In vitro fertilization and embryonic development of post-thawed buffalo oocytes vitrified with soybean lecithin

(Received: 15. 04. 2018; Accepted: 15.05.2018)

Hussein Y. S. [1], Ashour A. M. [2], Badr M. R. [3], and Gele M. A. [2]
[1]Biotechnology Research Department, Animal Production Research Institution, Dokki, ARC, Giza, Egypt.
[2]Animal Production Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.
[3]Artificial Insemination and Embryo Transfer Department, Animal Reproduction Research Institute, Al-Haram, ARC, Giza, Egypt.

ABSTRACT

Current study was carried out to the evaluate the impact of addition of zero (control), 1, 2.5, 5, 10% soybean lecithin to the vitrification solution media and its subsequent in vitro fertilization and embryo development of post-thawed immature and matured buffalo oocytes cryopreserved with stepwise vitrification. Although the addition of lecithin to the vitrification solution did not significantly affect in vitro fertilization(IVF) of both immature and mature oocytes, lecithin increased penetration and fertilization rates of immature (50.0 and 33.3%) and mature oocytes (52.6 and 36.8%) at the level of 5 and 2.5% respectively. Trials of embryo In Vitro culture(IVC) revealed that 2.5% addition of lecithin significantly improved ($p<0.05$) both blastocyst formation and total embryonic development (9.5 and 28.6% resp.) of vitrified-thawed mature oocyte than control ones after 7 days of culture. Similar insignificant trend was observed for vitrified-thawed immature oocytes at the level of 5% lecithin. Our results revealed that, soybean lecithin addition to vitrification solution might have a protective potential on vitrified buffalo oocytes after warming and their subsequent embryo development in vitro.

Keywords: *lecithin, buffalo oocytes, vitrification, IVF, IVC.*

INTRODUCTION

Widespread use of livestock oocytes for procedures such as *in vitro* embryo production, nuclear transfer or gene banking has dramatically increased interest in oocyte cryopreservation in the agricultural and scientific communities (Arcarons *et al.*, 2017). Oocytes are much more difficult to cryopreserve than cleavage-stage embryos (Aono *et al.*, 2013) which are particularly susceptible to cryodamage because of its size and water content (Moussa *et al.*, 2014). The large volume of oocyte decreases the surface-to-volume ratio, making it very sensitive to chilling and highly susceptible to intracellular ice formation and osmotic injury (Saragusty and Arav, 2011). Both of which are the main reasons of cellular disruption and low survival rate after warming (Mahmoud and El-Sokary, 2013). Post-warming survival rate of vitrified oocytes is the key success of *in vitro* embryo production (IVEP). Such production carried out through oocyte collection and a combination of *in vitro* maturation (IVM), fertilization (IVF) and culture (IVC), beside to each technique conditions (Kumar and Anand, 2012 and Alvarez *et al.*, 2013). These

techniques are severely hampered and still limited in buffalo because of its relatively low yield of oocyte per ovary (Purohit *et al.*, 2003) and poor recovery of total oocytes after warming (Waheed *et al.*, 2016).

The cryoinjery of oocytes may result in abnormal spindle associated with disorganized microtubules and chromosomes, altered distribution of cortical granules and increased polyspermy or on the contrary, zona pellucida (ZP) hardening that impairing fertilization (Rojas *et al.,* 2004). For these reasons, a practical and successful freezing method of buffalo oocytes is the key factor in buffalo IVEP and future of the commercial embryo transfer (Abd Allah, 2009). It was reported that in mouse (Jung *et al.,* 2014) and bovine oocytes (Aono *et al.*, 2013), 31 type of oocyte phospholipids (PLs) decreased and 5 increased with a decrease to several triglycerides in response to vitrification. Including membrane PLs, nine of which showed a significant reduction after vitrification-thawing processes that affects total number of survived oocytes available for *in vitro* maturation, fertilization and culture process (IVMFC).

