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IMPACT OF NOVEL MODIFIED FILTER STANDARD STRAW ON ULTRA-STRUCTURE AND VIABILITY OF VITRIFIED-WARMED SHE-CAMEL OOCYTES

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SUMMARY

The present study was carried out to test a new method of cryo-devices called a Novel Modified Filter Standard Straw (MFSS), as a tool for immature camel oocyte vitrification, and to compare its efficacy with Cryotop (CT). MFSS was utilized either as an open or closed device. Collected COCs of slaughtered She-Camel were divided into three groups ; ***group 1*** *(Cryotop),* ***group 2*** *(MFSS-open method) or* ***group 3*** *(MFSS-close method) and exposed to vitrification media of TCM 199 +0.1 M sucrose + 0.5% (w/v) bovine albumin, containing 7.5% ethylene glycol (EG) and 7.5% dimethylsulfoxide (DMSO) for a duration of 2 min (**VS-I**), and subsequent to vitrification media of TCM 199 + 0.1 M sucrose + containing 15% EG and 15% DMSO for a duration of 45s. (**VS-II**) and immersed in liquid nitrogen. Oocytes recovery rates of the three groups were not significantly different (91.3%, 97.1% and 98.0%, respectively) and post-thawing survival rates were (88.8%, 91.3 and 86.1%, respectively). While, oocytes with normal morphology were higher in MFSS-open method and CT than in MFSS-close method were (87.6%, 83.6% and 82.6%, respectively, P<0.05). Moreover, ultra-structural observation showed that the MFSS appears suitable for verification as indicated by the good ultrastructural preservation of cumulus cells, zonapellucida and cortical granules distribution.*

Keywords: Camel, oocytes, cryodevice, verification, Transmission Electron Microscopy

INTRODUCTION

Cryopreservation of mammalian oocytes is proved to be affected by method of preservation, media, protocol of cryopreservation and thawing procedure,time of exposure in vitrification, and thawing solutions (Panagopoulou *et al*., 2013), but limited studies gave attention to cryo-devices. Vitrification became the most widely used technique for cryopreservation of oocytes, however, during vetrification processes, the oocytes are subjected to stresses and chilling injury (Martino *et al*., 1996) and osmotic effects (Arav *et al*., 1993) lead to morphological changes associated with molecular alterations and subsequent functional damage (Paynter, 2005). Damage in oocytes caused by vitrification may be dependent on the cryopreservation procedures and the used vitrification devices (Khalili *et al*., 2012). To overcome these drawbacks, numerous investigations were carried out in the last years to improve and develop cryo-devices, i.e. to utilize small volumes of solution to minimize the toxicity effect of cryoprotectants, also to avoid damaging of ice crystal formation. Moreover, the development of reliable cryo-device for oocytes preservation may facilitate the vitrification procedure during dehydration/and rehydration process. Conventional plastic straw is the common tool for vitrification of human oocytes (Çetin and Baştan, 2006). Plastic straw provides a cooling rate of 2500°C/min. and a warming rate of 1300°C/min. (Palasz and Mapletoft, 1996). Recently numerous investigations carried out to improve carrier system for oocyte cryopreservation e.g. electron microscope grids; Open Pulled Straw; Cryoloop; Glass micropipettes; Gel loading tips; Closed pulled straw (CPS); Nylon mesh; Sterile stripper tips; Flexipet denuding pipette (FDP); Cryotip and Cryotop (Elnahas *et al*., 2010).

Cryotop is another cryo-device for oocytes vitrification procedure (Kuwayama *et al*., 2005). This device is rapid in cooling rate which may reach up to 20,000°C/ min and warming rate up to 40,000°C/min with a minimum volume ~ 1 µL of vitrification solution. While, the main risk of this device is the oocytes contamination due to direct contact of oocytes with LN_2 (Vajta *et al*., 2015), In addition to they are less adequately in protecting the cumulus cells specially during step-wise procedure in the vitrification solutions. Directly exposure of COCs to LN_2 led to the boiling movement of LN_2 around the COCs and this may cause damage to the gap junctions between cumulus and COCs. Therefore, the current innovation of a new carrier system for oocyte cryopreservation named a Novel Modified- Filter Standard Straw (MFSS), simply, is a conventional plastic straw Fitted with a filter to load oocytes with least volume of solution to avoid ice crystal formation, prevent toxicity from cryoprotectant agents (CPA_s) exposure and for facilitating bypass the current step-wise procedure, and can be utilize either as an open or closed device system. Therefore, this study was carried out for the first time to compare the efficacy of my innovation -MFSS with Cryotop by analyzing the subsequent viability and

ultrastructure change in camel cumulus oocyte-complexes (COCs).

MATERIALS AND METHODS

The study was conducted in the Laboratory of Animal Physiology and Biotechnology, Sheep and Goats Research Department, Animal Production Research Institute.

Media:

Except where otherwise indicated, all media were prepared from analytical grade chemicals (Sigma-Aldrich Co., St Louis, MO, USA).

