Tropical and Subtropical Agroecosystems

REVIEW [REVISIÓN DE LITERATURA]

ARTIFICIAL INSEMINATION IN *CAMELIDAE*

[INSEMINACIÓN ARTIFICIAL EN CAMELIDAE]

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SUMMARY

The most important problems of Artificial Insemination (AI) in *Camelidae* is its timing in relation to ovulation in the she-camel. The present article reviewed collection of semen, processing of semen, manipulation of the female and semen deposition technique in *Camelidae* species. Commonly, semen is collected by electroejaculation, artificial vagina (AV), flushing of the epididymus with saline solution, while the more accepted methods are the former two methods. Semen is usually used in raw condition or after extension, depending on the method of semen processing. In the fresh raw method, whole semen is used within minutes or after few hours. Extension of the semen ejaculate is carried out by adding extenders and it is required in more efficient use of AI, in short-term preservation or liquid semen (within a few hours or days) and long-term preservation or frozen semen (months or years). In short-term preservation, semen is used extended under different temperatures (30, 25 or 4°C). Long-term preservation is carried out by cryopreservation. Packaging methods such as pellets, ampoules or in plastic straws with different volumes (0.25, 0.5 or 4 ml) represent different freezing procedures. The quality and survival of spermatozoa of post-thaw semen are highly variable from one male to the other, even after using the same freezing technique. To ensure that the inseminated females ovulate, hormonal manipulation of ovarian activity is used such as the induction of follicular activity and ovulation, as well as, synchronization of these phases in a group of females. The best time for insemination can only be determined by ultrasonography and/or rectal palpation of the ovaries. The other alternative is to inseminate at known intervals following induction of ovulation by hormonal treatment with human-chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (Gn-RH).

Keywords: *Camelidae*, semen collection, semen preservation, female breeding management, artificial insemination

RESUMEN

El problema más importante de la inseminación artificial (AI) en Camelidae es su sincronización con la ovulación en la hembra. La presente revisión aborda la colecta de semen, procesamiento, manipulación de la hembra y las técnicas para deposición de semen en camélidos. Comúnmente el semen es colectado por electroeyaculación, vagina artificial y lavado del epidídimo con solución salina, siendo los dos primeros los métodos más aceptados. El semen usualmente es empleado sin tratamiento alguno o después de ser procesado. Cuando se emplea fresco, el semen es empleado después de algunos minutos o pocas horas. Se usan como diluyentes para un uso más eficiente de la AI, preservarlo por tiempos reducidos en forma líquida (horas o días) o preservación a largo plazo o semen congelado (meses o años). Cuando se usa en el corto plazo, las temperaturas de uso fluctuan (20, 25 o 4 °C). La conservación a largo plazo es a través de la criopreservación. Se han empleado diferentes métodos de empacado como los pellets, ampolletas o pojillas de diferentes volúmenes (0.25, 0.5 o 4 ml) con diversos protocolos de congelado. La calidad y sobrevivencia del esperma descongelado es altamente variable de un individuo a otro, incluso cuando se ha empleado el mismo protocolo de conservación para ambos. Para asegurarse que la hembra inseminada ovule es necesario emplear manipulación hormonal de la actividad ovárica. Los tratamientos incluyen inducción de actividad folicular y ovulación, así como también la sincronización de estas fases en grupos de hembras. El mejor momento para inseminar sólo puede ser determinado por ultrasonografía y/o palpación rectal de los ovarios. Otra alternativa es inseminar a intervalos preestablecidos después del tratamiento hormonal (usualmente gonadotropina corionica humana (hCG) ó factor liberaro de gonadotropina (Gn-RH).

Palabras clave: *Camelidae*, colecta de semen, preservación, manejo reproductivo de la hembra, inseminación artificial.

INTRODUCTION

Camelidae (*Dromedary, Bactrian, guanaco, Llama, alpaca* and *vicuna*) as induced ovulators offer great prospects of natural synchronization of oestrus, solve problems of oestrous detection and has made AI more convenient and attractive (Helmy, 1991, Minoia *et al*., 1992).

However, a major difficulty with camel AI is to ensure that the inseminated females ovulate (Chaudhary, 1995). In other words, the most important aspect of AI in *Camelidae* is its timing in relation to ovulation in the she-camel.

