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Influence of the Number of Cumulus Cells and the Expression of LH Receptor, Caspase 3, and P53 in Various Patterns of Cumulus Cells with the Success in Oocyte Maturation in the Process of in Vitro Maturation after Vitrification

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Abstract : To analyze the influence of cumulus cell and the expression of LH receptor, caspase 3, p53 on the success in oocyte maturation in the process of in vitro maturation after vitrification. Method: The research subjects were 60 oocyte of germinal vesicle stadium of cows (*Bos taurus*), divided into two groups: control group consisted of non-vitrified oocyte and exposure group consisted of vitrified oocyte (30% of v/v ethylene glycol, 18% of w/v Ficoll-0, and 0.3 M sucrose). Oocyte was divided into 3 groups (A,B and C) based on the cells pattern of oocyte cumulus in the germinal vesicle stadium of 2-8 mm with three layers of cumulus cells. The examination of the number of cumulus cells, using neubauwer calculating room and the expression of LH receptor, caspase 3, and p53 with ihc method, was done. After that, ivm were performed and their development were evaluated in 24 hours (first polar body). Result: Statistically, number of cumulus cells and expression of LH receptor had no significant correlation with maturity *($p>0.05$)*. Statistically, expression of caspase 3 and p53 had significant correlation with maturity *($p<0.05$)*. Conclusion, The expression of caspase 3 and p53 determined the success in oocyte maturation in the process of ivm after the vitrification
Keywords: Number of cumulus cell, lh receptor, caspase 3, p53, ivm,vitrification.

Introduction

Assisted Reproductive Technology (ART) facilitates natural conception which involves technology. ART includes conception process manipulation technique which one of them is direct manipulation on ooycite outside of the body with In Vitro Maturation (IVM).

In ART, in this case IVM technique, frozen storage method of human gamete and embryo has become an integral part of ART. One of the methods of frozen storage technological development was vitrification which reflected quick expansion in reproduction technology in the last two decades. The result was that various research methods of frozen storage in ART which evaluated the influence of oocyte and cumulus cell on oocyte maturation and prediction of oocyte quality had rapidly developed.

Some studies reported that oocyte cumulus cell indicated various patterns. Oocyte cumulus cell is influenced by intake pressure angles of pumpers during ovum pick up (OPU).[1,2] The pattern influenced the capacity of oocyte maturity in IVM technique.[2]

In vitrification, cryoprotectant concentration should be 3 to 4 times higher. This concentration can probably increase toxicity of the components which are contained in it.[3,4] The content of Reactive Oxygen Species (ROS) in oocyte which has been vitrified will increase.[5]Men. Monson and Rutledge[6] reported the existence of DNA fragmentation in oocytes in the post vitrification. DNA fragmentation and apoptosis was also reported to exist in cumulus cell.

DNA damage probably causes the increase in the expression of p53 and caspase 3 proteins as caspase executioner. Caspase 3 is the most important caspase in creating apoptosis in cells. It is the key of the main medium of apoptosis which is needed for tissue development and homeostasis.[7,8]

Moreover, CPA can also cause the incidence of cracked zone in cumulus cell as the result of vitrification. The incidence of cracked zone causes the decrease in the number of cumulus cells (NCC).[9,10]

This probably causes the decrease in LH receptor so that it influences oocyte maturity as the result of vitrification.

Materials and Method

The research used experimental method which was aimed to find out the influence of the number of cumulus cells, the expression of LH receptor, caspase 3, and p53 in various patterns of cumulus cell on the success in oocyte maturation in the process of in vitro maturation by conducting matching process in oocyte control group in the laboratory of Gladiol Infertility Clinic, Magelang, Central Java, Indonesia. The research subjects were 60 oocyte of germinal vesicle stadium of cows (*Bos taurus*), divided into two groups: control group consisted of non-vitrified oocyte and exposure group consisted of vitrified oocyte (30% of v/v ethylene glycol, 18% of w/v Ficoll-0, and 0.3 M sucrose).

Oocyte was divided into 3 groups, based on the cells pattern of oocyte cumulus in the germinal vesicle stadium of 2-8 mm with three layers of cumulus cells: (A) all cumulus cells (100%) which mostly covered oocyte, (B) almost all cumulus cells (>50%) covered oocyte, and (C) some of cumulus cells (<50%) covered oocyte.

Denuded technique by mechanical absorption, using pipette repeatedly was used to examine the number of cumulus cells, the expression of LH receptor, caspase 3, and p53 samples which had matched with the three patterns of oocyte cumulus cell. After that, examination on the number of cumulus cells was carried out by using neubauwer calculation room and the expression of LH receptor, caspase 3, and p53 by using immunohistochemical method.

