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Prospects of Improving Semen Collection and Preservation from Elite Dromedary Camel Breeds

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ABSTRACT

Low reproductive performance is one of the most important factors affecting camel productivity. The genetic improvement of dromedary camels is still less progressed compared with other productive farm animals. In order to improve productive and reproductive performance of camels, monitoring and increasing efficient selection strategies in concurrent with developing assisted reproductive technologies and establishing a semen bank for elite camel breeds is urgently needed. To this date, semen preservation and its related techniques in dromedary camels has been slow due to the continual preference of natural breeding with elite males, the difficulty of semen collection, the highly viscous nature of camel semen and the low sperm resilience, unable to withstand processing for preservation. The present article is aimed in surveying of the fundamental challenges of semen collection, processing and preservation from dromedary camels. Developing a collection process, improving the rheological characteristics of delivered semen, modification of extenders and optimizations of the preservation protocols have also been discussed.

Key words: Breeding management, Electroejaculation, Camel-dummy, Semen viscosity, Processing, Chilling, Freezing.

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INTRODUCTION

More than 70% of dromedary camels (C. *dromerdarius*) are raised in Arabian countries (FAO, 2009). This Godgiven gift is praised for its strategic stockpile of meat, milk, culture and traditions in Arabian deserts especially under the predicted hazardous effects of climatic changes forthcoming within the next 3-5 decades (Trondalen, 2009). Camels are known to have peculiar physiological features that enable them to survive and reproduce under the harsh desert environmental conditions (El-hassanein, 1989). However, low reproductive performance is one of the most important factors affecting camel productivity. Compared to other productive farm animals, there are still many potential ways available to improve productivity and reproductive performance of Arabian camels. Many and complex hereditary and environmental factors are contributed to low fertility in camels (El-Hassanein, 2003). This low reproductive efficiency could be improved by a better understanding of the reproductive cycle and increased use of assisted reproduction techniques such as Artificial Insemination (AI) and Embryo Transfer (ET) (Skidmore, 2013). Applying AI, particularly with frozen semen, has been by far the most effective reproductive technology for selective genetic improvement in farm animal production. It has many advantages over natural breeding, such as reduction of injuries (during natural mating), facilitation of rapid dissemination of superior genetics, restriction of spreading infectious and transmitted venereal diseases, in addition to prolongation of the reproductive lifespan of males even beyond their death. For this reason, there is a considerable interest in the use of AI in Camel breeding programs. During the last two decades, several scientific efforts have been conducted to utilize AI and ET techniques in improving reproductive performance of elite camel breeds (race and milch breeds). Embryo transfer has been tremendously developed in racing camels since 1990 (Anouassi and Tibary, 2013). However, AI technique has not developed well as a routine method for breeding camels compared with its fast and universal application in other farm animals. This may be due to difficulty in collecting semen from aggressive males in rut, the viscous nature of camel semen (Bravo and Johnson, 1994; Bravo et al., 2000a) and the continued use of traditional natural breeding system in most breeding herds (Skidmore, 2013). It is aimed at articulation of different natural and managerial constraints limiting improvement of productivity and reproductive performance of camels. The need to put into practice a persistent and steady technique for semen collection and

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processing into valuable frozen doses has been discussed. Also, the urgency of establishing a sperm bank for semen of genetically superior camel breeds has been debated for conservation and efficient dissemination of superior genotypes purposes.

Breeding Management in Camel Herds

Under pastoral conditions, the breeding management is usually focused on the male selection at 3-4 years age (3 years in average) to become the new replacement breeding males (Ishag and Ahmed, 2011). Most of the herders select these males from within the herd. Typically, herders select these prospective breeding males according to specific criteria, the most important of which are the male' dam potency in milk production and fitness and the fitness of the male's sire, in addition to their body confirmation, fitness, racing ability, disease resistance and drought tolerance (Farah et al., 2004). As the selected males reach maturity and start to exhibit rutting behavior, herders should isolate them from the herd as the leader breeding male becomes very aggressive towards other competitor males and even humans. The age of sexual maturity of breeding males is averaged seven years while the average age of keeping breeding males in herds is 15 years (14-18 years age) (Ishag and Ahmed, 2011). A rutting male is normally able to mate with about 50 females in a breeding season depending on his *libido*, fecundity and the fertility level of the served females (Elmi, 1989). The male is typically proficient to locate the vulval opening of the female during mating, however, it is common that herders aid the entrance of the male's penis into the female genitalia. Practically, herders forbid the breeding male in herds from mating with his mother; however, they did not prevent the male from mating with his sisters and daughters (Kuria et al., 2011). The practice of selection of breeding males from within the herd and mating the male with his related females may expose the herd to the risks of inbreeding depression and weakness or malformation of calves at birth.

In race camels, herders are usually eager to mate their camels with racing legend studs. Owners of famous males are usually obliged to share breeding with herders with no studding charges. Before the beginning of the autumn breeding season, herders with their females often start to travel hundreds of kilometers to reserve close to a breeding farm having some famous champion males. They may camp there for months to give their females the chance of being mated with these legendary males. Actually, there is a deficiency in formal pedigree records for most of the racing camel herds however; herders can be able to recite the pedigree of their camels for several generations. Missing formal pedigree records makes it difficult to analyze the herd reproductive performance. In addition, mixing of herds from different regions during the breeding season may increase the possibility of transmission of *Brucella* infection which is likely the cause of abortion and reproduction loss in camels.

A conventional system of reproductive management in camel breeding is considered one of the major limits of improving reproductive performance of dromedary camels. These age-old methods make it difficult to ensure an optimum number of females are pregnant at the end of the season and can also lead to widespread of venereal infections with a consequent lowering of fertility (Skidmore, 2013). In a personal experience extended for 15 months (Dec. 2011 - Feb. 2013) working on a royal flock of race camels in Qatar, several constraints have led to submission of my resignation and insistence to accepting it. The main reason for this decision was the inability to accord with the persistent traditional natural breeding of elite males during two successive breeding seasons and the powerlessness to realize an established semen collection and preservation system for the priceless royal male camels. Few days after joining the work in Leawaina Hejen Hospital (LHH) (Dec. 6, 2011), a field visit to the royal breeding farm (about 15 km to the northwest of LHH) was executed. The main impression of this visit was the shock from the emulative congregations of several hundreds of female camels around the farm to have the best chance of being fertilized by any of the royal male camels having a champion reputation. This breeding festival extends from September to April every year for females coming from different regions of the state and from several neighboring states without any care or precautions against infectious and venereal diseases. About 40 famous champion males were subjected to intensive breeding sessions with the possibility of becoming infected with sexually transmitted diseases. Throughout two consecutive breeding seasons, the responders of the breeding farm insistently refused to assign a group of the breeding males to be subjected to a persistent training for collection and preservation of semen in LHH during the period from November to April (the optimal period for collection and processing of camel semen). Their infinite priority was the employment of all the race legend studs in natural breeding rather than their attendance to the newly established AI and ET unit in LHH. The responders start to bring males to LHH near the end of April. Most of the incoming males were often suffering from extremely sexual exhaustion and weakness and many of them had clinically proven to be infected with brucellosis. It is well known that the fertility of males is obviously reduced with increasing number of their consecutive use in mating and the infection of breeding studs with venereal diseases will help in wide spreading of these diseases within the served camel herds.

In the first breeding season (2011-2012), farm responders have been hardly satisfied to bring about 6 of the newly retired males from racing (7-8 years age) to LHH (Feb. 2012) rather than their inclusion to the breeding farm. These males have shortly mastered to mount with the camel dummy and gradually produced semen of a very good quality for

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few weeks before their pulling to the breeding farm in a response to the forceful demand for natural breeding with them. These males were not brought to LHH again.

