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VITRIFICATION AND IN VITRO MATURATION AND FERTILIZATION OF BOVINE OOCYTES WITH OR WITHOUT CUMULUS CELL LAYER.

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ABSTRACT

Aim of this study was to evaluate the effect of presence or absence of cumulus cells around oocytes, on post-vitrification survival rate, maturation and developmental capacity of bovine oocytes. Ovaries were collected from abattoirs and all visible follicles were aspirated and examined. Only, cumulus cell complex (COCs) and natural (NDOs) or mechanically denuded (MDOs) were vitrified/warmed, matured, fertilized and cultured for 7 days to calculate post-thawing survival, maturation, fertilization and blastocyst production rates. Results showed that the proportions of total and morphological normal oocytes recovered post-vitrification were greater (P<0.05) for COCs (79.0 and 76.0%) than those obtained for NDOs (56.0 and 51.4%). Also, post-vitrification recovery rate and proportion of morphological normal oocytes was greater (P<0.05) for MDOs (67.8 and 63.2%) than those for NDOs (56.0 and 51.4%). Proportion of damaged oocytes post-vitrification was not affected significantly by oocyte type, but leakage of cellular contents represented the highest frequency of damaged oocytes, while splitting in two halves was the lowest frequent abnormality observed. The COCs yielded the highest (P<0.05) percentages of oocytes at M II (maturation rate) and the lowest percentages of degenerated oocytes, followed by MDOs, while NDOs showed the lowest percentages, being 60; 10% for COCs, 46.9; 20.5% for MDOs and 38.9; 30.4% for NDOs, respectively. Cleavage rate was higher (P<0.05) for COCs than those of MDOs and NDOs (40.0 vs. 26.3 and 21.3%, respectively). Production rate of embryos at morula and blastocyst stages was not affected significantly by oocyte type. In conclusion**,** cumulus cell layer surrounding the bovine oocytes recovered from ovaries collected from abattoirs play very important roles for maintain viability of oocytes during vitrification, and successfully *in vitro* matured, fertilized and developed to blastocyst stage.

Keywords: Bovine oocytes, cumulus, denudation, vitrification, maturation, fertilization

INTRODUCTION

Achievement a high efficiency of bovine oocyte cryopreservation remains for years of great interest of researchers and practitioners working on *in vitro* production of bovine embryos and somatic cloning (Papis *et al.* 2013). Cryopreservation of oocytes from slaughtered animals has great value in increasing the availability of materials for basic research and their subsequent utilization for embryos production may provide an opportunity to replenish the endangered species gene banking and the genetic improvement of the livestock species (Nucharin Sripunya, 2011).

Several cryopreservation methods such as conventional (slow), equilibrium rapid freezing (vitrification) and ultra-rapid freezing have been used to preserve embryos and oocytes of many animal species resulting in the birth of live offspring (El-Shahat and Hammam, 2014). These methods

are highly dependent upon cryoprotectant agents (CPAs) that protect oocytes from damage during the freezing process (Sanchez-Partida *et al.* 2011). A higher number of competent oocytes for *in vitro* maturation and *in vitro* fertilization (IVM – IVF) to obtain superior transferable bovine embryos coupled with development of freezing technique through vitrification will entail more productivity from the non-descript animals (Dutta *et al.* 2013).

One of the factors that could affect oocyte quality following vitrification is the presence or absence of cumulus cells around the oocyte prior to cryopreservation (Karima *et al.* 2014). It have been reported that presence of cumulus cells is beneficial to the oocyte survival after cryopreservation (Li *et al.* 2006); as it may minimize the release of cortical granules and prevent premature zona reaction, thereby cumulus cell improve the *in vitro* fertilization rates after cryopreservation (Vincent *et al.* 1990). Cumulus cell removal prior to *in vitro* maturation or vitrification have shown to have a detrimental effect on oocyte morphology for immature vitrified buffalo (Gasparrini *et al.* 2007), equine (Tharasanit *et al.* 2009), mouse (Suo *et al.* 2009), bovine (Zhou *et al.* 2010), and goat (Purohit *et al.* 2012) oocytes. In goat, Purohit *et al.* (2012) found that cumulus compact oocytes are less vulnerable to cryo-injuries compared to their denuded counterparts.

