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EMBRYO TRANSFER IN CAMELIDS

Transferencia de embriones en camélidos

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INTRODUCCION

La ventaja de la transferencia de embriones (ET) y las técnicas asociadas es el mejoramiento genético a través de la selección de las hembras, y reducción del intervalo entre generaciones (Nagy *et al.*, 2013; Tibary, 2001; Tibary y Anouassi, 1997; Tibary *et al.*, 2013). Además, estas técnicas son utilizadas para prologar la vida reproductiva de hembras genéticamente superiores con problemas de fertilidad o incapaces de llevar a término la gestación. El desarrollo de técnicas de ovulación múltiple, transferencia de embriones (MOET) y producción de embriones in vitro viables son muy importantes para el movimiento de genética a nivel internacional y la conservación de especies en peligro o raros, tales como vicuñas, guanacos y camellos bactrianos silvestres (Tibary *et al.*, 2005). Asimismo, estas biotecnologías ofrecen una herramienta para el estudio de los mecanismos de fecundación, desarrollo embrionario e interacción embrión-útero (Huanca, 2015).

Las primeras experiencias en la recuperación de embriones en camélidos es a finales de los años 1950 y principios de los años1960 (véase revisión Sumar, 2013). Sin embargo, el éxito de la transferencia de embriones no quirúrgico en camélidos con un nacimiento de la llama, no fue reportado hasta los años 1980s (Wiepz and Chapman, 1985). Muchos progresos se han realizado en el desarrollo de MOET en camélidos, a inicios de los años 1990 con el financiamiento de la industria privada (Mckinnon *et al.*, 1994; Tibary and Anouassi, 1997). La transferencia de embriones comercial a gran escala se extendió por primera vez en el camello dromedario y más tarde en la industria de la alpaca (Anouassi y Tibary, 2013; Vaughan *et al.*, 2013).

El objetivo del presente trabajo es discutir los estudios fisiológicos de importancia que han llevado a la desarrollo de MOET en camélidos y los factores que afectan el éxito de los programas de ET utilizando

embriones producidos *in vivo* e *in vitro*, y señalar las principales limitaciones y áreas donde se necesita más investigación. Muchos de estos aspectos han sido objeto de una excelente (Anouassi y Tibary, 2013; Sumar, 2013; Trasorras *et al.*, 2013; Vaughan *et al.*, 2013).

En este trabajo, a menos que se menciona una especie específica, vamos a utilizar el término "Camélidos Sudamericanos" (SAC) para referirse a la alpaca (*Vicugna pacos*), llamas (*Lama glama*), Guanaco (*Lama guanicoe*) y vicuñas (*Vicugna vicugna*). El término "camellos" se referirán a todos los camélidos del viejo mundo (*Camelus dromedarius* y *Camelus bactrianus*). El término "camélidos" se utiliza en todo el documento para incluir todas estas especies.

INTRODUCTION

Advantages of embryo transfer (ET) and associated techniques include improved genetics through selection from the female gene pool and reduction of generational intervals (Nagy *et al.*, 2013; Tibary, 2001; Tibary and Anoussi, 1997; Tibary *et al.,* 2013). Additionally, these techniques are useful for prolonging the reproductive life of genetically superior females that became infertile or unable to carry a pregnancy to term. Development of reliable multiple ovulations and embryo transfer (MOET) and in-vitro production of embryos are of particular importance for international movement of genetics and the preservation of endangered or rare species such as vicunas, guanacos and wild Bactrian camels (Tibary *et al.*, 2005). These biotechnologies also offer a tool for the study of mechanisms of fertilization, embryo development, and embryo-uterus interaction (Huanca, 2015).

The earliest attempts to collect embryos from camelidae date back to the late 1950's and early 1960's (see Review, Sumar, 2013). However, successful non-surgical embryo transfer in camelidae with a birth of a live offspring was not reported in the llama until the 1980s (Wiepz and Chapman, 10985). Much progress has been made in the development of MOET in camelids starting in the early 1990s due largely to private funding support from the camel racing industry (Mckinnon *et al.*, 1994; Tibary and Anouassi, 1997). Large scale commercial embryo transfer became widespread first in the dromedary camel and later in the alpaca industry (Anouassi and Tibary, 2013; Vaughan *et al.*, 2013).

The objective of the present paper is to discuss the fundamental physiological studies that have led to the development of MOET in camelids, factors affecting success of ET programs using *in vivo* and *in vitro* produced embryos, and point out the major limitations and areas where further research is needed. Many of these aspects have been the subject of excellent recent reviews (Anouassi and Tibary, 2013; Sumar, 2013; Trasorras *et al.*, 2013; Vaughan et al., 2013). Unless a specific species is mentioned, we will used the term "South American Camelids" (SAC) to refer to alpaca (*Vicugna pacos*), llamas (*Lama glama*), Guanaco (*Lama ganacoe)* and vicunas (*V. vicugna*). The term "camels" will refer to all old world camelids (*Camelus dromedarius* and *Camelus bactrianus*). The term "camelids" will be used throughout the paper to include all these species.

PHYSIOLOGICAL FUNDAMENTALS FOR EMBRYO TRANSFER IN CAMELIDS

Optimization of ET programs requires a thorough understanding of the physiological mechanisms controlling follicular dynamics, ovulation, fertilization, early embryo development, and maternal recognition of pregnancy. The first attempts at embryo transfer were made without this knowledge, often leading to conflicting data and sometimes even publication of erroneous information.

Follicular dynamics

Ultrasonographic and hormonal studies in the mid to late 1990s have helped define the follicular dynamics in most of the camelid species (Skidmore, 2001; Tibary *et al.*, 2007; Vaughan, 2011). Field and experimental observations have shown that ovarian activity in the female camelid is not seasonal under optimal nutritional conditions. Follicular waves occur in an overlapping manner. The duration of follicular waves is variable (Table 1). It is important to note that some of these follicular waves lead to the development of large anovulatory follicles that may become hemorrhagic or luteinized. Anovulatory hemorrhagic follicles (AHF) seem to be more frequent in camels and llamas than in other camelid species and seem to be individual female dependent. The pathophysiology of development of AHF is poorly understood (Tibary *et al.*, 2007).

A major difference between SAC and camels is the timing of postpartum resumption of ovarian activity. While SAC can resume ovarian activity with 10 days postpartum, camels have a long lactational anestrus. This long lactational anestrus is one of the reasons why MOET can have a huge impact on the generation interval and genetic improvement in camels (Tibary *et al.*, 2007).

Ovulation

The induced nature of ovulation in camelids has long been suspected based on clinical and hormonal studies

(Tibary *et al.*, 2007). However, the major breakthroughs in defining the mechanisms of induction of ovulation came in two main groups of studies. The first demonstrated the hypothalamic response to mating in camels (Chen *et al.*, 1984; Marie and Anouassi, 1986) and SAC (Bravo et al., 1992). Luteinizing hormone was shown to increase sharply within minutes following mating when a mature follicle was present. The second group of studies led to the hypothesis of the presence of an ovulation-inducing factor (OIF) within the seminal plasma (Chen *et al.*, 1983; Chen *et al.*, 1985; Pan *et al.*, 2001; Li *et al.*, 2002; Li *et al.*, 2004). Recent studies in llamas and alpacas identified the OIF as β nerve growth factor (βNGF) (Adams and Ratto, 2013; Kershaw-Young *et al.*, 2012). Both βNGF and endometrial inflammation are required to maximize ovulation rate (see review Admas and ratto, 2013). In addition, the βNGF seems to have a luteotrophic effect on the corpus luteum (Silva *et al.*, 2014; Ulloa-Leal, 2014).

Ovulation occurs on average 30 hours after mating. Both ovaries are equally active and alternating of ovulation between ovaries occurs occasionally (Tibary *et al.*, 2007). Double ovulations are not uncommon in most domestic camelids in good health and nutritional status (Campbell *et al.*, 2015). Triple and quadruple ovulations have also been documented in the dromedary camel (Tibary and Anouassi, 1996).

Table 1: Reproductive parameters in female camelids (Tibary et al., 2007; Skidmore, 2011; Vaughan, 2011).

Parameter	*C. dromedarius*	*C. bactrianus*	*V. pacos*	*L. glama*
Follicular wave phases duration				
Growth (days)	10.5±0.5	10.9 ± 3	3-9	3-9
Maturation (days)	7.6 ± 0.8	7 ± 4.2	2-8	2-8
Regression (days)	11.9±0.8	11.9 ± 4.2	3-8	3-8
Ovulatory follicle characteristic				
Minimum size (mm)	9	9	6	8
Growth rate (mm/day)	1.8	1.8	0.43	0.5-0.9
Average size (mm)	10-18	10-18	8-10	9-12
Maximum size (mm)	25	22	12	13
Incidence of anovulatory follicles (%)	40-50	?	5	10-40
Anovulatory follicle regression (days)	8-45	?	?	4-22
Corpus luteum characteristics				
Interval from mating to ovulation (hours)	32 to 40	30 to 48	28 to 30	27-36
Size (mm)	15-25	15-25	11-15	11-18
Day at CL maximum size	7.2±1.7	7.3	7-8	8
Luteolysis day	10 ± 1.2	10.5	10-12	10-12

*Extreme variation in onset of postpartum ovarian follicular activity is primarily due to nutritional condition and effect on lactation anestrus and seasonality

Fertilization and early embryonic development

During mating, sperm is deposited deep into the uterine horns. Studies on in vivo sperm migration and fertilization mechanisms in camelids are scarce (Bravo *et al.*, 1996; Tibary *et al.*, 2007; Vaughan and Tibary, 2006). Molecular and ultrastructural studies in llamas have shown that a sperm reservoir is formed in the isthmus region of the uterine tube following mating (Figure 1). (Apichela *et al.*, 2009; Apichela *et al.*, 2011; Apichela *et al.*, 2010; Apichela *et al.*, 2014; Apichela *et al.*, 2015). Sperm may be stored in this region for up to 5 days (Stekleniov, 1968; Thibault, 1973; Tibary et al., 2007). It is hypothesized that presence of seminal plasma (secretions from the testis, epididymis, bulbourethral glands and prostate) is fundamental in the formation of the sperm reservoir and may be one of the reasons why artificial insemination trials have had very low success thus far.

Oocyte maturation is completed within a few hours following copulation and LH release. The LH surge triggers resumption of meiosis in the oocyte, disruption of cumulus cell cohesiveness and rupture of the follicular wall. Cumulus dispersion is observed in cumulus-oocyte complexes (COC) recovered by aspiration from follicles 18 to 24 hours after hCG injection (Del Campo *et al.*, 1998; Tibary *et al.*, 2007).

Fertilization rates are very high (>80%) (Tibary *et al.*, 2007). Higher fertilization rates for ovulation from the left ovary than from the right ovary have been reported in alpacas (Fernandez-Vaca *et al.*, 1970). However, we

have not seen this difference in camels and alpacas (Tibary and Anouassi, 1996; Picha *et al.*, 2013; Campbell *et al.*, 2015). Conception rates per mating are reportedly lower (50 to 75%) when females are mated based on receptivity than when mating is based on presence of a mature follicle (Tibary *et al.*, 2007; Campbell *et al.*, 2015).

The chronology of early embryo development in camelids has been poorly studied until recently. The pre-hatching stages of embryo development occur in the uterine tube during the first 6 days post-ovulation (Picha *et al.*, 2013; Tibary *et al.*, 2013). The camelid embryo reaches the uterine cavity between 6 and 6.5 days after ovulation and fertilization at the hatched blastocyst stage (Figure 2). This is a unique feature of early camelid embryo development compared to other domestic species (Tibary *et al.*, 2013). Non-fertilized oocytes are not retained in the uterine tube as in the equine (Picha *et al.*, 2013).