The first offspring from AI in *Camelidae* was reported in a *Bactrian* camel inseminated with frozen semen collected by electroejaculation (Elliot, 1961). However, it was not until the last 2 decades that this breeding technique started to be used more frequently in the mentioned breed (Chen and Yuen, 1984; Chen *et al.,* 1984, 1985; Xu *et al.,* 1985, 1987; Zhao *et al.,* 1990, 1992, 1994, 1996b). In the *Dromedary*, many authors have studied semen preservation but actual artificial insemination trials were very rare (Anouassi *et al.,* 1992).

In the present article, collection and preservation of semen, manipulation of the female and semen deposition technique in *Camelidae*, will be discussed.

Semen collection

Collection of semen from the male camel is complicated by the position during mating and long duration of copulation (5-50 min). Copulation takes place with the female in recumbent position, while the male squats behind and dictates the duration of copulation.

Commonly, semen is collected by electroejaculation, artificial vagina (AV), flushing of the epididymus with saline solution, intraviginal spongs or passeries, postcoital vaginal aspiration and fistulation of the urethra (Sumar, 1991), while the more accepted methods are the former two methods.

For semen collection using AV, a shortened bull vagina (30 cm long, 5 cm internal diameter) gave the best results (Zeidan, 1999, 2002; Bravo *et al*., 2000). Such a shortened AV allows the semen to pass directly into the collection flask and avoids the ejaculate contact with the rubber liner of the AV which adversely affects sperm mobility. Alternatively, an additional disposable plastic inner liner may be inserted to avoid direct contact with the rubber material. However, it has been revealed at National Research Centre on Camel, Bikaner, India, that there is no significant difference in sperm motility when semen is collected with traditional AV where ejaculate comes in contact with rubber liner or modified AV where ejaculate pass directly into the collection flask. Particularly, the AV is filled with water at 55-60°C to give an internal temperature of 41-43°C. A clear glass water-jacketed (35-37°C) semen vessel is attached to the apex of the cone-shaped internal latex rubber liner to enable visualization of the ejaculation. The male is then led up from behind to the sitting female with the operator sitting on the left side of the female. As soon as the male has sat down on the female and makes a few thrusts, the operator grasps the male's sheath and directs his penis into the artificial vagina (AV) and holds it there by manual pressure at the base of the scrotum. The male will make several thrusts, interspersed by periods of rest, until ejaculation is completed. The ejaculate usually occurs in fractions and this whole process can take between 5 and 10 min, although it may occasionally last for 20 min or even longer. The method that provides the most natural and physiological sample of semen is the use of AV mounted inside a dummy (Sumer and Leyva, 1981). This AV is similar to the AV for sheep, but stimulating the cervix by means of a coil spring mounted inside the lower part of a dummy or phantom. The coil spring was a "sine qua non requisite" to obtain semen (Franco *et al*., 1981). The warm water was changed each 10 min or the AV was wrapped with a heated pad (called modified AV by some authors), to maintain the temperature.

Observation of natural mating suggested that the highly mobile urethral process of the camel penis may need to gain entry to the cervix to stimulate ejaculation during the extended copulatory olfactory contact. Observation of natural mating suggested that the highly mobile urethral process of the camel penis may need to gain entry to the cervix to stimulate ejaculation during the extended copulatory olfactory.

Electroejaculation may be employed if collection by AV cannot be achieved, using a standard bovine ejaculator (Standard Precision Electronics, Denver). The male is secured in sternal recumbence and then turned on his side as described by Tingari *et al*. (1986). Collection of semen can be done with or without the use of sedative and analgesic, depending on the temperament of the individual male. Domosedan [(Detomidine hydrochloride, 30-35μg/kg body weight (bwt), intravenous (i.v.), or 70-80μg/kg bwt, intramuscular (i.m.)] was found to be superior to other sedatives such as xylazine and acepromazine (Jochle *et al.,* 1990). Ejaculation can be achieved by using the rectal probe lubricated with a copious amount of jelly to ensure good contact with the mucosa with using two sets of stimulation, each of 10-15 pulses of 3-4 s duration at 12 V and 180 mA with a rest of 2-3 min between the two series of impulses. The semen is collected into a flask held at the prepuce

orifice with occasional milking of the prepuce to expel all semen. The volume of semen collected by electroejaculation is usually less than that obtained by AV, but the other semen parameters are similar. Regarding the epididymal semen collection, each epididymal caudal region was cut to allow the escape of its contents in buffer citrate solution (0.9% NaCl) as the method described by Zeidan *et al.* (2000).