The persistence on annual appending of the newly retired famous champion males to the natural breeding mission is certainly in opposition to the establishment a sufficient and enduring semen collection and preservation system. Considering the customary principles in any AI-center for productive farm animals (semen cryobank), male camels of desired genetic merits should be fully dedicated to semen collection and preservation program all through their breeding potency rather than to be employed in natural mating. These males will have the greatest contribution to the genetic progress of elite camel breeds if they can be accurately evaluated and selected for applying AI-program in accordance with their sound progeny testing and proper genetic evaluation (Hermas, 1998).

Nature of Copulation in Camels

Coitus in camels differs completely from that process which is carried out in farm animals. In cattle, for example, copulation occurs in the standing posture. The sexually excited male in a full penile erection aggressively jumps on the rear back of a standing female. Just after resting his chest on the female back, he starts to intercourse the erected penis into inside the vagina and suddenly expels semen in one shot. This process is terminated within few seconds and is called "an ejaculation". On the contrary, copulation in camels takes place in the sitting position (sternal recombency). The male in rut leads up from behind over the pelvis of the kneeling female until his front legs are on either side of the female shoulders. Some males start to flex all joints of their hind legs to squat behind the female and then emplace their chests on the female hump, while the others firstly drop their chests on the hump before sitting behind the female. After mounting the kneeling female, the male starts to protrude the forepart of his penis in a verminous movement searching for the vulval cleft of the female. Then, the male starts to erect and direct his penis into inside the vagina and penetrates the cervix with his penis (intromission), followed by pushing himself forward to embrace the female pelvis between his thigh of hindlegs. After full intromission, the male starts to carry out continual rhythmic mild urethral pulses intermediated with several consecutive clusters of a strong whole body strain with urethral pulses (Lichtenwalner et. al., 1996a). These rhythmic urethral pulses and penile strokes can be followed by watching contractions of pelvic, hind legs and abdominal muscles of the male during copulation. Furthermore, if we are using a camel dummy for semen collection, we can observe from underneath the dummy the repeatedly entrance of the penis and influx of semen into the bulbous neck of the semen collection tube (Figure 1) with the corresponding penile strokes. This means that each cluster is considered as a distinct ejaculation, i. e. during the copulation of a male camel, several ejaculations are carried out and the amount of semen and sperm concentration well increase as copulation period is prolonged (Lichtenwalner et. al., 1996b). Naturally, copulation duration in camels may extend for more than 40 minutes depending on the male libido, sexual potency, breed, age and frequency of use. Practically, the male himself will dictate the duration of copulation. Regarding the pattern of semen flood during copulation, it is better to indentify the delivered semen after collection from a male camel as "a collection" rather than "an ejaculation".

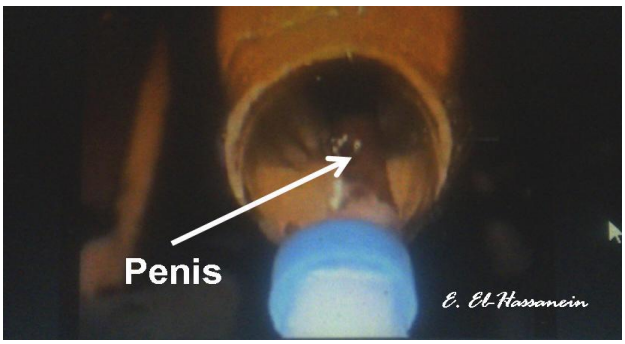

Figure 1a. Entrance of penis into inside the collection tube during consecutive penile strokes.

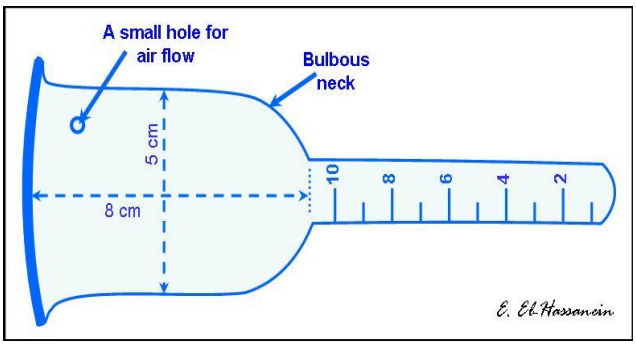

Figure 1b. Modified tube for semen collection using a camel dummy.

Semen Collection from Male Camels

Semen collection from genetically superior males is considered the main component in establishing an AI program. Consistent collection and evaluation of the male semen helps in allowing selection of suitable ejaculates of a reasonable quality to be processed and preserved for AI programs. Only males with excellent reproductive performance that produce high quality semen and have good production potential should be selected for AI. Consistent semen collection from breeding males in AI-centers helps in accurate evaluation of males, testing and formulation of standard extenders protocols and achieving optimal dilution, freezing and thawing rates for processing delivered semen into preserved semen doses of good quality post-thaw.

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By the onset of the breeding (rutting) season, the sexually mature male camel exhibits several peculiar morphological, behavioral and endocrinological traits (Yagil and Etzion, 1980; El-Hassanein et al., 2004; Bhakat et al., 2005; Deen, 2008; El-Bahrawy and El-Hassanein, 2011; Padalino et al., 2015). Additionally, males in rut are almost restless, aggressive, difficult to handle and often tend to bite other animals and humans (Abu-Zidan et al., 2012). The behavior of males in rut is mostly unpredictable and experience in handling them is important to avoid injuries. Therefore, semen collection in dromedary camels is often relatively long, unsafe, and tedious (Ziapour et al., 2014). Inability to consistently collect semen has been one of the most serious impediments to the application of AI programs in camels. Semen collection in camels is complicated by their recumbent mating posture, long duration of copulation and intrauterine deposition of semen (Arthur and Tigani, 1990; Bravo et. al., 2000a) in addition to the dripping manner of ejaculation and the challenge of handling the violently males in rut (El-Hassanein, 2003). Nowadays, attempts to collect semen from dromedary camels are carried out by using either electroejaculation or using a modified artificial vagina (AV) either in combination with a receptive (teaser) female or formerly fixed inside the newly invented device "*EL-Hassanein Camel Dummy*" (El-Hassanein, 2003).

Collecting semen by electroejaculation (EE)

Semen collection by using EE technique requires to kneel down the male, binding his front legs to shoulder and neck, forcing him to turn on his side and binding his hindlegs from the shin region together, followed by his sedation or general anesthesia (Hemeida et al., 2001; El-Hassanein, 2003; Mostafa et al., 2014).

Several studies have obtained less semen volume by using EE than that obtained by using an AV and a teaser female (Abdel-Raouf and El-Naggar, 1978; Tingari et al., 1986; Tibary and Memon, 1999; Bravo et al., 2000a; El-Hassanein, 2003; Marai and Zeidan, 2007), but other semen parameters were nearly similar in both techniques (Tibary and Memon, 1999; Marai and Zeidan 2007) or negatively affected by using EE (El-Hassanein, 2003). Due to the short duration of ejaculation using EE technique, the semen obtained is often of poor quality (Bravo et al., 2000a). On the contrary, other studies found an increase in ejaculate volume after using EE technique as compared to using an AV and a teaser female in Llama (Giuliano et al., 2008) and in dromedary camels (Mostafa et al., 2014). These studies have elucidated this increase in ejaculate volume by EE as an increase in volume of accessory fluids associated with electric stimulation of the accessory sex glands.

It is clear that the sedated or anesthetized male is forcibly ejaculated by using EE technique without any sexual stimuli for his gonads as it normally occurs in natural mating process. This may reflect that semen delivered by this technique does not resemble the semen that can be naturally produced. In general, EE technique is advices to be used only when semen collection by AV is not possible, as the practice of this technique has demonstrated many disadvantages (El-Hassanein, 2003), the most important of which are: consuming a lot of time and effort, restraining males by sedation or general anesthesia, needs a lot of laborers for tightening the males and removing ties, exposing males to bleeding injuries and even fractures, causing males to be fearful and unable to mount normally again, in addition to the possibility of contamination of the delivered semen with urine and cellular debris (Tibary and Memom, 1999).

