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EFFECT OF CRYOPROTECTANTS ON THE QUALITY OF DROMEDARY CAMEL OOCYTES WITH PARTICULAR REFERENCE TO ITS ULTRA-STRUCTURE UNDER VITRIFICATION TECHNIQUE

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SUMMARY

Cryopreservation of mammalian oocytes is becoming a valuable technique in assisted reproduction technology particularly in vitro fertilization and embryo transfer. Two experiments were performed to establish the optimum condition for vitrification of immature camel oocytes. Experiment 1: examined the effect of different cryoprotectants Ethylene Glycol (EG), Propandiol (PROH) Dimethyl sulfoxide (DMSO) or their combinations on post-thaw survival rate and ultra-structural changes of she-camel oocytes. The immature oocytes of slaughtered dromedary camel were divided into four groups and exposed to different vitrification media of TCM 199 + 0.1 M sucrose + 20% (v/v) fetal camel calf serum containing 30% EG(group 1); 30% PROH(group 2); 30% DMSO (group3) or 10% EG+10% PROH +10% DMSO (group 4) and cryopreservedin straws. Experiment 2: evaluated the efficiency of two vitrification protocols: direct vitrification (DV), or solid surface vitrification (SSV) for in vitro maturation of immature camel oocytes. Results of the first experiment revealed that ethylene glycol or mixture of the three cryoprotctants showed better survival rate than other treatment groups (PROH and DMSO). Post-thawing survival rate of camel oocytes of four groups was 83.7, 75.3, 72.6 and 82.9%, respectively. Moreover, ultra-structural observation showed that ZP, oolemma, and mitochondria distribution of EG group were found similar to fresh controls. Also, results of the second experiment indicated that the differences in maturation rate of both SSV and DV protocols were not significant, yet both protocols provedcomparable results of usedcryoprotectants.

Keywords: *Dromedary Camel, oocyte, cryopresevation, maturation, transmission electron microscopy*

INTRODUCTION

Recent advances in cryopreservation technology and modifications of vitrification protocols, oocytes remain challenge for cryobiologists. Two principal cryopreservation methods are currently utilized, the first is the conventional freezing method (slow freezing) and the second (fast freezing) vitrification method (Kuwayama *et al*., 2005). Development of vitrification methods depends on use of sub-microlitre volumes, which increases rates of cooling and warming. Other studies have employed open pulled straws (Vajta *et al*., 1998), solid surface vitrification (Dinnyes *et al*., 2000), and the cryotop (Kuwayama *et al*., 2005). During the freezing process oocytes are gradually exposed to cryoprotectant solutions consistes of penetrating cryoprotectants such as glycerol, dimethyl sulfoxide (DMSO), ethylene glycol (EG), propandiol and non-penetratingcryoprotectants (sucrose, glucose, Ficoll, proteins, lipoproteins). Success using each type of permeatingcryoprotectants depends in part on the speed at which they can cross cellular membranes.

Permeability of oocytes varies among development stages when same cryopreservation protocol is used. Membrane permeability of oocytes varies among individuals of certain species. Several studies have indicated that solution containing mixtures of permeable cryoprotectants seem to be more advantageous than solutions containing one cryoprotectant (Vicente *et al*., 1994). Mahmoud *et al*. (2010) found that the best combination of cryoprotectant was EG + DMSO for vitrification of immature buffalo oocytes using either straw or open pulled strows (OPS) methods.

Previous studies demonstrated that, ethylene glycol would be the ideal cryoprotectant because it penetrates membranes faster than glycerol (Cha *et al*., 2000) and is less toxic than other permeable cryoprotectants (Dinnyes *et al*., 2000). Freezing media, containing permeable and nonpermeable cryoprotectants, seem to be more efficient than a single permeable cryoprotectant (Shaw *et al*., 2000).

Many studies used electron microscopy as a tool to evaluate the damage or changes on the oocyte ultrastructure followingvitrification (Kacinskis *et al.,* 2005 and Kafi*et al.,* 2005). No studies on the ultrastructure of dromedary camel cumulus-oocyte complexes (COCs) exposed to different cryoprotectants followed by vitrification have been found.

It is known that vitrification causes ultrastructural modifications to microvilli, mitochondria, oolemma, cortical granules and damages of the meiotic spindle apparatus in the oocyte of several species (Boonkusol *et al*., 2007and Diez *et al*., 2005). On the other hand, many problems have been reported in the cryopreservation of the germinal vesicle (GV) and metaphase II stage oocytes. Especially, GV stage oocytes have a relatively little microtubule (MT) and

oocytes at this stage might be less injured from the osmotic and cytotoxic effects (Szell and Shelton, 1987).

The purposes of this study is aimed at defining the ultrastructural changes in thawed/warmed cumulus oocyte complex of dromedary camel vitrified at different cryoprotectants using direct vitrification method Also, a comparative study between both direct vitrification method and solid surface vitrification method was carried out.

MATERIALS AND METHODS

This study was carried out at the Laboratory of Animal Physiology and Biotechnology, Sheep and Goats Research Department, Animal Production Research Institute, during the period from July, 2014 to February, 2015.

