Cryopreservation of immature and mature camel (*Camelus dromedarius*) oocytes by open pulled straw vitrification

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ABSTRACT

The aim of the present investigation was to establish a reliable vitrification protocol for camel oocytes. A total of 540 cumulus oocyte complexes (COCs) were collected by manual slicing and divided into two groups. Three hundred oocytes were used to investigate the effect of the open pulled straw (OPS) vitrification procedure on the maturation rate of vitrified immature camel oocytes compared to control (non-vitrified). The rest (240 oocytes) were employed to evaluate the effect of vitrification on the morphological characteristics of immature and mature camel oocytes. COCs were treated with vitrification solution-1 (VS-1) [10% Dimethyl sulfoxide (DMSO) and 10% ethylene glycol (EG)] for 30-45 sec and transferred to vitrification solution-2 (VS2) [20% DMSO, 2 0% EG and 0.5M sucrose]. Oocytes were loaded into the OPS and then directly submerged into liquid nitrogen (LN_2) for 1h. The average value of maturation rate between the vitrified immature camel oocytes and the non-vitrified group (control) revealed non-significant differences as indicated by expansion of oocytes (88% (132/150), and 85.3% (128/150), respectively). However, the average rate of extrusion of the first polar body was significantly reduced in the vitrified immature oocytes (20.3%) compared to control group (40.1%). In addition, the morphological abnormalities occurred at higher rate in vitrified immature (GV stage) oocytes than in vitrified mature oocytes [16/99 (13.6%) vs, 6/106 (6.4%), respectively]. Consequently, the survival rate was significantly reduced in vitrified immature oocytes compared to vitrified mature oocytes (83.9% vs., 92.4%, respectively). In conclusion, the present study revealed that the camel oocytes could be successfully cryopreserved by OPS vitrification using EG and DMSO as cryoprotectant. Moreover, our results demonstrated that in camel mature oocytes are more resistant to cryoinjuries than immature oocytes and could produce a high percentage of normal oocytes that could be useful for future use in in vitro fertilization and camel improvement programs.

Keywords: *Camel, Camelus dromedarius, Oocyte, Vitrification, Open pulled straw, Cryopreservation.*

INTRODUCTION

The dromedary or one humped camel (*Camelus dromedarius*) belongs to the family *Camelidae*. Together with the Bactrian or two humped camel (*Camelus bactrianus*), they constitute the genus *Camelus*, where, the dromedary represents 90% of the genus *Camelus* (Al-swailem *et al*., 2010). Camel might be an exceptional animal which can work ideally under nourishment instability

condition. It is well documented that camel is one of the best animals to combat the climate change and future food insecurity problem (Tariq *et al*., 2014). Camel has unique physiological characteristics that enable it to adapt with its desert environment, such as fluctuation of its body temperature, tolerance of water loss and capability of drinking more water in little time (Schmidt-Nielsen, 1979 and Duehlmeier *et al*., 2007).

In Egypt, camels are economically important as they are considered dual purpose animals. In the Nile Valley and Delta, they are mainly raised for meat production and for some agricultural labor. In the desert, they are raised equally for meat and milk production, while some for labor and transport, and some are especially for camel racing (Abd El-aziem *et al*., 2015). The main camel breeds reared in Egypt are Maghrabi, a dual purpose animal (meat and milk), Falahy (Baladi), Sudani, Somali and Mowaled (hybrid between Maghrabi and Falahi) (El-Seoudy *et al*., 2008).