Collection by an AV in combination with a receptive female

The pronounced and accustomed method of semen collection in camels is the use of an AV and a receptive female. A bull AV of 30 cm long and 5 cm internal diameter has been modified by adding a foam imitation cervix (8 cm in length) inside it in the part at which the rubber cone and the collection tube well be attached (Bravo et al., 2000a). For collection using an AV, a teaser female should be kneeled and her fore legs are tied with a rope to the neck while her hind legs are tied with a rope around the lumber region (El-Hassanein, 2003). Proper tying of the teaser before collection prevents her sudden movements during copulation that can cause injuries or even fractures for the male. As the male is fully squatted down behind the teaser and starts to protrude and erect his penis, the technician instantly sits beside the hind legs of the teaser, grasps the male's sheath and deviates the penis into inside the AV. The technician should hold the erected penis inside the AV throughout the copulation period (Figure 2).

Under the conditions of semen collection using an AV and a teaser female, the male camel is often excited and stressed by the intervention of the technician in mating process throughout the copulation period and also by the presence of several persons in the collection area. Therefore, males mostly have the tendency to dismount and slip sideways off the teaser without completion of the copulation. As long as the male has been used for natural mating for several consecutive breeding seasons, the male will dismount faster refusing to complete the copulation process. Elder breeding males can almost dismount just after the intervention of the technician for diverting the male's penis towards the AV. This mating behavior during semen collection using an AV and a teaser may reflect the reason for the reported wide ranges of the copulation durations and the volumes of semen delivered by this technique. Ejaculate volume can vary from 2 to 10 ml as there is great variation between males and even between ejaculates from the same male (El-

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Hassanein, 2003; Wani et al., 2008; Morton et al., 2013; Skidmore et al., 2013; Mostafa et al., 2014). Good quality semen can be collected by using AV at weekly intervals from dromedary camels, but the overall efficiency of semen collection by AV is low and needs to be further improved to adopt camel males for artificial insemination programs (Al-Bulushi et al., 2014). The larger volume samples of dromedary camel semen (16 ml) collected by Kutty and Koroth (2012) using AV were white, homogeneous, viscous and sperm rich. These values of semen volume were recorded for males after their copulation for durations ranged from 5 to 15 minutes, however, under natural mating, copulation duration may extend to more than 40 minutes. It is obvious that the elongation of male camel copulation duration is almost attained with donation of more semen volume and sperm concentration.

Figure 2. Semen collection by an artificial vagina and a teaser female.

Although collection of semen from male camels by AV and teaser females is considered to be the most standard and repeatable procedure, practicing the technique has however revealed several disadvantages, the most important of which are:- occurrence of incised bleeding injuries on the back of the teaser female, disturbance and excitation of the male by intervention of the technician in the mating process, exhaustion of the technician during the long copulation period, the risk of injuries to the operators, frequent failure of ejaculation in the AV, dismounting of males without completion of copulation (Tibary and Anouassi, 1997; El-Hassanein, 2003) in addition to refusal to serve the AV and sand contamination (Deen et al., 2003).

Collection by using an AV fitted inside the camel dummy

Several attempts to overcome the difficulties of semen collection from camelids were noticed early (Garnica et al., 1993). The most striking of these attempts was the construction of an alpaca or llama dummy of the same shape and size of a recumbent female for semen collection (Garnica et al., 1993; Lichtenwalner et al., 1996b; Bravo et al., 1997). The practical use of an AV mounted inside a dummy for semen collection from alpaca and llama has proven to yield copulation periods, mating behaviors and semen output mostly representative to that obtained in natural mating. Similar attempt to overcome different constraints of semen collection from dromedary camels was also conducted later in Egypt. El-Hassanein (2003) has successfully devised and tested a camel-dummy that resembles the shape and size of a natural female dromedary camel in the recumbent posture. The design and the structural formation of this devise has been discussed earlier in details (El-Hassanein, 2003). The dummy was designed to become visible as a recumbent natural female camel on the ground of the collection area when firmly fixed on a definite aperture in the ceiling of a small lab underneath it. It was also designed to mount an AV inside it such that the front edge of which is fitted in the same position of the vulva of the natural female. Fixation of AVs and exchanging them between different males are promptly carried out from the small lab underneath the dummy (Figure 3). Practically, using the dummy for semen collection from well trained males has proven that it is the most natural and reliable technique for semen collection from dromedary camels.

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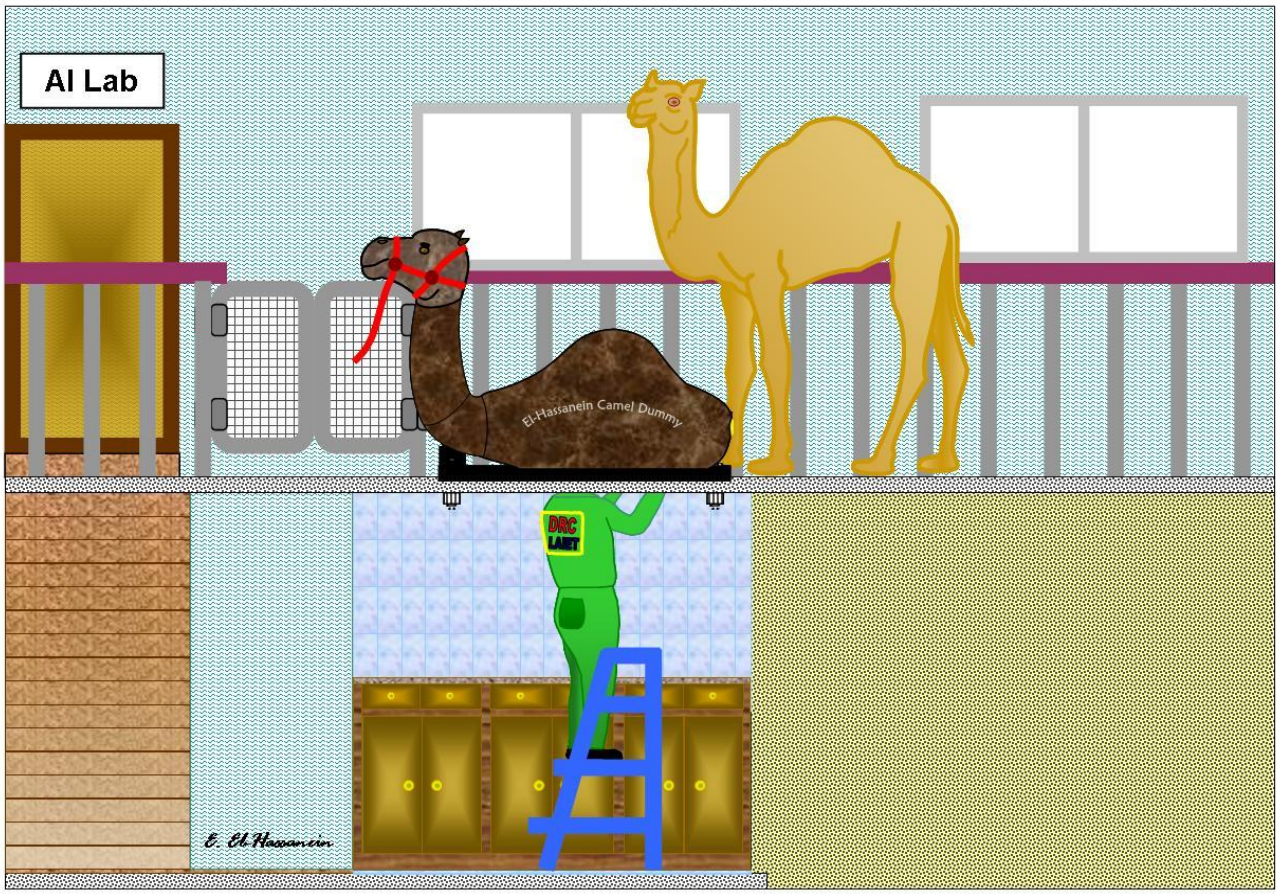

Figure 3. The technician promptly fixes and exchanges artificial vaginae for different males from the lab underneath the dummy.