Collection of ovaries and recovery of oocytes:

Camel ovaries were collected from slaughtered adult females (Cairo abattoir) and transported to the laboratory within 3h after the slaughtering in phosphate buffered saline (PBS, Sigma) supplemented with 100 IU mL-1 penicillin and 100 µL mL-1 streptomycin at 30-35°C. At the laboratory, the ovaries were sliced according to Dutta *et al*. (1993). Follicular fluid was collected and transferred into 50 ml sterile conical tube and kept at 37°C in a water bath for 10 min for the oocytes to settle at the bottom by gravity. The supernatant was then discarded and the pellet was diluted with washing solution (WS) and transferred to 90 mm Petri dish for oocyte screening and grading. Cumulus-oocyte-complexes (COCs) with moderate to slightly dark appearance, and homogeneously pigmented ooplasm were washed 3 times in WS.

Washing solution (WS) consisted of TCM-199-Hepes modified with Earle's salt and L-glutamine, (Sigma, USA) plus 10% (v/v) Fetal camel calf serum (FCCS) and 1% antimycotic antibiotic(Gibco, Switzerland).

Based medium (BM) was prepared as; TCM199-Hepes supplemented with 20% (v/v) fetal camel calf serum (FCCS) supplemented with 0.1 M sucrose.

The present study included two experiments:

Experiment 1.

To evaluate the effect of three cryoprotectants and/or their mixtures on ultra-structural changes of camel immature oocytes, COCs (n = 380) were randomly allocated to four groups.

Group 1: oocytes were equilibrated in 15% Ethylene Glycol-based medium (15% EG -BM) for 2 min and then in 30% Ethylene Glycol-vitrification based medium (30% EG-BM) for 45 sec.

Group 2: oocytes were equilibrated in 15% Glycol Propandiol-based medium (15% PROH -BM) for 2 min and then in 30% Glycol Propandiol-based medium (30% PROH-BM) for 45 sec.

Group 3: oocytes were equilibrated in 15% Dimethyl sulfoxide-based medium (15% DMSO-BM) for 2 min and then in 30% Dimethyl sulfoxide-based medium (30% DMSO-BM) for 45 sec.

Group 4: oocytes were equilibrated in 5% Ethylene Glycol+5% GlycolPropandiol+5% Dimethyl sulfoxide-based medium (5% EG+5% PROH+5% DMSO-BM) for 2 min. and then in mixture consisted of 10% Ethylene Glycol+10% GlycolPropandiol+10Dimethyl sulfoxide-based medium (10% EG+10% PROH+10% DMSO-BM) for 45 sec.

Oocytes vitrification and thawing method:

The procedures for cryopreservation of oocytes were performed at room temperature. Immature oocytes (germinal vesicle stage) of four experimental groups were loaded into 0.25 ml straws and directly plunged into liquid nitrogen (Vajta *et al*., 1998). After 3 to 5 days, the straws were warmed initially by waving in air for 5sec and then thawed in a 37°C water bath for 20 sec. and cryoprotectants were removed serially in sucrose diluting solutions (Vajta *et al*., 1998).

Microscopic evaluation:

Oocytes of each group were examined under an inverted lightphase contrast microscope (Olympus, Tokyo, Japan) and evaluated according to Dhali *et al.* (2000) based on the transparency and shape of the cytoplasm. The oocytes were morphological classified as normal or abnormal according to (Wani and Wernery, 2010). Moreover, the post-aspiration and immediately post-thawing oocytes were examined for viability using trypan blue stain, exclusion test according to Abd-Allah (2010).

Transmission electron microscopy:

Oocytes were fixed in 2.5% buffered glutaraldehyde (pH 7.2) at room temperature for 5 min, kept at 4°C for an additional an hour and then post-fixed using 1% osmium tetroxide (OsO4; Plano, Wetzlar, Germany) for 1h at 4°C. Sample dehydration was performed in ascending grades of acetone (35, 50, 75, 95, and 100%) for 10 min. A mixture of resin and acetone was used for sample infiltration. Subsequently, samples were polymerized in 100% resin at 60ºC for 20 hours.

For histological changes, blocks were sectioned using an ultra microtome (Leica Ultracut, UCT), ultrathin sections (60-80 nm) were collected on glass slides or 200-mesh thin bar copper grids, and stained with uranyl acetate and lead citrate and then washed in double distilled water. Samples were examined under a transmission electron microscope (LEO 912AB EFTEM, Omega Filtering System, Germany) at 80 kV.

Experiment 2:

The objective of this experiment was vitrification of immature camel oocyte by using 0.25 ml straw and solid surface vitrification protocols in order to determine the optimum protocol. Based on the results of experiment I, EG-based medium was better than

other media. Therefore, EG solution was used with both protocols.

