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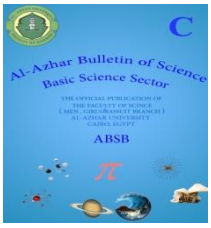

EFFECT OF CRYOPROTECTANTS ON CAMEL OOCYTES VITRIFICATION

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ABSTRACT

The objective of this study was to show how two cryoprotectants affect the vitrification rate in immature camel oocytes when used in a separate or a mixed form. As a result, their ability to mature after being warmed was recorded. The cumulus oocytes complexes (COCs) were divided into four groups, control; EG (30 percent ethylene glycol) in vitrification solution (VS); DMSO (30 percent dimethyl sulfoxide); MIX (15 percent EG + 15 percent DMSO) in vitrification solution (VS). COCs were impeded in equilibration solution (ES) for 2 minutes before being transferred to a vitrification solution containing 30% cryoprotecting agent (EG, DMSO, or MIX), 20% FBS, and 0.5 M sucrose in the medium in each vitrification group. Oocytes were put into the open pulled straw (OPS) and subsequently frozen for one hour in liquid nitrogen (LN2). The oocytes were then thawed and warmed for 2.5 minutes at 37°C in four warming solutions (WS) containing varying amounts of sucrose. The findings revealed that the oocyte maturation rate was high. In comparison, DMSO treatment had a better recovery rate, viability, and more negligible damage effect than EG and MIX.

Key words: Vitrification; Cryoprotectants; Oocytes; Dromedary camel; IVM.

1. INTRODUCTION

Governments are working to increase camel numbers, and there are many problems facing camel breeding, including a long time between breeding seasons. Assisted reproductive technologies (*ARTs*) can help with increasing offspring by using modern laboratory methods. [1]. The previous authors listed the reproductive biotechnologies nowadays used in female camels, including dynamic follicular synchronization, artificial insemination, embryo recovery and transfer, oocyte recovery for in vitro maturation (IVM), in vitro fertilization (IVF), intra-cytoplasmic sperm injection (ICSI), nuclear transfer (cloning), and embryo cryopreservation [2].

In this regard, vitrification, as a method of cell cryopreservation, has been considered an essential tool for Assisted Reproduction Technologies (*ART*), improving the reproductive quality of economically important species for animal production and in humans for subfertility treatments [3-5], as vitrification is used in oocytes and embryos of different livestock species such as sheep [6], cattle [7]. *IVM* has increased the importance of establishing a mature oocyte cryobank for large-scale embryo production programs in animals [8]. Many problems are associated with oocyte cryopreservation related to the injury or sensitivity due to chilling and the toxicity of cryoprotectants that cause considerable

morphological and functional damage [9,10]. Several studies demonstrated that Ethylene Glycol (EG) would be the ideal cryoprotectant [11] because it penetrates membranes faster than glycerol [12] and is less toxic than other permeable cryoprotectants [13]. Also, Dimethylsulfoxide (DMSO) is a non-toxic and low-cost permeating cryoprotectant that protects cells from intracellular ice crystal formation [14]. Although successful vitrification of alpaca oocytes [15] and camel [16, 17, 18 and 19] and llama [20] embryos was reported, there was scarce information on the vitrification of camel oocytes, explained by previous authors' some reliable vitrification protocols for camel oocytes [21- 27].

The present study was designed to focus on the effect of EG, DMSO cryoprotectants or the combination on recovery rate, viability, Cumulus Oocytes Complex (COC) morphology, types of COC damage, and maturation rates (expansion polar body extrusion) of immature vitrified or warmed dromedary camel oocytes.

2. MATERIAL AND METHODS

The present study was registered at Al-Azhar University - Faculty of Science - Department of Practical Zoology, and the practical design was performed for two consecutive years (2018/2019 and 2019/2020) at the Desert Research Center (DRC), Cairo, Department of Animal and Poultry Production, Embryology Processing Unit (EMU).

2.1. Chemicals and media:

All of the reagents and culture components (Sigma-Aldrich Chemicals, Germany) were prepared daily from a stock solution of each compound and sterilizing until the use by passing through a Millipore filter with only a width of 0.22 μm fitted to a 10 ml syringe **[28].**

2.2. Biological material:

Dromedary camel ovaries of unknown reproductive history were used as the source of oocytes that collected from the slaughterhouse in El-Bassatine, Cairo, and placed in a thermos flask containing sterilized pre-warm physiological saline solution (NSS, 0.9% NaCl) at 30 - 35 ^{o}C. It was supplemented with 100 IU penicillin and 100 μg streptomycin/ml as antibiotic antimycotic [29] and transported to the laboratory within 2 - 3 hrs.

