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A Review: Assessment of Artificial Insemination (AI) in dromedary camels (*Camelus dromedaries*)

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Abstract

Artificial Insemination (AI) is an important tool in farm animals to maximize the use of genetically superior males and thereby insure rapid genetic improvement. The use of AI has been reported in camelids although insemination trials are rare. This could be because of the constraints include collection as well as handling the semen due to the gelatinous nature of the seminal plasma. In addition, all camelids are induced ovulators, the females need to be induced to ovulate before being inseminated. This review discusses the different methods of collection, evaluation, dilution, and preservation of semen in camels. Various methods for inducing ovulation in the female camels are also discussed as well as the different techniques and ideal timing of insemination.

Keywords: Dromedary camel, semen, collection, preservation, insemination

1. Introduction

Artificial insemination (AI) is a technique used in domestic animals such as the cow, sheep, goat and horse to enhance the productive and reproductive performance (Skidmore *et al.*, 2018) [46]. AI technique has not evolved well as a method of breeding in camels compared with in other farm animals. This may be due to many reasons such as difficulty of semen collection from aggressive males in rutting season, semen viscosity is high in camel (Bravo and Johnson, 1994; Bravo *et al.*, 2000) [7, 8] and the continuous use of traditional natural breeding methods in most breeding herds (Skidmore, 2013) [45].

The use of AI as a breeding technique has been reported in camels since the 1960s. The first calf delivered using AI in Bactrian camel was registered in 1961 (Elliot, 1961) [17]. But during the last 25 years, the technique started to use more frequently in trying to improve genetic traits such as milk, meat and wool production as well as racing ability in the Middle East (Skidmore *et al.*, 2013) [45].

Some essential demands are required before AI programme can start. Firstly, the male camel must be trained to use of an artificial vagina (AV) for semen collection, and it should be diluted in appropriate extender to increase the use of each ejaculate. Secondly, as camels are induced ovulators, ovulation occurrence correlated with mating, each female must be induced ovulation for inseminating (Skidmore *et al.*, 2013) [45].

In the dromedary camel, many studies were conducted on semen preservation and insemination (Anouassi *et al.*, 1992; Bravo *et al.*, 2000; Skidmore and Billaha, 2006; Wani *et al.*, 2008) [5, 8, 44, 57], however, the most of authors reported that post-thaw sperm motility was low and pregnancies with AI of chilled or frozen semen were little (Deen *et al.*, 2003) [10].

This paper established to highlight methods of collection, evaluation, dilution, preservation and insemination of semen in dromedary camels.

2. Collection of semen

Semen collection from highly genetically males is considered substantial component in establishment an AI program. Proper collection and evaluation of the semen permits selection of high quality ejaculates for AI programs (El-Hassanein, 2017) [16].

Semen collection in dromedary camels is often relatively long, dangerous, and exhausted (Ziapour *et al.*, 2014) ^[58]. Semen collection in camels has many difficulties partially because their copulation in sternal recumbency, which makes it practically difficult for the collector, and the prolonged ejaculation period that may take from 5-10 min (Skidmore *et al.*, 2018) ^[46].

There are four major methods for semen collection in camels, including artificial vagina (AV) (Tibary and Anouassi, 1997a; Mosaferi *et al.*, 2005) ^[38, 49], electroejaculation (Tingari *et al.*, 1986) ^[53], phantom or dummy (Ziapour *et al.*, 2014) ^[58], and intra-vaginal condoms (Tibary and Anouassi, 2018; Mansour, 2022) ^[30, 52]. These approaches have advantages and disadvantages among which semen quality and animal welfare are the important challenges (Niasari-Naslaji, 2023) ^[40].

Artificial vagina (AV) is the most common approach for semen collection in camels. A modified bull vagina (30 cm long, 5 cm internal diameter) has given the best results (Bravo *et al.*, 2000; Skidmore *et al.*, 2013, 2020) ^[8, 45, 47]. However, the rubber liner of AV may have side effect, if sperm contacts to it, on sperm motility. Consequently, a shortened AV is more commonly allowing the semen to pass immediately into the collection tube. The AV is prepared by filling with water at 55-60 °C to adjust internal temperature for 38-40 °C and pressure inside the AV, to stimulate ejaculation, is acquired by inflate air between the inner liner and outer strong wall. A clear, glass tube (35-37 °C) semen vessel is attached to the apex of the internal latex rubber liner to preserve the semen warm during the prolonged ejaculation process and easy observation of ejaculation. Noticing of natural copulation proposed that the highly moving urethral process of the camel penis may need to introduce into the cervix for stimulating ejaculation during the extended copulatory process. Therefore, a foam imitation cervix of about 8 cm in length is putted inside the AV and the entrance of the AV is greased with KY jelly before use (Skidmore *et al.*, 2013) ^[45]. The volume of semen collected may differ between 2-10 ml but is occasionally contaminated with sand due to the mating behaviour of the male in sternal recumbency (Skidmore *et al.*, 2018) ^[46].