Experimental design: Immature oocytes were vitrified either by:

a) using 0.25 ml straws protocol (DV), oocytes were divided into three groups: control (G1), exposure to vitrification medium (G2) oocytes were exposed to vitrification medium without loading into straws or storage in liquid nitrogen, and vitrified-warmed(G3), oocytes were vitrified, loaded into straws, stored in liquid nitrogen for 7 days and warmed at 38.5°C.

b) Using solid surface vitrification protocol, immature oocytes were vitrified using SSV as described by Dinnyes*et al*. (2000). Briefly, equilibrated oocytes were dropped with about 10 µl vitrification solution onto the cold dry surface of a hollow metal cube partly submerged into liquid nitrogen. Vitrification of oocytes was carried out throughout 30sec. The vitrified micro drops including oocytes were plunged into liquid nitrogen. After storage in liquid nitrogen for three weeks, oocytes were thawed by transfer with cooled forceps into 200 µl droplets of 1.25 M sucrose in BM for 3 min. To promote removal of intracellular cryoprotectants, oocytes were transferred stepwise for1min. periods into 200 µl droplets of decreasing sucrose solution (0.2 M, 0.1 M, or 0.05 M in BM), and then equilibrated for 10 min in BM. Abnormal oocytes were discarded. While, the remaining oocytes were submitted to IVM. One to 3 min later, oocytes were consecutively transferred for 1min into 500-µl droplets of BM supplemented with 0.2 M, 0.1 M, or 0.05 M sucrose, thereafter they were then washed three times in BM.

In vitro maturation*:*

In vitro maturation procedure was conducted as described by Wani and Wernery (2010). The three groupswere: control, exposure to vitrification medium and vitrified- warming immature oocytes of both protocols were washed three times in BM at 37^{o}C and cultured in TCM-199 supplemented with 5% fetal camel calf serum (FCCS), 10ng/ml FSH, 10 IU /ml LH, 1 µg/ml estradiol 17β, 0.8 mg/ml Na bicarbonate, 0.25 mg/ml Na pyruvate and 0.05 µg/ml gentamicinsulphate. *In vitro* maturation (IVM) were carried out in culture dishes (35mm) that contained maturation medium previously incubated for 2 h at 38.5^{o}C, groups of 15 COCs were placed in 100µl droplets of maturation media, covered with mineral oil and incubated for 40 -44 h at 38.5°C, 5% $CO_2$and 90-95% humidity.

Assessment of cumulus expansion and nuclear maturation:

Assessment of the cumulus expansion was determined after incubation for 44 hours by the criteria of Pavlok *et al.* (1992). While, nuclear maturation was assessed according to the procedures described by Beker *et al*. (2000). Vitrified-warmed oocytes of both protocols were evaluated morphologically under an inverted lightphase contrast microscope (Olympus, Tokyo, Japan).The cumulus cells of COCs were removed by vortexing for 5 min in TCM-199 supplemented with 0.05% (w/v) hyaluronidase (H3506; Sigma), the free cumulus oocytes were mounted on a glass slide with a cover slip supported by droplets of Paraffin/Vaseline mixture, then fixed with acetic acid/ethanol (1:3) for 24 h. They were subsequently stained with aceto-orcein (2% orcein in 45% acetic acid solution) for 30 min, rewashed with a fresh fixative and assessed for the nuclear stage of meiosis under a light microscope (100 x magnification) (Costa *et al*., 1997).

Statistical Analysis*:*

All data from experiment 1for morphologically normal follicles were analyzed using a factorial design in an ANOVA test of SAS (1996), combined with Duncan's Multiple Range test. The comparison between means of maturation rates (Experiment 2) for every maturation phase was carried out individually using Tukey's test. Values were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Experiment1:

The survival rate and morphological damage of fresh non-vitrified and vitrified GV oocytes are given in Table (1). Light microscopy (LM) revealed that the fresh non-vitrified oocytes were surrounded by intact zonapellucida with a regular round shape and a clear homogeneous ooplasm (Figure 1;A). While, the highest survival rate of cryopreserved oocytes with normal-shaped cytoplasm and zonapellucida was recorded with 30% EG-BM and with a combination of 10% EG+10% PROH+10% DMSO-BM .

Similarly, the morphological study revealed that the post-thaw abnormal percentages of total GV (damages percentage of post-thawing immature oocytes) were significantly higher ($P<0.01$) with PROH and DMSO than with EG or combination of CPAs (EG, PROH, and DMSO). Similar trend was reported by Yang *et al.* (2003), who obtained 64.2, 91.5 and 79.7% of viable oocytes with immature bovine oocyte vitrification using 0.25 ml straw method, and vitrification solution consisted of 40% Ethylene Glycol, 0.5 M sucrose, and 10% FBS dissolved in DPBS.

Different types of damaged oocytes were observed as cracked zonapellucida, leaking of cellular contents and partially or fully removal of cumulus cells layer (Figure 1;C,D,E and F).