The nonpermeating macromolecules is known to increase the vitrification solution viscosity, less toxic than other cryoprotectants (CPA),avoids formation of extracellular ice crystals and protect oocytes against ZP cracking (Moore and Bonilla, 2006). Soybean lecithin contains high contents of membrane PLs including phosphatidylcholine (PC), phosphatidylglycerol (PG), sphingomyelin (SM) and Cholesterol (Col) that might work as membrane stabilizer (Pitangui-Molina *et al.*, 2017).

The objective of the current research study is to separately evaluate the subsequent *in vitro* fertilization and embryonic development of post-thawed buffalo oocytes cryopreserved using stepwise vitrification procedure with addition of Soybean Lecithin (SL) to the vitrification solution (VS) media.

MATERIALS AND METHODS

Current study was carried out at Animal Reproduction Research Institute, Egypt. Soybean lecithin (SL, MC041) was purchased from Lab M Ltd. Co., Lancashire, UK. Meanwhile, the rest of all chemicals used in this study were purchased from Sigma Aldrich Co. St. Louis, Mo, USA. The pH value for all media was adjusted at 7.3-7.4 and osmolarity at 280-300 mOsmol/L and filtrated twice by 0.22-µm filter (Millipore, Germany) before usage.

Retrieval of oocytes

Briefly, ovaries were collected from slaughtered buffaloes, transformed to laboratory in NaCl solution (9 mg/ml) + antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin), washed twice in washing medium contains phosphate buffer saline (PBS) + antibiotics and rinsed in 70% ethyl alcohol for a few minutes. Compact cumulus-oocytes complexes (COC's, 2-8 mm follicle) were aspirated using a 18-gauge needle in 5ml washing media +3% bovine serum albumin (BSA).

Vitrification and thawing of oocytes

According to Mahmoud *et al.* (2013), the vitrification-warming procedure was done using basal medium (BM) containing PBS +20 % fetal calf serum (FCS) and antibiotics. A stepwise vitrification was carried out to all experimental groups as follows: step one, oocytes were loaded into vitrification solution 1; [VS1, BM + 12.5% (v:v) ethylene glycol (EG) + 12.5% (v:v) dimethyl-sulfoxide (DMSO)] for 2 min. step two, oocytes were loaded into VS2 [BM + 20% (v:v) EG+20% (v:v) DMSO] for 30 sec. Then, 3-7 oocytes

were loaded into 0.25 ml French straws with VS2, sealed and plunged directly into liquid nitrogen. Thawing was done seven days later by transferring straws into 37°C water bath for 30sec.; rehydrated thereafter in BM+0.25M and BM+0.15M sucrose for one min and 5 min respectively. The rehydrated oocytes were washed four times and transferred to fresh BM.

***In vitro* maturation (IVM)**

Both post-thawed and fresh oocytes with normal morphology were recruited for IVM. Oocytes were cultured in 100 µl of TCM-199+ 20% FCS+ 1 µg/ml oestradiol-17β + 50 µg/ml gentamicin covered with mineral oil in four-well culture plates at 38.5ºC, 5% $Co_2$for 22-24 h in humidified air. Maturation was assessed by expansion of cumulus cell mass (El-Shahat and Hammam, 2014).

Sperm preparation and *in vitro* fertilization (IVF)

Procedures of sperm capacitation and *in vitro* fertilization (IVF) were done according to (Hammad *et al.*, 2015). Swim up technique was used to separate the most motile spermatozoa in Tyrode's albumin lactate pyruvate medium (Sperm TALP) that was incubated in an atmosphere of 5% CO_2 in air at 38.5°C for 2 hours prior usage using the contents of two straws (0.25 ml) which were thawed at 37 ºC for a minute in the bottom of 15 ml of size centrifuge tube and washed twice by centrifugation in 5 ml of sperm-TALP+ 6 mg/ml bovine serum albumin (BSA) for 5 min. The supernatant was discarded leaving 0.25-0.5 ml of Sp-TALP and sperm pellet. The sediment of spermatozoa was suspended in 5 ml of IVF-TALP +10 µg/ml heparin and then incubated finally with an angle of 45^o in CO_2 incubator at 38.5°C. After 30 min of incubation, the entire supernatant was aspirated in IVF-TALP to adjusted sperm concentration to reach $2x10^6$ sperm/ml.