2.3. Ovaries manipulation and oocyte retrieving:

Immediately after camel ovaries reached the EMU, they were washed three times with warm (30 ^{o}C) NSS to remove the blood and debris. Then, all ovaries were rapidly washed once with ethanol (70%) to remove any contamination on the ovarian surface and washed using warmed (30 ^{o}C) phosphate buffer saline (PBS) supplemented with antibiotics (100 IU penicillin and 100 μg streptomycin/ml) as final wash [28]. After that, the ovaries were preserved in glass gars containing PBS and kept in a 30 oC adjusted water bath during oocyte recovery [30].

By slicing method, (COCs) were harvested from ovaries in a 90 mm petri dish, rinsing with warm (30 ^{o}C) PBS supplemented with 50 μg/ ml gentamicin [30].

The COCs were then assessed under a stereomicroscope (GXmicroscope, UK, Range: 8x to 50x) using the criteria described by Nowshari and Wernery [31]. There were three types oocytes' grades which are illustrated as follow; Three layers of compact cumulus cells with homogenous granulated ooplasm (Grade I); three layers of cumulus cells with homogenous granular cytoplasm (Grade II) or denuded ooplasm (Grade III). In the present study, Grade I oocytes were used. Oocyte collection medium (OCM) was used for searching, cleaning, grading, and choosing oocytes (TCM-199 supplemented with 25 mM HEPES, 10% FBS). Before IVM or vitrification, the oocytes were washed three times in OCM.

2.4. Experimental design:

Dromedary camel cumulus-oocyte complexes (COCs) were collected and grouped as follow:

Group 1: control group, COCs were *in vitro* matured without vitrification.

Group 2: EG group, COCs were exposed to ES (50% of VS) for 2 min and transferred to VS (TCM 199, 20% FBS, 30% EG (v/v) and 0.5 M sucrose) for 45 s.

Group 3: DMSO group, COCs were exposed to ES (50% of VS) for 2 min and transferred to VS (TCM 199, 20% FBS, 30% DMSO (v/v) and 0.5 M sucrose) for 45 s.

Group 4: MIX group, COCs were exposed to ES (50% of VS) for 2 min and transferred to VS (TCM 199, 20% FBS, 15% DMSO (v/v) + 15% EG (v/v) and 0.5 M sucrose) for 45 s.

2.5. Oocytes vitrification:

In this study, vitrification of COCs was achieved in two steps [23]. Initially, COCs were equilibrated in ES (2 min), which consisted of 50% VS [32]. Then vitrification in VS, composed of TCM-199, was completed with 20% v/v fetal bovine serum, 30% Cryo-Protectant Additives CPA, and 0.5 M sucrose for 45 seconds. A group of 5 COCs in 1-2 µl VS in OPS. Then immediately after loading the oocytes, straws were plunged into LN2 (- 196 oC) for 1hr [22]. The process from vitrification to dropping in LN2 takes 45 s.

2.6. Open pulled straw (OPS) method:

The straw must be slightly melted over a flame to become more flexible, then pulled to reach half its original diameter (Figure 1). Finally, the straw was broken at the narrow end and exposed to the air for a few seconds. Five COCs were loaded into the mini straws by capillary action after the proper exposure to the VS [33].

2.7. Oocytes warming:

After one hr. in LN2, oocytes were warmed in 4 sequential warming solutions (WS) with different concentrations of sucrose as follows: WS1, WS2, WS3, and WS4 were prepared from TCM-199 supplemented with 0.5 M, 0.25 M, 0.125 M, and 0 M sucrose respectively, at intervals of 2.5 min at 37 ^{o}C [34].

2.8. Oocyte evaluation:

The viability of vitrified/warmed COCs had been assessed according to Gupta, *et al* the previous authors' technique. The oocytes were transferred to (50 µl) of essential medium (BM) consisting of TCM 199 supplemented with 20% v/v FBS and examined using inverted phase-contrast microscopy. The dead oocytes looked like a blue stain, but the life remained unstained [35].

