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Review

Semen of dromedary camel: a review

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ABSTRACT:

This review aims to highlight the characteristics of semen based on season, age, nutrition, the effect of diluents and preservation by dilution, cooling, freezing and thawing in dromedary camels. Various studies showed that the semen characteristics of camels were found to be: volume: 5-20 (mL), colour: creamy white, consistency: highly viscous, pH: 7.0- 8.0, Individual motility: 40-60 (%), sperm concentration: 100-350 (million/mL), total sperm (billion/ ejaculate): 0.5- 6 and abnormal sperm: 5- 20 (%). During the seasons of the year, the highest percentages of dead spermatozoa and sperm abnormalities were recorded during summer and the lowest during winter. It has been recorded that during cold months (December, January and February) the Iraqi mature camel shows higher percentage of sperm parameters live and individual motility. It was observed that the age between 4-5 years was better for semen collection. The mean of ejaculate volume has been reported to be highest during the copulation time of 395.95 Sec was 4.76 mL and lowest of 1.7 mL in 216.8 Sec. The method of collecting semen by the dummy camel was better in semen characteristics than that of electrical-ejaculation and artificial collection vagina and teaser methods. Semen liquefaction was achieved by leaving the ejaculate in a water bath at 25-37°C for a period of 4.5 min to 2 h. Tris and green buffer with the addition of 20% of egg yolks were the best diluents used to save semen between 4-25 °C for 48 h. Supplementation of tris based extender with 7% glycerol preserves the post-thaw quality and fertility of camel bull spermatozoa using 0.25 mL straws for long term semen preservation by dipping in liquid nitrogen. In conclusion, the semen characteristics of dromedary camels can be affected by several factors, in addition to many diluents that maintain sperm life during the period of dilution, cooling, freezing and after thawing.

Keywords:
Semen, Dromedary camel, Spermatozoa.

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INTRODUCTION

The camelidae family consists of two genera, *Camelus* and *Lama*. Within the genus *Camelus* is the dromedary camel (*Camelus dromedarius*) with one hump, and the Bactrian camel (*Camelus bactrianus*) with two humps. Both species are also known as old world camelids (Bravo *et al*., 2000). There are very few complete accounts of reproduction in the Camelidae. Most of the published data deal with *Camelus dromedarius* (Arabian camel) but reproduction is comparable across the whole Camelidae family: *Camelus bactrianus* (two humped camel), *Lama glama* (llama), *Lama pacos* (alpaca), *Lama guanicoe* (guanaco) and *Vicugna vicugna* (vicuna) (Ptaszynska, 2003). Camels are known to have exceptional physiological characters that empower them to endure and reproduce in the desert (El-Hassanein, 1989) (Table 1). Anyhow, low performance of reproduction is one of the most significant variables influencing productivity of camel compared to other farm livestock, there are as yet numerous potential ways accessible to improve profitability and reproductive performance of Arabian camels.

Numerous complex genetic and environmental variables influence low fertility in camels (El-Hassanein, 2003). Anyhow, low reproductive performance could be tackled by a good knowledge of the reproductive cycle and expanded utilization of assisted reproduction strategies, for example, Artificial Insemination (AI) and Embryo Transfer (ET) (Skidmore *et al*., 2013). In the male dromedary camels semen fluctuates as indicated by the concentration of spermatozoa and consistency of semen in addition to age, season, nourishment, copulation time and strategies for collection of semen (Marai *et al*., 2009). Collection of semen from the male camel is a significant step for semen assessment and handling. Many techniques for collection of semen for example, electrical-ejaculation, artificial vagina and dummy camel for various species were adjusted to adopt to the male copulating behavior (the sitting position and enduring mating time) (El-Bahrawy, 2005). 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.

This technique uses two extenders a cooling extender and a freezing extender. The cooling extender is added to the semen immediately after collection. The freezing extender contains the cooling extender in addition to a cryoprotectant (glycerol) that plays a role in the stabilization of the sperm plasma membrane (Marai and Zeidan, 2007).

Many researchers have considered semen preservation and insemination in the dromedary camel (Anouassi *et al*., 1992; Bravo *et al*., 2000; Skidmore and Billah, 2006), yet most of studies report reduced post-thaw motilities and barely any, pregnancies with AI of frozen or chilled semen (Deen *et al*., 2003). This could be because of the challenges associated with collecting semen from male camels and that the discharges are of low volume, low sperm count and are profoundly viscous. The spermatozoa are entangled inside this thick seminal plasma which makes the processing dilution and cryopreservation troublesome (Bravo *et al*., 2000; Deen and Sahani, 2006). Because of the enhanced enthusiasm for applying present day reproductive strategies in camels, particularly Artificial Insemination (AI), optimizing the dosage of is vital semen. Diverse extenders and cryoprotectants for camel semen have been tried and assessed for such a reason. Semen diluents especially; tris-sucrose, tris-citrate, lactose, skim-milk, sucrose I (15% egg-yolk) and sucrose II (20% egg-yolk) were assessed with regard to post-thaw semen quality (El-Bahrawy *et al*., 2006). Cryopreservation is a non-

physiological technique that includes an elevated level of adjustment of natural cells to the osmotic and thermic shock that happens during the dilution, cooling-freezing and the defrosting strategies (Watson, 2000) and the latter one influences cellular membrane damage (plasma and mitochondrial) and in the worst case, the nucleus (Ahmed *et al*., 2017). Cryopreservation of sperm in camelids is a waste, essentially attributable to an absence of information with respect to camelid sperm physiology and the viscous nature of the semen (Morton *et al*., 2010; Bravo *et al*., 2013). The aim of this review is to study about the dilution and preservation of cooled and frozen camel (*Camelus dromedarius*) semen.

Characteristics of camel (*Camelus dromedarius*) semen

One of the fundamental qualities of camel semen is high viscosity. The spermatozoa are entangled in a viscous seminal plasma that ends up in handling and estimation of sperm parameters troublesome. The gel comprises of mucopolysaccharides from the secretions of the bulbourethral gland or the prostate, in spite of the fact that the level of consistency relies upon the individual male (Skidmore *et al*., 2013). Liquefaction of the semen can be accomplished by tenderly pipetting the semen to break down the gel, or by the utilization of enzymes.

Thick, gel like ejaculate can be considered as satisfactory, while thin ejaculates even though might measure more in volume may indicate an incomplete ejaculate. An intriguing aspect is that semen ejaculate does not mix with the semen extenders. A thick characteristic semen ejaculate liquefy only if it is kept at room temperature, while it does not liquefy if it is extended, cooled and preserved at refrigeratory temperature immediately after collection (Deen, 2008a). Colour of the semen relies upon the proportion of the gelatinous part which is grey, to the sperm part which is white. The colour turns out to be somewhat yellow if the sample is polluted by urine. Especially, such data can give a prelimimary assessment by visible examination of semen. Based on the age and season, the semen concentration and consistency differed in the male dromedary (Marai *et al*., 2009).