Electro-ejaculation may be used when collection by AV cannot be done, utilizing a standard bovine ejaculator (12 V and 180 mA; Standard Precision Electronics, Denver, CO). Electro-ejaculation can be done by the rectal probe greased with a plenteous amount of jelly, to ensure good contact with

the mucosa, and applying electrical impulses of increasing strength until ejaculation occurs. Two groups of stimulation are commonly used, each of 10-15 pulses of 3-4 s duration, with a rest of 2-3 min between the two impulses. The ejaculate is made into a flask grasped at the preputial orifice with sometimes milking of the prepuce to eject all the semen (Skidmore *et al.*, 2013) ^[45]. Electro-ejaculation is very exhausted for the animal and does not produce an enough semen sample (Tingari *et al.*, 1986) ^[53]. This method needs sedation ((Detomidine hydrochloride 80 µg/kg BW, i.m.; Al-Qarawi *et al.*, 2002) ^[3] or even general anesthesia, and may cause a hazard to the life and welfare of camels. Furthermore, semen collected by an electroejaculator may contain high volume and low concentration (Tibary and Anouassi, 2018) ^[52].

Phantom could be alternative method for semen collection in camel. Phantom prevents the danger for the collector, makes easy collection process and provides more suitable conditions to collect good quality of semen samples (Ziapour *et al.*, 2014; Panahi *et al.*, 2017) ^[41, 58]. Ziapour *et al.* (2014) ^[58] compared semen harvested from four males using the dummy with six males collected by AV and reported that no variations in an interval of semen collection or semen parameters like volume, motility, osmolarity and concentration. Furthermore, the number of samples tainted with visible particles was higher in the samples collected by AV (72.7%) compared to those collected by the dummy (0%). The major point in using phantom for semen collection in camels is training the bull. If one male is trained, it can be used to stimulate others. Many strategies could be taken to train the male to accept the camel dummy. To stimulate the male camels, entire isolation of them from herd during the season, sprinkle urine of estrous she-camel around the back and perineal area of the camel dummy, and playing the recorded sound of camel through copulation may be useful. EL-Hassanien (2003) ^[15] in which a female dummy camel resembles to the teaser female is utilized in a sitting mating position, where the artificial vagina is placed towards the end of the phantom. The dummy is in a same form and size of a female camel in sternal decumbency and has a solid structure of iron, with an empty space in center, to bear the heavy weight of the male (around 400-500 Kg) during mating. The iron skeleton is supported with a solid smooth wooden sheath that was a similar shape of female camel and the whole dummy surface, along with the head and neck, are secured with a camel hide. (Figure 1), (El-Hassanein, 2017) ^[16].



Fig 1: Semen collection by using dummy camel dummy (phantom) (Courtesy: El-Hassanein, 2017) ^[16]

Recently, intra-vaginal condoms used for semen collection in camels (Tibary and Anouassi, 2018; Mansour, 2022) [30, 52]. Main limitations in using condoms for semen collection in camels involve problems related to the installation and fixation of the device inside the vagina and around the vulva. In addition, displacing the installed condom due to clockwise and anti-clockwise movement of the penis (Niasari-Naslaji, 2023) [40].

3. Semen evaluation

After collection, the semen should be evaluated and the following parameters are measured.

3.1 Volume

The volume of the ejaculate can be examined immediately from the graduated collection tube. It may differ from 2 to 10 ml due to individual variation between males and even between semen from the same male (Skidmore *et al.*, 2018) [46].

3.2 Colour

The colour of the ejaculate may differ from a grayish translucent, when the ejaculate is gelatinous seminal plasma fraction and not very concentrated, to a creamy white colour as highly concentrated (Skidmore *et al.*, 2018) [46].

