarse fiber, prognathism), and should be ≥ 30 days post-partum. In a recent study in alpacas (Sumar et al., 2010), the pregnancy rate was higher ($P < 0.001$) for non-lactating (44%) than lactating (18.2%) recipients. An ideal recipient at the time of the transfer will have a CL ≥ 12 mm, soft uterine tone, a closed cervix, no vaginal or vulvar exudates, and a body condition score of ≥ 3 (out of 5).

4. Time of embryo entry into the uterus

In early studies, flushing the oviducts 3 days after mating resulted in the collection of embryos from two blastomeres to the morula stage (Novoa and Sumar, 1968). In alpacas slaughtered at 4, 7 and 10 days after copulation, respectively, Bravo et al. (1996) reported the collection of ova at the morula stage and compacted morulae by 7 days after mating from the oviducts, and blastocysts from the uterus at 10 days. Flushing of the excised reproductive tract of alpacas after slaughter at 2–15 days after mating ($n = 3-4$ each day) resulted in the collection of 2–4 cell embryos on Day 2, 4–8 cell embryos on Day 3, early morulae on Day 4, compact morulae and early blastocysts on Day 5, ovoid to tubular blastocysts on Day 10, and filamentous blastocysts on Days 11–15, but for unknown reasons, no embryos were recovered from alpacas on Days 6–9 after mating (Cardenas, 1997). Authors of a recent study (Cervantes et al., 2008) reported that the alpaca embryo (hatched blastocyst) does not enter the uterus until 6 days after mating and it develops very rapidly to almost double in diameter from Days 6 to 7 after mating.

Results of embryo transfer studies are consistent with studies of excised tracts; i.e., embryos enter the uterus

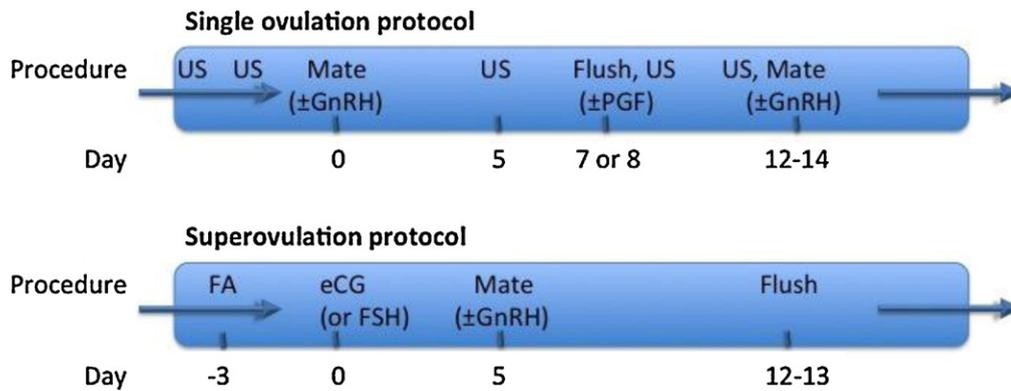


Fig. 2. Single ovulation vs. superovulation protocols used for embryo production in domestic South American camelids.

6–6.5 days after mating at the hatched blastocyst stage (Bourke et al., 1991; Del Campo et al., 1995). In a llama study (Taylor et al., 2000), embryos were collected from 55%, 79%, and 100% of donors at 7, 8 and 10 days after mating, respectively; all were at the hatched blastocyst stage.

