Emirates Journal of Food and Agriculture. 2022. 34(4): 339-345
doi: 10.9755/ejfa.2022.v34.i4.2850
http://www.ejfa.me/

RESEARCH ARTICLE

An easy, safe, and practical method for semen collection in dromedary camels

Nabil Mansour[1,2]*, Ahmed El-Ramah[1], Marcia C. Silveira[3], Lucia A.M. Bernardes[3]

[1]Bulaida Farms, Raibal Co., and Fujairah Research Center (FRC), Al-Hilal Tower 3003, P.O. Box 666, Fujairah, UAE, [2]Department of Theriogenology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt, [3]Fertilife, Fertilização, In Vitro FIV, Av. Castelo Branco, 1531 - Coimbra, Goiânia - GO, 74530-010, Brazil

ABSTRACT

In the current study, we have developed an easy, practical, and reliable method to collect semen from camel bulls. Simply, intromission of a super-sensitive artificial insemination glove intravaginally with an outer fixation in the perineal region from lateral and dorsal sides of the vulva, mating, glove withdrawal and semen collection were the successive steps. Bull behaviour before and during the mating process was observed. The collected semen was evaluated in aspects of semen colour, volume and sperm cell concentration. Also, individual sperm motility and semen fertility by applying *in-vitro* fertilization were investigated after semen dilution in a new developed extender for camel semen (100 mmol/l Tris, 65 mmol/l lactose, 50 mmol/l glucose, 40 mmol/l galactose, 125 mg/100ml BSA, 50 IU/ml catalase enzyme, 1000 IU/ml penicillin G and 500µg/ml streptomycin: pH 7.0). Presence of the intravaginal glove and its fixation by the external adhesive tape did not affect the bull willingness and did not interrupt the mating process. Semen collection was successful in 112 out of total 128 trials (87.5%) and the lengthy copulation was (10.3 ± 6.2 min - Mean ± SD). Mean parameters of the collected ejaculates were: volume of 8.3 ± 3.6 ml (Mean ± SD); sperm cell concentration of 437.6 ± 62.4 X 10^6 (Mean ± SD) sperm/ml; and sperm individual motility after liquefaction for 45 min was 68.2 ± 11.4 % (Mean ± SD). Semen was grayish white to creamy white in colour depending on sperm cell concentration. Finally, using of the collected diluted semen in IVF technique showed cleaved, morula and blastocyst rates 66.1%, 43.5% and 25.8%, respectively. In conclusion, using of intravaginal super-sensitive AI gloves is a practical technique for semen collection and will facilitate the application of the new reproductive technologies on a large scale in dromedary camels.

Keywords: Semen collection, Camel, *In-vitro* fertilization

INTRODUCTION

In the last three decades, camels in Arabian Gulf area have a special interest due to financial support by the local governments for racing and beauty contests. In comparison to other domestic animals, application of some reproductive technologies such as artificial insemination and *in-vitro* fertilization cannot be applied on a large scale (Skidmore, 2018) due to the difficulty in semen collection from camel bulls. Prolonged mating time, mating in the sternal recumbency, difficulty in bull handling and complicated mating behaviour are the biggest limitation factors to collect reliable clean semen samples from dromedary camel bulls (Bravo et al., 2000; Deen et al., 2003). Up till now, there are two main methods for semen collection in camels, either by using the bovine artificial vagina, either the normal or the modified one, or by the electro-ejaculation (Musa et al., 1993; Bravo et.al., 2000; Skidmore eta l., 2013). Although, the modified bovine AV was more widespread method and can be handled by an operator or fixed in a developed camel dummy or phantom (El-Hassanein 2003; Ziapour et al., 2014), a previous long-term training of camel bulls to accept the AV is essential (Ziapour et al., 2014). Also, using the electroejaculation technique is more complicated than the use of modified bovine AV and the collected semen samples are characterised by low sperm cell concentration with high risk of contamination with urine (Tingari et al., 1986). Additionally, the frequent usage of electro-ejaculation in camel is very stressful with a negative effect on camel bulls (Tharwat et al., 2014; Tharwat and Al-Sobayil, 2018). In the current study, we have developed an easy, safe, and practical method to collect the semen without a prior training of camel bulls. Intra-vaginal intromission of a super-sensitive artificial insemination