3.3 Viscosity

Evaluation of the semen facing a main problem concerns with high viscosity which makes handling and assessment of sperm parameters very difficult. Semen consistency is highly viscous by nature (Deen *et al.*, 2003) [10], with mass vibration at the initial raw semen measurement (Panahi *et al.*, 2017) [41] and liquefaction time is prolonged (18-41 h), (Mal *et al.*, 2016) [24]. Proteins responsible from the liquefaction of camel semen may belonging to the β -nerve growth factors family (Mal *et al.*, 2016) [24]. The viscosity is attributed to the bulbo-urethral glands and the prostate secretions, (as camelids do not have seminal vesicles), which form the seminal plasma and help copulation (Tibary and Anouassi *et al.*, 1997b; Kershaw-Young and Maxwell, 2012) [20, 50]. Viscosity is ordinarily assessed using the thread technique, *i.e.* measuring the strand formed between a pipette tip and a semen sample placed on a glass slide (Tibary and Anouassi *et al.*, 1997b) [50] (between 4-8 cm) (Figure 2). The viscosity may have been role in the lubrication of the vagina and remaining of semen in the female genital tract after coitus, thus preventing loss sperm. Since ovulation happen at 28-48 h following copulation (Anouassi *et al.*, 1992; Vaughan and Tibary, 2006) [5, 54] this viscous seminal plasma may guarantee that the spermatozoa are freed slowly as the semen liquefies to improve the time taken for them to reach and fertilize the egg in the oviduct (Deen *et al.*, 2003) [10].



Fig 2: Measurement of semen viscosity by Thread test (Courtesy: Tibary and Anouassi *et al.*, 1997b) [50].

Evaluation and processing of camel ejaculates need liquefaction so as to liberate trapped spermatozoa. In dromedary camel, many experiments on semen preservation showed that the spontaneous liquefaction after incubation of the ejaculate at 30-37 °C for 15-30 min were just partially (Al-Qarawi *et al.*, 2002; Wani *et al.*, 2008) [3, 57]. The viscosity of semen can be minimized by adding cysteine protease, such as papain, present in papaya (Kershaw-Young *et al.*, 2013, 2017; Monaco *et al.*, 2016; El-Bahrawy *et al.*, 2017) [14, 21, 22, 33]. Skidmore *et al.* (2018) [46] found that extending the semen sample 1:5-1:10 (depending on the viscosity) in tris-citrate-fructose extender, at room temperature, and gentle aspiration with a sterile transfer pipette for 30-60 min decreases viscosity, releases sperm and could be efficiently evaluated.

3.4 Sperm concentration

After liquefaction of the semen is diluted 1:100 or 1:200 in formal citrate the concentration should be assessed using a haematocytometer. There are few studies on the normal average of sperm concentrations in camels but it is proposed to be between 200 and 300 $\times 10^6 \text{ ml}^{-1}$ (Anouassi *et al.*, 1992) [5].

3.5 Motility

Motility is one of the most essential aspects of fertile spermatozoa. This parameter can be examined by a visual examination under the microscope. Placing a drop of diluted semen (1:1 in appropriate diluent) on a pre-warmed slide and estimating it under the microscope. The primary motility can

be very low basing on the viscosity of the semen but increases as the semen liquefies, so a real measurement can only be performed after semen liquefaction (Skidmore *et al.*, 2018) ^[46]. Other methods can be used to analyze sperm motility such as computer assisted sperm analysis (CASA). It evaluates several motility parameters to characterize individual movement like velocity average path (VAP; $\mu\text{m/s}$), velocity straight line (VSL; $\mu\text{m/s}$), velocity curvilinear (VCL; $\mu\text{m/s}$), amplitude of lateral head deviation (ALH, μm), straightness (STR,%), linearity (LIN,%), and beat cross frequency (BCF, Hz) (Skidmore *et al.*, 2018) ^[46]. On the other hand, Al-Bulushi *et al.* (2016) ^[2] and Malo *et al.* (2016a, 2017a, b, 2018) ^[25, 27-29] reported that no correlations between CASA results and fertility in camels when it was used to analyze sperm motility.

3.6 Morphology

Measurement of sperm morphology is conducted using smears prepared and stained with vital stains such as nigrosin

and eosin or Giemsa (Deen *et al.*, 2003; Morton *et al.*, 2011; Malo *et al.*, 2016a; El-Badry *et al.*, 2017) ^[10, 12, 25, 37] on a microscope slide. At least numbers of 100 spermatozoa are then assessed under high power magnification (1000 $\times$) as follows: normal, abnormal heads (large, small, tapering, pyriform, vacuolated, and double head), abnormal midpiece (distended or irregular, abnormally thick/thin), abnormal tails (short, multiple, broken, coiled, absent, bent, presence of cytoplasmic droplets) (Skidmore *et al.*, 2018) ^[46].