The immature oocyte should be considered as a one functional unit with cumulus cells communicating each other via cellular projections penetrating across a zona pellucida. Undisturbed oocyte-cumulus communication and co-operation seem essential for adequate maturation process (Gilchrist, 2011), so an optimum cryopreservation method should provide protection for oocyte, cumulus cells and their intercommunication system.

Although immature oocytes vitrified without cumulus were matured and fertilized *in vitro* with acceptable efficiency (Modina *et al.* 2004; Luciano *et al.* 2009; Zhou *et al.* 2010), their further development as embryos was compromised (Modina *et al.* 2004). However, less information is known about the efficiency and the consequences of cryopreservation on immature and *in vitro* matured oocytes (Fausta *et al.* 2013).

Therefore, the present study aimed to evaluate the effect of, presence or absence of cumulus cells on immature bovine oocytes, on post-vitrification survival rate and developmental capacity.

MATERIALS AND METHODS

This study was carried out at the International Livestock Management Training Center (ILMTC), belonging to the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, in cooperation with Department of Animal Production, Faculty of Agriculture, Tanta University.

Oocte collection:

Bovine ovaries were collected from abattoirs and transported within 3 h to the laboratory in normal saline (0.9% NaCl) containing gentamicin (50 µg/ml) at 27-30°C. In the laboratory, extraneous tissues on the ovarian

surface were removed and the ovaries were washed three times in phosphate buffer saline (PBS, pH 7.3). All visible follicles (3-8 mm in diameter) were aspirated using 18-gauge needle attached to a 5 ml syringe containing 2 ml of DPBS with 20% fetal calve serum (FCS, sigma) and antibiotics (50 µg/ml gentamicin**)**.

Oocytes were examined under stereomicroscopy and classified according to their compaction, number of cumulus cell layers and homogeneity of ooplasm according to Ravindranatha *et al.* (2003) into 4 categories namely cumulus oocytes–complexes (COCs), expanded cumulus cells oocytes, natural denuded oocytes (NDOs), partial denuded oocytes. Only, COCs and NDOs (natural or mechanically denuted) were used in this study.

Mechanical denudation of oocytes:

Natural COCs were mechanically denuded (MDOs) by repeated pipetting in PBS supplemented 0.025% hyaluronidase solution (SIGMA, St. Louis, MO) till the complete separation of the cluster of cumulus cells according to Papis *et al.* (2013).

Vitrification of oocytes:

Different type of oocytes (COCs, NDOs or MDOs) were vitrified by open-pulled straw cryodevice (OPS). The vitrification procedures employed throughout this experiment were based on the methods originally designed by (Shayegh and Barati, 2011) with minor modifications. TCM-199 medium (Sigma) supplemented with 20% (v. v) of FCS as a basic medium (BM) as well as ethylin glycol (EG) and dimethyl sulfoxide (DMSO) as cryoprotectants were used. Different types of oocytes were vitrified by placing them in the first vitrification solution (V1, 10% EG+10% DMSO in BM) for 5 min, then they were transferred into the second vitrification solution (VS2: 20% DMSO, 20% EG, and 0.5M sucrose in BM) for 30 s, instantly oocytes were loaded in OPS and plunged in liquid nitrogen (LN2).

Thawing and evaluation of oocyte viability:

After storage for at least 2 weeks in LN2, all types of vitrified oocytes (COCs, NDOs and MDOs) were warmed by holding the OPS for 6 s in air and then agitating them in water bath at 20 °C for at least 10 s. The contents of OPS were expelled into Petri dish. To remove of intracellular cryoprotectants effects, oocytes were transferred in BM plus 0.25M sucrose for 5 min and then transferred to buffer solution (BS) plus 0.125M sucrose solution for 5 min and finally, the oocytes were washed twice in BS without sucrose for 5 min according to Hajarian *et al.* (2011) with minor modifications.

