Study on the Effect of Cysteine and Myo-inositol on *In Vitro* Maturation of Porcine Oocytes

B. K. Lee[1] and S. K. Kim*

College of Veterinary Medicine, Chungnam National University, Daejeon 305-764, South Korea
[1]Dept. of Animal & Animal Resource Science, Joonbu University

ABSTRACT

This study was carried out to investigate the effect of morphology of oocytes, kinds of media, cysteine and myo-inositol supplementation on IVM rate of porcine oocytes. Cumulus- enclosed oocytes were incubated in maturation NCSU-23 and TCM-199 medium with supplementation with 3, 5, 10, 20 mM myo-inositol and 0.05, 0.1, 0.5, 1.0 mM cysteine.

1. When classified by morphology, excellent, good and fair of cumulus-enclosed oocytes were incubated for 48 hrs and the IVM rate were 14.2±3.7%~58.7±4.0%, respectively. The rate were greater in oocytes with excellent cumulus cells than those without cumulus cells.
2. The IVM rate of oocytes cultured in TCM-199 and NCSU- 23 medium supplementation or non-supplementation with 1.0 mM myo-inositol were 7.5±4.5%, 45.0±4.8% and 4.4%, 42.5±4.2%, 18.0±5.2%, respectively. Supplementation with myo-inositol significantly increased the IVM rate of oocytes.
3. The IVM rate of oocytes cultured in NCSU-23 medium supplementation of 3, 5, 10, 20 mM myo-inositol for 48 hrs were 47.5±4.5%, 57.5±4.2%, 62.5±4.9%, 50.0±5.2%, respectively. The IVM rate of oocytes in NCSU-23 medium supplemented with 10 mM myo-inositol were significantly increased compared to control (42.5±4.0%).
4. The IVM rate of oocytes cultured for 48 hrs in NCSU-23 media supplement with 0.3, 0.5, 1.0, 2.0 mM myo-inositol were 50.0±4.5%, 62.5±4.2%, 52.5±4.9%, 45.0±4.2%, respectively. The IVM rate of oocytes in NCSU-23 medium supplemented with 10 mM cysteine were significantly increased compared to control (42.5±4.0%).

(Key words : porcine oocytes, cysteine, myo-inositol, kinds of media, IVM rates)

INTRODUCTION

Cysteine and myo-inositol is important for cytoplasmic maturation; the addition of cysteine, myi-inositol, EGF and β-ME to a maturation medium stimulated meiotic maturation (Chance *et al.*, 1979; Ding and Foxcroft, 1993; Berridge MJ. 1993; Abeydeera *et al.*, 1998; Yang, 2002; Quan *et al.*, 2004).

Investigations into factors affecting oocyte quality in conventional IVF cycles usually assess the oocyte maturity during its retrieval by examining the morphological appearance of the oocyte-cumulus complex (Imthurn *et al.*, 1996). Recent studies reveal that morphology of oocyte-corona-cumulus complex bears little relationship to oocyte maturity (Rattanachaiyanont *et al.*, 1999).

Myo-inositol is essential for the growth of eukaryotic cells and serves as a precursor for biosynthesis of inositol-containing phospholipids (Downes, 1989). Although previous reports suggest that the metabolism of phosphoinositides may be important in the induction of meiotic maturation in mammalian oocytes (Homa, 1991; Downes and Macphee, 1990). Results from the present study confirm and extend the findings of Pesty *et al.* (1994) by demonstrating a dose-dependent effect of MI on the normal kinetics of GVBD and a significant increase in the proportion of mouse oocytes that can progress to full meiotic maturation (Ishii *et al.*, 1981; Yoshida *et al.*, 1993; Foote and Simkin, 1993; Lafleur *et al.*, 1994; Hynes, 2000).

The cysteine supplementation into the medium for oocyte maturation *in vitro* plays important role on the rates of maturation, fertilization and development, indicating that increased GSH that is synthesized by the γ-glutamyl cycle and its synthesis is dependent on the availability of cysteine in the medium (Meister and Tate, 1976). It is similar to other observations reported by Chance *et al.* (1979) and de Matos *et al.* (1995). de Matos *et al.* (1996, 1997) reported that supplementation of

* Correspondence : E-mail : kskkim@cnu.ac.kr

β-mercaptoethanol, cysteine and cystine during *in vitro* maturation increased the GSH concentration. Particularly, an experiment (De Matos *et al.*, 1996) observed a 3-fold increase in GSH concentration when bovine oocytes were matured in the medium supplemented with 0.1 mM cysteamine. Similar was the observation by Takahashi *et al.* (1993) with the use of bovine oocyte in which a 3.6-fold increase in intracellular GSH after 24 hrs of culture in the presence of 50 uM cysteamine. However, an urgent subject need to be increasement of the developmental rate porcine immature oocytes are low than that of other animals.

