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THE ROLE OF VETERINARY SCIENCE TO SUPPORT MILLENNIUM DEVELOPMENT GOALs *and* THE 12th ASIAN ASSOCIATION OF VETERINARY SCHOOLS CONGRESS

FACULTY OF VETERINARY MEDICINE
UNIVERSITAS AIRLANGGA

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THE ROLE OF VETERINARY SCIENCE TO SUPPORT MILLENNIUM DEVELOPMENT GOALs AND THE 12th ASIAN ASSOCIATION OF VETERINARY SCHOOLS CONGRESS

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THE ROLE OF EXTERNAL HEAT SHOCK PROTEIN 70 (HSP70) SUPPLEMENTATION ON EXPRESSION OF CASPASE3 IN OOCYTE DURING VITRIFICATION

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ABSTRACT

The cryopreservation of animal and human oocytes has potential prospect for the development of Assisted Reproductive Technology (ART). One of the techniques used for oocyte cryopreservation is vitrification procedure to avoid the critical temperature and reduce cell damage caused by the formation of ice crystals on oocyte cryopreservation. The aim of this study was to evaluate the role of external HSP70 supplementation on the expression of pro-apoptosis related gen Caspase 3 in oocyte during vitrification. In Vitro Matured bovine oocytes were exposed to vitrificationcryoprotectant consisting of 40% Ethylene Glycol (EG) + 0.3M Sucrose (S) + different dose of HSP70 supplementation (0.25 µg/ml, 0.5 µg/ml, 1.0 µg/ml, and 2.5 µg/ml). Oocytes were kept in Open Pulled Straw, and then plunged into liquid nitrogen rapidly. After keeping the oocytes for 2-4 weeks, the frozen oocytes were thawed in a 30 C water bath and washed twice in 0.5M Sucrose. Examination of caspase 3 expression was done to fresh oocytes, in vitro maturated oocytes, and post-thawing oocytes with different dose of HSP70 supplementation by using immunofluorescence technique. In conclusion, supplementation of HSP70 into vitrification medium supressed the activation of caspase 3.

Keywords : bovine oocyte, vitrification, HSP70, caspase 3

INTRODUCTION

Various program and research related to provision of embryo have been conducted for the development of reproductive biotechnology especially in animal husbandry. Further studies are intended as providing frozen oocyte for alternative of embryos cryopreservation. Especially for the field of animal husbandry and veterinary, oocyte cryopreservation has potential prospect for developing Assisted Reproductive Technology (ART), particularly provision gamete bank for preservation and conservation of endangered animal oocytes (Gautam et al., 2008), as well as provides oocytes for embryo transfer (Sudardjad, 2003). The success of oocyte cryopreservation is not only important for preservation or conservation of endangered species, but also valuable to the availability of materials for research & development (Zhang et al., 2009).

The risks in cryopreservation process is stress temperature that will be happened when temperature return to normal after thawing or rewarming (Sonna et al., 2002;Gasparinni et al., 2007). Cryopreservation procedures can cause damage due to fragmentation of DNA and transcription of apoptosis - related genes, thereby reducing the ability of developing oocytes or embryos after freezing and thawing process (Park et al., 2006). Injury related to the changes in temperature of the warm - cold - and return to warm in vitrification process, in vitro maturation, vitrification, and especially during rewarming or thawing need to look out for possible damage to the structure of cell organelles and cell proteins.

Extreme temperature will cause stress oocytes. Presence of excessive stress inducesapoptosis and gene expression changes in response to condition of cells beyond the normal limits (Sonna et al.,2002). Apoptosis occurs through multiple pathways (Li et al., 2000;Lewin, 2004; Nasronudin,

2007). Pathway that leads to apoptosis through on-going interaction between the death ligand FasL and Tumor Necrosis Factor α (TNF-α) and its receptors, namely Fas receptor and TNF receptor will trigger the activation of protease. Procaspase 8 activity will break Bid causing release of cytochrome c from mitochondria that will trigger the formation of complex Apaf-1 and caspase 9, and protease activity will last until end in destruction of cell structure or apoptosis. In addition apoptosis can also occur when mitochondria is damaged so Mitochondria Permeability Transition Pore (MPTP) opens. The opening of MPTP will cause secretion of cytochrome c and other molecules. Apaf-1 together with caspase 9 activates caspase 3 as a pro-apoptosis related gen.