Oocyte viability (survival) was evaluated morphologically based on the integrity of the oolemma and zona pellucida; loss of membrane integrity (lysis) was obvious upon visual inspection as the sharp demarcation of the membrane disappeared and the appearance of the cytoplasm changed. The criteria used for assessing the post-thaw morphology of vitrified/warmed oocytes were as follows: Normal oocytes with spherical and symmetrical shape with no sing of lysis, and damaged oocytes (abnormal) with crack in zona pellucida, split in two halves, change in shape and leakage of contents.

The survival rate was calculated as the proportion of normal morphology oocytes against the total number of vitrified oocytes. Thereafter, morphologically normal oocytes were matured and fertilized *in vitro*.

***In vitro maturation**:*

All types of oocytes (COCs, NDO and MDO), morphologically normal post-thawing, were cultured in 100 µl of TCM-199+20% FCS+1 µg/ml oestradiol-17β (Sigma) and 50 µg/ml gentamicin covered with mineral oil (Sigma) in four-well culture plates (8-10 oocytes per droplet) for 24 h in a CO_2 incubator (5% CO_2 and in humidified air at 38°C). Oocytes were washed using phosphate buffer solution (PBS) containing 1 mg /ml hyaluronidase to remove the cumulus cells. Then, oocytes were washed two times with PBS supplemented with 2% bovine serum albumin (BSA), and loaded on clean slide. Slides were placed into fixation solution (3 ethanol: 1 glacial acetic acid) for 24 h and stained with 1% orcein in 45% glacial acetic acid. The nuclear status of oocytes was evaluated under a microscope and considered to be matured if they were at metaphase II stage with reduced number of chromatin, metaphase plate and extrusion of the 1st polar body (Purohit *et al.* 2012).

***Sperm preparation and in vitro fertilization**:*

Spermatozoa were recovered from frozen semen by swim-up separation in Tyrode's Albumin Lactate Pyruvate medium (TALP) previously described by (Parrish *et al.* 1988) with some modifications. One straw of frozen Holstein bull (0.25 ml) was thawed in a water bath at 37.5 ^{o}C for 1 min. The contents were washed twice in 5 ml of sperm-TALP supplemented by 6 mg/ml BSA (fraction V, A- sigma) by centrifugation at room temperature for 5 min to remove extender and cryoprotectants. The sediment of spermatozoa was resuspended in 5 ml of IVF-TALP containing 10 µg/ml heparin and then centrifuged for 5 min. The supernatant was removed leaving 0.25-0.5 ml of IVF-TALP and sperm pellet. The concentration of sperm was adjusted by adding IVF-TALP medium to reach 1x10^{6} sperm/ml as tested by a haemocytometer.

Fertilization Process:

After *in vitro* maturation, all matured oocytes from each type were washed two times in sperm-TALP, followed by final washing in fertilization medium (IVF-TALP). *In vitro* matured oocytes were transferred into Petri dish containing 100 µl droplets of fertilization medium at the rates of (10 oocytes per drop). Aliquots of the sperm suspension (8 µl) were added to each droplet containing matured oocytes. The oocytes and spermatozoa were co-cultured in an CO_2 incubator at 38.5˚C in 5% CO_2 in air, with saturated humidity for 24 h.

In vitro culture and embryo development:

After 24 h co-incubation of spermatozoa and oocytes from each type, the presumptive embryos were washed in sperm-TALP medium .The final washing was done in a culture medium consisting of TCM-199 supplemented with 3 mg/ml BSA, 20 µg/ml Na-pyruvate and 50 µg/ml gentamycin.

After co-incubation, presumptive embryos were placed in petri dish in the culture medium covered with mineral oil and incubated at 38.5^{o}C under 5% CO_2 in humidified air for 7 days. The frequency of morula and/or

blastocyst was recorded. Medium was replaced with fresh medium after every 48 h of culture (Dutta *et al.* 2013).