5. Single ovulation vs. superovulation protocols

There are two basic strategies for producing embryos for transfer (Fig. 2). The simplest involves collection after a single ovulation, with no ovarian superstimulatory treatment. Daily transrectal ultrasonography has been used to verify the presence of a viable, mature dominant follicle (i.e., between 7 and 12 mm) at the time of mating (Day 0). Some recommend administration of GnRH immediately after mating to ensure ovulation (Bourke et al., 1991; Del Campo et al., 1995). Transrectal ultrasonography may be done on Day 5 after mating to confirm the presence of a CL (i.e., that ovulation occurred). Alternatively, confirmation of ovulation may be done by ultrasonographic examination at the time of flushing. Embryo collection is carried out ≥ 7 days after mating, when embryos are generally in the expanded blastocyst stage (Ratto and Adams, 2007). Embryo collection rate from the uterus is low at Day 6, inconsistent at Day 6.5 or 7, and optimal at ≥ 7.5 days after mating (Sumar, 2008). Although, the embryo collection rate at 8 days after mating is very high, the embryos are usually very fragile because they are large and beginning to elongate (Taylor et al., 2000). Donors may be given prostaglandin after uterine flushing to ensure luteolysis and receptivity by the time of the next mating and collection cycle, although luteolysis will normally be complete by that time without treatment. Since ovarian follicular development and ovulatory capability are present year-around in healthy, well-nourished alpacas (Fernandez-Baca et al., 1972; Sumar et al., 2010), a donor may be induced to ovulate every 12–14 days using a single-ovulation protocol. A single embryo may be expected from about 85% of these flushes (Sumar, 2008), and 12–18 embryos per donor per year have been produced using the continuous single-ovulation technique (Taylor et al., 2000; Sumar, 2008).

The second approach involves superovulation. A review of the topic is provided in this publication by Ratto et al.

and will not be recapitulated here. Briefly, the response to superovulatory treatment protocols in South American camelids may be characterized as extremely variable and unpredictable. Although critical studies are sparse, perhaps the greatest variability is a consequence of initiating superstimulatory treatment irrespective of the status of the follicular wave. The dominant follicle suppresses the subordinate follicles within the same wave (Adams et al., 1990), an effect that has been shown to dramatically affect the superovulatory response in cattle (Mapletoft et al., 2007) and in camelids (Ratto et al., 1997). Protocols to electively induce follicular wave emergence so that superstimulatory treatment may be initiated at that time have been attempted using a combination of estrogen and progesterone, GnRH or LH, and transvaginal ultrasound-guided follicle ablation (Ratto et al., 2003; Ratto and Adams, 2007; Huanca et al., 2009). In this regard, results of recent studies in the author's laboratory (unpublished) suggest that the superovulatory response is improved by initiating superstimulatory treatment (eCG) 2 or 3 days after manual rupture of the dominant follicle (via rectal palpation) (Fig. 3). Many issues remain to be addressed to optimize the superovulatory response in domestic South American camelids, including species (alpaca vs. llama), breed (huacaya vs. suri), age (yearlings vs. adults), lactational state, body condition, environmental conditions, and hormone preparations and protocols. Furthermore, there is no information in domestic South American camelids about the optimal time interval between successive superovulatory cycles. Cattle may be superovulated at 2-month intervals for three treatments without an appreciable decrease in embryo recovery (Farin et al., 2007).

6. Embryo collection

The non-surgical method for collection of embryos is used most widely because it is non-invasive and does not require specialized equipment. Avoiding surgical intervention eliminates complications caused by adhesions and scarring. Ultrasonography has also been implemented in the non-surgical method to monitor follicular activity and diagnose pregnancy. The anatomical characteristics of the cervix (three irregular annular or spiral folds) and rectum

