Copyright © 2014, American-Eurasian Network for Scientific Information publisher

American-Eurasian Journal of Sustainable Agriculture
ISSN: 1995-0748

JOURNAL home page: http://www.aensiweb.com/aejsa.html 2014 Special; 8(7): pages 39-43.

Published Online 2014 February 30. **Research Article**

Supplementation of Goat Follicular Fluid in TCM 199 Based Medium on In Vitro Matured Oocyte

Sri Wahjuningsih, Nurul Isnaini and Nolasco da Costa

Department of Animal Production, Faculty of Animal Husbandry, University of Brawijaya, Indonesia

Received: 25 June 2014; **Received**: 8July 2014; **Accepted**: 10 August May 2014; **Available online:** 30 August 2014

© 2014 AENSI PUBLISHER All rights reserved

ABSTRACT

During the process of in vitro maturation, oocyte requires proteins, hormones and growth factors for the survival and development. Unfortunetely hormones such as FSH, LH and estrogen are very expensive and imported. So alternative materials to replace these hormones with satisfactory results are being Follicular Fluid The research objective were to analyze the supplementation of Goat Follicular Fluid in medium on cumulus cell expansion and metaphase-II of the oocyte nucleus. The best quality of immature oocytes were matured in vitro for 26 h with basic medium TCM 199 + 10% FCS supplemented with different concentrations of Goat Follicular Fluid : 0%, 5%, 7.5%, 10%, 12.5% [v/v]. Observation of completely expanded of cumulus cells and the level of metaphase II were made on 26 h after in vitro maturation. The level of metaphase II was determined by staining 1 % aceto-orcein and viewing under an inverted microscope with 400 X magnification. The results showed that supplementation of GFF 0%, 5%, 7.5%, 10 %,and 12.5% yielded completely expanded of cumulus cells of 13.51%, 63.23%, 70.24%, 85.23%, and 65.42% , respectively while the oocyte reached to Metaphase-II was 12.45%, 52%, 66.42%, 75.20%, 67.27% for the respective GFF levels. It was concluded that supplementation 10% Goat Follicular Fluid yielded the highest rate of matured oocyte in vitro.

Keywords: In vitro maturation, Cumulus cells, Metaphase II, Goat oocytes.

INTRODUCTION

One of the obstacles in the implementation of embryo transfer in Indonesia is the availability of embryos Embryo production cost is still relatively high, especially if it produces embryos collected from superovulated donor female. In fact, there is a source of embryos that can be used and relatively cheap to produce embryos in vitro [1]. The technology of embryo production in vitro includes in vitro maturation [IVM], in vitro fertilization [IVF] and embryo culture to the morula or blastocyst stage. Immature oocytes are very helpful to the process of fertilization in vitro maturation. Ovaries can be obtained from slaughterhouse. Oocytes obtained from females ovarian cycle phase regardless of lust, straight oocytes retrieved from follicles diameter 2-6 mm and stored in a suitable medium and then stimulated maturation process. During the process of in vitro maturation, oocyte requires proteins, hormones and growth factors for the survival and development. Medium used should be made as closely as possible to the conditions of oocyte maturation in vivo so as to keep the gap junctions between oocytes and cumulus cells that surround it. Supplementation of gonadotrophin and steroids in the maturation medium will increase the oocytes potential for fertilization and embryonic development so conditions were similar to those in vivo [2]. Gonadotropin and estrogen hormone accelerate the germinal vesicle fusion and enable the nucleus division [3]. Supplementation of Follicle Stimulating Hormone [FSH], Luteinizing Hormone [LH] and estradiol 17 B also caused expansion of cumulus cells [4]. Previous research showed that goat follicular fluid contained p90rsk protein expression with a molecular weight of 22 kDa and ERK 2 with a molecular weight of 45 kDa, FSH and estrogen concentrations [1.42 ± 0:15 IU / L and 11:44 ± 0.08 pmol / L] in the small follicle [1.45 ±

Corresponding Author: Sri Wahjuningsih, Department of Animal Production, Faculty of Animal Husbandry, University of Brawijaya,, Indonesia 65142,
Tel: 62341553513; Fax: 62341584727; E-mail: yuning@ub.ac.id

0:16 IU / L ± 0:26 and 13:32 pmol / L] in large follicles. This study proves GFF role in activating Maturation Promoting Factor [MPF] through the Mitogen-Activated Protein Kinase [MAPK] so GFF has the opportunity for supplementation of oocyte maturation medium [5]. Follicular fluid contains many ingredients that stimulate oocyte maturation [6,7].

Objecties:

The research objective were to analyze the supplementation of Goat Follicular Fluid [GFF] in medium on cumulus cell expansion and metaphase-II of the oocyte nucleus.