***Corresponding author:**
Nabil Mansour, Bulaida Farms, Raibal Company and Fujairah Research Center, FRC, Al Hilal Tower 3003, P.O. Box 666, Fujairah, United Arab Emirates. **Tel:** +971 50 6191799. **E-mail:** nabil@raibal.ae

Received: 16 December 2021; **Accepted:** 04 April 2022

glove followed by mating, glove withdrawal and semen collection were successfully done. Camel bull response and behaviour before and during the mating process of the females with the intravaginal glove or without for the natural mating were observed. Finally, the collected semen was evaluated in aspect of semen volume, sperm cell concentration and sperm individual motility. Also, semen fertility after dilution in a new developed extender by applying *in-vitro* fertilization was tested.

MATERIALS AND METHODS

Animals and keeping

Eight camel bulls were used in the current study for both semen collection and natural mating. However, 27 parous she-camels were used for semen collection experiments and another 25 she-camels were used for natural mating. Each male was housed in a single pen of 9X9 m and the females were kept in two large pens, each of 60X60 m and was housed by 26 she-camels. The camels were fed on wheat bran, crushed maize, crushed barley, hay, and dried Alfalfa with a rate of 1.2, 0.8, 0.4, 4.0 and 2.0 Kg/head/day, respectively.

Natural mating and semen collection

To prepare the she-camels for either natural mating or semen collection, they were slightly tranquilized by administration of xylazin 2% (Alfasan, The Netherland) at a dose of 0.1 mg/kg I/V. The females were examined in the standing position by trans-rectal ultrasonography using SonoScape S9V scanner (SonoScape Medical Corp, China) equipped with an endorectal linear array transducer of 5.5 MHz. She-camels showed ovarian follicular activity of 1.2-1.6 mm in diameter were used for either the natural mating or semen collection.

Semen was collected 4 times weekly during February 2021 to have a total of 128 semen collection trials. For semen collection, the perineal region of she-camel was cleaned, webbed, and disinfected with 70% alcohol. The main principle of this technique was to introduce a supersensitive artificial insemination glove deeply intravaginal without folding. To apply that, firstly the operator completely wore a normal gynaecological glove followed by incomplete wore of the supersensitive artificial insemination glove (WTA Watanabe Technologia Aplicada Ltda, Cravinhos, Brazil). The fingers of the supersensitive gloves were hold in the operator hand (Fig. 1a). Few drops of normal saline were sprayed over the supersensitive glove to facilitate its introduction inside the vagina. Then, the operator introduced his hand deep in the vagina till touching the cervix and slowly withdrew his hand, leaving the supersensitive glove inside. Care was taken to leave the

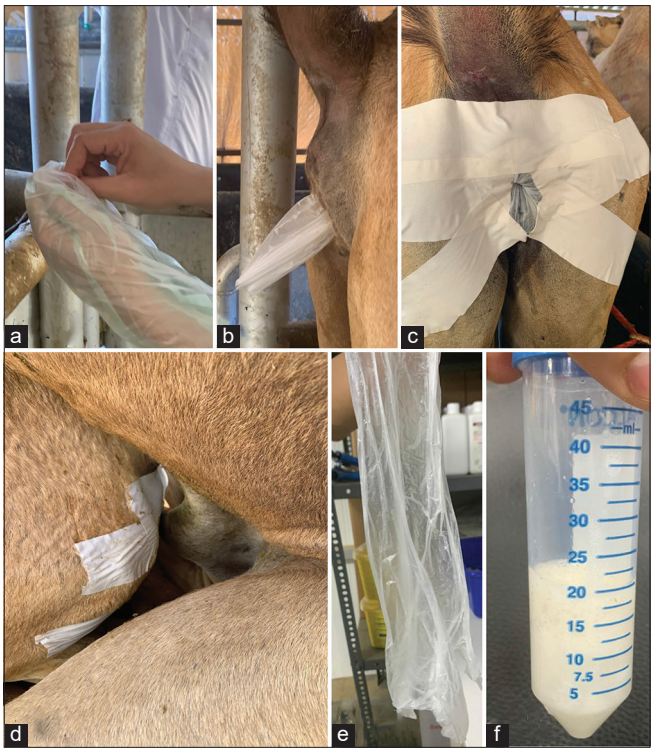

Fig 1. (a) Supersensitive glove over a gynaecological glove and the fingers of the supersensitive glove were hold in the operator hand. (b) intravaginal supersensitive glove and its extra length of the glove was cut off. (c) Fixation of the glove in the perineal region from lateral and dorsal sides of the vulva. (d) mating of a she-camel with the intravaginal supersensitive glove. (e) the glove containing semen after mating. (f) collected semen in a graduated tube.