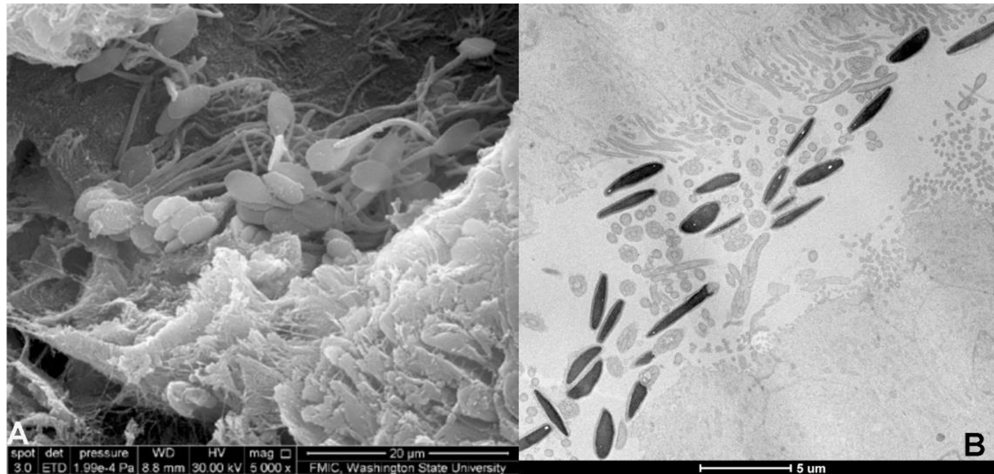

Figure 1: Scanning electron microscopy (A) and Transmission electron microscopy images of the sperm reservoir in alpaca 26 hours after mating.

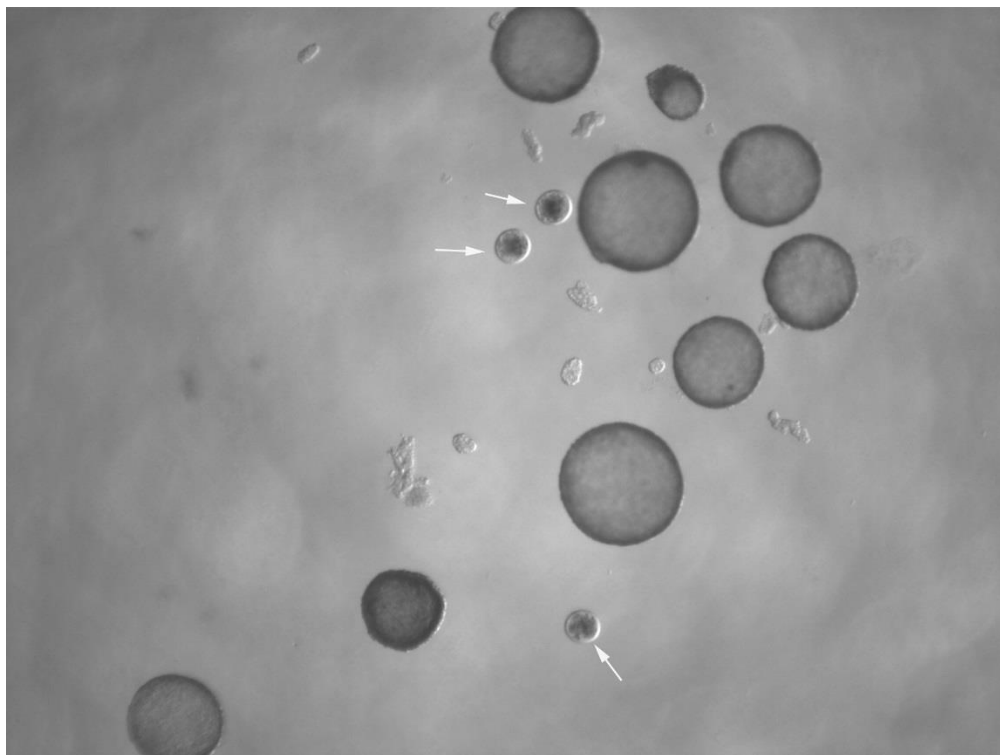

Figure 2: Group of 12 embryos collected on day 7.5 post-mating from a superstimulated female dromedary. Note the variation of size of the hatched blastocysts and the presence of degenerating embryos (arrows)

Maternal recognition of pregnancy

The hatched blastocyst expands rapidly and starts to elongate on day 9, then grows rapidly to occupy the entire uterine cavity by day 12 (Figure 3). (Picha *et al*., 2013; Tibary and Pearson, 2015). During the process of elongation, the embryo migrates from the right uterine horn to the left uterine horn for females that had a right-sided ovulation. In alpacas, 83.3% of the embryos resulting from right ovulations were found in the left uterine horn on day 9 (Picha *et al*., 2013). This suggests that embryo migration to the left horn is an important mechanism in prevention of luteolysis in camelids.

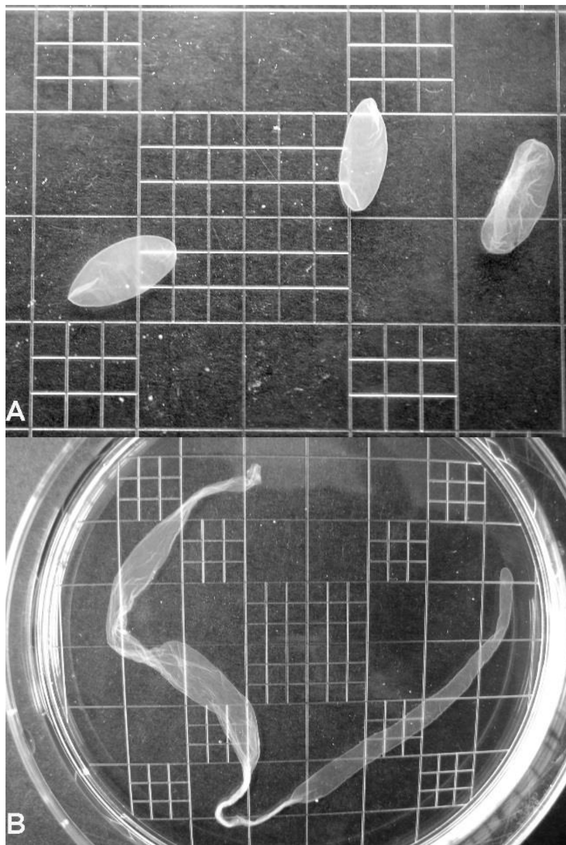

Figure 3: Elongating (A) and elongated (B) alpaca embryos collected from alpacas at Day 9 and Day 11.5 post-mating, respectively.

The mechanism of maternal recognition of pregnancy (MRP) remains poorly studied in camelids. Clinical and endocrine studies suggest that MRP in camelids has to take place relatively early after mating (day 8 to day 10) in order to prevent luteolysis. Even in presence of a conceptus, a pulsatile release of PGF2a is observed between day 7 and 15 post-mating resulting in a transient decrease in progesterone concentrations by day 9 post-mating (Aba *et al*., 1997; Aba *et al*., 2000). During the same period, endometrial expression of cyclooxygenase-2 in the luminal epithelium decreases and reaches one-third of the level observed during luteolysis in non-pregnant animals by day 12 (Bianchi, 2012). It is important to note that MRP occurs at a time when the embryo undergoes a rapid elongation as is observed in ruminants. Attempts to identify a substance such as interferon tau (INFτ) were not successful. Similarly to the equine embryo, camelid embryos have a high aromatizing ability between days 10 and 15 of pregnancy (Powell *et al*., 2007; Skidmore *et al*., 1994). Additionally, administration of estradiol benzoate (10 mg, IM) to llamas from day 7 to day 15 after induction of ovulation with hCG, resulted in an extension of the corpus luteum lifespan and progesterone production (Powell *et al*., 2007). In alpacas, administration of estradiol on days 8 and 9 post-ovulation improved embryo survival by 30 to 50% (Chipayo, 2003; Palomino *et al.,* 2006). However, a similar protocol in llamas resulted in a decrease in pregnancy rate (Trasorras *et al*., 2011). The involvement of estrogens in MRP in camelids is further supported by the increase in estrogen receptor a (ERa) between days 8 to 12 post mating in pregnant animals and a reduction in the expression of progesterone receptors by day 12 post-mating (Bianchi *et al*., 2011).

One of the major peculiarities of pregnancy in camelids is that nearly all fetuses are located in the left uterine horn despite the fact that ovulation is equally distributed between the left and right ovary (Tibary *et al*., 2007; Tibary and Pearson, 2015). The side of ovulation does not influence pregnancy rate (Ratto *et al*., 2011; Tibary and Anouassi, 1996). The preponderance of left-horn pregnancies in camelids is attributed to a difference in PGF2a release between the two uterine horns. PGF2a release from the right uterine horn is local whereas its release from the left horn is systemic. The migration of the embryo originating from an ovulation in the right ovary to the left horn is therefore required to prevent PGF2a release into the general circulation and may even exert a luteotrophic effect making possible the survival of the embryo (Picha *et al*., 2013).

The exact mechanism of embryo migration is not known. Estrogen receptor β (ERβ) expression was found to be greater in pregnant than in sterile-mated llamas, particularly on day 13 (Powell *et al*., 2007). Endometrial expression of ERa and ERβ was not affected by uterine horn side, reproductive status, or days post-mating. However, the presence of a CL seems to upregulate ERβ expression in the uterus mostly in non-endometrial tissue (myometrium and perimetrium). While endometrial ERβ was not affected by pregnancy status, uterine ERβ was significantly increased. This led to the hypothesis that the embryo exerts a direct effect on non-endometrial tissue. These effects may operate through estradiol secretion. Because all embryos migrate to the left horn, a greater

expression of ER in the right horn to promote its contraction was expected but could not be demonstrated. This suggests that a more complex mechanism with differential expression of ER subtypes may be involved in embryo migration from the right to the left horn (Powell *et al.*, 2007).

The corpus luteum (CL) is necessary for maintenance of pregnancy throughout gestation. The CL size varies between 11 and 20 mm in SAC and 15 to 25 mm in camels and may be cavitary (see reviews Tibary *et al.*, 2007; Tibary *et al.*, 2015).

Donor management

From a practical point of view donor management can be divided into 2 broad categories: embryo collection on a per cycle basis (without manipulation of the number of follicles) and MOET. Results from large embryo transfer programs using both approaches have been published recently (Anouassi and Tibary, 2013; Vaughan *et al.*, 2013).

Donor management without ovarian stimulation

Collection of embryos without ovarian superstimulation offers several practical advantages such as eliminating the need for synchronization of a large number of recipients. In addition, embryo recovery results are more reliable and in the case of alpacas it may allow easier manipulation of the genital tract for non-surgical collection of embryos. Females are mated when the follicle has reached the mature size and the uterus presents maximum tone and edema. Although mating is sufficient for induction of ovulation, in most situations hCG or GnRH is administered immediately after mating in order to have a predictable response. Embryo collection is scheduled 8 days after mating (approximately 7 days after ovulation) (Anouassi and Tibary *et al.*, 2013; Sumar, 2013; Vaughan *et al.*, 2013).

Studies in our laboratory on dromedaries showed that the collection rate using this system ranges from 60 to 100% depending on female fertility. In alpacas, the embryo recovery rate varies between 58 and 100% (Campbell *et al.*, 2015; Pacheco *et al.*, 2014; Picha *et al.*, 2013). It is important to note that in this system, collection of 2 embryos is not rare (25% of the collections in the dromedary (Anouassi and Tibary 2013) and 8% of the collections in the alpaca Vaughan *et al.*, 2013). In a recent study on alpacas, the incidence of double ovulation and presence of two embryos in the uterine cavity was 30% and 20%, respectively (Campbell *et al.*, 2015).

There are no controlled studies on the effect of frequency of collection from the same females in alpaca. In the dromedary camel, collection every 2 weeks from the same female throughout the breeding season did not result in any adverse effect on fertility. The number of collections per season was 10±4.2 resulting in an average number of transferrable embryos per season of 8.5±3.1 and a mean number of pregnancies per donor per season of 4.1±1.2 (Anouassi and Tibary, 2013).