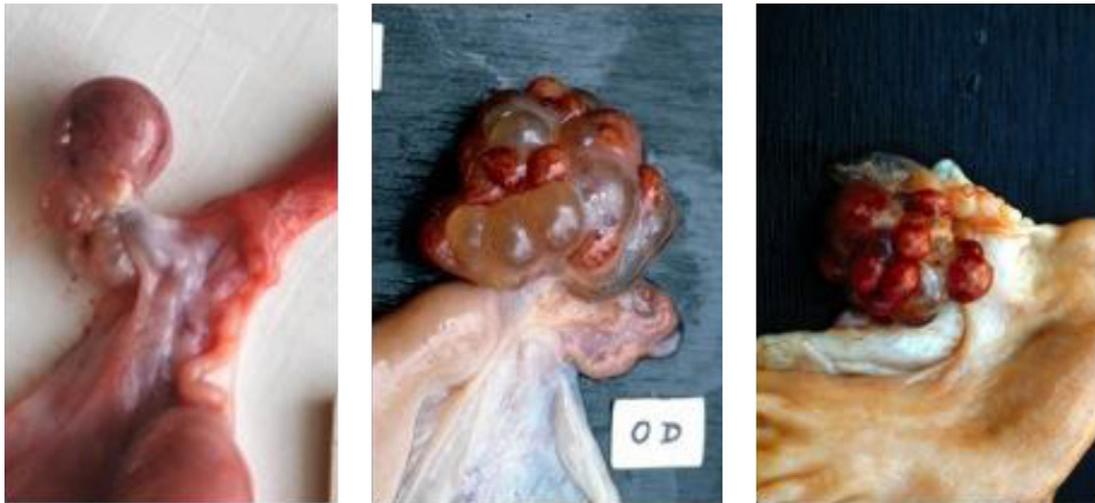


Fig. 3. Ovaries of alpacas induced to ovulate a single follicle (left) or multiple follicles (middle and right). Photographs were taken 8 days after mating/GnRH. Superstimulation was induced with 800 IU eCG. Ablation of the dominant follicle prior to superstimulatory treatment appeared to induce a more uniform ovulatory response (right) compared with no ablation (middle, showing a large number of large non-ovulated follicles).

permit relatively easy canulation of the cervix during both the estrogen-dominant and progesterone-dominant phases.

6.1. Materials for embryo collection

The materials for embryo collection consist of:

- i. A two-way Foley catheter (14–18 FR).
- ii. A stylet 6.2 in. length with a clip to hold it within the catheter.
- iii. A sanitary-plastic bag to prevent any possible contamination during the passage of the catheter through the vulva and the first portion of the vagina.
- iv. A 10 ml syringe with sterile distilled water or saline to fill the Foley catheter balloon.
- v. An embryo collection filter placed on a graduated container. The container allows the collector to measure the volume of flushing media recovered from the uterus.
- vi. Exit and entry tubes for flushing medium. The author suggests reducing the length of commercially available hoses to reduce the risk of losing the embryos during passage through the tubes.
- vii. Petri dishes into which the contents of the embryo filter are emptied to search for the embryo.
- viii. A pipette or syringe to rinse the filter in case the embryo is attached to the wall of the filter.

6.2. Donor restraint and uterine flushing

Collection of embryos is done by maintaining the donor in sternal recumbency (Fig. 4). The donor may be sedated (Acepromazine, 0.02–0.05 mg/kg, IM, Boeringer-Ingelheim, Germany; Promazil, Lab Chile, Santiago, Chile) and given caudal epidural anesthesia (Lidocaine 2%, 1 ml/100 kg body weight; United Lab SA, USA). The tail is

wrapped with an elastic bandage and fixed to the dorsum with a clip to minimize contamination of the vulva. For the purposes of transrectal manipulation of the reproductive tract during embryo collection and transfer in alpacas, the ideal circumference of the operator's hand is ≤ 20 cm (Fig. 4). Soft latex examination gloves and generous amounts of lubricant are recommended for transrectal manipulation. When introducing the hand into the rectum, the operator must gently rotate his/her hand during passage through the anal sphincter. Feces are removed from the rectum and the perineal area is cleaned with soap and water, and rinsed with 70% ethanol.