introduced part of the super-sensitive glove straight in the vagina with a low chance of folding. The extra length of the glove was cut off (Fig. 1b) and fixed from lateral and dorsal sides of the vulva by zinc oxide tape (Max Medical Product LTD, London, UK). Fixation from the dorsal side of the vulva could also prevent the defecation during the mating process and therefore reduced the possibility of contamination (Fig. 1c). From the ventral side, no fixation tape was applied to facilitate passing the urine which usually happens shortly before the actual mating. Then the she-camel was driven to the male pen for natural mating (Fig. 1d). After mating, the glove was withdrawn from the vagina, cleaned from vaginal secretion (Fig. 1e) and squeezed to collect the semen in a graduated collection tube (Fig. 1f).

Bull response

Subjectively, the bull response to the she-camels used for semen collection with the intravaginal supersensitive gloves was compared to the response to the she-camels used for natural mating. Twenty-eight natural mating were observed and recorded as a control. Bull behaviour and eagerness before, and during mating process were observed and the lengthy copulation period was recorded.

Semen evaluation

All the chemicals and hormones used in this study were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). Once semen was collected, semen volume and colour were recorded. To measure the sperm individual motility, semen was quickly transferred to a water bath previously adjusted to 35 ºC. Then semen was diluted 1:2 (V: V, semen: diluent) with a diluent composed of 100 mmol/l Tris, 65 mmol/l lactose, 50 mmol/l glucose, 40 mmol/l galactose, 125 mg/100 ml BSA, 50 IU/ml catalase enzyme, 1000 IU/ml penicillin G and 500µg/ml streptomycin: pH 7.0, followed by a continuous pipetting for 5 min. Because it was difficult to assess sperm motility shortly after semen collection and dilution, semen was left for 45 min in the water bath with regular pipetting every 5 min for reduction of semen viscosity (Wani et al., 2008). Before measuring the sperm motility, semen was further diluted 1:2 with same diluent (final dilution 1:6, V: V, semen: diluent). Then 2 µl of diluted semen was pipetted on a 37ºC pre-warmed Leja ® Standard Count Slide (Leja Products B.V., The Netherlands) and sperm motility was examined under a hotplate Olympus CX41RF (Olympus Corporation, Tokyo, Japan). Sperm motility was evaluated by computer assisted sperm assessment (CASA -Sperm Vision® SAR software, Minitube GmbH, Tiefenbach, Germany). Three randomly selected microscopic fields were scanned and approximately 200 spermatozoa counted. The following sperm motility parameters were measured: spermatozoa with a velocity<5 µm/s were defined as immotile, spermatozoa with a velocity of 5–20 µm/s as locally motile, spermatozoa with a velocity>20 µm/s as motile. Sperm cell concentration was evaluated by using Bürker Türk Counting Chamber after complete semen liquefaction and dilution 1:100 with 0.5% formaldehyde solution (Monaco et al., 2018).

Fertility testing- Application of IVF with collected semen

Ovum pick-up (OPU) and *in-vitro* maturation (IVM)