Deen *et al*. (2005) connected between the high failure of fertilization and the viscous camel semen, which may assume a job as a reservoir of sperm and secure the viability of spermatozoa in the female genital tract by capturing sperm. Discharge consistency likewise makes it hard to deal with semen, to assess the concentration of sperm and motility just as to dilute semen in extender (El-Zanaty *et al*., 2004) and hence messing up the technical progress while filling straws. The high consistency in camelid semen brings about oscillatory motility of sperms (Bravo *et al*., 2000). This is because of entrapment of spermatozoa in mucus, and sperm can move simply after its liquefaction. Few mucolytic agents may thoroughly eliminate viscosity in camel semen with clear improvement of sperm forward motility as contrasted with untreated semen. Notwithstanding, these mucolytic agents may deleteriously affect acrosomal integrity after equilibration (El-Bahrawy and El-Hassanein, 2009). Bahrawy (2011) revealed that 5, 10 and 15 µL/mL concentrations of alpha-amylase were extremely powerful in overcoming seminal plasma viscosity, upgrading the post-thaw forward motility of camel sperm with no critical discernible impact on both acrosomal integrity and other uncommon variations.

Abdel-Raouf and El-Naggar (1976a) and Musa *et al*. (1993) revealed that the semen will liquefy partially whenever stored at 25-37°C for 10-20 min. However, different investigations showed it can take up to 8 h (Tibary and Anouassi, 1997). Assessment of the viscosity can be evaluated by estimating the strand formed between the glass slide and a pipette (Abdel- Raouf and El-Naggar, 1976a). Semen can be collected for AI with an artificial vagina or electro-ejaculator, the ejaculate taking 5 to 10 min to liquefy. It has the following characteristics (Al-Eknah, 2009): Volume: 5- 20 mL, colour:

creamy white to greyish white, consistency: highly viscous, pH: 7.0- 8.0, individual motility: 40- 60%, sperm concentration: 100- 350 million/mL, total sperm: 0.5-6 billion/ejaculate and abnormal sperm: 5- 20%.

Factors affecting sperm characteristics

Season

The seasonal variations in semen constitute differences with the highest values in winter and spring (Ptaszynska, 2003). Spermatogenic activity and diameter of seminiferous tubules is highest in breeding season. During the nonbreeding season, the seminiferous epithelial cells undergo shift in the stages of frequency. Low number of tubules go into final development and meiotic stages, and less tubules go into conclusive development and meiotic periods of primary spermatocytes. Spermatids elongation requires a more extended time. Sexual action of males in the non-rearing season is invigorated by GnRH treatment; pheromonal "male effect" initiates female camels to cycle prior in reproducing season. Adjustment in the semen consistency with administration of GnRH ends up in poll glands (found uniquely in males) that are 20-55 mm long/10-30 mm wide in the center during non-reproducing season and mostly twofold in size during rearing season. Glands are directed by testosterone, since they relapse upon castration. Poll glands secrete abundant colourless and transparent fluids (Hafez and Hafez, 2001).

In a year, the most noteworthy rates of dead spermatozoa and sperm a abnormalities were recorded during summer and the least during winter (Rai *et al*., 1997). Zeidan *et al*. (2001) found that semen colour of the male camels varied from yellowish white to grayish white, creamish white to watery, white and milky white to light milky white during winters, spring, summer and autumn and the sperm motility values were 73.5, 70.1, 61.6 and 65.0% during winter, spring, summer and autumn, respectively and no significance differences were observed in pH value of the male camels' semen due to different seasons. Deen *et al*. (2003) observed donation of semen improved from December onwards and reached a peak after mid January with peak performance persisting until April. It declined during May. The majority of camels had lost libido and refuse to donate semen by the end of May.

Normal estimations of ejaculate volume has been accounted for to be most noteworthy during February (7.9 mL) and least during December (3.9 mL) with an intermediate during January (5.1 mL). Motility of spermatozoa was significantly higher in February (60.7%) as compared with December (40.2%) or January (43.7%) in fertile Egyptian camels (El-Bhrawi, 2005). Deen (2008a) observed the mean of ejaculate volume to be highest during March (4.76 mL) and lowest during December (1.7 mL). Deen (2008b) noticed overall average of ejaculate volume to be highest during February (3.41 mL) and lowest during July, August and September (0 mL). Tajik and Lamsoo (2008) observed that the proportion of live sperms in breeding and nonbreeding seasons was not significantly different for, caput (83 vs 80%), corpus (90 vs 82%) and/or cauda epididymis (86 vs 90.5%) for breeding and nonbreeding seasons, respectively.

Abd and Ibrahim (2014) reported that during cold months (December, January and February) the Iraqi mature camel shows higher percentage of epididymal sperm parameters which are live and with individual motility. Moreover, Fatnassi *et al*. (2017) found that ejaculates collected in January and February showed good quality, especially regarding sperm motility and was more suitable for further program of cryopreservation or artificial insemination.

Age

The diameter of seminiferous tubule increases till nine years old and the quantity of spermatozoa enhances during the next years, which decreases progressively after. The total germinal cells-spermatogonia, primary spermatocytes and spermatids varied between 6 and 18 years of age (Abdel-Raouf *et al*., 1975). Testicu-

lar weight and dimensions enhance with age and attain their maximum at 10 to 15 years old, which later decline marginally post 15 years (Ismail, 1982). The ejaculate volume of 7.82, 8.12 and 7.94 ml during 2.5 to 5, more than 5 to 10 and more than 10 to 20 years, respectively was recorded by Zeidan (1999) and Ahmadi (2001). Percentages of each of dead spermatozoa, sperm abnormalities and acrosomal damage were recorded in the male dromedary camels at ages of 2.5 to 5, over 5 to 10 and over 10 to 20 years, respectively.

Zeidan *et al*. (2001) studied the effect of age on camel semen collected from corpus epididymal region at slaughtering. They reported that semen colour of the male camels varied from yellowish white to grayish white at 3 to 5 years, creamish white to watery white at 6 to 11 years and milky white to light milky white at 12 to 20 years of age, and no significant differences were observed in pH value of the semen due to age.

Nutrition

Semen quality has been seen connected with the general wellbeing and the nutritional status of the males (Musa *et al*., 1993). It is known that severe nutritional deficiencies are associated with a delay in puberty, testicular atrophy and a reduction in sperm production (Tibary and Anouassi, 1997). Severe deficiencies in vitamin A are specifically associated with a reduction in the testis weight and spermatogenesis (Ismail *et al*., 1988). Iodine and selenium deficiency have additionally been related with stamped decrease in the ejaculated volume and concentration (Ahmed and Nada, 1993; Barsham *et al*., 2002). Obesity, associated with excess fat deposition within the scrotum and perineal region, will result in increase in testes temperature, loss of libido and marked reduction in spermatogenesis (Wilson, 1992).

Copulation time

Copulation begins in a sitting female until his front legs are on her shoulders. The male flexes all joints in the sitting position behind the female. At that point, he propels himself gradually forward until his genitalia are close to hers and the tip of the penis, presently coordinated cranially, is looking for the vulval cleft. The penis is turned until the tip enters the vulva and is embedded into the vagina. The male makes many pushing movements with resting stages in the middle. He drools and grunts constantly. The female flexes her head more than once towards the male's head. The female squeals and grunts consistently (Yagil, 1985).

Normally, intercourse length in camels may stretch out for over 40 min relying upon the male libido, sexual intensity, breed, age and recurrence. Essentially, the male himself will direct the length of sexual intercourse. With regard to semen flood pattern during sex, it is smarter to indentify the semen after delivered from a male camel as "an assortment" as opposed to “a discharge” (El-Hassanein, 2017).