With the help of an assistant, the vulvar lips are opened so that the operator can introduce the uterine lavage catheter. The catheter is directed toward the dorsum of the vagina, after which the plastic cover that protects the catheter tip is pulled back. Once the catheter is within the vagina, the operator places a pre-lubricated hand into the rectum of the donor and guides the catheter through the cervix and into the uterine body. The entire uterus may be flushed at once; hence, no attempt is made to catheterize an individual uterine horn. The stylet is withdrawn slightly so that the tip is caudal to the balloon of the Foley catheter. The balloon is then inflated with 5–10 ml of distilled water or uterine flush medium just cranial to the interval cervical os to secure the catheter within the body of the uterus. The stylet is removed from the catheter and a Y-junction is fitted to the Foley catheter to connect a bag of warm flush medium. With the help of assistant controlling thumb-clamps on the tubing, the operator guides the filling and emptying of the uterus flush medium, being careful to avoid overfilling of the uterus. The uterus is flushed 3 times, with an interval of at least 30 s between each lavage. Each lavage involves gentle massage of the uterine horns to evacuate the fluid. At the end of the third uterine lavage, the balloon is deflated, the catheter is removed, and its contents carefully flushed into the embryo filter. After uterine flushing,

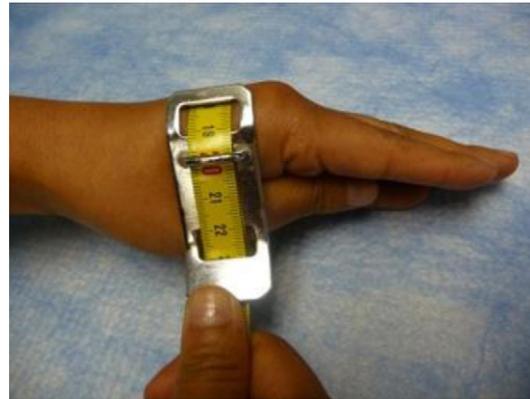


Fig. 4. Collection and transfer room: two chutes with one operator in each and an assistant to record ultrasound findings. The two windows in the back wall permit controlled access to the embryo manipulation laboratory, isolated from the collection room. The ideal circumference of the operator's hand for rectal palpation in alpacas is ≤ 20 cm.

the donor may be given a luteolytic dose of prostaglandin to prevent unwanted pregnancy in the donor in the event that the embryo is not collected during flush.

7. Embryo evaluation and handling

The contents of the filter are poured into a petri dish, and the filter is rinsed with flush medium using a syringe. If the content of the filter is abundant and/or cloudy, a portion of the flush may be placed in a second petri dish. Embryos are located and evaluated using a stereo-microscope, according to the recommendations of [Springfellow and Siedel \(1990\)](#). Embryos collected ≥ 7.5 days after mating range in size from 0.5 to 1.0 mm, and are usually found in the expanded blastocyst stage. In another study, the average embryo diameter of llama and alpaca were similar at 8 days after mating: $527.1 \pm 168.0 \mu\text{m}$ ($n=6$) and $534 \pm 151.4 \mu\text{m}$ ($n=8$), respectively. There is, however, considerable variation in the size of embryos collected among and within donors ([Vasquez et al., 2007](#)).

A tuberculin syringe attached to a pipette tip is used for embryo handling, and care is taken to aspirate a small amount of medium before contacting the embryo to minimize sticking and contact with air. Washing medium (Hyclone, DPBS/Modified + Embiotic III) is placed in separate dishes before the embryo is removed from the search dish. The embryo is placed in the washing dish after introducing the pipette tip into the medium, to prevent the loss of the embryo and air contact. Morphologic characteristics of embryo quality in South American camelids have not been critically investigated, so the classification system for bovine embryos published by the International Embryo Transfer Society is used at present ([Springfellow and Siedel, 1990](#)). Good quality embryos for embryo transfer are identified and washed several times in the flush medium, enriched with fetal calf serum or in a maintenance commercial medium (Vigro Holding medium, Bioniche, WA, USA), to remove cervical mucus, debris, and bacterial contamination. Washed embryos are placed in a small petri dish, at 37°C in the flushing medium supplemented with 3% BSA, until loaded into a transfer straw.