Oocytes were collected *in-vivo* from 8 she-camels by trans-vaginal ultrasound-guided aspiration (ovum pick-up: OPU) technique. Animals were tranquilized by administration of xylazin 2% (Alfasan, The Netherland) at a dose of 0.1 to 0.2 mg/Kg I/V. After emptying the rectum and thoroughly cleaning the vulva and perineal area with a mild antiseptic solution Betadine®, the vulva was dried with cotton and wiped with 70% ethanol. The transvaginal OPU probe was inserted into the vagina and the ovaries were manipulated per rectum and positioned in front of the probe, to obtain a clear image on the ultrasonographic monitor. Before, and after OPU, the needle and Teflon tubing were thoroughly rinsed with normal saline containing 3 µg/ml heparin to prevent blood from clotting or oocytes from sticking to the Teflon tubing. All visible follicles were aspirated, and the contents collected in a 50 ml Falcon tube. Immediately following recovery, the aspirated fluid was filtered through an Emcon Oocyte Filter 3mm Tubing Port (Partnar Animal Health, USA), which washed with warm saline of 38ºC and oocytes or cumulus oocyte complex (COC) were picked up under a stero- microscope Nikon SMZ800 (Nikon Instruments Inc., USA) and morphologically graded according to Hasler and Barfield, (2021) into: Grade I, several layers of cumulus cells; Grade II, 1-3 layers of cumulus cells; Grade III, nude oocyte without cumulus cells; Grade 4, oocyte surrounded with expanded and loose cumulus cells (Fig. 2a). A total number of 162 oocytes were collected. COCs of Grade I & 2 (n= 94) were washed and transferred to the maturation media TCM199 media with Hepes buffer contained 26 mmol/l $NaHCO_3$; 0.2 mmol/l sodium pyruvate; 16.7ug/ml amikacin; 5.0 µg/ml of luteinizing hormone (LH); 0.5 µg/ml of follicle stimulating hormone (FSH) and 10% fetal bovine serum. The maturation medium contained the selected COCs was covered with mineral oil and incubated at 38°C in 5% CO_2 in humidified air for 32h according to Yaqoob et al., 2017. Nuclear maturation was assessed using aceto-orcein 30 h post-IVM according to the previously method of Fathi et al., (2014 & 2021). In brief, after stripping off the cumulus cells from COCs by repeat pipetting, denuded oocytes were incubated in hypotonic sodium citrate solution for 3 minutes. Oocytes were then placed on a

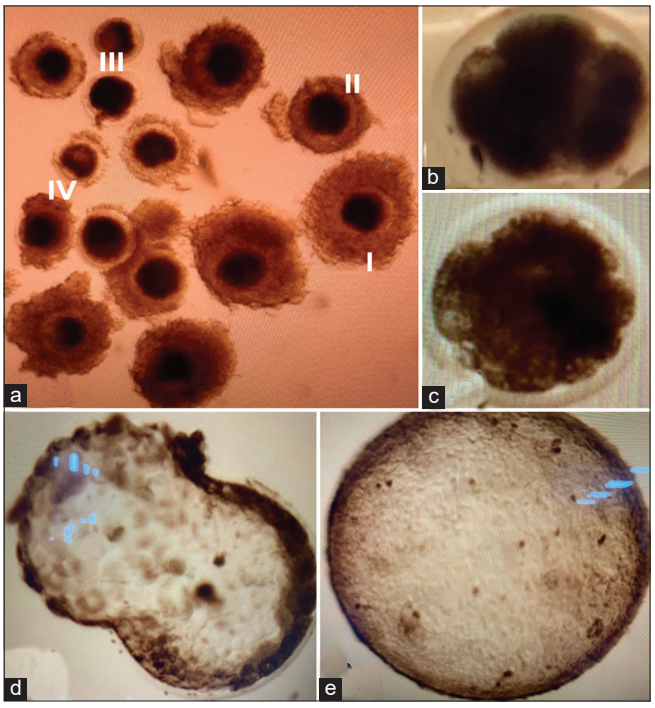

Fig 2. (a) Camel oocytes or cumulus oocyte complex (COC); I, II, III, and IV are grades of oocytes. (b) Cleaved embryo, 4 cell stage 2 days post-fertilization. (c) Morula stage, 4 days post-fertilization. (d) Hatching blastocyst, 6 days post-fertilization. (e) Hatched blastocyst of Grade I, 7 days post-fertilization.

glass slide and covered with a glass coverslip. The slides were fixed in ethanol: acetic acid (3:1) overnight and the oocytes were then stained with a 1% aceto-orcein stain. The slides were examined under phase contrast microscope for evaluation of nuclear maturation. Oocytes at the stage of metaphase II (MII) were recorded as mature.

Sperm preparation and oocyte fertilisation

To prepare sperm for IVF, collected semen was diluted 1:3 in the previously mentioned diluent and kept for 45 min at 35 ºC, then centrifuged at 1000 rpm for 4 min. Supernatant was discarded and sperm pellet was resuspended in the fertilization medium (Tyrode's albumin lactate pyruvate (TALP) supplemented with 6 mg/mL BSA, 30 µmol/l penicillamine, 15 µmol/l hypotaurine, 1 µmol/l epinephrine and 5 mmol caffeine according to Fathi et al., 2014) and kept one hour at 38 ºC in 5% Co_2. For fertilization, matured COCs were washed two times in the fertilization medium and transferred to 40 µl droplets (10 COCs per droplet) of fertilization medium covered with mineral oil. Insemination was done with the previously prepared spermatozoa at a concentration of $2X10^6$/ml.

