Impact of Various Serum Concentrations and Cryoprotectants on The *in vitro* Maturation of Vitrified Dromedary Camel Oocytes

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ABSTRACT

This study aimed to investigate the effects of two different doses (10% and 20%) of fetal bovine serum (FBS) with various cryo-protectant additives (CPAs; Ethylene glycol (EG) and Dimethyl sulfoxide (DEMSO)) on the maturation rate of dromedary camel oocytes. A total number of 2515 cumulus-oocyte complexes (COCs) were divided into seven experimental groups. As a control group (G1), the first group was rinsed with an *in vitro* maturation (*IVM*) medium and allowed to incubate for 30 hours. The remaining COCs were divided into six groups and treated with varying concentrations of vitrification solution (VS). First, COCs were transferred to an equilibration solution (ES) for two minutes. Afterward, the experimental groups were given 45 seconds in various VSs. Five COCs were put into 1-2 µl VS and loaded into open-pulled straws (OPS). The straws were immediately submerged in LN_2 (-196 ^{o}C) for one hour. Following warming, the percentage of morphologically damaged oocytes revealed that G2 had the highest percentage (67.39%) and G6 had the lowest percentage (23.46%). The G1–G7 groups had a maturation rate of 78.24% 30.49%, 48.61%, 39.33%, 56.25%, 50.36%, and 68.75%, respectively, due to the expansion of cumulus cells. For the G1–G7 groups, the polar body extrusion results were 22.35%, 0%, 5.56%, 0%, 11.25%, 8.63%, and 6.25%, in that order. In conclusion, 20% FBS and DMSO as a single combination (G5) or combined with EG (G7) were the best methods for vitrifying dromedary camel oocytes.

Keywords: Vitrification, oocyte, dromedary, *IVM*, cryoprotectants.

INTRODUCTION:

The versatile and indispensable dromedary camel (Camelus dromedarius) is a kind of livestock that is especially well-suited to challenging climates. It is capable of yielding wool, milk, and meat. In addition, it serves as a means of transportation as well as a venue for competition, celebration, and entertainment (Sharma *et al.*, 2018). That being said, most people consider camel reproduction to be poor (El-Hassanein, 2017; Skidmore, 2013). When it comes to integrating assisted reproductive technologies (ARTs) into the standard breeding system, most farm animals have made significant progress. Therefore, it is likely that camelids are the last large domestic species to benefit from these technologies (Agag *et al*., 2021; Hemeida, 2014; Shahin *et al.*, 2021). Genetic material can be transferred between populations without requiring the transportation of animals thanks to artificial insemination (AI), *in vitro* fertilization (*IVF*), embryo transfer (ET), and cryopreservation of gametes (Ferré *et al*., 2020; Howard *et al*., 2016; Loskutoff, 2003; Sudano *et al.*, 2012). Additionally, they remove physical barriers to

reproduction, get rid of behavioral incompatibilities, and lessen the chance of disease transmission. The damage or sensitivity brought on by cooling and the toxicity of cryoprotectants, which seriously harm oocyte morphology and function, are the root causes of many problems with oocyte cryopreservation (Succu *et al.*, 2007; Tharasanit and Thuwanut, 2021). Because oocytes have a different lipid content than embryos (oocytes have less polyunsaturated fatty acids than embryos), oocyte cryopreservation is more technically difficult than cryopreservation of sperm and embryos (Moawad *et al.*, 2019). According to Dias *et al.* (2022), the main issue with oocyte cryopreservation is the limited proportion of oocytes that can still go through normal maturation and fertilization. Both vitrification and cryoprotectant treatment had a significant negative impact on the functional ability of oocytes (Chandra and Sharma, 2020; Sharma *et al.*, 2006). Because some concentrations of CPA solution may be harmful to the oocytes, the study of various cryoprotectants and their combinations is, therefore, crucial for successful vitrification (Dujíčková *et al.*, 2021). According to several published data, immature oocytes degenerate more quickly than mature oocytes since they still need to mature after warming up before being fertilized (Cocchia *et al.*, 2010; Sowińska *et al.*, 2020). Following cryopreservation, oocytes exhibiting reduced developmental competence could be linked to damage to the meiotic machinery, such as spindle breakage and microtubule loss, in addition to other ultrastructural irregularities (Fernández-Reyez *et al.*, 2012). Furthermore, cytosolic lipids are essential for the growth and maturation of oocytes (Huang *et al.*, 2019), but because they make freezing more sensitive, they are thought to be the largest barrier to cryopreservation. As a result, the recommended method for cryopreservation is now vitrification.

Therefore, the purpose of this study was to determine how different cryo-protectant additives (CPAs) combined with two different doses of fetal bovine serum (FBS) (10% and 20%) affected the post-thawing maturation rate of vitrified dromedary camel oocytes.

MATERIALS AND METHODS

Chemicals and media components were acquired from Sigma-Aldrich: (St. Louis, MI, USA). unless specified otherwise. According to Russo *et al.* (2014), all media were made overnight from stock solutions of each component and sterilized before use by passing through a Millipore filter with a 0.22 µm diameter fitted on a 10-milliliter syringe. The Embryo Manipulation Unit (EMU), Desert Research Centre (DRC) in Cairo, Egypt, has been the site of all laboratory activity.

1. Biological Material

This work was conducted during camel breeding season from February to March. More than 200 dromedary camel ovaries with unknown reproductive history were gathered from the nearby abattoir in El-Bassatine, Cairo, Egypt. Samples were transferred from a nearby slaughterhouse to the laboratory in a thermos flask filled with sterilized pre-warm physiological saline solution (SS, 0.9% NaCl) at 35 to 37 °C (Ashour *et al.*, 2021). The solution was supplemented with antibiotic antimycotic (AA: 100 IU penicillin and 100 µg streptomycin/mL). The process took two to three hours.

1.1.Ovaries manipulation and oocyte retrieving

The camel ovaries were immediately washed three times with warm (30 °C) saline solution upon arrival at the EMU. Subsequently, all ovaries underwent a quick cleaning with 70% ethanol to eliminate any contamination from their surface. Finally, ovaries were washed with a 30 °C heated phosphate buffer saline (PBS) enhanced with antibiotics (100 µg streptomycin and 100 IU penicillin/ml) as described by Russo *et al.* (2014). After that, during the oocyte recovery process,

the ovaries were maintained in glass jars with PBS and kept in a water bath at an adjusted temperature of 30 °C. According to Ashour *et al.* (2021), the COCs were collected from ovaries in a 90 mm petri dish using the slicing method and then rinsed with warm (30 °C) PBS supplemented with 50 µg/mL gentamicin.

The COCs were evaluated using the standards outlined by Nowshari and Wernery (2003) using a stereomicroscope (GX microscope, UK, Range: 8x to 50x). The three different oocyte grades were depicted as follows: Gradations I and II of the COCs consisted of more than three layers of compact cumulus cells with homogenous ooplasm, while grade III COCs were denuded or had irregular cytoplasm. Only grade I and II oocytes were used in this investigation. Oocytes were washed, graded, and chosen using the washing medium (WM) which consisted of TCM-199 medium supplemented with 25 mM HEPES and 10% FBS. The oocytes were cleaned three times before IVM or vitrification using the wash media.

2. Experimental Design

The purpose of this experiment was to examine the effects of two different types of cryoprotectants (EG & DMSO) and two different amounts of FBS (10 and 20%) on dromedary camel oocyte vitrification. The COCs (total number of 2515) were randomly distributed into seven groups, which were as follows:

G1: As a control group, 170 COCs were rinsed, put into *in vitro* maturation (IVM) media, and incubated for 30 hours without being vitrified.

Regarding G2 – G7, after washing, the COCs (G2: 362; G3: 364; G4: 388; G5: 350; G6: 501; G7: 380) were moved for two minutes to the equilibration solution (ES), which consisted of 50% of the vitrification solution. The COCs were kept for forty-five seconds in vitrification solution (VS), which in turn differed according to the experimental groups as follows:

G2: TCM-199 + 10% FBS + EG 30% (v/v) + 0.5 M sucrose.
G3: TCM-199 + 20% FBS + EG 30% (v/v) + 0.5 M sucrose.
G4: TCM-199 + 10% FBS + DMSO 30% (v/v) + 0.5 M sucrose.
G5: TCM-199 + 20% FBS + DMSO 30% (v/v) + 0.5 M sucrose.
G6: TCM-199 + 10% FBS + DMSO 15% (v/v) + EG 15% (v/v) 0.5 M sucrose.
G7: TCM-199 + 20% FBS + 15% (v/v) DMSO + 15% (v/v) EG + 0.5 M sucrose.