The next step was that IVM was done in the three groups (TCM plus HMG 0.1 IU/mL plus 10% of Follicle Fluid) by evaluating its development within 24 hours. Its maturity quality was assessed by the appearance of the first body polar (IPB). Wilcoxon signed ranks test was used to find out the changes in non-vitrified and vitrified.

Logistic regression analysis would be used to test the correlation of the number of cumulus cells, the expression of LH receptor, caspase 3, and p53 vitrified toward maturity.

Results

This research would present the result of data analysis which included, mean and median of the number of cumulus cells, expression of LH receptor, caspase 3, and p53, correlation toward maturity.

Table 1. Mean and Median of the number of cumulus cells, expression of LH receptor, caspase 3, p53

Group	Variant	Pre	Post	Sig (2-tailed)*
A (n=17)	NCC	1.776.470 (2.400.000)	1.447.058 (1.000.000)	0,102
	LH	88,767 (96,050)	83,201 (86,600)	0,017
	p53	0,915 (0,250)	2,560 (2,690)	< 0,01
	Cas 3	5,034 (2,120)	16,926 (14,280)	0,002
B (n=15)	NCC	1.573.333 (1.600.000)	1.333.333 (1600.000)	0,102
	LH	93,894 (97,980)	79,714 (76,990)	0,001
	p53	1,126 (0,280)	2,892 (2,800)	0,004
	Cas 3	6,066 (0,235)	20,949 (24,940)	0,001
C (n=13)	NCC	1.323.076 (1.000.000)	1.046.153 (1.000.000)	0,109
	LH	88,197 (85,900)	71,123 (71,420)	0,001
	p53	2,306 (2,630)	2,895 (2,910)	0,249
	Cas 3	12,436 (14,280)	28,166 (28,920)	0,001
Combination (n=45)	NCC	1.577.777 (1.600.000)	1.293.333 (1.000.000)	0,008
	LH	90,311 (94,800)	78,550 (78,880)	< 0,01
	p53	1,387 (0,280)	2,767 (2,770)	< 0,01
	Cas 3	7,516 (2,530)	21,514 (22,530)	< 0,01

* Median Disparity Wilcoxon Signed Ranks Test.

Note: NCC = the Number of Cumulus Cells

The analysis on the correlation of the vitrified number of cumulus cells, LH receptor, caspase 3 and p53 with maturity was presented in Table 2 below:

Table 2. Variables in Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 5[b]	dispa_p53	-3.391	1.338	6.429	1	.011	.034
	dispa-cas3	.705	.278	6.434	1	.011	2.024
	Constant	5.892	3.023	3.799	1	.051	362.283

Table 2 which was indicated in five steps showed that expression of caspase 3, p53 statistically had significant correlation ($p < 0.05$) with maturity. Based on Table 2, it was also found that logistic equation was as follows:

It was also found in Table 2 that the most dominant factor (Exp (β)-value) was caspase 3 disparity. The next dominant is p53 disparity.

Discussion

This research which was referred to Table 1 indicated that the number of cumulus cells decreased in the three groups (A, B, C) non-vitrified and vitrified: the group A, 1.776.470 non-vitrified and 1.447.058 vitrified, group B, 1.573.333 non-vitrified and 1.333.333 vitrified, group C, 1.323.076 non-vitrfied and 1.046.153 vitrfied, that there was no significant difference in the three groups ($p>0.05$)

This indicated in this research that the effect which could cause big cracked zone in the cumulus cell did not occur. It also indicated that vitrification and warming technique conducted in this research was fairly good. However, it was also found in this research the number of cumulus cells which statistically had no significant correlation with maturity at $p > 0.05$.

In IVM, the number of LH receptors had the correlation with morphological pattern of oocyte cumulus cells and oocyte maturity.[2] Granulocyte cells were connected by large gap junction that effectively connects them into an integrated and functional sinsitium. This specialized cell connection is important for metabolic exchange and small molecule transportation among these adjacent cells. Besides that, granulocyte cells expand the process of its cytoplasm through pelucydezone in order to form gap junction by oocyte plasma membrane. Cyclic mono-phosphate adenosine (cAMP) produced by granulocyte cells could be a factor which is passed through oocyte through gap junction in order to maintain oocyte in maturation stop phase.[11]

In this research, referring to Table 1, it was found that the expression of LH receptors decreased in the three groups (A, B, and C) in non-vitrified and vitrified: the group A, 88.76% non-vitrified and 83.20% vitrified, group B, 93.89% non-vitrified and 79.71% vitrified, group C, 88.19% non-vitrified and 71.12% vitrified. There was significant difference in the three groups ($p<0.05$). However, in this research, it was found that the expression of LH receptor statistically did not have any significant correlation with maturity ($p>0.05$).