Similar reports were also recorded in the South American camelids by Adams et al. (2009). Males that were trained well and accustomed to mating with the dummy have entirely reacted well throughout their copulation periods which exceeded up to 45 minutes and produced a collection semen volume of up to 35 ml. (Figure 4). These obtained results may reflect to how much the semen collection from dromedary camels can be advanced by applying the camel dummy technique. Replication of the male's copulation duration by this technique and hence the multiplication of the delivered semen quantity may imitate to a great extent the natural mating process in dromedary camels. The duration of copulation (15-45 min) and the volume of delivered semen in a collection varied between males (12-35 ml) depending on their sexual stamina and to how much they were trained well to savour mating with the dummy.

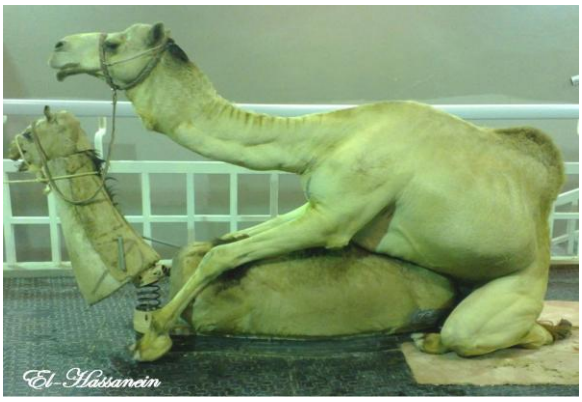

Figure 4a. A newly retired racing-male (8 years age) acting exclusively well with the camel dummy.

Figure 4b. A partially diluted collection of semen (1 semen : 1 extender) of a total volume of about 70 ml.

Management and training of males for semen collection using AVs

Under semi extensive herding production, pastoralists either release the desired breeding male to graze together with the breeding females during the breeding season (free mating) or confine the breeding male lonely in a captivity yard for managing the breeding activity of their herds (controlled breeding). Under intensive breeding management in

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AI-centers, where males are selected and intended for standard semen collection and preservation, each desired male camel is often kept in a well ventilated and half-shaded pen of about 75 m2 space.

Regarding the confinement-related problems in intensive management systems, several reports have proven that animals in captivity can develop abnormal stereotypical behaviors which reflect the impact of captivity on the animal welfare and hence on their performance (Broom, 1991; Mason, 1991; Inglis and Langton, 2006; Cooper and McGreevy, 2007). A recent striking study on the negative effect of captivity on the housed dromedary male camels in rut has been reported by Padalino et al. (2014). This study has asserted that male camels in full captivity housing systems have obviously revealed several oral and locomotor stereotypical behaviors. In our practice, we have also recorded two abnormal sexual behaviors. Some males in captivity were occasionally exhibited self-ejaculation. Other males were rhythmically protrude their penis when they were recumbent on the sandy ground and then retract it back into inside the scrotum after being contaminated with sand grains (Figure 5). These abnormal sexual behaviors were negatively reflected on the delivered semen either as a reduction in its volume or a contamination with sand particles.

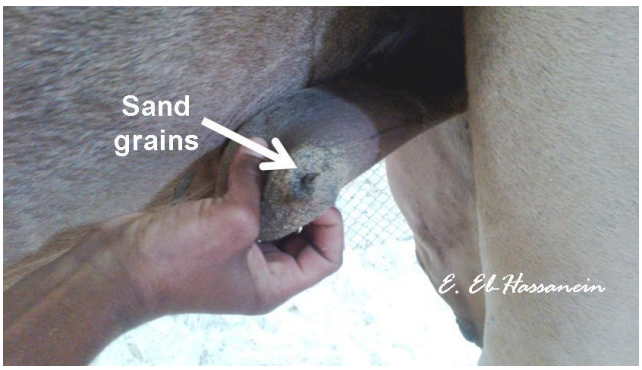

Figure 5a. Scrotal orifice filled with sand granules.

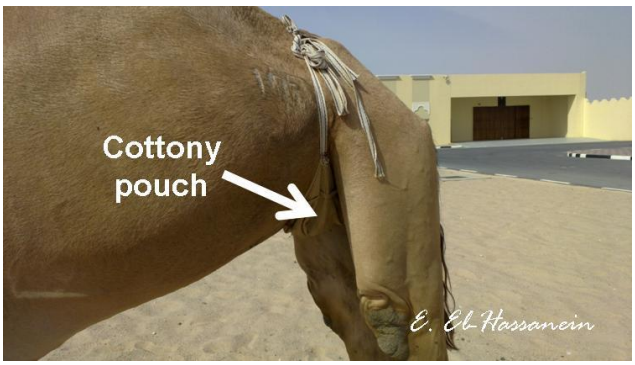

Figure 5b. Scrotum enclosed with a cottony pouch.

It is generally considered that the major effects of confinement on the male camels in rut are the limited opportunity for locomotion, the social isolation and the inability to forage on pasture. These captivity stressors may negatively affect their welfare status and their reproductive performance. It has been noticed that male camels in herds mostly show earlier sexual behaviors and longer breeding season compared to housed males. Recent reports have asserted that the allowance of a daily free movement for 1 hour and the social interaction with frequent female parade could improve the welfare and reproductive performance of male camels housed for semen collection (Vyas et al., 2001; Bhakat et al., 2005; Fatnassi et al., 2014a,b; Padalino et al., 2014).

By the onset of the breeding season, male camels housed for semen collection programs must be allowed to have the opportunity to daily regular movement for about 1 hour to improve the captive animal's physical fitness and muscle tone. This exercise regime should be carried out daily or bi-daily throughout the breeding season. On the scheduled days of training or actual semen collection, and just before the time of starting the training or collection process, a parade of receptive females (5-10 animals) should be carried out for about 1 hour in front of the confined males to enhance their olfactory and ocular stimuli before training or collection of semen.

Males adopted for semen collection by AV techniques (either in combination with a natural female or mounted inside a camel dummy) must be habituated to the collection area at the beginning of the natural breeding season (Sep.-Oct.). Each male should enter the collection area and spend about 20-30 minutes in the presence of the expected operators of the collection process and of either a restrained kneeling natural female or a fixed camel dummy. This accustoming program must be carried out bi-daily after a female parade and nearly at the expected time of actual semen collection.

Training males to accept mating with the AVs must be start during the course of acclimatization to the collection environment. The modified AVs must be prepared and filled with warm water and incubated for 2-3 hours inside an AVs-Incubator pre-switched on and adjusted at 50-55^{o}C. Attention must be given to the adjustment of temperature inside the AV at 40-45^{o}C throughout the training and collection periods.

For semen collection using an AV and a teaser female, intervention of the technician should be carried out only after squatting down of the male behind the teaser and as he begins to erect his penis towards the female's vulva. Operators of the collection process should be careful of sudden biting and their movement inside the collection area must be limited during male copulation.