3. Oocytes Vitrification

Two stages were taken to achieve the vitrification of COCs (Madboly and El-Sayed, 2017). COCs were first equilibrated in ES (50% VS) for two minutes (El-Shahat and Hammam, 2014). Subsequently, COCs were treated with 10 or 20% v/v FBS, 30% CPAs, and 0.5 M sucrose added to TCM-199 medium as VSs for 45 seconds, according to the experimental group. A set of five COCs was loaded as per the guidelines in the prior study (Yassin *et al.*, 2022) using 1-2 µL VS in open-pulled straw (OPS). Right after the loading of oocytes, the straws were submerged in LN_2 (-196 °C) for one hour (Al-Soudy *et al.*, 2016).

4. Oocytes Warming

Following an hour in LN_2, the oocytes were thawed in four successive warming solutions (WS) containing varying amounts of sucrose. These WS, namely WS1, WS2, WS3, and WS4 were composed of TCM-199 and supplemented with 0.5 M, 0.25 M, 0.125 M, and 0 M sucrose, respectively. The COCs were translocated between these WSs at intervals of 2.5 minutes at 37 °C (Yoon *et al.*, 2003).

5. Oocyte Evaluation

The oocytes were next inspected with an inverted phase-contrast microscope (Leitz Fluovert FU; Leica Microsystems, Wetzlar, Germany) to check for any morphological damage (Fig. 1). The viability of vitrified/warmed COCs had been assessed (Fig. 2), according to Gupta *et al.* (2002). Briefly, trypan blue powder was dissolved in BM (HEPES in TCM-199 +10% FBS) at pH 7.0 to create a trypan blue solution (0.4%). One drop of trypan blue (0.4%) was combined with one drop of the basic media in equal volumes. Oocyte staining was done at room temperature for five minutes. Using the same inverted phase-contrast microscope, the oocytes were transfused into a 50 µL drop of TCM 199 supplemented with 20% v/v FBS and inspected. The dead oocytes looked like a blue stain, and the viable oocytes remained unstained (Gupta *et al.*, 2002).

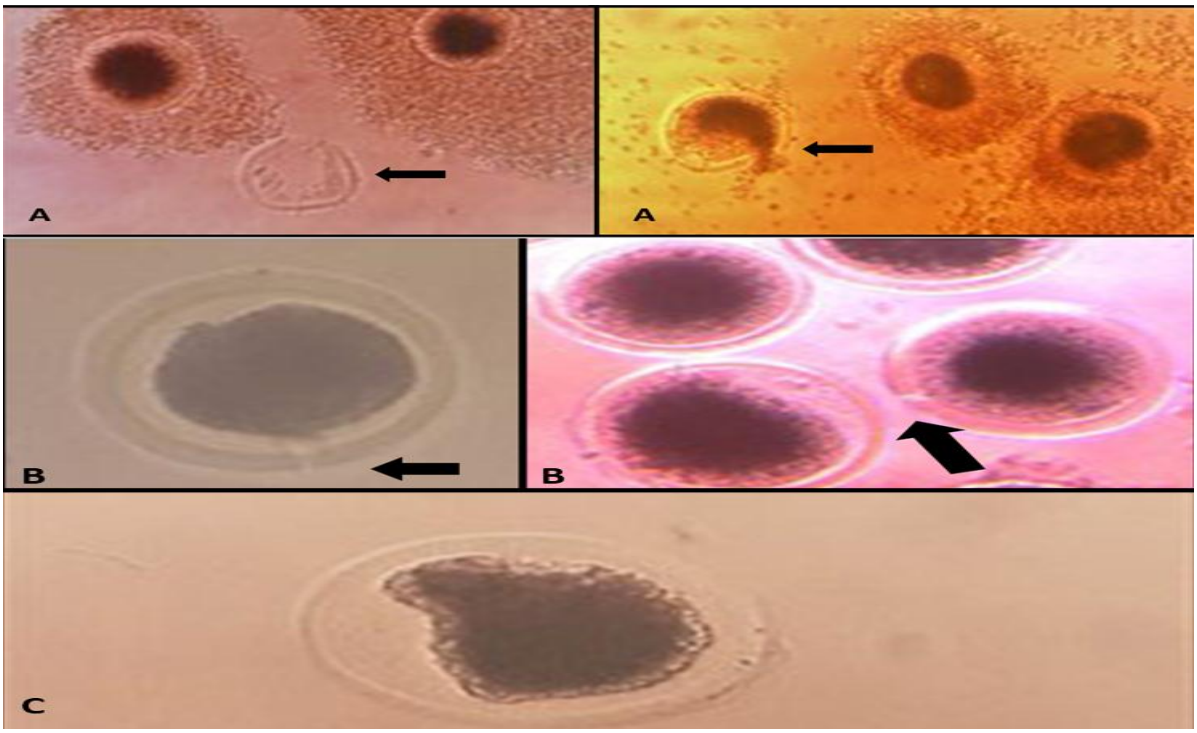

Fig. 1. Some of the post-warming morphologically damaged oocytes. **(A)**. Leak of cytoplasm. **(B)**. Crack in zona pellucida. **(C)**. Shrinkage of cytoplasm.

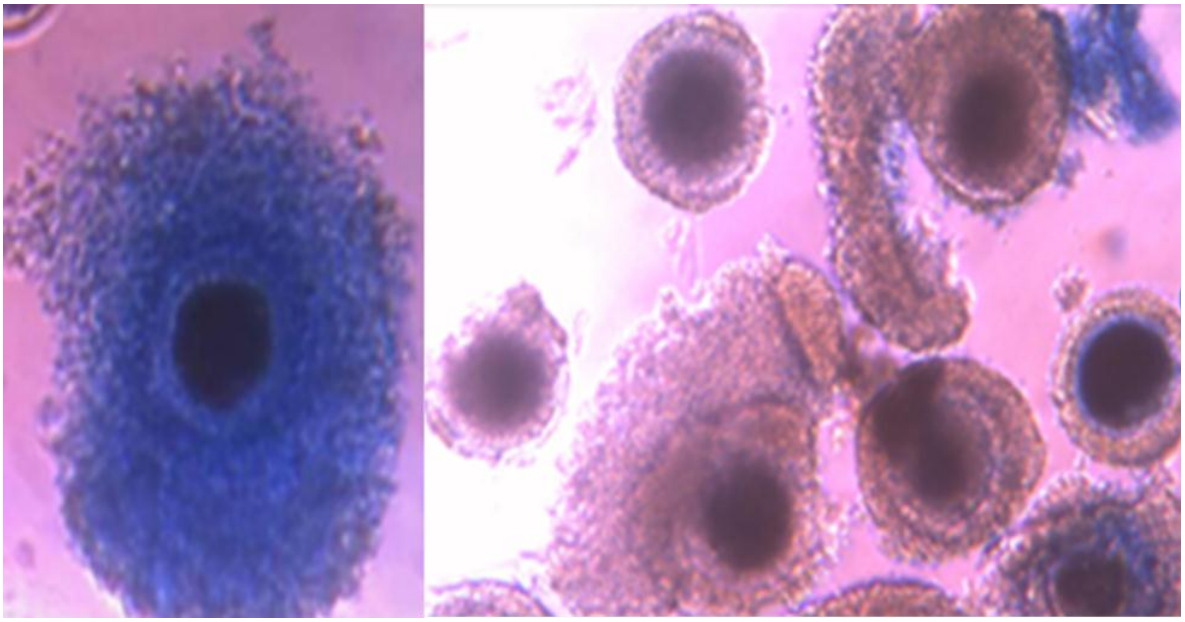

Fig. 2. Viability of vitrified/warmed dromedary camel's COCs assessed by trypan blue (Stained: dead oocyte. Unstained: viable oocyte.)