3.7 Sperm plasma membrane integrity (PMI)

The hypo-osmotic swelling test (HOS) is used to examine sperm PMI. Neat semen is diluted (1:4) by a hypo-osmotic solution (Fructose-sodium citrate, 60mOsm/kg) and incubated (35 °C for 45 min). Displaying of sperm with coiled tails indicate that the plasma membrane of them is intact and those with straight tails have non-intact membranes. (Figure 3) (Skidmore *et al.*, 2013) ^[45].

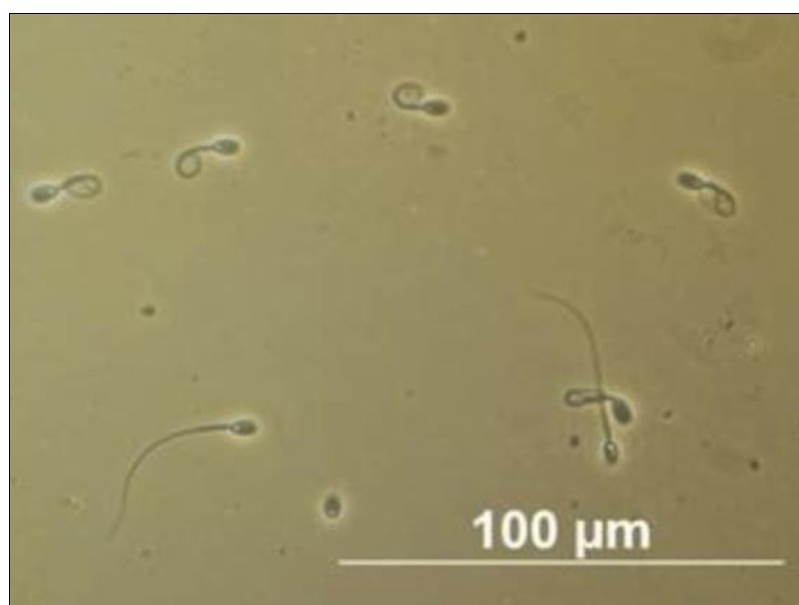


Fig 3: Hypo-osmotic swelling test (HOS): Coiled sperm-intact plasma membrane. Straight sperm, non-intact plasma membrane (Courtesy: Skidmore *et al.*, 2013) ^[45]

3.8 Sperm acrosome membrane integrity

Giemsa or FITC-PNA stain is used to evaluate sperm acrosome integrity. Smears are made with neat or diluted semen, dried with air, stained with Giemsa (10%, V:V in dd H₂O for 40 min) rinsed and dried. When using FITC-PNA stain, smears are prepared, fixed with ethanol (96% for 30 s) and then stained (10 $\mu\text{g/mL}$; 37 °C for 30 min). Slides are then rinsed in phosphate buffer saline (PBS) and measured (Morton *et al.*, 2008) ^[34].

3.9. Sperm viability

Sperm viability is examined by elimination of dyes such as eosin-nigrosin, eosin-fast green or propidium iodide. Stained smears are prepared by putting 1-2 drops of eosin-nigrosin stain and semen on one end of a slide, after that mixing the drops and smeared establishing a thin film. Live sperm have an intact membrane, pink color and exclude the stain, while the dead sperm have non intact membranes and purple color due stain penetration. (Skidmore *et al.*, 2013) ^[45].

4. Semen dilution

Semen extenders have a critical role in conserving spermatozoa and their functional activity while semen is

