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Effect of Addition Vitamin C and Zinc Chloride in Vitrification Medium on Viability, *in vitro* Maturation and Ultrastructure Changes of Vitrified Immature Bovine Oocytes

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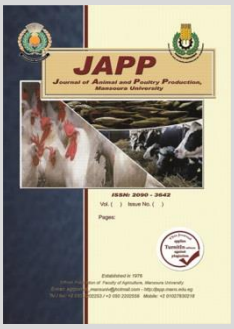

ABSTRACT

This study aimed to evaluate the impact of adding vitamin C and zinc chloride to vitrification medium on viability *in vitro* maturation and ultrastructure changes of vitrified immature Baladi cow oocytes. Compact cumulus oocytes (COCs) (n=1370) were obtained from slaughtered bovine ovaries. Then the morphology of oocytes was examined using a stereomicroscope. Staining with trypan, the *in vitro* maturation and ultrastructural changes were studied. The results revealed significant (P<0.05) increase of total and normal survival rate of bovine oocytes vitrified with zinc chloride (90.28 and 81.11%) than in vitamin C media (82.5 and 65.65%) or control medium (74.44 and 54.72%). Recovery rate of abnormal bovine oocytes showed significantly an opposite trend (9.17 vs. 16.94 and 19.72%). Proportion of oocytes with viable cytoplasm and viable cumulus (VOVC) was increased significantly (93.75%, P<0.05) in fresh (control) than in medium supplemented with vitamin C and untreated medium (74.55 and 68.63%), respectively. There were non-significant differences among zinc chloride, control and vitamin C media in oocytes with vaible cytoplasm and unvaiable cumulus (VOUC). Supplementation of the vitrification medium with zinc chloride and vitamin C significantly (P<0.05) improved maturation rates (MII) of recovered cumulus oocyte complexes (COCs) than medium without supplementation. The percentage of ultrastructural alterations in most organelles bovine oocytes significantly (P<0.05) increased in oocytes vitrified without supplementation followed by vitamin C, then zinc chloride medium. Conclusion, supplementation of vitamin C or zinc chloride to the vitrification medium improved survival rate, morphologically and ultrastructural, as well as maturation rate of bovine oocytes

Keywords:*Antioxidants,vitrification, bovine oocyts, ultrastructure*

INTRODUCTION

Low fertilization rates in cryopreserved oocytes have been related to freeze damage, including solidification of zona pellucida as a result of releasing of premature cortical granules, spindle disorganization and microtubule loss or agglutination (Carroll *et al.*, 1990; Hwang and Hochi, 2014). Furthermore, reactive oxygen species (ROS) such as superoxide (O_2), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) may disrupt the cellular function of oocytes and embryos that survive cryopreservation (Vajta *et al.*, 1997; Dobrinsky, 2002).Oocytes preserved via slow freezing suffer from osmotic shock and intracellular ice crystallization as reported by Ledda *et al.* (2006) and those undergone vitrification are exposed to toxic concentrations of the cryoprotectant agents (Sripunya *et al.*, 2010).

Vitrification is still remaining an effective protocol for cryopreservation of gametes and embryos. In this regard, several trials have been conducted to reduce its side effects on the quality and developmental potential of oocytes (Wani *et al.*, 2004; Sripunya *et al.*, 2010; Mostagir *et al.*, 2019).

Several studies showed that increasing ROS and H_2O_2 levels and decreasing glutathione (GSH) content following oocyte vitrification (Somfai *et al.*, 2007; Gupta *et al.*, 2010). The cryopreservation lead to lowering the rate of *in vitro* oocyte maturation, fertilization and production of embryo might be due to damaging the cytoskeleton of mature oocytes through disrupting the sub-cortical actin network, microtubule depolymerization, abnormal spindle configuration and chromosome scattering (Prentice and Anzar, 2011; Brambillasca *et al.*, 2013; Dutta *et al.*, 2013; Hwang and Hochi, 2014).

The beneficial effects of L-carnitine (LC) which can be included in the vitrification media of bovine oocytes and embryos to protect against harmful effects of vitrification Were reported by Badr *et al.* (2020). Mostagir *et al.* (2019) conducted a study to determine the efficiency of different vitrifying solutions, Dimethyl sulphoxide (DMSO) and ethylene glycol (EG) with or without supplementation of three concentrations of LC (5, 10 and 15 mM) on post-thawing viability of vitrified immature buffalo oocytes. They found that using EG or a mixture of EG and DMSO supplemented with LC at 5 and 10 mM increased oocyte recovery and survival rates.

Ascorbic acid (AA) addition also seemed to protect embryos against oxidative stress during *in vitro* culture (IVC) and improve development of embryos competence after either *in vitro* fertilization (Hossein *et al.* (2007). Supplementation with AA during IVC improves the quality

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of blastocysts in terms of cell numbers and increases the survival rate after vitrification (Hu *et al.*, 2012; Castillo-Martín *et al.*, 2014).

This study aimed to evaluate the effect of addition of vitamin C or zinc chloride to vitrification medium on viability, ultrastructural changes and *in vitro* maturation of vitrified immature bovine oocytes.

MATERIALS AND METHODS

The present study was done at the lab of Physiology and Biotechnology, Department of Animal production, Faculty of Agriculture, Mansoura University during the period of April 2018 to March 2020.

Ovaries collection:

In this study, the ovaries were collected immediately, after slaughtering, from the local Balidi cows in Elbagor slaughterhouse, Minufia government. The collected ovaries ere placed into thermos (35 ºC) which contains saline solution (0.9% NaCl), penicillin G (100 IU/mL) and streptomycin sulfate (100 µg/mL) and transported to the lab within 3-5 h.