6. Oocytes Maturation:

According to El-Shahat and Hammam (2014), the oocytes underwent three washings in WM. Afterward, they were washed once in IVM medium (TCM-199) that was supplemented with 15% (v/v) heat-treated (56 °C for 30 min), fetal bovine serum (FBS), 20 ng/mL epidermal growth factor (EGF), 40 IU/mL PMSG, 0.25 mg/mL Na+ pyruvate, 1 µg/mL estradiol (E_2), and 100 µg/mL ascorbic acid (Khattab *et al.*, 2020). Before use, the medium was brought to a pH of 7.4, filtered, and incubated for at least two hours at 38.5 °C with 5% CO_2 and 95% relative humidity. The oocytes were cultured in drops of maturation media (10 to 15 oocytes/100 µL drop) in a CO_2 incubator for 30 hours at the prior conditions. The COCs expansion (Fig. 3) was evaluated by a stereomicroscope (UK, GX microscope: 8x–50x). For the polar body (PB) evaluation, COCs were denuded by gentle pipetting with 80 IU/mL of hyaluronidase (Moura *et al.*, 2017) in FertiCult flushing medium (Fertipro©, Beernerm, Belgium) before being washed twice in WM and examined under the inverted microscope (Amer and Moosa, 2009).

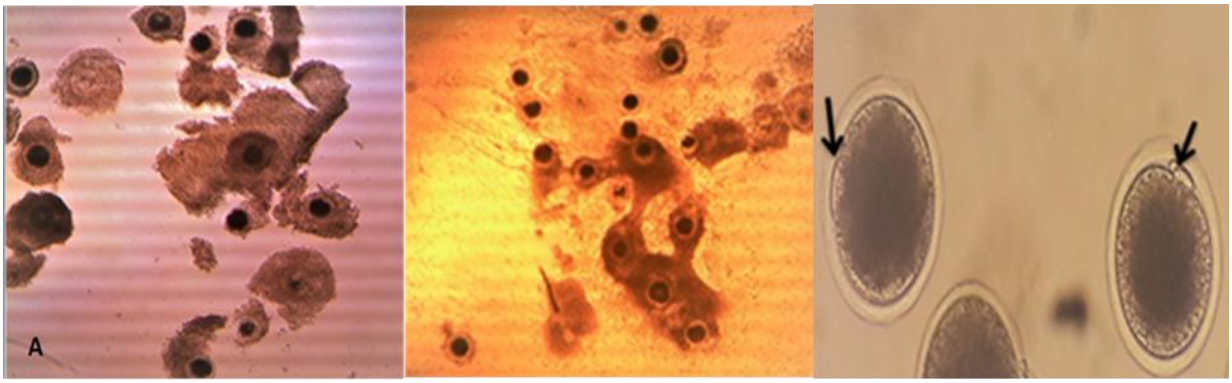

Fig. 3. Maturation of dromedary camel's oocytes. (**A**) immature oocytes. (**B**) Expanded oocytes. (**C**) Black arrows point to the first polar body.

7. Statistical Analysis

The Chi-square test was used in the SAS software (2004) to statistically assess the data from this study for post-thawing recovery rate, viability, morphological damage, and maturation rate (owing to expansion and polar body).

RESULTS

The purpose of this experiment was to compare the effects of adding 10% or 20% FBS with various CPAs (EG, DMSO, and a mix of both 50% EG + 50% DMSO) on the after-warming parameters: recovery rate, viability, and maturation rates (as determined by the expansion of cumulus cells and polar body extrusion) of camel oocytes vitrified at the GV stage, as well as the morphological damage to COCs as shown in Table 1. Following warming, the recovery rates of COCs were 93.18%, 93.75%, 89%, 100%, 87%, and 100% for G2, G3, G4, G5, G6, and G7, in that order. According to these findings, there was no significant difference between 10% and 20% of FBS in the EG groups (G2 (93.18%) and G3 (93.75%), respectively). However, in the DMSO groups (G4 89% and G5 100%), a highly significant difference was observed between these groups. In the same trend, MIX groups (G6 87% and G7 100%) showed a highly significant difference. It is worth noting that there was no significant difference between G5 and G7.

The viability rate of COCs after warming was 32%, 62%, 44%, 74%, 52%, and 68% for G2, G3, G4, G5, G6, and G7, respectively. These results showed that the highest viability rate of COCs after warming was 74% in the G5 group treated with DMSO and 20% FBS. However, the lowest oocyte viability rate was recorded when EG and 10% FBS were used in G2.

The percentage of observed morphological damage in oocytes after warming was 67.39%, 39.29%, 50.51%, 50%, 23.46%, and 40% for G2, G3, G4, G5, G6, and G7, respectively. This data showed that the highest percentage of oocyte damage occurred in G2, and the lowest percentage of damage occurred in G6.

Table (1): Effect of 10% and 20% of fetal bovine serum (FBS) supplementation on post-warming recovery rate, viability rate, and morphological damage rate of camel oocytes vitrified at GV stage

CPAs	FBS con.	Group	Recovery rate%	Viability%	Morphologically damaged %
EG	10%	G2	93.18[a] (82/88)	32[b] (32/100)	67.39[a] (62/92)
	20%	G3	93.75[a] (75/80)	62[a] (62/100)	39.29[b] (44/112)
DMSO	10%	G4	89[b] (89/100)	44[b] (44/100)	50.51[a] (50/99)
	20%	G5	100[a] (80/80)	74[a] (74/100)	50[a] (45/90)
MIX	10%	G6	87[b] (87/100)	52[b] (52/100)	23.46[b] (38/162)
	20%	G7	100[a] (80/80)	68[a] (68/100)	40[a] (48/120)

The letters a, b, and c differ significantly within the same column for each CPA. EG: Ethelene glycol 30%. DMSO: Dimethyl sulfoxide 30%. MIX: Ethelene glycol 15% + Dimethyl sulfoxide 15%.

The maturation rate due to the expansion of cumulus cells (Table 2) in the control group (G1) was significantly higher than the EG (G2 and G3), DMSO (G4 and G5), and MIX (G6 and G7) groups (78.24% vs. 30.49%, 48.61%, 39.33%, 56.25%, 50.36%, and 68.75%, respectively).

Table (2): Effect of 10% and 20% of fetal bovine serum (FBS) supplementation on post-warming maturation rate of camel oocytes vitrified at GV stage

CPAs	FBS con. In vitrification solution	Group	Total number of COCs	Expansion rate%	First polar body rate%
Control		G1	170	78.24 (133/170)	22.35 (38/170)
EG	10%	G2	82	30.49 (25/82)	0 (0/82)
	20%	G3	72	48.61 (35/72)	5.56 (4/72)
DMSO	10%	G4	89	39.33 (35/89)	0 (0/89)
	20%	G5	80	56.25 (45/80)	11.25 (9/80)
MIX	10%	G6	139	50.36 (70/139)	8.63 (12/139)
	20%	G7	80	68.75 (55/80)	6.25 (5/80)

The letters a, b, and c differ significantly within the same column for each CPA. EG: Ethelene glycol 30%. DMSO: Dimethyl sulfoxide 30%. MIX: Ethelene glycol 15% + Dimethyl sulfoxide 15%.

There was no significant difference among groups treated with vitrification G3, G4, G5, and G6. However, there was a significant difference between the G7 and G2 groups (50.36% and 30.49%, respectively). On the other hand, the results of maturation rate due to polar body extrusion (Table 2) were 22.35%, 0%, 5.56%, 0%, 11.25%, 8.63%, and 6.25% for G1, G2, G3, G4, G5, G6, and G7, respectively. These results indicated that there was a significant difference between the control group and all other groups. However, it is worth noting that G5 recorded the highest proportion of polar bodies (11.25%) among vitrified/warmed treatments.