8. Non-surgical transfer to recipients

8.1. Loading transfer straws

The petri dish containing the embryo is placed under the stereoscope to load in a 0.25 ml transfer straw. Using a tuberculin syringe attached to the cotton-filled end of the straw, succeeding columns of medium and air are drawn into the straw as follows: medium, air bubble, medium, air bubble, medium with the embryo, air bubble, and medium ([Fig. 5](#)). Care should be taken to ensure that the first column of medium contacts the plug of cotton and polyvinyl alcohol to form a water-proof seal. The straw is then loaded into a transfer gun and covered with a transfer sheath.

In a fashion similar to that described for uterine flushing, the transfer gun is introduced into the vagina of recipient. The tip of the transfer gun is guided through the cervix by transrectal manipulation and positioned into the uterine horn. Some authors recommend placement of the embryo in the left uterine horn regardless of the side of the CL, left or right ([Picha et al., 2010](#)), while others recommend placement in the horn ipsilateral to the corpus luteum ([Bourke et al., 1995](#); [Trasorras et al., 2010](#)). Manipulation of the reproductive tract is minimized to avoid trauma and inflammation of the endometrium. After expelling the contents of the straw, the transfer gun is withdrawn slowly and the tip of the gun is checked to confirm that the cotton plug effectively expelled the contents and the contents did not leak between the straw and sheath.

8.2. Recipient synchronization and pregnancy diagnosis

Using a similar examination and treatment schedule as for single-ovulating donors ([Fig. 2](#)), recipients are examined ultrasonographically to confirm the presence of a growing dominant follicle between 7 and 12 mm, and ovulation is induced by administering GnRH. Ovulation may be confirmed by ultrasonography on Day 5 after GnRH, or at the time of embryo collection on Day ≥ 7 . No reports have been published on the degree of synchrony required between donors and recipients for optimal embryo survival.



Fig. 5. Schematic drawing of a loaded embryo transfer straw.

in domestic South American camelids. At present, the goal is to have the recipient ovulate within ± 1 day of the donor. To minimize the luteolytic effects of uterine inflammation resulting from manipulations, a prostaglandin synthase inhibitor (Flunixin meglumine) may be administered to recipients 30–60 min before embryo transfer. Nervous recipients may be tranquilized before transfer and epidural anesthesia may be induced, as described for donors. The tail is wrapped and tied out of the way, feces are removed from the rectum, and the perineal area is cleaned as previously described. Pregnancy diagnosis may be done by transrectal ultrasonography by 12–13 days after donor mating (presence of an embryonic vesicle), and confirmed by detection of an embryonic heartbeat at ≥ 25 days after donor mating (reviewed in Adams and Domínguez, 2007).

9. Cryopreservation of embryos

Cryopreservation of embryos offers several important logistical and economic advantages. It permits preservation of embryos in excess of the number of available recipients, and it facilitates national and international movement of embryos. Two methods of cryopreservation have been attempted in South American camelids, conventional slow-freezing and vitrification.

9.1. Conventional “equilibrium” or slow freezing method

Very few studies are reported about alpaca or llama frozen by conventional methods. Palasz and Adams (2000) studied the exposure of llama trophoblastic vesicles (13 days) to permeating cryoprotectant, exposing to a 10% solution of ethylene glycol, propylene glycol, or glycerol supplemented with 10% fetal calf serum or 0.1% sodium hyaluronate at 22 °C for 15 min. Cryoprotectant was removed with 0.5M sucrose solution. There was no effect of supplement or method of cryoprotectant removal on trophoblastic vesicle survival in culture. There were no differences in trophoblastic vesicle survival after 24 h in control groups and those exposed to ethylene glycol or propylene glycol. The embryos frozen in ethylene glycol progressively expanded to 90% of original volume in culture, but none of the embryos frozen in propylene glycol re-expanded in culture.