Oocyte recovery

An eighteen-gauge needle joined to a 10 ml syringe were used for aspiration of cumulus oocyte complexes (COCs) from the follicles that have 2-8 mm diameter. Only e high quality immature oocytes that characterized by the homogeneous distribution of the cytoplasmic granules and enclitic by compact layers of cumulus cells were used for vitrification.

Experimental design

This experiment aimed to study the effect of cryopreservation of Baladi cow oocytes by vitrification method (supplemented with vitamin C 1mg or zinc chloride 1.5µg/mLvitrification solution) on survival; changes in ultrastructure of oocytes and *in vitro* maturation rates (IVM) as well as compared with fresh oocytes.

Vitrification of oocytes:

The HEPES-buffered TCM 199 plus 20% (v/v) fetal bovine serum was used as a holding medium (HM) for handling oocytes during vitrification and warming

All operations are performed on a heating plate at 37°C in a warm room at 25°C to 27°C. Except for the heating solution used at 37°C, all media are used at room temperature. The straw method is used for vitrification and thawing of immature oocytes. The vitrification process includes two main steps: equilibrium and vitrification. First, the immature COCs is placed on the middle surface of the equilibrium solution, which is obtained by adding both of ethylene glycol (EG) and dimethyl sulfoxide (DMSO) at a concentration of 7.5% (v:v) to the HM. After five minutes of equilibration, the shrunken oocytes restore its original size, which refers to the time for vitrification. Ten oocytes were transferred to a vitrification solution prepared using both of EG, DMSO at a concentration of 15% (v:v) and 0.5-M sucrose dissolved in the HM. Within one minute, the oocytes must be thoroughly rinsed with vitrification solution, loaded on the straw with a volume of less than 0.01 mL, and finally immersed in liquid nitrogen.

Warming of oocytes and assessment of oocyte viability

Two weeks post storing, the oocytes warms process was done by placing the straws in the air for 6 second and then stirring in a 38°C water bath for at least 20 second. The content of each straw was discharged into a petri dish, and the oocytes were transferred to three diluent solutions. The HM solution contained 1 M sucrose for 1 minute, 0.5 M sucrose for 3 minutes, and then washed twice in HM for 5 minutes respectively. The viability of oocytes was investigated morphologically by using the stereomicroscope according to its integrity of oolemma, zona pellucida and the loss of membrane (lysis).

The criteria that were used to evaluate the morphology of vitrified warmed oocytes after thawing were as follows: (a) Normal oocytes with spherical and symmetrical shapes without signs of lysis. (b) Abnormal oocytes having fissure of the zona pellucida, disintegration or leakage of cell contents, changes in the shape and shrunken of the oocytes.

Evaluation of oocytes viability:

The trypan blue staining was used to evaluate the viability of oocytes according to Abd Allah *et al.* (2008).

***In Vitro* maturation:**

Prepare maturation medium

The main tissue culture medium (TCM –199) used in this study contained 10% (v/v) FCS and 50 µg/mL Gentamycin and supplemented with 10 µg/mL epidermal growth factor.

The pH value of the medium was adjusted to 7.2-7.3, and the osmotic pressure was 280-300 mOsm/kg. It was filtered through a 0.22 µM Millipore filter. About 50 µL of the prepared culture medium were put into a sterile disposable plastic petri dish (3.5 cm in diameter) and covered with sterile mineral oil. Before using the Petri dishes must be incubated in a CO_2 incubator with 38.5°C, 5% CO_2 and 95% relative humidity for at least one hour for equilibration.

Oocytes maturation

After warming, the high-quality of vitrified oocytes and non-vitrified oocytes (control), were washed 3 times in washing medium and twice in the prepared maturation medium to remove the preventing substances maturation. Finally, the oocytes were incubated for 24 hours in the medium in the previously prepared Petri dish at 38.5°C, 5% CO_2 and high humidity.

Fixation and staining of oocytes:

At the end of the maturation, the compact cumulus oocytes were taken from the maturation medium and the oocytes were isolated from the cumulus cells by using 2.9% sodium citrate for five minutes and then repeat pipetting. Seven to ten oocytes in a drop of the medium were pippeted and mounted on a glass slide. The coverslip with inert paraffin spots on each of its four corners was placed directly above the center of the droplet of the oocyte-containing medium. After that, the oocytes were investigated under microscope magnification. The coverslip was pressed down on the oocyte until it was firmly fixed in place.

Fixation of oocytes was done by putting the slides in a freshly prepared mixture of acetic acid and ethanol (1:3) overnight. The slides were stained with 1% aceto orcein (1% orcein in 40% acetic acid w/v) for a few minutes and washed with acetyl-glycerol (3:1). The low power and oil immersion were used for detailed examination.

Criteria of maturation:

After maturation, oocytes (vitrified and control) were categorized into 5 groups as follows:

a - Germinal vesicle (GV): Interphase chromosomes enclosed within a nuclear membrane.

b - Germinal vesicle breakdown (GVBD): An absence of a visible nuclear membrane and chromatin condensation characterized by a cluster of DNA material without individual chromosomes.

c- Oocytes at metaphase I stage (MI): Chromosomes were condensed in pairs and without detected polar body (immature oocytes).

d - Oocytes at metaphase II stage (MII): One large group of chromosome formed an equatorial plate and the remaining chromosomes are highly condensed or had extruded a polar body (oocytes mature).

e- Degenerated oocytes (Deg): Oocytes were vacuolated or had scattered.