Deen (2008a) recorded the mean of ejaculate volume to be highest during the copulation time of 395.95 Sec as 4.76 ml and lowest in 216.8 Sec as 1.7 mL. In another study, the same researcher Deen (2008b) noticed the ejaculate volume has been increased to 3.41 mL in the copulation time 294.41 Sec.

Methods of semen collection

There are many semen collection strategies utilized for various species that are altered and adopted, all with fluctuating degrees of success. The techniques utilized were artificial vagina mounted inside a dummy (El -Hassanien, 2003), artificial vagina (Skidmore *et al*., 2013) and electro-“ejaculation” (Mostafa *et al*., 2014). (Table 2). Semen collection from the male camel utilizing electro- ejaculations requires sedation and extraordinary limitation. The ejaculate may be lower in volume and poor in quality and there is the probability of urine and cellular debris contamination. The AV is viewed as the most reasonable methods for collection, with a few examinations attempted to improve the technique. As the male camel in the reproducing season can be awful and forceful, the most significant consideration for se-

men collection in designing facilities is the security of the handler and collector (Tingari *et al*., 1986; Chaudhary, 1995; Tibary and Memon, 1999; Noakes *et al*., 2019).

Electrical-Ejaculation (EE)

Semen collection by utilizing electrical ejaculation method requires the male to kneel down, restricting his front legs to shoulder and neck, constraining him to turn on his side and binding his hind legs from the shin area together, trailed by his sedation or general anesthesia (El-Hassanein, 2003; Mostafa *et al*., 2014). Tingari *et al*. (1986) utilized the electro-ejaculator; the technique utilizes a greased up rectal test embedded into the anus and a 12 volt current is applied for semen ejaculation. Two electric stuns were applied, each with 10-15 pulse of 3-4 seconds span, with 2-3 min rest in the middle. Jöchle *et al*. (1990) announced that the utilization of the electro-ejaculator is constantly associated with sedation. Musa *et al*. (1992) prescribed a technique to instigate ejaculation by rectal manage of the ampullae before beginning collection of semen with the electro-ejaculator.

Many investigations reported less semen volume by utilizing electrical discharge than that by utilizing an AV and a teaser female (Bravo *et al*., 2000; El-Hassanein, 2003; Marai and Zeidan, 2007). However, other semen parameters were similar in the two procedures (Tibary and Memon, 1999; Marai and Zeidan 2007) or contrarily influenced by utilizing electrical ejaculation (El-Hassanein, 2003). Because of the brief term of ejaculation utilizing electrical ejaculation method, the semen got is frequently of low quality (Bravo *et al*., 2000). Unexpectedly, other investigations found an expansion in ejaculation volume in the wake of utilizing electrical ejaculation strategy when contrasted with utilizing an AV and a teaser female in dromedary camels (Mostafa *et al*., 2014). These investigations have explained this expansion in ejaculation volume by electrical ejaculation as an expansion in volume of accessory liquids related with electric stimulation of the accessory sex glands.

As it is clear, the sedated or anesthetized male is persuasively ejaculated by utilizing electrical ejaculation procedure with no sexual stimuli for his gonads as it typically happens in normal mating process. This may mirror that semen delivered by this strategy doesn't look like the semen that are normally produced. Electrical ejaculation method is advised to be utilized just when semen collection by AV is beyond the realm of imagination, as the act of this strategy has exhibited numerous disadvantages (El-Hassanein, 2003), the most significant of which are: expending a ton of time and effect, limiting males by sedation or general anesthesia, needs a great deal of workers for fixing the males and expelling ties, presenting males to bleeding wounds and fractures, making males to be frightful and incapable to mount normally once more, along with the probability of delivered semen contamination with cellular debris and urine (Tibary and Memom, 1999). Tibary and Anouassi (1997) declined the utilization of the electro-ejaculator for semen collection in camelids in light of the fact that they found that it was distressing and doesn't yield a test sample of semen and doesn't permit assessment of sexual conduct. Moreover, procedure might be morally unsuitable in certain social communities. Alfuraiji (1999) found to enhance productivity in camel by using artificial insemination, semen must be collected by electro-ejaculation.

Artificial Vagina (AV)

The most common and acclimated technique for semen collection in camels is the utilization of an artificial vagina and a responsive female (Figure 1). A bull artificial vagina of around 40 cm long is generally utilized for collecting semen from male adults (more than 10 years old), while a shorter artificial vagina of around 30-35 cm length, is proposed for the collection from more younger adults (under 10 years old). The coat of the AV is loaded up with water warmed to around 55 -

60ºC (Skidmore *et al*., 2013). For collection using an artificial vagina, 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).

Khan and Kohli (1973) reported the use of a artificial vagina of a female teaser for collecting the camel semen. While collecting the semen samples, utmost care should be taken to avoid sand and dirt contamination. Hence, the penis should be kept inside the artificial vagina before sample collection. (Tibary and Anouassi, 1997). By using a disposable plastic inner liner in the artificial vagina, the contact of the ejaculate with the inner rubber liner could be avoided and the reduction in sperm mobility could be prevented (Skidmore, 2004). Due to ground level copulation and long duration, the semen collection using artificial vagina was considered impossible by Arthus and Tigani (1990). In addition to this, contamination by sand, deficient ejaculation and refusal towards artificial vagina were also reported by Deen and Sahani (2006).

Hemeida *et al*. (2001) formulated a more secure and progressively helpful technique for collecting semen from the male dromedary, wherein there was an excavated pit underneath the collection region where the individual collecting the discharge utilizing a artificial vagina could be seated. This empowered suitable positioning of the artificial vagina underneath the teaser's perineum and alignment with the base of the penis so semen collection was generally simple. Also, ventral or horizontal bending of the penis was avoided, permitting better semen release just as giving the authority a superior perspective on the mating procedure and ejaculation. The primary drawback of utilizing such an facility, or to which the artificial vagina is joined, is the necessity for the male to be prepared. In any case, when this is accomplished, collection is quick and productive.

Ejaculate volume changes from 2 to 10 mL as there is incredible variation among males and even between ejaculates from a similar male (Morton *et al*., 2013; Skidmore *et al*., 2013; Mostafa *et al*., 2014). Great quality semen can be collected by utilizing fake vagina at week by week interims from dromedary camels, however the general productivity of semen collection by artificial vagina is low and should be additionally improved to adopt male camels for artificial insemination programs (Al-Bulushi *et al*., 2014). The semen volume of 16 mL from dromedary camel gathered by Kutty and Koroth, (2012) utilizing artificial vagina were white, homogeneous, thick and sperm rich. These estimations of semen volume were recorded for males after their sexual intercourse for a time lapse ranging from 5 to 15 min, any how, under natural mating, sex length may stretch out to over 40 min. Moreover, the higher copulation time by male camels resulted in increased semen volume and concentration.