GVBD is started by the sudden increase in LH gonadotropic hormones; LH induces the loss of communication between oocyte and cumulus cell so that molecule regulator flow into oocyte stops and GVBD occurs.[12] This condition can occur if LH receptor in cumulus cell is adequate. A research conducted by Amansyah, Anwar & Fibrianto [2] reported that the number of LH receptors in cow oocytes in the post-IVM was 24-183 / glance space and maturation rate of 12% - 74% so that it could be concluded that LH receptors were correlated with oocyte maturity.

In this research, referring to Table 1, it was found that the expressions of caspase 3 increased in the three groups (A, B, and C) in non-vitrified and vitrified: group A, 5,03% non-vitrified and 16,92% in vitrified, group B, 6,06% non-vitrified and 20,94% vitrified, group C, 12,43% non-vitrfied and 28.16% vitrfied, There was significant difference in the three groups ($p < 0.05$).

In this research, it was found that caspase 3 had significant correlation ($p < 0.05$) with maturity, where vitrified oocyte cumulus cell increased in expression of caspase 3, and this increase reflected the incidence of the death of cumulus cell as the result of vitrification which would cause the decrease in maturity rate.

This was in accordance with the result of the research conducted by Lopes *et al*[13]on comparing the number of devitrified cumulus cells with the number of vitrified cumulus cells. Vitrification increased in the number of cumulus cells which die 13.7% proportionate with 2.6% ($p < 0.05$).

DNA damage in cells caused the increase in the expression of p53 which caused the incidence of GI arrest or apoptosis. The members of Apoptosis Stimulating Protein p53 (ASPP) specifically stimulated the function of p53 transactivation in proapoptotic gene promoter like Bax and p53 Inducible Gene 3 (PIG 3) and the increase in caspase 3 and the incidence of apoptosis in cells.[8,14]

The analysis on Confocal laser scanning microscope, conducted by Tharasanit [15]revealed that all cumulus cells underwent death in vitrified oocyte on the outer layers of cumulus cell, and there was no death of cell on the deepest layers. Using oocyte in Group A = cumulus cell which covered the whole (100%) oocyte.

In this research, oocyte used was different – Group B and Group C did not have complete cumulus cells and the area of cumulus cell surface was smaller which caused the exposure to cryoproptectant, vitrification, and warming process, and crystal were bigger in cumulus cell and oocyte. It probably brought about the death

rate in cumulus cell and the damage in cytoplasma and DNA, and all of these caused the decrease in maturity rate.

Moreover, cumulus cell also acts as a prevention from damage in oocyte because of vitrification, but we cannot certain what has caused the death of cumulus cell, whether it is caused by vitrification, warming, or CPA. CPA exposure to cumulus cell without vitrification could not influence the viability of cumulus cell: only 2.7% of cumulus cells died.[15]

In this research, referring to Table 1, it was found that the expression of p53 increased in the three groups (A, B, and C) non-vitrified and vitrified: the group A, 0,91% non-vitrified and 2,56% in vitrified, group B, 1,12% non-vitrified and 2,89% vitrified, group C, 2,30% non-vitrfied and 2.89% vitrfied, There was significant difference in Group A and B ($p < 0.05$). but there was no significant difference for group C ($p > 0.05$). statistically expression of P53 had significant correlation ($p < 0.05$) with maturity.

This was in accordance with the result of the research conducted by Leon *et al* [16]which compared morphologically the expression of p53 oocyte gene with cumulus cell, healthy cell and unhealthy cell in horse's oocyte in In vitro maturation (IMV) and was evaluated with Polymerase chain reaction (PCR). There was increased of p53 in cumulus cell.

This p53 protein was found in a very small number of non-exposed cells by stressor. However, when stressor occurred, whether in hypoplasia, damage in cellular integrity, and inappropriate oncogen, p53 protein would be expressed in a larger number in order to activate various channels toward the modification of post-protein translation and apoptosis stabilization.[8,17]

Protein p53 is a polypeptide which is expressed and coded by p53 gene that plays its role in guarding the completeness of cell integrated genome through transcription process. p53 gene is a tumor suppressor gene or tumor suppressor.[8] The accumulation of p53 will activate transcription as a gene which is involved in arousing the effect of anti proliferation or cycle stop and apoptosis activation. The result is that p53 protein is considered as a central monitor toward stressor which can direct cells to give appropriate response, either as a cycle stop or apoptosis.[8,17]

Conclusion

The expression of caspase 3 and p53 determined the success in oocyte maturation in the process of ivm after the vitrification.