***Collection of ovaries and recovery of oocytes*:**

Cumulus-oocyte complexes (COCs) were harvested by slicing technique (Dutta *et al*. 1993), from ovaries of slaughtered adult femalecamels. Ovaries were transported to the laboratory within 2−3 h in a thermos flask filled with a pre-warmed (37°C) sterile normal saline solution (0.9 % NaCl). Ovaries were washed twice in normal saline solution and sliced using a micro blade. Content of follicles released in culture medium-199 (TCM-199) as described by Modina (2004) Oocytes surrounded by compact cumulus cells and homogenous ooplasm were selected for experiment execution (Madison and Fraser, 1992).

Experimental Design:

Experimental 1: Effect of cryo- devices on recovery rates and oocyte viability:

A total of 746 COCs were randomly divided into three experimental groups:

G1: vitrified in Cryotops (CT, n=248) (Kuwayama and Kato, 2000); G2: MFSS-open method (n=240) and G3: MFSS-closed method (n=258).

Oocytes vitrification and thawing method

Vitrification protocol:

Two solutions were used for vitrification protocol. Vitrification solution 1; **VS-I)** was composed of TCM-199 as a base medium with 7.5% dimethyl sulfoxide (DMSO) (Carl Roth, Denmark), 7.5% ethylene glycol (EG) (Carl Roth, Denmark), 0.5% (w/v) bovine albumin (Octapharma, Germany), the second vitrification solution **(VS-II)** was composed of base medium (TCM-199) with 15% DMSO, 15% EG, 0.5% (w/v) and bovine albumin. All solutions were kept at room temperature.

Modified- Filter Standard Straws (MFSS):

The self-constructed Modified- Filter Standard Straw (MFSS) was made from electron microscope grids (1.9 mm diameter) fixed on a top of plastic straw (1.7 mm inner diameter, 0.15 wall thickness and 3 cm length) and the other end of this straw was drawn over the flame of alcohol burner in order to achieve a diameter equal to half of their original diameter " *first part"*. Another plastic straw (1.7 mm inner diameter, 0.15 wall thickness and 10 cm length) was processed through filler to facilitate insertion of the first part into the second part.

MFSSs were designed for vitrification, and are categorized as either MFSS-open method 'open system' or MFSS-closed method 'closed system' according to the occurrence of direct contact between the oocytes and liquid nitrogen(LN_2) during cryopreservation.

a) MFSS-closed method: Group of 5 COCs in 50µL BM was loaded by negative pressure using syringe 1 ml in a filter region of the MFSS, and separated by an air bubble, 50µl of **VS-I** was loaded by negative pressure until COCs reach filter region. During this step COCs were submitted to **VS-II** for 2 min. Thereafter, and by the procedure of the previous step, **VS-II** was submitted to COCs for 45 s (Fig.1), followed by negative pressure to exclude the COCs surrounding VS-II. After sealing with polyvinyl alcohol powder, straws were pre-cooled in LN_2vapour for at least one min, and then they were directly plunged into LN_2 (G 1): and stored for two weeks.

b) MFSS- open method: is similar to MFSS closed system except COCs were exposed directly to LN_2 (through loading it by negative pressure).

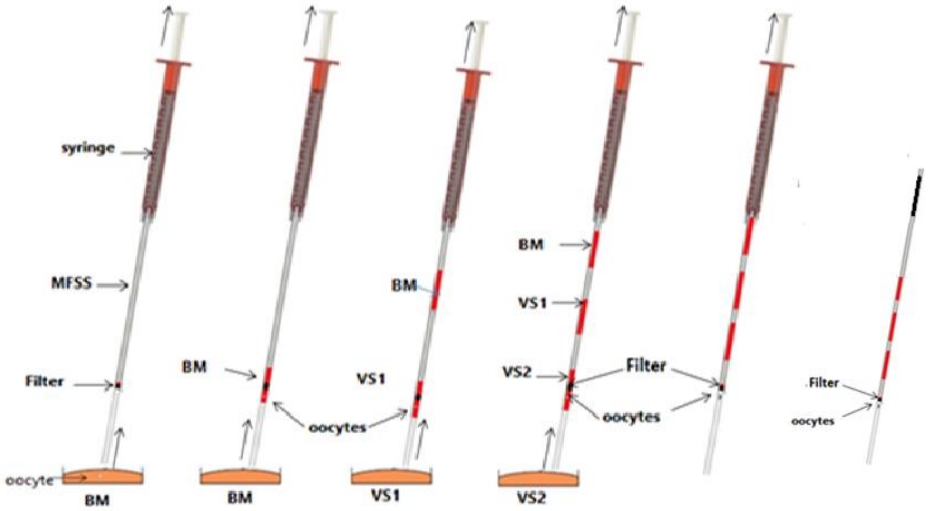


Figure 1. Vitrification procedure of she-camel oocytes using Modified Filter standard Straw (MFSS)

Thawing procedure:

After storage for two weeks, the COCs were warmed initially by loading BM at 39°C into MFSS by negative pressure for 10 seconds. Thereafter, cryoprotectants were removed serially by loading descending concentrations of sucrose (0.5, 0.25, and 0.125 M) for 60 s at 37 °C, then COCs exposed to BM for 1 min two times. Final solution with COCs of each straw was emptied into Petri dish for evaluation.