Statistical analysis:

Data were analyzed by analysis of variance using computer program of SAS (2000). The significant differences among group means were preformed using Duncan Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of oocyte type on:

Post-thawing recovery rate and quality of vitrified oocytes:

Data in Table (1) showed that the proportions of total and morphological normal oocytes found to be recovered post-vitrification were significantly (P<0.05) greater for COCs (79.0 and 76.0%) than those obtained for NDOs (56.0 and 51.4%). Also, post-vitrification recovery rate and proportion of morphological normal oocytes was significantly (P<0.05) greater for MDOs (67.8 and 63.2%) than those for NDOs (56.0 and 51.4%). However, proportion of damaged oocytes post-vitrification showed an opposite trend.

Table: (1): Effect of oocyte type on recovery and quality of vitrified oocytes.

Oocyte type	Vitrified oocytes (n)	Recovery rate (%)	Oocytes viability (%)	
			Viable	Damage
COCs	100	79.00±1.00[a]	76.00±1.00[a]	24.00±1.00[c]
NDOs	125	56.00±0.00[c]	51.43±1.43[c]	48.57±1.43[a]
MDOs	115	67.80±0.80[b]	63.17±1.98[b]	36.82±1.98[b]

[a, b] and [c]: Means within the same column with different superscripts are significantly different at P<0.05.

In accordance with the present results, Prentice *et al.* (2012) reported that the survival and developmental rate of bovine oocytes were higher when vitrified with enclosed cumulus cells than partially denuded cells. They showed that the survival rate and normal morphologically immature oocytes of goats was significantly higher for COCs than MDOs (86.73 and 89.22% vs. 80.31 and 94.12%, respectively). In buffalo, Karima *et al.* (2004) found that percentage of morphological normal buffalo oocytes (COCs) after vitrifying, was non-significantly differing with partially DOs, while there were significant (P<0.01) difference between COCs and NDOs (91.6 vs. 78.0%). Dhali *et al.* (2000) reported that the percentage of buffalo COCs found to be morphologically normal which varied from 89 to 96% for the two equilibration solutions and the two exposure times was not significantly different.

In this respect, Zhou *et al.* (2010) studied the effects of cumulus cells on bovine oocyte vitrification at the GV stage. They reported that the survival rate of vitrified oocytes was significantly higher for COCs than partially-denuded vitrified and control oocytes. Also, Babaei *et al.* (2006) reported a

high proportion of morphologically normal bovine oocytes (90%) were recovered after vitrification-warming using glass capillary micropipette.

Moreover, Nikseresht *et al.* (2015) showed that the survival rate of mice oocytes in the stepwise cumulus oocytes complexes and denuded oocytes group were significantly higher than those for the single-step NDOs. Mahmoud *et al.* (2013) reported that the rate of morphologically intact oocytes following vitrification / warming was high, ranging from 87.7% in straws to 90.8% in cryotops using a mixture of 3 M DMSO + 3 M EG.

It was suggested that the presence of the cumulus cells can reduce the adverse effects of DMSO on the oocytes (Johnson and Packering, 1987). Also, the presence of cumulus cells can minimize the release of cortical granules and premature zona reaction for zona hardening resulting in low fertilization rates (Vincent *et al.* 1990).

On the other hand, Chian *et al.* (2004) reported that bovine oocytes survival rates following vitrification are not affected by the presence or absence of cumulus cells. Also, Zhang *et al.* (2009) observed no difference in the survival rate of vitrified mature ovine oocytes with or without cumulus cells. The difficulty, in obtaining acceptable rates of survival and functionality for oocytes after cryopreservation, is due to the size of this cell and its unique morphologic characteristics (Martins *et al.* 2005).

Classification of damaged oocytes post-vitrification:

Various degree of damage were observed in abnormal oocytes post- vitrification included cracking zona pellucida, split into two halve, change in shape and leakage of cellular content (Table 2). Similar findings were observed by Dhali *et al.* (2000) for morphological abnormalities observed in buffalo oocytes after vitrification-thawing.