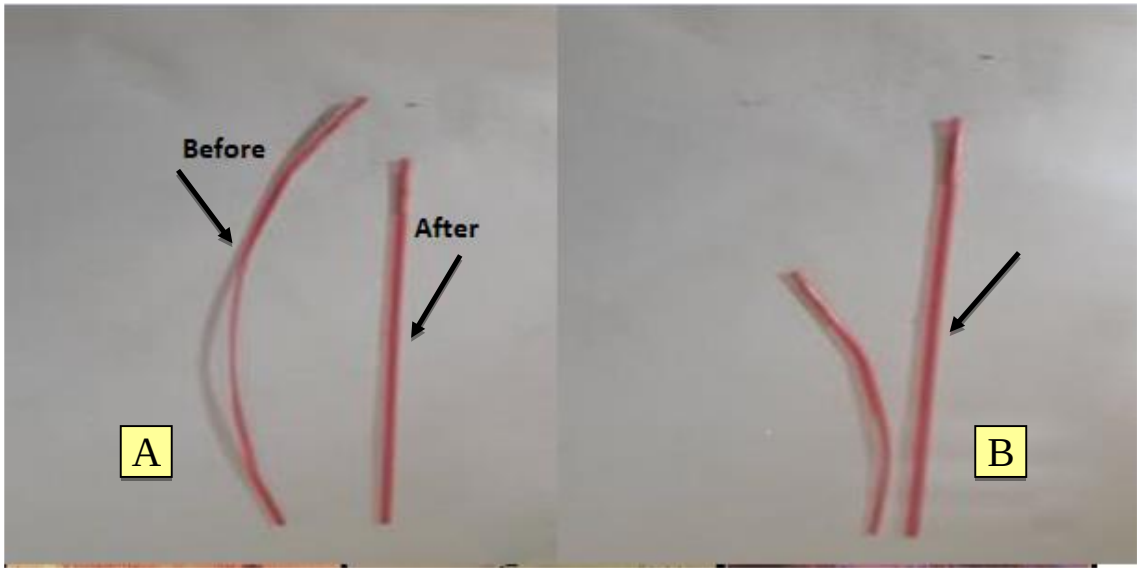

Fig.1. A. Open pulled straw before and after cutting the narrowest point of the pulled portion with a diameter half of their original (French mini straws) diameter. B. French mini straw with standard diameter before pulling.

2.9. Oocytes maturation:

According to El-Shahat and Hammam [32]**,** oocytes were washed three times in TCM-199 washing medium (WM) supplemented with 25 mM HEPES and 10% FBS) and once in maturation medium (TCM-199) supplemented with 15% (v/v) heat-treated (56 oC- 30 min), foetal bovine serum (FBS), 20 ng/ml epidermal growth factor (EGF), 40 IU PMSG, 0.25 mg/ml Na+ pyruvate, 1 µg/ml estradiol (E2) and 100 µg/ml ascorbic acid [36]. The media pH was adjusted to 7.4 and incubated at 38.5 oC under 5% CO2 and 95% relative humidity for at least 2 hours before use. Oocytes were incubated in a CO2 incubator for 30 hrs at the previous condition in drops of maturation media (10 to 15 oocytes/ 100 µl drop). The COCs expansion was evaluated by a stereomicroscope (the UK, GX microscope: 8x–50x). For PB evaluation, COCs were denuded by gentle pipetting with 80 IU of hyaluronidase before being washed twice in HEPES-TCM 199 and examined under the inverted microscope [37]**.**

Statistical analysis:

Data of recovery rate, viability, oocytes morphology, type of damages, and maturation rate (due to expansion and polar body) were statistically analyzed by Chi-square test using the SAS program, 2004.

3. RESULTS

In the present study, the recovery rate in DMSO and the MIX groups were significantly higher than in the EG group. The viability % was significantly higher in the control group than DMSO, MIX, and EG groups. Regarding the camel COCs, phenotype showed no significant difference between all treatments. In comparison, the DMSO group was significantly higher than the EG group or the MIX group in leakage of ooplasm. But the EG and DMSO groups were significantly higher than the mixed group in partial cumulus loss. Generally, the maturation rate in the control group was significantly higher than in all treatments.

3.1. After warming recovery rate:

As shown in Table 1, the recovery rate percent for the DMSO and the MIX groups was significantly higher than the EG group (100%, 100 %, and 93.33 %, respectively). At the same time, no significant difference was observed within the DMSO and MIX group (100 % and 100 %, respectively).

3.2. After warming COCs viability

The viability % of cumulus cells was significantly higher in the control group than DMSO, MIX, and EG groups (**Table 2** and **Fig.2),** with detected values of 88.42 %, 74.00%, 68.00%, and 62.00%, respectively. But the viability % declared insignificant difference between EG, DMSO, and MIX (62%, 74%, and 68 %, respectively).

3.3. Effect on COCs morphology:

Morphological appearance (phenotype) of camel COCs are presented in **Table 3** showing insignificant difference (P≥0.05) between EG group, DMSO group and MIX group (39.29%, 50 % and 40 % respectively).

Table 1: Effect of different CPAs and CAPs combination after warming of dromedary camel COCs recovery rate

Treatment	Total no. of vitrified COCs	Recovery rate (%)	Loss rate (%)	Standard error	Chi square value
EG	60	93.33 b (56/60)	6.67 (4/60)	±12.84	8.18* (0.0167)
DMSO	60	100 a (60/60)	0 (0/60)		
MIX	60	100 a (60/60)	0 (0/60)		

Means with different alphabetical superscripts within columns or * are significantly different at (P≤0.05).
EG: Ethylene Glycol; DMSO: Dimethyle Sulphoxide; Mix: EG+ DMSO mixture.

Table 2: Effect of different CPAs and CAPs combination on viability of vitrified camel oocytes

Treatment	Total no. of vitrified COCs	Live COCs %	Dead COCs %	Standard error	Chi square value
Control	51	88.42	11.76	±4.78	9.76* (0.02)
EG	50	62	38		
DMSO	50	74	26		
MIX	50	68	32		

Means with different alphabetical superscripts within columns are significantly different at least at P < 0.05. EG: Ethylene Glycol; **DMSO:** Dimethyle Sulphoxide; **Mix:** EG+ DMSO mixture.