Twenty-two hours after insemination, the oocytes were stripped of their cumulus cells by several pipetting and transferred into 100 µl droplets of culture medium (10 oocytes per droplet) and cultured at 38 °C in 5% CO2 in air. The culture medium was synthetic oviduct fluid (SOF) with amino acids supplemented with 2.7 mmol/l myo-inositol, 0.2 mmol/l pyruvate, 5% FCS, 5 mg/ml BSA, and 16.7 µg/ml amikacin. The number of cleaved embryos, morula and blastocyst stages were determined on days 2, 4 and 6-7 from fertilization, respectively. At day 7 post-fertilization, blastocytes were morphologically classified, according to Skidmore (2000) with modification, into: Grade 1: Excellent quality hatched spherical blastocyte with a smooth surface, Grade II: Medium quality, hatched blastocyte with some dark patches and irregularity in its outer contour, Grade III, Bad quality with either unhatched blastocyte or hatched dark blastocyte with some extruded cells.

Statistical analysis

Data of lengthy copulation period, semen parameters (semen volume, concentration, and sperm individual motility) and different developmental stages of embryos were checked for normality by using Anderson-Darling test. Chi-square test was used to compare the lengthy copulation period in females subjected to natural mating and the others with intravaginal gloves used for semen collection. The results were presented as mean ± standard deviation (SD) and the significance was set at $P < 0.05$. All statistical analysis were done using SPSS® 22 for Windows® (SPSS Worldwide Headquarters, Chicago, IL, USA).

RESULTS

There was no difference in bull reaction and willingness toward the female either in natural mating or those used for semen collection with the intravaginal supersensitive gloves. Initial reaction of the bull was to run eagerly toward the female followed by smelling the female particularly at the vulvar region followed by mounting. Presence of the intravaginal glove with its outer fixation by the external adhesive tape did not affect the bull willingness and did not interrupt the mating process. Semen collection was successful in 112 (87.5%), while in 5 trials (3.9%), the penis penetrated the glove, and the ejaculation was done inside the female. The lengthy copulation period did not show a significant difference ($P < 0.05$) for the females used for natural mating (11.7± 4.2 min- mean ± SD; Min: 6.8 min; Max: 15.3 min) and the females with the intravaginal gloves used for semen collection (10.3 ± 6.2 min - mean ± SD; Min: 4.9 min; Max: 17.1 min).

Semen parameters of the collected ejaculates were shown in Table 1. Semen was highly viscous, and the colour varied from greyish white to creamy white depended on sperm cell concentration.

A number of 62 oocytes, out of 94 collected oocytes of Grade 1 & 2, were matured (66%) and used for the fertilization with the collected diluted semen. After fertilization, a cleavage rate of 66.1% (41 out of 62 matured oocytes-Fig. 2b) was recorded. Twenty-seven (43.5%) were developed to the morula stage (Fig. 2c) and only 16 (25.8%) were completely developed to the blastocyte stage (Figs. 2d & 2e). Nine, 4 and 3 blastocytes were of Grade I, II, and III, respectively.

DISCUSSION

This study showed an easy, safe, and practical method to collect semen from camel bulls without a manpower interference during the collection process. Additionally, training of the bulls was not necessary, and semen could be successfully collected from all the used camel bulls in the first or second collection trials. The most important considerations in establishing a method for semen collection is safety of the handler and collection of reliable clean semen sample (Bearden et al., 2004). In dromedary camels, the difficulties primarily in semen collection and the ejaculates are of low volume, low sperm concentration, highly viscous and of high contamination risk (Skidmore et. al., 2013). Additionally, the complex mating behaviour and long copulation period in camels decrease the chance of male acceptance to many collection methods (Tibary and Anouassi, 1997).