Proportion of damaged oocytes post-vitrification was not affected significantly by oocyte type, but leakage of cellular contents represented the highest frequency of damaged oocytes, while splitting in two halves was the lowest frequent abnormality observed. In agreement with the present results, Dhali *et al.* (2000) showed that morphological abnormalities observed in buffalo oocytes after vitrification-thawing were, crack in zona pellucida (69%), split into two halves (3%), change in shape (5%) and leakage of cellular contents (36%). Also in buffalo, Hammam and El-Shahat (2005) found that types of damages observed after vitrification-thawing were, 16.6% crack in zona pellucida, 50% shrinkage of cytoplasm and 33.3% leakage of cellular contents. However, Babaei *et al.* (2006) found that the percentage of morphologically damaged bovine oocytes was 10% and mostly cracking zona pellucid. Recently, El-Shahat and Hammam (2014) reported that among the damaged oocytes, cracking of zona pellucida was the most frequent abnormality observed compared to shrinkage of cytoplasm and leakage of cellular content (53.3 % *vs*. 20.0 and 26.6%).

Table: (2): Effect of oocyte type on classification of damaged oocytes post-vitrification.

Oocyte type	Damaged oocytes (n)	Oocyte classification (%)			
		Crack in zona pellucida	Splitting into two halves	Change in shape	Leakage of cellular contents
COCs	19.00	20.00±5.00	15.00±6.12	21.67±5.65	43.33±8.07
NDOs	34.00	26.67±3.23	17.62±2.77	29.52±0.95	26.19±2.38
MDOs	29.00	32.10±4.96	13.05±3.42	18.05±2.02	36.80±3.42

***In vitro* maturation of vitrified oocytes:**

Results in Table (3) revealed that COCs yielded significantly (P<0.05) the highest percentages of oocytes at M II (maturation rate) and the lowest percentages of degenerated oocytes, followed by MDOs, while NDOs showed the lowest percentages, being 60; 10% for COCs, 46.9; 20.5% for MDOs and 38.9; 30.4% for NDOs, respectively.

Table: (3): Effect of type of vitrified on *in vitro* maturation of vitrified oocytes post-thawing.

Item	N	Oocyte stage (%)				
		GV	GVBD	M I	M II	Degenerated
COCs	60	8.3±0.00	8.3±0.00	13.3±2.04	60.0±1.66[a]	10.0±1.66[c]
NDOs	36	11.4±2.85	11.1±2.78	8.2±3.36	38.9±2.78[c]	30.4±1.78[a]
MDOs	49	10.3±0.38	12.1±1.54	10.26±0.38	46.9±1.29[b]	20.5±0.76[b]

[a, b] and [c]: Means within the same column with different superscripts are significantly different at P<0.05. N: Number of viable oocytes. GV: Germinal vesicle. GVB: Germinal vesicle breakdown. M I: Metaphase I. M II: Metaphase II.

In harmony with the present results, Karima *et al.* (2014) showed the highest maturation rate of COCs, followed by partial DOs (73.7 vs. 64.8%), while NDOs showed the lowest maturation rate (53.0%). In goat, Purohit *et al.* (2012) found that immature COCs had significantly higher maturation rates compared to their MDOs (41.25 vs. 27.48%, respectively). In mice, Nikseresht *et al.* (2015) found that maturation rate in the stepwise COCs was significantly higher than in single step NDOs (73.2 vs. 50.8%).

Cumulus cells are closely related to oocytes, forming in fact one functional unit reflecting each other on auto- or paracrine manner (Gilchrist, 2011). Cumulus-oocyte interrelationship during oocyte maturation process seems crucial for achievement of optimum oocyte maturation and developmental capacity (Papis *et al.* 2013). The interactions between the oocyte and its surrounding cumulus cells at this stage are crucial for development of a matured oocyte (MII), the only cell type that can be fertilized to initiate a new organism (Sutton *et al.* 2003). It was reported that cumulus cell removal prior to *in vitro* maturation or vitrification has a detrimental effect on oocyte morphology for both immature and mature vitrified buffalo (Gasparrini *et al.* 2007), equine (Tharasanit *et al.* 2009), mouse (Suo *et al.* 2009) and bovine (Modina *et al.* 2004) oocytes. Cumulus cell removal increases the maturation promoting factor activity and accelerates the transition to metaphase stage and the redistribution of cortical

granules (Ge *et al.* 2008). Therefore, according to these findings and those reported in the present study, immature bovine oocytes can be vitrified after cumulus cell removal, and that they successfully mature (Modina *et al.* 2004). Finally, It has been reported that the absence of cumulus cells could provoke a possible shortcoming in protein synthesis and could reflect the levels of molecules involved in the regulation of meiotic and mitotic cell cycles (Combelles *et al.* 2005).