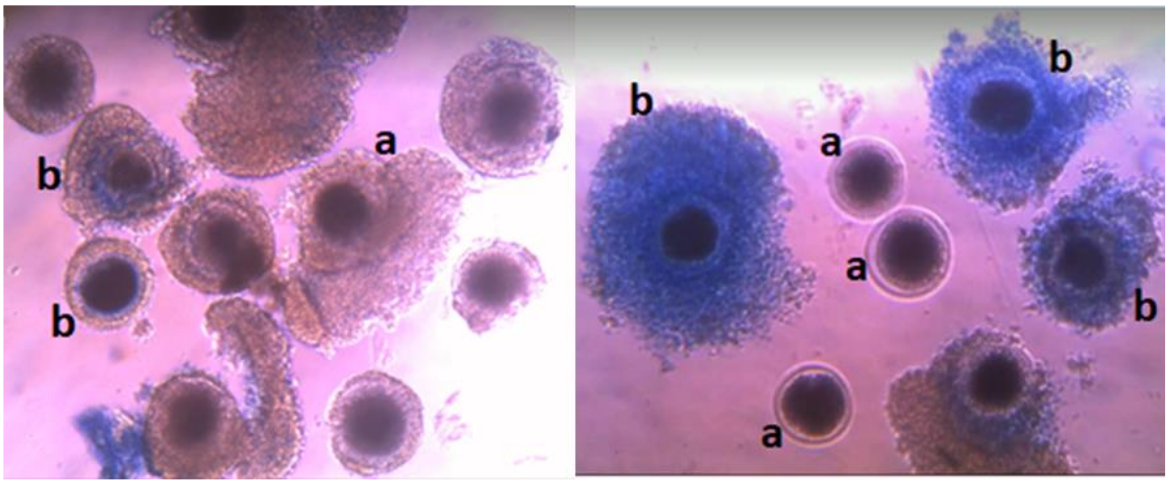

Fig. 2. Immature Cumulus oocytes complex stained by trypan blue stain A. non vitrified oocytes (Control). B. oocytes vitrified with EG and showing a: viable unstained oocytes; b: dead stained oocytes.

Table 3: Effect of different CPAs and CAPs combination on morphological appearance (phenotype) of camel COCs

Treatment	Total no. of vitrified COCs	Morphologically normal COCs%	damaged COCs%	Standard error	Chi square value
EG	56	60.71(34/56)	39.29(22/56)	2.23±	1.7309 (0.4209)
DMSO	60	50(30/60)	50(30/60)		
MIX	60	60(36/60)	40(24/60)		

Means with different alphabetical superscripts within columns are significantly different at least at P < 0.05.

3.4. Types of COCs damages

The leakage of cellular contents (**Fig. 4-A**) declares that the DMSO group was significantly higher than the EG group or the MIX group (13.33 %, 0.0%, and 0.0%, respectively). At the same time, there was no significant difference between the EG and the MIX group (0.0% and 0.0%, respectively). As for the crack in zona pellucida (**Fig. 4-B**), the results showed no significant difference between the EG, DMSO, and MIX groups (9.09%, 6.67%, and 0.0%, respectively). No significant difference was observed between all groups in the shrinkage of cytoplasm (**Fig. 4–C**). On the other hand, the partial cumulus loss (**Fig.4-D**) for the EG and DMSO groups was significantly higher than the mixed group (29.03%, 26.67%, and 16.67%, respectively). Thus, no significant difference between the EG and DMSO groups was

observed (29.03%and 26.67%, respectively). However, in complete cumulus loss (**Fig.4-E**), there was no significant difference between all groups EG, DMSO, and MIX (18.18 %, 20%, and 33.33%, respectively), similarly to the change in shape loss (Fig.4-F) showing insignificant difference between all groups EG, DMSO and MIX (**Table 4 and Fig. 3**).

3.5. Maturation rates

a-Expansion rate

As presented in **Table 4**, the maturation rate due to expansion of cumulus cells (**Fig. 5-B**) was significantly higher in the control group than MIX, DMSO and EG groups with detected values of (78.24%, 68.75%, 56.25%, and 48.61%, respectively).

a- Polar body extrusion rate

Results in **Table 5** presents the maturation rate due to the polar body extrusion rate (**Fig. 5-C**) the control group was significantly higher than the DMSO group, MIX and EG group (22.35%, 11.25%, 6.25%, and 5.56%, respectively. There was no significant difference between the MIX group and EG group (6.25 % and 5.56 %, respectively).