Semen processing and preservation

Semen is usually used raw or after extension, depending on the method of semen processing. Particularly, semen–ejaculate volume of the male dromedary camel ranges, in general, between 5 and 22 ml (Wilson, 1984) and between 5.3 and 3.5 ml in breeding to non-breeding seasons, respectively (Rai et al., 1997). Zeidan (1999), Ahmadi (2001) and Zeidan *et al.* (2001) found that semen – ejaculate volume was 7.82, 8.12 and 7.94 ml at ages of 2.5 to 5, over 5 to 10 and over 10 to 20 years, respectively. When semen was collected by artificial vagina and electro–ejaculation, semen-ejaculate volume values were 3.92 and 8.47 ml, respectively (Alfuraiji, 1999). In the Bactrian camel, variability of semen volume amongst males is well established. Average ejaculate volume values obtained by an artificial vagina was 4.35 ml (range 1.0-12.5 ml) (Chen *et al.*, 1990 and Zhao *et al.*, 1992 and 1994). Chen *et al.* (1990) estimated the mean volume values of the ejaculate were 2.55, 2.62, 3.12, 4.44 and 4.94 ml for 5 different Bacterian males. Sieme *et al.* (1990) and Willmen *et al.*(1993) also reported that ejaculate volume is significantly lower if collected by electroejaculation.

Whole semen

In the whole semen method, semen is used raw within minutes or after few hours. Such method is usually used to overcome behavioural problems or size incompatibility in natural breeding (Tibary and Anouassi, 1997).

Semen can be kept at room temperature (37°C) until insemination, to be used within a few minutes of collection (Anouassi *et al.,* 1992).

Short-term semen preservation (liquid semen)

In short-term preservation (a few hours or days) of *Camelidae,* semen is extended at different temperatures (30, 25 or 4°C). Extension of the ejaculate is required in more efficient use of AI and in short-term (few days) and long-term preservation (months or years).

Semen extension is carried out to obtain a known concentration of spermatozoa such as 50 x 10^{6}/ml (Anouassi *et al.,* 1992), by adding the extender at a ratio of 1 : 1 to 1 : 3 (semen : extender) depending on the concentration of the ejaculate, in the *Dromedary* and *Bactrian* camels (Anouassi *et al.,* 1992; Musa *et al.,* 1992). Extenders are added at a temperature of 30 to 35°C to the semen, but after completely liquefaction before adding the extender (in order to obtain a good mixture).

Various extenders have showed good results for liquid preservation of camel semen. Such extenders include Laiciphos^{TM} (Cassou, 1959), Androlep^{TM} (Waberski *et al.,* 1989), sodium citrate egg yolk extender (Kupper, 1954) and the dimitropolus^{TM} (Rasbech, 1984). Egg yolk and lactose extenders appeared to be more suitable to preserve camel semen in the liquid state (Sieme *et al.,* 1990; Anouassi *et al.,* 1992; Musa *et al.,* 1992; Zeidan, 2002; Vyas *et al.*, 1998). In general, all extenders containing lactose or egg yolk are suitable for the short-term preservation of *Camelidae* semen. However, the authors have not come across research articles regarding effect of physical and chemical properties of the extender, i.e. pH, ionic strength and osmotic pressure on the motility and fertilizing ability of preserved semen.

Extension of freshly collected semen can be carried out by several extenders (Table 1). Most of these extenders are adapted from that applied in other species and usually contain a source of energy (glucose or fructose), a protein for protection against cold shock (lipoprotein from egg yolk or casein from milk), buffer and antibiotics.

Extended fresh semen has been preserved in plastic tubes and stored at 4°C for a maximum of 26 hrs (Musa *et al.,* 1993). For longer preservation in a liquid form (up to 48 hrs), semen should be cooled slowly to 4 or 5°C. Slow cooling of the semen can be achieved by placing the tube containing extended semen into a water bath at room temperature and placing it in the refrigerator. This system allows cooling of the extended semen to 5°C during 1.5 to 2.0 hrs. Equitainer system used for chilling equine semen was found to be suitable to keep good motility for at least 18 hrs.

Table 1. Extenders used for extension of *Camelidae* semen for short term preservation.

Species	Extender	Remarks	Reference
Llama pacos, V. vicugna	Heat treated milk	Semen collected by flushing of the epididymis	Fernandez-Baca (1993)
Camelus dromedarius	Commercial bull extender Dimitropolous 11 Sodium citrate Glucose-EDTA Skim milk	Semen collected by electroejaculation or artificial vagina	Sieme *et al.* (1990) Musa *et al.* (1992) Musa *et al.* (1993) Zeidan (2002)
	Sodium-citrate-EY Lactose-egg yolk	Semen collected with artificial vagina	Anouassi *et al.* (1992) Zeidan (2002)

Long-term preservation (frozen semen)

Semen cryopreservation is the best preservation technique in any species, due to the following advantages (Chen *et al*., 1990): a. preservation for almost indefinite time, b. extended use of semen, even long after the male's death, c. easiness of transportation and international exchange and d. more efficient use of male. Most of the information available on semen cryopreservation are on the *bactrian* and *dromedary* camels.