preserved at different temperatures (Medan *et al.*, 2008) ^[32]. Most of diluents comprise protein protects sperms from cold shock, a buffering system, an energy source such as fructose or glucose, and antibiotics (Skidmore, 2013) ^[45]. After collection, semen is diluted in a ratio of 1:1-3:1 (extender: semen) basing on the concentration of the ejaculate, with warmed (30-35 °C) extender added slowly to the semen. For précising assessments of concentration and motility the semen is liquefied, before insemination, to improve mixing of the semen with the extender (Skidmore *et al.*, 2013) ^[45]. Successful dilution of camel semen held at 5 °C was done using a diluent of tris-egg yolk-fructose enhanced with 500 IU/ml catalase (Medan *et al.*, 2008) ^[32]. According to Hafez and Hafez (2001) ^[19], glucose-milk diluent or sucrose-sodium citrate, lactose sucrose, and glycerol may be used for the cryopreservation of camel semen. Anouassi *et al.* (1992) ^[5] attained pregnancy rates of 50% with diluent including 11% lactose and 20% egg yolk. In further study, good pregnancy rates were obtained with laciphos + 20% egg yolk (53%) and green buffer + 20% egg yolk (47%) compared with other diluents like skimmed milk extender (0%) (Skidmore *et al.*, 2000) ^[43]. Recent studies compared the extender INRA-96 (I.M.V.) with green buffer. Findings observed that whilst

motility was higher after dilution in green buffer (67%) compared with INRA-96 (59%), membrane integrity was higher after dilution in INRA-96 (65%) compared with green buffer (56%). In contrast, sperm viability and acrosome integrity were identical for both buffers and pregnancy rates were not affected by diluent, green buffer (34%) and INRA-96 (34%), (Morton *et al.*, 2010a) ^[35].

5. Semen preservation

There are many constraints must be taken as considered in processing and preservation of camel semen. The high viscosity of seminal plasma, the harm effects of cold-shock through short-term preservation (chilling form) and the damage during long-term preservation (frozen form) are the main problems in preservation of camel semen (El-Hassanein, 2017) ^[16].

5.1. Short term preservation (liquid or chilled form)

Semen of camel can be preserved in a liquid (chilled) form at 4-5 °C to use during 1-2 days without a significant effect in its quality. Extenders used for short preservation of semen must be involved an energy source (glucose, lactose, sucrose or fructose), a non-permeating protein to protect sperm cell membrane against cold-shock (lipoprotein from or casein), buffer medium (to maintain pH and tonicity) and the antibiotics (El-Hassanein, 2017) ^[16].

In dromedary camels, previous studies reported that dilution of semen with a tris-based buffer persisted sperm viability for up to 48 h at 5°C compared by dilution in lactose buffer (Vyas *et al.*, 1998) ^[55], Biociphos (Deen *et al.*, 2004) ^[11] or in citrate or sucrose based extenders (Wani *et al.*, 2008) ^[57]. Other reports stated that green buffer was better than tris-buffer for preserving sperm motility, integrity and viability in dromedary semen stored at 5 °C for up to 48 hours (Ghoneim *et al.*, 2010; Waheed *et al.*, 2010) ^[18,56]. Recently, Al-Bulushi *et al.* (2016) ^[2] showed that there were no variations in motility, viability and acrosome integrity of dromedary sperm preserved at 5 °C for 48 h in optixcell, green buffer or triladyl. However, samples diluted with EquiPlus were recorded significant lower motility at 0, 24 and 48 h compared with other extenders.

Many studies recorded that the pregnancy rates were 25-33% after insemination of chilled semen diluted in green buffer + 20% egg yolk. ^[8] In contrast, authors reported that pregnancy rate was 50-55% in camels inseminated with fresh, diluted semen.

In subsequent studies, percentages of fertile sperm in chilled semen diluted with INRA were 23.5% compared with that diluted by green buffer (0.0%). This result showed that sperm DNA was damaged during chilled semen diluted with green buffer (Alvarez, 2003) ^[4]. Medan *et al.* (2008) ^[32] registered that the addition of catalase enzyme at concentrations of 500 IU/ml to chilled dromedary semen (at 5 °C for 5 days) diluted in tris-fructose-egg yolk extender significantly enhanced sperm parameters (increase motility, viability and decrease sperm abnormalities, acrosomal damage). Furthermore, the pregnancy rates of female camels inseminated with whole fresh, diluted cooled catalase-free and diluted chilled semen supplemented with 500 IU/ml catalase enzyme were 46.2, 22.2 and 37.5%, respectively.

5.2 Long term preservation (cryopreservation or frozen form)

The goal of cryopreservation of semen is the production of frozen sperm with high genetics to use during several years

for AI-programs and other reproductive technologies (*in vitro* and *in-vivo* embryo production). During freezing semen, sperm cells are susceptible to many stressful exhausted stages that may cause biochemical and anatomical changes in the parts of spermatozoa (acrosome, DNA, mitochondria, axoneme and plasma membrane), (El-Hassanein, 2017) ^[16].