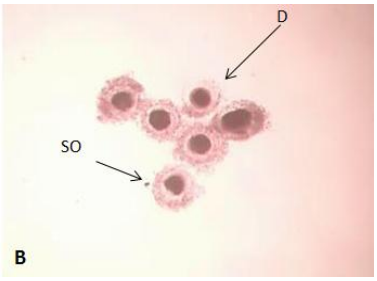
The most frequent types of damages were partially or fully removal of cumulus cells layer from oocyte and cracked zonapellucida (Table 2). The number of morphological abnormal oocytes results were significantly higher ($P<0.05$) with PROH or DMSO than with either combination of CPAs or EG. In

swine, Hettig*et.al.* (2011) the mean percentages of morphologically normal and viable vitrified oocytes using SSV method with 45% EG in the vitrification media were 76.47 and 42.5%, respectively. Moreover, Babaei *et al.* (2006) demonstrated that the most of the vitrified bovine oocytes (90%) using 20% EG + 20% DMSO solution had morphologically normal appearance after thawing.

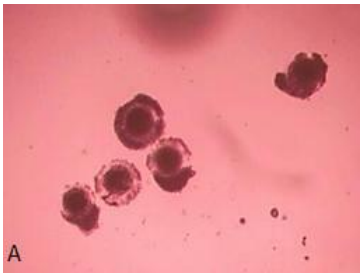
Table1. Post-thaw survival rate (P.T) of total oocytes and abnormal percentages of vitrified immature camel oocytes

Cryoprotectants	No. of vitrified oocytes	P.T survival rate (%)	Survival oocytes (%)		Dead oocyte with abnormal type (%)	Total abnormal of vitrified oocytes (%)
			in normal type	abnormal type		
30% EG-BM	104	83.7 ±1.6[a] (n=87)	78.8 ±1.6[a] (n=82)	4.88 ±1.6[a] (n=5)	16.3±1.6[b] (17)	21.2 ±1.6[b] (n=22)
30% PROH-BM	97	75.3 ±1.6[b] (n=73)	66.0±1.6[b] (n=64)	9.24±1.6[a] (n=9)	24.7±1.6[a] (n=24)	34.6±1.6[a] (n=33)
30% DMSO-BM	95	72.6±1.6[b] (n=67)	65.3±1.6[b] (n=62)	5.26±1.6[a] (n=5)	29.4±1.6[a] (n=28)	34.6±1.6[a] (n=33)
10% EG+10% PROH+10% DMSO-BM	111	82.9±1.6[a] (n=92)	75.7±1.6[a] (n=84)	7.2±1.6[a] (n=8)	17.1±1.6[b] (n=19)	24.3±1.6[b] (n=27)
Control (non- vitrified)	93	90.5	82.9	8.5	8.49	16.1

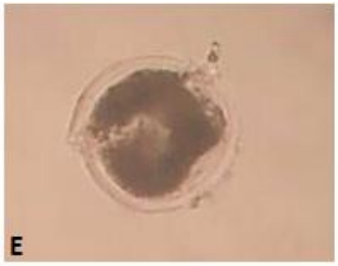
30% EG-BM ;30% Ethylene Glycol-vitrification based medium
30% PROH-BM ;30% Glycol Propandiol -based solution
30% DMSO-BM ;30% Dimethyl sulfoxide -based medium
10% EG+10% PROH+10% DMSO-BM; 10% Ethylene Glycol +10% Glycol Propandiol+10 Dimethyl sulfoxide-based medium
Data are displayed as the mean±SE.Values with different superscripts (a, b) differ within column (P < 0:05)



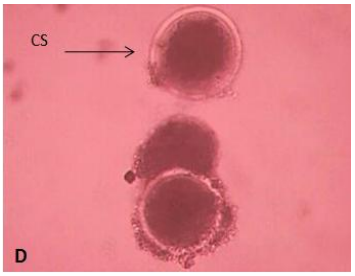
Partially denuded oocyte**(D)** and **s**hrinkage of ooplasm (**SO)**



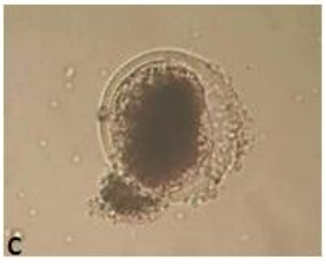
Control



Oocyte with cracked zona.



Change in shape **(CS)**



Ooocyte with cracked zona and leaking of cellular contents

Figures 1. Different forms of oocyte abnormalities observed after post thawed-warmed procedure C,D,E and F. and untreated oocyte A

Table 2.Types of damages (%) observed in immature camel oocytes after vitrification-warming in Different cryoprotectants

Cryoprotectants	No. of vitrified oocytes	Number (%) of abnormal oocytes	Morphological damages (%)			
			D	SO	CZ	CS
30% EG-BM	104	21.15% (n=22)	45.8±3.8^{Aa} (n=10)	8.3±3.8^{Ba} (n=2)	22.9±3.8^{Ba} (n=5)	22.9±3.8^{Ba} (n=5)
30% PROH-BM	97	34.02% (n=33)	45.9±3.8^{Aa} (n=15)	20.9±3.8^{Ba} (n=7)	20.9±3.8^{Ba} (n=7)	12.4±3.8^{Ba} (n=4)
30% DMSO-BM	95	34.74% (n=33)	45.4±3.8^{Aa} (n=15)	18.6±3.8^{Ba} (n=6)	20.9±3.8^{Ba} (n=7)	15.0±3.8^{Ba} (n=5
10% EG+10% PROH+10% DMSO-BM	111	24.32% (n=27)	37.04±3.8^{Aa} (n=10)	19.1±3.8^{Ba} (n=5)	22.0±3.8^{ABa} (n=6)	22.0±3.8^{ABa} (n=6)