Ovarian superstimulation

Approaches to ovarian superstimulation in camelids have been largely adapted from protocols used in ruminants. The primary hormones used are FSH and eCG alone or in combination. As for other species, response to these hormones depends on timing of initiation of treatment in relationship to follicular dynamics, dose and schedule of administration, and individual variation. Response to gonadotropin treatment is largely affected by the stage of follicular wave recruitment and the presence of a dominant follicle. Although FSH and eCG treatments have been initiated during the receptive or luteal phase of the cycle with some success, better results are obtained when the treatment is initiated in absence of any follicles greater than 2 mm (Tibary and Anouassi, 1997; Tibary *et al.*, 2007). Several approaches have been used to control ovarian follicular dynamics and eliminate dominant follicles prior to gonadotropin treatment. These include manual ablation, ultrasound guided aspiration of the dominant follicles or initiation of treatment following a period of progestogen treatment (McKinon *et al.*, 1994; Tibary and Anouassi, 1997; Sansinena *et al.*, 2007; Ratto *et al.*, 2013; Sansinena *et al.*, 2013). Progestogen treatments include daily progesterone injection (50 to 100 mg in SAC and 100 to 150 mg in camels), intravaginal devices (PRID or CIDR with 1.38 mg progesterone in camels, CIDR with 0.3 g progesterone or Medroxyprogesterone acetate (MAP, 60 mg) sponges in llamas and alpacas) or subcutaneous implants of norgestomet (3mg) in llamas and alpacas (Ratto *et al.*, 2013; Hussein *et al.*, 2015). The length of treatment varies from 7 to 14 days. However, it is clear that progesterone alone does not always control follicular wave emergence (Tibary and Anouassi, 1997; Tibary *et al.*, 2007; Hussein *et al.*, 2015). The combination of estradiol and progesterone has been more effective in the control of the follicular wave in some studies (Aller *et al.*, 2010) but not others (Ratto *et al.*, 2013). In a study on llamas, daily treatment with 100 or 150 mg progesterone for 5 days after a single injection of estradiol benzoate (1mg) resulted in a higher embryo recovery rate (Carretero et al., 2010). In our laboratory, daily administration of estradiol and progesterone for 7 to 10 days produced a more uniform response however the ovulatory response was poor (Picha *et al.*, 2009). Studies in llamas and alpacas have shown improved embryo recovery rates when gonadotropin treatment

was started after administration of LH, supposedly to synchronize follicular wave emergence (Huanca *et al.*, 2009; Hussein *et al.*, 2015). Initiation of gonadotropin treatment at a specific time (2 to 4 days) following induction of ovulation has been shown to be more reliable (Rodriguez *et al.*, 2014).

Follicle stimulating hormone (FSH)

Both ovine (oFSH) and porcine (pFSH) FSH have been used in the stimulation of follicular development with variable success (Tibary *et al.*, 2007). The manner of administration of FSH (dose, frequency, and timing during the cycle) has been investigated to some degree. Unfortunately, detailed description of the treatment protocol is often not clearly presented in many publications. In the dromedary, a total dose of 20 to 30 mg of oFSH is given over 6 days (two injections daily) starting 2 days before and up to 1 day after completion of a 7-day course of progesterone treatment by intravaginal device (PRID) (Cooper *et al.*, 1992). FSH was also given in a single small dose (3.3 units) followed by an injection of 3500 IU of eCG, resulting in an average of 7 embryos recovered per treated female (Skidmore *et al.*, 1992). In another study, oFSH was given twice a day (1 to 3 mg per injection) for 3 to 5 days following a 10 to 15 day course of progesterone treatment (100 mg per day for 10 to 15 days) (Mckinnon *et al.*, 1994). Porcine FSH given twice daily in decreasing doses over 3, 5, or 7 days after a 10 to 15 day progesterone treatment resulted in ovarian superstimulation of dromedary females (Mckinnon *et al.*, 1994; Anouassi and Ali, 2013; Anouassi and Tibary *et al.*, 2013). A single subcutaneous dose of oFSH was tested with variable results. The interval from pFSH treatment to development of a mature follicle (10 to 16 mm in diameter) varied between 6 and 8 days (Tibary and Anouassi, 1997; Tibary *et al.*, 2007). Similar protocols have been used for superstimulation of Bactrian camels (Niasari-Naslaji *et al.*, 2009; Nikjou *et al.*, 2008). The number of embryos collected following superstimulation with FSH is variable (8 embryos on average) (Anouassi and Tibary, 2013).

In llamas and alpacas, FSH alone or in combination with eCG has been used following a 12 day progesterone treatment (Correa *et al.*, 1994; Correa *et al.*, 1997). The best superovulation and embryo collection results were obtained following administration of pFSH twice a day for 5 days in decreasing doses (32, 27, 22, 17 and 12 mg, IM) (Correa *et al.*, 1997; Ratto *et al.*, 1997; Aller *et al.*, 2002). The number of embryos obtained after pFSH stimulation is generally low. Alpacas reportedly produce a more variable response to superstimulation protocols than llamas (Ratto *et al.*, 2013).

Equine Chorionic Gonadotropin (eCG)

Superstimulation with eCG has been extensively used in camelids. In general, a single dose is administered intramuscularly one day before or on the day of completion of a 5 to 15 days progesterone regime. The dose of eCG used varied from 1500 to 6000 in camels (Yaguil and van Creveld, 1990; Amouassi and Ali, 1993; Cooper *et al.*, 1993; Mckinnon *et al.*, 1994), 500 to 2000 IU in the llama (Agüero *et al.*, 2001; Bourke *et al.*, 1992; Bourke *et al.*, 1995; Bravo *et al.*, 1995; Correa *et al.*, 1994; Correa *et al.*, 1997; Ratto *et al.*, 2013) and 500 to 750 in alpaca (Ancco and Olivera, 2013; Correa *et al.*, 1994; Mendoza *et al.*, 2012; Ratto *et al.*, 2013) and vicuñas (Aller *et al.*, 2000).

In the dromedary, eCG given as a single injection of 2000 IU, 2500 IU or 4000 IU, one day before or one day after PRID removal, resulted in ovulation in 40% of treated animals. Only 42% of ovulating females yielded one or more embryos. The interval from PRID removal to mating was 5 and 4.5 days respectively for females receiving 2500 IU and 4000 IU of eCG. This interval was one day shorter in females treated with eCG one day before removal of PRID (Cooper *et al.*, 1993; Skidmore *et al.*, 1992). When eCG (2000 to 3000 IU) is administered to females with no dominant follicle, the interval from treatment to mating (follicular diameter of 12 mm) is relatively constant (8 days) (Tibary and Anouassi, 1997; Tibary *et al.*, 2007). Follicular response is variable (0 to 19) with about 20% of the females not responding (Anouasi and Ali, 1993). In the llama, eCG (1000 IU) was used following progesterone priming either in the form of a natural corpus luteum (ovulation induced by hCG or GnRH injection), CIDR, or subcutaneous implants (Bourke *et al.*, 1992; Bourke *et al.*, 1995). Follicular response was variable (0 to 13 follicles) and the number of ovulations ranged from 0 to 7 with a mean number of embryo collected of 1.3 to 2.3 per donor (range 0 to 6). Follicles reached the mature size (9 to 13 mm) 5 to 11 days following eCG treatment. Many females show premature luteinization 7 to 9 days following eCG treatment. Increasing the dose of eCG to 2000 IU results in an increase in incidence of anovulatory follicles. In the alpaca, injection of 750 IU of eCG resulted in an average of 3.7 embryos per ovulating female (Correa *et al.*, 1994; Correa *et al.*, 1997).

The main disadvantage of eCG is the high incidence of follicular luteinization and disturbance of ovulation, probably due to its long half-life. Dromedary females tend to become refractory to eCG following multiple administrations. This suggests that at least in the camel,

the risk of inducing anti-eCG antibodies is real (Tibary and Anouassi, 1997). Superstimulation protocols combining both FSH and eCG have been published in SAC ((Agüero *et al.*, 2001; Ratto *et al.*, 2013) and camels (Tibary and Anouassi, 1997; Tibary *et al.*, 2007; Ismail *et al.*, 2008).

Immunization against inhibin

Immunization again inhibin results in very high levels of circulating FSH and consequently an increase in the number or recruited follicles which will continue to grow until maturation. Preliminary experiments in the dromedary using synthetic peptide fragment from N-terminal sequence of Inhibin-a as an antigen are very encouraging. An increase in ovulation number (4 to 10) was observed in 60% of the immunized females (Tibary and Anouassi, 1997).

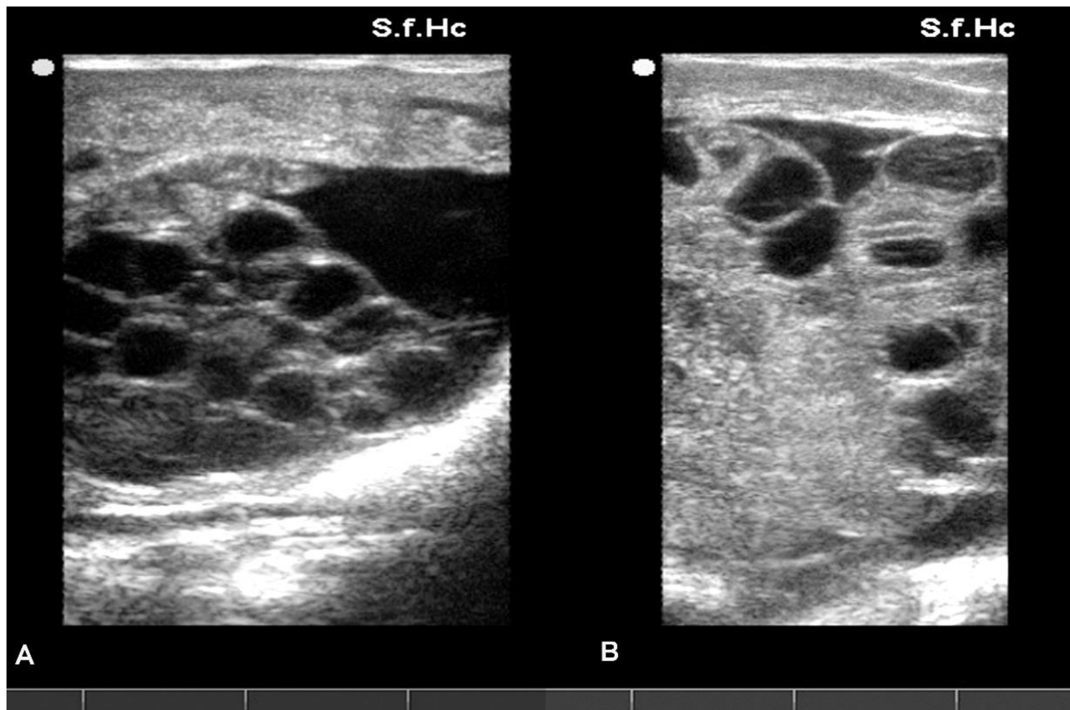

Figure 4: Ultrasonography of a hyperstimulated ovary in a dromedary (pFSH supertimualtion protocol). A) prior to ovulation; B) at 8 days post-mating.

Problems with superstimualtion in camelids

Superstimulation treatments of the female camelidae are far from being perfect. Ovulation response and embryo yield remain highly variable (Anouassi and Tibary, 2013; Ratto et al., 2013; Tibary *et al.*, 2007; Vaughan *et al.*, 2013). The major problems that need to be addressed are: the high incidence of non-responsive females (20 to 30%), incidence of follicular luteinization, hyperstimulation in some females (Figure 4), and loss of pharmaceutical efficacy after multiple treatments. In addition, the recovery rate (number of embryos recovered / number of corpora lutea) is low (40%). Sources of variation that need to be investigated in response to superstimulation include species, individual animal variation, and breed variation. In alapacas, the response to superstimulation seems to be repeatable within a female (Vivanco *et al.*, 2014). We observed a similar trend in the dromedary camel.