In the case of using the camel dummy in training and for semen collection, the males can get excited and attracted to the dummy by trembling its head and neck, through pulling a long robe looped around its head, in combination with emission of a recorded female bleat inside the collection area as an audiovisual stimulus. Furthermore, sprinkling a little bit of urine of a receptive female around the tip of the AV at the rear end of the dummy is also practiced as an olfactory

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stimulus to arouse males for mating with the dummy. As in natural breeding practice of herders, some males may need assistance in directing their penis into inside the AV of the dummy.

In general, to optimize semen collection from dromedary camels using AV techniques (either in combination with a teaser female or fitted inside a camel dummy), males can be practically categorized into three brands:

1. Males which have recently reached sexual maturity (7-8 years age) and have not practiced natural mating yet: These males are usually acceptable to serve AVs after a short period of training and give excellent results in semen collection, i. e. semen volume and quality. These trained males will be the main source of semen in AI-program throughout their breeding lifespan.
2. Males which have practiced natural mating for 4-5 breeding seasons (8-12 years age): These males take a relatively longer time and more effort for training to serve AVs. Most males of this type often accept mating with AVs after a training course of about 2-3 weeks at the beginning of the breeding season.
3. Elder males which have professionally practiced natural mating for 5-10 breeding seasons (12-20 years age): These kinds of males are most likely to refuse mating with AVs. However, a small fraction of these males can accept training and collection with AVs after a long period of training (4-6 weeks of a condensed training course).

It is very essential to emphasize that alternation of the males between practicing natural mating and their employment in semen collection by the AVs is usually attended with an obvious mutiny of males to mate with the AVs. Consequently, to establish a persistent AI program, males which have been selected and trained well for semen collection by AVs (either in combination with a teaser female or formerly fitted inside a camel dummy) must be completely devoted for this mission in AI-centers and preventing their reuse in natural mating.

Processing and Preservation of Camel Semen

Successful in-vitro preservation of superior genetic materials from elite male camels will obviously facilitate its dissemination over a wide range of female herds through AI-programs. A collection from a male camel will be diluted by 3-5 folds (according to the collection concentration) and then processed into multiple standard semen doses to be used for fertilizing many of receptive females rather than the deposition of this collection in one female.

There are many challenges should be taken into consideration in processing and preserving camelid semen. The prominent viscous nature of seminal plasma and the detrimental effects of cold-shock during short-term preservation (chilling form) and the cryo-damage during long-term preservation (frozen form) are the major constraints in processing and preservation of camelid semen.

Elimination of viscosity in camelid semen

Viscosity of camelid semen causes difficulty in handling and evaluation of the delivered semen immediately after collection. It obviously hinders the interaction of the components of the used diluents with the sperm membranes, and consequently, exposes sperm to cold shock and cryodamage during processing and preservation. In camelids, the whole collection is mainly composed of highly viscous seminal plasma in which spermatozoa are totally embedded. Conversely, in stallions and boars as examples, the delivered semen is mostly composed of two distinct fractions: a sperm-rich fraction (75-90% of sperm) and a sperm-poor fraction (the bulbourethral gel fraction). The poor fraction is about 5-10% and 20-25% of the total ejaculate volume in stallions and boars, respectively. This gel fraction is practically filtered and excluded from the delivered semen and AI doses have been prepared by diluting and processing the sperm-rich fraction.

Freshly collected dromedary camel semen undergoes spontaneous liquefaction (Skidmore, 2005). Dilution of dromedary semen (1:1) in Tris-lactose-yolk extender revealed best liquefaction within 60-90 minutes of incubation at 37^{o}C and highest progressive sperm motility after storage for 24 hours either refrigerated or at room temperature (23^{o}C) (Wani et al., 2008). Several enzymes have been used to improve the rheological characteristics of camelid semen however; all the enzymes used have proven to have detrimental effects on sperm plasma membrane and acrosomal integrity. Bravo et al. (2000b) used trypsin, fibrinolysin, hyaluronidase and collagenase enzymes to reduce viscosity of llama and alpaca seminal plasma and found that collagenase was superior in reducing viscosity but all enzyme treatments had detrimental effects on sperm function and integrity, less of which was recorded in collagenase treatment. Similar findings were also reported by Giuliano et al. (2010) that when they used collagenase at 0.1% in H-TALP-BSA medium to eliminate viscosity in llama semen. Deen et al. (2003) found that adding 0.2 mM of caffaeine to a Tris buffer and dilution of dromedary camel semen at 1:1 ratio it significantly improved motility of individual spermatozoa while the addition of 1% α-chymotrypsin did not reduce semen viscosity. El-Bahrawy and El-Hassanein (2009) tested five different mucolytic agents to remove viscosity in dromedary camel semen after dilution in one step at rate of 1:3 with a Tris-lactose based extender. They found that α-amylase superiorly eliminated seminal viscosity and improved individual sperm motility post-dilution (46.0%) compared to control (27.5%), however, all mucolytic agents had deleterious effects on acrosomal integrity (11.8% detached) after 4 hours of incubation at 37^{o}C. Whilst, addition of α-amylase (10-15 µl/ml)

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to a Tris buffer for diluting dromedary camel semen significantly eliminates viscosity and improves sperm motility and acrosomal integrity post-thaw (El-Bahrawy, 2010), while lower concentrations of α-amylase (5 µl/ml) had no effect on viscosity (El-Bahrawy, 2010; Monaco et al., 2016).

In a proteomic analytical study on several mammalian seminal plasmas, dromedary camel and alpaca had proven to display the least complex seminal plasma proteome of the species studied (Druart et al., 2013). The authors found that the highly abundant camelid seminal plasma proteins (recently identified as beta nerve growth factors, ß-NGF) were rarely shared with other species and if so, were observed in relatively lower abundance. During liquefaction of camelid seminal plasma, abundant proteins with high-molecular mass in fresh semen start to degrade and gradually disappear in seminal plasma in conjunction with production of two low-molecular mass proteins in liquefied seminal plasma (Mal et al., 2016). It has been confirmed that ß-NGF is an ovulation-inducing factor for alpacas and causes 80% ovulation associated with a marked elevation in plasma progesterone concentration after alpaca treatment (Kershaw-Young et al., 2012a).

Earlier reports have attributed the viscous consistency in camelid seminal plasma to the abundance of mucopolysaccharides, recently named glycosaminoglycans (GAGs), which in camels can only come from secretions of the bulbourethral and the prostate glands (Garnica et al., 1993; Hassan et al., 1995). Also, Kershaw-Young et al. (2012b) suggested that GAGs (mainly keratan sulphate), that are secreted from the bulbourethral gland of alpaca, may be the cause of seminal plasma's prominent viscosity. The authors found also that keratan sulphate was correlated with viscosity. However, Kershaw-Young and Maxwell (2012) have proven that proteins, mainly mucin 5B which is secreted from the bulbourethral gland, are mainly responsible for the viscosity in camelid seminal plasma rather than GAGs. Mucin5B protein was found to be about five times more abundant in highly viscous seminal plasma and its concentration decreased considerably by liquefaction of semen. The authors found that keratanase enzyme that specifically degrades keratan sulphate, which constitutes about 85% of the total GAGs in camelid seminal plasma, did not completely reduce viscosity within 2 hours of incubation. Conversely, protease papain was found to be most effective in the complete elimination of viscosity within 30 minutes and in preservation of sperm viability and acrosomal integrity compared to GAGs enzymes and even to the proteinase k (Kershaw-Young et al., 2013). However, Morton et al. (2012) found that papain alone or supplemented with Tris-buffer efficiently reduced alpaca semen viscosity but detrimental effects on sperm integrity were observed. Also, Monaco et al. (2016) found that dilution of dromedary camel semen in Tris based buffer containing the protease papain underwent complete liquefaction within 90 minutes of incubation at 37^{o}C, however, higher percentage of head-to-head sperm agglutination was recorded and is supposed to be due to papain-induced protein degradation. In order to eliminate seminal plasma viscosity in camelids, semen can be diluted immediately following collection with a suitable extender at 1:1 ratio, then treated with 0.1 mg/ml papain for about 20 min at 37^{o}C (or until complete removal of viscosity) followed by addition of 10 uM E-64 (papain inhibitor) to halt the digestion of proteins by papain to conserve sperm cell-membrane integrity and viability (Kershaw-Yong and Maxwell, 2011).