30% EG-BM**:**30% Ethylene Glycol-vitrification based medium
30% PROH-BM**:**30% Glycol Propandiol -based solution
30% DMSO-BM**:**30% Dimethyl sulfoxide -based medium
10% EG+10% PROH+10% DMSO-BM**:** 10% Ethylene Glycol +10% Glycol Propandiol+10 Dimethyl sulfoxide-based medium
D: Partially/fully denuded. **SO:**Shrinkage of ooplasm. **CZ:** Cracked zona and / or leaking of cellular contents. **CS:** change in shape
Data are displayed as the mean±SE.Values with different superscripts (a, b) differ within column (P < 0:05)
Values with different superscripts (A, B) differ within row (P < 0:05).

With regard to electron micrograph, the vitrified immature camel oocytes showed good preservation with EG or combination of CPAs (Figures 2 and 5), and showed better arrangements of the ooplasm organelles surrounded by regular and a continuous zonapellucida, also, appeared similar in shape and dimensions and overall arrangement of the ooplasm to non-vitrified oocytes (Figure 2). In contrast, marked alterations in perivitelline space and vesicles were observed in oocytes treated with PROH and DMSO. However, some cytoplasmic vacuoles were detected in the ooplasm of a part of vitrified-warmed immature oocytes of four treated groups (Figure 2).These results were similar to those observed in immature bovine oocytes that exposed to vitrification solution (DMSO 2M, acetamide 1M, PROH 3M) for 1.5 h. (Thibault *et al*., 1987).On the other hand, distortion and shrinkage of the oocyte vacuoles as a consequence of vitrification were observed by using either DMSO or PROH- based VS, accompanied by some disorganization of the cytoplasm organelles. Moreover, vitrification with DMSO or PROH resulted in immature oocytes showing massive lysis within ooplasm (Figures 4 and 5), while, slight lysiswas observed with either EG or mixture of EG, PROH and DMSO. Similar findings were described by Diez *et al.* (2005).

However, in all treatment groups, most of the cumulus cells were separated from the immature oocytes and the gap junctions between cumulus cells were ruptured, and microvilli were disrupted or disappeared (Figures 3, 4, 5 and 6). Also, the present results showed that the most common ultrastructural feature observed in the thickness of the ZP which appeared to be less in all the vitrified oocytes 5.6 µm than those reported in fresh COCs 6.15 µm. Gordon, (2003) demonstrated that the zonapellucida and cumulus cells are closely associated with structures and thus cryopreservation process might have caused volume changes and alterations in these structures. Such changes resulted in immature oocytes showing 9-15% denuded oocytes, and this may be due to the negative influence intercellular communication between cumulus cells and oocyte subsequently, poor *in vitro* maturation and developmental competence of immature oocytes.

In vitrified oocytes, the change in the physical properties of the inner surface of ZP was associated with the decrease of CGs density and with disappearing of perivitelline space (PVS). The present result is in agreement with the report by Ghetler *et al.* (2006) who reported that the massive decrease in the number of CG as an effect of cryopreservation may be resulted from the premature release of cortical granules. In vitrified oocytes, CGs are located abutting the oolemma. In some sub-oolemmal domains, CG appeared less abundant and/or electron-dense (Figures 5 and 6).

Also, homogeneous lipid droplets were observed in fresh non vitrified GV, while irregular nonhomogeneous lipid droplets surrounding large vacuoles were observed after vitrification. There is no available information about the ultrastructure evaluation of vitrified immature camel oocytes. However, non-vitrified immature camel oocytes showed typical structure previously described in camel (Kafi *et al.*, 2005) and bovine (Kacinskis *et al.,* 2005). Confirming previous observations (Mondadori *et al.,* 2008), the most important difference that was observed among the species is the larger number of lipid droplets in camel ooplasm. However, the mechanisms of cell damage as a consequence of

cryopreservation have been explained by numerous authors. Mazur *et al.* (1972) indicated that hyperosmotic stress might lead to an influx of non-permeating solutes when cells are returned to iso-osmotic conditions, they swell beyond their isotonic volume and lyse. Otoi*et al.* (1997) demonstrated that the damage of oocytes during cryopreservation could be attributed to large lipid-like material found in oocytes of many species. A study by Schalkoff*et al.* (1989) showed that the human oocytes exposed to either 1, 2-propanediol or DMSO at ambient temperatures showed ultrastructure changes such premature cortical granule release.