Stress cells increase the need of Heat Shock Protein 70 (HSP70) as a stress protein. The response of HSP70 inductive or synthesize is thought to be protective mechanism of cells to stress condition such as an extreme change of temperature by inhibiting activation of pro-apoptosisrelated gen (Jurisicova and Acton, 2004). Oocytes post vitrification with good viability was not followed by successful IVF. The low of IVF rate is presumably because HSP70 is produced by oocytes was not quite able to give protection during vitrification process, so it is considered necessary to add HSP70 supplementation in vitrificationcryoprotectant.Assessment needs to be done regarding the role of HSP70 external in cell protection against the extreme changes of temperature during oocyte vitrification by inhibiting activity of pro-apoptosis related gen, caspase 3.

MATERIALS AND METHOD

Ovaries were obtained from slaughtered house. Immature oocytes were aspirated from antral follicles. After being washed in medium TCM 199 for 3 times, then transferred to oocyte maturation medium TCM 199 + 3% BSA, FSH 0.01 mg/ml, LH 5 μ/ml, and gentamicin sulphate 50 mg/ml. In Vitro Maturation of the oocytes was carried out in 50 ml medium drops filled with 15 oocytes per drop, and then matured for 22 hours in an incubator containing 5% CO2 with temperature 38.5°C.Mature oocytes were exposed to vitrificationcryoprotectant 40% Ethylene Glycol (EG), 0.3M Sucrose (S) with different dose of HSP70 supplementation in formulations of P0 : EG+S, P1 : EG+S+HSP70 0.25 μg/ml, P2 : EG+S+HSP70 0.5 μg/ml, P3 : EG+S+HSP70 1.0 μg/ml, and P4 : EG+S+HSP70 2.5 μg/ml. Oocytes were kept in Open Pulled Straw and then plunged into liquid nitrogen rapidly. After keeping the oocytes for 2-4 weeks, the frozen oocytes were thawed in a 30°C water bath and washed twice in 0.5M Sucrose (Sun et al., 1995).Examination of caspase 3 expression was done to fresh oocytes, post IVM oocytes, and post-thawing oocytes with different dose of HSP70 supplementation by using immunofluorescence technique to determine the expression of caspase 3 in oocytes (Rantam, 2003). Data obtained were statistically analysed by Fisher's Exact Test.

RESULT AND DISCUSSION

An expression of HSP70 (green in colour) wasapparent in the oocyte examined by using immuno fluorescens technique (Fig. 1).

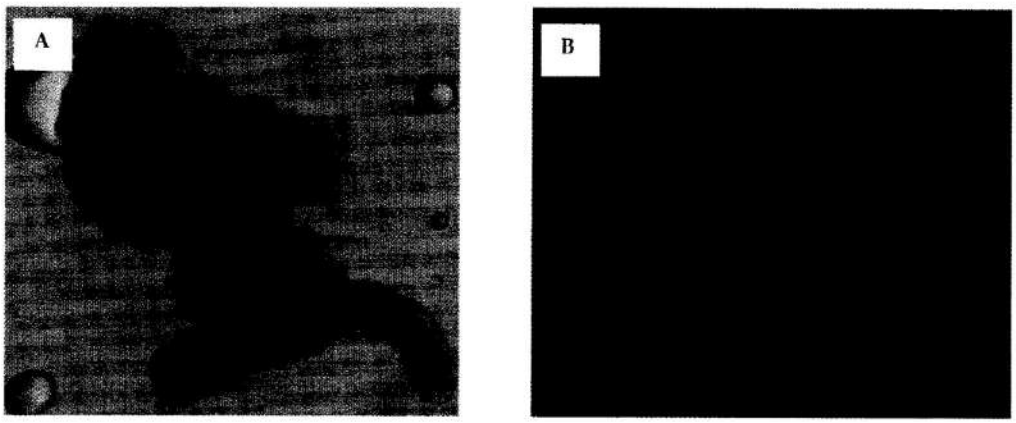

Fig. 1.Expression of caspase 3 in oocytes by immunofluorescenttechniqueusing FITC marker. Normal light (A) and fluorescencelight (B). Arrow (➔ oocytes (➔): cumuluscells. 100x magnification.