Consequently, it is obviously needed to identify a reliable enzymatic protocol (type, concentration, incubation temperature and exposure time) that can be used to eliminate the viscosity of camel semen whereas sufficiently preserving sperm viability and integrity, i. e. to protect sperm function.

Short-term preservation (Liquid or Chilled form)

Camelid semen can be preserved in a liquid (chilled) form at 4-5^{o}C for use within 1-2 days without a significant decrease in its quality. Accordingly, a reliable daily ovarian scanning of naturally receptive or hormonally induced females should be conducted to assure the existence of the dominant follicle(s) at the optimal size (1.5-2.0 cm in diameter) for ovulation and timed-AI within the limited period of preservation. Diluents used for chilling-storage of semen must be containing a sugar as an energy source (glucose, lactose, sucrose or fructose), a non-permeating protein for protecting sperm cell membrane against cold-shock and damage (lipoprotein from egg yolk or casein from milk) in addition to the buffer medium (to maintain pH and tonicity) and the antibiotics.

Over the years, several extenders have been tested for chilling-storage of camelid semen including conventional (sugar-based, citrate-based, Tris- and Tris-tes-based buffers in addition to skimmed-milk) and commercial extenders (Green buffer, Biladyl, Androhep, Triladyl, Laiciphos, Biocephos, OptiXcell, EquiPlus and INRA-96) and have revealed obvious conflicting results.

In alpacas, dilution of epididymal sperm cells with lactose-yolk extender superiorly maintained sperm progressive motility and longevity (62%) for up to 24 hours at 4^{o}C as compared with dilution in citrate- and Tris- based extenders (Morton et al., 2007). However, dilution of collected alpaca semen with citrate-glucose-yolk (reviewed in Bravo et al., 2013) or with Biladyl (Morton et al., 2009) had improved sperm viability for up to 48 hours at 4^{o}C as compared with dilution in citrate, Tris-based, lactose, Androhep, Triladyl and skim-milk extenders. In Bactrian camel, Niasari-Naslaji et al. (2006) found that dilution of semen with SHOTOR (a Tris-based) extender or with Green buffer had revealed higher

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progressive forward motility (65.5 and 60.5%, respectively) after dilution as compared with dilution in lactose (31%) and sucrose (28%). In another study, Niasari-Naslaji et al. (2007) had recorded 72.3% progressive forward motility, 63% membrane integrity and 87.5% live sperm after dilution with SHOTOR, however, fast cooling after dilution to 4°C and storage for 24 hours revealed a progressive motility of 47% compared with slow cooling (31%).

The marked reduction in sperm progressive motility after cooling of diluted Bactrian semen either at slow (57%) or fast (35%) rate, compared with the post-dilution related value (72.3%), may reflect the need to optimize the cooling rate. It is noteworthy to mention that too fast-cooling of sperm will susceptible them to damage by intracellular ice formation, while too slow-cooling may lead to sperm damage by long exposure to concentrated solutions resulting from progressive conversion of extracellular water to ice. Optimization of pH value and osmotic pressure in diluted semen and the rate of cooling of it from 35 to 5°C is considered crucial for successful retaining of sperm viability and longevity post-chilling.

In dromedary camels, earlier reports had stated that dilution of semen with a Tris-based buffer superiorly maintained sperm viability for up to 48 hours at 5°C compared with dilution in lactose buffer (Vyas et al., 1998), Biociphos (Deen et al., 2004) or in citrate or sucrose based extenders (Wani et al., 2008). However, Zeidan et al. (2008) found that dilution of camel semen with fructose-yolk-citrate, lactose-yolk-citrate, sucrose-yolk-citrate or Tris-yolk-fructose extender revealed better sperm motility and longevity after storage at 5°C for 3 days compared with dilution in glucose-yolk-citrate, skim-cow-milk and skim-camel-milk extenders. Other studies showed that Green buffer was found to be superior to Tris-buffer in preserving sperm motility, integrity and viability in dromedary semen preserved at 5°C for up to 48 hours (Ghoneim et al., 2010; Waheed et al., 2010). Recently, Al-Bulushi et al. (2016) recommended dilution of dromedary semen with OptiXcell, Green buffer or Triladyl for storing at 4°C for up to 48 hours.

An attempt was conducted to compare the effect of diluting dromedary camel semen with freshly prepared (4°C) or stored frozen (-20°C) Green buffer extender on sperm parameters after dilution and on the *in-vivo* fertility after AI (Morton et al., 2011). Dilution in fresh or frozen-thawed Green buffer revealed higher motility (70.7 and 68.8%, respectively) compared with sperm motility in neat semen (35%), pregnancy rates were higher for single and multiple ovulating camels inseminated with semen diluted in fresh buffer (72.7 and 83.3%, respectively) compared to camels inseminated with semen diluted in frozen-thawed buffer (27.3 and 11.1%, respectively). The reported AI results may reflect alteration in the buffer components by freezing and thawing that led to deprivation of its protecting ability for sperm membrane and DNA against cold-shock.

Recent studies have been focused on using commercial Green buffer and INRA-96 (IMV, France) extenders for dilution and chilling-storage of dromedary semen. It is reviewed that both extenders were found to be comparably able to maintain sperm motility and integrity after dilution (56%), but sperm viability was higher after chilling in INRA-96 (65%) compared with Green buffer (56%) and pregnancy rates were similar with the two extenders (34%) (Skidmore et al., 2013). Also Morton et al. (2013) reported that dilution of dromedary semen with Green buffer or with INRA-96 significantly improved sperm motility after dilution (67.4 and 59.1%, respectively) compared with motility in neat semen (45.5%) and both extenders comparably retained sperm motility (47.6 and 48.3%, respectively), sperm viability (58.9 and 62.2%, respectively), sperm membrane integrity (54.9 and 57.6%, respectively) and acrosomal integrity (84.8 and 84.6%, respectively) after being stored at 4°C for 24 hours. However, chilling-stored semen diluted with INRA revealed superior sperm fertilizability (23.5%) than that diluted with Green buffer (0.0%) which may reflect sperm DNA damage during chilling-storage of semen diluted with Green buffer. It has been reported that sperm with damaged DNA, when succeeded to fertilize oocytes, the resulting embryos may fail to develop or implant in the uterus or may be naturally aborted at a later stage (Alvarez, 2003).

The noticeable reduction in sperm progressive motility, after cooling and storage for 24 hours, by up to 30-35% of corresponding post-dilution values (Niasari-Naslaji et al., 2007; Morton et al., 2013) may reflect susceptibility of sperm cells to cold-shock and membrane damage during cooling of semen from body temperature to near the freezing point of water (from 35 to 5°C). The primary lesions associated with cold-shock damage are the morphological alterations in sperm plasma membrane and changes in membrane permeability (Barrios et al., 2000).

It is known that storing semen in a liquid form will be associated with sperm ageing and a decline in their incubation lifespan due to accumulation of toxic metabolic products mainly in the form of reactive oxygen species (ROS) resulting from the lipid peroxidation of sperm membranes (Salamon and Maxwell, 2000). During processing and preservation of semen, sperm membrane will be susceptible to lipid peroxidation due to oxidation of the membrane polyunsaturated fatty acids and thereby high production of hydrogen peroxide. ROS accumulation leads to oxidative stress which may cause damage of sperm membrane, reduction in motility, viability, DNA integrity and lowering fertility (Gavella et al., 1996; Aitken et al., 1998, 2010; Kumer et al., 2011).