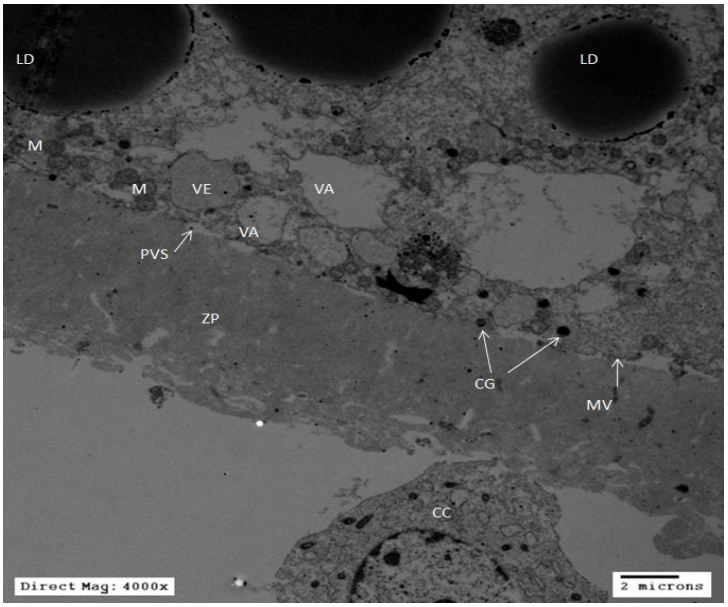


Figure 2. Electron micrograph of untreated control (fresh) immature camel oocyte, showing; Cumulus cells (CC), zonapellucida (ZP), vesicles (VS), and lipid droplets (LD); Mitochondria (M) , Microvilli (Mv) vacuole (VA), and cortical granules (CG).

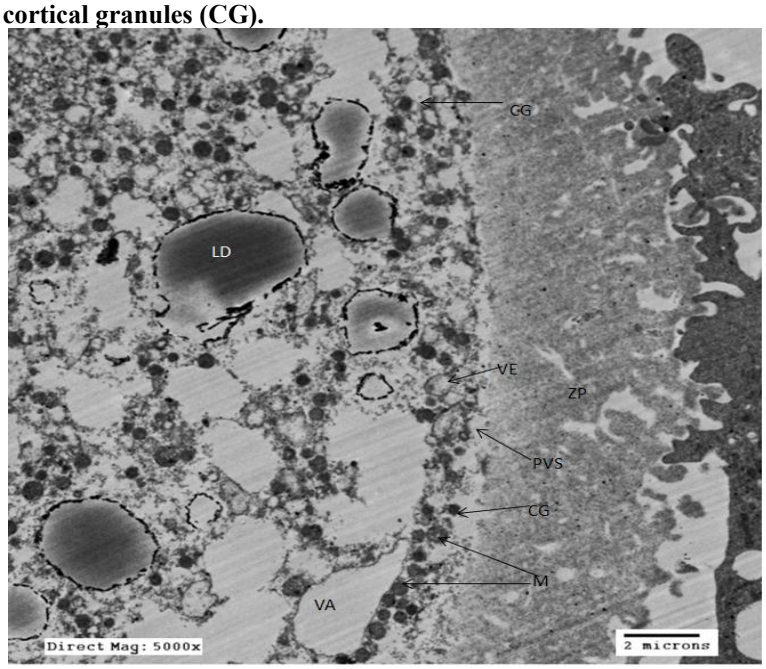


Figure 3. Electron micrograph of vitrified-thawed immature camel oocytes using 30% EG, showing; Zonapellucida (ZP), Cortical granules (CG), Mitochondria (M) , Vesicles (VE), Vacule (VA) and Lipid droplets (LD).

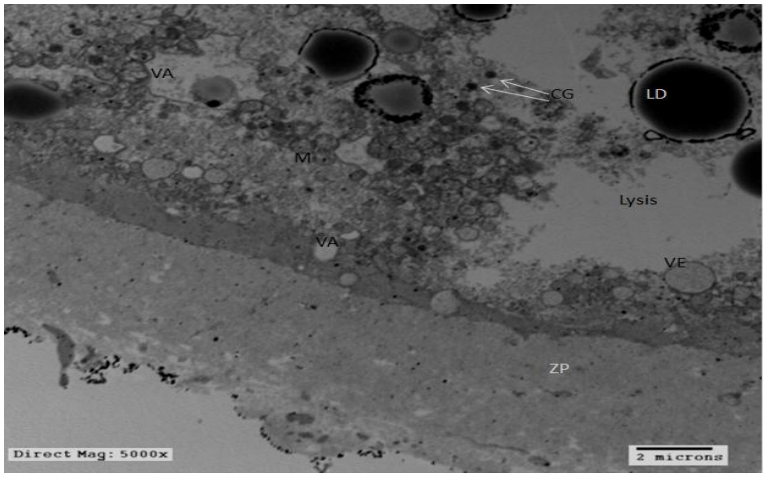


Figure 4. Electron micrograph (TEM) of vitrified-thawed immature camel oocytes using 30%PROH, showing; Zonapellucida (ZP),Cortical granules (CG), Mitochondria (M) , Vesicles (VE), Vacule (VA) and Lipid droplets (LD).