9.2. Vitrification

Vitrification is a rapid process that consists of dehydration of the embryo at room temperature by a very highly concentrated vitrification media and a very rapid freeze that avoids the formation of ice crystals, allowing the solution to change from a liquid to a glassy state. A low toxicity vitrification solution consists of three cryoprotective agents (Vajta, 2000; Kassai, 1996). In a comparison of the slow freezing method and vitrification, Lattanzi et al. (2002) tested the viability of hatched

blastocyst using two methods: vitrification and slow freezing. Embryo viability was tested by culture in SOFaa + BSA (bovine serum albumin) medium. After 48 h of culture, re-expansion of vitrified and slow-frozen embryos was 54% and 57%, respectively, not significantly different from those frozen using the conventional method. Another study was designed to determine the effect of vitrification by open pull straw (OPS) on the morphology and survival of llama hatched blastocysts (Von Baer and Del Campo, 2002). Embryos ranging from 300 μm to 800 μm were exposed in one step to high 40% ethylene glycol and submerged directly into liquid nitrogen. Results showed that re-expansion of embryos after thawing was acceptable, but no pregnancies were obtained. Some of the embryos that survived freezing were examined by transmission electron microscope (TEM), revealing a high lipid content in the cytoplasm of llama oocytes and embryos may contribute to low survival after vitrification. With expanded llama blastocyst, Aller et al. (2002) reported 50% of pregnancy in recipients receiving vitrified embryos (2/4) and 33.3% (2/6), in those receiving fresh embryos.

10. Interspecies embryo transfer

The first two reports of interspecies transfer in South American camelids were published by Taylor et al. (2001) and von Baer (personal communication). In the first case, a llama gives birth to an alpaca cria. In the second case, a llama gives birth to a guanaco cria. In a more recent study (Sumar, 2008), embryos were recovered from alpacas and llamas by non-surgical flush 7.5 days post-mating and transferred to recipients of the opposite species. In llama recipients with alpaca embryos, 4/7 (57%) were detected pregnant at 15, 25 and 35 days after donor mating, but one aborted at 6 months of pregnancy. Three healthy alpaca crias were born from llamas (Fig. 6), with an average body weight of 10.5 kg; i.e., 3.5 kg more than that of alpaca crias born from alpacas (7.0 kg). At 6 months of age, the alpaca crias born to llama recipients gained 12 kg more than their counterparts born to alpacas, but body weights were similar by 1 year of age. In alpaca recipients with llama embryos, 3 of 6 (50%) alpacas were detected pregnant at 15, 30, and 45 days after donor mating, but one alpaca aborted at 4.5 months of pregnancy. The body weight of the 2 llama crias born to alpacas was about 8.0 kg (Fig. 6).

11. Future of the embryo transfer in alpacas and llamas

Examples of the potential application of embryo transfer in llamas and alpacas are: (1) embryos from valuable females could be transferred to less valuable recipients, thus freeing the valuable animal for the production of more embryos than would otherwise be possible (Fig. 6), (2) acquired infertility problems in valuable animals may be by-passed by embryo transfer, (3) preservation of



Fig. 6. Top (from left to right): Elite breeding male alpaca, elite embryo donor alpaca, embryo recipient alpaca, and an elite cria from the recipient. Middle: Three llama recipients with alpaca crias. The cria in the center is of the Huacaya breed, and the other two are of the Suri breed. Bottom: Alpaca recipient with a llama cria.

endangered camelid species (vicuña and guanaco), and (4) genetic improvement may be accelerated in South American countries that have suffered genetic erosion in the last 30 years through indiscriminate exportation.

Despite the commercial potential of embryo transfer in domestic South American camelids, the technology has not been critically and systematically studied in these species. In addition research is needed regarding the mechanisms involved in the time of entry of the embryo to the uterus, embryo migration to the left uterine horn, the nature of embryonic loss, and the loss of twin pregnancies. Development of in vitro embryo production, embryo splitting, gene transfer, and embryo sexing early will all depend on successful development of the embryo transfer technique.

Conflict of interest

No conflict of interest.

Acknowledgement

I am grateful to Dr. Gregg Adams for the critical reading of the manuscript.

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