After warming, all matured oocytes subjected to IVF were washed two times in Sp-TALP, followed by final washing in IVF-TALP, transferred into Petri dish containing 50 µl droplets of IVF medium (5-7 oocytes/drop). Aliquots of the sperm suspension (10µl) were added to each droplet containing oocytes. Spermatozoa and oocytes were co-incubated (insemination, day 0) under mineral oil (38.5ºC and 5% CO_2 in humidified air). Eighteen hours post-insemination (pi), oocytes were washed with fresh medium and vortexed for a minute to separate the cumulus mass. Fixing and staining for fertilization assessment was done according to Purohit *et al.* (2012) with 1% orcein in 45% glacial acetic acid. Oocytes showed sperm head, swollen head in the vitellus (penetration) or penetrating head, with male and female pronucleus were considered as normal fertilization; meanwhile, occurrence of three or more pronuclei was considered as polyspermic (Nagina *et al.*, 2016).

***In vitro* culture and embryo development (IVC)**

All presumptive buffalo's zygotes were washed in Sp-TALP medium after IVF then washed with *in vitro* culture medium (IVC); consists of TCM-199 + 3 mg/ml BSA+20 µg/ml Na-pyruvate+50 µg/ml gentamycin, cultured thereafter in petri dish covered with mineral oil at 38.5ºC under 5% CO_2 in humidified air for 7 days. The media was replaced with fresh one every 48 h of culture. The frequency of total embryonic development (TED) was calculated from the number of morula and/or blastocyst that cleaved once at least after day 7 pi.

Experimental design

Current study was carried out in two individual experiments; which evaluate the impression of SL supplementation to VS media on post-thawed buffalo's oocytes *in vitro* fertilization and embryonic developmental competence. For each experiment fresh oocytes enclosed in a compact, more than three layers, of cumulus cells, and uniform cytoplasm were selected under stereomicroscope and divided into two main groups. The first group was assigned for direct immature oocyte vitrification with addition of zero (control), 1, 2.5, 5, 10% SL to VS media. In the meantime, the rest of oocytes underwent direct IVM process which were vitrified thereafter with the same levels of SL added to VS media. After warming, both major groups were divided in two subgroups to separately evaluate oocyte fertilization and embryonic development *in vitro*. The major group of immature oocytes was underwent IVM before both of IVF and IVC experiments.

Statistical analysis:

The experiment was replicated three times. Data were analyzed statistically with Chi-square analysis using Statistical Package for Social Sciences (SPSS V.20).

RESULTS

Influence of soybean lecithin addition to vitrifcation solution on *in vitro* fertilization of buffalo's oocytes.

***In vitro* fertilization of Post-warming immature oocytes.**

Addition of SL to the VS; did not significantly alter neither penetration nor fertilization rates of immature oocytes after thawing, *in vitro* maturation and *in vitro* fertilization (IVMF) compared untreated group (Table 1). However oocytes vitrified with 5% SL showed better penetration and fertilization rates with low polyspermia that showed non-significant decrease when compared with control and Lecithin 1%.than other groups.

Post warming mature oocytes *in vitro* fertilization

Similarly to immature oocytes, no such effect of SL added to VS observed on the post-thawed mature oocytes subjected to IVF as shown in Table 2. However, the level of 2.5% SL added to mature oocytes VS showed similar rates of penetration and fertilization close to those obtained from immature oocyte IVF trail.

Table (1): Impact of soybean lecithin added to vitrification solution on in vitro fertilization of immature buffalo's oocytes after warming.