Materials and Methods

Goat follicular fluid collection:

Goat follicular collection was performed by aspiration of 2-6 mm diameter follicles using a syringe needle size 18 G, centrifuged 600Xg for 10 min , filtered using milipore 0,22 μm, in-activated at a temperature of 56^0 C for 30 min and stored in the freezer.

Preparation of oocyte aspiration medium:

Oocyte aspiration was TCM199 medium [Gibco, Cat no. 21,200,076] were added Hepes [Sigma], $NaHCO_3$ [Sigma] and 10% Fetal Calf Serum [Gibco, Cat no.39N8255]. medium pH of 7.2-7.4. This media is filtered using a 0.22 μm membrane filter [Sartorius], incubated in 5% CO_2 incubator, temperature 38.5 ^{0}C overnight

Oocyte maturation:

Goat ovaries was brought to the laboratory within no more than 3 h after slaughtered in 0.9 % saline solution at a temperature of 25-35^{o}C. Ovaries were washed with medium and aspirated selected collection of ovaries. Oocytes aspirated from follicles with a diameter of 2-6 mm by using a syringe with a needle size of 18G mixed with Tissue Culture Media [TCM] 199 +FCS 10 %, incubated in waterbath at 38 ^{o}C for 10 min. Oocytes were selected based on layers of cumulus cells,morphologically bright and uniform cytoplasm, then maturated in TCM 199 + FCS 10% basic medium and supplemented with 0%,5 %,7.5 %,10% and 12.5 % GFF. Maturation was performed in groups of 10 oocytes in 50 μL of each maturation medium for 26 h in incubator at 38.5 ^{o}C, 5 % CO_2 Evaluation of oocyte maturation

Evaluation of oocyte maturation was done by observing completely expanded cumulus cell by inverted microscope with 400 X magnification. The level of metaphase II was done by staining with 1% aceto-orcein [8].

Statistical analysis:

Each treatment was 10 replicated ten times. The data of the completely expanded cumulus cells. Analysis of variance was used to analyze significant difference among treatment [p<0.05]. Duncan's multiple range test was used to compare the means of each treatment.

Completely Expanded Cumulus Cells:

There were significant differences in percentage of completely expanded cumulus cells among concentration of goat follicular fluid [Table 1].

Table 1: Effect of different concentration of goat follicular fluid on Completely Expanded Cumulus Cells.

Concentration of Goat Follicular Fluid	Completely expanded cumulus cells[%]
0 %	$13.51 \pm 2{,}13^a$
5 %	63.23 ± 6.63^b
7.5 %	70.24 ± 5.71^c
10 %	85.23 ± 7.52^d
12.5 %	65.42 ± 4.25^b

Different notations of the same column indicate significantly different [P <0.05]

The study showed that supplementation of GFF gives significant effect on completely expanded cumulus cells [P <0.05]. It can be seen that when oocytes were cultured in TCM 199 + 10% FCS only a few cumulus cells were completely expanded. While the oocytes were cultured with TCM 199 + 10% FCS + GFF 10% had the highest number of completely cumulus cells [85.23%]. Supplementation goat follicular fluid responded positively to the expansion of cumulus cells. Cumulus cells will expand and bonding among the cumulus oophorus will be loosened. Cumulus cells have a role as a specific tool to transfer the signal transduction mechanism of gonadotrophin into the oocyte through the gap junction. Contact between granulosa cells with oocytes through gap junctions to inhibit oocyte maturation by inhibiting the meiotic process. Reducing the number of gap junctions may increase the frequency of oocyte maturation. The close relationship between the oocyte cumulus cell oocyte maturation resulted stuck to not undergo meiosis. If the expanded cumulus cells will lead to decreased gap junction number, then the course of meiosis inhibition is reduced. The cumulus cells play a role in the maturation process of cumulus cell expansion because of the nucleus role in creating a microenvironment for oocyte and cytoplasm. Cumulus cells not only play a role in the process of oocyte meiosis but also can be an indicator of maturation [9].

Cumulus cells are granulose cells attached to oocytes and walls serve as an agent of communication between cells and hormonal mechanisms because there are many FSH and LH receptors in the cumulus cells. Cumulus cells will be stimulated due to increased gonadotrophin hormone activity and cellular metabolism. There are FSH and estrogen present in GFF. High levels of FSH and LH in the GFF to stimulate follicular development and granulosa cells and theca cells so as to increase the secretion of estrogen as well as LH and FSH stimulates the maturation of acolytes contained within follicles.

Moreover leptin also found in GFF. Leptin is a 16-kDa peptide hormone known to play a role as a modulator of reproductive functions especially the maturation of oocytes [10]. Leptin significantly increases the proportion of oocytes reaching metaphase II and an increase in the content of cyclin-B1 M II oocytes at this stage, it was proved that leptin acts on the nucleus and cytoplasmic maturation. Besides hormones, GFF also contain ingredients that can stimulate oocyte maturation, the Insulin Growth Factor [IGF-1], Transforming growth factor [TGF] [6].