DISCUSSION

The results of the current investigation demonstrated that there was no significant difference in the after-warming recovery rate with 10% FBS across all treatments (EG, DMSO, and MIX) (93.18%, 89%, and 87.42%, respectively). These outcomes agreed with those of Moawad *et al.* (2019), who found no significant difference between EG, DMSO, and MIX (92%5, 94.44%, and 94.71%, respectively). In addition, when EG is compared to DMSO and MIX with 20% FBS, it has the lowest after-warming recovery rate (93.33%, 100%, and 100%, respectively). This result was similar to the results that Moawad *et al.* (2019) published, which were 92.43%, 94.44%, and 94.71%. Additionally, the 20% FBS of DMSO and MIX groups exhibited comparable values (100% and 100%, respectively), which were in close agreement with the 94.44% and 94.71%, respectively, reported by Moawad *et al.* (2019). Nonetheless, the after-warming recovery rate of COCs in DMSO with 20% FBS was 100.0%, exceeding the findings (94.44%) of Moawad *et al.* (2019). Furthermore, the MIX group with 20% FBS surpassed the findings of Ali *et al.* (2014) in caprine**,** Fathi *et al.* (2018) and Moawad *et al.* (2019) in dromedary camel, and El-Shalofy (2016) in bovine (79.6%, 94%, 94.71%, and 73.46%, respectively).

Several species, including buffaloes (El-Shalofy *et al.*, 2017), goats (Begin *et al.*, 2003), sheep (Moawad *et al.*, 2013), and mice (Gomes *et al.*, 2008), have reported experiencing oocyte loss during vitrification and warming procedures. Nearly all studies pointed out that the loss of oocytes following straws or OPS vitrification could be ascribed to sticking oocytes on the straws' inner wall, adherence to cracks or rough surfaces, or damage to oocytes during the vitrification/warming process (Sharma and Loganathasamy, 2007). Additionally, osmotic damage may result in this loss (Gomes *et al.*, 2008). Also, the number of oocytes per cryo-device may be related to oocyte loss; as numbers rise, more time is needed for the movement of cells through the vitrification solutions. This causes cells to be exposed to chemical substances for an extended period of time, and it may lead to subsequent cell damage or an increase in the frequency of oocyte loss (El-Shalofy, 2016). During the cryopreservation process, oocytes are subjected to unfavorable physiological circumstances that result in various cytological damage to the oocytes (Men *et al.*, 2003). According to the current work, the addition of sucrose to the vitrification solutions (equilibration and vitrification solutions) could be the reason for the greater recovery rate. The vitrification solution's viscosity may be increased by adding sugars. By incubating cells in this solution prior to vitrification, more water is removed from the cells, and their exposure to the harmful effects of the cryoprotectants is minimized (Orief *et al.*, 2005). Furthermore, high CPA concentrations before cryopreservation increase viscosity, which raises the possibility of oocytes adhering to the pipette's or cryo-carrier's inner wall. When macromolecules like fetal bovine serum (FBS) are present, the temperature at which the solution vitrifies is changed (Shaw *et al.*, 1997). This lowers the amount of intracellular cryoprotectant needed to achieve vitrification (Kuleshova *et al.*, 2001), which consequently lowers the solution's toxicity. Furthermore, according to George *et al.* (1992), it stops the zona pellucida proteins from

changing into a state called zona hardening. Which is responsible for maintaining the conditions necessary for normal fertilization, following oocyte cryopreservation (Shirazi *et al.*, 2014).

The after-warming viability percentage of dromedary camel oocytes was significantly higher in the control group than EG, DMSO, and MIX supplemented with 20% FBS (88.24%, 62%, 74%, and 68 %, respectively). However, there was no significant difference between EG, DMSO, and MIX (62%, 74%, and 68 %, respectively). These results were incompatible with the results of Madboly and EL-Sayed (2017) for control, EG, DMSO, and MIX (90.5%, 83.7%, 72.6%, and 82.9%, respectively). The post-thaw viability rate in the EG group with 10% was (32%), which was lower than that reported by Yang *et al.* 2003 in bovine (64.2%) using VS consisting of 40% EG +0.5M Sucrose +10%FBS. This pattern was also lower than Moawad *et al.* (2019) in dromedary camel and Shirazi *et al.* (2014) in ovine. The post-thaw viability rate in the EG group with 20% was 62%; this was lower than that reported by Madboly and EL-Sayed (2017) and Moawad *et al.* (2019) in dromedary camels when using 30% and 40% EG, respectively. In alpaca-developed oocytes, Ruiz *et al.* (2013) found a similar trend (70.2%) when using 25% EG, whereas results were nearly identical (66.5% and 60.6%) when using 35% and 45% EG, respectively, with 20% FBS. Additionally, these outcomes closely matched those of Ruiz *et al.* (2013), who observed values of 56.5% and 63.5%, respectively, after vitrifying mature alpaca oocytes for 45 and 30 seconds using 30% EG. However, Wang *et al.* (2014) found that combining 30% EG with 20% FBS for 45 seconds had better outcomes 93.6%. While in camelids, EG has been effectively applied to the vitrification of alpaca oocytes and embryos (Ruiz *et al.*, 2013 and Vivanco-Mackie, 2013), llama (Aller *et al.*, 2002), and dromedary (Nowshari, 2005). There was no clear information available regarding its function in the vitrification of dromedary oocytes. However, in the DMSO group, the post-thaw survival rate was 74%, which is extremely similar to the 73.91% published by El-Badry *et al.* (2015) in dromedary camels using 20% EG + 20% DMSO and 20% FCS, and also very similar to the 72.6% reported by Madboly and EL-Sayed (2017) in dromedary camels using 30% DMSO and 20% FBS. According to our results, DMSO surpassed EG with 20% FBS (74% and 62%) and 10% FBS (44% and 32%). These results appeared to be lower than Yang *et al.* (2003), who used 10% FBS with EG, PROH, and DMSO to achieve 64.2%, 91.5, and 79.7% of viable bovine immature oocytes, respectively. Furthermore, Gautam *et al.* (2008) stated that for the gradual freezing of immature buffalo oocytes, DMSO was superior to EG or PROH. Furthermore, the data from the same study indicated that the DMSO group outperformed the MIX and EG groups in terms of results. In the meantime, El-Badry *et al.* (2015) pointed out that the MIX group was greater than EG and DMSO. When compared to DMSO and EG in the current investigation, the MIX group has the highest viability rates with both 10% and 20% FBS. These outcomes concur with the findings published by Moawad *et al.* (2019). The after-warming COC viability in the MIX group (68%) with 20% FBS was very close to that reported by Fathi *et al.* (2018) in dromedary camel and Shirazi *et al.* (2014) in goats' oocytes (68.2% and 72%, respectively) when using 40% mix with conventional straw and 30% mix with OPS, respectively. Conversely, the results in the MIX group were lower than those reported by Ali *et al.* (2014), Sharma and Purohit (2008), Moawad *et al.* (2019), Fathi *et al.* (2018), El-Badry *et al.* (2015), and Sofi *et al.* (2021) (90.4%, 85.6%, 90.16%, 78.7%, 90.54%, and 84.4%, respectively). Furthermore, in oocyte cryopreservation, 20% FBS concentration in VSs with all CPAs (EG, DMSO, and MIX) was more protective than 10% FBS; our findings were consistent with those published by Shirazi *et al.* (2014). Comparing our results to non-vitrified controls, we often found lower viability rates. According to earlier research, oocyte viability was considerably

reduced when alpaca *in vitro*-developed oocytes were vitrified using solid-surface vitrification (SSV) and EG-based vitrification solutions as opposed to non-vitrified controls (Ruiz *et al.*, 2013). Furthermore, the small volume of vitrification fluid employed in the OPS method, which provides faster cooling and warming rates than conventional straws, further justifies the method's greater survivability rate (Ali *et al.*, 2014). These broad restrictions are required, but for a variety of reasons, they might not be enough. Cold shock and other mechanisms unrelated to ice formation or CPA damage can cause harm to cells. Another explanation is that an intact plasma membrane with its typical, semipermeable characteristics serves as the primary reference point for defining cell viability limitations. Conditions that permit the plasma membrane to "survive" might not permit the "survival" of vital organelles inside cells, according to Gao and Critser (2000). Finally, variations in species and media composition could account for our study's low survivability rates. The current study generally agreed with Shirazi *et al.* (2014) in that the after-warming morphological damage in VSs with all CPAs, EG, DMSO, and MIX (39.29%, 50%, and 40%, respectively) was lower in 20% FBS concentration in VSs than in 10% FBS with all CPAs, EG, DMSO, and MIX (67.39 %, 50.51 %, and 23.46 %, respectively). These findings suggest that the presence of macromolecules like FBS modifies the vitrification temperature of the solution (Shaw *et al.*, 1997) and, as a result, lowers the amount of intracellular cryoprotectant needed to achieve vitrification (Kuleshova *et al.*, 2001), thereby lowering the solution's toxicity. It also maintains the conditions for normal fertilization after egg cryopreservation intact by preventing the zona pellucida proteins from changing into a state known as zona hardening (George *et al.*, 1992).