***In vitro* fertilization of vitrified-matured oocytes:**

Data presented in Table (4) showed that cleavage rate was significantly (P<0.05) higher for COCs than those of MDOs and NDOs (40.0 vs. 26.3 and 21.3%, respectively). However, production rate of embryos at morula and blastocyst stages was not affected significantly by oocyte type.

In accordance with the present results, Purohit *et al.* (2012) showed significantly (P<0.05) higher proportion of fertilized oocytes for immature COCs of goat than immature MDOs (31.7 *vs.* 25.0%, respectively).

Table: (4): Effect of category of vitrified oocytes on cleavage and development rates.

Item	Type of oocytes		
	COCs	**NDOs**	**MDOs**
Vitrified oocytes, n	80	75	80
Cleaved oocytes, n	32	16	21
Cleaved oocytes, %	40.00±0.00[a]	21.33±1.25[b]	26.25±1.25[b]
Embryonic stage:			
2-cell embryos, n	25	14	20
2-cell embryos, %	78.13±3.12	87.50±5.00	95.24±6.57
Morulae, n	4	1	1
Morulae, %	12.50±0.00	6.25±5.00	4.76±5.00
Blastocysts, n	3	1	0
Blastocysts, %	9.37±3.12	6.25±6.25	0

[a, b and c:] **Means denoted within the same row with different superscripts are significantly different at P<0.05.**

Also, Modina *et al.* (2004) observed a lower percentage of fresh NDOs reached the blastocyst stage in comparison with intact COCs (23.9 vs. 35.4, P<0.05). The blastocyst rate was 4.3%, being lower than those reported in the current study (6.25-9.37%). In fact, this is one of the evidences of successful oocyte cryopreservation for which, to date, only controversial and sporadic data are available for the bovine species (Hochi, 2003). Moreover, Zhou *et al.* (2014) achieved cleavage (63.5%) and blastocyst development (20.0%) after parthenogenetic activation of vitrified-warmed bovine oocytes similar to that from oocytes vitrified by the open-pulled straw method (57.0%) cleavage and 23.0% blastocyst development, respectively (Hou *et al.* 2005). Low blastocyst development rates (less than 10%) was reported by Martins *et al.* (2005). In buffalo, Hammam and El-Shahat (2005) found that oocytes vitrified at the immature cleaved and developed into morula and blastocyst stage after thawing were 20.0, 3.3 and 2.0%, respectively).The developmental capacity of the vitrified-thawed immature buffalo oocytes was

significantly lower compared to control (Hammam and El-Shahat., 2014; Yadav *et al.* 2008). Furthermore, Mahmoudi *et al.* (2005) reported that intact mouse oocytes had a higher developmental competence than denuded oocytes. In this respect, Hochi *et al.* (1998) vitrified immature bovine oocytes in straws by using a mixture of 40% EG, ficoll and sucrose as a vitrification medium. They reported 47.5% fertilization rate from the vitrified bovine oocytes. Abe *et al.* (2005) reported developmental rates to blastocyst of bovine GV-COCs, using Nylon-Mesh and exposer with stepwise cryoprotctant, being significantly higher than with the single-step vitrification.

In this respect, Zhou *et al.* (2010) reported that cleavage and blastocyst rates of cumulus-enclosed vitrified bovine oocytes of GV were significantly higher than those of partially-denuded vitrified and control oocytes.

Studies have shown that GV-stage oocytes which are stripped of cumulus cells have a reduced developmental capacity compared with that of cumulus-enclosed GV-stage oocytes (Mahmoudi *et al.* 2009). Therefore, Cumulus cells play an important role in oocyte maturation since they provide and transfer several known and unknown factors that are essential for normal nuclear and cytoplasmic maturation of oocytes and subsequent embryonic development after fertilization (Mahmoudi *et al.* 2012).