Extenders used for deep freezing of the *Dromedary* and *Bactrian* camels semen have been adapted from other species (Table 2). The major difference between extenders used for preservation of semen at the liquid state and those used for freezing is the presence of cryoprotectant substances, although lipoproteins from egg yolk or milk provide same protection from the cold temperatures. Particularly, glycerol as a cryoprotectant has been added to protect semen from chilling injury during cryopreservation. However, although that the final concentration of glycerol in the extender and the method of addition to the semen are critical for the survival of spermatozoa and maintenance of its fertilizing ability, there are factors such other cryoprotectants or methods (fast or slow cryoprotection) used to cryoconserve semen which can affect survival rate of spermatozoa or fertilization, but no studies have been carried out for determination of these factors in semen cropreservataion of *Camelidae*.

The best extender for cryopreservation (freezing) *dromedary* and *bactrian* camel semen was found to be a modified boar or stallion semen extender, depending on assessment of post-thaw motility and morphology (Sieme *et al.,* 1990). This technique uses two extenders (a cooling extender and a freezing extender). The cooling extender is added to the semen immediately after collection (Table 2). The freezing extender contains the cooling extender in addition to a cryoprotectant (glycerol) and an emulsifying agent (orvus paste) that plays a role in the stabilization of the sperm plasma membrane. Particularly, Zhao *et al.* (1996b) obtained the best post-thaw motility and acrosomal integrity for freezing of *Bactrian* camel semen by using the sucrose extender containing 7% glycerol, followed by stallion, swine and sucrose extender, with only 3.5% glycerol (Table 2). Extension rates of semen vary from 1 : 1 to 1 : 8 (semen : extender) in the Llama and from 1 : 2 to 1 : 3 in the *Dromedary* and *Bactrian* camels (Chen *et al.,* 1990; Zhao *et al.,* 1994, 1996b).

Cryopreservation procedures

Cryopreservation procedure depends on the packaging method used, whether it is. pellets (Graham *et al.,* 1978), ampoules (Chen *et al.,* 1990; Zhao *et al.,* 1994, 1996b) or in plastic straws with different volumes (0.25, 0.5 or 4ml) (Sieme *et al.,* 1990; Musa *et al.,* 1992; Willmen *et al.,* 1993). The packaging method affects both the freezing and the thawing rates.

Semen pellets are obtained by dropping a known volume (0.1 or 0.2 ml) of extended semen into depressions made in dry ice. The pellets formed by freezing for a few seconds after contact of semen with the dry ice. Changing the volume of semen, is the only way to change the freezing rate of pellets (Graham *et al.,* 1978). However, this technique is rarely used due to the difficulties in labeling the semen, the inability to modify the freezing rate and the impossibility of properly labeling the semen.

Packaging in 1.5 ml ampoules is the most commonly used technique for freezing *Bactrian* camel semen in China (Chen and Yuan, 1979; Zhao *et al.,* 1994, 1996b). The technique is carried out in the following steps: equilibration at each of 37°C for 10 minutes, 20°C for 10 minutes, 10°C for 10 minutes and 4°C for 4 hours. Freezing is carried out on a wire grid placed above liquid nitrogen at 3cm for 3 minutes, at 2 cm (-75°C) for 2 minutes, at 1cm (-175°C) for 1 minute, then plunging into liquid nitrogen (-196°C).

Table 2. Extenders used for the freezing of *Camelidae* semen.