Electron microscopy

Twenty-four oocytes in each group were fixed and subjected to TEM treatment as described by Nottola *et al.* (2009). The oocytes were fixed in 1.5% glutaraldehyde at 4°C for 2-5 days, embedded in 1% gelose, and exposed to 1% osmium tetroxide. Then, the samples were dehydrated in increasingly higher concentrations of ethanol, immersed in propylene oxide for solvent replacement, and then respectively embedded in Epon 812. Then the oocytes were ultrathin sectioned (60–80 nm), stained with uranyl acetate and lead citrate, examined and photographed with a TEM.

Statistical analysis

Statistical analysis of the data was carried out through analysis of variance (ANOVA) after arcsine transformation. Dunacn's multi-range test was subsequently used to test for significant differences among treatments (Duncan, 1955). Chi-square test ($\chi 2$) tests the changes in ultrastructure. The statistical procedure used is the Statistical Analysis System (SAS, 2004).

RESULTS AND DISCUSSION

Survival and viability rates (proportion of normal and abnormal) of bovine oocytes vitrified in medium supplemented with vitamin C or Zinc chloride are showed in Table 1. Data in table 1 showed significant (P<0.05) increase of survival and normal percentages of bovine oocytes cryopreserved using vitrification solution supplementation with Zinc chloride (90.28 and 81.11%) as compared to vitamin C (82.5 and 65.65%) and free (74.44 and 54.72%) media. However, the survival rate of abnormalities bovine oocytes showed significantly an opposite trend (9.17 vs. 16.94 and 19.72%, Table 1).

Table 1. Survival and viability rates of bovine oocytes as affected by different supplementation.

Vitrification medium	Total number of oocytes	Post-thawing survival oocytes					
		Survival		Normal		Abnormal	
		n	%	n	%	n	%
Free nedium	360	268	74.44^{c}	197	54.72^{c}	71	19.72^{a}
Vitamin C	360	297	82.5^{b}	236	65.56^{b}	61	16.94^{a}
Zinc chloride	360	325	90.28^{a}	292	81.11^{a}	33	9.17^{b}

a, b and c. in the same row, the values with different superscripts are significantly different (P < 0.05).

Selection of oocytes for *in vitro* culture based only on the morphological features is not enough to obtain better *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) (Blanco *et al.*, 2011). Unknown factors seem to affect the 1st step of embryo development accumulated throughout the oogenesis period. The *in vitro* production of cryopreserved oocyte-derived embryos is also important in a commercial environment, as an aid to embryo transfer programs and as a guarantee for future reproductive biotechnology (Pomp and Critser, 1988).

The most morphological abnormalities noticed in the vitrified warmed oocytes in this study (19.72, 16.94 and 9.17%) could be attributed to cooling and warming that damage cytoskeleton and inducing degenerative cellular changes (Elsden, 1988). Also, osmotic stress is consider another factor that can induce damage in oocytes and causing changes in its volume and adversely affect their viability (Shaw *et al.*, 2000).

Categories of oocytes viability using trypan blue after cryopreservation immature bovine oocytes supplementation with vitamin C and Zinc chloride are illustrated in Table 2. The proportion of viable cytoplasm and viable cumulus (VOVC) oocytes was significantly (P<0.05) higher significant (93.75%, P<0.05) in fresh group than in those vitrified with medium supplemented with vitamin C and untreated medium (74.55 and 68.63%), respectively. However, no significant differences between Zinc chloride and fresh group (Table 2).

Table 2. Viability of cytoplasm and cumulus of different types of fresh and vitrified cow oocytes as affected by treatments using trypan blue staining.

Vitrification medium	N	Post-thawing survival oocytes							
		VOVC		VOUC		UOVC		UOUC	
		n	%	n	%	n	%	n	%
Fresh oocytes (control)	64	60	93.75^{a}	1	1.56	2	3.13	1	1.56^{c}
free medium	51	35	68.63^{c}	3	5.88	5	9.80	8	15.69^{a}
Vitamin C	55	41	74.55^{bc}	3	5.45	5	9.09	6	10.91^{ab}
Zinc chloride	85	73	85.88^{ab}	2	2.35	3	3.53	7	8.24^{b}

a, b and c. in the same row, the values with different superscripts are significantly different (P < 0.05).

N: Total number of oocytes

VOVC= Viable cytoplasm and viable cumulus

VOUC= Viable cytoplasm and unviable cumulus

UOVC= Unviable cytoplasm and viable cumulus

UOUC= Unviable cytoplasm and Unviable cumulus

Statically analysis showed insignificant (P>0.05) difference among treatments in the proportion of VOUC and UOVC oocytes, being nearly similar for vitamin C and free media, and also between zinc chloride and control medium. The proportion of UOUC were significantly (P<0.05) lower in fresh group (1.56%) than in other treatments, followed by zinc chloride medium. It was observed that the percentage oocytes with viable cumulus cells was higher in all groups as compared to in oocytes with viable ooplasm, that may be refer to smaller sizes of cumulus cells and lead to easily and quickly inlet and outlet cryoprotectants from cells during (Freezing/thawing).

Vital dyes are chemical compounds bind to tissues or cells and widely used in basic and applied research to evaluate specific characteristics of a cell or tissue. It can be used as an an alternative method for quality assessment of female gametes in mammalians (Rodrigues *et al.*, 2009).

Trypan blue staining has previously been used to asses quality and the viability of oocytes. The dead oocytes show a dark blue egg mass with translucent cumulus cells (Jewgenow and Göritz, 1995; Abd Allah *et al.*, 2008).