The fast development of assisted reproductive technologies such as *in vitro* maturation, *in vitro* fertilization and *in vitro* cultures has led to an increase in the need of mammalian oocytes. The easiest way of accessing oocytes at any time is by preserving them at very low temperatures (Cetin and Bastan, 2006). The development of a reliable method for the cryopreservation of mammalian oocytes would be an important advance in the field of reproductive biology for the preservation and management of genetic resources (Bogliolo *et al*., 2007). Also, conservation of oocytes would enable a more efficient management of livestock and laboratory animal species (Go and Mun, 2012). The cryopreservation technology is particularly valuable for establishing an oocyte bank to preserve the pure animal genetic resource (Gautam *et al*., 2008). The purpose of cryopreservation procedures is to minimize the damage and help cells to regenerate. However, there are many problems associated with oocyte cryopreservation related to the injury or sensitivity due to chilling and to the toxicity of cryoprotectants that cause considerable morphological and functional damages (Succu *et al*., 2007; Zeron *et al*., 1999). In recent years, vitrification has been adopted by many researchers as an alternative promising method assuring minimum freezing injuries to the cells and higher survival rates of oocytes after thawing in compared to slow rate freezing method. The vitrification method relies on the use of high concentration of cryoprotectants which inhibits the formation of ice-crystal and leads to the formation of a solid glasslike state. The advantages of the vitrification technology compared to slow-rate freezing are the low price of equipment, the simplicity of the procedure, and the short time required (Palasz and Mapletoft, 1996). Different vitrification procedures have been applied to cryopreserve embryos or oocytes. One of the most efficient methods for vitrification is open pulled straw (OPS). In the vitrification, oocytes are exposed to a high concentration of cryoprotectants for short periods of time, and the freezing/thawing procedures are very rapid (Vajta *et al*., 1997). The OPS provides additional advantages by reducing the cryoprotectant volume in the narrow part of the straw down to 0.5 µl, low heat insulation characteristics of the straw wall, and more than tenfold acceleration of freezing when the straws are immersed into liquid nitrogen (Vajta *et al*., 1998). Also, rapid freezing (vitification) by OPS inhibits zona fracturing, and damage of the cytoskeleton, chromosomes and meiotic spindle. In addition, toxic and osmotic stress at thawing is minimized by immersion of the capillary containing the embryo and oocytes in thawing solution (Vajta and Kuwayama, 2006 and Vajta and Nagy, 2006). Also, the OPS vitrification minimizes the time delay during warming thus

prevents exposing oocytes to unsuitable conditions (Ali *et al*., 2014).

Despite the widespread use of embryos and oocytes cryopreservation in cattle, sheep and goat, very limited information are available regarding the vitrification of camel oocytes. Therefore, the main objective of the present study was to establish a reliable vitrification protocol for immature and mature camel oocytes using the OPS procedure, and to investigate the effects of the vitrification procedure on the maturation rate. In addition, the efficacy of the OPS vitrification technique in terms of cryosurvivability and damages caused by vitrification to camel oocytes was assessed.

MATERIALS AND METHODS

Chemicals and media

All the chemicals and media were purchased from Sigma – Aldrich (Carlsbad, CA, USA) unless otherwise indicated.

Experimental design

In the present investigation two experiments were designed. The first experiment was conducted on a total of 300 oocytes, where half of the oocytes underwent vitrification before maturation, while the remaining oocytes were used as control (non-vitrified). This was performed to assess the effect of vitrification on the maturation rate of the camel oocytes. The second experiment aimed to investigate the effect of vitrification on the morphological characters of the *in-vitro* matured and immature oocytes. 240 oocytes were divided to two groups, *i.e. in-vitro* matured and immature oocytes. Each group included four replicates, each comprised of 30 oocytes.

Source of camel ovaries

Ovaries were collected randomly from Egyptian reared camels at local slaughter house (Bassateen, Cairo, Egypt) and transported within 2h to the laboratory (Embryology laboratory, Cairo University Research Park) in physiological saline solution (NSS, 0.9% NaCl) supplemented with streptomycin(0.1 mg/ml) at 25-30 ˚C. In the laboratory, the ovaries were washed once with 70% ethanol and three times with phosphate buffer saline (PBS).

Camel oocytes collection

Cumulus oocyte Complexes (COCs) (n= 540) were collected by manual slicing of follicles. Oocytes were collected in 4-(2-hydroxyethyl)-1-piperazineezanosulfonic acid (HEPES) buffered Tissue Culture Medium 199 [(TCM-199, 22340; GIBCO, UK) containing 25mM HEPES, Earl's salts; L-glutamine and 1-amion acids)] and supplemented with streptomycin sulfate (100µg/ml) and penicillin (100IU/ml).Then, the oocytes were counted and graded under a stereo microscope based on the combined morphology of the cumulus and homogeneity. According to Kharche *et al.* (2008) oocytes were graded based on the cumulus layers and the appearance of ooplasm. Oocyte with smooth and more than 5 cumulus layers were qualified as grade 1, oocytes with 3-5 cumulus grade 2, oocytes with 1-2 cumulus grade 3, and denuded oocytes were qualified as grade 4. Only COCs with three or more layers of cumulus cells and homogeneous ooplasm were used in this study.

Preparation of OPS

The method used for OPS vitrification was as described by Rao *et al.* (2012). French mini straws were slightly melted over a 100 ˚C hot plate and pulled by hand to achieve a diameter that was half of their original diameter. The straws were then held in air for

few seconds prior to cutting at the narrowest point of the pulled portion.