Species	Extender		Reference
Camelus batriamus	SYG 1 :		
	Sucrose 12%	85.5ml	Chen *et al.* (1990)
	Egg yolk	10.0ml	Sun *et al.* (1990)
	Glycerol	3.5ml	Zhao *et al.* (1994)
	Penicillin	1000 IU	Zhao *et al* (1996b)
	Streptomycin	1000µg/ml	
	SYG 2 :		
	Sucrose 12%	73.0ml	Zhao *et al.* (1991)
	Egg yolk	20.0ml	Zhao *et al.* (1996b)
	Glycerol	7.0ml	
	Penicillin	1000IU/ml	
	Streptomycin	1000 µg/ml	
Camelus dromedarius	Cooling extender :		
Camelus bactrianus	Lactose 11%	80ml	Sieme *et al.* (1990)
	Egg yolk	20ml	Musa *et al.* (1992)
	Freezing extender :		Musa *et al.* (1993)
	Cooling extender	95.5ml	Willmen *et al.* (1993)
	Glycerol	6.0ml	
	OEP-Equex	1.5ml	
L. lama, C. dromedarius	Tris-glycerol-egg yolk		Graham *et al.* (1978)

Straws are usually frozen by placing them on a rack at known distances above the surface of liquid nitrogen. The fastest freezing rates are obtained by the use of a small volume (0.25 or 0.5 ml). Freezing rates can be modified by adjustment of the elevation of the straws. Precise freezing rates can be achieved in semen packaged in straws by using a computerized freezer that can be programmed to follow a precise freezing curve. The samples are placed on a platform which is lowered stepwise toward the liquid nitrogen surface. The temperature inside the samples is monitored by a thermocouple which guides the movement of the platform. Packaging the semen of the *Dromedary* and *Bactrian* camels is carried out in 4-ml straws according to Sieme *et al.* (1990), Musa *et al.* (1992) and Willmen *et al.* (1993) as follows. Equilibration of raw semen at 25 to 37°C until liquefaction which occurs during 7 to 20 min (Musa *et al.*, 1993), after 90 min (Hassan *et al.*, 1995) or after 8 hr (Tibary and Anouassi, 1997), initial extension with the cooling extender as shown in Table 2 (1 volume semen : 1 volume extender), then cooling to 15°C over a 2 hr period. Second extension with the freezing extender to a concentration of 150 x 10^6/ml, then cooling to 5°C during a period of 1.5 hrs. Final extension with the freezing extender to a concentration of 100 x 10^6/ml and packaging in large straws (4ml) and freezing by placing the straws in liquid nitrogen vapour for 20 minutes (allowing them to reach a temperature of 120°C, then plunging into liquid nitrogen). A simplified technique of freezing of semen packaged in 0.25 or 0.5 ml straws can be carried out as follows. Cooling the extended semen during one hour and keeping it at this temperature for 2 hours before packaging in straws. The straws are placed on a rack, 4 cm above the liquid nitrogen surface for 10 minutes, then inserted directly into liquid nitrogen.

Thawing

Thawing is carried out in a water bath. Thawing rates vary according to packaging technique used. Pellets are usually thawed by dropping into heated receptacles or by mixing in a warm thawing extender. Semen frozen in ampoules is thawed by placing in a water bath set at 45 to 55°C for 30 seconds to 1 minute (Chen *et al.,* 1990; Zhao *et al.,* 1996b). Small straws are thawed in a water bath at 37°C for 30 to 40 seconds or 40°C for 8 seconds. Large straws are thawed by continuous agitation in a water bath at 40°C for 50 seconds. The quality of thawed *Dromedary* camel semen is improved by freezing in small straws (0.25ml), but the use of larger straws is recommended to ensure deposition of an adequate amount of semen in the uterus, which is necessary for inducing ovulation (Bravo *et al.,* 2000).

Post-thaw semen quality and survival of spermatozoa are highly variable from one male to the other, even after using the same freezing technique. Llama semen

frozen in pellets and thawed in warm extender showed 45% post-thaw motility (pre-freezing motility 50%). Motility of frozen semen remained acceptable even after 8 years of storage (Graham *et al.,* 1978). In the *Dromedary*, very little loss is caused by freezing if the initial semen motility is very good. Semen frozen in lactose-egg-yolk extenders has maintained the same post-thaw motility after 6 years of storage in liquid nitrogen. In the *Bactrian* camel, it is recommended that only semen showing 30% or more motility should be used for insemination (Chen *et al.,* 1990). Motility usually remains unchanged after extension, but decreases after equilibration and freezing. Motility before freezing ranges between 75 and 85% and decreases to 67 to 79% after equilibration in SYG1 and SYG2 extenders, respectively (Chen *et al.,* 1990; Zhao *et al.,* 1994, 1996b). Survival of spermatozoa after thawing by incubation of the thawed semen at 37°C or 4°C was very variable from one male to another (Chen *et al.,* 1990). In addition to post-thaw motility, acrosome integrity should be evaluated in all frozen-thawed samples (Sieme *et al.,* 1990; Zhao *et al.,* 1996b). Factors that influence the quality of semen after freezing and thawing have not yet been thoroughly investigated (Zhao *et al.*, 1996b).