Maturation rates of vitrified/thawed bovine oocytes

Supplementation of the vitrification medium with zinc chloride or vitamin C significantly (P<0.05) improved maturation rates (MII) of recovered COCs than without supplementation as depicted in Table 3. Fresh COCs (Fig. 1) achieved the highest significant (P<0.05) maturation rates (71.15%), while the lowest maturation rate was obtained for COCs matured in vitrification medium without supplementation (39.34%). The moderate maturation rate was obtained by supplementation of vitamin C (49.41%) and zinc chloride (52.17%) in vitrification medium of immature bovine oocytes without significant differences between them**.**

The reduction of maturation rate of oocyte in vitrification medium without supplementation (untreated) than fresh COCs may be due to the harmful effect of cryopreservation such as cytoskeleton damage (Hwang and Hochi, 2014). This is may be also attributed to oxidative stress, higher levels of hydrogen peroxide and lower concentrations of glutathione as a result of vitrifiction process (Kelly *et al.*, 2005). The improvement in maturation rate after supplementation of antioxidants like vitamin C and zinc may be effective in protecting the oocytes from harmful ROS (Kere *et al.*, 2012).

In addition, Jeon *et al.* (2014) and Geravandi *et al.* (2017) reported that supplementation with adequate zinc concentrations to vitrified medium regulates the intracellular GSH concentration, the ROS level, transcription factor expression and meiosis during maturation of oocytes. In this context, Jeon *et al.* (2015) revealed that supplementation with zinc during IVC improved the viability of embryos might be due to increasing PCNA, POU5F1, and Bcl2 gene expression. Furthermore the supplementation with vitamin C promotes the growth of vitrified-thawed bovine oocytes through inhibiting lipid peroxidation and biosynthesis of collagen (Geesin *et al.*, 1991; Sonowal *et al.*, 2017).

The present data showed that the proportion of GV oocytes were significantly (P<0.05) decreased after vitrification as shown in Table 3. These results are in agreement with the results obtained by Huang *et al.* (2018).

Table 3. Effect of vitamin C or Zinc chloride in vitrification medium of immature bovine oocytes on *in vitro* maturation rate.

Vitrification	Total	GV		GVBD		M1		M11		DEG.	
Medium	N	N	%	N	%	N	%	N	%	N	%
Fresh (control)	104	4	3.85^{b}	8	7.69	11	10.58^{b}	74	71.15^{a}	7	6.73^{c}
Untreated	61	7	11.48^{a}	5	8.2	12	19.67^{a}	24	39.34^{c}	13	21.31^{a}
Vitamin C	85	8	9.41^{a}	7	8.24	15	17.65^{a}	42	49.41^{b}	13	15.29^{ab}
Zinc chloride	92	8	8.7^{a}	9	9.78	14	15.22^{ab}	48	52.17^{b}	13	14.13^{b}

a, b and c. in the same row, the values with different superscripts are significantly different (P < 0.05).

Ultrastructural change after *in vitro* maturation of vitrified of immature bovine oocytes.

Table 4 summarizes the ultrastructural changes of immature bovine oocytes supplemented with vitamin C and zinc chloride in vitrification medium after in vitro maturation. In the fresh control group (Figure 1) and all treatment groups, most of the cumulus cells around mature oocytes appeared to be normal, and very few cumulus cells showed cytoplasmic vacuoles. There are no zona cracks in all oocytes.

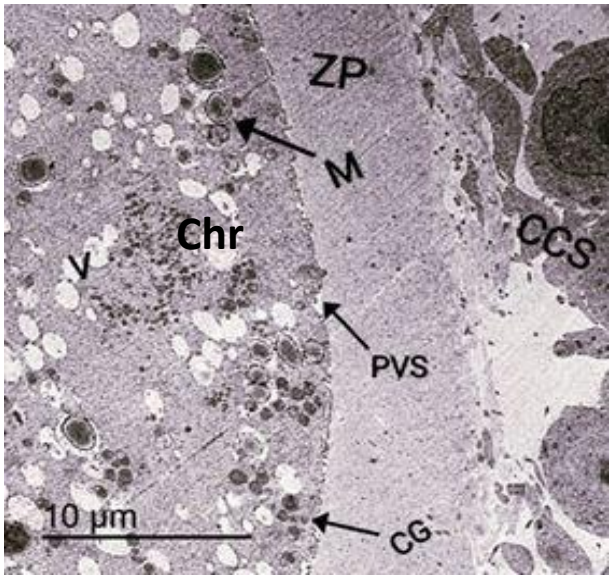

Fig. 1. Ultrastructure of cumulus cells surrounding mature bovine oocyte in control fresh group showing chromosome (Chr) at metaphase I stage (MI), zona pellucida (ZP), perivitelline space (PVS), mitochondria (M), vesicle (V), cortical granules (CG), and cumulus cells (CCS). (6000X)

The percentage of ultrastructural alterations in Perivitelline space and cortical granulosa were showed highly significant (P<0.05) in bovine oocytes vitrified control followed by vitamin C and Zinc chloride group (Fig. 2). However, the ultrastructural alterations in mitochondria was significantly higher (P<0.05) in vitrified oocytes without supplementation than other groups (Table 4 and Fig 3).