Additionally, the current investigation showed that, compared to the DMSO and MIX groups, the EG group with 10% FBS had substantially more morphological damage. These findings contrast with those of Madboly and El-Sayed (2017), who discovered that either PROH or DMSO significantly increased the number of morphologically aberrant oocyte outcomes compared to using either a combination of CPAs or EG. While there was no significant difference ($P \leq 0.05$) between the EG group, DMSO group, and MIX group with 20% FBS, these results contradict Moawad *et al.* (2019). With the same concentrations of CPAs (30%), FBS (20%), and OPS, our study's results were higher than those of Madboly and El-Sayed (2017) in the oocytes of dromedary camels, following the same general pattern. This discrepancy could result from Madboly and El-Sayed (2017) using a greater sugar concentration in their investigation. Although sucrose cannot cross a cell's membrane, it does aid in osmosis, which helps extract more water from cells and reduces the amount of time the cells are exposed to the harmful effects of the cryoprotectants. In addition, the non-permeating sucrose serves as an osmotic buffer to lessen the possibility of osmotic shock from the cryoprotectant's dilution following cryo-storage (Liebermann, 2012). This value was likewise greater than the results in dromedary camels reported by Moawad *et al.* (2019) and Al-Soudy *et al.* (2016), and it was higher than the results in bovines reported by Sharma *et al.* (2010) and El-Shahat and Hammam (2014). Additionally, it was greater than the values for ovine published by Ali *et al.* (2014) and Sofi *et al.* (2021). This discrepancy can result from employing various CPA concentrations and animal species. Additionally, EG with 20% FBS had fewer after-warming morphologically damaged COCs than DMSO, which tended to contradict the findings of Madboly and EL-Sayed (2017). Simultaneously, 10% FBS in EG groups had larger percentages than DMSO groups (67.39% and 50.51%, respectively). In comparison to MIX and EG, DMSO with 20% FBS was greater; this conclusion was in agreement with the results of Moawad *et al.* (2019). According to the current data, DMSO with 20% FBS was higher than EG (50% and 39.29%, respectively),

showing a general agreement with El-Shahat and Hammam (2014). However, EG tended to be greater than DMSO with 10% FBS. These findings clarify that EG responds more favorably to morphology with high FBS concentration.

Immature oocytes were usually treated as objects better suited for cryopreservation because they had fewer vulnerable structures, such as condensed chromosomes, and genetic material protected by a nuclear envelope (Prentice and Anzar, 2011). However, the general efficiency of their cryopreservation is relatively low (Papis *et al.*, 2013). Regardless of the kind of CPA or concentration of FBS used in the vitrification, the control group (G1) in this study had a significantly higher maturation rate due to the expansion of cumulus cells and the extrusion of the first polar body than the other groups (G2:G7). This result was in line with earlier findings in cattle (El-Shalofy, 2016). El-Badry *et al.* (2015) findings on the vitrification of immature camel oocytes were consistent with the maturation rate reduction in the vitrification groups relative to the control group. Vitrification of immature oocytes has also resulted in lower maturation rates in other species, including cattle (Kim *et al.*, 2007**)**, buffaloes **(**Wani *et al.*, 2004**)**, goats **(**Kharche *et al.*, 2005**),** sheep **(**Moawad *et al.*, 2012**),** buffalos (El-Shalofy, 2016), cats (Apparicio *et al.*, 2013), mice (Moawad *et al.*, 2014), humans (Shahedi *et al.*, 2013), and horses **(**Tharasanit *et al.*, 2006**)**. Owing to the lower membrane stability and susceptibility of the cytoskeleton of immature oocytes, they were more sensitive to chilling injury (Bogliolo *et al.*, 2007). As well as the greater osmotic sensitivity in the immature oocytes which may be due to their two-fold lower hydraulic conductivity than *in vitro* mature oocytes (Agca *et al.*, 2000). In contrast to 10% FBS, our results also showed that 20% FBS enhanced the expansion rate regardless of the kind of CPA, which is consistent with findings published by Shirazi *et al.* (2014). The expansion rate in the (G7) MIX group with 20% FBS was (68.75%), which was higher than Moawad *et al.* (2019) (58.95%), Fathi *et al.* (2018) (30.7% and 37.9%), El-Badry *et al.* (2015) (37.50%) in the dromedary camel, and El-Shalofy (2016) in Bovine (42.11%). Colombo *et al.* (2019) in cats (21.25%, 19.46%, 21.89, 47.44%, and 52.16%) used 15% EG + 15% DMSO and 20% FBS. At the same time, the expansion rate (50.35%) in the (G6) MIX group with 10% FBS was lower than the previously mentioned studies except for Colombo *et al.* (2019) in cats. Compared to the EG, DMSO, and MIX groups with 10% and 20% FBS, the average rate of extrusion of the first polar body in the control group was significantly higher. These outcomes were in line with those of El-Shalofy (2016), who used 20%, 40%, and 10% EG doses as well as CPA combinations (20% EG + 20% DMSO and 20% glycerol + 20% DMSO) with 20% FBS in Buffalo.

According to our findings, all treatments result in better polar body extrusion when 20% FBS is used as opposed to 10% FBS, which is consistent with the cleavage rate data published by Shirazi *et al.* (2014). This could be a result of the complex mixture of nutrients found in FBS, which includes growth factors, sugars, lipids, vitamins, trace elements, and hormones. These components are necessary for the development and maintenance of the majority of cultured cells and tissue types (van der Valk *et al.*, 2010; Verdanova *et al.*, 2014). Because FBS comprises proteins that give the plasma membrane mechanical protection and buffering qualities, it has been used to reduce the danger of ice melting, crystallization, or recrystallization during the freeze-thaw process (Garzón *et al.*, 2008; Peñaranda *et al.*, 2009). Also, FBS proved to be effective in elevating antioxidant transcript levels and reducing reactive oxygen species (ROS) in porcine embryos cultured *in vitro* (Mun *et al.*, 2017). Furthermore, as EG is more likely to diffuse out of the cell quickly than DMSO, which is less permeable, the combination of EG and DMSO is expected to lower both the osmotic damage at warming as well as the toxicity of each cryoprotectant (Taniguchi *et al.*, 2007). Al-Soudy (2016) observed a similar pattern, reporting

that cryopreservation dramatically decreased the extrusion of the first polar body to 10.6% from 34.1% in the control group. Furthermore, our results based on the extrusion of the first polar body were in agreement with those reported by Cetin and Bastan (2006). They discovered that the maturation rates of immature bovine oocytes that had been vitrified using DMSO and EG were lower than those of the control group (13.3% vs. 74.7%, respectively). According to Jin *et al.* (2011), because oocytes expel water rapidly (within 20 seconds) while CPA influx takes longer, cumulus cells may hinder the passage of CPAs into the oocyte, potentially resulting in an unsuitable intracellular CPA concentration. Exposing oocytes to CPAs during vitrification induces osmotic volume changes due to the migration of water and CPAs. Therefore, the low maturation rate in this study may be due to the high percent of partial and complete loss of cumulus-oocyte complexes. Furthermore, the disruption of specific cytoplasmic organelles that are essential for oocyte maturation can be the reason for the decreased maturation rates following vitrification and warming of GV-oocytes (Moawad *et al.*, 2014). The fact that the maturation rate is mostly dependent on the quality, quantity, and composition of the medium, as well as the incubation environment, may also be the cause of the disparity in the results (Dutta *et al.*, 2013). As well, it was noted that the freeze-thaw process is known to induce an alteration in the physicochemical properties of the intracellular lipids, and such damages may render the oocyte incapable of retaining its developmental competence. This means that the increased lipid content of dromedary camel oocytes could have a negative effect on the after-warming *IVM* results. In addition, as it has been noted that high lipid content in oocytes makes them more sensitive to chilling injury by cryopreservation, the higher lipid content present in oocytes may also be one of the factors responsible for decreasing maturation rates (Dutta *et al.*, 2013; Diez *et al.*, 2012).