Vitrification and warming by the Cryotop method:

The Cryotopvitrification and warming procedure, originally reported by Kuwayama and Kato (2000). Briefly, oocytes were exposed to VS-I for 2 min. and then transferred to VS-II for 45 s ; 3 oocytes were placed on a sheet of Cryotop (Kitazato Supply, Tokyo, Japan) in a small volume of the vitrification solution (<1 µL). The Cryotop was plunged into liquid nitrogen and storage for two weeks. For warming of the Cryotop, the protective the protective cover was removed in liquid nitrogen and the end of the polypropylene strip was immersed directly into BM following with a descending concentrations of sucrose (0.5, 0.25, and 0.125 M) for 60 s at 37 °C, Finally, oocytes were washed three times in BM and evaluated.

Morphological evaluation:

Oocytes from each group were evaluated by stereomicroscope (Olympus SZ 40, Japan) according to Wani and Wernery (2010). Oocytes were considered abnormal when there was a change in shape, cumulus cell loss (Cumulus dispersed – partial cumulus loss- complete loss) and breakage of zonapellucida (Garg and Purohit, 2007). However, the viability of vitrified and warmed COCs were evaluated using trypan blue stain, exclusion test according to Madboly (2017). Unstained cumulus cells and ooplasm were classified as viable and fully stained oocytes as dead. The recovery rate was defined as the number of oocytes counted after the end of rehydration, in relation to the total of oocytes vitrified.

Experimental 2: Effect of cryo- devices on ultrastructural alterations in COCs:

Similar treatments in the first experiment were used for 15 immature oocytes. Ultrastructural alterations in zonapellucida, perivitelline space, mitochondria, cortical granules, cytoplasmic vesicles, lipid droplets and vacuoles were examined, interpreted and assessed according to by Barakat *et al.* (2012) and Khalili *et al*. (2012) and taken into consideration the qualitative morphological assessment (including shape and dimensions) of lipid droplets and vacuoles we considered the vacuoles with a diameter < 1.5µm as "small vacuoles" and those with a diameter > 1.5 µm as "large vacuoles."

Transmission electron microscopy

Fifteen oocytes were randomly collected (n=5 from each groups) and fixed and processed for Transmission electron microscopy (TEM) as described by Nottola *et al*. (2009). For light microscope examination, the oocytes were sectioned at a thickness of 0.5 to 1 µm and stained with Toluidine blue, and then examined by light microscopy (Zeiss Axioskop). Ultrathin sections (60 - 80 nm) were cut and stained with uranyl acetate (7 min) and lead citrate (13 min). These sections were observed and photographed with a TEM at 80KV (LEO 912AB EFTEM, Omega Filtering System, Germany).

Statistical analysis

Experiments were replicated three times in each treatment group. Data were analyzed by ANOVA (Duncan-test) of SAS (2006), and differences among groups were considered statistically significant $P \leq 0.05$ and are presented as mean ± S.E.M. calculated from three replicates.

$Y_{ij} = \mu + T_i + e_{ij}$

Y = an observation, (recovery rates, viability and oocyte abnormality)

μ = overall mean

T_i = effect of cryo- devices

e = random error

RESULTS AND DISCUSSION

***Recovery rates and oocyte viability*:**

No significant differences were observed among the studied groups in both recovery and survival rates (Table 1).

Morphologically disorder traits showed significant difference (P0.05) among the three studied groups, It is of interested to underline that the cracking of zona pellucid was the highest in G1 (Table 2). Also, alterations in shape were clearly observed especially in complete cumulus loss (denueded) or partial cumulus loss (partly denueded) COCs of the three studied groups (Fig. 2).

Table 1. Oocyte recovery and survival rates of the three techniques of cryopreservation

Vitrification Technique	Vitrified	Recovered		Survived	
	n	n	(%) ± SE	n	(%)± SE
CT	248	226	91.3(±6.3)[a]	201	88.8(±4.1)[a]
MFSS-open method	240	233	97.1(±0.9)[a]	213	91.3(±5.5)[a]
MFSS-closed method	258	253	98.03(±0.4)[a]	218	86.1(±3.5)[a]

CTCryotop, MFSS: Modified Filter Standard Straw

Means with different superscripts within a column vary significantly ($P < 0.05$).