Treatment	N*	Penetration (%)	Fertilization (%)	Polyspermia (%)
Control	32	13(40.6)	6(18.8)	7(21.9)
Lecithin 1%	36	15(41.7)	8(22.2)	7(19.4)
Lecithin 2.5%	39	17(43.6)	12(30.8)	5(12.8)
Lecithin 5%	36	18(50.0)	12(33.3)	7(19.4)
Lecithin 10%	38	18(47.4)	8(21.1)	10(26.3)

*N total number of post thawed IVMF live oocytes

Table (2): In vitro fertilization of post-thawed mature live oocytes vitrified with soybean lecithin.

Treatment	N*	Penetration (%)	Fertilization (%)	Polyspermia (%)
Control	39	15(38.5)	8(20.5)	7(17.9)
Lecithin 1%	36	17(47.2)	10(27.8)	7(19.4)
Lecithin 2.5%	38	20(52.6)	14(36.8)	6(15.8)
Lecithin 5%	35	17(48.6)	11(31.4)	6(17.1)
Lecithin 10%	37	16(43.2)	9(24.3)	7(18.9)

N* total number of post thawed mature live IVF-oocytes

Developmental competence of thawed buffalo oocytes vitrified with soybean lecithin

***In vitro* culture of embryos obtained from immature oocytes**

As presented in Table 3, current trail of IVMFC immature oocytes vitrified with SL, exhibited insignificant progress of cleavage, blastocyst formation and TED for 5% SL IVMFC vitrified oocytes.

Table (3): Developmental competence of post-thawed IVMFC buffalo's immature oocytes vitrified with soybean lecithin.

Treatment	N*	Cleavage (%)	Morula (%)	Blastocyst (%)	TED (%)
Control	24	4(11.8%)[a]	1(2.9%)	0(0)[a]	1(2.9%)[a]
Lecithin 1%	31	6(19.4%)[a]	2(6.5%)	0(0)[a]	2(6.5%)[a]
Lecithin 2.5%	33	7(21.2%)[a]	3(9.1%)	1(3.0)[a]	4(12.1%)[a]
Lecithin 5%	32	11(34.4%)[b]	4(12.5%)	2(6.3)[b]	6(18.8%)[b]
Lecithin 10%	28	4(14.3%)[a]	1(3.6%)	0(0)[a]	1(3.6%)[a]

N* total number of post thawed IVMFC oocytes

Developmental competence of post thawing buffaloe's matured oocytes

In contrary of post warmed IVMFC immature oocytes, although fresh matured and vitrified oocytes showed no significant advance of cleavage and morula stages, both blastocyst formation and TED (Table 4) exhibited significant developmental improvement ($p<0.05$) especially for the level of 2.5% SL (9.5 and 28.6% resp.) when compared with control oocytes after 7 days of IVC.

Table (4): Embryonic development of recovered mature, IVF and IVC buffalo's oocytes vitrified with soybean lecithin.

Treatment	N*	Cleavage (%)	Morula (%)	Blastocyst (%) **	TED (%) **
Control	41	8(19.5)	2(4.9%)	0(0)[a]	2(4.9)[a]
Lecithin 1%	47	13(27.7)	4(8.5%)	1(2.1%)[a]	5(10.6)[a]
Lecithin 2.5%	42	16(38.1%)	8(19.0%)	4(9.5%)[b]	12(28.6)[b]
Lecithin 5%	38	11(28.9)	5(13.2%)	2(5.3%)[a]	7(18.4)[a]
Lecithin 10%	40	7(17.5%)	2(10.0%)	0(0)[a]	2(10.0%)[a]

*N total number of post-thawed live mature IVF and IVC oocytes

**Different superscripts indicate significant differences within columns (Chi-Square $P < 0.05$)

DISCUSSION

Although, immature germinal vesicle (GV) stage oocytes tolerate the cryodamage more efficiently compared to matured oocytes (Purohit *et al.*, 2012), current results showed that using of SL added to VS of immature or mature oocytes did not significantly affect neither penetration nor fertilization rates after thawing. Pitangui-Molina *et al.* (2017) established that oocytes supplemented with PC during IVM process has no potential detrimental effect on oocyte maturation and subsequent *in vitro* fertilization.