MATING MANAGEMENT AND EMBRYO COLLECTION

Mating management

In non-superstimulated as well as superstimulated females the best breeding management is based on ultrasonographic monitoring of follicular size (Tibary and Anouassi, 1997; Tibary, 2001; Anouassi and Tibary, 2013; Vaughan *et al.*, 2013). In camels, mating or artificial insemination is performed when follicles are between 12 and 16 mm in diameter. Breeding should never be delayed beyond the 20 mm follicular size because of the increased incidence of luteinization or failure of ovulation at this stage. Although some authors suggested two matings at 12 to 24 hours interval (Mckinnon *et al.*, 1994; Skidmore et al., 1992) our

results show that a single mating is sufficient. A second breeding is needed only if the copulation time at the fist mating was less than 3 minutes (Anouassi and Ali, 1993; Tibary and Anouassi, 1997; Tibary *et al.*, 2007). In SAC, mating is performed when the follicles become mature (8 to 10 mm in llamas, 7 to 10 mm in alpacas) (Sumar, 2013; Vaughan *et al.*, 2013).

Donors are treated following mating with hCG (1500 to 3000 IU for camels and 750 to 1000 IU for SAC), GnRH (100 µg in camels, 50 µg in SAC) or its agonist Buserelin (20 µg in camels, 4 to 8 µg in SAC) (Wiepz and Chapman, 1985; McEvoy, 1992; Adam *et al.*, 1992; Correa *et al.*, 1994; Bourke *et al.*, 1995; Correa *et al.*, 1997; Ratto *et al.*, 2007; Tibary *et al.*, 2007; Vaughan *et al.*, 2013). In one study, incidence of ovulation after breeding alone, breeding + hCG, and breeding + GnRH was respectively 80%, 90% and 81% (Adam *et al.*, 1992). The main advantage of treatment with GnRH or hCG is the synchronization of ovulations. Ovulation occurs on the average 30 to 40 hours after breeding, 27 ± 0.31 hours after hCG or 28.6 ± 0.36 hours after GnRH (Adam *et al.*, 1992).

Ovulation of the donor is detected by ultrasonography or serum progesterone levels. Plasma progesterone levels start to increase 2 to 3 days after ovulation and reach high levels (> 2 ng/ml) by day 5 after ovulation. Plasma progesterone levels are highly correlated with the number of corpora lutea but can also be elevated in the case of luteinization. In large scale embryo transfer operations, donors are examined by ultrasonography a day or two before embryo collection to estimate number of corpora lutea (Anouassi and Tibary, 2013).

Embryo collection and evaluation

Non-surgical collection of embryos is the standard method used in commercial operations. The best timing for recovery of blastocysts from the uterus has been a subject of controversy. This is due to the fact that in most studies the timing of embryo flushing is based on time of mating and not time of ovulation. Although blastocysts can be recovered from the uterine cavity as early as 6 days post-copulation (Carretero *et al.*, 2010), for better recovery rates it is preferable to wait until day 7 or day 7.5. In camels, the rectovaginal technique of catheterization of the cervix is performed in the standing position. Sedation may be necessary in some females (Tibary and Anouassi, 1997; Anouassi and Tibary, 2013). The same technique is used in SAC, however, alpacas and small llamas require sedation and sometimes caudal epidural (Wieps and Chapman, 1985). In alpacas the uterus is flushed with the animal sitting in a sternal position (Picha *et al.*, 2009; Sumar, 2013; Vaughan *et al.*, 2013). Catheterization of the cervix in alpacas may be achieved through a vaginal speculum. Foley catheters (12 to 16 Fr gauge for llamas and 18 to 22 Fr gauge for camels) are used for flushing the uterus (Tibary and Anouassi, 1997; Sumar, 2013). Very small catheters (5-8 Fr) have been used in alpacas by some authors (da Silva *et al.*, 2012).

The uterine horns are flushed either separately or both at the same time using commercial complete embryo flushing medium (Vigro® complete flush, Vetoquinol, Pullman, WA) in small quantities (20 to 60 mL in SAC and 60 to 200 mL in camels) (Tibary and Anouassi, 1997; Tibary *et al.*, 2011; Sumar, 2013). The uterus is flushed with a total volume of 500 mL in llamas and alpaca and 1 liter in camels. The flushing medium is recuperated into an embryo filter (Tibary and Anouassi, 1997; Anouassi and Tibary, 2013; Sumar, 2013).

Embryos recovered from the uterus in camelids are at the hatched blastocyst stage (Wiepz and Chapman, 1995; Adam *et al.*, 1992; Mckinnon *et al.*, 1994; Tibary and Anouassi, 1997). The size of the embryo is highly variable. In llamas, the average diameter of the embryo is 0.3 mm (0.1 to 0.7 mm), 0.9 mm (0.5 to 1.0 mm), and 7.5 mm (4.0 to 16.0 mm) respectively at 6.0 to 6.5 days, 6.5 to 7.0 days, and 8.5 to 9.0 days post-ovulation (Adam *et al.,* 1992). Similar sizes are reported for alpaca embryos (Picha *et al.,* 2013). Dromedary blastocysts range in diameter from 175 to 500 µm at 7 days (Mckinnon *et al.*, 1994; Tibary and Anouassi, 1997). Camelid blastocysts lose their spherical form and start elongating by day 8.5 or 9 post ovulation (Tibary and Anouassi, 1997; Tibary *et al.*, 2007; Picha *et al.*, 2013). The evaluation system used by the authors classifies the embryos into 5 grades according to their morphological characteristics and stage of development (Table 2) (Figure 5) (Tibary and Anouassi, 1997).

Recipient selection and management

Selection of recipients based on health and fertility is likely the most important factor in the success of a commercial embryo transfer program (Anouassi and Ali, 1993; Vaughan *et al.*, 2013). Recipients should be screened for contagious diseases (Brucellosis, BVD, Trypanosomiais) (Anouassi and Tibary, 2013). Ideally, only females that have successfully carried at least one pregnancy should be used. Maiden and aged females should not be used. Recipients should be in good body condition and on a good nutritional plane. Trace mineral supplementation should be provided where needed. In our large camel MOET program, all females undergo a complete breeding soundness examination. Females with unknown history are submitted for an endometrial culture and biopsy before being accepted into the program (Anouassi and Tibary, 2013). Synchronization between the recipient and donor is extremely important for pregnancy establishment and maintenance after

transfer of the embryo. Studies on SAC and camels indicate that the best pregnancy rates are obtained when the embryo recipient has ovulated a day or 2 after the donor (Anouassi and tibary, 2013; Mckinnon *et al.*, 1994; Skidmore *et al.*, 2002; Vaughan *et al.*, 2013). Unfortunately, there are no easy and reliable methods for synchronization of the donor and recipient ovulation. Synchronization of follicular development in recipients with eCG when superstimulation is started in the donor is possible. However pregnancy rates after ET are usually low if the number of corpora lutea is greater than 6 or lower than 2 in the recipient (Mckinnon et al., 1994).

Table 2: Classification of uterine stage of camelid embryos

Grade	Characteristics
I	Excellent quality: Hatched blastocyst. Should be almost perfectly spherical between day 7 and day 8
II	Good quality: Some irregularities with very few extruded cells
III	Medium quality: Small with dark patches, irregularly shaped, extruded cells
IV	Poor quality: collapsed blastocysts, irregular contour with dark areas, light areas or tears
V	Non transferrable embryos: collapsed very dark blastocysts, all stages of embryonic development still with zona pellucida (non-hatched)

Ovarian progesterone is required for maintenance of pregnancy in all camelids. Use of asynchronous recipients supplemented with progesterone or after administration of meclofenamic acid to prevent luteolysis is possible but not practical (Skidmore and Billah, 2015; Tibary *et al.*, 2007). Contrary to what has been suggested (Skidmore *et al.*, 1992) camelids cannot give birth in presence of progesterone therapy and the decision to stop progesterone treatment is very difficult to make based only on pregnancy length alone as it does not correlate well with readiness for birth. Pregnancy length after transfer varies from 349 to 420 days in dromedaries (Anouassi and Tibary, 2013) and from 319 to 387 days in alpacas (Vaughan *et al.*, 2013). Maintenance of pregnancy with exogenous progesterone has also been associated with dystocia primarily due to failure of cervical dilation (Pearson and Tibary, 2014; Tibary *et al.*, 2015).

To avoid maintenance of pregnancy with exogenous progesterone in non-ovulating progesterone-treated females, formation of new corpora lutea can be induced with eCG followed by hCG treatment. However, the success of this technique depends on the number of corpora lutea induced (Tibary and Anouassi, 1999; Skidmore and Billah, 2011). All commercial embryo transfers are performed using a direct non-surgical technique. Embryos are loaded in 0.25 mL or 0.5 mL straws and are transferred using a standard bovine ET gun (Anouassi and Tibary, 2013; Vaughan *et al.*, 2013). The effect of site of embryo deposition on pregnancy rate has been a subject of research (see below). We usually administer a dose of flunixin meglumine to the recipient prior to transfer (Tibary and Anouassi, 1997).

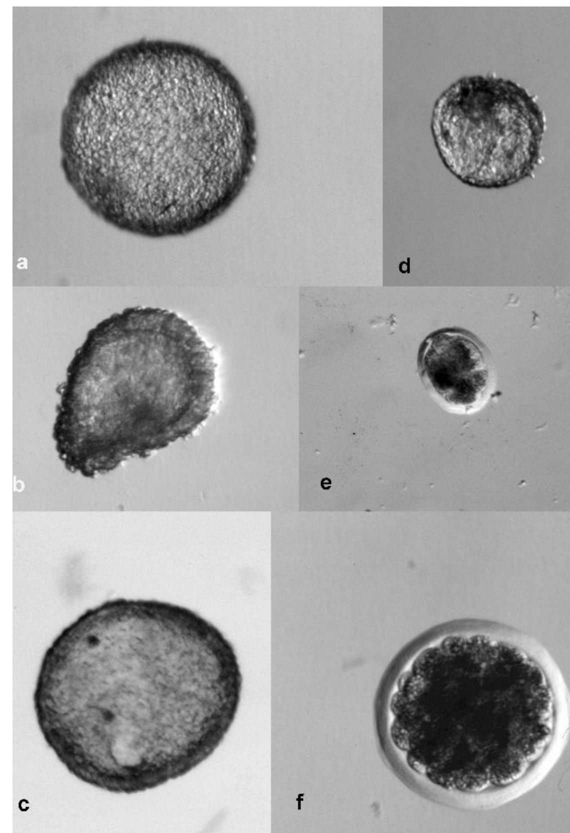

Figure 5: Different quality of dromedary embryos collected 7.5 to 8 days post-mating. a) Grade I blastocyst, b-c) Grade III embryos both resulted in pregnancies, d) Grade IV, resulted in early pregnancy loss, e) Grade V, degenerating, f) Grade V, morula, no pregnancy after transfer

FACTORS AFFECTING EMBRYO TRANSFER RESULTS

The overall performance of an embryo transfer program is based on the percentage of offspring born or weaned for all treated donors. Very little data of this sort has been available until recently. In a large commercial embryo transfer operation in alpacas (4516 embryos transferred), the overall pregnancy and birthing rates

were 43.2% and 41.9%, respectively (Vaughan *et al.*, 2013). The same retrospective study showed that the major factors that significantly affected success rate were: day of collection, embryo quality, recipient age, and recipient body condition score. The effect of lactation and poor body condition score has been reported by others as well (Sumar *et al.*, 2010). The embryo recovery rate was extremely variable and was lower in stimulated females than in non-stimulated females. Finally, recovery and pregnancy rates were significantly affected by the season in single ovulation donors with winter and spring transfers producing less pregnancies (Vaughan *et al.*, 2013). In a similar retrospective study on dromedaries (total of 11,477 ET over 18 years), the overall pregnancy rate at 60 days post-transfer varies from 20 to 92%. The overall weaning rate for pregnant females varies from 70 to 100% (Anouassi and Tibary, 2013). The embryo recovery and pregnancy rates in non-stimulated female were 94.6% and 42%, respectively. The mean number of collections per female per season was 10 ±4.2 which produced a total of 8.5 ±3.1 transferrable embryos and 4.1±1.2 pregnancy per season per donor. For superstimulated animals, the mean number of embryos collected was 8.2 ± 6.1 (0-36) in FSH-treated females and 7.1 ± 4.3 (range 0-19) for eCG-treated females. Pregnancy rate was significantly affected by quality of embryos and age of the donor (Anouassi and Tibary, 2013).