Manipulation of females

A major difficulty with *Camelidae* AI is ensuring that the inseminated females ovulate (Chaudhary, 1995), i.e. harmonization timing of insemination and ovulation.

Ovulation in she-camel is induced by copulation and/or semen deposition in the female genital tract. Studies in the *bactrian* camel showed that the Gn-RH-like factor present in semen is responsible for the induction of ovulation. This means that a sufficient amount of semen has to be deposited in the uterus at insemination in order to obtain ovulation. In the *bactrian* camel, at least 1 ml of semen is needed in order to achieve ovulation (although this is not a viable solution, due to that using a high volume of semen will reduce the number of females inseminated per ejaculate).

In this respect, hormonal treatment can be used in breeding management of the she-camel. Hormonal manipulation of ovarian activity treatments include the induction of follicular activity and ovulation, as well as, synchronization of these phases in a group of females.

Induction of follicular activity

Induction of follicular growth can be obtained by administration of follicle stimulating hormone (FSH) or hormones with FSH-like activity such as equine chorionic gonadotropin (eCG). Further, FSH or eCG can be used to advance puberty (Yagil and Etzion, 1984; Rai *et al.,* 1990), induce ovarian activity during the postpartum period (Elias, 1990; Dahir *et al.,* 1990) or during seasonal anoestrus (Arthur and Al-Rahim, 1982; Elias *et al.,* 1985; Dahir *et al.,* 1990). Treatments of eCG are based on either single or double intramuscular injections of 1500 to 7000 IU. Appearance of mature follicles usually occurs between 5 and 10 days post-treatment.

Induction of ovulation

Ovulation is normally induced by copulation in the *Camelidae* female during mating with either an intact or vasectomized male (Marie and Anouassi, 1987). Stimulation of the release of sufficient luteinizing hormone (LH) from the anterior pituitary gland to cause ovulation could also be carried out by manual stimulation of the cervix and inrauterine injection of either whole semen, seminal plasma or prostaglandin (Musa and Abusineina, 1978; Sheldrick *et al.,* 1992; Abu Zead, 1995).

The ovulatory response in the camel could be a result to a combination of stimuli, including a chemical factor in the seminal plasma, neurohormonal responses to the chemical stimuli of the coitus and the male effect (Marie and Anouassi, 1987; Anouassi *et al.,* 1992; Moslah *et al.,* 1992; Sheldrick *et al.,* 1992), since the mechanical stimulation of the cervix which triggers ovulation in the cat and rabbit species were not useful in induction of ovulation in the camel (Musa and Abusineina, 1978; Elias *et al.,* 1984; Musa *et al.,* 1990).

Ovulation rates and pregnancy were found to be significantly higher in inseminated she-camels that had been mated by vasectomized male (Anouassi *et al.,* 1992). However, this is not a practical method because of the risk of transmission of venereal and other diseases.

Mckinnon and Tinson (1992) suggested to induce ovulation following AI with either 3000 IU HCG, 20µg GnRH analogue or its analogue Busereline. Other studies showed that ovulation can be induced 26 to 28 hrs following injection of either 0.5 to 1.0 mg Gn-RH, in the *Dromedary* and *Bactrian* camels (Bono *et al.,* 1985; Elias, 1990; Anouassi *et al.,* 1994) or 15 to 20 µg Busereline (Cooper *et al.,* 1990, 1992; Mckinnon and Tinson, 1992; Skidmore *et al.,* 1992; Musa *et al.,* 1993; Skidmore *et al.,* 1996). Ovulation can also be induced by intravenous or intramuscular administration of HCG with dosages of 2500 to 4000 IU.

Response rates in these treatments vary between 85 and 100% in the *dromedary* and *bactrian* camels depending on the basis of follicular size and uterine

tone (Chen and Yuen, 1984; Chen *et al.,* 1985; Anouassi and Ali, 1990; Cooper *et al.,* 1990, 1992, McKinnon and Tinson, 1992; Skidmore *et al.,* 1992; Ismail *et al.,* 1993; Anouassi *et al.,* 1994; Skidmore *et al.,* 1996).

Synchronization of follicular development and ovulation

Several techniques such as treatment with progesterone, prostaglandin or a combination of both have been used for synchronization of oestrus and ovulation in the she-camel herds.

