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Embryo production by superovulation and dual siring in alpacas (*Vicugna pacos*)

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ABSTRACT

Alpacas can only produce one offspring per year. In order to accelerate the genetic gain of a herd, superovulation and embryo transfer can be used to produce multiple embryos from superior females. We hypothesized that the use of dual siring with superovulation would result in the production of multiple embryos sired by different males. After administration of the superovulation protocol, receptive females were bred to two proven males (A and B) 8–12 h apart and ovulation was induced by gonadotropin at the time of the first breeding. Growth of multiple dominant follicles was successfully achieved in 95% of cycles. Females that were receptive after FSH treatment and were bred with both males (order A–B or B–A). Embryo collections were performed 8–9 days post-breeding on 15 cycles and 73% of collections recovered ≥1 embryo. A total of 46 embryos, were recovered for an average of 3.13 ± 3.1 (range 0–10) embryos/flush. Parentage analysis was performed for 23 embryos (6 from A to B, 17 from B to A). Twenty-two of the 23 embryos were determined to be sired by male B, being six embryos from breeding A-B and 16 embryos from breeding B-A. A single embryo from breeding B-A was sired by male A. In conclusion, FSH administered at decreasing doses can be used to promote superovulation resulting in collection of multiple embryos per cycle. However, slight differences in male fertility may affect the frequency of embryos sired by each male.

1. Introduction

South American camelids have a gestation length of approximately 340 days, and therefore females can only produce one offspring per year with regards to natural breeding (Vaughan et al., 2013). In order to accelerate the genetic gain of a herd, assisted reproductive techniques (ART) such as embryo transfer (ET), can be used to produce multiple offspring from genetically valuable females every year (Trasorrasa et al., 2013). During ET, an embryo is harvested and transferred into surrogate females that carry the pregnancy to term. In many species, such as bovine and equine, ART has been mainly used for two reasons: 1) for rapid multiplication of certain genetics lines, and 2) to produce offspring from animals that otherwise would be infertile (Rader et al., 2016; Phillips and Jahnke, 2016). In New World camelids, the advance of ART has been very slow due to the lack of financial support for research and breeding registry regulations. Currently, very few veterinary practices around the world perform ET in alpacas, and almost all are located in South America and South West Pacific (Australia and New Zealand).

Embryo transfer has been successfully performed in alpacas by applying similar techniques used in other species including cattle and sheep (Ratto et al., 2013). Despite the lack of a standardized protocol for ET in alpacas and low success rates, very few prospective studies have been performed on ET in alpacas (Vaughan et al., 2013). Moreover, the few veterinary practices performing ET do not publish their procedures and/or results frequently, resulting in a scarcity of information in the literature from a commercial standpoint.

Superovulation (SO) is one strategy that has been used in association with ET to increase the number of embryos produced from a valuable female per cycle (Ratto et al., 2013). SO and ET (SOET) have been extensively studied in bovine and efficient methods to induce multiple ovulations and the production of multiple embryos have been established (Hasler, 2014). In camelids, there have been limited studies on SOET and to this date, there is no consensus on an effective

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treatment protocol. As a result, most protocols used for SOET in camelids have been adapted from bovine species (Huanca et al., 2009a).

In an attempt to increase the genetic diversity of embryos produced from a single SOET cycle, females can be bred with different males during estrus, a procedure termed dual siring. This technique has been used in many species including swine, rabbits, chickens, cattle, and most recently in canines (Flowers et al., 2016; Berger, 1995). In bulls, rabbits, and chickens, dual siring has been used experimentally to assess differences in fertility between two males (Flint et al., 2003; Parrish and Foote, 1985; Tajima et al., 1989). In dogs however, the use of dual siring has been aimed at the production of puppies from different males within the same litter. Currently, there is no published information on the use of dual siring in combination with SOET in alpacas. The use of dual siring could be very advantageous to the alpaca industry by allowing production of multiple genetically diverse embryos during a single cycle. Therefore, it was hypothesized that the use of SOET and dual siring would result in the production of multiple embryos from different sires in the same cycle. The objectives of this study were to determine the effects of: 1) decreasing doses of FSH on alpaca superovulation success, 2) the use of dual siring on the paternity rates of embryos produced by SOET and 3) time of breeding (first vs second) on the frequency of paternity.

2. Materials and methods

2.1. Animals

Twenty-one female Huacaya alpacas (50–80 kg), ranging from 3 to 15 years old, and two sexually mature Huacaya males (male A and male B, ages 7 and 4 years old, respectively) of good fertility were used during this study. Fertility was determined in a previous study by mating individual males to single ovulating females. Males were judged to have similar fertility based on embryo collection rates between 6 to 8 days post-mating. Male A had a 49% recovery rate and male B had a 43% recover rate after a single breeding (Coutinho da Silva et al., 2012). Females were group-housed in stalls while the males were housed individually. All procedures were previously approved by the IACUC at The Ohio State University (2011A00000039; 4/08/2011).