Table 3: Pregnancy rate (PR %) obtained following transfer of embryo to the horn ipsilateral or opposite horn to the CL bearing ovary (Anouassi and Tibary, 2013).

CL side /Horn side	Right/Right	Left/Left	Right/Left	Left/Right
Year 1 PR (n)	69a (103)	47b (90)	53b (30)	50b (28)
Year 2 PR (n)	60a (39)	57a (21)	52a (78)	47a (97)
Year 3 PR (n)	68a (70)	71a (32)	57a (28)	82a (62)
Total PR (n)	65.6a (239)	58b (143)	54b (136)	59.6b (187)

Different letters within the same row represent significant difference (P<0.05).

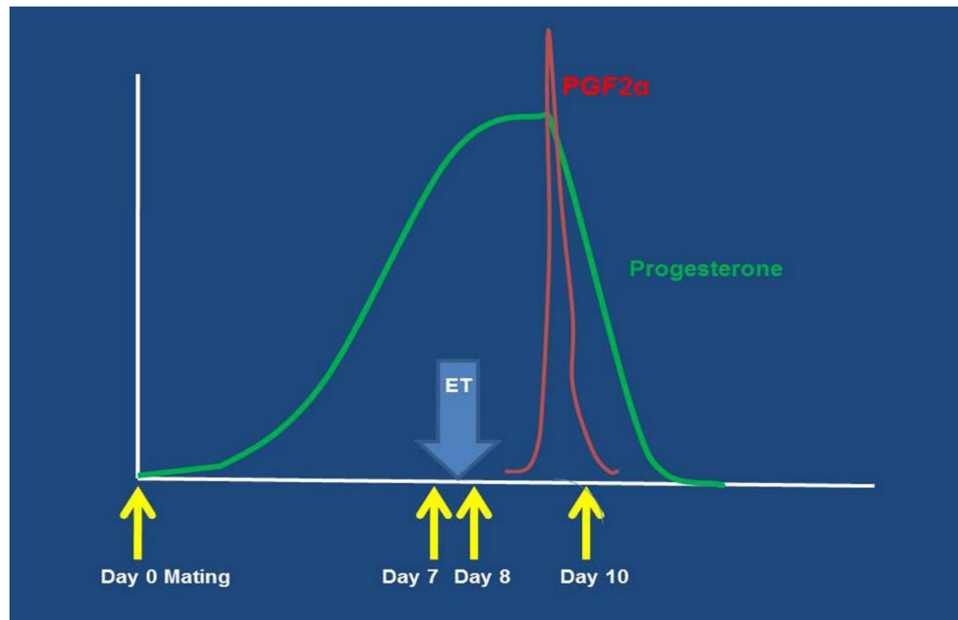

Figure 6: Schematic drawing illustrating the challenge with embryo transfer in camelids. Embryo reach the uterine cavity at the hatched blastocyst stage on day 7 post-mating, by day 9 postmating PGF2a is released by the endometrium if there is no maternal recognition of pregnancy. This gives a very small widow for collection and transfer of embryos

One of the major factors affecting overall success rate in large embryo transfer programs is the high rate of embryo loss between transfer and 60 days (Anouassi and Tibary, 2013). Early pregnancy losses have also been reported in SAC and are generally attributed to luteolysis or failure of maternal recognition of

pregnancy. Luteolysis was suspected to be due to overt cervical manipulation during transfer. However, a study in llamas did not show any difference in luteal function between untreated females and females that received an infusion of PBS in utero transcervically (Trasorras *et al.*, 2010). Failure of MRP and release of PGF2a from the endometrium could be due to embryo quality and a lag between embryo signaling and CL age. Indeed, one of the major challenges in embryo transfer in camelids compared to other domestic species is that the window for MRP before luteolysis occurs is very narrow (Figure 6). The effect of the side of transfer of the embryo in relationship to the corpus luteum location on pregnancy rate has been investigated in some studies. In llamas, pregnancy rate was higher when embryos were transferred into the uterine horn ipsilateral to the corpus luteum (Carnero *et al.*, 2011). Others have shown that the best pregnancy rate (50%) is achieved when 8-day embryos (postmating) are deposited in a 6-day uterus (post-GnRH treatment) in the left horn when the CL is on the left. Deposition of the embryo in the right horn when the CL was on the right resulted in only 30% pregnancy rates and transfer into the body of the uterus resulted in only 10% pregnancy rates no matter where the CL was located (Trasorras *et al.*, 2010). However, in a large data set from dromedaries, there was a significantly better pregnancy rate when the embryos were deposited in the right horn with an ipsilaterally located CL (Table 3) (Anouassi and Tibary, 2013).

A transient endometritis has been described following embryo transfer in mares that may justify the administration of an NSAID at the time of transfer (Koblischke *et al.,* 2008). In camels, recipients receiving flunixin meglumine prior to transfer had lower serum levels of PGF2a metabolite (PGFM) after transfer than non-treated recipients (Skidmore *et al.,* 2002). The effect of technician and stress during all the steps of embryo transfer merits further investigation (Tibary and Anouassi, 1997; Anouassi and Tibary, 2013).

Interspecies embryo transfer

Interspecies embryo transfer is an attractive technique for the preservation and multiplication of endangered species. Interspecies ET has been performed successfully between SAC species (guanaco in llama, alpaca in llama, and llama in alpaca) (Sumar, 2013). Transfer of alpaca embryos into llamas results in normal pregnancies. The offspring (alpaca) from llamas are usually heavier at birth and up to weaning. Similarly, interspecies pregnancies were achieved in camels (Bactrian in dromedary and vice-versa) (Tibary and Anouassi, 1997; Niasari-Naslaji *et al.*, 2009; Niasari-Naslaji *et al.*, 2014). Attempt of embryo transfer from SAC into camels have resulted in pregnancies but none have continued beyond 4 months of gestation.

Manipulation of in vivo produced embryos

Although not used extensively, pregnancies have been reported from embryos following short term preservation (24 hours at 4°C) (Tibary and Anouassi, 1997; Skidmore *et al.*, 2002; Tibary *et al.*, 2007). Successful transfers were also obtained after short term culture (12 to 24 hours) (Khatir *et al.*, 2009).

One of the main challenges in cryopreservation of camelid blastocysts is their large size and the fact that they are already hatched. Cryopreservation of camelid embryos has been attempted using both slow freezing and vitrification protocols. Slow freezing seems to better preserve the cytoskeleton of camel embryos (Skidmore *et al.*, 2009). Transfer of camel embryos cryopreserved in in 1.5 M ethanediol followed by stepwise thawing and rehydration in sucrose resulted in 6.5% to 19% pregnancy rate (Skidmore *et al.*, 2004). Vitrification of camel embryos (20% glycerol+20% ethylene glycol + 0.3M sucrose + 0.375M glucose + 3% polyethylene glycol) showed a better viability of day 6 embryos compared to day 7 and 8 embryos. A pregnancy rate of 38% was obtained with vitrified camel embryos (Skidmore *et al.*, 2005). Vitrification of dromedary blastocysts with a high concentration of ethylene glycol (7.0 mol/L) with sucrose (0.5 mol/L) and direct plunging in liquid nitrogen resulted in the birth of one offspring but overall low pregnancy rates (Nowshari *et al.*, 2005).

Transfer of vitrified llama blastocysts (vitrification solution; 20% glycerol + 20% ethylene glycol + 0.3 M sucrose + 0.375 M glucose + 3% polyethylene glycol in three steps) resulted in a 50% pregnancy rate (Aller *et al.*, 2002; Aller *et al.*, 2002b). Vitrification and slow freezing of llama embryos resulted in recovery rates of 75% and 57% respectively however no transfers were performed (Vasquez *et al.*, 2011). Alpaca embryos were successfully vitrified using commercial equine or bovine vitrification kits but no transfers were performed.

Aspects of in vivo embryo manipulation that would be beneficial for commercial use that have not been thoroughly studied are trophoblastic biopsies for gender determination and genetic testing.

EMBRYO TRANSFER USING IN VITRO PRODUCED EMBRYOS

In vitro production of embryos in camelids has been studied intensely in the last decade (for reviews see Miragaya *et al.*, 2006; Tibary *et al.*, 2007; Tibary *et al.*, 2011; Trasorras *et al.*, 2013).

Oocyte collection, maturation and activation

Oocyte collection has been attempted using postmortem and in vivo surgical techniques (Gomez *et al.*, 2013; Tibary *et al.*, 2005; Trasorras *et al.*, 2013). Laparoscopic and transvaginal ultrasound-guided (TUGA) ovum pickup yield variable results depending on the species (Brogliatti *et al.*, 2000; Berland *et al.*, 2011; Ruso *et al.*, 2014). In the dromedary, TUGA resulted in 77% oocyte recovery rate with more than 80% of mature oocytes when performed in superstimulated females 26 to 28 h after GnRH administration (Wani and Skidmore, 2010). Similarly, in vivo maturation rates have been obtained following gonadotropin superstimulation and administration of hCG or GnRH in SAC (Ratto *et al.*, 2005; Ratto *et al.*, 2007).

In vitro maturation techniques have been developed and are relatively similar to methods used in ruminants (Kafi *et al.*, 2005; Tibary *et al.*, 2005; Tibary *et al.*, 2007; Abdoon *et al.*, 2011; Trasorras *et al.*, 2013). Culture time varies from 27 to 42 hours depending on studies (Tibary *et al.*, 2005; Trasorras *et al.*, 2013; Ayuque *et al.*, 2014). Maturation rates achieved are very high (Khatir *et al.*, 2007; Khatir *et al.*, 2009; Wani and Wernery, 2010). Ovaries can be stored for up to 16 hours at room temperature (22-25°C) prior to processing (Arriaga *et al.*, 2014). Vitrification of in vitro matured oocytes has been achieved with good post-thaw in vitro fertilization in alpacas (Ruiz *et al.* 2011; Ruiz *et al.*, 2013).

In vitro fertilization

The first dromedary offspring obtained from in vitro matured, in vitro fertilized, and in vitro cultured oocytes was from abattoir oocytes (Khatir and Anouassi, 2006). Most studies on in vitro fertilization have used epididymal sperm (Del Campo *et al.*, 1994; Tibary *et al.*, 2005; Trasorras *et al.*, 2013). In SAC, blastocyst formation rates vary from 20 to 30% following fertilization with epididymal sperm (Berland *et al.*, 2011; Arriaga *et al.*, 2014; Huanca *et al.*, 2014). Similar results have been obtained with epididymal sperm in the dromedary (Wani, 2009; Badr *et al.*, 2010; Fathi *et al.*, 2014) and Bactrian camel (Bou *et al.*, 1993). To our knowledge only two groups have attempted in vitro production of embryo using ejaculated sperm (Camels (Khatir *et al.*, 2004) and llamas (Conde *et al.*, 2008; Trasorras *et al.*, 2012; Trasorras *et al.* 2014)). In llamas, embryo production by IVF with semen obtained by electroejaculation resulted in a blastocyst rate of 34 to 36% and hatched blastocyst rate of 20% after culture in semi-defined oviductal fluid with amino – acids (SOFaa). A single pregnancy was obtained out of 7 transferred embryos but was lost at 42 days (Trasoras *et al.*, 2012; 2014). In the dromedary, various protocols for embryo production by IVF using semen collected by artificial vagina were tested. These include culture with semi-defined media, and co-culture with oviductal or granulosa cells (Khatir and Anouassi, 2006; Khatir *et al.*, 2007; Khatir *et al.*, 2009; Khatir et al., 2009b). Despite these advances, in vitro production of embryos by IVF remains limited as far as commercial use.

Intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT)

Development of protocols for chemical activation of camelid oocytes has led to development of ICSI and SCNT techniques (Abdoon *et al.*, 2007; Khatir and Anouassi, 2008; Silva *et al.*, 2014; Trasoras *et al.*, 2013). Embryos have been produced by ICSI in llamas (Miragaya *et al.*, 2003; Sansinena *et al.*, 2007; Conde *et al.*, 2008) and camels (Wani, 2008). However, these techniques have not yet been optimized for commercial use.

Camel oocyte parthenogenesis has been described previously (Khatir and Anouassi, 2008). However, in vitro production of camelid embryos by SCNT is limited to a few studies in llamas and camels. In llamas, fusion and cleavage rates of reconstructed embryos obtained were 62.5% and 32 to 40%, respectively. However, no pregnancies were obtained (Sansinena *et al.*, 2003). In the dromedary, blastocyst formation rates of 29 to 46% were obtained from nuclear transfer embryos and one viable offspring was produced following transfer of 26 embryos (8%) (Wani *et al.*, 2010). The overall efficiency of SCNT in camelids is still extremely low to justify use in production. Camelus bactrianus embryos have been used from skin fibroblast using xenonuclear transfer with rabbit, ovine and bovine oocytes (Zhao *et al.*, 2006; Zhou *et al.*, 2006).

CONCLUSION

Embryo transfer using in vivo produced embryos is now commercially available in some countries. The overall efficiency of these programs is still very difficult to appreciate. Recently published results from the largest commercial programs [alpacas in Australia (Vaughan *et al.*, 2013) and dromedaries in UAE (Anouassi and Tibary *et al.*, 2013)] have shed some light on the most important factors affecting results. More studies are needed to evaluate sources of variation in the superstimulation response, particularly with regards to breed, age, and nutritional management. A more uniform reporting system for superstimulation protocols, donor mating management and embryo collection timing is necessary in order to evaluate these treatments. Factors affecting embryo quality and their effect on MRP

following transfer need further elucidation. The effect of changes in the uterine environment following superstimulation on embryo quality needs further investigation. Recent research on oviductal function may shed some light on the poor embryo recovery following superstimulation (Apichela *et al.*, 2015; Arganaraz et al., 2015). A recent study in vicunas demonstrate a difference in glycosylation patterns between superstimulated and non-stimulated females. This finding indicates that embryos from superstimulation may be in a different uterine environement than that observed in non-stimulated females (Lopez *et al.*, 2014). The effect of recipient health and nutrition (trace mineral supplementation in particular) on pregnancy maintenance need to be further investigated. Also, factors affecting corpus luteum quality need to be studied in more detail. The risk for disease transmission through embryo transfer is said to be low (Sutmoller, 1999). However, no studies have been performed to determine the risk for transmission of reproductive infectious diseases such as BVD, Brucellosis, and Chlamydiosis. This is even more important as camelid embryos are collected at the hatched blastocyst stage which is not easy to wash. Although some progress has been achieved in the manipulation of camelid embryos (cryopreservation) and their in vitro production by IVF, ICSI, and SCNT, these biotechnologies are not yet optimized and results have poor repeatability.

REFERENCES

- Aba MA, Bravo PW, Forsberg M, Kindahl H. Endocrine changes during early pregnancy in the alpaca. Anim Reprod Sci 1997. 47:273-279.
- Aba MA, Kindahl H, Forsberg M, Quiroga M, Auza N. Levels of progesterone and changes in prostaglandin F-2 alpha release during luteolysis and early pregnancy in llamas and the effect of treatment with flunixin meglumine. Anim Reprod Sci; 2000. 59:87-97.
- Abdoon ASS, Kandil OM, Zeng SM, Cui MS. Mitochondrial distribution, ATP-GSH contents, calcium [Ca2+] oscillation during in vitro maturation of dromedary camel oocytes. Theriogenology; 2011. 76:1207-1214.
- Abdoon ASS, Kandil OM, Berisha B, Kliem H, Schams D. Morphology of dromedary camel oocytes and their ability to spontaneous and chemical parthenogenetic activation. Reprod Domest Anim; 2007. 42:88-93.
- Adam CL, Bourka DA, Kyle CE, Young P, McEvoy TG. Ovulation and embryo recovery in the llama. First international camel conference, Dubai 1992:125-127.
- Adams GP, Ratto MH. Ovulation-inducing factor in seminal plasma: a review. Anim Reprod Sci; 2013. 136:148-156.
- Agüero A, Chaves MG, Capdevielle EF, Russo AF, Aba MA. Superovulation in llamas: comparison of two treatments. InVet - Investigación Veterinaria; 2001. 3:13-18.
- Aller JF, Cancino AK, Rebuffi GE, Alberio RH. Superovulatory response to progestagen-eCG in vicuñas (*Vicugna vicugna*) in semicaptive conditions. Veterinaria Argentina; 2000.17:652-562.
- Aller JF, Rebuffi GE, Cancino AK, Alberio RH. Successful transfer of vitrified llama (*Lama glama*) embryos. Anim Reprod Sci; 2002. 73:121-127.
- Aller JF, Cancino AK, Rebuffi GE, Alberio RH. Transfer of vitrified embryos of the llama (*Lama glama*) on the altiplano of Argentina. Veterinaria Argentina; 2002b. 19:363-386.
- Aller JF, Cancino AK, Rebuffi GE, Alberio RH. Effect of estradiol benzoate used at the start of a progestagen treatment on superovulatory response and embryo yield in lactating and non-lactating llamas. Anim Reprod Sci; 2010. 119:322-328.
- Ancco E, Olivera L. Superovulatory response of alpaca suri with two doses of eCG during thebreeding season. Spermova; 2013. 3:99-100.
- Anouassi A, Ali AC. Embryo transfer in camels. Études et Synthèses de l'IEMVT. 1993:327-32.
- Anouassi A, Tibary A. Development of a large commercial camel embryo transfer program: 20 years of scientific research. Anim Reprod Sci; 2013.136:211-221.
- Apichela S, Jiménez-Díaz MA, Roldan-Olarte M, Valz-Gianinet JN, Miceli DC. In vivo and in vitro sperm interaction with oviductal epithelial cells of llama. Reprod Domest Anim. 2009; 44:943-51.
- Apichela SA. Sperm reservoir in the llama oviduct and advances in the study of the proteolytic systems involved. Archivos Latinoamericanos de Producción Animal. 2011; 19:55-7.
- Apichela SA, Valz-Gianinet JN, Schuster S, Jiménez-Díaz MA, Roldán-Olarte EM, Miceli DC. Lectin binding patterns and carbohydrate mediation of sperm binding to llama oviductal cells in vitro. Anim Reprod Sci; 2010. 118:344-353.
- Apichela SA, Arganaraz ME, Zampini R, Vencato J, Miceli DC, Stelletta C. Biochemical composition and protein profile of alpaca (*Vicugna pacos*) oviductal fluid. Anim Reprod Sci; 2015. 154:79-85.
- Apichela SA, Argañaraz ME, Giuliano S, Zampini R, Carretero I, Miragaya M, *et al.* Llama oviductal sperm reservoirs: involvement of bulbourethral glands. Andrologia; 2014. 46:290-295.
- Arganaraz ME, Apichela SA, Zampini R, Vencato J, Stelletta C. Biochemical and Protein Profile of Alpaca (*Vicugna pacos*) Uterine Horn Fluid During Early

Pregnancy. Reprod Domest Anim; 2015.50:121-128.

- Arriaga C I, Huanca L W, Terreros C M, Becerra G JJ, García H P, Ampuero B A. Effect of temperature and time of storage of alpaca ovaries on in vitro maturation and cleavage rate of oocytes. Revista de Investigaciones Veterinarias del Perú (RIVEP); 2014. 25:477-486.
- Ayuque A, Justiniano E, Mendoza J, Landeo J L, Ruiz J. Effect of time in *in vitro* maturation in the meiotic ability and embryonic development of llama. Spermova; 2014. 4:99-101.
- Badr MR, Abdel-Malak MG. In vitro fertilization and embryo production in dromedary camel using epididymal spermatozoa. Global Veterinaria; 2010. 4:271-276.
- Berland MA, Baer Av, Ruiz J, Parraguez VH, Morales P, Adams GP, *et al*. In vitro fertilization and development of cumulus oocytes complexes collected by ultrasound-guided follicle aspiration in superstimulated llamas. Theriogenology; 2011. 75:1482-1488.
- Bianchi CP. Estudios sobre el control endocrino de la luteólisis y reconocimiento materno de la preñez en llamas (*Lama glama*). Doctoral thesis. Universidad Nacional del Centro de la Provincia de Buenos Aires, Argentina. 2011.
- Bou SG, Pang YF, Zhang SL, Xue XX. Preliminary study on in vitro fertilization in the domestic camel (*Camelus bactrianus*). Chinese Journal of Zoology; 1993. 28:35-37.
- Bourke DA, Adam CL, Kyle CE, Young P, McEvoy TG. Superovulation and embryo transfer in the llama. 1992:183-185.
- Bourke DA, Kyle CE, McEvoy TG, Young P, Adam CL. Superovulatory responses to eCG in llamas (*Lama glama*). Theriogenology. 1995; 44:255-68.
- Bravo PW, Tsutsui T, Lasley BL. Dose response to equine chorionic gonadotropin and subsequent ovulation in llamas. Small Ruminant Res; 1995. 18:157-163.
- Bravo PW, Stabenfeldt GH, Fowler ME, Lasley BL. Pituitary response to repeated copulation and/or gonadotropin-releasing hormone administration in llamas and alpacas. Biol Reprod; 1992, 47: 884-888.
- Bravo PW, Moscoso J, Ordoñez C, Alarcon V. Transport of spermatozoa and ova in female alpaca. Anim Reprod Sci; 1996, 43:173-179.
- Brogliatti GM, Palasz AT, Rodriguez-Martinez H, Mapletoft RJ, Adams GP. Transvaginal collection and ultrastructure of llama (*Lama glama*) oocytes. Theriogenology; 2000, 54:1269-1279.
- Campbell AJ, Pearson LK, Spencer TE, Tibary A. Double ovulation and occurrence of twinning in alpacas (*Vicugna pacos*). Theriogenology; 2015. 84:421-424.
- Carnero S S, Huanca L W, Cordero R A, Vásquez E M, Huanca M T. Embryo transfer ipsilateral and contralateral to the position of the corpus luteum and embryo survival in Llamas. Revista de Investigaciones Veterinarias del Perú (RIVEP); 2011. 22:114-120.
- Carretero MI, Miragaya M, Chaves MG, Gambarotta M, Aguero A. Embryo production in superstimulated llamas pre-treated to inhibit follicular growth. Small Ruminant Res; 2010. 88:32-37.
- Cervantes FM, Huanca LW, Gonzales CM, Palomino CM, Leyva VV. Relationship between day of collection and embryo recovery in superovulated alpacas. Revista de Investigaciones Veterinarias del Perú (RIVEP); 2011, 22:125-132.
- Chen BX, Yuen ZX, Pan CW. Factors inducing ovulation in the bactrian camel. The Camelid: an all-purpose animal / editors, W. Ross Cockrill. Uppsala: Scandinavian Institute of African Studies. Pp. 1984.387-398.
- 38. Chen BX, Yuen ZX, Pan GW. Semen-induced ovulation in the bactrian camel (*Camelus bactrianus*). J Reprod Fert.; 1985. 73:335-339.
- Chen BX, Yuen ZX, Pan GW, Huang YM, Gao YH. Studies on the ovulation mechanism in the bactrian camel. II. The role of semen in induction of ovulation. Acta vet zoo-tech sin; 1983. 14:161-166.
- Chipayo Y. Efecto del estradiol en el periodo de reconocimiento materno de la preñez sobre la supervivencia embrionaria en alpacas. Rev Inv Vet Perú; 2003. 14:111-118.
- Conde PA, Herrera C, Trasorras VL, Giuliano SM, Director A, Miragaya MH, *et al*. In vitro production of llama (*Lama glama*) embryos by IVF and ICSI with fresh semen. Anim Reprod Sci; 2008. 109:298-308.
- Cooper MJ, Skidmore JA, Allen WR, Wensvoort S, Billah M, Chaudhry MA, *et al*. Attempts to stimulate and synchronise ovulation and superovulation in dromedary camels for embryo transfer. Proc 1st int Camel Conf, Dubai, UAE. pp. 1992. 187-91.
- Cooper MJ, Skidmore JA, Allen WR, Wensvoort S, Billah M, Chaudhry MA, *et al*. Attempts to stimulate and synchronise ovulation and superovulation in dromedary camels for embryo transfer. Etude de synthèse de l'IEMVT, 1993. 313-316.
- Correa JE, Ratto MV, Gatica R. Oestrous activity and ovarian response in llamas and alpacas treated with progesterone and gonadotropins. Arch Med Vet; 1994. 26:59-64.
- Correa JE, Ratto MH, Gatica R. Estrus Activity and Ovarian Response in Llamas and Alpacas Treated with Progesterone and Pmsg or Fsh. Arch Med Vet; 1994. 26:59-64.