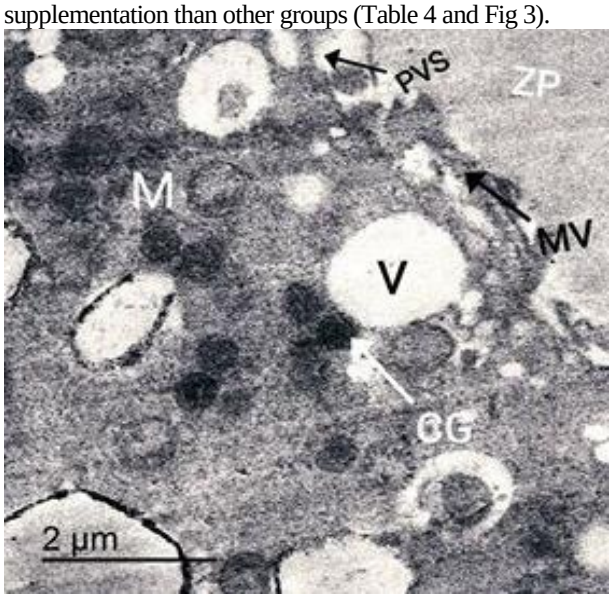

Fig. 2. Ultrastructure of mature bovine oocyte from vitrified without supplementation showing microvilli (MV), zona pellucida (ZP), Perivitelline space (PVS), mitochondria (M), vesicle (V), cortical granules (CG). (6000X).

Compared with the fresh control group, the ultrastructural alterations that includes the microvilli/oolema, vesicles and lipid droplets were significantly higher in all vitrified ooccytes (P<0.05), while there was no significant differences among the vitrified treatments (Table 4 and Fig. 4).

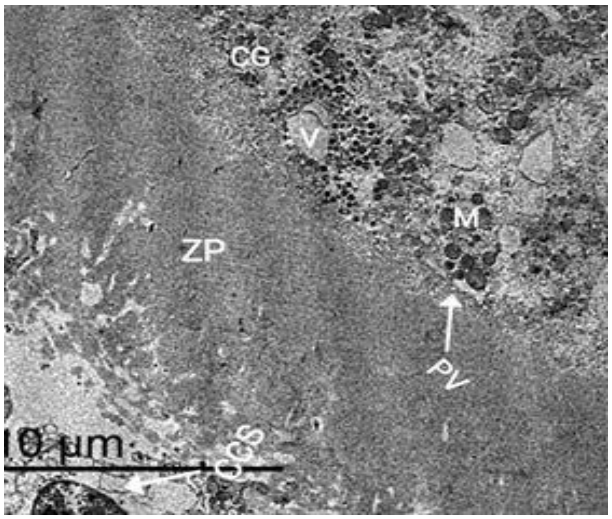

Fig. 3. Ultrastructure of mature bovine oocyte from vitrified without supplementation showing mitochondria (M), zona pellucida (ZP), Perivitelline space (PVS), vesicle (V), cortical granules (CG), and cumulus cells (CCS). (6000X)

Table 4. Effect of vitamin C and Zinc chloride in vitrification medium of immature bovine oocytes on ultrastructural changes after (IVM).

Ultrastructure	Antioxidant supplementation			
	Control fresh	Control vitrified	Vitamin C	Zinc Chloride
Zona pellucida	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Perivitelline space	4 (16.67)^{c}	24 (100.0)^{a}	20(83.33)^{ab}	19 (79.17^{b}
Microvilli/oolema	2 (8.33)^{b}	22 (91.67)^{a}	20 (83.33)^{a}	18 (75.0)^{a}
Mitochondria	0 (0.0)^{b}	3 (12.5)^{a}	0 (0.0)^{b}	0 (0.0)^{b}
Cortical granulosa	2 (8.33)^{b}	8 (33.33)^{a}	5 (20.83)^{ab}	3 (12.5)^{ab}
Vesicles	4(16.67)^{b}	8(33.33)^{a}	8(33.33)^{a}	8(33.33)^{a}
Lipid droplets	0 (0.0)^{b}	5(20.83)^{a}	5 (20.83)^{a}	5 (20.83)^{a}

a, b and c. Within rows, values with different superscripts are different (P<0.05).

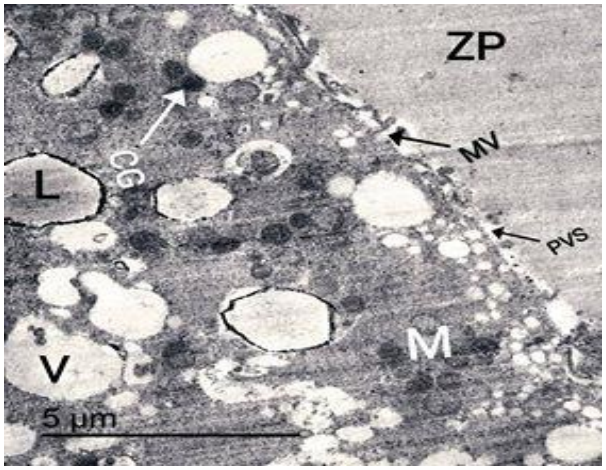

Fig. 4. Ultrastructure of vitrified mature bovine oocyte showing. mitochondria (M), zona pellucida (ZP), Perivitelline space (PVS), microvillie (MV), Vesicle (V), cortical granules (CG), and lipid droplets (L). (6000X).

Vitrification is an effective protocol for cryopreservation of gametes and embryos that assist in reproduction treatments (Borges and Vireque, 2019). Oocytes are exposed to high concentrations and toxic of the cryoprotectant agents during vitrification method (Sripunya *et al.*, 2010). Several studies have been conducted to reduce its harmful side effects on the quality and developmental potential of oocytes (Wani *et al.*, 2004; Sripunya *et al.*, 2010; Mostagir *et al.*, 2019).