***In vitro* maturation (IVM) of camel oocytes**

Camel COCs were washed three times in HEPES-buffered TCM-199 (22340; GIBCO, UK) and then washed three times in IVM medium on a heating stage at 37 ˚C in the sterile cabinet. The maturation medium consisted of TCM-199 medium supplemented with 15% Fetal Bovine Serum (FBS; F7524,Sigma,Germany), 10µg/ml Follicle Stimulating Hormone (FSH; F2293,Sigma, USA) , 1µg/ml estradiol 17β (E2;E2758,Sigma ,USA), 20ng/ml Epidermal Growth Factor (EGF) , 0.25mg/ ml Na- pyruvate (P-4562; Sigma, USA), 0.3 mM cysteine and 50µg/ml gentamicine. Then, oocytes were cultured in petri dishes (30 COCs/Petri dish) containing 500 µl of maturation medium, overlaid with mineral oil, and placed in an incubator for 42 h at 38.5 ˚C under 5% CO_2 in humidified atmosphere . Oocytes maturation was assessed at the end of the culture period (42 h) based on cumulus cells expansion and the extrusion of the 1st polar body (PB I) after treating the oocytes with hyaluronidase (100 IU/ml). Cumulus cell expansion was determined and measured by three levels in same magnification of x 20; 1: indicating less expansion of COCs; 2: indicating moderate expansion; and 3: indicating high expansion of cumulus cells with a compact layer.

Vitrification / Warming procedures

The holding vitrification medium (HM) used for handling oocytes during vitrification was TCM-199 containing HEPES + 20% fetal bovine serum. All vitrification solutions were prepared using this holding medium. Briefly, oocytes were placed in the HM. Then the oocytes were transferred to vitrification solution-1 (VS1; HM with 10% Dimethyl sulfoxide (DMSO; Me2SO) and 10% ethylene glycol (EG)) for 30-45sec and transferred to vitrification solution-2 (VS2; HM with 20%DMSO,20% EG and 0.5M sucrose) for 25-30 sec. Single droplet (approximately 1.5 µl) containing 3–4 oocytes was loaded into the OPS by the capillary effect and then directly submerged into liquid nitrogen (LN**2**) after a quick passage in VS2. The time interval between the contacts with VS2 and (LN_2) did not exceed 30 sec .For warming, the OPS containing the vitrified COCs were taken out from liquid nitrogen and immersed in warming solution 1 (WS1; HM with 1.250 M sucrose).The oocytes were transferred to WS1 by gently tapping on free one end of the OPS to push the oocytes and left in WS1 for 1 min. The oocytes were then transferred to warming solution 2 (WS2; HM with 0.625M sucrose) for 30 sec and then transferred to warming solution 3 (WS3; HM with 0.310 M sucrose) for 30 sec. Finally, the oocytes were washed twice in HM for 5 min each and processed. The immature and mature oocytes were morphologically evaluated after warming.

Morphological evaluation of oocytes

Camel oocytes were washed twice with holding medium and examined using an inverted microscope (Leica, Wetzlar, Germany). Oocytes with spherical and symmetrical shape with no sign of membrane damage, swelling, vacuolization, leakage of the cellular content, ruptured zona pellucida or vitelline membrane, and fragmented cytoplasm were considered normal. Four replicates each comprised of 30 oocytes were analyzed.

Statistical analysis

The experiment was designed as completely randomized design. The analysis of data was according to Snedecor and Chochran (1980) using chi-square. Data were presented as means. The M Stat-C Program (1989) was used to calculate the least significant

differences (L S D) and compare among means according to Duncan (1955) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Vitrification refers to the physical phenomena describing the solidification of water or water based solution into a glass like amorphous liquid state (called the vitrification state), due to extreme elevation in viscosity during cooling, without the formation of ice crystal (Gupta and Lee, 2010 and Ali *et al*., 2014). In the present study, EG and Me2SO were chosen as favorable cryoprotectant combination since several investigators demonstrated that short exposure time to a vitrification solution containing EG + Me2SO with different concentration gradients supports cryoprotectant equilibrium between intra- and extracellular environments (Dhali *et al*., 2000, Wani *et al*., 2004 and Yadav *et al*., 2008) in buffaloes and in immature bovine oocytes by Massip, (2003). EG is suitable for less permeable immature oocytes, as demonstrated in goats and cattle (Agca *et al*.,1998). Also, Mahmoud *et al*. (2013) employed EG and DMSO for the cryopreservation of buffalo oocytes with the straw method. They referred the successful use of EG and DMSO to their low molecular weight, therefore, they can permeate through cell membranes rapidly (25-30 sec) to achieve concentration equilibrium across the cell membrane. Moreover, EG has been found to be convenient for vitrification of bovine and equine oocytes (Hurtt *et al*., 2000 and Cetin and Bastan, 2006) and immature human oocytes (Cha *et al*., 2000), due to its high permeability and low cytotoxicity.