- Correa JE, Ratto MH, Gatica R. Superovulation in llamas (*Lama glama)* with pFSH and equine chorionic gonadotrophin used individually or in combination. Anim Reprod Sci; 1997. 46:289-296.
- da Silva MAC, Pinto CRF, Forshey BS, Messerschmidt CA, Coffman EA, Lakritz J. Factors affecting embryo collection and transfer in alpacas. Reprod Domest Anim; 2012.47:458.
- Del Campo MR, Del Campo CH, Donoso MX, Berland M, Mapletoft RJ. 1. In vitro fertilization and development of llama (*Lama glama*) oocytes using epididymal spermatozoa and oviductal cell co-culture. Theriogenology; 99441:1219-1229.
- Fathi M, Seida AA, Sobhy RR, Darwish GM, Badr MR, Moawad AR. Caffeine supplementation during IVM improves frequencies of nuclear maturation and preimplantation development of dromedary camel oocytes following IVF. Theriogenology; 2014. 81:1286-1292.
- Fernandez Baca S, Hansel W, Novoa C. Embryonic mortality in the alpaca. Biology of Reprod. 1970. 3:243-251.
- Gomez O, Choque D, Henao F, Escobedo M, Valverde N. Comparison of three recovery methods of alpaca cumulus-oocyte complexes (COCs) harvested from postmortem ovaries in a slaughterhouse. Spermova; 2013. 3:103-104.
- Huanca L W, Condori P R, Chileno M M, García H P, Cainzo C J, Becerra G JJ. Effect of time of incubation on nuclear maturation and cleavage post in vitro fertilization of alpaca oocytes. Revista de Investigaciones Veterinarias del Perú (RIVEP); 2014. 25:468-476.
- Huanca W. Reproductive biotechnologies in domestic South American camelids as alternatives for genetic improvement. Archivos Latinoamericanos de Producción Animal; 2015. 23:1-4.
- Huanca W, Cordero A, Huanca T, Cardenas O, Adams GP, Ratto MH. Ovarian response and embryo production in llamas treated with equine chorionic gonadotropin alone or with a progestin-releasing vaginal sponge at the time of follicular wave emergence. Theriogenology; 2009. 72:803-808.
- Hussein FM, Metwelly KK, Mona, Mahmoud A, Ragab MH. Effect of CIDR application duration (7-10-14 days) on circulating estrogen and progesterone during breeding and non-breeding season in she-camels. Alexandria Journal of Veterinary Sciences; 2015. 44:125-129.
- 56. Ismail ST, Al-Eknah MM, Al-Busadah KA. Hormonal changes during busserlin (GnRH) priming regimen for superovulation in the camel (*Camelus dromedarius*). Scientific Journal of King Faisal University (Basic and Applied Sciences); 2008. 9:103-17.
- Kafi M, Mesbah F, Nili H, Khalili A. Chronological and ultrastructural changes in camel (*Camelus dromedarius*) oocytes during in vitro maturation. Theriogenology; 2005. 63:2458-2470.
- Kershaw-Young CM, Druart X, Vaughan J, Maxwell WMC. β-Nerve growth factor is a major component of alpaca seminal plasma and induces ovulation in female alpacas. Reproduction, Fertility and Development; 2012. 24:1093-1097.
- Khatir H, Anouassi A. The first dromedary (*Camelus dromedarius*) offspring obtained from in vitro matured, in vitro fertilized and in vitro cultured abattoir-derived oocytes. Theriogenology; 2006. 65:1727-1236.
- Khatir H, Anouassi A. Preliminary assessment of somatic cell nuclear transfer in the dromedary (*Camelus dromedarius*). Theriogenology; 2008, 70:1471-1477.
- Khatir H, Anouassi A, Tibary A. Production of dromedary (Camelus dromedarius) embryos by IVM and IVF and co-culture with oviductal or granulosa cells. Theriogenology; 2004. , 62:1175-1185.
- Khatir H, Anouassi A, Tibary A. Quality and developmental ability of dromedary (Camelus dromedarius) embryos obtained by IVM/IVF, in vivo matured/IVF or in vivo matured/fertilized oocytes. Reprod Domest Anim; 2007. 42:263-270.
- Khatir H, Anouassi A, Tibary A. In vitro and in vivo developmental competence of dromedary (Camelus dromedarius) oocytes following in vitro fertilization or parthenogenetic activation. Anim Reprod Sci; 2009. 113:212-9.
- Khatir H, Anouassi A, Tibary A. Effect of different conditions of in vitro culture on the quality of the dromedary (Camelus dromedarius) embryos produced in vivo and their viability after transfer to recipients. Clinical Theriogenology; 2009b. 1:241.
- Koblischke P, Kindahl H, Budik S, Aurich J, Palm F, Walter I, *et al*. Embryo transfer induces a subclinical endometritis in recipient mares which can be prevented by treatment with non-steroid anti-inflammatory drugs. Theriogenology; 2008. 70:1147-1158.
- Li XL, Zhao XX. Separation and purification of ovulation-inducing factors in the seminal plasma of the Bactrian camel (Camelus bactrianus). Vet Res Commun; 2004. 28:235-245.
- Li XL, Zhang Y, Chen BX, Zhao XX. 2002. The concentrations of LH, FSH, oestradiol-17 beta and progesterone in the blood plasma of the female Bactrian camel (Camelus bactrianus) before and after intramuscular injection of seminal plasma. Vet Res Commun; 2002; 26:571-576.
- López C V, Vásquez C M, Huanca L W, Santiani A A, Barbeito C, Canuzzi A C, *et al*. Lectinhistochemistry study of the uterus of alpacas

(*Vicugna pacos*) under superovulation treatment. Revista de Investigaciones Veterinarias del Perú (RIVEP). 2014; 25:1-15.

- Marie M, Anouassi A. Mating-Induced Luteinizing Hormone Surge and Ovulation. Biol Repro; 1986. 35:792-798.
- McEvoy TG. Ovulation and embryo recovery in the llama. Proc 1st int Camel conf, Dubai, UAE. Pages, 125-127. 1992.
- Mckinnon AO, Tinson AH, Nation G. 1994. Embryo-Transfer in Dromedary Camels. Theriogenology; 41:145-150.
- Mendoza R, Pozo A, Huanca T, Naveros M. Production and evaluation of embryos by superovulation using the eCG and FSH in alpacas (*Vicugna pacos*) Huacaya. Spermova; 2012. 2:49-50.
- Miragaya MH, Chaves MG, Agüero A. Reproductive biotechnology in South American camelids. Small Ruminant Res; 2006. 61:299-310.
- Miragaya MH, Herrera C, Quintans CJ, Chaves MG, Capdevielle EF, Giuliano SM, *et al.* Producción in vitro de embriones de llama (*Lama glama*) por la técnica de ICSI: resultados preliminares. In: Proc. III Congreso Mundial sobre Camélidos, Bolivia, pp. 267–270. 2003.
- Nagy P, Skidmore JA, Juhasz J. Use of assisted reproduction for the improvement of milk production in dairy camels (*Camelus dromedarius*). Anim Reprod Sci; 2013. 136:205-210.
- Niasari-Naslaji A, Barani M, Adel HR, Ghanbari A, Moosavi-Movahedi AA. Adaptation of Bactrian Camel to Hot Desert Climate Using Interspecies Embryo Transfer. J Camel Pract Res; 21:161-164. 2014
- Niasari-Naslaji A, Nikjou D, Skidmore JA, Moghiseh A, Mostafaey M, Razavi K, *et al.* Interspecies embryo transfer in camelids: the birth of the first Bactrian camel calves (*Camelus bactrianus*) from dromedary camels (*Camelus dromedarius*). Reprod Fert Develop; 2009. 21:333-337.
- Nikjou D, Niasari-Naslaji A, Skidmore JA, Mogheiseh A, Razavi K, Gerami A, *et al.* Synchronization of follicular wave emergence prior to superovulation in Bactrian camel (*Camelus bactrianus*). Theriogenology; 2008. 69:491-500.
- Nowshari MA, Ali SA, Saleem S. Offspring resulting from transfer of 2005. cryopreserved embryos in camel (*Camelus dromedarius*). Theriogenology;63:2513-2522.
- Pacheco J, Pezo S, Velez V. Efficiency of the collection and the quality of embryos of alpacas obtained from simple successive ovulations. Spermova; 2014. 4:159-161.
- Pan G, Chen Z, Liu X, Li D, Xie Q, Ling F, *et al.* Isolation and purification of the ovulation-inducing factor from seminal plasma in the bactrian camel (*Camelus bactrianus*). Theriogenology; 2001. 55:1863-1879.
- Pearson LK, Tibary A. Clinical management of postpartum hemorrhage following failure of cervical dilation in an alpaca. Clinical Theriogenology; 2014. 6:111-117.
- Picha Y, Tibary A, Memon M, Kasimanickam R, Sumar J. Chronology of early embryonic development and embryo uterine migration in alpacas. Theriogenology; 2013. 79:702-708.
- Picha Y, Nielsen S, Rodriguez JS, Bott NI, Sandoval S, Sumar J, *et al.* Synchronization of follicular waves with progesterone/estrogen combination before superstimulation with pFSH in alpacas. Clinical Theriogenology; 2009. 1:218.
- Powell SA, Smith BB, Timm KI, Menino AR, Jr. Expression of estrogen receptors α and β in the corpus luteum and uterus from non-pregnant and pregnant llamas. Molecular Reproduction and Development; 2007. 74:1043-952.
- Powell SA, Smith BB, Timm KI, Menino AR. Estradiol production by preimplantation blastocysts and increased serum progesterone following estradiol treatment in llamas. Anim Reprod Sci; 2007. 102:66-75.
- Ratto M, Gomez C, Berland M, Adams GP. Effect of ovarian superstimulation on COC collection and maturation in alpacas. Anim Reprod Sci; 2007. 97:246-256.
- Ratto M, Berland M, Huanca W, Singh J, Adams GP. In vitro and in vivo maturation of llama oocytes. Theriogenology; 2005. 63:2445-2457.
- Ratto M, Cervantes M, Norambuena C, Silva M, Miragaya M, Huanca W. Effect of location and stage of development of dominant follicle on ovulation and embryo survival rate in alpacas. Anim Reprod Sci; 2011. 127:100-105.
- Ratto MH, Gatica R, Correa JE. Timing of mating and ovarian response in llamas (*Lama glama*) treated with pFSH. Anim Reprod Sci; 1997. 48:325-330.
- Ratto MH, Silva ME, Huanca W, Huanca T, Adams GP. Induction of superovulation in South American camelids. Anim Reprod Sci; 2013. 136:164-169.
- Rodriguez J, Pearson L, Campbell A, Ciccarelli M, Tibary A. Comparison of two superovulation treatments in the dromedary camel (*Camelus dromedarius*). Clinical Theriogenology; 2014.6: 371.
- Ruiz B J, Landeo J L, Artica F M, Ratto F M, Correa S J. Chemical activation of alpaca oocytes vitrified after in vitro maturation. Revista de Investigaciones Veterinarias del Perú (RIVEP); 2011. 22:206-212.
- Ruiz J, Landeo L, Mendoza J, Artica M, Correa JE, Silva M, *et al.* Vitrification of in vitro mature alpaca oocyte: effect of ethylene glycol concentration and

time of exposure in the equilibration and vitrification solutions. Anim Reprod Sci; 2013. 143:72-8.