Table 2. Effect of vitrification techniques on morphology of camel COCs

Vitrification technique	No. of Recov. COCs	No. (%) of abnormal COCs(live and dead)	Types of morphological damages; number (%).				
			Cumulus dispersed	Partial cumulus loss	Complete cumulus loss	Cracked Zona	Shrinkage of ooplasm
CT	226	37(16.4) ^{A}	6(16,31±0.9)^{A}	8(21.6±1.6)^{A}	8(21.6±3.5)^{AB}	9(24.4±0) ^{A}	6(16.3±0.9)^{A}
MFSS-open	233	29(12.4)^{B}	6(20.7±2.9) ^{A}	8(27.8±6.3)^{A}	6(20.7±2.9)^{B}	4(14.1±3.2)^{B}	5(17.4±2.9)^{A}
MFSS-closed	253	44(18.1) ^{A}	8(18.5±1.9) ^{A}	9(21.1±0.5)^{A}	11(25.4±1.7)^{A}	8(15.8±1.9)^{AB}	8(20.9±1.9)^{A}

CT:Cryotop, MFSS: Modified Filter Standard Straw

Means with different superscripts within aculumn vary significantly (P < 0.05).

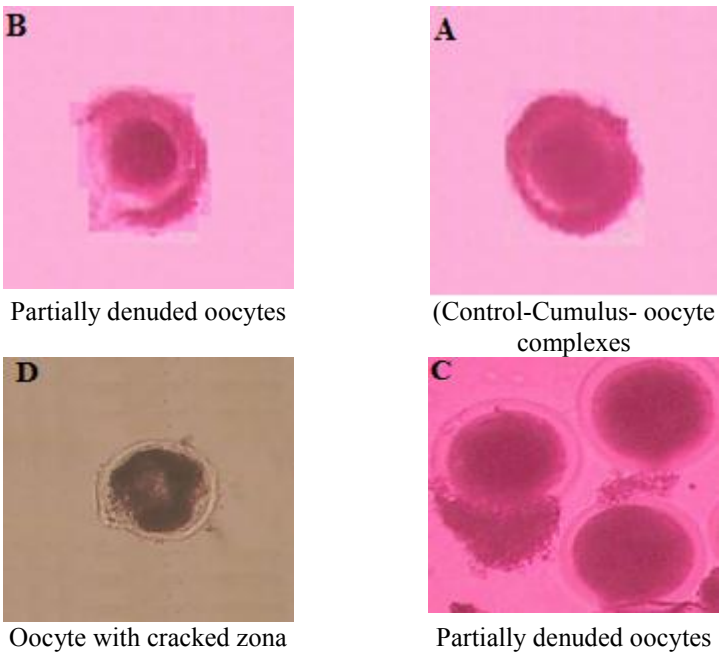
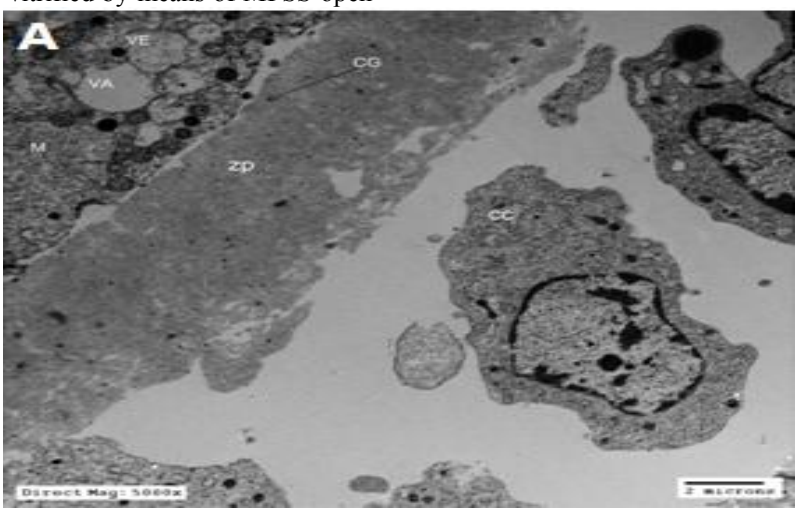


Figure 2. Different forms of abnormalities observed after post thawing – warmed procedure B,C and D and untreated oocyte A

Confirming previous light microscopy (LM) observations, TEM study showed that, few of the cumulus cells were separated from the immature oocytes in all treatment groups, and the gap junctions between cumulus cells were ruptured (Figures 3, 4 and 5). However, all COCs; irrespective of vitrification methods; showed a distribution of small membrane-bound vacuoles was located in the cytoplasmic periphery. However, large vacuoles (diameter > 1.5 µm)distributed in the cortical area, were rare in COCs vitrified by means of MFSS-open comparing to CT group (Figures 3 and 4). In the same context, homogeneous lipid droplets were observed in Cryotop and MFSS-closed method, while large lipid droplets surrounding small vacuoles (diameter < 1.5 µm) were observed in the MFSS-open method after vitrification. In all treated groups, light scarcely electron-dense CGs were found scattered in the ooplasm, and most of them were aligned just beneath the column.