Ziapour *et al*. (2014) reported semen collection in two different ways using phantom and artificial vagina, *viz*., the respective time of semen collection (411.2 vs 326 sec), volume (6.6 vs 6 mL), osmolarity (328 vs 319.4 mOsm/kg H_2O), pH (7.7 vs 7.9) of semen, concentration (161.4 vs 160.2)× 10^6/mL, total motility (84.1 versus 78.3%), dynamic forward motility (45.5 versus 44.3%), live rate (72.2 versus 76%) and plasma membrane integrity (61.5 versus 58.9)%. Monaco *et al*. (2018) found no distinction between an artificial vagina with or without a silicone inward liner utilized during the standard semen collection technique. Hence, the impact of silicone internal liner was found to be nil with regard to the mating behavior and the ejaculates. The utilization of this modified artificial vagina subsequently stays easily proven wrong, except if further examinations can distinguish potential advantages utilizing this or other artificial vagina types or changes. Such investigations, anyhow, ought to be performed utilizing a bigger dataset, applying an standard semen collection approach and an appropriate assessment of mating and semen parameters. Musa *et al*. (1993) observed differ-

Table 1. Semen characteristics in dromedary camels

S. No	Researchers	Volume (mL)	pH	Mass motility (%)	Individual motility (%)	Sperm concentration $\times 10^6$ (sperm/ mL)	Sperm concentration $\times 10^6$ (ejaculate/ mL)	Abnormal sperm (%)	live sperm (%)
1	Ismail (1988)	8.49	8.60	-	-	396	-	-	55.36
2	Noakes, *et al*. (2001)	3	-	-	-	300	-	-	-
3	Hafez and Hafez (2001)	4	-	-	80	600	2500	-	-
4	Deen *et al*. (2003)	4.30	-	-	35.9	300	-	-	73.3
5	Al-Qarawi and El-Belely (2004)	-	-	-	68.2	12	-	3.3	73.2
6	Agarwal *et al*. (2004)	6.7	7.3	-	-	566.4	-	-	-
7	El-Bhrawi (2005)	8.4	-	-	46.7	296.8	-	16	-
8	El-Bahrawy *et al*. (2006)	8.43	8.72	-	46.7	296.76	-	16	-
9	Skidmore and Billah (2006)	4	-	-	72.6	185- 350	-	-	-
10	El-Hassanein *et al*. (2010)	13.45	-	64.16	23.04	296.3	-	13.16	-
11	Kutty and Koroth (2012)	4.5	-	-	74.2	364.66	-	8.34	-
12	Bahrawy *et al*. (2012)	10.4	-	50.15	-	382.67	-	28.5	-
13	Fatnassi *et al*. (2017)	12.6	-	40	47.8	450.6	5122.4	-	-

ence between artificial vagina and electro-ejaculation in the characteristics of semen volume of ejaculate (7.5 and 3.9 mL), sperm concentration (325 and 331×10^6 mL), percentage of motile sperm (50.5 and 49.7%), pH (7.4 and 7.4), dead sperm (18 and 19%), abnormalities (27.7 and 27.4%) and acrosome abnormalities (8.5 and 8.1%).

Modified methods for semen collection (dummy)

Homeida *et al*. (2001) portrayed a more secure and increasingly proficient semen collection method. Semen is gathered from underneath the male camel utilizing an under-ground room with a square loop hole in the room to gather semen with a altered ox-like artificial vagina through the loop hole utilizing a teaser female. Another alternative strategy was created by El-Hassanien (2003) in which a female dummy camel identical typically to the teaser female is utilized in a sitting mating position, where the artificial vagina is set towards the end of the dummy. The dummy is in a similar shape and size of a female camel in sternal decumbency and has a solid iron skeleton, with an empty center, to withstand the heaviness of the male (around 400-500 Kg) during sex. The iron skeleton is secured with a solid smooth wooden sheath that was a similar symmetrical shape as a typical female camel and the whole dummy surface, along with the head and neck, are secured with a camel hide. Semen is gathered from underneath the dummy, as a well-prepared lab for immediate semen investigation is found under the dummy. Using a dummy had a better result over many semen collection issues especially the injury of the female, the anxiety of the male and above all very secure for the collectors providing high quality semen (El-Hassanein, 2017) (Figure 2).

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 deliv-

ered 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 (El-Hassanein, 2017). El-Bhrawi (2005) observed collection of semen by three methods *viz*: female teaser, electro-ejaculator and dummy. The results showed that nil effect of semen collection method on reaction time when using a female teaser or the dummy (37.06, 37.7 sec). Significant decrease of semen volume (5.15 mL) for electro-ejaculator collection method versus 8.21, 8.43 mL for female teaser and dummy respectively, pH for female teaser, dummy and electric ejaculation was 8.56, 8.72 and 7.96 respectively, while semen osmolarity was 0.37, 0.36 and 0.51 (Osmol/kg) and semen freezing point depression was -0.637, -0.681 and -0.946 (-0°C), respectively.

Dilution and freezing of camel semen

Liquefaction time

Semen comprises of spermatozoa contained in a watery liquid known as seminal plasma representing the consolidated secretions of the diverse accessory glands, for example, the seminal vesicles, bulbourethral and prostate gland. The relative commitments of these various organs fluctuate between species. Camel semen is thick and gels coagulated following collection. The gel comprises of mucopolysaccharides from discharges from the bulbourethral or the prostate gland, in spite of the fact that the level of viscosity relies upon the individual male. Semen liquefaction was accomplished by leaving the ejaculate in a water bath set at 25-37°C for 4.5 min to 2 h (Abdel-Raouf and El-Naggar, 1976b; Hafez and Hafez, 2001; El-Bhrawi, 2005; Marai and Zeidan, 2007; Skidmore *et al*., 2013).

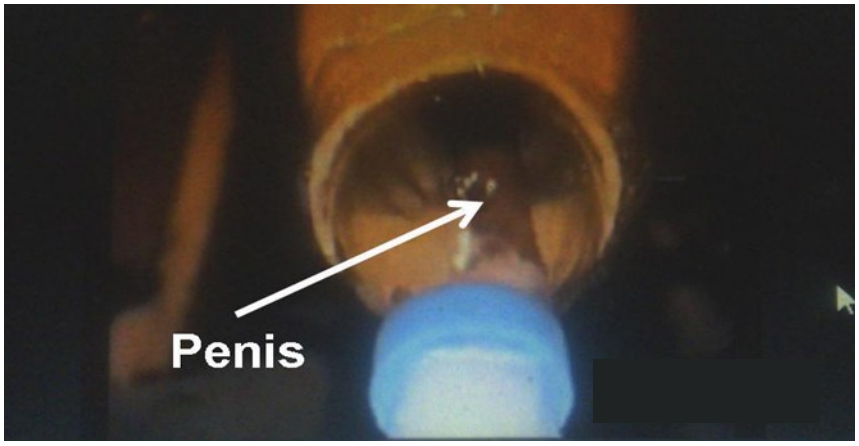
Kershaw-Young and Maxwell (2012) have demonstrated that proteins, primarily mucin 5B which is secreted from the bulbourethral gland, are for the most part liable for the viscosity in camelid seminal plasma instead of GAGs. Mucin 5B protein was about five fold in highly viscous seminal plasma and its concentration diminished significantly by semen liquefaction. The researchers found that the enzyme keratanase explicitly degrades keratan sulfate, which contributes about 85% of total GAGs in camelid seminal plasma and didn't totally decrease viscosity, while incubating for 2 h. On contrary, protease papain was significant in the total degradation of viscosity in about 30 min along with conservation of sperm viability and acrosomal integrity contrasted with GAGs enzymes and proteinase k (Kershaw-Young *et al*., 2013).