- Russo R, Monaco D, Rubessa M, El-Bahrawy KA, El-Sayed A, Martino NA, *et al*. Confocal fluorescence assessment of bioenergy/redox status of dromedary camel (*Camelus dromedarius*) oocytes before and after in vitro maturation. Reprod Biol Endocrin; 2014. 12.
- Sansinena MJ, Taylor SA, Taylor PJ, Denniston RS, Godke R. Production of nuclear transfer llama (*Lama glama*) embryos from in vitro matured llama oocytes. Cloning Stem Cells; 2003. 5:191-198.
- Sansinena MJ, Taylor SA, Taylor PJ, Schmidt EE, Denniston RS, Godke RA. In vitro production of llama (*Lama glama*) embryos by intracytoplasmic sperm injection: Effect of chemical activation treatments and culture conditions. Anim Reprod Sci; 2007. 99:342-353.
- Silva M, Ulloa-Leal C, Norambuena C, Fernández A, Adams GP, Ratto MH. Ovulation-inducing factor (OIF/NGF) from seminal plasma origin enhances Corpus Luteum function in llamas regardless the preovulatory follicle diameter. Anim Reprod Sci; 2014. 148:221-227.
- Skidmore J, Allen WR, Cooper MJ, Chaudhry MA, Billah M, Billah AM. The recovery and transfer of embryos in the dromedary camel: results of preliminary experiments. Proc 1st int Camel Conf, Dubai, UAE. 1992:137-42.
- Skidmore JA. Reproductive physiology in female Old World Camelids. Anim Reprod Sci; 2011. 124:148-154.
- Skidmore JA, Billah M. Embryo transfer in the dromedary camel (*Camelus dromedarius*) using asynchronous, meclofenamic acid-treated recipients. Reprod Fertil Dev; 2005. 17:417-421.
- Skidmore JA, Billah M. Embryo transfer in the dromedary camel (*Camelus dromedarius*) using non-ovulated and ovulated, asynchronous progesterone-treated recipients. Reprod Fert Develop; 2011. 23:438-443.
- Skidmore JA, Allen WR, Heap RB. Oestrogen synthesis by the peri-implantation conceptus of the one-humped camel (*Camelus dromedarius*). Journal of Reproduction and Fertility; 1994. 101:363-367.
- Skidmore JA, Billah M, Allen WR. Investigation of factors affecting pregnancy rate after embryo transfer in the dromedary camel. Reprod Fert Develop; 2002. 14:109-116.
- Skidmore JA, Billah M, Loskutoff NM. Developmental competence in vitro and in vivo of cryopreserved, hatched blastocysts from the dromedary camel (*Camelus dromedarius*). Reprod Fert Develop; 2004. 16:605-609.
- Skidmore JA, Billah M, Loskutoff NM. Comparison of two different methods for the vitrification of hatched blastocysts from the dromedary camel (*Camelus dromedarius*). Reprod Fert Develop; 2005.17:523-527.
- Skidmore JA, Schoevers E, Stout TAE. Effect of different methods of cryopreservation on the cytoskeletal integrity of dromedary camel (*Camelus dromedarius*) embryos. Anim Reprod Sci; 2009. 113:196-204.
- Stekleniov EP. Des particularites anatomo-morphologiques de la structure et des fonctions physiologiques des trompes de Fallope chez les camelides (genres Lama et Camelus). 6th ICAR. 1968;1:74.
- Sumar J, Picha Y, Arellano P, Montenegro V, Landone P, Rodriguez C, *et al*. Effect of recipient lactation status on pregnancy rate following embryo transfer in alpacas (*Vicugna Pacos*). Clinical Theriogenology; 2010. 2:399.
- Sumar JB. Embryo transfer in domestic South American camelids. Anim Reprod Sci; 2013.136:170-177.
- Sutmoller P. Risk of disease transmission by llama embryos. Rev Sci Tech Oie; 1999. 18:719-728.
- Thibault C. Sperm Transport and Storage in Vertebrates. J Reprod Fert; Suppl. 1973.18:39-53.
- Tibary A. Embryo Transfer in Camelids. Proceedings of the Annual Conference of the Society for Theriogenology. Vancouver, BC, Canada, September 12-15, 2001. p. 379-86.
- Tibary A, Anouassi A. Ultrasonographic changes of the reproductive tract in the female camel (*Camelus dromedarius*) during the follicular cycle and pregnancy. J Camel Pract Res; 1996. 3:71-90.
- Tibary A, Anouassi A. Artificial Breeding and Manipulation of Reproduction in Camelidae. In: Tibary A, editor. Theriogenology in Camelidae: Anatomy, Physiology, BSE, Pathology and artificial breeding: Actes Editions, Institut Agronomique et Veterinaire Hassan II; 1997. p. 413-452.
- Tibary A, Anouassi A. Maintenance of pregnancy in dromedary embryo recipients following induction of new corpora lutea. Proceeding of the annual meeting of the Society for Theriogenology Sptember 22-24, 1999, Nashville TN P 14. 1999.
- Tibary A, Pearson L. Pregnancy and pregnancy loss in camelids. Proceedings of the First Latin American Society for Animal Reproduction Congress. Buenos Aires, Argentina, March 25-28, 2015.pp 67-95
- Tibary A, Anouassi A, Khatir H. Update on reproductive biotechnologies in small ruminants and camelids. Theriogenology; 2005. 64:618-638.
- Tibary A, Picha Y, Pearson LK. Embryo transfer in camelids. Large animal Proceedings of the North American Veterinary Conference, Orlando, Florida, USA, 15-19 January 2011. 2011:316-319.

- Tibary A, Campbell A, Pearson L. Evolution of embryo transfer in domestic animals. Spermova; 2013. 3:1-9.
- Tibary A, Pearson LK, Anouassi A. Cesarean section in camels (*Camelus dromedarius*): Complications and post-surgical fertility. Proceedings of the Society for Theriogenology and American College of Theriogenologists Annual Conference, San Antonio, TX, Aug 5-8, 2015.
- Tibary A, Anouassi A, Sghiri A, Khatir H. Current knowledge and future challenges in camelid reproduction. Society of Reproduction and Fertility, Volume 64. Nottingham: Nottingham University Press; 2007. p. 297-313.
- Trasorras V, Giuliano S, Miragaya M. In vitro production of embryos in South American camelids. Anim Reprod Sci; 2013. 136:187-193.
- Trasorras V, Chaves MG, Neild D, Gambarotta M, Aba M, Aguero A. Embryo transfer technique: Factors affecting the viability of the corpus luteum in llamas. Anim Reprod Sci; 2010. 121:279-285.
- Trasorras V, Chaves G, Miragaya M, Neild D, Gambarotta M, Agüero A. Effect of exogenous estradiol on maternal recognition of pregnancy after embryo transfer in llamas. Journal of Camelid Science; 2011. 4:79-82.
- Trasorras V, Castex CB, Alonso A, Giuliano S, Cruz RS, Arraztoa C, *et al*. First llama (*Lama glama*) pregnancy obtained after in vitro fertilization and in vitro culture of gametes from live animals. Anim Reprod Sci; 2014. 148:83-89.
- Trasorras V, Giuliano S, Chaves G, Neild D, Aguero A, Carretero M, *et al*. In vitro Embryo Production in Llamas (*Lama glama)* from In vivo Matured Oocytes with Raw Semen Processed with Androcoll-E using Defined Embryo Culture Media. Reprod Domest Anim; 2012. 47:562-567.
- Ulloa-Leal C, Bogle OA, Adams GP, Ratto MH. Luteotrophic effect of ovulation-inducing factor/nerve growth factor present in the seminal plasma of llamas. Theriogenology; 2014. 81:1101-1117.
- Vásquez E M, Cueva M S, Cordero R A, Gonzales C ML, Huanca L W. Evaluation of two embryo cryopreservation methods in llama on the in vivo e in vitro embryonic survival rates. Revista de Investigaciones Veterinarias del Perú (RIVEP); 2011. 22:190-198.
- Vaughan J. Ovarian function in South American camelids (alpacas, llamas, vicunas, guanacos). Anim Reprod Sci; 2011.124:237-243.
- Vaughan J, Mihm M, Wittek T. Factors influencing embryo transfer success in alpacas-A retrospective study. Anim Reprod Sci; 2013. 136:194-204.
- Vaughan JL. Ovarian function in South American camelids (alpacas, llamas, vicunas, guanacos). Anim Reprod Sci; 2011.124:237-243.
- Vaughan JL, Tibary A. Reproduction in female South American camelids: a review and clinical observations. Small Ruminant Res; 2006. 61:259-281.
- Vivanco W, Ponce D, Miguel M, Youngs C, Huaman E, Leon S, *et al*. Repeatability of the orvairan reponse and embryo production in superovulated Huacaya alpacas. Spermova; 2014. 4:77-79.
- Wani NA. Chemical activation of in vitro matured dromedary camel (*Camelus dromedarius*) oocytes: optimization of protocols. Theriogenology; 2008. 69:591-602.
- Wani NA. In vitro embryo production in camel (*Camelus dromedarius*) from in vitro matured oocytes fertilized with epididymal spermatozoa stored at 4 degrees C. Anim Reprod Sci; 2009. 111:69-79.
- Wani NA, Wernery U. In Vitro Maturation of Dromedary (*Camelus dromedarius*) Oocytes: Effect of Different Protein Supplementations and Epidermal Growth Factor. Reprod Domest Anim; 2010. 45:E189-E93.
- Wani NA, Skidmore JA. Ultrasonographic-guided retrieval of cumulus oocyte complexes after super-stimulation in dromedary camel (*Camelus dromedarius*). Theriogenology; 2010. 74:436-442.
- Wani NA, Wernery U, Hassan FAH, Wernery R, Skidmore JA. Production of the First Cloned Camel by Somatic Cell Nuclear Transfer. Biol Reprod; 2010. 82:373-379.
- Wiepz DW, Chapman RJ. Non-Surgical Embryo Transfer and Live Birth in a Llama. Theriogenology; 1985. 24:251-257.
- Yagil R, van Creveld C. Embryo Transfer Technology in Camels (*C. dromedarius*). Why and How. Proceedings of the workshop 'Is Possible to Improve the Reproductive Performance of the Camel?' - Paris. 1990. pages293-311.
- Zhao ZJ, Ouyang YC, Nan CL, Lei ZL, Song XF, Sun QY, *et al*. Rabbit oocyte cytoplasm supports development of nuclear transfer embryos derived from the somatic cells of the camel and Tibetan antelope. J Reprod Develop; 2006. 52:449-459.
- Zhou HM, Guo ZH. Heterogeneous nuclear-transferred-embryos reconstructed with camel (*Camelus bactrianus*) skin fibroblasts and enucleated ovine oocytes and their development H-M. Anim Reprod Sci; 2006. 95:324-330.
- Zhou HM, Guo ZH. Heterogeneous nuclear transfer embryos reconstructed by bovine oocytes and camel (*Camelus bactrianus*) skin fibroblasts and their subsequent development. In Vitro Cell Dev-An; 2006. 42:16-19.