Effect of OPS vitrification by DMSO- EG combination on maturation rate of camel oocytes

The goal of any IVM and *in vitro* fertilization program is to produce a high number of good quality embryos capable of delivering live birth after transfer to recipient animals (Skidmor, 2005). Therefore, in the present study we investigated the effect of the OPS vitrification procedure using a combination of EG and DMSO as cryoprotectants on the maturation rate of the camel oocytes. A total of 300 immature oocytes were collected, 150 oocytes were subjected to OPS vitrification, while the remaining 150 oocytes were non-vitrified and used as control. After 42 h on *in vitro* maturation the camel oocytes were examined. Maturation rates were assessed either by the presence of the 1st polar body (Hurtt *et al*., 2000) or roughly by expansion of cumulus cell mass (Schellander *et al*., 1989) as shown in Fig (1).The present study revealed non-significant differences between the vitrified immature camel oocytes using 20% DMSO and 20% EG and the control groups as indicated by cumulus expansion of oocytes (Expansion rate of 88 % (132/150), and 85.3 % (128/150), respectively) as shown in Table (1) and Fig (3). However, extrusion of the first polar body was significantly reduced in the vitrified- thawed immature camel oocytes (20.3%) compared to control group (40.1%). Therefore, the results showed that cryopreservation of immature camel oocytes by OPS vitrification has adverse effects on maturation rate at the nuclear level but no effect at the cytoplasmic level. This could be due to the damaging effect of the cryoprotectants on the chromosomes and the ultrastructure of the oocytes (Shahedi *et al*., 2013). Our results are highly concordant with those of El-Sayed *et al*. (2015). They demonstrated that cryopreservation of immature buffalo oocytes did not affect cytoplasmic maturation (based on expansion rate). The maturation rate in vitrified and control groups was 90.9% and 91.1%, respectively. However, cryopreservation significantly reduced the extrusion of the first

polar body to 10.6 % compared to 34.1% in the control group. Also, our results based on extrusion of the first polar body are in good agreement with those reported by Cetin and Bastan (2006). They found that maturation rates of vitrified –immature bovine oocytes with DMSO and EG were less than the control group (13.3% *vs.* 74.7%, respectively). Similarly, these findings come in accordance with those of Hammam and El-shahat (2005). They found that the maturation rate of immature buffalo oocytes after vitrification-warming was much lower than that for the control groups (30.0 and 80.0 %, respectively). Also, our results are in agreement with Mohsenzadeh *et al*.(2012) who found that the rate of maturation in vitrified – thawed human oocytes (33.3%) was significantly lower than that of non- vitrified- thawed human oocytes as control (61.2%). Moreover, Park and Kim (2011) reported that the *in vitro* maturation rate of vitrified- thawed canine oocytes were significantly ($p \leq 0.05$) lower than those of fresh canine oocytes (17.5% *vs*.43.6%, respectively). Also, Lee *et al.* (2013) reported a negative influence of cryopreservation on the efficiency of maturation rate of cryopreserved GV-stage oocytes, compared to the control in human (25 % *vs.* 50 %). Furthermore, Yamada *et al.* (2007) showed differences among maturation rates of immature bovine oocytes vitrified in the presence of EG + DMSO at different concentrations. The highest maturation rates were obtained after the exposure for 30 sec to 25% EG + 25% DMSO before vitrification. In contrast , our results were not in accordance with Dutta *et al*. (2013). Based on cumulus expansion they found that there was significantly ($p \leq 0.05$) higher *in-vitro* maturation rate of bovine oocytes in non-vitrified group (93.85%) than in vitrified group (81.58 %).

The reduction in maturation rate of vitrified immature camel oocytes could be due to a possible multifactorial cause including toxic effect of cryoprotectants, ultrastructural damage to the oocytes, and deleterious effects on chromosomes and other cytoplasmic structures as mentioned by Dobrinsky, (1996). In this respect, Dutta *et al*. (2013) reported that the freeze thaw process is known to induce an alteration in the physico-chemical properties in the intra-cellular lipids and such damages may render the oocyte incapable of retaining its developmental competence. In addition, the greater lipid content present in oocytes may also be one of the factors responsible for decreasing maturation rates, since it has been reported that high lipid content in oocytes makes them more sensitive to chilling injury by cryopreservation (Go and Mun, 2012). Moreover, the cryoprotectant agents (CPAs), despite their protective effect during freezing, may impose concentration- time and temperature-dependent toxicity (Fahy *et al.*, 1990). CPAs have been shown to have adverse effects on the organization of the microtubule system in mouse oocytes (Vincent *et al.*, 1990).