Table 1: Evaluated parameters of the dromedary camel semen (n=112)

Semen parameters	Minimum	Maximum	Mean ± SD
Semen volume (ml)	3.5	15	8.3 ± 3.6
Sperm cell concentration (sperm/ml)	379.3 X 10^6	489.5 X 10^6	437.6 ± 62.4 X 10^6
Semen colour	Greyish white	Creamy white	-
Sperm individual motility (%)	47.9	80.4	68.2 ± 11.4

Previously to collect camel semen, the bovine artificial vagina (AV) or the bovine electro-ejaculator were used. Using the modified shortened bovine AV provided internally with a foam imitation cervix, to stimulate the motile urethral process of camel penis for ejaculation (Bravo et al., 2000), was the most routinely used method. To collect the semen by AV, a sexually receptive female is first teased by the male to make olfactory contact and get him aroused before the bull is lead up behind the sitting female (Skidmore et al., 2013). The used AV was either handled by a person or fixed inside a dummy (Hassanein et al., 2003; Ziapour et al., 2014; Skidmore et al. 2013). Fixation inside a camel dummy with an internal warming system to maintain a constant water temperature inside AV was preferred than handling by an operator (Ziapour et al., 2014). However, collection of semen by AV handled by an operator or incorporated in a camel dummy necessitates a former training of the camel bulls (Skidmore et al., 2013 & 2018; Ziapour et al., 2014). In contrary to our new technique, high percentages of semen sample collected by AV displayed a visible contamination with a subsequent low or no motile spermatozoa (Ziapour et al., 2014). Generally, there are 3–4 times of penis withdrawal from AV throughout semen collection in camel which act as the main source of semen contamination (El-Wishy, 1988).

Electro-ejaculation in camel is very stressful and does not yield a good representative semen sample (Tingari et al. 1986; Tharwat et al., 2014). Moreover, response of camel bulls to the electrical impulses of electro-ejaculator is variable and failure to obtain an ejaculate or only get few sperm cells contaminated with urine and cellular debris is common (Tingari et al. 1986; Skidmore et al., 2018). Additionally, using electroejaculation in camels is not recommended and causes reversible myocardial injury, changes in the acid-base status, and an increase in serum concentrations of lactic acid (Tharwat et al., 2014), as well as the indicators of inflammatory disturbances such as acute phase proteins and bone metabolism biomarkers (Tharwat and Al-Sobayil, 2018).

In the present study, the mean lengthy copulation period was non-significantly different between females in natural mating (11.7 ± 4.2 min) or those used for semen collection (10.3 ± 6.2 min). This is indicating that the bulls have fully accepted the used materials and mating course was not interrupted. Naturally, camel bull makes several thrusts, interspersed by some rests, and so the ejaculation comes in fractions and the whole process can takes between 5-20 min (Rai et al., 1988; Skidmore et al., 2013). Also, semen volume in the current study (8.3 ± 3.6 ml) was in the range of 3.5-15 ml and like the previous studies (El-Hassanien, 2003; Skidmore et al., 2013; Ziapour et al., 2014).

In the present study, variations in sperm cell concentration (379.3 - 489.5 X 10^6) and semen colour from a greyish white in colour, if the sperm cell concentration was low, to a creamy white colour, as the concentration of spermatozoa increased were also reported in other previous studies (Musa et al., 1993; Skidmore et al., 2013).

Sperm individual motility (68.2 ± 11.4) in the present study is higher than 30-50 % of (Billah and Skidmore, 1992; Deen et al., 2003), and slightly lower than 78.3-84.1 % of (Ziapour et al., 2014). Generally, semen of dromedary camel is highly viscous, and spermatozoa are entrapped in a gel like substance of the seminal plasma. Recording of sperm individual motility in dromedary camel necessitates a previous liquefaction of the gel content of seminal plasma which lasts from 25 min to 8 hrs (Tibary and Anouassi, 1997; Skidmore et al., 2013). Therefore, evaluation of sperm motility after its partial liquefaction might be the main reason for high differences in sperm motility between the various studies.

This is the first study applied IVF in dromedary camels using the oocytes collected by OPU technique without superovulation. In the most previous studies done on IVF in camel, Oocytes were collected either directly from ovaries after slaughtering (Moawad et al., 2012; Fathi et al., 2021) or from live she-camels through surgical aspiration (Tinson et al., 2001) or after superovulation (Wani and Skidmore, 2010). Also sperm cells were collected directly from cauda epididymis (Wani 2009; Moawad et al., 2012; Fathi et al., 2018; 2021). The used semen diluent was developed for camel sperm in our laboratory after several trials and testing on sperm motility and fertility. Also, using the diluted semen in the current study was successful in IVF and showed similar rates of cleavage, morula, and blastocyst formation in comparison to sperm cells collected directly from cauda epididymis (Moawad et al., 2012; Abdelkhalek et al., 2017). Generally, *in-vitro* production system of camel embryos is not well developed. Low number of collected oocytes and low rate of oocyte maturation in camelids in comparison to cattle are the main obstacles (Moawad et al., 2020; Wani, 2021).