Camel semen does not show high mortality. After semen liquefaction, semen motility was observed individually (Deen *et al*., 2003). Liquefaction of coagulum released spermatozoa from their arrested position in coagulum and acetyl cholinesterase mediated breakdown of ATP appeared to be an essential prerequisite for sperm motility (Salisbury *et al*., 1985). Calcium (Ca), zinc (Zn), and iron (Fe) in high concentration may assume a significant impact during coagulation and liquefaction of dromedary camel semen (Mal *et al*., 2014). It has been suggested that citric acid is connected with coagulation and liquefaction of semen and also with calcium binding capacity of seminal plasma (Huggins, 1945). Lundquist (1947) suggested that citrate may act as an activator of the prostatic acid phosphatase. Another significance of citric acid has been shown to be in the maintenance of osmotic equilibrium in semen with sodium and potassium ions.

SgI and SgII discharged from seminal, are missing in camel. On account of dromedary semen, the spermatozoa are captured in a fibrinous network and in this manner it should be liquefied before the spermatozoa are free and the samples gets homogenous. Collagenase, fibrinolysin, hyaluronidase, and trypsin have all been utilized to decrease the viscosity of camelid semen; anyhow, every one of the catalysts have been believed to cause acrosomal damage in spermatozoa (Tibary and Anouassi, 1997). Semen liquifies with collagenase (Wani et al., 2008) yet there was diminished motility of

(A)

(B)



(C)

Figure 1. Semen collection by an artificial vagina and a teaser female (Courtesy: El-Hassanein, 2017)

spermatozoa post dilution with various extenders and at room or cooling temperature during storage demonstrating that the enzymes do have some negative impacts on the spermatozoa. Collagenase type I enhanced the rheological qualities of dromedary camel semen (Shekhar *et al*., 2012). Ismail (1988) observed that semen lasts 7.68 min before liquefaction. Motility examined 15 min after collection (liquefaction time) ranged from 30 to 50% (Billah and Skidmore, 1992).

Deen *et al*. (2003) found that adding 0.2 µm of caffeine to a tris buffer and dilution of dromedary camel semen at 1:1 ratio, significantly improved motility of individual spermatozoa while the addition of 1% α-chymotrypsin did not reduce semen viscosity. Wani *et al*. (2008) found that there was in every case moderate liquefaction in the camel semen without an additional extender as contrasted with diluted semen revealing that the best liquefaction was seen in tris-lactose extender. Fresh semen was immediately diluted (1:1) in INRA 96 and kept in a water bath (37°C) for liquefaction. It was observed that complete liquefaction of semen occurred within 30 min after extending camel semen with INRA 96 and motion characteristics of sperm easily evaluated by CASA (Pratap *et al*., 2012).

Collagenase enzyme (2% and 4%) melted 100% of the semen in 15 min. The impact of tris-fructose egg yolk, Androhep®, Laciphose®, collagenase (1%, 2% and 4%) and Green buffer® on semen liquefaction was

restricted distinctly to the initial 15 min of incubation. The motility of semen sample varied between 10 to 70%. During incubation, tris fructose egg yolk, Laciphose®, Green buffer® and collagenase (4%) invigorated the sperm motility significantly ($P<0.01$) in the initial 15 minutes (Ghoneim *et al*., 2010). The initial motility of semen samples ranged between 10 to 70%. Tris fructose egg yolk, Laciphose®, Green buffer® and collagenase (4%) stimulated the sperm motility significantly ($P<0.01$) within the first 15 min of incubation.

Viscous semen liquefaction was noticed at 23.89±1.49 h (Mal *et al*., 2016). In the process of liquefaction, 24.55kDa and 22.07 kDa proteins showed conjunction whereas intact 26.00 kDa protein disappeared. These proteins were identified as β-nerve growth factors (β-NGFs) in liquefied camel semen. Guanidine-HCL improves the rheological characteristics of dromedary camel semen along with significant ($P<0.01$) increase in sperm motility. Monaco *et al*. (2016) found that dilution of dromedary camel semen in tris based buffer containing the protease papain underwent complete liquefaction within 90 min of incubation at 37°C. However, higher percentage of head-to-head sperm agglutination was recorded and is supposed to be due to papain-induced protein degradation. 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°C (or until complete removal of viscosity) followed by addition of 10 μM E-64 (papain inhibitor) to halt the digestion of proteins by papain to conserve sperm cell-membrane integrity and viability (Kershaw-Young and Maxwell, 2011).

Short-term preservation (liquid or chilled form)

El-Hassanein (2017) reported that camel semen can be stored in a fluid (chilled) form at 4-5°C for use until 1-2 days without a noteworthy reduction in its quality. Extenders utilized for storage by chilling of semen must contain a carbohydrate as a energy source (glucose, lactose, sucrose or fructose), a non-penetrating protein for ensuring sperm cell membrane against cold-shock and damage (lipoprotein from egg yolk or casein from milk) along with the buffer (to keep up pH and tonicity) and the anti-biotics.

Extension of semen is done to get a known concentration of spermatozoa, for example, 50×10^6/mL (Anouassi *et al*., 1992), by including the extender at a proportion of 1: 1 to 1: 3 (semen: extender) contingent upon the concentration of the ejaculation, in the dromedary camels (Anouassi *et al*., 1992; Musa *et al*., 1992). Extenders are included at a temperature of 30 to 35°C to the semen, however after total liquefaction before including the extender (so as to get a good blend) (Marai and Zeidan, 2007). Diluted fresh semen has been preserved in plastic tubes and stored at 48°C for a maximum of 36 h (Musa *et al*., 1993).

The recognizable decrease in sperm progressive motility, subsequent to cooling and storage for 24 h, by up to 30-35% of related post-dilution values (Morton *et al*., 2013) may reflect defenselessness of sperm cells to cold-shock and membrane damage during cooling of semen from body heat level to the freezing point of water (from 35 to 5°C). The primary lesions related with cold-shock damage are the morphological modifications in sperm plasma membrane and modifications in the membrane permeability (Barrios *et al*., 2000).

It is realized that storing semen in a fluid form are related with sperm maturing and a decrease in their incubation lifespan because of deposition of toxic metabolic items for the most part as Reactive Oxygen Species (ROS) produced because of the lipid peroxidation of sperm membrane (Salamon and Maxwell, 2000). During handling and storage of semen, sperm membrane will be more prone to lipid peroxidation because of oxidation of the membrane polyunsaturated fatty acids and consequently increased generation of hydrogen peroxide. ROS deposition prompts oxidative stress which may cause damage to sperm membrane, decrease

Figure 2. Semen collection by using an artificial vagina dummy camel dummy (Courtesy: El-Hassanein, 2017)

in motility, viability, DNA integrity and reducing fertility (Kumer *et al*., 2011).

Oxidative stress assumes a significant part in the decay of sperm quality during chilled preservation through the synthesis of hydrogen peroxide and supplementation with an antioxidant additive, (for example, thioglycol, adenosine, prolactin, lycopene, catalase, cysteine, ascorbic acid, Vitamin C and E etc.,) is frequently used to protect oxidative stress in stored liquid semen. In addition, it was proposed that bringing down the final egg yolk concentration in the diluent, as a substrate for the dead sperm enzyme aromatic L-amino acid oxidase, it will likewise diminish the generation of hydrogen peroxide during diluted semen storage (El-Hassanein, 2017).