The use of progesterone releasing intravaginal devices (PRID) alone was not satisfactory for controlling ovarian function (Cooper *et al.,* 1992). It was also found that the use of eCG in doses ranging between 1000 to 8000 IU resulted in a very low number of pregnancies (Elias *et al.,* 1985; Yagil and Etzion, 1984; Rai *et al.,* 1990). Similarly, Progestagen with the use of Progesterone Releasing Intravaginal Devices (PRID, CIRD) and subcutaneous implants (Norgestomet) showed no much success, due to failure to arrest follicular development.

Mckinnon and Tinson (1992) reported that synchronization of oestrus in the *Dromedary* could be successfully carried out by progestin injections. Similarly, natural progesterone in oil seems to give better results at least in the *Dromedary* camel, if administered daily (100 mg per day) for 10 to 15 days. Synchronization of follicular development and ovulation is also improved by administration of eCG (1500 to 2000 IU) one day before, or on the last day of progestagen treatment.

The change in oestradiol-17β and progesterone (during the non-breeding season) is indicative of actual induction and follicular growth, post-treatment (Ismail, 1993).

Semen deposition technique

The above information necessitate to combine reproductive behavioural signs of the female with assessment of ovarian activity by rectal palpation or ultrasonography, before insemination (Anouassi *et al.,* 1992). In other words, the best time for insemination can only be determined by ultrasonography and palpation of the ovaries.

The best alternative is to inseminate at known intervals following induction of ovulation by hormonal treatment (hCG or Gn-RH) (Tibary and Anouassi, 1997).

The optimum time for insemination is the first day of reproductive behavioural when the uterus has a good tone and the follicle is between 12 and 18 mm in diameter in the *Dromedary* and 12 mm in *bactrian* camels (Anouassi and Ali, 1990).

Semen is deposited into uterus by using an insemination gun or a pipette in the same manner described for the bovine or equine species, although the instrument can be guided directly into the cervix by vaginal manipulation it is preferred to use the rectovaginal technique in order to eliminate the risk of contamination. Rectovaginal manipulation is easier in the estrus female, because the uterus is contracted and the cervix is open (Anouassi *et al.,* 1992). Semen should be deposited just cranial to the internal cervical os. In the *bactrian* camel, it is reported that insemination is done using a porcine rubber insemination tube heated to 38°C to prevent cold shock (Chen *et al.,* 1990). When frozen semen is used, a second insemination is performed 24 hrs after first one.

The minimal number of spermatozoa to be used for insemination (semen dosage) has not been determined yet, in *Camelidae*. Chinese authors suggested to use 400 million spermatozoa in the *Bactrian* camel (Chen *et al.,* 1985, 1990; Zhao *et al.,* 1991, 1994, 1996b). However, it is almost certain that these doses can be reduced if ovulation is induced by hormonal treatment and the timing of insemination is determined precisely.

Fertility

In whole semen, fertility rates obtained are variable. In the *dromedary* camel, pregnancy rates with whole semen vary from 30 to 80% (Table 3) depending on the male, ovulation treatment used and timing of insemination (Anouassi *et al.,* 1992; McKinnon and Tinson, 1992; McKinnon *et al.,* 1994). In Alpacas, a pregnancy rate of 2.3% was obtained following intracervical insemination with vicuna semen. The latter low fertility rate was explained by the poor quality of the semen used (Fernandez-Baca and Calderon, 1968). Deposition of semen by rectoraginal mainipulation resulted in a pregnancy rate of 38%. In the South American *Camelidec*, it was found that fertilization rates were the highest when insemination was carried out at 35 to 45 hrs after induction of ovulation treatment. Breeding was to vasectomized males or injection of 500 to 1000 IU hCG or 50 mg Gn-RH (Fernandez-Baca and Colderon, 1968).

Insemination with frozen-thawed semen showed a remarkable fertility in *bactrian*s. The pregnancy rates obtained with frozen-thawed semen after different “systems” of insemination are shown in Table 3. It can be seen that the mean pregnancy rate was higher after

insemination with frozen-thawed semen (95%) than after the natural mating (60-65%) during over 8 years. In addition, it could also be noted that it was possible to use the semen of one male for 450 inseminations by supplementation of semen with Gn-RH (Zhao, 1995; Zhao *et al.,* 1996a). However, the above results are questionable because of very high percentage (95%) with frozen semen than natural mating. In no other domestic species it is above 90% whereas the AI in Camelidae is already being reffered as difficult due to "induced ovulator" factor. In the same Table 4, it is clear that, a double insemination at 24 hrs intervals or a single insemination of 400 million spermatozoa after hCG treatment resulted in very high conception rates (80 to 90%; Zhao *et al.*, 1991, 1992, 1994; 1996b). In *dromedaries*, however, the low pregnancy rates did not reflect the promising post-thaw motility.