Few reports registered on cryopreservation of camel semen. The ideal long term preservation technique includes the controlled cooling, freezing and storage of sperm in liquid nitrogen. But freezing of dromedary semen facing some challenges such as low volume and concentration of ejaculates, the viscous nature of camel semen causes initial difficulties of liquefaction to release the sperm and permit access of cryoprotectants during freezing (Skidmore *et al.*, 2018) ^[46]. One of the main elements to success AI with frozen semen is the freezing protocol used. The major variation between diluents used for chilling storage and cryopreservation is the addition of a cryoprotectant (such as glycerol or DMSO) to the freezing diluent (Skidmore *et al.*, 2013) ^[45].

Many studies carried out for recognizing the ideal diluents and cryoprotectants to support sperm viability (Malo *et al.*, 2017b; Morton *et al.*, 2010a) ^[28, 35]. In dromedary camels, El-Bahrawy *et al.* (2006) ^[13] recorded that the dilution of dromedary semen with Tris-citrate-yolk-glycerol, tris-sucrose-yolk-glycerol, tris-lactose-yolk-glycerol, lactose-yolk-glycerol and skim milk-yolk-glycerol diluents showed comparable pre-freezing sperm motility (63.3-68.7%), however, dilution in tris-lactose-yolk-glycerol extender was the highest post-thaw motility (62.3%) and sperm survival (93.2%) compared with the other extenders. In bacterian camel, Niasari-Naslaji *et al.* (2007) ^[39] reported that the Shotor+6% glycerol diluent improved post thaw motility (30%) compared to the green and clear buffers (4.2%).

El Badry *et al.* (2017) ^[12] studied the impact of cryoprotectants on frozen-thawed semen of dromedary camel. In this experiment, the semen was primary diluted in SHOTOR extender (Sh) supplemented with 6% of glycerol (Sh-G), dimethyl formamide (Sh-DMF), dimethyl sulfoxide (Sh-DMSO) or ethylene glycol (Sh-EG). These findings indicated that the Sh-DMF recorded highest post-thaw motility compared to the Sh-G and Sh-DMSO (55.8, 47.5 and 45.0% respectively), sperm membrane (49.0, 39.3 and 42.7% respectively) and acrosomal integrities (53.0, 57.3, 52.3% respectively), while the Sh-EG registered the lowest post-thaw motility, plasma membrane and acrosome integrities (12.5, 22.7 and 30.7%, respectively).

Addition of 6% DMSO in tris-fructose-yolk buffer for dilution and cryopreservation of dromedary camel semen significantly enhanced 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) ^[1].

A two-step cooling/ freezing method using green buffer produced good motility (average 47% total motility, range 38-64%). Sperm restored after centrifugation of liquefied semen through a colloid are primarily diluted in 20% egg yolk-green buffer, cooled to 5 °C, over 2 h before addition of green buffer containing 6% glycerol at a ratio of 1:1. Sperm are then balanced for 30 min, during which time the semen is filled into 0.5 ml straws and frozen at 1 cm above liquid nitrogen for 15 min (Skidmore *et al.*, 2018) ^[46]. The straws are suspended in liquid nitrogen vapour for 20 min (58 °C-120 °C), then plunged into liquid nitrogen (-120 to-196 °C) (Skidmore *et al.*, 2013) ^[45].

Thawing rates based on the packaging method used. The most popular method of packaging semen is in 0.5 ml straws, however, pellets have also been used for freezing semen of camel with good results (Morton *et al.*, 2010b)^[36]. Pellets are putted in a container held in a water bath until they thaw, while straws are commonly thawed in a water bath 37 °C for 10 s or 40 °C for 8 s (Bravo *et al.*, 2000)^[8].

Malo *et al.*, (2016b)^[26] compared samples thawed at 37 °C for 30 s with samples thawed at 60 °C for 10 s and the motility of the sperm at 0 h and 1 h was measured after thawing. Results showed that semen thawed at 60 °C was higher total motility at 0 h, progressive motility and acrosome integrity at 1 h compared with that thawed at 37 °C.

6. Induction of ovulation in female camels

Several methods to induce ovulation in dromedary camel have been reported such as mating to a vasectomized male, intramuscular injection of seminal plasma or injection with LH-like gonadotrophic hormones (GnRH or hCG) (Chen *et al.*, 1985; Marie and Anouassi, 1987)^[9,31]. But, copulation to a vasectomized male or injection of seminal plasma is impractical due to the difficulty of semen collection and the hazard of dissemination of venereal diseases (Skidmore *et al.*, 2013)^[45].

