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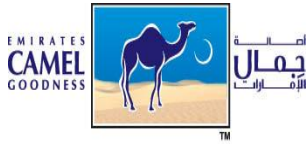
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# ICAR 2012 SATELLITE MEETING ON CAMELID REPRODUCTION

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# **Twin reciprocal embryo transfer between alpacas and llamas**

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## **Introduction**

Twin crias from alpacas or llamas are very seldom born alive under the natural breeding conditions in the highlands of Peru (Sumar, 1980), and there are no reports of twin parturitions in the wild camelids (Koford, 1957; Raedecke, 1978). Previous reports about llamas and alpacas in USA indicated a very low twinning frequency in both species (Fowler, 1990; Sweig, 2002) and similarly, studies carried out in Peru reported low double ovulation rates of only 3 % and 0.9 % after natural mating in alpacas and llamas respectively (Calderon, 1968; Sumar and Leyva, 1979). However, Bravo et al. (2000) reported a high incidence of twin pregnancies (12.5%) in alpacas, which were detected between days 21 and 33 after copulation, but the conceptus identified in the right uterine horn became undetectable between days 28 and 33. Fernandez-Baca et al. (1970) and Sumar, (1980) have also observed twin pregnancies in alpacas between 38 to 42 days after mating with one live foetus being located in the left uterine horn and one dead foetus in the right uterine horn. There are no reports of twin calves in dromedary camels, although some abortions have resulted in two fetuses being expelled (Yagil, 1994) and reports of twinning in Bactrian camels are controversial. In a study of single and twin embryo transfers from Bactrian camel donors into recipient dromedaries, four healthy calves were born but there were no twin births (Niasari-Naslaji et al., 2009). Interestingly, the pregnancy rate following twin embryo transfer seemed to be better than that after single embryo transfer, although the number of transfers were not sufficient for definitive conclusions to be reached. In ewes, Nancarrow et al. (1982) proposed that the greater the number of transferred embryos, the greater the luteal resistance to the luteolytic effect of prostaglandin F<sub>2</sub> $\alpha$ .

The aims of this study were therefore: 1) to study twin reciprocal embryo transfer pregnancy rates between alpacas and llamas; 2) the proposed hypothesis that the bigger and taller llama has a larger uterus and therefore a better chance of supporting twin fetuses, and 3) the greater the number of transferred embryos, the greater the luteal resistance to the luteolytic effects of PGF<sub>2</sub> $\alpha$ .

## Material and Methods

The study was carried out at the Quimsachata Research Station, Camelid Research Program, INIA, Puno, Peru, which is located at about 4,100 meters above sea level, 55 km west of the city of Puno. The alpacas and llamas used in this study were kept on good quality natural pastures with free access to water. Non-pregnant, non-lactating adult female alpacas (n = 47), and non-pregnant, non-lactating adult female llamas (n = 55) with an average body condition score of 3 (on a scale of 1 to 5) were selected for these experiments. The ovarian activity was monitored daily by ultrasound (ALOKA 500D, with a 7.5 MHz transducer, Japan) and when a dominant follicle (diameter  $\geq 7$  mm in alpacas and  $\geq 9$  mm in llamas) was detected in their ovaries, donors from both species were mated and then immediately received a single injection of 1.0 (alpacas) or 1.5 ml (llamas) GnRH (0.0042 mg of buserelin acetate/ml, Conceptal®, Intervet) intramuscularly (i.m) to aid ovulation. Simultaneously, alpaca and llama recipients were synchronized with the donors by injecting similar doses of GnRH on the same day that the donors were mated. For superovulation donors from both species, with only small follicles and no CL in their ovaries, were selected and injected with either 400 or 800 IU of eCG (Folligon, Bioniche, Canada) for alpacas and llamas respectively. In both species embryos were collected on Day 7.5 post-mating (single and superovulated) and transferred into the left uterine horn of either the synchronized alpaca or llama recipient according to the protocol. Pregnancy diagnosis was carried out by ultrasonography, at Days 25, 35, 38 and 45 post breeding.

A total of four experiments were performed; **Experiment I**, each llama recipient (n=15) received one alpaca embryo from alpacas (n=20) flushed during their natural follicular wave cycle. **Experiment II**, each alpaca recipient (n=14) received one llama embryo that had been collected from llamas (n=22) during their natural follicular wave cycle. **Experiment III**, eight female llamas were superovulated and two embryos were transferred into each alpaca recipient (n=10). **Experiment IV**, six female alpacas were superovulated and two embryos were transferred to each llama recipient (n=7).

## Results

In Experiments I and II, the embryo recovery rates from single ovulation uterine flushes were 70% and 80% for alpacas and llamas respectively. The average number of embryos recovered from superovulated llamas was 3.5 embryos per female (4 small embryos were discarded), and in alpacas the average recovery rate was 4 embryos per female (10 embryos were discarded for poor quality).

The pregnancy results for each experiment were as follows: Experiment 1, alpaca embryos into llamas resulted in a 53.3% pregnancy rate. Experiment II, llama embryos into alpacas resulted in a 42.8% pregnancy rate. Experiment III, twin llama embryos into alpacas resulted in 70.0 % single pregnancies, and two alpacas conceived twins (one embryo in each uterine horn) but the fetuses in the right horn died at 42 and 45 days post-mating. In Experiment IV, twin alpaca embryos into llamas resulted in 71.4 % single pregnancies, but one fetus died at 45 days of gestation. There was no statistical significant difference between Experiments I and II ( $P \leq 0.05$ ), or between Experiments III and IV ( $P \leq 0.05$ ). However, there was a significant difference in pregnancy rate, between Experiments II and IV ( $P \leq 0.01$ ), in favor of twin embryo transfer. No live twins were born in Experiment III and IV.

## **Discussion**

The embryo recovery rates in both species were similar to those obtained in other studies carried out in different countries under different management systems (Del Campo, 1995; Huanca et al, 2007; Miragaya et al, 2006; Sumar, 2008). The new superovulation protocol using eCG in low doses in alpacas and llamas that had no mature or active CL in their ovaries, yielded a moderate number of embryos. The protocol using lower doses of eCG had the added advantage that it did not change the anatomical and physiological structure of the ovaries and thus problems of bleeding, rupture of the infundibulum and adhesions with other surrounded organs than can result from using higher doses of hormones, did not occur (Sumar, in press). Results from Experiment I and II, are in agreement with results presented in this Camelid Satellite Meeting (Sumar et al., 2012). The natural and artificial interbreeding of alpacas and llamas, tells us about the very close “genetic distance” between these species and, the similarity between their placentas, and glycosylation patterns at the fetomaternal interface. The pregnancy rate of Experiments II and IV (42.8% vs.71.4 %), favors twin transfers and the luteotrophic effect of twin conceptuses on the uterine environment during early pregnancy (12 to approximately 38 days). However as the twin conspectuses develop beyond 38-40 days they compete for space within the uterus and therefore the right horn fetus is frequently aborted.

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