References

1. Seong-Ho Yang, Weon-Young Son, San-Hyun Yoon , Yong Ko, Jin-Ho Lim. Correlation between *in vitro* maturation and expression of LH receptor in cumulus cellsof the oocytes collected from PCOS patients in HCG-primed IVM cycles. Human Reproduction, 2005, 20(8): 2097-2103.
2. Adek Amansyah, Moch Anwar, Yudha H Febrianto. The number of lh receptor could predict the success of oocyte maturity in the process of in vitro maturation, Indonesian J Obstetrics Gynecology, 2013, 1(4): 183-187.
3. Mahmoudi R, Ragardi Kashani I, Abbasi M, Amidi F, Sobhani A, Abolhasani F Saadipour Kh, Amiri I. In vitro maturation and fertilization capacity of mouse GV-stage oocyte following stepwise vitrification, Journal of Clinical and Diagnnostic Research 2008; 2: 1234-1239.
4. Juergen Liebermann, Vitrification of Oocytes and Embryos, Current Frontiers in Cryobiology, Prof. Igor Katkov (Ed.), In Tech. ISBN 978-953-51-0191-8, 2012, 169-184.
5. Somfai T, Ozawa M, Noguchi J, Kaneko H, Kuriani Karja NW, Farhudin M, Dinnyés A, Nagai T, Kikuchi K. 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 2007, 55(2):115-126.
6. Men H, Monson RL, Rutledge JJ. Effect of Meiotic Stages and Maturation Protocols on Bovine Oocyte's Resistance to Cryopreservation, Theriogenology 2002, 57: 1095–1103.

7. Berthelot-Ricou A, Perrin J, di Giorgio C,de Meo, M Botta A, Courbiere, B.Genotoxicity assessment of mouse oocytes by comet assay before vitrification and after warming with three vitrification protocols.Fertility and sterility 2013; 100(3): 882-888
8. Kumar V,Abbas KA,Fausto N & AsterCJ. General pathology in pathologic basis of disease Elsevier Saunders. 2010, 3-42.
9. Satish Kumar Ȧ, S. K. Gautam Ḃ and S.K Gahlawat. Cryobiological effects of cryoprotectants on morphology of cumulus oocyte complexes (COCs) of sheep oocytes using vitrification. International Journal of Animal Biotecnologyí 2013; 3 : 14-19.
10. Rameez Ali, Waquar Razzaque , Utsav Sharma, Anil Pandey, Kafil Hussain. Survivability of caprine oocytes vitrifed in conventional and open pulled straws, Turkish Journal of Veterinary and Animal Sciences 2014; 38: 707-711.
11. Kwang-Yul Chaand Ri-Cheng Chian, R.C.Maturation in vitro of immature human oocytes for clinical use, Human Reproduction 1998, 4 (2): 103-120.
12. Hang Yin H &Roger Gosden, In vitro growth and differentiation of oocytes, in Alan Trounson, Roger Gosden, Ursula Eichenlaub-Ritter, UE (ed.), Biology and pathology of the oocyte role in fertility, medicine, and nuclear reprogramming, 2nd edition, Cambridge University Press New York, 2013.
13. G Lopes, Leen Vandaele, Tom Rijsselaere, Ann Van Soom˙ A Rocha. DNA Fragmentation in Canine Immature Grade 1 Cumulus-Oocyte Complexes. Reproduction in Domestic Animals 2010; 45(6): 275–281.
14. BitaEbrahimi, Mojtaba Rezazadeh Valojerdi, Poopak Eftekhari- Yazdiand Hossein Baharvand.In vitro maturation, apoptotic gene expression and incidence of numerical chromosomal abnormalities following cryotop vitrification of sheep cumulus-oocyte complexes, J Assist Reprod Genet 2010; 27(5): 239–246.
15. T Tharasanit, S Colleoni, C Galli, B Colenbrander and TAE Stout. Protective effects of the cumulus-corona radiata complex during vitrification of horse oocytes.The journal of The Society for Reproduction and Fertility 2009; March 1(137) 391-401.
16. P.M.M Leon, V.F Campos, C. Kaefer, K.R Begnini, A.J.A. McBride, O.A. Dellagostin, F.K Seixas, J.C. Deschamps and T. Collares. Expression of apoptotic genes in immature and in vitro matured equine and cumulus cells. Zygote Cambridge University Press 2013; 21(03): 279-285.
17. TP Stricker & Vinay Kumar. Neoplasia. In:Vinay Kumar, Abul K. Abbas, Nelson Fausto, Jon C. Aster., editors, Robbins and Cotran Pathologic Basis of Disease, 8th Edition, Philadelpihia: Saunders Elsevier. ISBN 9781416031215, 2010, 284-292.

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