Observational data and statisticalanalysis of the expression of caspase3 by using Fisher's Exact Test, while the results of these tests are presented in the table. Caspase 3 expression in fresh oocytes, oocyte post IVM, P0, P1, P2, P3 and P4 respectively 12.5%, 20%, 80%, 57.1%, 33.3%, 28.6% and 16.7% oftotal sample. The table shows that there are significant differences ($p < 0.05$) the expression of caspase 3 amongst oocyte treatments. Expression of caspase 3 highest 80% wasfound at P0 which did not differ significantly ($p > 0.05$) with P1 and P2 of 57.1% and 33.3%, but showed a significant difference ($p < 0.05$) with P3, P4, oocytes post IVM and freshoocytes, respectively 28.6%, 16.7%, 20.0%, and 12.5%. Lowest expression of caspase 3 wasfound in fresh oocytes were significantly different ($p < 0.05$) with P0 butdidnot show significantdifferences in oocytepostIVM, P1, P2, P3 (Table 1)

In cells undergoing apoptosis, in addition to the release of cytochrome c from mitochondria into cytoplasm, the ongoing activation of caspase 3 is also a biomarker of cell death or apoptosis (Abu-Qare and Abou-Donia, 2001). As said by Kregel (2002) that when there is stress on the cell physiological conditions such as hyperthermia, hypothermia, hypoxia, hyperoxia, virus infection, the condition of acidosis,

Table 1 Expression ofCaspase 3 in Different Treatment of Oocytes

Oocytes	Number of Sample (n)	Expression of Caspase 3	Percentage of sample (%)
FreshOocytes	8	1	12.5[a]
Post IVMOocytes	10	2	20.0[a]
Post Vitrification Oocytes:			
P0	10	8	80.0[b]
P1	7	4	57.1[ab]
P2	9	3	33.3[ab]
P3	14	4	28.6[a]
P4	18	3	16.7[a]

Withincolumn, values with differentlettersarediffersignificantly($p > 0.05$).

Reactive Oxygen Species (ROS), loss of energy, ischemia-reperfusion, and others will activate inductive forms of HSP70 and increases expression in the cell.But Edward and Hansen (1996) revealed that mature oocytes can be found in large numbers of HSP70, however HSP70 content did not increase when there was temperature stress. Reduced ability to synthesize HSP70 at the end of oocyte growth and oocyte no longer induces HSP70 synthesis during temperature stress. According to Ju (2005) increased synthesis of HSPs inhibit apoptosis that occurs due to changes in temperature as termotolerans activity. Cleavageofcaspase 3 polymerase were inhibited by HSP70 showed that HSP7 0will block the process ofapoptosis in the cells.

The resultsofthisstudy show extreme temperaturechangesthatoccur in the process ofvitrification is suspectedofcausing HSP70 thatis stored and prepared by the oocytetoprotect cells fromenvironmentalinfluencesare not capable enough to provide protection against stress such as chillingtemperature and rewarminginjury (warm - cold - warm), so that the highest expression ofcaspase 3 werefound at P0 can not be inhibited by HSP70 stored in the oocyte. Suchconditionsrequiresupplementation of HSP70 is thoughtto be the inhibition ofcaspase 3 activation.HSP70 supplementation of 0.25 µg/ml for P1 and 0.5 µg/ml for P2 was able to activate caspase 3 inhibition, although not significantly different with P0. While the expression ofcaspase 3 in freshoocytes, post IVMoocytes, post vitrification oocytes with HSP70 supplementation as 1.0 µg/ml for P3 and 2.5 µg/ml for P4 showed significant differences in the post-vitrified oocytes without supplementation HSP70 in cryoprotectants, thuscould be expected to inhibit the incidence of apoptosis.

CONCLUSION

HSP70 supplementation into vitrification cryoprotectant is possible to be applied regarding the role of HSP70 external in cell protection against the extreme changes of temperature during oocyte vitrification by inhibiting activity of pro-apoptosis related gen, caspase 3.

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