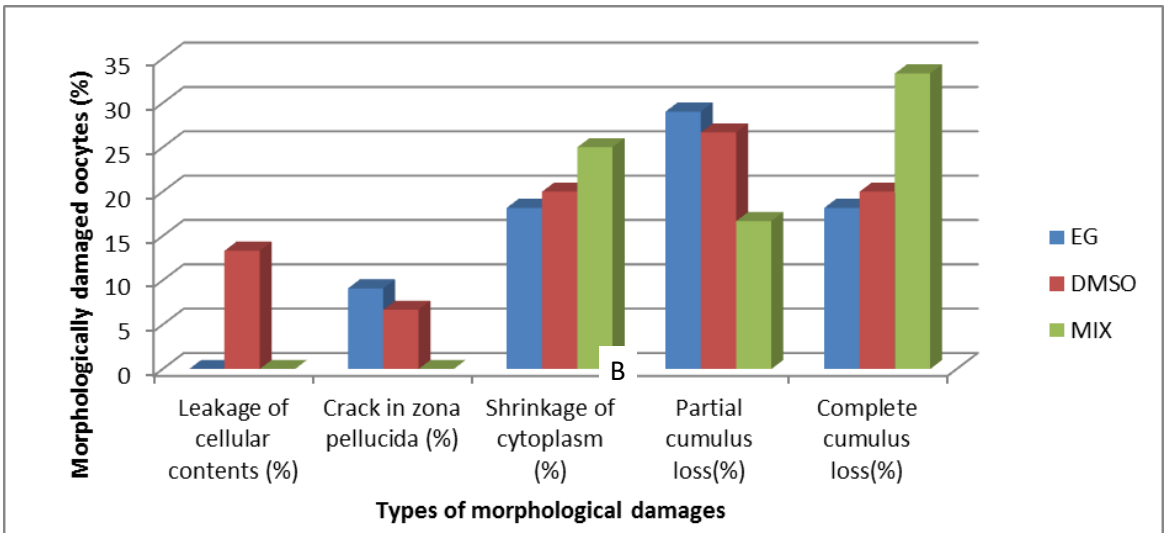

Fig. 3: Morphological damage parameters of dromedary camel oocytes as affected by different CPAs (EG, DMSO and MIX).

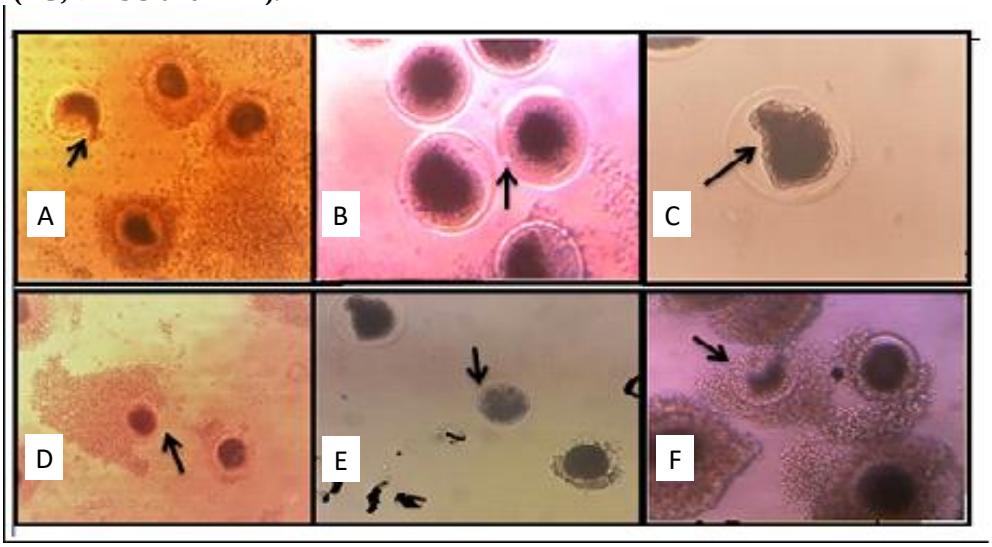

Fig. 4: Damages of cumulus oocytes complex after vitrification: A-Leaking of cytoplasm; B- Crack in zona pelucida; C- Shrinking in cytoplasm; D- Partial loss of cumulus cells; E- Complete/total loss of cumulus cells (denuded); F- Change in oocyte shape.

Table 4: Effect of different CPAs and CAPs combination on maturation rate of dromedary camel COCs according to expansion:

Treatment	Total number of COCs	Expansion rate%	Standard error	Chi square value
Control	170	78.24 [a] (133/170)	12.21±	24.86 ** (<0.0001)
EG	72.0	48.61 [d] (35/72)		
DMSO	80.0	56.25 [c] (45/80)		
MIX	80.0	68.75 [b] (55/80)		

The values of different letter superscripts (a, b, c and d) are significantly different (P≤0.05) within the same column. **: Highly significant at (P≤0.01). EG: Ethylene Glycol; DMSO: Dimethyle Sulphoxide; Mix: EG+ DMSO mixture.