In order to determine better conditions for *in vitro* maturation of porcine oocytes, this study also evaluated the effect of morphology of cumulus-oocytes-complexes, kinds of media, cysteine and myo-inositol supplementation during *in vitro* maturation of porcine oocytes.

MATERIALS AND METHODS

1. Collection of Oocytes

Porcine ovaries were collected at a local slaughterhouse and transported to the laboratory in sterile physiological saline containing 100 IU/ml penicillin G and 100 ug/ml streptomycin sulfate at 25~30℃. Oocytes were aspirated from medium size follicles with an 21 gauge fixed to a 10 ml disposable syringe. The cumulus-oocytes complexes (COCs) that had an evenly distributed cytoplasm and washed three times in oocyte maturation medium containing hormonal supplements.

2. *In Vitro* Maturation of Oocytes

Then each group of 50 COCs was cultured in 500 μl of TCM-199 (Whittaker, U.S.A.) medium supplement with 2 IU/ml hCG (Sigma, U.S.A.) and 10% FCS (Sigma, U.S.A.) which had previously been covered with mineral oil and equilibrated in a humidified atmosphere of 5% CO_2 and 95% air at 38.5℃.

Follicular oocytes was classified by morphology of oocytes as excellent (with multiple layers of compact cumulus cells and evenly granulated cytoplasm), good (with unevenly distribute cumulus cells) and fair (with free zoan pellucida). Cumulus-enclosed oocytes were incubated in maturation TCM-199 medium with supplemented with 3, 5, 10, 20 mM of myo-inositol and 0.05, 0.1, 0.5, 1.0 mM cysteine.

3. Assessment of Meiotic Stage

Oocytes were fixed in acetic acid : ethanol (1:3) solution for 24 h then stained using with 1% acetoorcein (Sigma, U.S.A.) or 10 μg/ml bisbenzimide (Hoechst 33342, Sigma, U.S.A.) and observed under an fluorescence microscope. The judgement of oocytes maturation *in vitro* was carried out depending on the criteria of maturation by cell and nuclear division, and survival rate or *in vitro* development by investigating embryo development.

4. Statistical Analysis

The results were expressed by treatment as mean±SD. For comparison of means, Duncan's multiple verification was performed using SAS package of general Linears Model procedures (SAS Institute).

RESULT AND DISCUSSION

1. Morphology and Media on IVM Rate

This experiment was carried out to investigate the effect of morphology and media on IVM rate of oocytes and the results were shown in Fig. 1. Oocytes was classified by morphology as excellent (with multiple layers of compact cumulus cells and evenly granulated cytoplasm), good (with unevenly distribute cumulus cells) and fair (with free zoan pellucida).

When the immature oocytes cultured in NCSU-23 for 48 hrs, excellent, good, fair class by morphological classification were 14.2±3.7%~58.7±4.0%, respectively. The IVM rate between control and excellent group of oocytes differed significantly ($p<0.05$). This result was similar to Jang *et al.* (2006) reported that the IVM rate of porcine oocyte cultured in TCM-199 for 24~48 hrs higher than cultured in good and fair group oocytes.

2. Medium and Myo-inositol on IVM Rate

This experiment was carried out to investigate the effect of kinds of media and medium supplementation with myo-inositol

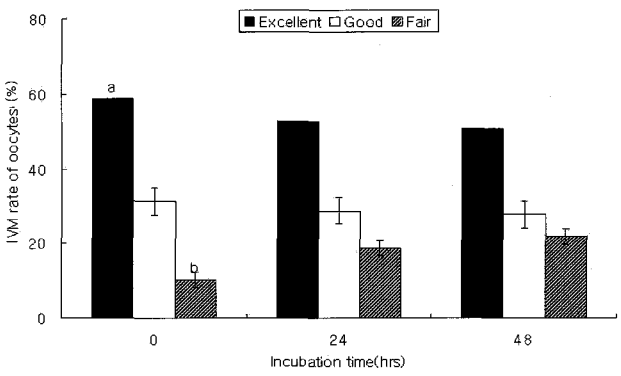

Fig. 1. Effect of morphological classification on IVM oocytes.

on IVM rate of oocytes were shown in Table 1.