Despite the low percentage of the maturation rate at the polar body level in vitrified immature camel oocytes, the results of the present study revealed that cryopreservation of camel oocytes does not cause injury to oocytes membranes or cumulus cells which play an important role in oocyte competency to development *via* the gap junction. Through gap junctions, cumulus cells facilitate the passage of nutrients, inhibitory substances and small molecules from follicle cells to the oocyte and are essential for oocyte growth and differentiation. Thus, the cryopreserved mature oocytes could serve as good sources for *in vitro* embryo production and assisted reproductive technology. This has been also reported by Cocchia *et al*. (2010). Therefore, these findings encourage further studies on camel oocytes cryotechnology.

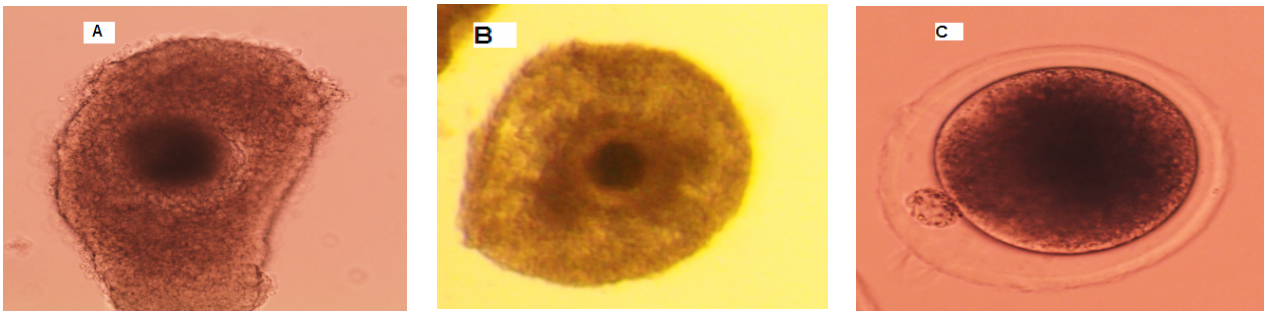

Fig. (1): Steps of oocytes maturation: (A) cumulus oocyte complex, (B) mature camel oocyte judged by expansion of cumulus cells, (C) extrusion of the first polar body.

Table (1): Effect of open-pulled-straw vitrification using DMSO-EG on maturation rate of immature camel oocytes compared to control.

Group	No. of COCs	Maturation rate	
		Expansion rate n(% ±SE)*	Polar body rate n(% ±SE)*
Non- Vitrified (Control)	150	132(88% ±0.64)[a]	53 (40.1%±0.45)[a]
Vitrified- thawed	150	128(85.3%±0.64)[a]	26(20.3%±0.45)[b]

*n= Number of Cumulus oocyte Complexes (COCs), SE= standard error.
Values with different superscripts within columns are significantly different at p ≤0.05.

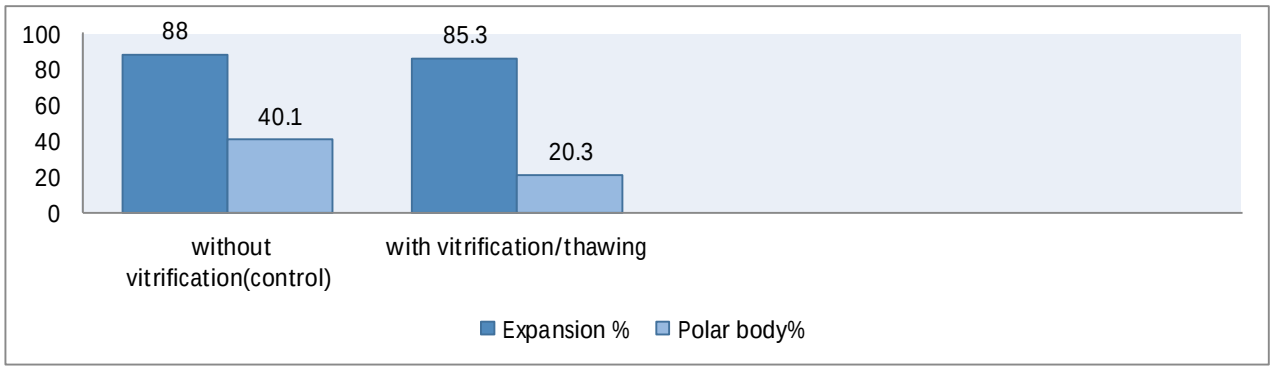

Fig. (2): In vitro maturation rates of vitrified and non-vitrified immature camel oocytes.