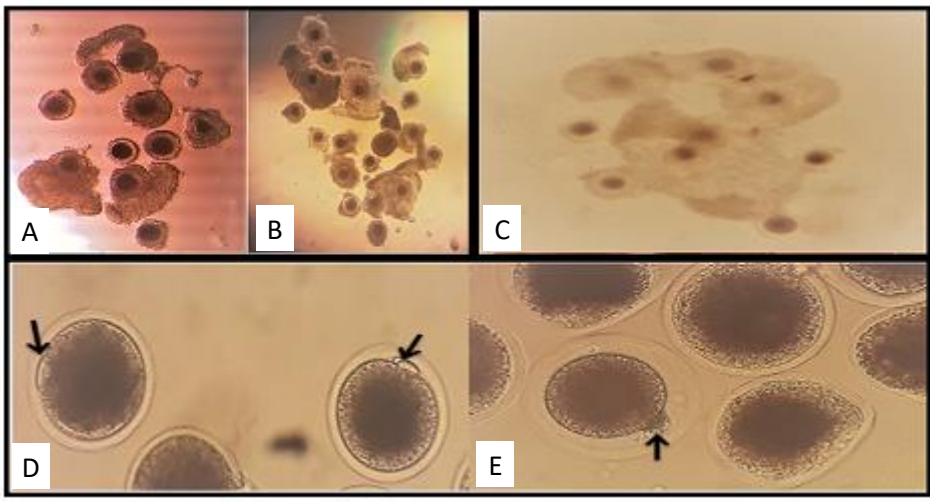

Fig. 5: Developmental stages of IVM (control) A- GV and M1 (immature oocytes IM) with the condensed cumulus cells (30X). B- MII (mature oocytes) with expanded cumulus cells (50X). C- The 1st polar body was excluded after IVM and denudation of oocytes (black arrows, 400X)

Table 5: Effect of different CPAs and CAPs combination on maturation rate of dromedary camel COCs according to polar body extrusion:

Treatment	Total number of Oocytes	Maturation rate%	Standard error	Chi square value
Control	170	22.35 [a] (38/170)	15.86±	18.69** (0.0003)
EG	72.0	5.56 [c] (4/72)		
DMSO	80.0	11.25 [b] (9/80)		
MIX	80.0	6.25 [c] (5/80)		

The values of different letter superscripts (a, b, c and d) are significantly different (P≤0.05) within the same column. **: Highly significant at (P≤0.01). EG: Ethylene Glycol; DMSO: Dimethyle Sulphoxide; Mix: EG+ DMSO mixture.

4. DISCUSSIONS

Ethylene Glycol (EG) had the lowest recovery rate after warming when compared with DMSO and MIX (93.33%, 100%, and 100%, respectively), which were consistent with the results obtained by the previous authors [26], who obtained 92.43 %, 94.44 %, and 94.71 %, respectively. Our findings confirmed the findings of Fathi et al. [25], who reported 94.44% and 79.6%, respectively, and the others [38], who reported 94.0%, 84.9%, 94.71%, and 91.0%, respectively, for MIX.

In the current work, the results showed that the higher recovery rate in the OPS method might be due to the low volume of vitrification solution used to preserve COCS. Also, the probability of oocyte loss increased with the increase in vitrification medium volume and

time, as was the case with the 0.25-mL French mini straws used in the conventional straw method [38, 39].

Oocyte loss during vitrification and warming procedures has been reported in different species, including buffaloes [40], goats [41], sheep [42, 43, and 44], and mice [45].

Nearly all studies pointed to the loss of oocytes following straws, or OPS vitrification could be attributed to sticking of oocytes on the inner wall of the straws, adherence to cracks or rough surfaces, or damage of oocytes during the vitrification/warming process [46,47]. Moreover, this loss could occur due to osmotic injuries and/or due to the sticking of oocytes to the pipette or caryo-carrier [48].

As reported in this study, the percentage of cumulus layer viability was higher in the control group than in the DMSO, MIX, and EG groups with detected values of **88.42%,** 74.00%, 68.00%, and 62.00%, respectively. These results were compatible with the previous authors [23] who reported in the dromedary camel (90.5%, 72.60%, 82.90%, and 83.70%, respectively) when using 30% CPA in VSs.

The viability in our study showed no significant difference between all treatments, EG, DMSO, and MIX (62%, 74%, and 68 %, respectively), also referred that DMSO has the higher viability rate. In the same trend, the previous authors [49] reported that the viable immature bovine oocyte ratio after vitrification was higher in DMSO treatment than in EG treatment (79.7% and 64.2%, respectively). The post-thaw survival rate in the DMSO group was higher than in the MIX and EG groups (74%, 68 %, and 62%, respectively), which differed from the results reported by the previous authors as 62.79%, 90.16%, and 86.11%, respectively [26], while the mixed group had higher values than EG (68% and 62%). This finding confirms the results of the previous authors [26]. Earlier studies declared that DMSO was more effective than EG or PROH for the slow freezing of immature buffalo oocytes [50]. Also, the present data reflects that the DMSO group showed higher results than both the MIX and EG groups. Meanwhile, the previous authors noted that the MIX group was higher than EG and DMSO [21].