Table 3. Pregnancy rates in Bactrian camel after AI with frozen-thawed semen (Semen frozen with SYG-2 diluent; Zhao *et al.,* 1996a).

Insemination system	Number of females inseminated	Number of females ovulated	Number of females pregnant at days 60-90 (%)
Double AI (4+4ml) at 24-h interval[b]	71	68	68 (95.8)
Single AI 24 h after 1000 IU hCG injection	10	10	10 (100)
Single AI 24h after 200 IU LH injection	10	10	10 (100)
Single AI with double dose (8ml)	10	10	10 (100)
Single AI with single dose (4ml)	5	4	4 (80)
Single AI with semen extended 10-fold+200µg Gn-RH/dose[c]	45	45	45 (100)
Single AI with semen extended 15-fold + 200 µg Gn-RH/dose[d]	149	149	138 (93)
Totals and mean	300	287	285 (95)

[a]Dose of inseminate : 4 ml (two 2.0ml ampoules).
[b]Total number of motile sperm inseminated : 1.48×10^9.
[c]Total number of motile sperm inseminated : 290×10^6.
[d]Total number of motile sperm inseminated : 150×10^6.

Table 4. Results of artificial insemination in *Camelidae*.

Species	Extender	Doses (Number of spermatozoa)	Results	Reference
Camelus dromedarius	Raw, whole ejaculate	40 to 300 x 10^7	Ovulation (33%) Pregnancy 1/6	Anouassi *et al.* (1994)
	Lactose-Egg yolk	2ml, 100 x 10^6	Ovulated (20%) Pregnant (10%)	
	Lactose-Egg yolk	2ml, 100 x 10^6, 24 hours after mating with vasectomized male	Ovulated (60%) Pregnant (50%)	
Camelus bactrianus	SYG 1 or SYG 2	400 x 10^6, 37% motility 55% motility	Pregnant, 86% Pregnant, 100%	Chen *et al.* (1990)
	SGY 2	400 x 10^6, AI twice at 24 hour intervals AI once after hCG (1000 IU) 800 x 10^6 single AI 400 x 10^6 single AI	Pregnant, 96% Pregnant, 100% Pregnant, 100% Pregnant, 100%	Zhao *et al.* (1994)
	SYG 2	400 x 10^6	Pregnant, 96.2%	Zhao *et al.* (1996b)

Problems facing development of AI in *Camelidae*

The use of AI in *Camelidae* is, generally, limited. This is due to the problems concerning AI which should to be resolved. These problems can be summarized as follows:

- Lack of adequate methods for semen collection: Discovery of improved semen collection techniques allows a more reliable semen collection (Lichtenwalner *et al.,* 1996).
- Poor post ejaculation sperm motility: This is mainly due to that liquefaction of semen can take several hours, because of the gelatinous nature of *Camelidae* semen (Garnica *et al.,* 1993; Hassan *et al.,* 1995).
- Lack of standard techniques for freezing camel semen: Difficulty in transcervical pipette passage suggest adapting some techniques used in the sheep and goat, such as direct cervical handling and laparoscopic insemination, may help in overcoming such difficulty.

Areas of further research.

In initial semen quality, it is needed to develop reliable semen collection techniques, procedures for semen handling, appropriate frequency of semen collection and reliable semen evaluation techniques. In semen preservation, it is needed to define the suitable properties of extenders, pH, osmotic pressure, glycerol level and ionic strength, optimal semen cryopreservation procedure, cooling rate, equilibration time and method of glycerolization, freezing rate and thawing rates. In management of insemination, it is needed to define the best time for induction of ovulation (Fernandez-Baca *et al.*, 1968; Tibary and Anouassi, 1997), the optimal timing of insemination in relationship to ovulation and the optimal number of live spermatozoa per insemination.

CONCLUSIONS

Under the traditional systems of reproductive management in most *Camelidae* breeding herds, reproductive efficiency is limited, not only by the long gestation period and the short breeding season, but also by the continuing use of the natural mating. Such systems do not result in an optimum number of pregnant females at the end of the season and may also lead to widespread of venereal infections which consequently decrease fertility. The use of AI technique can be employed to impregnate as much females as possible at the start of the breeding season, that permits to be impregnated again, after parturition. In addition, successful freezing of semen and its transport to different countries would lead to the genetic improvement of *Camelidae* stock worldwide.

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