2.2. Management of females and superovulation

Each morning, females were teased by a male alpaca, known to have high libido, who was allowed to mount the females for approximately 5 min per pen. Receptivity was determined when the female assumed sternal recumbency (cushed position) and allowed the male to mount; however, breeding was not allowed at that time. Transrectal ultrasonography (US) utilizing a linear 7.5 MHz prostate transducer (UST-660-7.5, Aloka, Tokyo, Japan) was performed on receptive females to evaluate follicular activity and ovulation. During US evaluations, digital still images and videos were recorded, and the size and number of all follicles as well as the time and number of ovulations were recorded. Receptive females displaying a dominant follicle ≥7 mm in diameter were enrolled in the study and received gonadotropin, either 1000 IU hCG IV (Chorulon, 1000 IU/mL, Merck Animal Health, Millsboro, DE) or 100 ug GnRH IM (Cystorelin, IM, Merial, Duluth, GA), at the time of US evaluation (day 0; Fig. 1) to induce ovulation of the dominant follicle and emergence of a new follicular wave. Females were then monitored twice daily by US to confirm ovulation of the dominant follicle.

Table 1
Schedule of pFSH treatment.

Day	Period	Dose (mg)
2	PM	50
3	AM/PM	50/40
4	AM/PM	40/30
5	AM/PM	30/20
6	AM/PM	20/20
7	AM/PM	20/20
8	AM/PM	20/20
9	AM	20

Approximately 60 h after induction of ovulation, treatment with porcine FSH (pFSH, Folltropin, 20 mg/mL, Bioniche, Montreal, Canada) was initiated to induce growth of multiple pre-ovulatory follicles. pFSH was administered IM in the semimembranosus or semitendinosus muscle twice daily at decreasing doses (Table 1). Treatment was discontinued when half of the growing follicles in the cohort reached ≥7 mm in diameter or after 7 days of treatment, whichever occurred first, as previously done in other species to standardize follicular size (Araujo et al., 2009).

On the last day of pFSH treatment, females received two doses of cloprostenol (PGF2α; 187 ug, IM; Estrumate, Merck Animal Health) approximately 8 h apart to induce luteolysis of any luteal tissue. Females were then exposed to males starting 24 h after the first PGF2α administration. If the female was not receptive to the male, additional doses of PGF2α were administered twice daily until breeding, or for a maximum of 3 days.

2.3. Management of males and breeding

Dual siring was performed by breeding each female with both males, approximately 8–12 h apart (10 ± 2.2 h). Breeding order (first or second breeding) was randomly assigned at the beginning of the study, and then alternated for subsequent females. Sixteen females were hand-bred and successful breeding was determined by visual and manual inspection. The length of each breeding and the interval between the first and second breedings were recorded. The breeding act proceeded until the male ceased voluntarily. Immediately after the first breeding, females received gonadotropin (2000 IU hCG IV or 100 ug GnRH IM) to assist with induction of ovulation.

2.4. Embryo collection

Embryo collections were performed approximately 8–9 days after the first breeding. Females were sedated (Xylazine, 0.25 mg/kg, TranquiVed, 20 mg/mL, Vedco, St. Joseph, MO) and secured on a hydraulic table in the cushed position. Additional sedation was given as needed. The perineum was aseptically prepared and dried with a clean towel. Then, a sterile, 25 cm sigmoidoscope (Welch Allyn, KleenSpec®, Skaneateles Falls, NY) connected to a light source was inserted through the vulva into the vagina and the external os of the cervix was visualized.

A metal stylet was inserted into a 12 fr catheter (Cook® Medical,

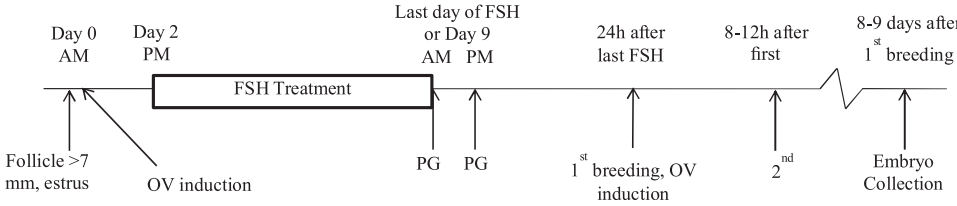

Fig. 1. Experimental design. OV: ovulation PG: prostaglandin FSH: follicle stimulating hormone.