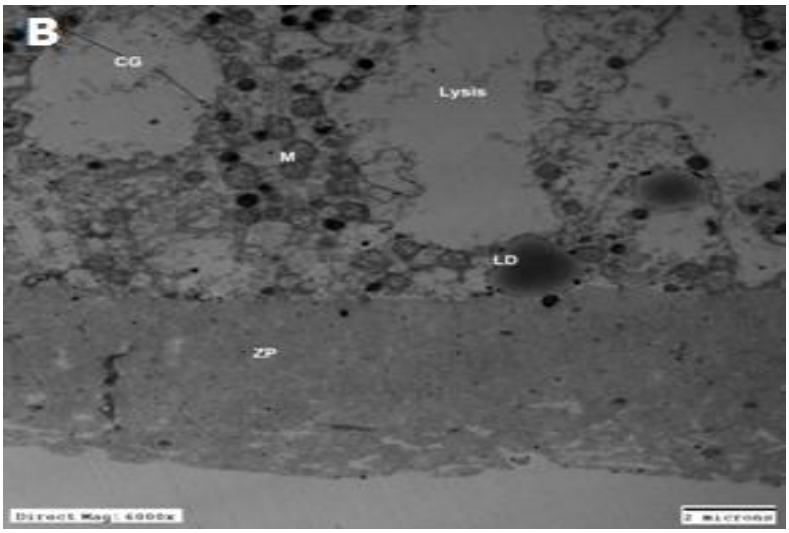


Figure 3 (A and B): Electron micrograph (TEM) of vitrified-thawed immature camel oocytes using Cryotop method, showing; Zona-pellucida (ZP),Cortical granules (CG), Mitochondria (M) , Vesicles (VE), Vacule (VA) and Lipid droplets (LD), 5000X.

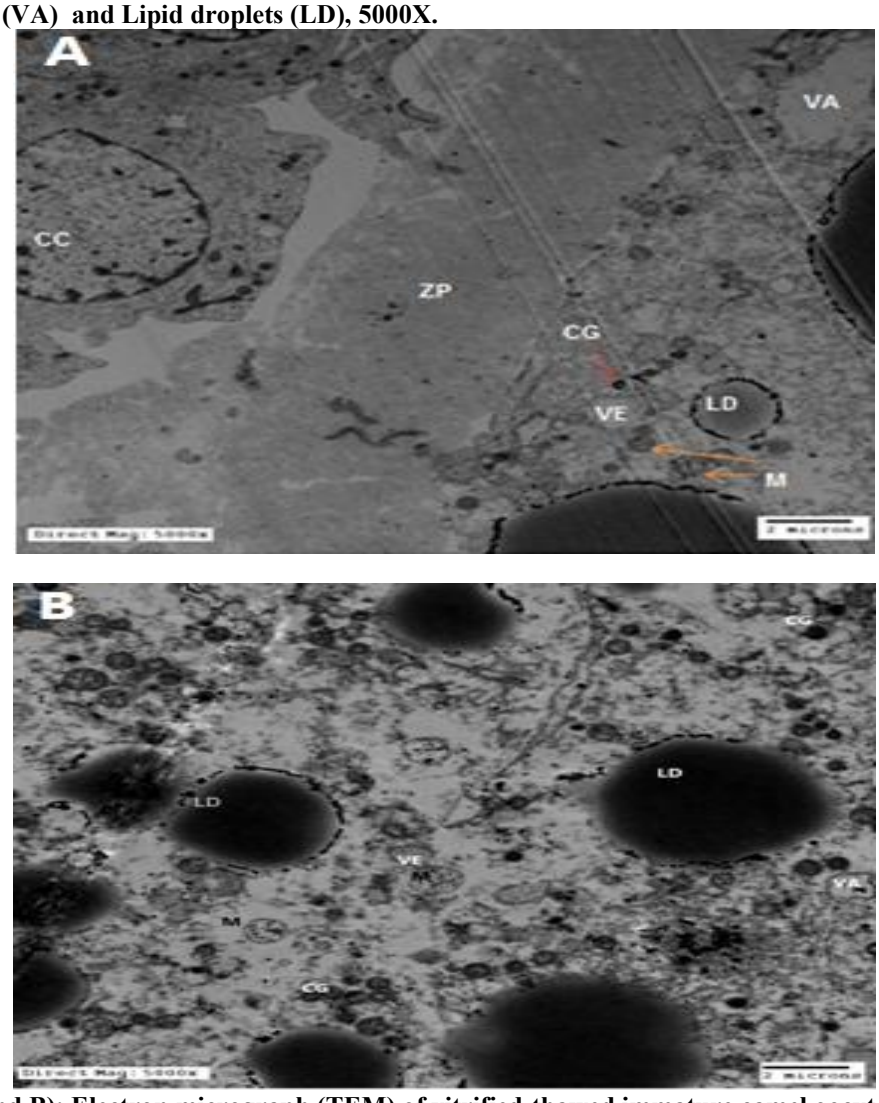


Figure 4 (A and B): Electron micrograph (TEM) of vitrified-thawed immature camel oocytes using MFSS-open method, showing; Zona-pellucida (ZP),Cortical granules (CG), Mitochondria (M) , Vesicles (VE), Vacule (VA) and Lipid droplets (LD) , 5000X.