CONCLUSION

Using of super-sensitive AI gloves intravaginally is an easy, useful, and practical technique for semen collection in camels. Using of the current technique will facilitate and encourage the application of the new reproductive technologies in a wide range in dromedary camels.

Declaration of competing interest

The authors declare that there is no conflict of interest.

Authors' contributions

Nabil Mansour: Methodology, investigations, statistical analysis, revision, writing and editing. Ahmed El-Ramah: helped in the practical part of semen collection and investigations. Marcia C. Silveira: application of ovum pickup. Lucia A.M. Bernardes: laboratory IVF technique.

Acknowledgment

The authors wish to thank the crown prince of Fujairah Emirate, Sheikh Mohammed bin Hamad Al-Sharqi, for offering the research facilities and for his continuous encouragement.

REFERENCES

Abdelkhalek, A. E., S. A. Gabr, W. A. Khalil, S. M. Shamiah, L. Pan, G. Qin, M. H. Farouk. 2017. *In vitro* production of Sudanese camel (*Camelus dromedarius*) embryos from epididymal spermatozoa and follicular oocytes of slaughtered animals. Pol. J. Vet. Sci. 20: 95-101.

Bearden, H. J., J. W. Fuquay and S. T. Willard. 2004. Artificial insemination. In: Applied Animal Reproduction. 6th ed. Pearson Prentice Hall, Hoboken, New Jersey.

Billah, M. and J. A. Skidmore. 1992. The Collection, Evaluation and Deep-freezing of Dromedary Camel Semen. In: Proceeding 1st International Camel Conference Dubai, UAE, February 2-6, p. 61.

Bravo, P. W., J. a. Skidmore and X. X. Zhao. 2000. Reproductive aspects and storage of semen in Camelidae. Anim. Reprod. Sci. 62: 173-193.

Deen, A., S. Vyas and M. S. Sahani. 2003. Semen collection, cryopreservation, and artificial insemination in the dromedary camel. Anim. Reprod. Sci. 77: 223-233.

El-Hassanein, E. E. 2003. An Invention for Easy Semen Collection from Dromedary Camels, El-Hassanein Camel Dummy. Recent Advances in Camelid Reproduction, Ithaca. International Veterinary Information Service, New York, USA, pp. 1-7.

El-Wishy, A. B. 1988. Reproduction in the male dromedary (*Camelus dromedarius*): A Reviews. Anim. Reprod. Sci. 17: 217-241.

Fathi, M., A. R. Moawad and M. R. Badr. 2018. Production of blastocysts following in vitro maturation and fertilization of dromedary camel oocytes vitrified at the germinal vesicle stage. PLoS One. 13: e0194602.

Fathi, M., A. Salama, K. El-Shahat, H. R. El-Sherbiny and E. A. Abdelnaby. 2021. Effect of melatonin supplementation during IVM of dromedary camel oocytes (*Camelus dromedarius*) on their maturation, fertilization, and developmental rates *in vitro*. Theriogenology. 172: 187-192.

Fathi, M., A. A. Seida, R. R. Sobhy, G. M. Darwish, M. R. Badr and R. Moawad. 2014. Caffeine supplementation during IVM improves frequencies of nuclear maturation and preimplantation development of dromedary camel oocytes following IVF. Theriogenology. 81: 1286-1292.

Hasler, J. F. and J. P. Barfield. 2021. *In-vitro* fertilization. In: Bovine Reproduction, . 2nd ed. Wiley-Blackwell, USA, pp. 1124-1141.

Moawad, A. R., G. M. Darwish, M. R. Badr and A. B. El-Wishy. 2012. *In vitro* fertilization of dromedary camel (*Camelus dromedarius*) oocytes with epididymal spermatozoa. Reprod. Fertil. Dev. 24: 192-193.