Effect of egg yolk addition

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 reduce the chilling damages on sperm by attaching to sperm membrane and enhancing its permeability without altering its physiological and chemical properties (Holt, 2000). Anyhow, egg yolk had demonstrated to contain progesterone (Bowden *et al*., 2001) which assumes a significant part in the sperm capacitation during handling and storage. Additionally, a few parts in egg yolk meddle with biochemical tests and metabolic examinations (El-Hassanein, 2017).

As a rule, egg yolk is made out of plasma and granules fraction of the yolk plasma is for the most part comprised of 85% low-density lipoproteins and 15% livetin, while yolk granules by 70% high-density lipo-

Table 2. Techniques of semen collection from camels

S. No	Parameters	Method of collection		
		Electrical-ejaculation	Artificial vagina and Teaser	Camel dummy
1	Copulation duration (min)	-	5-12	20-45
2	Ejaculate volume (mL)	3.56	6.87	17.75
3	Concentration ($\times 10^6$)	320	370	850
4	Motility (%)	42.8	60.2	80.3
5	Dead sperm (%)	25	21	12
6	Abnormal sperm (%)	22	19	11
7	Abnormal acrosome (%)	15.5	12.2	7.5

Courtesy: El-Hassanein, 2003

proteins, 16% phosvitin and 12% Low-Density Lipoproteins (LDL) (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. However, 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 was 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). Moreover, it has been demonstrated that LDL contain less progesterone than egg yolk because of its extraction process (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. Hence, further researches are required to test a different anti-oxidant and replacement of egg yolk with LDL extracts in camel extenders so as to reduce oxidative stress created during chilling-storage of dromedary semen and to secure sperm membranes and DNA integrity against cold-shock damage (El-Hassanein, 2017).

Type of extender in the dilution of camel semen

Many extenders have been tried for chilling-storage of camel semen including conventional (sugar-based, citrate-based, tris-and tris-tes-based buffers along with skimmed-milk) and commercial extenders (Green buffer, Biladyl, Androhep, Triladyl, Laiciphos, Biocephos, OptiXcell, EquiPlus and INRA-96) (Figure 3). Except in the case of Androhep, extenders containing egg yolk and lactose were best suited for liquid preservation of camel semen and preservation for upto 36 h (Bravo *et al*., 2000; El-Hassanein, 2017). Sieme *et al*. (1990), Anouassi *et al*. (1992) and Musa *et al*. (1992) reported that extenders containing egg yolk and lactose showed up highly appropriate to store camel semen in the fluid state.

Anouassi *et al*. (1992) indicated that SYG-2 extender gave the best results specially when supplemented with GnRH (50 μg/mL). Willmen *et al*. (1992) studied the mean motility of 20 ejaculates collected from three males of one humped camels after treatment by three different extenders, namely; glucose-EDTA, Androhep and Laiciphos. They concluded that according to their split sample test, Laiciphos was the most suitable diluent for the liquid preservation of semen up to 72 h as compared with the other 2 extenders. Musa *et*

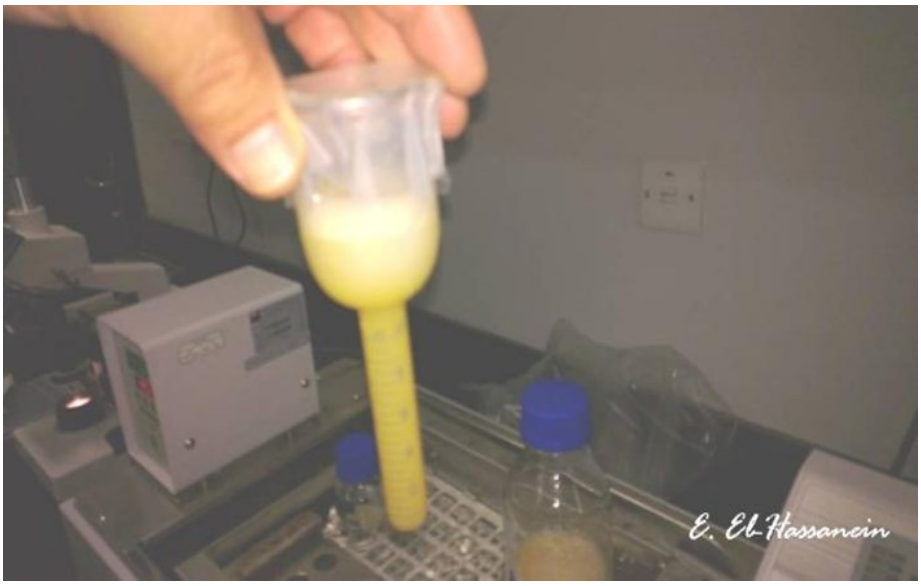


Figure 3. A partially diluted collection of semen (1 semen : 1 extender) of a total volume of about 70 mL (Courtesy: El-Hassanein, 2017)

al. (1992) used different extenders for diluting camel semen for short term preservation of liquid semen. They concluded that extenders containing egg-yolk and lactose were best suited for liquid preservation of the semen within 36 h. Sieme *et al*. (1993) showed that the Laiciphos extender gave the best quality followed by Androhep and Sodium citrate extenders.

Tibary and Anouassi (1997) reported that most camel extenders normally contain an energy source (glucose, lactose and fructose), a protein for security against cold shock (lipoprotein from egg yolk or caseine from milk), a buffering system and anti-microbial agents. They also indicated that no studies were conducted on the effect of physical and chemical properties (pH and osmotic pressure) of the extender on the motility and freezability of the preserved semen. Vyas *et al*. (1998) revealed that extender containing tris was accounted to be better than that containing 11% lactose.

Skidmore (2000) noticed that the best outcomes on conception rates have been accomplished with three commercial extenders, Green buffer (IMV, L'Aigle, France) with 20% (V:V) egg yolk, Laiciphos (IMV) and Androhep or an extender containing 11% (w:v) lactose and 20% (V:V) egg yolk, as contrasted with different extenders.

Deen *et al*. (2003) have showed the effect of α-chymotrypsin (1% α-chymotrypsin in tris buffer) and caffeine (0.2 µm caffeine supplemented in tris extender), added to split samples of semen at a ratio of 1:1, on spermatozoa motility and found that the addition of caffeine, but not of α-chymotrypsin, improved motility of individual spermatozoa.

Zeidan *et al*. (2004) showed that extending camel semen with lactose yolk citrate extender gave insignificant higher percentage of sperm motility and maintained the pH value, lower percent of dead and abnormal spermatozoa, lower activity of AST and ALT than those obtained with tris yolk extender. It was also reported that the addition of 10 µm caffeine to both extenders significantly improved motility and decreased dead sperm.

Deen *et al*. (2004) weakened split ejaculates with tris based and Biociphos extenders, cooled them to 4°C and inspected sperm motility at 24, 48, 72 and 96 hr. They found that none of the diluted samples in Biociphos showed any motility after 24 h while 40% of spermatozoa weakened in tris were motile after 24 h; anyway this was decreased to 10% by 96 h. Wani *et al*.

(2008) likewise utilized a split-sample procedure to examine the adequacy of five extenders for storing camel semen at 4°C for 24-48 h. Semen samples were diluted in tris + egg yolk, tris–lactose + egg yolk, citrate + egg yolk, sucrose + egg yolk, tris fructose + egg yolk before keeping in a fridge at 4°C and inspected post 24 and 48 h. The outcomes demonstrated that the most noteworthy extent of motile and viable sperm was accomplished with tris-lactose + egg yolk (80-90%) trailed by tris + egg yolk and sucrose + egg yolk, yet there was a steady decrease in motility in all extenders to <20% by 48 h. Half skimmed milk appeared to be a better extender for transient preservation of semen proposing that lactose is suited for spermatozoa of camel (Hammadi *et al*., 2008).