CONCLUSION

In conclusion, this study showed that the vitrification of immature camel oocytes is still a challenge and needs further investigation. However, the combination of 20% FBS and 30% DMSO alone (G5) or mixed with EG (G7) was the best-applied protocol for the vitrification of immature dromedary camel oocytes.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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REFERENCES

Agag, M., Abdel Fattah, A., Nasef, M., El-Raey, M., El-Khawagah, A., and Karen, A. (2021). Factors affecting the pregnancy rates post embryo transfer in dromedary camels: Quality and number of recovered embryos. *Benha Vete. Med. J.*, 41(1), 194-197.

Agca, Y., Liu, J., Rutledge, J., Critser, E. and Critser, J. 2000. Effect of osmotic stress on the developmental competence of germinal vesicle and metaphase II stage bovine cumulus oocyte complexes and its relevance to cryopreservation. *Mol. Reprod. Dev.*, 55(2): 212-219.

Ali, R., Razzaque, W., Sharma, U., Pandey, A. and Hussain, K. 2014. Survivability of caprine oocytes vitrified in conventional and open pulled straws. *Turkish J. Vet. Anim. Sci.*, 38(6): 707-711

Aller, J., Rebuffi, G., Cancino, A. and Alberio, R. 2002. Successful transfer of vitrified Ilama (*Lama glama*) embryos. *Anim. Reprod. Sci.*, 73(2): 121-127.

Al-Soudy, A., El-Sayed, A., El-Itriby, H.A. and Hussein, E. H. 2016. Cryopreservation of immature and mature camel (*Camelus dromedarius*) oocytes by open pulled straw vitrification. *Arab J. Biotech.,* 19(2): 1-8.

Amer, H. and Moosa, A. 2009. Effect of season and culture media on the competence of dromedary camel oocyte to mature *in vitro*. *Glob. Vet.*, 3(1): 1-8.

Apparicio, M., Ruggeri, E. and Luvoni, G. 2013. Vitrification of immature feline oocytes with a commercial kit for bovine embryo vitrification. *Reprod Domest. Anim.*, 48(2): 240-244.

Ashour, G., Kamel, A. M., El-Bahrawy, K. A., El-Sayed, A. and Ghanem, N. 2021. Effect of ascorbic acid supplementation on *in vitro* production of camel embryos cultured under oxidative stress. *J. Camelid Sci.*, 14 (1): 35-42.

Begin, I., Bhatia, B., Baldassarre, H., Dinnyes, A. and Keefer, C. 2003. Cryopreservation of goat oocytes and *in vivo* derived 2-to 4-cell embryos using the cryoloop (CLV) and solid-surface vitrification (SSV) methods. *Theriogenology*, 59(8): 1839-1850.

Bogliolo, L., Ariu, F., Fois, S., Rosati, I., Zedda, M., Leoni, G. and Ledda, S. 2007. Morphological and biochemical analysis of immature ovine oocytes vitrified with or without cumulus cells. *Theriogenology*, 68(8): 1138-1149.

Cetin, Y. and Bastan, A. 2006. Cryopreservation of immature bovine oocytes by vitrification in straws. *Anim. Reprod. Sci*., 92(2): 29-36.

Chandra, V. and Sharma, G. T. 2020. *In vitro* strategies to enhance oocyte developmental competence. *Front. Biosci. (Schol Ed)*, 12(1): 116-136.

Cocchia, N., Ciani, F., Russo, M., El Rass, R., Rosapane, I., Avallone, L. and Lorizio, R. 2010. Immature cat oocyte vitrification in open pulled straws (OPSs) using a cryoprotectant mixture. *Cryobiology*, 60(2): 229-234.

Colombo, M., Morselli, M., Tavares, M., Apparicio, M. and Luvoni, G. 2019. Developmental competence of domestic cat vitrified oocytes in 3D enriched culture conditions. *Animals (Basel)*, 9(6): 329-333.

Dias, S. D. D. F., Palmeira-de-Oliveira, A., Rolo, J., Gomes-Ruivo, P., Oliani, A. H., Palmeira-de-Oliveira, R., Martinez-de-Oliveira, J. and Pinto-de-Andrade, L. 2022. Parameters influencing the maturation of bovine oocyte: a review. *Anim. Prod. Sci.*, 62(8): 751.

Diez, C., Munoz, M., Caamano, J. and Gomez, E. 2012. Cryopreservation of the bovine oocyte: current status and perspectives. *Reprod. Domest. Anim.*, 47: 76-83.

Dujíčková, L., Makarevich, A. V., Olexiková, L., Kubovičová, E., and Strejček, F. 2021. Methodological approaches for vitrification of bovine oocytes. *Zygote*, 29(1): 1-11.

Dutta, D., Dev, H. and Raj, H. 2013. *In vitro* blastocyst development of post-thaw vitrified bovine oocytes. *Vet. World.*, 6 (10):325-329.

El-Badry, D., Scholkamy, T. nd Darwish, G. 2015. Vitrification of dromedary camel oocytes: effect of maturation state, equilibration and vitrification time and type of cryoprotectant. *J. Egypt. Med. Assoc.*, 75(1): 7-17.

El-Hassanein, E. E. 2017. Prospects of improving semen collection and preservation from elite dromedary camel breeds. *World Vete. J.*, 7(2), 47-64.

El-Shahat, K. H. and Hammam A. M. 2014. Effect of different types of cryoprotectants on developmental capacity of vitrified-thawed immature buffalo oocytes. *Anim. Reprod.*, 11(4): 543-548.

El-Shalofy, A. 2016. Some trials for cryobanking of Egyptian buffalo oocytes. Ph, Thesis, Cairo University, faculty of Veterinary Medicine.p_p112-132.

El-Shalofy, A., Moawad, A., Darwish, G., Ismail, S., Badawy, A. and Badr, M. 2017. Effect of different vitrification solutions and cryodevices on viability and subsequent development of buffalo oocytes vitrified at the germinal vesicle (GV) stage. *Cryobiology*, 74: 86-92.

Fathi, M., Moawad, A. and Badr, M. 2018. Production of blastocysts following *in vitro* maturation and fertilization of dromedary camel oocytes vitrified at the germinal vesicle stage. *Plos One*, 13(3): 194602-194612.

Fernández-Reyez, F., Ducolomb, Y., Romo, S., Casas, E., Salazar, Z. and Betancourt, M. 2012. Viability, maturation and embryo development *in vitro* of immature porcine and ovine oocytes vitrified in different devices. *Cryobiology*, 64(3): 261-266.

Ferré, L. B., Kjelland, M. E., Taiyeb, A. M., Campos-Chillon, F., and Ross, P. J. 2020. Recent progress in bovine *in vitro*-derived embryo cryotolerance: Impact of *in vitro* culture systems, advances in cryopreservation and future considerations. *Reprod. Domest. Anim*., 55(6): 659-676.

Gao, D. and Critser, J. (2000). Mechanisms of cryoinjury in living cells. *ILAR J.*, 41(4): 187-196.

Garzón, D. L., Peñaranda, D. S., Pérez, L., Marco-Jiménez, F., Espert, X., Müller, T., Jover, M. and Asturiano, J. F. 2008. Effects of pH, sodium bicarbonate, cryoprotectants and foetal bovine serum on the cryopreservation of European eel sperm. *Reprod. Domest. Anim*., 43(1): 99-105.

Gautam, S., Verma, V., Singh, B., Palta, P., Singla, S., Chauhan, M. and Manik, R. 2008. Effect of slow freezing on morphology and developmental competence of buffalo (*Bubalus bubalis*) immature oocytes. *Anim. Reprod. Sci*., 105(3-4): 311-318.

George, M., Johnson, M. and Vincent, C. 1992. Use of fetal bovine serum to protect against zona hardening during preparation of mouse oocytes for cryopreservation. *Hum. Reprod*., 7(3): 408-412.

Gomes, C., Silva, C., Acevedo, N., Baracat, E., Serafini, P. and Smith, G. 2008. Influence of vitrification on mouse metaphase II oocyte spindle dynamics and chromatin alignment. *Fertil. Steril.*, 90(4): 1396-1404.