On the other hand, current results revealed a significant embryo development of matured-vitrified oocytes with 2.5% SL compared to immature oocytes. Guyader-Joly *et al.* (1999) reported a significant (p<0.05) hatching rate (52 %) of post-thawed bovine embryos cryopreserved with addition of 1% SL, using slow freezing protocol, compared to control group (31.8%). All of presented data confirmed the theory of Pugh *et al.* (1998) who hypothesized that, although there was no effect of IVC of embryos with SM and/or Col containing-lecithin, both are important for embryos to survive post cryopreservation, since embryos that developed in the presence of lecithin failed to survive subsequent freezing. Moreover, embryo survival after cryopreservation is affected by both PC and SM modification which are structural units of functional membranes that determines the fluidity, permeability, and thermal phase behavior of cell membranes (Sudano *et al.,* 2013). Current IVC results of post-thawed mature oocyte turned the attention to the addition of 2.5% SL level that might equilibrate or stabilize membrane PLs compared to 5% SL-vitrified immature oocytes regardless of their early developmental stages. Additionally, SL thought as a better emulsifier that might promote CAP to distribute uniformly and reduce it's relieve toxicity of freeze-thawing process (Trotta *et al.*, 2002) which were obviously noticed during current IVC trails of the current study.

Earlier studies reported that, treatment of bovine oocytes with 2 mg/ml cholesterol-loaded methyl-β-cyclodextrin (CLC) before vitrification improved the cryotolerance of metaphase II (MII) oocytes than GV stage with relative differences in the expression of genes related to embryo development (Arcarons *et al.*, 2017). The expression of lipid metabolism related genes (CYP51) were down-regulated, meanwhile the expression of both apoptosis (BAX) and DNA methylation (DNMT3A) genes were up-regulated of bovine morula obtained from vitrified-thawed GV. Whereas the expression of the BAX gene in vitrified MII oocytes remained similar to control group regardless of CLC treatment with elevated expression of DNMT3A gene of untreated MII oocytes only. Last but not least, previous facts explain the current significant augmentation of embryonic development for post-thawed 2.5% SL treated mature oocytes compared to those 5% SL vitrifed-thawed immature oocytes. It might be that SL mimic the previously mentioned trend of CLC of up-regulation of DNA methylation genes with holding the expression of apoptosis genes in the steady state. Somehow, SL has a protective potential against oxidative stress and DNA deterioration of vitrified buffalo oocytes after warming, IVF and IVC which might be throughout the reduction of malondialdehyde production and increased total antioxidant capacity (Badr *et al.*, 2012).

CONCLUSION

Addition of Soyben Lecithin to vitrification media did not improve IVF of vitrified-thawed buffalo's oocytes. Meanwhile, addition of 2.5% SL to VS significantly enhance buffalo embryos developmental competence of vitrified-thawed mature oocytes paralleled to immature ones vitrified with 5% SL after warming and IVMFC process that exhibited insignificant progress of cleavage, blastocyst formation and TED. Hence, more studies are needed to prove whether SL alter the gene expression responsible of DNA methylation and apoptosis of IVPE diverted from post-thawed mature oocytes or not.

REFERENCES

Abd-Allah, S. M. (2009). *In vitro* production of buffalo embryos from stepwise vitrified immature oocytes. Veterinaria Italiana, 45, 3: 425-429.

Álvarez, C., García-Garrido, C. Tarongerand R. and González de Merlo G. (2013). In vitro maturation, fertilization, embryo development and clinical outcome of human metaphase-I oocytes retrieved from stimulated intra cytoplasmic sperm injection cycles. Indian J. Med. Res., 137(2): 331-338.

Aono, A., Nagatomo, H. Takuma, T. R. Nonaka, R. Ono, Y. Wada, Y. Abe, Y. Takahashi, M. Watanabe T. and M. Kawahara (2013). Dynamics of intracellular phospholipid membrane organization during oocyte maturation and successful vitrification of immature oocytes retrieved by ovum pick-up in cattle. Theriogenology. 79(8):1146-1152.