Moawad, A. R., I. M. Ghoneim, G. M. Darwish, M. R. Badr, D. A. El-Badry and A. B. El-Wishy. 2020. Factors affecting *in vitro* embryo production: Insights into dromedary camel. J. Anim. Reprod. Biotechnol. 35: 119-141.

Monaco, D., M. Fatnassi, D. Lamia, B. Padalino, M. M. Seddik, T. Khorchani, M. Hammadi and G. M. Lacalandra. 2018. Mating behaviour and semen parameters in dromedary camel bulls (*Camelus dromedarius*): A comparison between two types of artificial vagina. Emir. J. Food Agric. 30: 326-334.

Musa, B., H. Sieme, H. Merkt, B. E. D. Hago, M. J. Cooper, W. R. Allen and W. JoSchle. 1993. Manipulation of reproductive functions in male and female camels. Anim. Reprod. Sci. 33: 289-306.

Rai, A. K., S. N. Tandon and N. D. Khanna. 1988. Copulation time of Bikaneri male camels. Indian J. Anim. Sci. 58: 1202-1203.

Skidmore. 2000. Embryo Transfer in the Dromedary Camel (*Camelus dromedarius*) In: Recent Advances in Camelid Reproduction by Skidmore L. and Adams G.P. OCT 02, 2000.

Skidmore, J. A., C. M. Malo, E. G. Crichton, J. M. Morrell and B. S. Pukazhenthi. 2018. An update on semen collection, preservation, and artificial insemination in the dromedary camel (*Camelus dromedarius*). Anim. Reprod. Sci. 194: 11-18.

Skidmore, J. A., K. M. Morton and M. Billah. 2013. Artificial insemination in dromedary camels. Anim. Reprod. Sci. 136: 178-186.

Tharwat, M., A. Ali, F. Al-Sobayil, R. Derar and A. Al-Hawas. 2014. Influence of stimulation by electroejaculation on myocardial function, acid-base and electrolyte status, and hematobiochemical profiles in male dromedary camels. Theriogenology. 82(6): 800-806.

Tharwat, M. and F. Al-Sobayil. 2018. Influence of electro-ejaculator on serum concentrations of acute phase proteins and bone metabolism biomarkers in male dromedary camels (*Camelus dromedarius*). J. Appl. Anim. Res. 46: 1226-1232.

Tibary, A. and A. Anouassi. 1997. Theriogenology in Camelidae: Anatomy, Physiology, Pathology and Artificial Breeding. Acetes Editions, Rebat, Morocco.

Tingari, M. D., M. M. El-manna, A. T. A. Rahim, A. K. Ahmed and M. H. Hamed. 1986. Studies on camel semen, electroejaculation and some aspects of semen characteristics. Anim. Reprod. Sci. 12: 213-222.

Tinson, A. H., A. O. McKinnon, K. Singh, K. S. Kuhad and R. Sambyal. 2001. Oocyte collection techniques in the dromedary camel. Emir. J. Agric. Sci. 13: 39-45.

Wani, N. A. 2009. *In vitro* embryo production in camel (*Camelus dromedarius*) from *in vitro* matured oocytes fertilized with epididymal spermatozoa stored at 4 degrees C. Anim. Reprod. Sci. 111: 69-79.

Wani, N.A., 2021. *In vitro* embryo production (IVEP) in camelids: Present status and future prospectives. Reprod. Biol. 21, 100471.

Wani, N. A., M. Billah and J. A. Skidmore. 2008. Studies on liquefaction and storage of ejaculated dromedary camel (*Camelus dromedarius*) semen. Anim. Reprod. Sci. 109: 309-3018.

Wani, N. A. and J. A. Skidmore. 2010. Ultrasonographic-guided retrieval of cumulus oocyte complexes after super-stimulation in dromedary camel (*Camelus dromedarius*). Theriogenology. 74: 436-442.

Yaqoob, S. H., I. M. Saadeldin, A. B. Swelum and A. N. Alowaimer. 2017. Optimizing camel (*Camelus dromedarius*) oocytes *in vitro* maturation and early embryo culture after parthenogenetic activation. Small Rumin. Res. 153: 81-86.

Ziapour, S., A. Niasari-Naslaji, M. Mirtavousi, M. Keshavarz, A. Kalantari and H. Adel. 2014. Semen collection using phantom in dromedary camel. Anim. Reprod. Sci. 151: 15-21.