Gupta, P., Nandi, S., Ravindranatha, B., Raghu, H. and Sarma, P. 2002. Trypan blue staining to differentiate live and dead buffalo oocytes and its effect on embryo development *in vitro*. *Buffalo J.*, 18(3): 321-330.

Howard, J. G., Lynch, C., Santymire, R. M., Marinari, P. E., and Wildt, D. E. 2016. Recovery of gene diversity using long-term cryopreserved spermatozoa and artificial insemination in the endangered black-footed ferret. *Anim. Conserve.*, 19(2): 102-111.

Hemeida, N. 2014. Reproductive Technologies in Camelids. Review Article. *Vet. Med. J*., 60(1): 1-15.

Huang, Z., Gao, L., Hou, Y., Zhu, S. and Fu, X. 2019. Cryopreservation of farm animal gametes and embryos: recent updates and progress. *Front. Agric. Sci. Eng.*, 6(1): 42-53.

Jin, B., Kawai, Y., Hara, T., Takeda, S., Seki, S., Nakata, Y. and Edashige, K. 2011. Pathway for the movement of water and cryoprotectants in bovine oocytes and embryos. *Biol. Reprod*., 85(4), 834-847.

Kharche, S., Sharma, G. and Majumdar, A. 2005. *In vitro* maturation and fertilization of goat oocytes vitrified at the germinal vesicle stage. *Small Rumin. Res*., 57(1): 81-84.

Khattab, A. K, Abu Elnaga, N. A., Shedeed, H. A., Kamel, A. M., Abd Rabu, M. A. and El-Bahrawy, K. A. 2020. Antioxidant supplementation effects on *in vitro* maturation and fertilization of dromedary camel oocytes. *J. Camelid Sci.*, 13: 10-21.

Kim, D., Park, H., Kim, S., Hwang, I., Yang, B., Im, G. and Yang, B. 2007. Vitrification of immature bovine oocytes by the microdrop method. *J. Reprod. Develop*., 2:0704240080-0704240080.

Kuleshova, L., Shaw, J. and Trounson, A. 2001. Studies on replacing most of the penetrating cryoprotectant by polymers for embryo cryopreservation. *Cryobiology*, 43(1): 21-31.

Liebermann, J. 2012. Vitrification of Oocytes and Embryos. Current Frontiers in Cryobiology, pp. 213-574. DOI: 10.5772/35136.

Loskutoff, N. 2003. Role of embryo technologies in genetic management and conservation of wildlife. Conservation Biology Series 2: 183-194.

Madboly, M. M. and EL-Sayed E. 2017. Effect of cryoprotectants on the quality of dromedary camel oocytes with particular reference to its ultrastructure under vitrification technique. *Egyptian J. Anim. Prod.*, 54(3): 245-254.

Men, H., Monson, R., Parrish, J. J., and Rutledge, J. J. 2003. Degeneration of cryopreserved bovine oocytes via apoptosis during subsequent culture. *Cryobiology*, 47(1):73-81.

Moawad, A., Fisher, P., Zhu, J., Choi, I., Polgar, Z., Dinnyes, A. and Campbell, K. 2012. *In vitro* fertilization of ovine oocytes vitrified by solid surface vitrification at germinal vesicle stage. *Cryobiology*, 65(2): 139-144.

Moawad, A., Xu, B., Tan, S. and Taketo, T. 2014. L-carnitine supplementation during vitrification of mouse germinal vesicle stage–oocytes and their subsequent *in vitro* maturation improves meiotic spindle configuration and mitochondrial distribution in metaphase II oocytes. *Hum. Reprod*., 29(10): 2256-2268.

Moawad, A., Zhu, J., Choi, I., Amarnath, D., Chen, W. and Campbell, K. 2013. Production of good-quality blastocyst embryos following *IVF* of ovine oocytes vitrified at the germinal vesicle stage using a cryoloop. *Reprod. Fertil. Develop.*, 25(8): 1204-1215.

Moawad, M., Hussein, H., Abd El-Ghani, M., Darwish, G. and Badr, M. 2019. Effects of cryoprotectants and cryoprotectant combinations on viability and maturation rates of Camelus dromedarius oocytes vitrified at germinal vesicle stage. *Reprod. Domest. Anim.*, 54(1): 108-117.

Moura, B. R., Gurgel, M. C., Machado, S. P., Marques, P. A., Rolim, J. R., Lima, M. C. and Salgueiro, L.L. 2017. Low concentration of hyaluronidase for oocyte denudation can improve fertilization rates and embryo quality. *JBRA assist. reprod.*, 21(1): 27–30.

Mun, S. E., Sim, B. W., Yoon, S. B., Jeong, P. S., Yang, H. J., Choi, S. A., Park, Y. H., Kim, Y. H., Kang, P., Jeong, K. J., Lee, Y., Jin, Y. B., Song, B. S., Kim, J. S., Huh, J. W., Lee, S. R., Choo, Y. K., Kim, S. U. and Chang, K. T. 2017. Dual effect of fetal bovine serum on early development depends on stage-specific reactive oxygen species demands in pigs. *Plos One*, 12: e0175427.

Nowshari, M. A. 2005. The effect of harvesting technique on efficiency of oocyte collection and different maturation media on the nuclear maturation of oocytes in camels (*Camelus Dromedarius*). *Theriogenology*, 63(9): 2471-2481.

Nowshari, M. and Wernery, N. 2003. The effect of harvesting technique on efficiency of oocyte collection and their meiotic competence in dromedary camels. In 19th meeting AETE Rostock, pp. 12-13.

Orief, Y., Schultze-Mosgau, A., Dafopoulos, K. and Al-Hasani, S. 2005. Vitrification: will it replace the conventional gamete cryopreservation techniques. *Middle East Fertil. Soc. J.* 10(3): 171-184.

Papis, K., Shimizu, M., Saha, S., Izaike, Y. and Modlinski, J. 2013. Effects of vitrification of partially denuded bovine immature oocytes. *Anim. Sci. Pap. Rep.*, 31(1): 5-14.

Peñaranda, D. S., Pérez, L., Gallego, V., Jover, M. and Asturiano, J. F. 2009. Improvement of European eel sperm cryopreservation method by preventing spermatozoa movement activation caused by cryoprotectants. *Cryobiology*, 59: 119-126.

Prentice, J. and Anzar, M. 2011. Cryopreservation of mammalian oocyte for conservation of animal genetics. *Vet Med Int*., 146405.

Ruiz, J., Landeo, L., Mendoza, J., Artica, M., Correa, J., Silva, M. and Ratto, M. 2013. Vitrification of *in vitro* mature alpaca oocyte: effect of ethylene glycol concentration and time of exposure in the equilibration and vitrification solutions. *Anim. Reprod. Sci*., 143(1): 72-78.

Russo, R., Monaco, D., Rubessa, M., El-Bahrawy, K., El-Sayed, A., Martino, N. and Filioli Uranio, M. 2014. Confocal fluorescence assessment of bioenergy/redox status of dromedary camel (Camelus dromedarius) oocytes before and after *in vitro* maturation. *Reprod. Biol. Endocrinol.*, 12(1): 1-10.

Shahedi, A., Hosseini, A., Khalili, M., Norouzian, M., Salehi, M., Piriaei, A. and Nottola, S. 2013. The effect of vitrification on ultrastructure of human *in vitro* matured germinal vesicle oocytes. *Eur. J. Obstet. Gynecol. Reprod. Biol*., 167(1), 69-75.

Shahin, M. A., Khalil, W. A., Saadeldin, I. M., Swelum, A. A., and El-Harairy, M. A. 2021. Effects of vitamin C, vitamin E, selenium, zinc, or their nanoparticles on camel epididymal spermatozoa stored at 4 C. *Trop. Anim. Health. Prod.*, 53(86): 1-9.

Sharma, A. and Purohit, G. 2008. Vitrification of immature bubaline cumulus oocyte complexes by the open-pulled straw and conventional straw methods and their subsequent *in vitro* fertilization. Vet Med (Praha), 53(8): 427-433.

Sharma, G. and Loganathasamy, K. 2007. Effect of meiotic stages during *in vitro* maturation on the survival of vitrified-warmed buffalo oocytes. *Vet. Res. Commun.*, 31(7): 881-893.