Arcarons, N., Morato, R. Vendrel, M. Yeste, M. Pez-Bejar, M. L. Rajapaksha, K. Anzar M. and Mogas T. (2017). Cholesterol added prior to vitrification on the cryotolerance of immature and in vitro matured bovine oocytes. PLoS One; 12(9):e0184714.

Badr, M. R.; Abd El-Malak, M. G. Mohammed K.M. and Ibrahim E. A. (2012). Effect of soybean lecithin on freezability and fertilizing potentials of bovine spermatozoa. Assiut Vet. Med. J., 58 (133): 153-162.

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.

Guyader-Joly, C., Ponchon, S. Durand, M. Heyman, Y. Renard, J. P. Menezo Y. (1999). Effect of lecithin on in vitro and in vivo survival of in vitro produced bovine blastocysts after cryopreservation. Theriogenology, 52:1193-1202.

Hammad, M.E., Gabr, Sh. A. El-Ratel I. T. and Amin E. A. (2015). Vitrification and in vitro maturation and fertilization of bovine oocytes with or without cumulus cell layer. J. Animal and Poultry Prod., Mansoura Univ., Vol. 6 (6): 427 – 439.

Jung, J., Shin, H. Bang, S. Mok, H. J. Suh, C. S. Kim K. P. and Lim H. J. (2014). Analysis of the phospholipid profile of Metaphase II mouse oocytes undergoing vitrification. PLoS One, 9(7), e102620.

Kumar, D. and Anand T. (2012). In Vitro embryo production in buffalo: basic concepts. Journal of Buffalo Science, 1, 50-54.

Mahmoud K. Gh. M. and El-Sokary M.M.M. (2013). Improvement of the efficacy of buffalo oocytes vitrification. Global Veterinaria, 11 (4): 420-431.

Mahmoud, K. Gh. M., El-Sokary, M.M.M. Scholkamy, T. H. Abou El-Roos, M.E.A. Sosa G.A.M. and M. Nawito (2013). The effect of cryodevice and cryoprotectant concentration on buffalo oocytes vitrified at

MII stage. Anim. Reprod., vol. 10 (4):689-696.

Moore, K. and Bonilla, A.Q. (2006). Cryopreservation of mammalian embryos: The state of the Art. ARBS Annu. Rev. Biomed. Sci.; 8:19-32.

Moussa, Shu, M., J. Zhang, X. H. Zeng F. Y. (2014). Cryopreservation of mammalian oocytes and embryos: current problems and future perspectives. Sci. China Life Sci, 57: 903–914.

Nagina, G., A. Asima, U. Nemat and A. Shamim (2016). Effect of melatonin on maturation capacity and fertilization of Nili-Ravi buffalo (*Bubalus bubalis*) oocytes. Open Vet. J., 6(2): 128-134.

Pitangui-Molina, C. P., Vireque, A. A. Tata, A. Belaz, K. R. A. Santos, V. G. Ferreira, C. R. Eberlin, M. N. Silva-de-Sá, M. F. Ferriani, R. A. and Rosa-e-Silva J.S. (2017). Effect of soybean phosphatidylcholine on lipid profile of bovine oocytes matured in vitro, Chemistry and Physics of Lipids, 204: 76–84.

Pugh, P.A., Ankersmit, A. E. L. McGowan and L.T. Tervit H.R. (1998). Cryopreservation of in vitro-produced bovine embryos: effects of protein type and concentration during freezing or of liposomes during culture on post-thaw survival. Theriogenology 50:495-506.

Purohit, G. N., Meena H. and Solanki K. (2012). Effects of vitrification on immature and in vitro matured, denuded and cumulus compact goat oocytes and their subsequent fertilization. J. Reprod. Infertil. 13(1):53-59.

Purohit, G.N., Duggal, G. P. Dadarwal, D. Kumar, D. Yadav R. C. and S. Vyas (2003). Reproductive biotechnologies for improvement of buffalo: The current status. Asian Australasian Journal of Animal Science, 16, 1071–1086.