Effect of the EG-DMSO OPS vitrification procedure on the morphological characteristics of immature / mature camel oocytes

The camel oocytes were vitrified either immediately after collection (Immature group) or after 42 h of maturation in TCM-199 medium (mature group). The matured oocytes of both groups were then evaluated for survival rate by cumulus expansion and viability. The morphological evaluation of the camel oocytes post- thawing was performed using an inverted microscope (Leica). The camel oocytes were judged morphologically as normal (Fig3 A-E) when the spherical and symmetrical shape had no signs of lysis, membrane damage, swelling, degeneration or leakage of the cellular content. While, oocytes were considered abnormal (Fig.3 F-L) when a ruptured zona pellucida or a fragmented cytoplasm with signs of degeneration were present. As shown in Table (2) the results revealed that the morphological abnormalities occurred in immature (GV stage) camel oocytes at higher rate than in mature oocytes [16/99 (13.6%) *vs.*, 6/106 (6.4%), respectively]. This was reflected on the survival rate which was significantly reduced in immature oocytes compared to mature oocytes (83.9% *vs*., 92.4%, respectively). This suggests that in camel, the sensitivity of the oocytes to cryoinjuries is more accentuated at the immature stage (GV stage) than the mature stage. These findings are in agreement with Abd-Allah *et al*. (2009) who found that the proportion of post thawing morphologically normal vitrified mature oocytes were significantly higher ($P \leq 0.05$) than that of vitrified immature oocytes (70.4% *vs*, 55.4%). Similarly, Hammam and El-shahat (2005) revealed that the percentage of post thawing morphologically normal vitrified mature buffalo oocytes were significantly higher ($p \leq 0.01$) than that of vitrified immature oocytes. In addition, Fukui *et al*. (1992) and Lim *et al*. (1992) found that matured oocytes were resistant to freezing than immature ones. Moreover, Ruffing *et al*. (1993) demonstrated that the hydrolytic conductivity of immature bovine oocytes is only half that of *in vitro* matured oocytes. Agca *et al*. (1998) claimed that the permeability of bovine oocytes are significantly higher for cryoprotectant after *in vitro* maturation. However, Cao *et al*. (2009) reported that GV human oocytes are more resistant to cryoinjury because they have less depolymerization of the microtubules and are less prone to aneuploidy than mature oocytes. In addition, Le Gal and Massip (1999) mentioned that the ability of oocytes to survive cryopreservation is not affected by the meiotic stage in which the oocyte was cryopreserved.

In the present investigation several types of morphological abnormalities of camel oocytes were detected in each of the immature and mature stages. These included abnormal oocytes shape, zona pullecida, increase in privitelline space and heterogeneous cytoplasm (Fig.3). Shrinking of heterogeneous cytoplasm was the most frequent abnormality observed in the vitrified mature and immature oocytes (66.6% and 43.7%, respectively) indicating that the cytoplasm of camel oocytes is highly sensitive to cryopreservation. This could be due to the high concentration of CPAs (20%DMSO, 20% EG) that penetrate intracellular to avoid ice crystal formation, which have toxic effects on cells and may cause osmotic injury. In this respect Fuller and Paynter (2004) reported that cryoprotectants have both positive and negative effects on cell function depending on the kinetics of exposure. However, Luvoni (2006) suggested that the oocyte cryopreservation efficiency depends on different factors, including cryoprotectant type, cryopreservation method and cooling and thawing rates, each of which may be responsible for oocyte cryo-damage. Despite this, previous studies showed that

cryopreservation steps can be detrimental to the functionality of cumulus-oocyte communication in mouse (Lingham *et al*.,2006), cow (Martino *et al*.,1996), buffalo (Sharma and Loganathasamy.,2007) and porcine oocytes (Yuge *et al*.,2003) . In the present study, under *in vitro* conditions, the percentage of morphologically abnormal oocytes after vitrification was only 6.49% and 13.9% for vitrified mature and immature oocytes, respectively (Table2). This percentage is comparable to previous reports in other species such as, buffalo (Mahmoud *et al.*, 2014; Abd-allah *et al*., 2009 and Dhali *et al.*, 2000) and Human (Mohsenzadeh *et al*., 2012). While, in bovine, immature oocytes have been reported to be less permeable to cryoprotectants than mature oocytes (Agca *et al*., 1998). Also, immature bovine oocytes are more sensitive to osmotic stress than mature oocytes (Agca *et al*., 2000). While, in camel, to the best of our knowledge, there is no previous studies on oocytes cryopreservation. No degradation of cumulus cells was detected in vitrified mature oocytes while in vitrified immature oocytes the percentage of this abnormality was 31.2%. This confirms that immature camel oocytes are more cryosensitive than mature camel oocytes.