Deen (2008a) noticed that using tris and egg-yolk dilution, semen could be preserved at refrigerator temperature and sperm motility is maintained in cooled semen for 48 h in majority of the samples, 72 h and even for few weeks in some samples. 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^{o}C for 3 days compared with dilution in glucose-yolk-citrate, skim-cow-milk and skim-camel-milk extenders.

The effect of catalase enzyme (0, 250, 500 and 1000 IU/mL) at various concentration on the semen quality using tris-fructose-egg yolk extender when stored at 5°C for a period of 5 days was studied by Medan *et al*. (2008). The sperm motility was significantly enhanced using 500 IU/mL of catalase (from 53.2 to 62.7%) and declined the mortality of spermatozoa significantly (from 22.4 to 16.5%) abnormal sperm from (3.4 to 7.9%) and acrosomal damage from 7.5 to 4.5%, over the period of 5 days. However, sperm motility decreased from 62.7 to 30.1% and the percentages of dead sperm and abnormalities increased from 16.5 to 42.2% and 7.9 to 26.7% respectively.

Waheed *et al*. (2011) demonstrated that INRA-96® extender was the best for keeping dromedary epididymal spermatozoa at 5-30°C. Additionally, Morton *et al*. (2013) stated that dromedary semen dilution with green buffer or with INRA-96 fundamentally improved sperm motility post dilution (67.4 and 59.1%, respectively) contrasted with motility in semen (45.5%) and both extenders equivalently held 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) while being stored at 4^{o}C for 24 h. In any case, chilling-storage of semen diluted with INRA showed predominant sperm fertilizability (23.5%) than that weakened with green buffer (0.0%) reflecting sperm DNA damage during chilling-storage of semen diluted with Green buffer. It has been accounted that sperm with damaged DNA, when prevailing to fertilize oocytes, the subsequent embryos may neglect to develop or embed in the uterus or might be normally prematurely aborted later (Alvarez, 2003).

The Ovixcell® semen extender enhanced the dromedary camel epididymal sperm motility dynamically while storing at 4°C for 48 h, whereas sodium-citrate-egg yolk extenders was not best suited for the cold storage of epididymal sperms (Abdoon *et al*., 2013). Antioxidant supplementation *viz* 0.4 and 0.8 µm extender from glutathione and 0.5 and 1.0 gL^{-1} from ascorbic acid on the parameters of epididymal spermatozoa stored at 25°C or 5°C for 0-12 h was studied by El-Harairy *et al*. (2016). Al-Bulushi *et al*. (2016) prescribed dilution of dromedary semen with Optixcell, Green buffer or Triladyl for holding at 4^{o}C for up to 48 h. Al-Bulushi *et al*. (2019) observed that during the use of two types of diluents (Green buffer and Triladyl), the sperm have greater viability when preserved in liquid form for 48 h following dilution with Triladyl and storage at either 4 or 15°C.

Long-term preservation (frozen form)

The hypothesis of freezing semen is the prepara-

tion of a cryopreserved load of hereditarily elite sperm cells to be utilized many times for AI-programs and applying other technologies for reproduction. During semen handling for cryopreservation, sperm cells are vulnerable to many stress field stages that can cause biochemical and anatomical change in their compartments (acrosome, DNA, mitochondria, axoneme and plasma membrane). A flourishing sperm-freezing method ought to anticipate formation of lethal intracellular ice crystal and to decrease cell membrane harm during and subsequent to freezing of semen. The components of the semen extender assumes a basic role in securing sperm against chilling-shock and cryoinjury which happen during cooling, freezing and thawing at critical temperature phases during processing (El-Hassanein, 2017).

Cryoprotectants were routinely used along with the cryopreservation extender to diminish any stresses caused to the sperm cells by cooling, freezing and defrosting (Purdy, 2006). The effectiveness of the cryoprotectants used was analyzed by the pre-freezing and the immediate post-defrosting sperm viability and its ability to guarantee 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 cryoprotectants 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. Glycerol is considered as permeable agent which reduces the crystal formation in the inner as well as outer of sperm cell, preventing damage to plasma membrane and enhancing viability and function (Forero-Gonzalez *et al*., 2012). It caused higher sperm attributes when higher concentration was used in tris extender containing lower concentration of fructose. It might be theorized that it could be because of lower level of fructose in camel semen plasma. Dromedary camel sperm are not tolerant to higher fructose concentration. Because of sperm membrane protection and function with more elevated level of glycerol, this prompted enhanced motility and plasma membrane integrity. These discoveries could prompt better improvement of semen extenders for upgrading post-thawed quality of dromedary camel spermatozoa (Holt, 2000; Akbar *et al*., 2018).

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). Enough permeating cryoprotectant must quickly enter the sperm cells while freezing and furthermore to quickly leave the cells during defrosting. Amides, (for example, acetamide, methylacetamide, formamide, methylformamide and dimethylformamide) are penetrable cryoprotectants with lower molecular weight than glycerol and can cross sperm cell membrane quicker than glycerol, and hence apply less osmotic pressure than that can be produced by glycerol (Squires *et al*., 2004; Carretero *et al*., 2015).

Billah and Skidmore (1992) found that laiciphos was the best extender as compared with other media. They observed vigorous motility in high proportion of spermatozoa after one day to thirteen months of freezing. Deen and Sahani (2006) noticed post-thaw duration of survival of thawed spermatozoa by incubating at 37 and 4°C. At 37°C, the reduction in motility was about 50% at 0 h. At 2, 3, 4 and 24 h after incubation, almost 93, 99 and 100% spermatozoa lost motility respectively. At 4°C incubation, the percent decline was 17, 30, 35.8,

44.1 and 65.5%, at 1, 2, 3, 4 and 24 h of incubation respectively.

The inclusion of 2% glycerol to tris-lactose-yolk camel extender superiorly ensured post-defrost sperm motility (45.8%) and survival (73.3%) diverged from using 3% glycerol or 2% and 3% Dimethyl Sulfoxide (DMSO) (El-Bahrawy *et al*., 2006). Moreover, Abdel-Salaam (2013) reported that 6% DMSO in tris-fructose-yolk buffer addition as a diluent and cryopreservant of dromedary camel semen enhanced post-thaw motility (66.7%), freezability (95.2%) and acrosomal integrity (84.7%) significantly on par with 2, 4 and 6% glycerol or 2 and 4% DMSO.

Utilizing glycerol as a cryoprotectant with 2% final concentration had the most elevated post-thaw motility (45.83%) and sperm endurance rate (73.33%). The estimations of post-thaw motility were 30.0, 28.83 and 36.67% for 3% glycerol and 2% DMSO and 3% DMSO respectively, with endurance rate of 46.87, 40.7 and 53.82%, respectively. Level of intact acrosome was not influenced by either type or percent of the cryoprotectant (El-Bahrawy *et al*., 2006). Additionally, El-Hassanein (2006) found that tris-sucrose-yolk-glycerol extender essentially decreased the dilution impact on dromedary sperm viability and had improved their freezability after quick freezing as contrasted with dilution in sucrose-yolk-glycerol and tris-yolk-glycerol extenders.