The results obtained from this study indicate that there was no significant difference ($P \leq 0.05$) between the EG group, DMSO group, and MIX group concerning the after-warming morphologically damaged COCs. These results were compatible with those reported by the previous authors [23, 26]. The percentage of damaged oocytes was higher in the EG, DMSO, and MIX groups than in the previous authors' [22,23, and 26] camels, [32,51] bovine, and [39] ovines. Furthermore, the after-warming morphologically damaged COCs in DMSO were higher than in the MIX and EG groups, inconsistent with previous findings [23,26, and 32].

It is noteworthy that the damage of oocytes during cryopreservation could be attributed to the sizeable lipid-like material found in the oocytes of many species **[52, 53].** Also, homogeneous lipid droplets were observed in fresh nonvitrified GV, while irregular nonhomogeneous lipid droplets surrounding large vacuoles were observed after vitrification. To the best of our knowledge, there is no available information about the ultrastructure evaluation of vitrified immature camel oocytes. Nonvitrified immature camel oocytes, on the other hand, exhibited typical structures previously described in camel [54] and bovine [55]**.** As shown in the previous observations [56], the most significant difference observed among the species is the larger number of lipid droplets in the camel ooplasm.

In the present investigations, we recorded several morphological abnormalities of camel oocytes in immature stages. These included abnormal oocyte shape, crack of zona pullecida, shrinkage of ooplasm, leaking of ooplasm, partial loss of cumulus-oocyte complex, and complete partial loss of cumulus-oocyte complex.

The leakage of cellular content in DMSO was higher than EG (13.33% and 0.00%), which was controversial with the previous

observations [32] for the same media constituents (26.67% and 26.67%). Leakage of cellular content in EG (0.00%) was similar to that reported by the previous authors in bovine oocytes [51].

The present results demonstrated no significant difference between the EG, DMSO, and MIX groups cracks in the zona pellucida, which is compatible with previous authors' claims [23]. At the same time**,** it differs from that reported by the other authors [26], where the DMSO group was higher than the EG and mix groups (13.33 %, 8.27 %, and 4.11 %, respectively). However, the mixed group has the lowest value in both results.

There was no significant difference in cytoplasm shrinkage between all groups (**Fig. 4–C**), which differs from the previous authors' findings, where EG has the lower value [23].

Our results in the present study concerning shrinkage of ooplasm were higher than the previous observations [23, 26, and 32] for EG and DMSO groups but lower than the others [22, 57] in the MIX group.

The partial cumulus loss for the EG and DMSO groups was significantly higher than the mixed group (29.03%, 26.67%, and 16.67%, respectively). Thus, no significant difference between the EG and DMSO groups was observed (29.03% and 26.67%, respectively); this result was consistent with that reported by the previous authors as (45.8 %, 45.9%, and 37.04%) for EG, DMSO, and MIX, respectively [23].

The change in shape (**Fig.4-F**) showed no significant difference between all groups, EG, DMSO, and MIX (**Table 4 and Fig. 3**), which was suitable with the previous authors, who reported no significant difference between EG, DMSO, [23].

These results may be explained by the fact that immature oocytes at the germinal vesicle stage are more sensitive to cry injuries due to their low membrane stability and the susceptibility of their cytoskeleton **[38, 58,** and **59].** However, the efficiency of oocyte cryopreservation depends on different factors, including cryoprotectant type, cryopreservation method, and cooling and thawing rates, each of which may be responsible for oocyte cryo-damage [60, 61].

The chief problem associated with oocyte cryopreservation is the low percentage of oocytes retaining the ability to undergo normal maturation and fertilization [26, 62]**.** Still, the fresh or frozen testicular spermatozoa have similar results in ICSI treatments [5]. Both vitrification and cryoprotectant exposure severely impaired oocyte functional ability [63, 64]. Therefore, research on different cryoprotectants and combinations thereof is essential for effective vitrification. Specific concentrations of CPA solutions may be toxic to the oocytes [65, 66, and 67].

In the current study, the results showed that the EG group, DMSO group, and MIX group in after warming expansion rate as compared with control were (48.61%, 56.25%, 68.75 %, and 78.24%, respectively) where control was higher than all vitrified groups regardless of the type or concentration of the cryoprotectant. This result was compatible with the previous observations in Bovine as a result of using vitrification solutions with 10%, 20%, and 40% of EG and control, respectively [46]. In the same trend, this result was in agreement with that which had been reported by Al-Soudy et al. in dromedary camels as an expansion of vitrified oocytes and control were 85.3% and 88%, respectively [22]. Also, in the dromedary camel [21], the expansion rate was 37.50% and 54.55% for the treated group compared with the control, respectively. Additionally, the bovine oocytes' in vitro maturation rate following vitrification in glass capillary micropipette (GCM) was 40%, while the non-vitrified/ control was 61.29% [68].