Earlier studies stated that a single injection of either 20 µg of the GnRH analogue (Buserelin), or 3000 IU of hCG was induced ovulation with it is rate of approximately 80% when the dominant follicle measures between 1.0 and 1.9 cm in diameter. However, this was quickly reduced to < 20% if the follicle diameter increased to 2.0-2.9 cm and no follicle > 3.0 cm ovulated (Skidmore *et al.*, 1996)^[42]. More recent investigations revealed that the ideal time for insemination is when the diameter size of mature follicle between 1.2 and 1.8 cm (Skidmore and Billah, 2006; Tibary and Anouassi, 1997b)^[44, 50]. Ovulation happens 24-36 h (31 in average) h following the stimulation (Marie and Anouassi, 1987; Anouassi *et al.*, 1992; Vaughan and Tibary, 2006)^[5, 31, 54]. The corpus luteum (CL) begins growing 3-4 days after the inducing stimulus and reaches its utmost size during days 8-9 (Marie and Anouassi, 1987)^[31]. If pregnancy does not occur, the CL regresses 9-10 days post-mating (Skidmore *et al.*, 1996; Tibary and Anouassi, 1996)^[42, 48].

7. Methods of AI and optimum number of spermatozoa to inseminate

7.1 Cervical insemination

Fibrosopic examination of the camel's cervix pre and post mating has revealed that semen is deposited partially in anterior cervix and intra-uterine and partly (Tibary and Anouassi, 1997c)^[51]. Thus in AI, the semen is commonly placed directly into the uterus, just cranial to the cervix, by means of a manually guided bovine insemination catheter passed through the short, straight cervix of camel. Conceptions rates of 50% have been done following insemination with 300 × 10⁶ live spermatozoa (Bravo *et al.*, 2000)^[8] or as few as 100 × 10⁶ (Anouassi *et al.*, 1992)^[5] directly into the uterine body. But with a short, open cervix that occurs during oestrous there may be a considerable wastage of sperm result from backflow of semen through the cervix, when it is placed just into the uterine body. Therefore further studies registered that if the semen is deposited at the tip of the uterine horn can be obtained good findings compared to the body of the uterus (Skidmore and Billah, 2006)^[44].

7.2 Deep uterine insemination

The advantage of deep uterine insemination is decreased the required number of spermatozoa to achieve successful fertilization due to deposition the semen is nearer the utero-tubal junction (Lopez-Gatius, 2000)^[23]. Although the complicated anatomy of some domestic species hindered the development of non-surgical insemination into uterine horns, insemination of camels is easier as the cervix is shorter, straighter and the uterus less coiled, thus it is simpler to pass a catheter through the cervix and guide it up the horn of uterus per rectum (Skidmore *et al.*, 2013)^[45].

Pregnancy rate is also affected by the number of spermatozoa inseminated and site of insemination. Skidmore and Billah (2006)^[44] recorded that there was no variation in conception rate of 150 million motile spermatozoa were inseminated in the uterine body (53%) compared to the tip of the uterine horn in the same direction as that the ovary carries the mature follicle (43%). But, if the number of spermatozoa was reduced to 80 million so there was a dramatic decline in conception rates when semen was deposited into the uterus (7%) compare to the tip of the uterine horn (40%). Consequently, authors proposed that perhaps 80 million is the at least number of sperm required to conduct a pregnancy by deep intrauterine insemination. More recent report, registered that the conception rates were 48% and 58.3% after deep horn deposition of 24 million spermatozoa when insemination conducted at 0 and 24 h after GnRH injection, respectively (Anouassi and Tibary, 2010)^[6].

8. Conclusion

Artificial insemination is a simple procedure in camels and can be used to cope some constraints caused by traditional methods of breeding may lead to decrease reproductive efficiency (short breeding season, difficult to determine an optimum number of females are pregnant at the end of the season and prevalence venereal diseases with a subsequent decline in fertility). It also allows more efficient use of high genetic-mert males, leading to good quality neonates and genetic improvement of camel herds in worldwide. Further studies should be conducted to address many problems including viscosity of seminal plasma, induction of ovulation and to improve cryopreservation of camel semen, so that pregnancy rates can be increased with frozen semen.

Conflict of Interest

Not available

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Reference

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