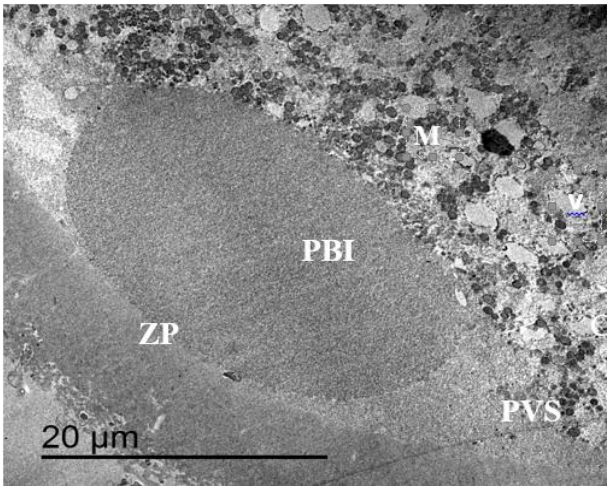

Fig. 5. Ultrastructure of mature bovine oocyte vetrified with zinc chloride Zona pellucida (ZP), and clusters mitochondria (M) and 1st polar body (PB1) in perivitelline space (PVS). (6300X).

Vitamin C is the most important antioxidant in extracellular fluid (Warren *et al.*, 2000). It has a positive effect on oocyte nuclear maturation (Tao *et al.*, 2004). In addition, it can also protect embryos from oxidative stress during *in vitro* culture. Also addition of ascorbic acid to the vitrified and serum-supplemented warm medium can improve the embryo survival rate of pig blastocysts produced *in vitro* (Castillo-Martín *et al.*, 2014).

The structural changes of lipid droplets in this study are consistent with previous reports in bovine and pig oocytes (Isachenko *et al.*, 1998; Isachenko *et al.*, 2001; Wu *et al.*, 2006). In this respect, Wu *et al.* (2006) proposed that the increased small lipid droplets come from broken larger lipid droplets, which exist in the form of smaller droplets during the vitrification of porcine oocytes. Isachenko *et al.* (2001) also reported that the lipid droplets in pig oocytes changed in shape during the cooling process. They become spherical with transparent stripes. Ghetler *et al.* (2006) found that the cryopreservation process resulted in the loss of cortical particles in the cortex, and the appearance of vesicles in the cytoplasm of unfrozen and mature human cryopreserved oocytes, which may indicate structural damage during freezing and warming. Gualtieri *et al.* (2009) reported that the mitochondria of freeze-thaw human oocytes have reduced matrix electron density or rupture of the outer and inner membranes. Mitochondria are the most abundant organelles in mammalian oocytes, and their disfunction or abnormalities would determine the oocytes and embryos developmental competence.

Conclusion, supplementation of vitamin C or Zinc chloride to the vitrification medium improved, morphologically and ultrastructural survival and viability rates as well as maturation rate of bovine oocytes.

REFERENCES

Abd Allah, S., Khalil, A. and Ali, K. (2008). The use of trypan blue staining to select the developmentally competent dromedary camel oocytes and its effect on in vitro maturation rate. In Proc. Twentieth Annual Scientific Conference, pp. 2-7.

Badr, A., Abdel-Gawad, E., Abdel-Halim, B., Mohamed, T. and Abdel, R. (2020). Effect of L-carnitine on Recovery and Maturation Rates and Quality of Embryos Produced from Vitrified Immature Bovine Oocytes. Curr. Sci. Int., 9: 8.

Blanco, M., Demyda, S., Moreno Millán, M. and Genero, E. (2011). Developmental competence of in vivo and in vitro matured oocytes: a review. Biotechnology and Molecular Biology Reviews, 6: 155-165.

Borges, E. D. and Vireque, A. A. (2019). Updating the Impact of Lipid Metabolism Modulation and Lipidomic Profiling on Oocyte Cryopreservation. EMJ, 4: 79-87.

Brambillasca, F., Guglielmo, M. C., Coticchio, G., Renzini, M. M., Dal Canto, M. and Fadini, R. (2013). The current challenges to efficient immature oocyte cryopreservation. Journal of assisted reproduction and genetics, 30: 1531-1539.

Carroll, J., Depypere, H. and Matthews, C. (1990). Freeze–thaw-induced changes of the zona pellucida explains decreased rates of fertilization in frozen–thawed mouse oocytes. Reproduction, 90: 547-553.

Castillo-Martín, M., Bonet, S., Morató, R. and Yeste, M. (2014). Comparative effects of adding β-mercaptoethanol or L-ascorbic acid to culture or vitrification–warming media on IVF porcine embryos. Reproduction, Fertility and Development, 26: 875-882.

Dobrinsky, J. (2002). Advancements in cryopreservation of domestic animal embryos. Theriogenology, 57: 285-302.

Duncan, D. B. (1955). Multiple range and multiple F tests. Biometrics, 11: 1-42.

Dutta, D., Dev, H. and Raj, H. (2013). In vitro blastocyst development of post-thaw vitrified bovine oocytes. Veterinary World, 6.

Elsden, R. (1988). Classification of embryos after thawing. 1988 Short Course Proceedings. Animal Reproduction Laboratory, Colorado State University: 51-55.

Geesin, J., Hendricks, L., Falkenstein, P., Gordon, J. and Berg, R. (1991). Regulation of collagen synthesis by ascorbic acid: characterization of the role of ascorbate-stimulated lipid peroxidation. Archives of biochemistry and biophysics, 290: 127-132.

Geravandi, S., Azadbakht, M., Pourmoradi, M. and Nowrouzi, F. (2017). Zinc supplementation of vitrification medium improves in vitro maturation and fertilization of oocytes derived from vitrified-warmed mouse ovaries. Cryobiology, 74: 31-35.