Vitrification of immature oocytes has also resulted in lower maturation rates in other species, including cattle [69], buffaloes [46,70], goats [71], sheep [42], horses [72], cats [73], mice [45] and humans [74, 75].

The average extrusion rate of the first polar body was significantly reduced in the vitrified

immature oocytes in EG, DMSO, and MIX compared to the control group. These results are compatible with those reported by the previous authors [22,32, and 46]. There was no significant difference between the MIX and EG groups (6.25 % and 5.56 %, respectively), which differs from the previous observations, where DMSO had an allowable value compared to EG and MIX [26, 32**].**

The dimethyl sulfoxide (DMSO) group has the highest extrusion value of the first polar body compared with the EG and MIX groups (11.25%, 5.56%, and 6.25%, respectively). However, all vitrified groups were significantly lower than the unvitrified control group (22.35%). In the dromedary camel, cryopreservation significantly reduced the extrusion of the first polar body to 10.6 percent using a 1:1 EG and DMSO mixture compared to 34.1% in the control group [22]. Also, our results based on the extrusion of the first polar body are in good agreement with those reported by the previous authors [11]. They found that maturation rates of vitrified-immature bovine oocytes with DMSO and EG were lower than those of the control group (13.3% vs. 74.7%, respectively).

The cumulus cells might impair the invasion of cryoprotectant agents (CPAs) into the oocyte, leading to an inappropriate intracellular CPA concentration, as the efflux of water from oocytes occurs quickly, within 20 s. Exposing oocytes to CPAs during vitrification induces osmotic volume changes due to the migration of H2O and CPAs [76]**.** So, the low maturation rate in this study may be due to the high percentage of partial and complete loss of cumulus-oocyte complexes.

5. CONCLUSION

The findings of this study showed that dromedary camel oocytes could be successfully cryopreserved and continued to retain their ability to undergo in vitro maturation after warming using a vitrification protocol that included EG, DMSO, or a combination of both.

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تأثير عوامل الحماية من التجمد على تزجيج بويضات الإبل

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الملخص

استهدفت الدراسة الحاليه تقييم تأثير الإيثيلين جلايكول (EG) , وثنائي ميثيل سلفوكسيد (DMSO) ، او المزيج بينهما على تزجيج بويضات الإبل غير الناضجة وتقييم قدرتهم على الإنضاج بعد الإساله. تم تجميع البويضات الركامية (COCs) بعد جمع المبايض من المسلخ وتم تقسيمها إلى أربع مجموعات تجريبية. وهي المجموعة الضابطة ، ومجموعة EG عولجت بمحلول التزجيج 30٪ إيثيلين جلايكول (VS) ، ومجموعة DMSO عولجت بثنائي ميثيل سلفوكسيد (30٪)، ومجموعة MIX عولجت بمزيج من 15%EG +15% DMSO. وفي كل مجموعة تزجيج ، تمت معالجة استرداد مجمعات البويضات الركامية (COCs بمحلول موازنة (ES) لمدة دقيقتين. ثم تم نقلها إلى محلول التزجيج بعامل حماية بنسبة 30٪ (EG، DMSO ، أو MIX) ، و 20٪ FBS ، و 0.5 مولار سكروز في وسط زراعة الأنسجة 199 (TCM-199). ثم تم تحميل البويضات في الماصة المسحوبة ثم غمرها مباشرة في النيتروجين السائل (LN2) لمدة ساعة واحدة. و تمت إذابة تجميد البويضات وتدفئتها في 4 محاليل تدفئة (WS) بتركيزات مختلفة من السكروز على فترات بينيه 2.5 دقيقة عند 37 درجة مئوية. أظهرت النتائج أن معدل نضج البويضات من حيث التمدد أو خروج الجسم القطبي كان أعلى بشكل ملحوظ في المجموعة الضابطة من مجموعة MIX و DMSO و EG. بينما ، لم يكن هناك فرق كبير في معدل النضج بين مجموعة MIX ومجموعة EG. لكن معدل الاسترداد بعد التزجيج لمجموعات DMSO و MIX كان أعلى بكثير من مجموعة EG. ولم يظهر فرق معنوي بين مجموعة EG ومجموعة DMSO ومجموعة MIX فى معدل الحيويه والمظهر المورفولوجي فى خلايا COC. في الختام ، أوضحت الدراسة أن بويضات الإبل يمكن حفظها بالتبريد عن طريق التزجيج فىOPS باستخدام EG و DMSO ومزيجهم كمواد واقية من التجمد. وقد حسنت مجموعه MIX معدلات نضج البويضات ، وقد سجلت المعالجه باستخدام DMSO معدل استرداد وحيوية أفضل من مجموعتي EG و MIX.