Small Ruminant Research 132 (2015) 99–102

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Short communication

Birth of live llama (*Lama glama*) derived from embryo transfer storage at 5 °C for 24 h

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ARTICLE INFO

Article history:
Received 5 March 2015
Received in revised form 3 October 2015
Accepted 5 October 2015
Available online 22 October 2015

Keywords:
Llama
Superovulation
Refrigeration
Embryo transfer
Live birth

ABSTRACT

The objective of the present work was to evaluate the efficacy of maintaining llama embryos metabolically inhibited but viable at 5 °C during 24 h. Adult females ($n = 20$) were used as embryo donors and were superstimulated with eCG. A total 61 embryos were non-surgically collected from 16 of the 20 llamas flushed at Day 7 after mating. Fourteen excellent hatched blastocysts were stored in sterile test tubes containing 1.5 ml of TCM 199-Hepes + 10% cow serum (v/v concentration) + antibiotics and cooled to 5 °C (cooling rate = 0.5 °C/min) and maintained at 5 °C during 24 h. After this, the embryos were warmed on warming plate (38 °C), washed three times and loaded individually into 0.25 ml French straws. Fourteen adult llamas were used as recipients and embryos were transferred only into the left uterine horn of the recipient regardless of side ovulation. All recipient llamas were pregnancy tested by transrectal ultrasonography at 45 days after transfer. Three (21.5%) pregnant females were detected and delivered three normal healthy male calves at term. To our knowledge, these offspring are the first llamas produced following transfer of refrigerated embryos. In summary, llama embryos can be cooled to 5 °C and maintained in storage for up to 24 h without significant losses of viability.

1. Introduction

The South American Camelids (SAC) are also known as the New World Camelidae and usually considered to be seasonal breeders and induced ovulators (Novoa, 1970). In their natural habitat, the breeding season normally occurs during the wet, warmer months when the forage is most abundant (San-Martin et al., 1968). In the South American countries exists programs for genetic improvement based on reproductive technologies, such as, artificial insemination with fresh semen and embryo transfer. The transfer of llama embryos generally consists of immediate, on-farm transfer of fresh embryos into closely synchronized llamas.

Cryopreservation of gametes and embryos and other related modern reproductive technologies offer many advantages to commercial animal production. Among the benefits of cryopreservation is the simplified exchange of genetic material between geographically separated groups.

There have been few attempts at cryopreservation of llama embryos (Palasz et al., 2000; Lattanzi et al., 2002; Vazquez et al., 2011); however, more than a decade ago, two pregnancies following transfer of eight vitrified embryos into four recipients were reported (Aller et al., 2002) and the same vitrification method was successfully replicated in dromedary camel (Skidmore et al., 2005). However, Nowshari et al. (2005) reported a successful transfer of vitrified embryo resulting in the birth of an offspring in camel (*Camelus dromedarius*).

At present, as an alternative to conventional freezing procedures and vitrification, the refrigeration embryos facilitates the domestic and perhaps the international trade of embryos between neighboring Andean countries. Embryos can be transported within a country or between countries with the same health status. The donor females should be held in isolation period (quarantine) before superovulation, embryo collection and shipment of the embryos to another country. This exchange of genetics may be very beneficial to the Andean countries and allows for a greater genetic diversity

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http://dx.doi.org/10.1016/j.smallrumres.2015.10.007
0921-4488/

within a group and for control of genetic traits. To commercial purposes the embryos should be transferred with zona pellucida to prevent health risk. However, Sutmoller (1999), established that if favourable epidemiological or ecological conditions exist in the region of origin of embryos, the risk of contamination of a batch of llama hatched embryos with the infectious agents (foot and mouth disease, vesicular stomatitis, bluetongue, tuberculosis) is close to zero. In addition, the risk of contamination with Mycobacterium or Brucella is very low when the results or diagnostic test of the herd and donor animals before and after collection of the embryos are negative.

Von Baer et al. (2002) reported only one pregnancy following transfer of three refrigerated embryos during 12 h, but no live birth was reported by these authors. To our knowledge, there are no reports of birth of an offspring in this species following transfer of refrigerated embryos. Therefore, the present work was undertaken to evaluate the efficacy of maintaining llama embryos metabolically inhibited but viable at 5 °C during 24 h.

2. Materials and methods

This work was carried out at Abra Pampa Experimental Station of Altitude of the National Institute of Agricultural Technology (22°49′S, 65°47′W) that is located in the High Andean plateau of north-west Argentina, in the Jujuy province (3484 m above sea level), termed the "dry puna", during non-reproductive spring season. The location is a natural dry grassland of "chillagua" (*Festuca scirpifolia*) crossed by a narrow river. Summer is the wet season in the puna, with about of the annual rainfall occurring between December and March. The annual rainfall ranges 250–350 mm. There is no season free from freezing. The average annual temperature is between 0 °C and 6 °C. In the dry winter season the temperature can drop to −25 °C; however, in the summer season the maximum temperature can be 25 °C. Animals were in good physical condition, each weighing approximately 100 kg and were kept on native pasture and water was provided *ad libitum*.