Rojas, C., M. J. Palomo, J. L. Albarracin and Mogas T. (2004). Vitrification of immature and in vitro matured pig oocytes: Study of distribution of chromosomes, microtubules, and actin microfilaments. Cryobiol.; 49:211–20.

Saragusty J. and Arav A. (2011). Current progress in oocyte and embryo cryopreservation by slow freezing and vitrification. Reproduction, 141 1–19.

Sudano, M.J, Paschoal, D.M. Maziero, T.S. Rascado, R.R.D. Guastali, M.D. Crocomo, L.F. Magalhães, L.C.O. Monteiro, B. A. Martins A. Jr, Machado R. and Landim-Alvarenga F.D.C. (2013). Improving post-cryopreservation survival capacity: an embryo-focused approach Anim. Reprod., 10 :(3), 160-167.

Trotta, M.; Pattarino, F. and Ignoni T. (2002). Stability of drug-carrier emulsions containing phosphatidylcholine mixtures. Eur. J. Pharm. Biopharm., 53: 203-208.

Waheed, M. M., El-Shahat K. H. and Hammam A. M. (2016). Developmental competence of buffalo (*bubalus bubalis*) oocytes: Effect of oocytes quality, protein additives, hormonal supplement and type of capacitating agents. Buffalo Bulletin, 35 (3): 427-435.

الملخص العربي

الأخصاب المعملي والتطور الجنيني لبويضات الجاموس بعد الإساله والمحفوظة بالتزجيج مع ليسيثين الصويا

ياسر صلاح حسين[١]، عبد الله محمد عاشور[٢]، مجدي رمضان بدر[٣] ومحمود عود جيلي[2]
[١] قسم التكنولوجيا الحيوية معهد بحوث الانتاج الحيواني ،الدقي ، مركز البحوث الزراعية
[٢] قسم الانتاج الحيواني كلية الزراعة، جامعة الأزهر، القاهرة، مصر.
[٣] قسم التلقيح الأصطناعي ونقل الأجنة معهد بحوث التناسليات الحيوانية بالهرم ، مركز البحوث الزراعية

اجريت الدراسة الحاليه بهدف تقييم تأثير إضافة تركيزات صفر (مقارنة) و ١و ٢.٥ و٥ و١٠% من ليسيثين الصويا الى بيئة التجميد وتأثير هذه المعاملة على الإخصاب المعملي والتطور الجنيني لبويضات الجاموس غير الناضجة والناضجة بعد عمليتي التجميد بالتزجيج والإسالة. بالرغم من ان إضافة الليسيثين لمحلول التجميد بالتزجيج لم يؤثر معنويا على الإخصاب المعملي لكلاً من نوعي البويضات إلا انه ادى إلى زيادة في معدلات إختراق الحيوان المنوي وكذلك معدل الإخصاب للبويضات الغير ناضجة (٥٠.٠ و ٣٣.٣%) والناضجة (٥٢.٦ و ٣٦.٨%) عند المستويات ٥% و ٢.٥% من الليسيثين على التوالي. أظهرت تجارب التطور الجنيني ان اضافة الليسيثين بتركيز ٢.٥% ادى الى تحسن معنوي ($p<0.05$) لكلا من معدل تكون طور البلاستوسيست واجمالي معدلى التطور الجنيني (٩.٥% و ٢٨.٦% على التوالي) لبويضات الجاموس الناضجة بعد التجميد والإسالة مقارنة بالمجموعة غير المعاملة بعد سبعة ايام من التحضين. لوحظ اتجاة مشابه ولكنه غير معنوي للبويضات غير الناضجة بعد التجميد والإساله عند مستوى ٥% من اضافة الليسيثين. النتائج الحاليه أظهرت انه قد يكون لليسيثين الصويا القدرة على حماية بويضات الجاموس بعد التجميد والإساله وما يتبعها من التطور الجنيني معمليا.