CONCLUSION

Based on the foregoing results in the present study, immature bovine oocytes can be vitrified after cumulus cell removal, and that they successfully mature and develop up to the blastocyst stage after *in vitro* fertilization as reported by Modina *et al.* (2004). Recently, El-Shahat and Hammam (2014) observed that the immature germinal vesicle stage oocytes tolerate the cryopreservation damage more efficiently compared to oocytes at metaphase-II and cumulus compact oocytes are less vulnerable to cryo-injuries compared to their denuded counterpart.

In conclusion, cumulus cell layer surrounding the bovine oocytes recovered from ovaries collected from abattoirs play very important roles for maintain viability of oocytes during vitrification, and successfully *in vitro* matured, fertilized and developed to blastocyst stage.

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التجميد بالتزجج والانضاج والاخصاب المعملى لبويضات الابقار مع أو بدون طبقات الخلايا الركامية

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تهدف هذه الدراسة الى تقييم تأثير وجود أو غياب طبقات الخلايا الركامية المحيطة بالبويضة على معدل الحيوية والإنضاج والقدرة التنموية لبويضات الابقار بعد التجميد. تم جمع المبايض من المجازر وشفط البويضات من الحويصلات المرئية الموجودة على المبيض ثم فحصها. تم فقط تجميد البويضات الجيدة والبويضات المعراة كليا طبيعيا والبويضات المعراة كليا ميكانيكيا ثم عمل اسالة لها ثم انضاجها واخصابها و زراعتها معمليا لمدة ٧ ايام لحساب معدلات الحيوية والانضاج والاخصاب وانتاج البلاستوسيست . وقد أظهرت النتائج أن العدد الكلى للبويضات المستردة ونسبة البويضات الطبيعية بعد التجميد بالتزجج كانت اعلى معنويا للبويضات الجيدة (٧٩.٠ و٧٦.٠ %) عن البويضات المعراة كليا طبيعيا (٥٦.٠ و٥١.٤ %). ايضا وجد ان معدل الاسترداد بعد التجميد بالتزجج ونسبة البويضات الطبيعية كانت اعلى معنويا للبويضات المعراة كليا ميكانيكيا (٦٧.٨ و٦٣.٢ %) عن البويضات المعراة كليا طبيعيا (٥٦.٠ و٥١.٤ %) . وجد ان نسبة البويضات المضمحلة بعد التجميد بالتزجج لم تتأثر بشكل معنوى بنوع البويضات ولكن لوحظ ارتفاع نسبة تسرب المحتويات الخلوية من البويضات المضمحلة و انخفاض نسبة البويضات المضمحلة المنقسمة الى نصفين. وقد اسفرت البويضات الجيدة عن ارتفاع معدل الانضاج المعلمى لها مع انخفاض نسبة البويضات المضمحلة (٦٠.٠ و ١٠.٠ %) تليها البويضات المعراة كليا ميكانيكيا (٤٦.٩ و٢٠.٥ %) بينما اظهرت البويضات المعراة كليا طبيعيا ادنى نسب لها (٣٨.٩ و ٣٠.٤ %) على التوالى . وكان معدل الانقسام للبويضات الجيدة اعلى معنويا عن كلا من البويضات المعراة كليا ميكانيكيا والبويضات المعراة كليا طبيعيا فكانت (٤٠.٠ مقابل ٢٦.٣ و٢١.٣ %) . ولم يتأثر معدل انتاج الاجنة فى مرحلة الموريولا والبلاستوسيست معنويا بنوع البويضات . وتشير هذه النتائج الى ان طبقات الخلايا الركامية المحيطة ببويضات الابقار المستردة من المبايض التى تم جمعها من المجازر تلعب ادوارا هامة جدا للمحافظة على حيوية البويضات اثناء التجميد ونجاح انضاجها واخصابها وتطورها معمليا حتى مرحلة البلاستوسيست.