The IVM rate of oocytes cultured for 48 hrs in TCM-199 and NCSU-23 media supplement with or without 1.0 mM myo-inositol were 57.5±4.5%, 55.0±4.4% and 45.0±4.8%, 42.5±4.2%, respectively. The IVM rate between control and myo-inositol supplementation group differed significantly ($p<0.05$). This result was similar to Chiu *et al.* (2002) reports that the GVBD rate of mouse oocyte cultured in MEM supplemented with 30 mM myo-inositol for 4 hrs was higher than cultured in control medium (94.1% and 68.7%).

3. Myo-inositol Concentration on IVM Rate

This experiment was carried out to investigate the effect of myo-inositol concentration on IVM rate of oocytes. As shown in Table 2, the IVM rate of oocytes cultured for 48 hrs in NCSU-23 media supplement with 3, 5, 10, 20 mM myo-inositol were 47.5±4.5%, 57.5±4.2%, 62.5±4.9%, 50.0±5.2%, respectively. The IVM rate of oocytes in NCSU-23 medium supplemented with 10 mM myo-inositol were significantly increased compared to the control (42.5±4.0%). Although the experimental animal was different, the above result was lower than Chiu *et al.* (2002) reports that the IVM rate of mouse oocyte cultured in MEM supplemented with 10, 20, 30 mM myo-inositol for 4 hrs were 78.0%, 83.1%, 94.1%, respectively.

4. Cysteine Concentration on IVM Rate

This experiment was carried out to investigate the effect of cysteine concentration on IVM rate of oocytes and the results were shown in Table 3.

The IVM rates of oocytes cultured for 48 hrs in NCSU-23 media supplementing with 0.05, 0.1, 0.5, 1.0 mM cysteine were 50.0±4.5%, 62.5±4.2%, 52.5±4.9%, 45.0±4.2%, respectively. The IVM rate of oocytes in NCSU-23 medium supplemented with 0.1 mM cysteine were significantly increased compared to the control (42.5±4.0%). This result was similar to Yang (2002) reports, in which the MII rate of bovine oocyte cultured in TCM-199 supplemented with 0.03 mM myo-inositol for 22 hrs were 66.03%, 63.0%, respectively.

REFERENCES

Abeydeera LR, Wang WH, Cantley TC, Prather RS and Dat

Table 1. Effect of with or without supplementation of myo-inositol in media on IVM rate

IVM condition		No. of oocytes examined	No. of at the stage of			Rate of IVM(%) (Mean±S.D)
Media	Myo-inositol		GV	GVBD	M Ⅱ	
TCM-199	−	40	5	12	23	57.5±4.5[a]
	+	40	8	14	18	45.0±4.8[b]
NCSU-23	−	40	7	11	22	55.0±4.4[a]
	+	40	9	14	17	42.5±4.2[b]

[a,b] Values within column with different superscript differ ($p<0.05$).

Table 2. IVM of oocytes after incubation for 48 hrs in myo-inositol- containing media

Myo-inositol (mM)	No. of oocytes examined	No. of at the stage of			Rate of IVM (%)
		GV	GVBD	M Ⅱ	
Control	40	8	15	17	42.5±4.0[a]
3	40	6	15	19	47.5±4.5
5	40	9	18	23	57.5±4.2[b]
10	40	4	11	25	62.5±4.9[b]
20	40	7	13	20	50.0±5.2

[a,b] Values within column with different superscript differ ($p<0.05$).

Table 3. IVM of oocytes after incubation for 48 hrs in cysteine-containing media

Cysteine (mM)	No. of oocytes examined	No. of at the stage of			Rate of IVM(%)
		GV	GVBD	MII	
Control	40	9	14	17	42.5±4.0[a]
0.05	40	4	16	20	50.0±4.5
0.1	40	5	10	25	62.5±4.2[b]
0.5	40	7	12	21	52.5±4.9
1.0	40	8	14	18	45.0±4.2

[a,b] Values within column with different superscript differ ($p<0.05$).

BN. 1998. Presence of β-mercaptoethanol can increase the glutathione content of pig oocytes matured *in vitro* and the rate of blastocyst development after *in vitro* fertilization. Theriogenology. 50(5):747-756.

Berridge MJ. 1993. Inositol triphosphate and calcium signalling. Nature, 361:315-325.

Chance B, Sies H and Boveris A. 1979. Hydroxy-peroxide metabolism in mammalian organs. Physiol. Rev., 59:527-605.