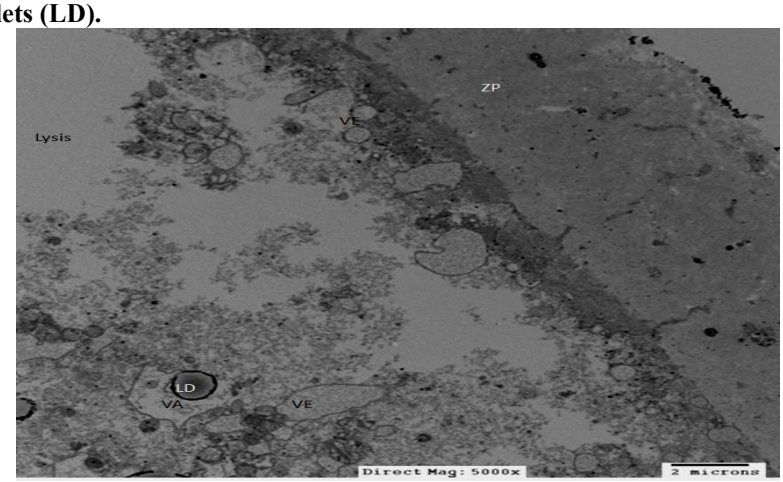


Figure 5. Electron micrograph (TEM) of vitrified-thawed immature camel oocytes using 30% DMSO, showing; Zonapellucida (ZP), Microvilli (Mv), Cortical granules (CG), Mitochondria (M) , Vesicles (VE), Vacule (VA) and Lipid droplets (LD), note the amount and density of CGs appeared abnormally reduced when compared with those of fresh controls.

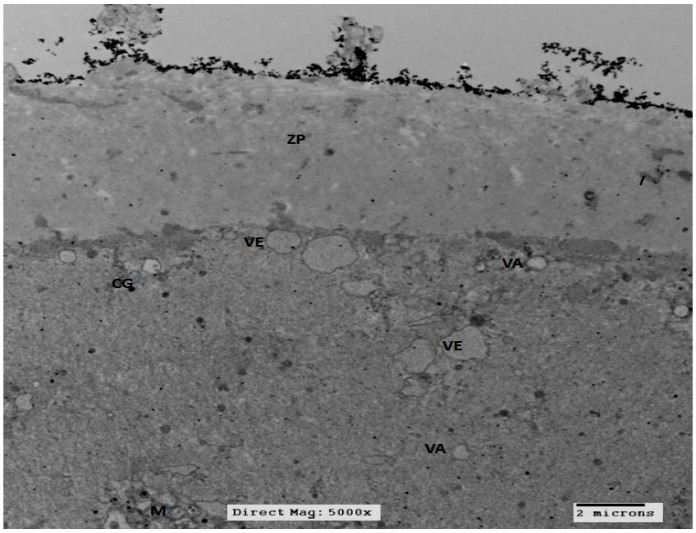


Figure 6. Electron micrograph (TEM) of vitrified-thawed immature camel oocytes using mixture of 10%EG + 10%PROH + 10%DMSO,, showing; Zonapellucida (ZP), Cortical granules (CG), Mitochondria (M), Vesicles (VE), Vacule (VA) and Lipid droplets (LD).note the abnormally dense appearance of the inner aspect of the ZP, and also a slight degree of vacuolization is seen in the oocyte.

Experiment 2:

Results in Table (3) shows that the stages of nuclear maturation reached to immature camel oocytes vitrified obtained with DV 0.25 ml straws or SSV protocols after 44 h of culture. The proportion of Telophase TI and Metaphase II formation of immature camel oocytes vitrified with either DV (0.25ml. Straw) or SSV (40.8% and 43.8%) were lower ((P<0.05) than those in fresh control oocytes (56.6%).While, the differences between both protocols DV (0.25ml. Straw) or SSV were insignificant.

This result was lower than those recorded with bovine immature oocytes, it was 75% (24/32) and 36.1% (13/36) for SSV or DV (0.25mlstraw) protocols, respectively (Amr*et al.*, 2017).

Table3. Recovery and maturation rates of thawed immature camel oocytes that were vitrified by the DV (0.25ml straw) or SSV method**.**

Vitrification protocol	Treatment	Oocyte No.	ES	VS	LN2	Stage of nuclear maturation					
						GV %	GVBD %	MI %	AI %	TI+ MII %	Deg. %
-	Fresh	166	-	-	-	4.8 ±1.4^{a}	4.8 ±0.7^{b}	15.8 ±0.8^{b}	11.5 ±0.3^{ab}	56.6 ±1.9^{a}	6.53 ±0.7^{b}
-	Exposed	168	+	+	-	4.8 ±1.2^{a}	4.7 ±0.6^{b}	16.1 ±0.8^{b}	7.7 ±1.4^{b}	53.7 ±1.4^{a}	13.1 ±1.0^{a}
0.25ml Straw	Vitrified	171	+	+	+	5.2 ±0.4^{a}	9.8 ±0.9^{a}	24.2 ±1.7^{a}	12.3 ±2.6^{ab}	40.8 ±1.2^{b}	6.66 ±1.2^{b}
SSV	Vitrified	178	+	+	+	6.2 ±0.3^{a}	10.6 ±0.8^{a}	16.3 ±0.7^{b}	15.8 ±0.9^{a}	43.8 ±2.1^{b}	7.9 ±0.9^{b}
Mean (SE)						5.2 ±0.1^{A}	7.5 ±.06^{D}	18.1 ±.06^{B}	11.8 ±.06^{C}	48.7 ±.06^{A}	8.4 ±.06^{D}