These findings are in accordance with Men *et al.* (2002) who reported that immature oocytes at the germinal vesicle stage are more sensitive to cryo-injuries due to their low membrane stability and the susceptibility of their cytoskeleton. In conclusion, the present study revealed that the camel oocytes could be successfully cryopreserved by open pulled straw vitrification using EG and DMSO as cryoprotectant. Moreover, our results demonstrated that in camel mature oocytes are more resistant to cryoinjuries that immature oocytes and could produce a high percentage of normal oocytes that could be useful for future use in *in vitro* fertilization and camel improvement programs.

Table (2): Effect of vitrification on morphological appearance (phenotype) of camel oocytes.

Group	No. of oocytes	No. of recovered oocytes	No. of morphologically normal oocytes (% ± SE)*	No. of morphologically abnormal oocytes (% ± SE)*	Survival rate post vitrified-thawed oocytes (% ± SE)*
Immature	120	115	99 (86% ± 0.89)[a]	16 (13.9% ± 0.64)[a]	83(83.9% ± 0.64)[a]
Mature	120	112	106(94.6% ± 0.89)[a]	6(6.4% ± 0.64)[b]	98(92.4% ± 0.64)[b]

* SE =standard error

Values with different superscript within columns are significantly different at $p < 0.05$.

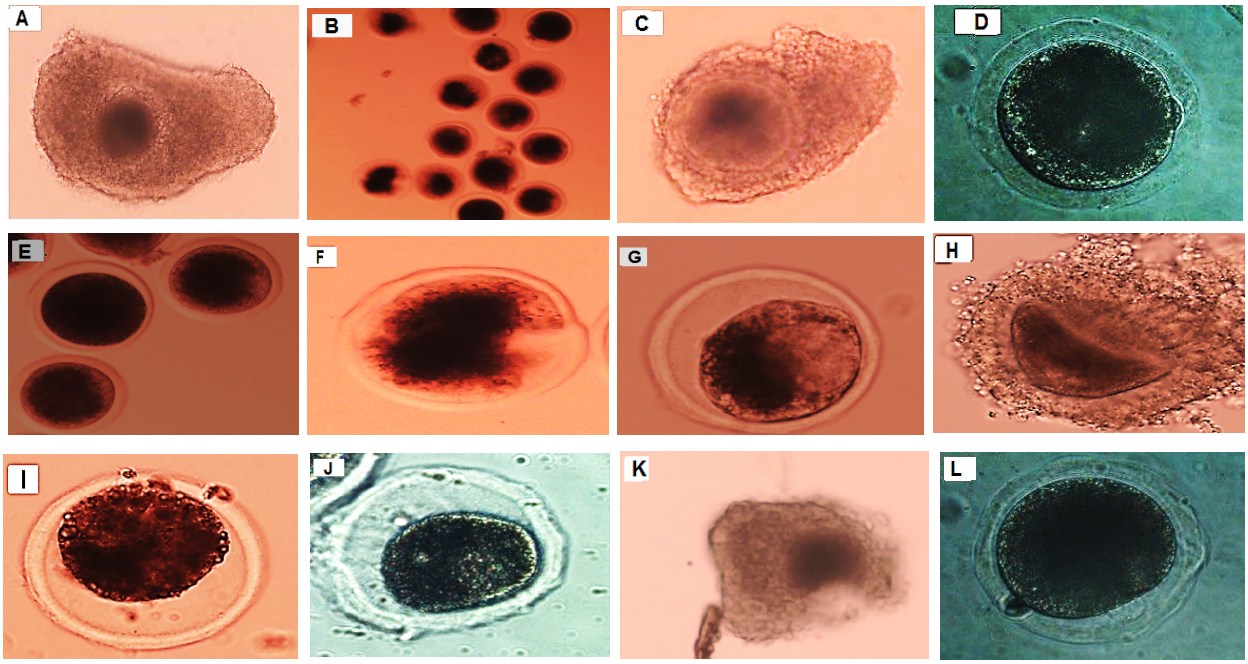

Fig. (3): Types of damages observed in camel oocytes post vitrification-thawing: (A) Normal camel cumulus oocyte complex COCs .(B) Oocyte after vitirifiction- thawing, (C) Normal immature oocyte after vitrification-thawing , (D) Normal vitrified mature oocytes with spherical shape, intact zona pellucida and homogeneous cytoplasm, (E) Normal vitrified immature oocyte, (F) Abnormal mature camel oocyte with heterogeneous cytoplasm, (G) Abnormal camel oocyte with shrinked dark-less cytoplasm, (H) Abnormal immature oocyte with abnormal shape, (I) Abnormal camel oocyte with wide privitelline space, (J) Abnormal mature camel oocyte with thinning of zona pullecida and shrinkage cytoplasm, (K) Abnormal immature camel oocyte with degenerated cumulus cell around it, (L) Abnormal vitrified mature oocyte with irregular zona pullecida.