Oxidative stress plays an important role in the decline of sperm quality during chilled storage through the production of hydrogen peroxide and supplementation with an antioxidant additive (such as: thioglycol, adenosine, prolactin, lycopene, catalaze, cysteine, ascorbic acid, Vitamin C, vitamin E) is often used to provide protection against oxidative stress in liquid-stored semen. Moreover, it is suggested that lowering the final concentration of egg yolk in the

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diluent, as a substrate for the dead sperm enzyme aromatic-L-amino acid oxidase, it will also reduce the production of hydrogen peroxide during storage of diluted semen. Medan et al. (2008) reported that the addition of catalaze enzyme at 500IU/ml significantly increased sperm motility (from 53.2 to 62.7%) and decreased dead sperm (from 22.4 to 16.5%), sperm abnormalities (from 13.4 to 7.9%) and acrosomal damage (from 7.5 to 4.5%) in dromedary camel semen diluted in Tris-fructose-yolk extender and stored at 5^{o}C for 5 days. The authors concluded that addition of catalaze enzyme to semen extender can be used to prolong camel sperm survival during storage at 5^{o}C.

Egg yolk is the most common non-permeable protein added to semen extender for protection of sperm against cold-shock. Phospholipids and low-density lipoproteins in egg yolk can lessen the chilling injuries on sperm by binding to sperm membrane and increasing its permeability whilst preserve its composition and physical properties (Holt, 2000). However, egg yolk had proven to contain progesterone (Bowden et al., 2001) which plays an important role in the sperm capacitation during processing and storage. Also, some components in egg yolk interfere with biochemical assays and metabolic investigations.

In general, egg yolk is composed of plasma and granules fraction. The yolk plasma is mainly constituted of 85% low-density lipoproteins and 15% livetin, whilst yolk granules by 70% high-density lipoproteins, 16% phosvitin and 12% low-density lipoproteins (McCully et al., 1962). Low-density lipoproteins are the main egg yolk constituent representing 2/3 of the yolk dry matter and 22% of the yolk proteins. It is considered the main contributor of yolk emulsifying properties (Martinet et al., 2002) and the most likely source of protection for sperm against effects of storage at 5^{o}C (Watson and Martin, 1975). It is also reported that extender containing purified low-density lipoproteins preserves the sperm membrane integrity after ejaculation and dilution by preventing seminal plasma proteins from binding with sperm surface and causing lipid efflux from sperm membrane (Bergeron et al., 2004). Low-density lipoproteins can be extracted from egg yolk at 97% purity according to the procedure of Moussa et al. (2002). It has also been proven that low-density lipoproteins contain a low progesterone level than egg yolk due to the filtering effect of the dialysis procedure during its extraction (Bencharif et al., 2008). Therefore, it is hypothesized that extender containing extracted low-density lipoproteins provides good protection for sperm membranes and acrosomes than the whole egg yolk.

Therefore, further research attempts are needed to test a wide range of antioxidants and substitution of egg yolk with extracted low-density lipoproteins in camel extenders in order to lessen oxidative stress produced during chilling-storage of dromedary semen and to protect sperm membranes and DNA integrity against cold-shock damage.

Long-term preservation (Frozen form)

The aim of freezing semen is the production of a cryopreserved stock of genetically elite sperm cells to be used throughout several decades for AI-programs and applying other reproductive technologies such as *in-vitro* and *in-vivo* embryo production and transfer. During semen processing for cryopreservation, sperm cells are susceptible to several stressful stages that can cause biochemical and anatomical alteration in their compartments (acrosome, DNA, mitochondria, axoneme and plasma membrane). A thriving sperm-freezing protocol should prevent lethal intracellular ice crystal formation and to reduce cell membrane damage during and after freezing of semen. The composition of the semen extender plays a critical role in protecting sperm against chilling-shock and cryoinjury which occur during cooling (from 15 to 5^{o}C), freezing (from -5 to -50^{o}C) and thawing (from -50 to -5^{o}C) critical temperature stages of processing.

Cryoprotectants are often included in the cryopreservation extender to reduce the physical and chemical stresses derived from cooling, freezing and thawing of sperm cells (Purdey, 2006). The efficacy of the utilized cryoprotectant agent is almost always based on comparing the pre-freezing and the immediate post-thawing sperm viability and its capability to protect sperm compartments against cryoinjury. Sperm cryopreservation extenders should contain a permeating cryoprotectant (glycerol, ethylene glycol, propylene glycol, dimethyl sulfoxide or amides) in addition to the non-permeating cold-shock protectants, the buffer medium, carbohydrates as energy source and antibiotics. The non-permeating cryoprotectors create an extracellular osmotic pressure that induces cell dehydration and lower the incidence of intracellular ice formation in addition to their interaction with phospholipids in the cell plasma membrane and increasing the sperm survival to cryopreservation. However, permeating cryoprotectors penetrate inside the sperm cells preventing the formation of ice crystals, stabilizing cell-membrane lipids and limiting shrinkage of the sperm cells at subzero temperatures. To this date, glycerol is the most common cryoprotectant in use for sperm cryopreservation for its osmotically permeating properties. It replaces intracellular water and maintains cellular volume, interacts with ions and macromolecules and depresses the freezing point of water so that less ice forms inside cells (Holt, 2000).

When alpaca and llama semen was liquefied after collection with collagenase (1 mg/ ml) and diluted in two-steps with citrate-yolk-glycerol extender and cryopreserved in liquid nitrogen, the percent of sperm motility reported after liquefaction, after final dilution (pre-freezing) and after thawing at 35^{o}C for 8 seconds were: 80% oscillatory motility, 60% progressive motility and 30-40% oscillatory movement, respectively (reviewed in Adams et al., 2009) and pregnancy rate recorded after insemination was 26% (5/19). In a previous study, alpaca semen was diluted in two-steps with either Tris-citrate-yolk or skimmilk-fructose-yolk extender, each of which was split to be supplemented either with

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glycerol or ethylene glycol as a cryoprotectant agent (Santiani et al., 2005). The authors reported that skimmilk-based extender containing either glycerol or ethylene glycol seems to promote the survival of more viable sperm with intact acrosomes than the Tris-based extender. Morton et al. (2007) reported higher pre-freezing and post-thawing motility and acrosomal integrity in epididymal alpaca semen diluted with lactose-glycerol based extender and frozen in pellet form than that diluted and frozen with citrate-glycerol and Tris-glycerol based extenders. Also, a study on the effect of glycerol concentration and EquexSTM® supplementation in diluent on the post-thaw viability of epididymal alpaca sperm was conducted by Morton et al. (2010). They reported that epididymal alpaca sperm can be cryopreserved after dilution in lactose (11%)-egg yolk (20%) extender containing 3-4% final glycerol and 1% final EquexSTM® paste concentration. EquexSTM® interacts with egg yolk in the extender, modifies the protein structure of lipoproteins and improves stability of plasma membrane lipids by adding cholesterol (Pena and Linde-Forsberg, 2000).