GV: germinal vesicle GVBD: germinal vesicle breakdown MI: metaphase I AI: anaphase I TI: telophase I MII : metaphase II Deg.: degenerated and oocytes were considered to have matured when they reached the (TI or M-II) stage.
Values with different superscripts (a, b, c) differ within column (P <0:05).
Data are displayed as the mean±SE.Values with different superscripts (A, B) differ within row (P < 0:05).
ES: equilibration solutions, VS: vitrification solutions, LN2 : liquid nitrogen

In the Dromedary camel, Abd-Allah (2010) studied the effect of equilibration time (3, 5 and 10 min.) on the IVM using ultra-rapid vitrification protocol with EG-based vitrification solution, and found that, the *in vitro* maturation was 43.8, 47.1 and 38.7, respectively.

The maturation of mammalian oocyte depends on the intact connection between the oocyte and surrounding cumulus, and changes or alterations in these structures after cryopreservation may lead to poor *in vitro* maturation of immature oocytes (Gordon, 2003). However, Babaei *et al*. (2006) reported that the *in vitro* maturation rate of bovine oocytes following vitrification in glass capillary micropipette (GCM) was 40% while, with non-vitrified/control was 61.29%.

CONCLUSION

Results of this study demonstrated that the optimal recovery of viable oocytes after freezing and thawing of she-camel oocytes is obtained using EG or mixture of CPAs, (EG, DMSO and PROH) and this was confirmed by oocyteshistological and ultrastructural observations. Also, thesubsequent camel oocytes *in vitro* maturation rate of both protocols SSV and DV was not significant different.

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تأثير مركبات الحماية على جودة بويضات الإبل مع إشارة خاصة إلى تركيبها البنائى الدقيق بإستخدام تقنية التزجيج

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تهدف هذه الدراسة إلى معرفة تاثير حفظ البويضات الغير ناضجة للابل العربية على التركيب البنائي الدقيق للبويضة وحيوية البويضات بعد الاسالة وقدرتها على استعادة نشاطها ونضجها تحت الظروف التحضين معمليًا، تم أجراء تجربتين لتحديد الحالة المثلى لتزجيج بويضات أنثى الجمل (الناقة) الغير ناضجة. التجربة ١: دراسة تاثير مركبات الحماية المختلفة: الإثيلين جليكول ; بروبانديول ; ثنائي ميثيل سلفوكسيد أو خليطها (١٠٪ الإثيلين جليكول + ١٠٪ بروبانديول + ١٠٪ ثنائي ميثيل سلفوكسيد) على معدل البقاء على قيد الحياة بعد الاسالة والتغيرات فى التركيب الهيكلي الدقيق لبويضات انثى الجمل (الناقة). تم تقسيم البويضات غير الناضجة من انثى الجمل (الناقة) إلى أربع مجموعات وتعرضت بيئة من ١٩٩ + ٠.١ M السكروز + ٢٠٪ (v / v) سيرمجنين الجمل تحتوي على ٣٠٪ الإثيلين جليكول (المجموعة ١). ٣٠٪ بروبانديول (مجموعة ٢)؛ ٣٠٪ ثنائي ميثيل سلفوكسيد (مجموعة ٣) أو ١٠٪ الإثيلين جليكول + ١٠٪ بروبانديول + ١٠٪ ثنائي ميثيل سلفوكسيد (مجموعة ٤) وحفظت في القشات. التجربة ٢: تقييم كفاءة اثنين من بروتوكولات التزجيج: التزجيج المباشر (باستخدام القشات) أو التزجيج باستخدام سطح صلب على نضج البويضات في المختبر. أظهرت نتائج التجربة الأولى أن الإثيلين جليكول او خليط من مركبات الحماية (الإثيلين جليكول ; بروبانديول ; ثنائي ميثيل سلفوكسيد) أظهرت معدل بقاء أفضل من مجموعات الحماية الأخرى (بروبانديول او ثنائي ميثيل سلفوكسيد).وكان معدل البقاء على قيد الحياة بعد الإسالة من البويضات انثى الجمل (الناقة) فى الاربع مجموعات ٨٣.٧، ٧٥.٣، ٧٢.٦ و ٨٢.٩٪ على التوالي. كما أظهرت الدراسة ان التركيب البنائى الهيكلي الدقيق لأغلفة البويضة ، وتوزيع الميتوكوندريا للمجموعة الإثيلين جليكول وجدت مماثلة للبويضات الغير معاملة. وأظهرت نتائج التجربة الثانية عدم وجود اختلافات معنوية في معدل الانضاج للبويضات بين الطريقتين (التزجيج المباشر أو التزجيج باستخدام سطح صلب). و توصى الدراسة بان مركب الاثيلين جلوكول هو أفضل مركب حماية في بيئة التجميد لحفظ بويضات انثى الجمل (الناقة) باستخدام اي من الطريقتين.