Table (3): Types of damage observed in camel oocytes post - vitrification-thawing.

Group	No.of damaged oocytes	Types of observed damage post- vitrification-thawing		
		Rupture of zona pellucida - wide privitelline space (%)	Shrinkage-of heterogeneous cytoplasm (%)	Degradation of Cumulus cells (%)
Immature	16	4/16 (25%)	7/16 (43.7%)	5 (31.2%)
Mature	6	2 /6 (33.3%)	4/6 (66.6%)	0(0.0%)

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الملخص العربي

حفظ بويضات الإبل الناضجة وغير الناضجة باستخدام طريقة التجميد فى الأنابيب المسحوبة المفتوحة

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حظيت عملية حفظ بويضات الحيوانات المزرعية بأولوية عالمية نتيجة للانخفاض الملحوظ فى التنوع البيولوجى للحيوانات، وكذلك الخطر الناتج عن انقراض الموارد الوراثية الحيوانية ولذا تتزايد الجهود البحثية العلمية للتركيز على انشاء بنوك للموارد الوراثية الحيوانية والعمل على تطوير بروتوكولات مناسبة لحفظ البويضات . والدراسة الحالية اجريت بهدف إعداد بروتوكول مناسب لحفظ بويضات الإبل . تم جمع ٥٤٠ بويضة ثم قسمت الى مجموعتين، ٣٠٠ بويضة استخدمت لدراسة تأثير عملية التجميد على معدلات النضج (السيتوبلازمى والنووى) لبويضات الجمال غير الناضجة مقارنة بالكنترول ، بينما استخدمت ٢٤٠ بويضة لتقييم تأثير عملية التجميد على الخصائص المظهرية لكل من البويضات الناضجة وغير الناضجة. حيث تم تجميد البويضات عن طريق وضعها فى سائل التجميد الأول المحتوى على ١٠% ايثيلين جيليكول + ١٠% داى ميثيلين سلفوكسيد لمدة ٣٠-٤٥ ثانية ونقلها الى سائل التجميد الثانى المحتوى على ضعف التركيزات السابقة مضافا اليه السكروز بتركيز مولار 0.5 وتم سحبها داخل أنابيب التجميد و غمرها مباشرة داخل النيتروجين السائل. تم حساب متوسط معدلات النضج للبويضات المجمدة فأوضحت النتائج انه لايوجد فرق معنوى ملحوظ فى معدلات نضج البويضات المجمدة (١٣٢/١٥٠ بنسبة ٨٨ %) مقارنة بالكنترول (١٢٨/ ١٥٠ بنسبة ٨٥.٣%) وذلك على مستوى النضج السيتوبلازمى ، بينما انخفضت نسب ظهور الجسم القطبى الاول (النضج النووى) للبويضات المجمدة الى (٢٠.٣%) مقارنة بالكنترول (٤٠.١ %). بالاضافة الى ذلك كان معدل ظهور بويضات ذات اشكال مظهرية غير طبيعية بعد عملية التجميد لبويضات الإبل غير الناضجة أكثر بشكل معنوى عن مثيلتها فى البويضات الناضجة (٦/ ١٦بنسبة ٦.٤%) مقابل (١٦ /٩٩ بنسبة ١٣.٦ %) ، وتبعاً لذلك انخفضت معدلات الحيوية للبويضات الغير الناضجة مقارنة بالناضجة. وبالتالى اوضحت النتائج نجاح عملية تجميد بويضات الأبل بإستخدام مادتى إثيلين جيليكول و داى ميثيل سلفوكسيد كمواد حماية للبويضات من أثر التجميد ، وأوضحت ايضاً ان بويضات الإبل الناضجة أكثر مقاومة للصدمات الناتجة من اثار التجميد من البويضات غير الناضجة مما قد يفيد فى الاستخدمات المستقبلية فى تداول وحفظ بويضات الإبل و زيادة البويضات الصالحة لإنتاج أجنة الأبل معملياً وكذلك فى برامج التحسين الوراثى للإبل.