In Bactrian camels, semen is diluted in two-steps with lactose (11%)-egg yolk (20%) + glycerol (6%) and OEP-Exquex (1.5%) to reach a final glycerol concentration of 2% after dilution (Seime et al., 1990) and achieved post-thaw motility rates of 70%, but morphological abnormalities reached 48%. Orvus ES Paste (OEP) is a detergent that causes the emulsion and dispersion of yolk-lipids in the extender facilitating their interaction with the sperm membrane surface to protect sperm cells against cryoinjury (Pursel et al., 1978). In Chinese studies, dilution of Bactrian semen in two-steps with sucrose (12%)-egg yolk (20%) and glycerol (7%) revealed higher post-thaw motility, viability and acrosomal integrity compared with different extenders (Zao et al., 1996) and realized a pregnancy rate of 93% (29/31) after insemination (Chen et al., 1990). However, Niasari-Naslaji et al. (2007) found that dilution of Bactrian semen in two-steps with SHOTOR (a Tris-based extender) containing 6% glycerol was superior in preserving progressive motility (35%), membrane integrity (49.5%) and sperm longevity (61.5%) post-thaw compared with 4 and 8% glycerol concentrations. The authors also compared SHOTOR containing 6% glycerol and Green/Clear buffers extenders on post-thaw viability and found that SHOTOR efficiently preserved progressive motility (29.9%) post-thaw compared with Green/Clear buffer (4.2%), however, the two extenders comparably preserved sperm membrane integrity (44.4 and 37.4%, respectively) and longevity (56.0 and 46.8%, respectively) post-thaw.

In dromedary camels, El-Bahrawy et al. (2006) found that the dilution of dromedary semen with Tris-citrate-yolk-glycerol, Tris-sucrose-yolk-glycerol, Tris-lactose-yolk-glycerol, lactose-yolk-glycerol and skimmilk-yolk-glycerol extenders revealed comparable pre-freezing sperm motility (63.3-68.7%), but dilution in Tris-lactose-yolk-glycerol extender superiorly maintained the highest post-thaw motility (62.3%) and sperm survival (93.2%) compared with dilution in the other extenders. Also, El-Hassanein (2006) found that Tris-sucrose-yolk-glycerol extender significantly reduced the dilution effect on dromedary sperm viability and had improved their freezability after fast freezing as compared with dilution in sucrose-yolk-glycerol and Tris-yolk-glycerol extenders. In a more recent study, El-Bahrawy et al. (2012) found that supplementation of Tris-citrate-yolk-glycerol camel extender with 15 µl/ml α-amylaze enzyme significantly improved sperm post-thaw sperm motility (61.6%) and decreased acrosomal damage (10.4%) and sperm primary and secondary abnormalities (5.0 and 7.0%, respectively). On the other hand, when dromedary semen was diluted and frozen with Triladyl, a post thaw sperm motility of >40% was recorded in 34% of freezing attempts and only 4 samples yielded a post-thaw sperm motility of >50%, however, a drastic reduction in post-thaw motility was recorded after 3 months of storage (Kutty and Koroth, 2012).

Regarding the type and concentration of the cryoprotectant used, El-Bahrawy et al. (2006) found that addition of 2% glycerol to Tris-lactose-yolk camel extender superiorly preserved post-thaw sperm motility (45.8%) and survival (73.3%) compared with using 3% glycerol or 2% and 3% dimethyl sulfoxide (DMSO). However, inclusion of 6% DMSO in Tris-fructose-yolk buffer for dilution and cryopreservation of dromedary camel semen had significantly improved post-thaw motility (66.7%), freezability (95.2%) and acrosomal integrity (84.7%) compared with addition of 2, 4 and 6% glycerol or 2 and 4% DMSO (Abdel-Salaam, 2013).

Dromedary epididymal sperm have been harvested and cryopreserved for using in in-vitro fertilization of dromedary oocytes. Abdoon et al. (2013) found that dilution of epididymal sperm with Ovixcell® (a soybean lecithin-based extender) or with Tris-fructose- yolk-glycerol extender significantly improved post-thaw sperm motility (47.5 and 45.0%, respectively) and viability (73.1 and 71.7%, respectively) and improved cleavage (37.3 and 83.8%, respectively) and Morulae and blastocyst (58.1 and 52.2%, respectively) rates. Also, El-Badry et al. (2015) diluted camel cauda epididymides sperm with a Tris- yolk-glycerol extender and reported post-thaw sperm motility, liveability and acrosomal integrity of 32.8, 67.6 and 71.2%, respectively. Frozen-thawed cauda epididymides sperm revealed higher fertilization, cleavage, morula and blastocyst rates (38.6, 28.4, 12.4 and 8.1%, respectively) when used for *in-vitro* fertilization of camel oocytes.

Earlier reports revealed that glycerol has deleterious effects on sperm cells due to its osmotic stress, changes in membrane organization, fluidity and permeability as well as changes in the membrane lipid composition (Watson, 1995). Adequate permeating cryoprotectant should be able to rapidly penetrate the sperm cells at freezing and also to rapidly exit cells at thawing. Amides (such as: acetamide, methylacetamide, formamide, methylformamide and

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dimethylformamide) are permeable cryoprotectants with lower molecular weight than glycerol and can cross sperm cell membrane faster than glycerol, thus exert less osmotic pressure than that can be exerted by glycerol (Carretero et al., 2015; Squires et al., 2004). Besides the crucial role of permeating cryoprotectants in preserving sperm integrity and viability during cryopreservation, substitution of egg yolk in extender by purified low-density lipoproteins has reported to improve the freezability and fertility in many species such as: cattle (Hu et al., 2011), buffalo (Akhter et al., 2011), dogs (Prapaiwan et al., 2016), boar (Jiang et al., 2007) and sheep (Tonieto et al., 2010).

To this date, results of semen freezing protocol and *in-vivo* pregnancy rates by using frozen-thawed semen are not yet satisfactory in the dromedary camels (Deen et al., 2003; Skidmore, 2003; Monaco et al., 2015). It is noteworthy that even when inseminations were carried out using equivalent amounts of motile and viable cryopreserved or raw spermatozoa, pregnancy rates were lower after AI with cryopreserved semen (0-26%) than with raw semen (50-80%) (Bravo et al., 2000a). Poor fertilizability of frozen semen may reflect a cryodamage of sperm DNA in frozen-thawed sperm even that revealing acceptable post-thaw motility and viability. Earlier report of Griveau and LeLannou (1997) demonstrated that sperm plasma membrane and DNA integrity are susceptible to damage during freezing-thawing process due to lipid peroxidation. Also, Bilodeau et al. (2000) reported that excessive generation of ROS molecules is evident during cryopreservation of mammalian semen that reduces the viability and fertilization capacity of sperm.

Therefore, further studies are needed to develop the cryodiluents for dromedary semen and the freezing and thawing protocols. Substitution of egg yolk with purified low-density lipoproteins in addition to utilizing amides as cryoprotectors may lessen the oxidative stress on the processed sperm and protects sperm membrane and DNA against cryodamage.

CONCLUSION

To establish a persistent AI-strategy for germblasm preservation from elite dromedary camel breeds, selected males must be dedicated to semen collection programs in AI-centers rather than permanently practicing natural mating. The dependence on the ease and sufficient semen collection with the camel dummy, as a technique resembling natural mating the most, is the optimal possibility to create a semen collection and storage strategy for preservation of semen from elite dromedary male camels. Persistent abundance of semen under controlled conditions in AI-centers provides excellent opportunities to develop extenders with optimal additives needed for elimination of viscosity and for proper protection of sperm against cold-shock and cryodamage during processing of semen.

Spermiogram assays after different stages of processing (neat semen, dilution, equilibration, post-thawing and incubation post-thaw) should include a set of both compensable and non-compensable traits (Saake, 2008). Including assays of non-compensable factors (such as chromatin or DNA consistency), which is important for predicting the male fertilizability and conception rate, helps to select suitable collections for processing and discarding processed collections of predicted low-fertilizing ability before using in AI (Oliveira et al., 2013; Saake, 2008).

Persistent preservation of successfully processed camel insemination-doses helps to initiate developing of other assisted reproductive technologies such as *in-vivo* and *in-vitro* embryo production and transfer as well as intra-cytoplasmic sperm injection regardless the limited male breeding season.

Competing interests

The author has no competing interest to declare regarding the publication of this article.

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