Ghetler, Y., Skutelsky, E., Nun, I. B., Dor, L. B., Amihai, D. and Shalgi, R. (2006). Human oocyte cryopreservation and the fate of cortical granules. Fertility and sterility, 86: 210-216.

Gualtieri, R., Iaccarino, M., Mollo, V., Prisco, M., Iaccarino, S. and Talevi, R. (2009). Slow cooling of human oocytes: ultrastructural injuries and apoptotic status. Fertility and Sterility, 91: 1023-1034.

Gupta, M. K., Uhm, S. J. and Lee, H. T. (2010). Effect of vitrification and beta-mercaptoethanol on reactive oxygen species activity and in vitro development of oocytes vitrified before or after in vitro fertilization. Fertility and sterility, 93: 2602-2607.

Hossein, M. S., Hashem, M. A., Jeong, Y. W., Lee, M. S., Kim, S., Kim, J. H., Koo, O. J., Park, S. M., Lee, E. G. and Park, S. W. (2007). Temporal effects of α-tocopherol and l-ascorbic acid on in vitro fertilized porcine embryo development. Animal reproduction science, 100: 107-117.

Hu, J., Cheng, D., Gao, X., Bao, J., Ma, X. and Wang, H. (2012). Vitamin C enhances the in vitro development of porcine pre-implantation embryos by reducing oxidative stress. Reproduction in domestic animals, 47: 873-879.

Huang, J., Ma, Y., Wei, S., Pan, B., Qi, Y., Hou, Y., Meng, Q., Zhou, G. and Han, H. (2018). Dynamic changes in the global transcriptome of bovine germinal vesicle oocytes after vitrification followed by in vitro maturation. Reproduction, Fertility and Development, 30: 1298-1313.

Hwang, I.-S. and Hochi, S. (2014). Recent progress in cryopreservation of bovine oocytes. BioMed research international, 2014.

Isachenko, V., Isachenko, E., Michelmann, H., Alabart, J., Vazquez, I., Bezugly, N. and Nawroth, F. (2001). Lipolysis and ultrastructural changes of intracellular lipid vesicles after cooling of bovine and porcine GV-oocytes. Anatomia, histologia, embryologia, 30: 333-338.

Isachenko, V., Soler, C., Isachenko, E., Perez-Sanchez, F. and Grishchenko, V. (1998). Vitrification of immature porcine oocytes: effects of lipid droplets, temperature, cytoskeleton, and addition and removal of cryoprotectant. Cryobiology, 36: 250-253.

Jeon, Y., Yoon, J. D., Cai, L., Hwang, S.-U., Kim, E., Lee, E., Jeung, E. B. and Hyun, S.-H. (2015). Effect of zinc on in vitro development of porcine embryos. Theriogenology, 84: 531-537.

Jeon, Y., Yoon, J. D., Cai, L., Hwang, S.-U., Kim, E., Zheng, Z., Lee, E., Kim, D. Y. and Hyun, S.-H. (2014). Supplementation of zinc on oocyte in vitro maturation improves preimplatation embryonic development in pigs. Theriogenology, 82: 866-874.

Jewgenow, K. and Göritz, F. (1995). The recovery of preantral follicles from ovaries of domestic cats and their characterisation before and after culture. Animal Reproduction Science, 39: 285-297.

Kelly, J., Kleemann, D., Kuwayama, M. and Walker, S. (2005). 99 Effect of Cysteamine on Survival of Bovine and Ovine Oocytes Vitrified Using the Minimum Volume Cooling (Mvc) Cryotop Method. Reproduction, Fertility and Development, 18: 158-158.

Kere, M., Siriboon, C., Lo, N.-W., Nguyen, N. T. and Ju, J.-C. (2012). Ascorbic acid improves the developmental competence of porcine oocytes after parthenogenetic activation and somatic cell nuclear transplantation. Journal of Reproduction and Development.

Ledda, S., Bogliolo, L., Succu, S., Ariu, F., Bebbere, D., Leoni, G. G. and Naitana, S. (2006). Oocyte cryopreservation: oocyte assessment and strategies for improving survival. Reproduction, Fertility and Development, 19: 13-23.

Mostagir, A. M., Elmetwally, M. A., Montaser, A. and Zaabel, S. M. (2019). Effects of L-Carnitine and Cryoprotectants on Viability Rate of Immature Buffalo Oocytes in Vitro After Vitrification. Alexandria Journal for Veterinary Sciences, 62.

Nottola, S., Coticchio, G., Sciajno, R., Gambardella, A., Maione, M., Scaravelli, G., Bianchi, S., Macchiarelli, G. and Borini, A. (2009). Ultrastructural markers of quality in human mature oocytes vitrified using cryoleaf and cryoloop. Reproductive biomedicine online, 19: 17-27.

Pomp, D. and Critser, J. K. (1988). Cryopreservation of Iian embryos. In: IS1 Atlas of Science. Journal of Animal an plant sciences: 7.

Prentice, J. R. and Anzar, M. (2011). Cryopreservation of mammalian oocyte for conservation of animal genetics. Veterinary medicine international, 2011.

Rodrigues, E. B., Costa, E. F., Penha, F. M., Melo, G. B., Bottós, J., Dib, E., Furlani, B., Lima, V. C., Maia, M. and Meyer, C. H. (2009). The use of vital dyes in ocular surgery. Survey of ophthalmology, 54: 576-617.

SAS (2004). SAS/QC 9.1: User's Guide, SAS Institute.

Shaw, J. M., Oranratnachai, A. and Trounson, A. O. (2000). Fundamental cryobiology of mammalian oocytes and ovarian tissue. Theriogenology, 53: 59-72.