Metaphase-II of oocyte:

Oocyte meiotic process can be observed through the nucleus in the form of phase transformation: Germinal vesicle [GV], germinal vesicle Break Down [GVBD], Metaphase I [MI] to Metaphase II-[M-II].

The level of supplementation GFF was significantly affected metaphase-II oocyte numbers [Table 2].

Table 2: Effect of different concentration of goat follicular fluid on metaphase-II oocyte [%].

Concentration of Goat Follicular Fluid	Metaphase-II [%]
0 %	$12,45 \pm 2,15^{a}$
5 %	$52,00 \pm 2,43^{b}$
7.5 %	$66.42 \pm 3,18^{c}$
10 %	75.20 ± 8.22^{d}
12.5 %	67.27 ± 3.29^{c}

Different notations of the same column indicate significantly different [P <0.05].

Supplementation 10% GFF gave the highest percentage of Mt-II oocytes [p <0.05]. The quality of cytoplasm and cumulus cells are both very important factors in the process of oocyte maturation. Morphological observations can be used for selecting the oocytes before fertilization. Nucleus and cytoplasm of matured oocytes are strongly influenced by the presence of cumulus cells that surround it. Oocytes were removed from cumulus cells when culture in vitro, a low level of maturation or incomplete oocyte maturation occurs [11]. Male pronucleus formation and early embryonic development after sperm penetration is inhibited if acolytes have no cumulus cells matured. In vitro maturation medium should be close to the same environmental conditions in vivo in the female reproductive tract of the animal.

Supplementation with 10 % GFF gave the highest percentage of Mt-II oocytes. Percentage of nucleus which reached metaphase II oocytes in TCM199 medium +FCS10% + 10% GFF was caused germinal vesicle fusion can accelerate and enable the process of reduction division nucleus. The process of meiotic maturation of oocytes involves activation of various signal transduction pathways to activate the Maturation Promoting Factor [MPF], which consists of the catalytic subunits, namely p 34cdc2 and regulatory subunits of cyclin-B. Recent research reveals that the regulation of oocyte maturation involves the mitogen-activated protein kinase [MAPK] cascade which is a major regulatory system that functions in parallel and interacts with the MPF in controlling the oocyte meiotic cell cycle progression. MAPK is also known as extracellular-regulated kinase [ERK] [12] then ERK2 and p90rsk are expressed [5]. At the cumulus cells, MAPK activity is likely to trigger the expression of Meiosis Activating Sterol [MAS] from cumulus to oocyte through gap junctions and will induce MPF.

Some opinions state that p90rsk have a major role in the maturation of oocytes that alleged that p90rsk activity should be high in the oocyte maturation process. Maturation Promoting Factor [MPF] plays an important role in encouraging the continuation of meiosis, other kinases are also activated prior to or simultaneously with MPF during meiosis reinisiation. Activation of two isoforms of the MAPK, ERK1, and ERK2 with different molecular weights during oocyte maturation and activation require an active protein synthesis [13].

Follicular fluid plays an important role in folliculogenesis, maturation of oocytes, granulosa cells, luteinizing and ovulation [14] Several studies have shown that pre ovulation, follicular fluid as a medium or as a supplement that is able to stimulate the natural environment on the maturation of oocytes [15,16]. Other researchers also mentioned that follicular fluid gives a favorable condition for granulosa cells [17,18,19] and endometrial cells by stimulating the maturation, growth and proliferation of cells. Results of this study indicate that there was a positive effect of follicular fluid on nucleus maturation.

Maturation in vitro of oocyte in culture media which supplemented GFF increased maturation rate

because it contains protein, glucose, fatty acids as a source of nutrients, gonadotropin hormones, estrogen and growth factors that are important to the process of oocyte maturation and embryo development in vitro. Growth factors has an important role in the regulation of oocyte maturation, particularly through cumulus cells such as Epidermal Growth Factors [EGF], Transforming Growth Factors [TGF alpha], and Insulin Growth Factors-I [IGF-I] were important for the maturation of oocytes in vitro.

The results of this study indicate that the level of cumulus expansion by increasing the achievement of metaphase II. The rate of expansion or proliferation of cumulus cells resulted in an increased number of high-metabolites that enter the oocyte such as inorganic ions, amino acids, sugars, nucleotides and cAMP through gap junctions. More important things in the process of oocyte maturation in vitro is the maturation of the oocyte nucleus, because the process of in vitro fertilization will be successful if it was supported by a nucleus maturation had reached metaphase-II

Completely maturation of the nucleus and the cytoplasm was strongly influenced by the presence of some of the surrounding cumulus cells. Oocytes were removed cumulus cells when cultured in vitro, rate of maturition level was low. Formation of the male pronucleus and early embryonic development after sperm penetration was inhibited if oocytes which have no cumulus cells matured [20].