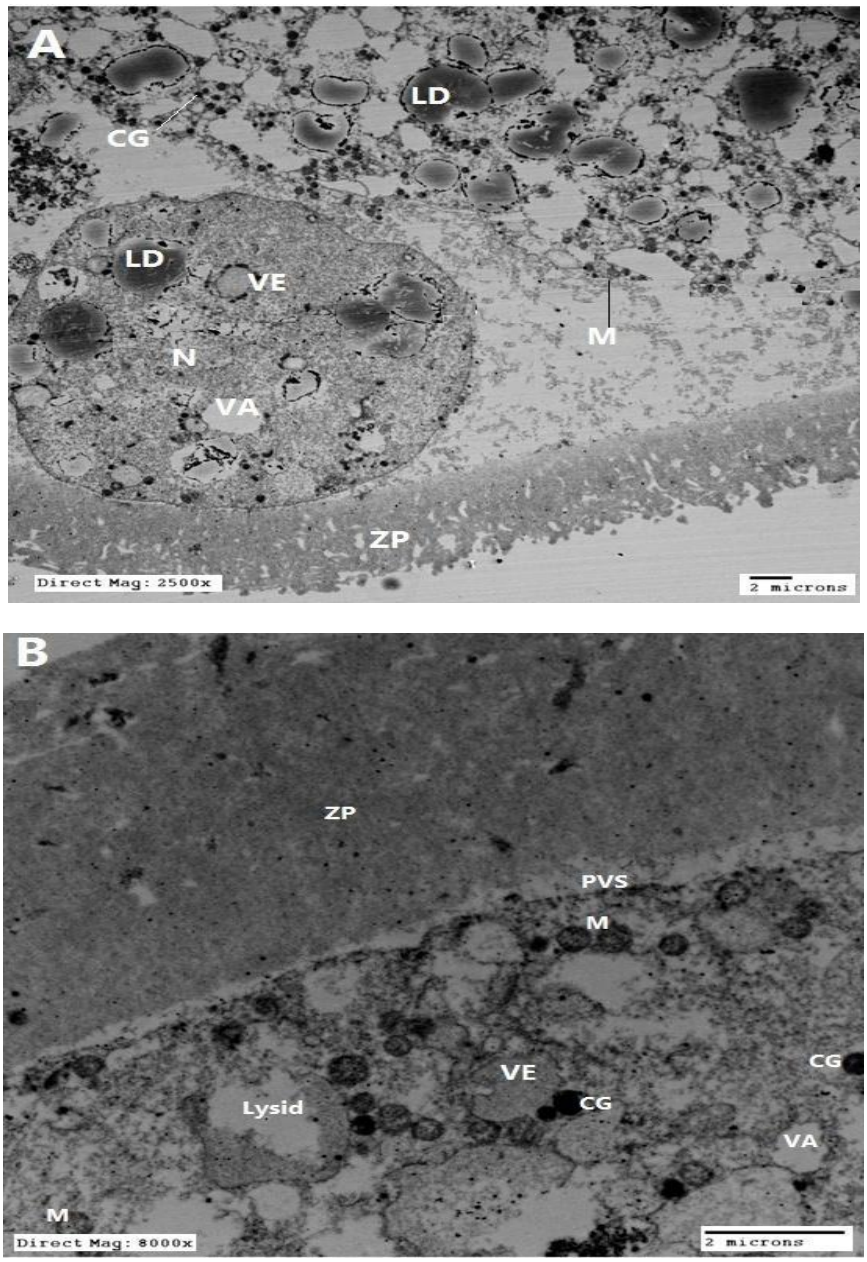


Figure 5 (A and B): Electron micrograph (TEM) of vitrified-thawed immature camel oocytes using MFSS-closed method showing; Zona-pellucida (ZP),Cortical granules (CG), Mitochondria (M) , Vesicles (VE), Vacule (VA), nucleus (N) and Lipid droplets (LD) 2500 and 5000X.

This study demonstrated that MFSS- vitrification procedure can be used to successfully vitrify she-camel oocytes resulting in high recovery rates, morphologically normal, and viable after vitrification. In the present study the high recovery rate of she-camel oocytes vitrified by the MFSS-open (97.1%), and MFSS-closed (98.0%), maybe the presence of due to bovine albumin in the vitrification medium which can prevent COCs adhesive with cryo-device, and this perhaps is a reason for decreasing the recovered rate that obtained in Cryotop method (91.3%). The recovery rates for the three methods in our study were higher than those measured by Chen *et al*. (2000) on vitrified mouse oocytes, in which the recovery rates were 62% in an open pulled straw (OPS) and 81% in a 0.25-mL plastic straw.