Somfai, T., Ozawa, M., Noguchi, J., Kaneko, H., Karja, N. W. K., Farhudin, M., Dinnyés, A., Nagai, T. and Kikuchi, K. (2007). Developmental competence of in vitro-fertilized porcine oocytes after in vitro maturation and solid surface vitrification: effect of cryopreservation on oocyte antioxidative system and cell cycle stage. Cryobiology, 55: 115-126.

Sonowal, J., Barua, P., Borah, P., Borgohain, I., Gogoi, C., Deuri, N., Das, A., Pathak, B., Singh, W. and Hazarika, G. (2017). Effect of L-Ascorbic acid on in-vitro maturation of vitrified-Thawed bovine oocytes. IJCS, 5: 1646-1648.

Sripunya, N., Somfai, T., Inaba, Y., Nagai, T., Imai, K. and Parnpai, R. (2010). A comparison of cryotop and solid surface vitrification methods for the cryopreservation of in vitro matured bovine oocytes. The Journal of reproduction and development, 56: 176.

Tao, Y., Zhou, B., Xia, G., Wang, F., Wu, Z. and Fu, M. (2004). Exposure to l-ascorbic acid or α-tocopherol facilitates the development of porcine denuded oocytes from metaphase I to metaphase II and prevents cumulus cells from fragmentation. Reproduction in Domestic Animals, 39: 52-57.

Vajta, G., Booth, P. J., Holm, P., Greve, T. and Callesen, H. (1997). Successful vitrification of early stage bovine in vitro produced embryos with the open pulled straw (OPS) method. CryoLetters, 18: 191-195.

Wani, N., Maurya, S., Misra, A., Saxena, V. and Lakhchaura, B. (2004). Effect of cryoprotectants and their concentration on in vitro development of vitrified-warmed immature oocytes in buffalo (Bubalus bubalis). Theriogenology, 61: 831-842.

Warren, S., Patel, S. and Kapron, C. (2000). The effect of vitamin E exposure on cadmium toxicity in mouse embryo cells in vitro. Toxicology, 142: 119-126.

Wu, D., Cheung, Q. C.-K., Wen, L. and Li, J. (2006). A growth-maturation system that enhances the meiotic and developmental competence of porcine oocytes isolated from small follicles. Biology of reproduction, 75: 547-554.

تأثير إضافة فيتامين ج وكلوريد الزنك في بيئة التزجيج على الحيوية والنضج المعملي والتغيرات في التركيب الدقيق لبويضات الأبقار غير الناضجة المزججة

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الهدف من هذه الدراسة تقييم تأثير إضافة فيتامين ج وكلوريد الزنك لبيئة االتزجيج على الحيوية والإنضاج المعملي والتغيرات في التركيب الدقيق لبويضات الابقار البلدي غير الناضجة المزججة. استخدم عدد من البويضات مكتملة التراكم للخلايا الوسادية ١٣٧٠ والتي تم الحصول عليها من مبايض الأبقار المذبوحة، فُحصت البويضات مورفولوجياً باستخدام الإستريوميكروسكوب مع صبغ البويضات بصبغة trypan الأزرق، وتم دراسة النضج المعملي والتغيرات في التركيب الدقيق للبويضات. وقد أظهرت النتائج أن إضافة كلوريد الزنك الي بيئة التزجيج أدي الي زيادة معنوية في العدد الكلي ومعدل البقاء على قيد الحياة الطبيعي لبويضات الأبقار المزججة (٩٠,٢٨، ٨١,١١ %) مقارنة بإضافة فيتامين ج الي بيئة (٨٢,٥، ٦٥,٦٥ %) أو المجموعة الضابطة (٧٤,٤٤، ٥٤,٧٢). بينما كان معدل الإسترداد للبويضات الغير طبيعية تميل للاتجاه العكسي (٩,١٧، ١٦,٩٤، ١٩,٧٢ %). وأظهرت النتائج زيادة معنوية في نسبة البويضات ذات السيتوبلازم الحيوي والركام الحيوي (VOVC) في المجموعة الطازجة (الضابطة) (٩٣,٧٥ %) عن كل من المجموعة المضاف لبيئتها فيتامين ج وتلك غير المعاملة (٧٤,٥٥، ٦٨,٦٣ %) على التوالي. بينما لم يكن هناك فروق معنوية بالنسبة للبويضات ذات السيتوبلازم الحيوي والركام غير الحيوي (VOUC) بين كل من المجموعة المضاف اليها كلوريد الزنك وفيتامين ج والمجموعة الضابطة. إضافة كلوريد الزنك وفيتامين ج إلى بيئة التزجيج أدى الى زيادة معنوية في معدل نضج البويضات معملياً وصولاً للطور الميتوزي الثاني للبويضات مكتملة التراكم المبيضي عن التي بدون إضافات. وأظهرت النتائج زيادة معنوية في النسبة المئوية للتغيرات في التركيب الدقيق لمعظم عضيات بويضات الأبقار المزججة بدون إضافات عن تلك المضاف اليها فيتامين ج وكلوريد الزنك. الخلاصة: نستخلص من هذه الدراسة أن إضافة فيتامين ج أوكلوريد الزنك الى بيئة التزجيج أدي الي تحسين في الشكل المورفولوجي والتركيب الدقيق ومعدل البقاء على قيد الحياة بالإضافة الى معدل نضج بويضات الأبقار معملياً.

الكلمات الدالة: مضادات الاكسدة , التزجيج , بويضات الابقار, التركيب الدقيق