Adult females ($n = 20$) were used as embryo donors. An intravaginal device (DIB 0.5g®, Syntex, Argentina or Cronipres M15®, Biogenesis Bago, Argentina) containing 0.5 g of vegetal progesterone was inserted into the vagina to control the ovarian dynamics during 7 days and the ovarian follicle development was stimulated by administration of 1000 IU of equine chorionic gonadotropin (eCG) (Day 3). At Day 9, donor females were mated with males of proven fertility and treated with 100 μg of GnRH analogue of gonadorelin (Gonasyn®, Syntex, Argentina) as an additional stimulus to induce ovulation. A second mating was allowed 24 h later. Seven days after first mating the uteri of the donor llamas were non-surgically flushed by transcervical *via* using 14-Fr Rusch two way catheter and 250 ml of Ringer lactate supplemented with 1% cow serum (previously heat-treated and filtered by 0.22 μm). The flushing medium was filtered (EmCon) and searching embryos was performed using stereomicroscope at magnification 40×. The recovered embryos were transferred to holding medium (Syngro holding medium®, Bioniche Animal Health, Canada) and kept at room temperature and classified according to IETS standards for cattle. After embryo collection, ovarian response in the donor females was monitored by transrectal ultrasonography using a real time scanner with a 5-MHz linear array transducer (Honda HS 101 V, Japan) to determine the number of anovulatory follicles and corpora lutea (CL). Fourteen excellent hatched blastocysts (400–600 μm in diameter) were immediately stored in sterile test tubes containing 1.5 ml of TCM 199-Hepes (N°L 4021-500, Microvet Argentina) + 10% cow serum (v/v concentration) + antibiotics and cooled to 5 °C (cooling rate = 0.5 °C/min) and maintained at 5 °C during 24 h. Embryos were recovered from the test tubes and morphologically assessed at 40× magnification. After this, the embryos were warmed on warming plate (38 °C), washed three times and individually loaded into 0.25 ml French straws. Fourteen adult llamas were used as recipients. The ovulation and stage of follicular wave were synchronized using GnRH analogue administered intramuscularly at the same time of donor females. A cattle embryo transfer device (Minitub, Germany) was used for transfer and the embryos were transferred (one embryo per recipient) only into the left uterine horn of the recipient regardless of side ovulation. All recipient llamas were pregnancy tested by transrectal ultrasonography at 45 days after transfer and those diagnosed as pregnant were again pregnancy tested by transrectal palpation and ultrasound scanning after four weeks.

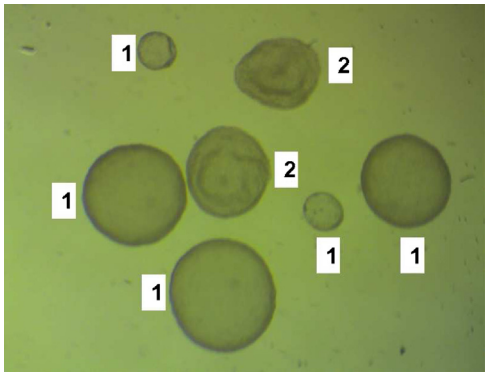

Fig. 1. Five Grade 1 (excellent) embryos and two Grade 2 (moderate to good) embryos.

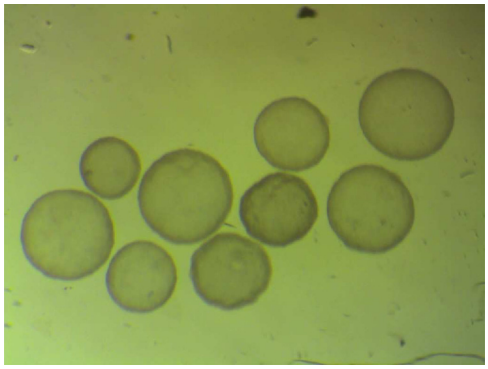

Fig. 2. Llama hatched blastocysts (Day 7) after refrigeration at 5 °C for 24 h.

3. Results

All llamas were sexually receptive and successfully mated. A total 61 embryos were recovered from 16 of the 20 llamas flushed, which indicate that approximately a fifth (20%) of the animals did not respond to the superovulatory treatment producing embryos. All recovered embryos were hatched blastocysts and were graded as excellent ($n = 52$) and moderate to good ($n = 9$) quality (Fig. 1). The numbers (mean ± SD) of follicles ≥7 mm, CL and recovered embryos were 2.9 ± 2.9, 4.5 ± 2.1 and 3.0 ± 2.8 on the total treated females. The number of recovered embryos per flush ranged from zero to ten. The pregnancy rate was 21.5% (3/14) for recipients that had received refrigerated embryos (Fig. 2) and three males live llamas were delivered (358, 365 and 366 days of gestation) (Fig. 3).

4. Discussion

This work provides the first published information on the birth of three offspring following transfer of refrigerated llama embryos