Chiu, TT, Rogers, MS, Law EL, Briton-Jones CM, Cheung LP and Haines CJ. 2002. Follicular fluid and serum concentrations of myo-inositol in patients undergoing IVF; relationship with oocyte quality. Hum. Reprod., 17:1591- 1596.

de Matos DG, Furnus CC, Moses DF and Balsassarre H. 1995. Effect of cysteamine on glutathione level and developmental capacity of bovine oocyte matured *in vitro*. Mol Reprod Dev., 42(4):432-436.

de Matos DG, Furnus CC, Moses DF, Martinez AG and Matkovic M. 1996. Stimulation of glutathione synthesis of *in vitro* matured bovine oocytes and its effect on embryo development and freezability. Mol Reprod Dev., 45(4):451-457.

de Matos DG, Furnus CC and Moses DF 1997. Glutathione synthesis during *in vitro* maturation of bovine oocytes: role of cumulus cells. Biol Reprod., 57(6):1420-1425.

Ding J and Foxcroft GR. 1993. Epidermal growth factor enhances oocyte maturation in pigs. Mol. Reprod. Dev., 39: 30-40.

Downes CP. 1989. Twenty-fifth Colworth medal lecture. The cellular functions of myo-inositol. Biochem. Soc. Trans., 17(2):259-268.

Downes CP and Macphee CH. 1990. Review: Myo-inositol metabolites as cellular signals. Eur. J. Biochem., 193:1-18

Homa ST. 1991. Neomycin, an inhibitor of phosphoinositide hydrolysis, inhibits the resumption of bovine oocyte spontaneous meiotic maturation. J. Exp. Zool.. 258(1):95-103.

Hynes AC, Sreenan JM, Kane MT. 2000. Uptake and incorporation of myo-inositol by bovine preimplantation embryos from two-cell to early blastocyst stages. Mol. Reprod. Dev., 55(3): 265-269.

Imthurn B, Macas E, Rosselli M and Keller PJ 1996. Nuclear maturity and oocyte morphology after stimulation with highly purified follicle stimulating hormone compared to human menopausal gonadotrophin. Hum. Reprod., 11(11):2387-2391.

Ishii T, Bannai S and Sugita Y. 1981. Mechanism of growth stimulation of L1210 cells by 2-mercaptoethanol *in vitro*. Role of the mixed disulfide of 2-ME and cysteine. J. Biol. Chem., 10;256(23):12387-12392.

Jang SH, Rhee MH and Kim SK. 2006. Study on effects of media, EGF, β-ME and hormones on IVM of porcine oocytes. Korean J. Emb. Trans., 21(3):247-253.

Lafleur MV, Hoorweg JJ, Joenje H, Westmijze EJ and Retel J. 1994. The ambivalent role of glutathione in the protection of DNA against singlet oxygen. Free. Radic. Res., 21(1):9-17.

Li J, Foote RH and Simkin M. 1993. Development of rabbit zygotes cultured in protein-free medium with catalase, taurine, or superoxide dismutase. Biol. Reprod,. 49(1):33-37.

Meister A and Tate SS. 1976. Glutathione and related gamma-glutamyl compounds: Biosynthesis and utilization. Annu. Rev. Biochem., 45:559-604.

Pesty A, Lefevre B, Kubiak J, Geraus G, Tesarik J and Maro B. 1994. Mouse oocyte maturation is affected by lithium via the polyphosphoinositide metabolism and the microtubule network. Mol. Reprod. Dev., 38(2):187-199.

Quan JH, Rhee MH and Kim SK. 2004. Localization of

angiotensin II in Korean bovine follicles and its effects on IVM/IVF of oocytes. Korean Dev. Biol., 28(1):59-63.

Rattanachaiyanont M, Leader A and Leveille MC. 1999. Lack of correlation between oocyte-corona-cumulus complex morphology and nuclear maturity of oocytes collected in stimulated cycles for intracytoplasmic sperm injection. Fertil. Steril., 71:937-940.

Takahashi M, Nagai T, Hamano S, Kuwayama M, Okamura N and Okani A. 1993. Effect of thiol compounds on *in vitro* development and intracellular glutathione content of bovine embryos. Biol. Reprod., 49(2):228-232.

Yang SY. 2002. Effects of cysteine supplementation during *in vitro* maturation on bovine embryo development and apoptosis. Graduate School of Kyeongsang National University Master thesis, 1-12.

Yoshida M, Ishigaki K, Nagai T and Chikyu M. 1993. Glutathione concentration during maturation and after fertilization in pig oocytes; Relevance to the ability of oocytes to form male pronucleus. Biol. Reprod., 49:89-94.

(접수일: 2007. 9. 5 / 채택일: 2007. 10. 2)