El-Bahrawy (2010a) inspected tris-lactose extender with five concentrations of α-amylase (0, 2.5, 5, 10, 15 µL/mL) on seminal plasma liquefaction before freezing. He revealed that including 5, 10 or 15 µL/mL α-amylase to semen extender adequately liquefied seminal plasma and improved post-thaw forward motility of camel sperm without a huge impact on either acrosomal integrity or sperm anomalies.

El-Bahrawy (2010b) detailed that tris-lactose at 3% glycerol concentration recorded the most noteworthy post-thaw motility (45.8%) with the most elevated survival rate (73.3%) yet didn't report pregnancy rates utilizing such freezing-thawing methods.

Bahrawy *et al*. (2012a) demonstrated a noteworthy difference ($P<0.01$) in post-thaw motility for rapid thawing (56.33%) contrasted with 47.66% for slow thawed cryopreserved specimens using α-amylase. No critical impact was distinguished for neither the detached acrosome rate nor the sperm abnormalities. Bahrawy *et al*. (2012b) found that supplementation of tris-citrate-yolk-glycerol camel extender with 15 µL/mL α-amylaze enzyme significantly improved post-thaw sperm motility (61.6%) and decreased acrosomal damage (10.4%) and sperm primary and secondary abnormalities (5.0 and 7.0%, respectively). Of course, when dromedary semen was debilitated and freezed with Triladyl, a post defrost sperm motility of >40% was recorded in 34% of freezing endeavors and only four samples yielded a post-defrost sperm motility of >50%. However, an unprecedented reduction in post-defrost motility was recorded after 3 months of capacity (Kutty and Koroth, 2012).

Dromedary epididymal sperm have been gathered and cryopreserved for using in *in vitro* preparation of dromedary oocytes. Weakening of epididymal sperm with Ovixcell® (a soybean lecithin-based extender) or with tris-fructose-yolk-glycerol extender basically improved post-defrost sperm motility (47.5 and 45.0%, respectivley) and suitability (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 (Abdoon *et al*., 2013).

Skidmore *et al*. (2013) detailed that green/clear buffers + 20% egg yolk + 6% glycerol is the best mix for freezing dromedary camel semen yet *albeit* half of spermatozoa were motile in the wake of cooling, this rate was decreased to 35% quickly post thawing and to 0%, at 3 h post thaw. Additionally, camel cauda epididymis sperm diluted with a tris-yolk-glycerol extender had post-thaw sperm motility, viability and acrosomal integrity of 32.8, 67.6 and 71.2%, respectively. Thawed

frozen cauda epididymis sperm had higher fertilization, cleavage, morula and blastocyst rates (38.6, 28.4, 12.4 and 8.1%, respectively) when utilized for *in vitro* fertilization of camel oocytes (El-Badry *et al*., 2015). As of late the effect of different freezing rates and defrosting temperatures on the nature of camel spermatozoa post defrosting were contemplated. Camel sperm exhibited a high resilience for freezing rates with little differentiations between the diverse freezing rates. Better reports for the sperm traits were found from the quickest freezing rate on par with slowest rate for total and progressive motility and some kinematic parameters, nonetheless, no treatment was viewed as significantly ($P<0.05$) better for all parameters of sperm quality. In any case, post thaw sperm quality was better with the fastest thawing rate (60°C for 10 sec) on par with the lower rates (37°C for 30 sec).

Shotor diluent blended in with glycerol (Sh-G), dimethyl formamide (DMF, Sh-DF), dimethyl sulfoxide (DMSO, Sh-DS) or ethylene glycol (EG, Sh-EG), at 6% concentration each along with the test samples were cryopreserved. The effect Sh-DF over Shotor diluent upgraded with glycerol and Sh-DS in regards to post-thaw motility (55.83 versus 47.50 and 45.00%, respectively) sperm membrane (49.00, 39.33 and 42.67%, respectively) and acrosomal integrities (53.00, 57.33 and 52.33%, respectively) were reported (El-Badry *et al*., 2017). Sh-EG group showed the lowest post-thaw motility, plasma membrane and acrosome integrities (12.50, 22.67 and 30.67, respectively). Supplementation of tris based extender with 7% glycerol retained the post-thaw quality and fertility of camel bull spermatozoa (Akbar *et al*., 2018).

Cryopreservation procedure depends on the packaging method used, whether it is pellets (Graham *et al*., 1978; El-Bahrawy *et al*., 2015), ampoules (Demissie, 2019) or in plastic straws with different volumes (0.25, 0.5 or 4 mL) (El-Bahrawy, 2010a; El-Bahrawy *et al*., 2015; Demissie, 2019). 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 were 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).

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. The technique is carried out in the following steps: equilibration 37^{o}C for 10 min, 20^{o}C for 10 min, 10^{o}C for 10 min and 4^{o}C for 4 h. Freezing is carried out on a wire grid placed above liquid nitrogen at 3 cm for 3 min, at 2 cm (-75^{o}C) for 2 min, at 1 cm (-175^{o}C) for 1 min, then plunging into liquid nitrogen (-196^{o}C). Straws are typically frozen by setting them on a rack at known distances above liquid nitrogen. The quickest freezing rates are observed by the utilization of a little volume (0.25 or 0.5 mL) (Marai and Zeidan, 2007; Skidmore *et al*., 2013).

Freezing rates can be changed by alteration of the elevation of the straws. Exact freezing rates can be accomplished in semen bundled in straws by utilizing a mechanized freezer that can be customized to pursue an exact freezing curve. The samples are put on a platform which is brought down stepwise toward the liquid nitrogen surface. The temperature inside the samples is observed by a thermocouple which manages the movement of the platform (Marai and Zeidan, 2007). Thawing is done in a water bath. Thawing rates shift as per packing methods utilized. Pellets are normally thawed by dropping into heated repositories or by blending in a warm thawing extender. Semen frozen in ampoules is thawed by setting in a water bath set at 45 to 55^{o}C for 30 sec to 1 min. Small straws are thawed in a water bath at 37^{o}C for 30 to 40 sec or on the other hand at 40^{o}C for 8 sec. Large straws are thawed by consistent agitation in a water bath at 40^{o}C for 50 sec (Marai and Zeidan,

2007; Skidmore *et al*., 2013).

The quality of thawed dromedary camel semen is improved by freezing in small straws (0.25 mL), 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. In the dromedary, negligible loss is brought about by freezing if the initial semen motility is excellent. Semen freezed in lactose-egg-yolk extenders has kept up a similar post-thaw motility following six years of storage in liquid nitrogen (Marai and Zeidan, 2007). Musa *et al.* (1992) used 0.25 mL straws for long term semen preservation by dipping in liquid nitrogen. They concluded that post-thaw motility and sperm morphology gave good results when thawed in 1% sodium chloride solution at 38°C.

CONCLUSION

Significant differences were observed in the characteristics of camel (*Camelus dromedarius*) semen. The factors such as season, age, nutrition, copulation time and methods of semen collection had an effect on the characteristics of camel semen. Liquefaction time affects the sperm activity before and after dilution and freezing. Differential diluents and egg yolk ratio affected the effectiveness of sperm during the dilution period. Extender containing tris was reported to be superior. The addition of glycerol to the diluent in different proportions has a role in the longevity of sperm during freezing.

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