Our results indicated that the survival rate of oocytes vitrified by the MSFF-open method (91.9%) using direct exposure approach to LN_2 was least deleterious and more beneficial compared with the MSFF- closed method (86.1) using indirect exposure to LN_2 approach. It seems that the chilling injury was decreased by increasing the cooling rate with the MFSS-open method compared with the MFSS-closed method. Compared to our previous study (Madboly *et al*., 2017), the present study showed that the viability of COCs was improved by applying a minimum volume of vitrification solution that is achievable with the Cryotop; less than 0.1 µL. (Kuwayama,

2005), and with MFSS-open method resulting in an increase of cooling/ warming rates. Also, this result was compatible with the results of (Rienzi *et al.*, 2012; and Cobo *et al.*, 2015) who reported that the survival rates obtained with the open storage Cryotop device ranging from 84.7 to 96.8%. In this context, Kuwayama *et al*. (2005) reported that both the open- (Cryotop) and closed- (Cryotip) vitrification system for human blastocysts resulted in similar survival rate (97% *vs*. 93%, respectively). Similarly, Debra *et al*. (2016) carried out a study which aimed to assess whether similar survival rates could be achieved using either a closed or open vitrification system for human oocytes vitrification, they reported that there was no difference in survival rate between the open (92.4 %) and closed 89.7 % vitrification of *in vitro* matured oocytes.Similar to their study, the present result found no difference in she-camel oocytes survival rate between open storage Cryotop device and closed/open storage MFSS device. Contrarily, Paffoni *et al.* (2011) found a significantly lower survival rate for the closed device: 57.9 *vs.* 82.8%, respectively; and 82.9 *vs.* 91.0%, respectively (Papatheodorou *et al.*, 2013).

The high survival rate in the three treatment groups may be also attributed to the presence of complete cumulus that might reduce the passage rate of cryoprotectants and avoid sudden osmotic changes/stresses and decrease subsequent toxicity Dhali *et al*. (2000). Although, these results demonstrated that even if the three methods allowed good overall survival rate of immature oocytes, it could not avoid the occurrence of the abnormal structure, and each of the vitrification methods negatively influenced cumulus integrity (Table 2). On the other hand, it is well known that the occurrence of devitrification during thawing brings about critical cooling levels in 0.25 ml straws (Çetin and Başţan, 2006). To tackle this problem in the present work, the MFSS was removed directly from LN_2 containers and immediately BM was loaded into MFSS device for direct exposed COCs to BM at 39^{o}C for 10 s that achieved a higher warming rate.

Although LM observation revealed that there were no differences in the morphological normality of the COCs camel oocytes among the CT and MFSS open method but COCs showed a mild expansion of cumulus cells and TEM showed differences among both methods where the damage of the gap junctions between cumulus and oocytes was higher in CT method compared to MFSS-open method (Fig.3 A and Fig. 4 A), these results suggest that, in Cryotop method, directly exposed of COCs to LN_2 led to boiling movement of LN_2 around the COCs and this may cause damage of the gap junctions between cumulus and COCs while, in MFSS-open method LN_2 was loaded into MFSS directly, in this cause once LN_2 touching the straw wall they are transforming to vapor and thus led to exposing initially of COCs to LN_2 vapor, and with decreased of straw temperature then COCs exposed to LN_2. In agreement with our results, previous studies revealed that the communication between cumulus cells and oocytes is very susceptible to the physical condition caused by cryopreservation and cumulus cell projections were disturbed by vitrification (Madboly, 2017). However, the percentage of oocytes maintaining ZP integrity in the MFSS-closed method was significantly higher than that in MFSS-open method and Cryotop groups ($P < 0.05$), under the present methodology, these results suggest that an MFSS-direct exposure and Cryotop as the cryo-device are good compared with an MFSS-closed method, for the vitrification of camel COCs. However, TEM images of vitrified COCs showed an abnormal appearance in MFSS or CT (groups) method, where many of the COCs were contained large empty spaces (lysis) with more severe vacuoles (vacuolization) (Fig. 3 A and B). However, large vacuole was frequently detected in COCs that have vitrified using the Cryotop and MFSS-closed method (Fig. 3 A and B- Fig. 4 A and B). These alterations are in agreement with a previous study showing evident vacuolization in human MII oocytes vitrified by means of closed Cryotip and open Cryotop devices (Bonetti *et al*., 2011). Also it could be argued that the presence of lysis in the ooplasm may be considered a sign of degeneration and could be a correlated to the cryo-device properties (heat exchange and cooling and warming rates). Moreover, the events occurring during vitrification (i.e. osmotic process) provoked ultrastructural changes including the disruption of lysosomes that release their proteolytic contents, inducing lysis of the cytoplasmic matrix (Paynter, 2005). Our results are in agreement with the data on she-camel frozen-thawed COCs (Madboly, 2017) and vitrified-warmed mature human oocytes (Nottola *et al*.2016). Thus, present different degrees of vacuolization in three vitrification methods; Cryotop, MFSS-closed and MFSS-closed methods may a considered the morphological expression of cryodamage (Khalili *et al*., 2012) and, it should not be ruled out that vacuolization may be dependent, at least in part, upon the type device. However, the occurrence of vacuolization as a consequence of cryopreservation has been explained by numerous authors (Bonetti *et al*., 2011 and Palmerini *et al*., 2014). Khalili *et al*. (2012) postulated that vacuolization in frozen-thawed human MII oocytes may be a consequence of osmotic stress, and claim that vacuolization is associated with the skill of the operator and may depend on the cryodevice type. Mullen*et al*. (2008) found a significantly higher number of vacuoles by using Cryotip device compared with non-vitrified oocytes.