Sharma, N. K., Rani, S., Dadhich, H., Mathur, M., and Kumar, S. 2018. Incidence and pathomorphology of myocardial lesions in camel in north-western Rajasthan, India. *Rumin Sci.*, 7(2):247-250.

Sharma, G., Kharche, S. and Majumdar, A. 2006. Vitrification of *in vitro* matured goat oocytes and the effect on *in vitro* fertilization. *Small Rumin. Res*., 64(2): 82-86.

Sharma, G., Duby, P. and Chndra, V. 2010. Morphological changes, DNA damage and developmental competence of *in vitro* matured, vitrified-thawed buffalo (Babalus bubalis) oocytes: A comparative study of two cryoprotectants and two cryodevices. *Cryobiology*, 60(3): 315-321.

Shaw, J., Kuleshova, L., Macfarlane, D. and Trounson, A. 1997. Vitrification properties of solutions of ethylene glycol in saline containing PVP, Ficoll, or dextran. *Cryobiology*, 35(3): 219-229.

Shirazi, A., Taheri, F., Nazari, H., Norbakhsh-Nia, M., Ahmadi, E. and Heidari, B. 2014. Developmental competence of ovine oocyte following vitrification: effect of oocyte developmental stage, cumulus cells, cytoskeleton stabiliser, FBS concentration, and equilibration time. *Zygote*, 22(2):165-173.

Skidmore, J. 2013. Key note presentation: Methods to Improve the Reproductive of Camel. In International Scientific Conference of Camel Research and Production (ISCCRP), Khartoum-Sudan, 17th-18th April.

Sofi, K., Qureshi, B., Shah, R. and Fazili, M. 2021. Post Thaw Quality and Viability of Vitrified Immature Sheep Oocytes Using Different Cryoprotectant Concentrations. *Cryo-Lett*., 42(6); 321-325.

Sowińska, N., Zahmel, J., Niżański, W., Hribal, R., Fernandez-Gonzalez, L. and Jewgenow, K. 2020. Meiotic status does not affect the vitrification effectiveness of domestic cat oocytes. *Animals*, 10(8):1371-1382.

Succu, S., Leoni, G., Bebbere, D., Berlinguer, F., Mossa, F., Bogliolo, L. and Naitana, S. 2007. Vitrification devices affect structural and molecular status of *in vitro* matured ovine oocytes. *Mol. Reprod. Develop*., 74(10): 1337-1344.

Sudano, M. J., Paschoal, D. M., Silva Rascado, T., Crocomo, L. F., Magalhães, L. C., Martins Junior, A., Machado, R. and Landim-Alvarenga, F. D. 2012. Crucial surviving aspects for vitrified *in vitro*-produced bovine embryos, *Zygote*, 11: 1–8.

Taniguchi, M., Ikeda, A., Arikawa, E., Wongsrikeao, P., Agung, B, Naoi, H. and Otoi, T. 2007. Effect of cryoprotectant composition on *in vitro* viability of *in vitro* fertilized and cloned bovine embryos following vitrification and in-straw dilution. *J. Reprod. Develop*., 2: 0704050073-0704050082.

Tharasanit, T., and Thuwanut, P. 2021. Oocyte cryopreservation in domestic animals and humans: principles, techniques and updated outcomes. *Animals*, 11(10): 2949.

Tharasanit, T., Colenbrander, B. and Stout, T. 2006. Effect of maturation stage at cryopreservation on post-thaw cytoskeleton quality and fertilizability of equine oocytes. *Mol Reprod Dev*., 73(5): 627-37.

van der Valk, J., Brunner, D., De Smet, K., Fex Svenningsen, A., Honegger, P., Knudsen, L. E., Lindl, T., Noraberg, J., Price, A., Scarino, M. L. and Gstraunthaler, G. 2010. Optimization of chemically defined cell-culture media – replacing fetal bovine serum in mammalian *in vitro* methods. *Toxicol. Vitro*, 24: 1053-1063.

Verdanova, M., Pyltik, R. and Kalbacova, M. H. 2014. Evaluation of sericin as a fetal bovine serum-replacing cryoprotectant during freezing of human mesenchymal stromal cells and human osteoblast-like cells. *Biopreserv. Biobanking*, 12 (2): 99-105.

Vivanco-Mackie, H. 2013. Strategies for superovulation, embryo production and transfer in sheep and alpacas. In Proc. 29th Annual Meeting AETE Istanbul, pp. 43-74.

Wang, Y., Okitsu, O., Zhao, X., Sun, Y., Di, W. and Chian, R. 2014. The effect of minimal concentration of ethylene glycol (EG) combined with polyvinylpyrrolidone (PVP) on mouse oocyte survival and subsequent embryonic development following vitrification. *J. Assist. Reprod. Genet.*, 31(1): 55-63.

Wani, N., Misra, A. and Maurya, S. 2004. Maturation rates of vitrified-thawed immature buffalo (Bubalus bubalis) oocytes: effect of different types of cryoprotectants. *Anim. Reprod. Sci*., 84(3):327-335.

Yang, B., Im, G., Chang, W., Lee, Y., Oh, S., Jin, D. and Lee, C. 2003. Survival and *in vitro* development of immature bovine oocytes cryopreserved by vitrification. *Asian-australas. J. Anim. Sci*., 16(1):23-28.

Yassin, H. B., Abu-Elnaga, N. A., Farrag B. and El-Bahrawy, K. A. 2022. Effect of cryoprotectants on camel oocytes vitrification. *Azhar Bull. Sci.*, Section C, 33(1): 1-14.

Yoon, T., Kim, T., Park, S., Hong, S., Ko, J., Chung, H. and Cha, K. 2003. Live births after vitrification of oocytes in a stimulated *in vitro* fertilization–embryo transfer program. *Fertil. Steril*., 79(6):1323-1326.

الملخص العربي

تأثير مستويات مختلفة من السيرم والمواد الواقية من التجميد على الانضاج المعملي لبويضات الجمل العربي المزججة

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تهدف هذه الدراسة إلى دراسة تأثير جرعتين مختلفتين (10% و20%) من مصل الأبقار الجنيني (FBS) مع مختلف الإضافات الواقية من التجميد (CPAs) مثل الـ إيثيلين جلايكول (EG) وثنائي ميثيل سلفوكسيد (DEMSO)، على معدل نضج بويضات الجمل العربي. تم تقسيم مجموعات البويضات (COCs) بإجمالي 2515 بويضة إلى سبع مجموعات تجريبية. المجموعة الأولى كمجموعة ضابطة (G1)، تم غسل المجموعة الأولى باستخدام بيئة النضج في المختبر (IVM) وتم تحضينها لمدة 30 ساعة. تم تقسيم البويضات المتبقية إلى ست مجموعات وتمت معاملتها بتركيزات مختلفة من محلول التزجيج (VS). أولاً، تم نقل COCs إلى محلول الموازنة (ES) لمدة دقيقتين. بعد ذلك، تم نقل المجموعات التجريبية الى VSs المختلفة لمدة 45 ثانية. تم وضع خمس بويضات في 1-2 ميكرولتر VS وتحميلها في قشات التجميد (OPS). تم غمر القشات على الفور في LN_2 (-196 درجة مئوية) لمدة ساعة واحدة. بعد الإسالة والتدفئة، أظهرت نسبة البويضات المتضررة شكليا أن G2 كانت لها أعلى نسبة (67.39٪) وG6 كانت لديها أقل نسبة (23.46٪). كان معدل النضج للمجموعات من المجموعة G1 الى المجموعة G7 كالتالي 78.24%، 30.49%، 48.61%، 39.33%، 56.25%، 50.36%، و68.75% على التوالي، اعتمادا على تباعد الخلايا المحيطة بالبويضات. بينما كانت نتائج طرد الجسم القطبي الاول 22.35%، 0%، 5.56%، 0%، 11.25%، 8.63%، و6.25%، بهذا الترتيب للمجموعات من المجموعة G1 الى المجموعة G7. من خلال ما تقدم يمكن ان نوصي بأن اضافة 20٪ FBS ومع الـ DMSO (G5) أو مجتمعا مع الـEG (G7) تعتبر أفضل الطرق لتزجيج بويضات الجمل العربي.

الكلمات الدالة: التزجيج، البويضات، الجمل العربي، IVM، واقيات التجميد.