In our study, morphological alteration in both mitochondria and CGs were rarely observed in the three treatment groups. However, TEM showed that the distribution pattern of CGs in the ooplasm

differed among treatment groups. Where, CGs were normal distribution in the sub-oolemmal of Cryotop and MFSS-open groups (Fig.3 A, B and Fig.4 A, B). While, in MFSS-closed the CGs were found scattered in the ooplasm of vitrified COCs group (Fig.5 A and B), and it is possible to infer that these could be a consequence of the vitrification process (Hyttel *et al*., 2000).

Finally, the methodology of the present study is another aspect that should be taken into account, where the present study declare for the first time that MFSS as a new innovation of cryo-device (open and closed-systems) can be used successfully for camel oocytes vitrification, with high recovery rate, normal morphology and viability of oocytes. The MFSS-closed device has a narrow capillary end, sealed after loading with a minimum volume solution, so there is no direct contact between the oocytes and liquid nitrogen. Although the successful use of Cryotop (open method) for cryopreservation of human embryos (Kuwayama *et al*., 2005), and bovine and buffalo embryos (Gasparrini *et al*., 2007), it may raise bio-safety concerns, where many viruses may survive exposure to liquid nitrogen and could potentially cause contamination (Vajta, 2015).

CONCLUSION

These study showed the high efficacy of new open/closed device (MFSS) as evidenced by the high percentages of survival and using MFSS as a cryo-device for camel oocytes vitrification provide a more optimistic outlook since the only little indication of damages was gained either by MFSS-closed or MFSS-open methods.

Moreover, the MFSS-closed method may elude some of the problems occurring during vitrification process particularly those related to oocyte manipulations. However, further studies are needed to confirm these results with immature/mature-oocyte and embryos of other species.

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تاثير القشات القياسية الجديد المزودة (المعدلة) بفلتر على التركيب البنائى الدقيق وحيوية بويضات إناث الأبل المجمدة

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تهدف هذه الدراسة الى اختبار طريقة جديدة من وسائل حفظ البويضات بالتزجيج باستخدام نوع جديد من القاشات المدعمة بفلتر حامل للبويضات تعرف بالقاشات القياسية الجديدة المعدلة بوجود بفلتر ومقارنتها بوسيلة اخرى وهى الكريوتوبز واستخدمت القاشات القياسية الجديدة بطريقتين :(أ) طريقة النظام المفتوح (حيث تتعرض لسائل النيتروجين بطريقة مباشرة) ، (ب) طريقة النظام المغلق (حيث تتعريض البويضات لسائل النيتروجين بطريقة غير مباشرة)٠ تم جمع البويضات الغير ناضجة من اناث الابل (النوق) المذبوحة ثم قسمت عشوائيا الى ثلاثة مجاميع – المجموعة الاولى (طريقة الكريوتوب – الطريقة الثانية (طريقة القشات القياسية بالنظام المفتوح) و الطريقة الثالثة (طريقة القشات القياسية بالنظام الغلق) – وتعرضت البويضات لبيئة الاستزراع (١٩٩) + ٠،١ مول سكروز + ٥% (وزن/حجم) البيومين سيرم دم العجول بالاضافة الى ٧,٥ % اثيلين جليكول و ٧,٥ % داى ميثايل سلفوكسيد (سائل التجميد ١) لمدة دقيقتين ثم البويضات لبيئة استزراع (بيئة تحضين – ١٩٩) + ٠،١ مول سكروز + ٥% (وزن/حجم) البيومين سيرم دم العجول بالاضافة الى ١٥ % اثيلين جليكول و ١٥ % داى ميثايل سلفوكسيد (سائل التجميد ١) لمدة ٤٥ ثانية (سائل التجميد ٢) ثم غمرت فى سائل النيتروجين٠ اظهرت النتائج الاتى : معدل استعادة البويضات بعد المعاملة فى المجاميع الثلاثة كان (٩١,٣% - ٩٧,١% - ٩٨%)٠ وان نسبة الحى (٨٨,٨ - ٩١,٣– ٨٦,٢ %) على التوالى مع عدم وجود اختلافات معنوية بين المعاملات الثلاثة وكانت نسبة البويضات ذات الاشكال الطبيعة (الغير شاذة) مرتفعة باستخدام طريقة القشات القياسية بالنظام المفتوح وطريقة الكريوتوب مقارنة بطريقة القشات القياسية بالنظام المغلق – مع وجود فروق معنوية بين الثلاثة مجاميع- وقد اكد التركيب البنائى الدقيق للبويضات اناث الابل قدرة القشات القياسية على حفظ مكونات البويضه وهى طريقة مناسبة لحفظ البويضات.