Conclusions:

1. Supplementation of goat follicular fluid 0%, 5%, 7.5%, 10%, 12.5% gave completely expanded cumulus cells of 13:51%, 63.23%, 70.24%, 85.23%, 65.42% respectively, while the oocyte reached Mt-II oocytes was 12.45% , 52%, 66.42%, 75.20%, 67.27% for the respective goat follicular fluid level.
2. Supplementation of goat follicular fluid 10 % in maturation medium in vitro gave the highest rate of matured oocyte.

Acknowledgements

The authors wish to thank the Directorate General of Higher Education, Ministry of Education and Culture, Indonesia which has financed the implementation of this research through the Research Grants Competency.

Authors' Contribution:

Dr. Sri Wahjuningsih and Dr. Nurul Isnaini developed the idea and had an important role in the result and material section. Nolasco da Costa, M.Sc. performed the statistical analysis, the discussion and the abstract submission.

Financial Disclosure:

There is no conflict of interest.

Funding/Support:

The project was funded by Directorate General of Higher Education, Ministry of Education and Culture, Indonesia which has financed

References

1. Hoque, S.A.M., S.K. Kabiraj, M.A.M. Khandoker, A.K. Mondal, M.A. Tareq, 2011. Effect of coolections techniques on cumulus oocytes complexes [COCs] recovery, in vitro maturation and fertilization of goat oocytes. *African Journal of Biotechnology*, 10: 9177-9181.
2. Gordon, I., 1994. Laboratory production of cattle embryos. Cab. International. Cambridge, pp: 55-65.
3. Eppig, J.J., M.M. Viveiros, C. Marin Bivens and R. De La Fuente, 2004. Regulation of mammalian oocyte maturation in : The Ovary. 2nd ed. San Diego,C.A. Elsevier Academic, pp: 1245-1256.
4. Edry, I., S. Sela-Abramovich and N. Dekel, 2006. Meiotic arrest of oocytes depends on cell-to-cell communication in the ovarian follicle. *J. Molecular Cell Endocrinology*, 252: 102-106.
5. Wahjuningsih, S., M.S. dan Dati, 2012. Protein Expression of Goat Follicular Fluid [GFF]. *J. of. Agriculture and Technology A*, 2(7): 917-920.
6. Man and Graves, 2001. Influence of hormones and follicular fluid on maturation of pig oocytes. Cient Rural, 31-1. Santa Maria.
7. Nicolas, M.O., Gopichandran, Leese, Picton, Harris, 2005. Fluctuations bovine ovarian follicular fluid composition throughout the oestrus cycle. Reproduction, 129: 219-228.
8. Mattioli, M., 1994. Transduction mechanisms for gonadotrophin-induced oocyte maturation in mammals. *Zygote*, 2: 247- 349. Cambridge University Press.
9. Mochizuki, M., S. Yamabe, M. Takeshi, 2004. Relationship between the inhibin in follicular fluid and the fertilizing capacity of the oocyte. *Advances in Obstetrics and Gynecology*, 56(2): 62-69.
10. Craig, J.A., H. Zhu, P.W. Dyce, J. Wen, 2004. Leptin enhances porcine pre implantation embryo development in vitro. *J. Molecular and Cellular Endocrinology*, 229: 141-147.
11. Appasami, M., P. Serhal, A. Al-Qahtani, N.P. Groome, S. Muttukhrisna, 2007. Evaluation of the relationship between follicular fluid oxidative strss,ovarian hormones. *Fertil. Steril*, 15: 440-443.
12. Fan, H.Y. and Q.Y. Sun, 2004. Involvement mitogen-activated protein kinase cascade during oocyte maturation and fertilization in

mammals. *J. Biology Reproduction*, 70: 535-547.
13. Meinecke, B., A. Wehrend and S. Meinecke-Tillmann, 1997. Effects og hnRNA and protein synthesis inhibition on chromosome condensation and H-1 and MAP kinase activities during in vitro maturation of porcine oocyte. *J. Reproduction in Domestic Animals*, 32: 20-25.
14. Aguilar, J.J., G.L. Woods, M.H. Miragaya, L.M. Olsen, D.K. Vanderwall, 2001. Effect of homologous preovulatory follicular fluid on *in vitro* maturation of equine cumulus–oocyte complexes. *Theriogenology*, **56:** 745-758.
15. Bogh, I.B., J. Beard, G. Duchamp, M. baltsen, N. Gerald, P. Daels, T. Greve, 2002. Pure preovulatory follicular fluid promotes in vitro maturation of in vitro maturation of in vitro aspirated equine oocyte. *Theriogenology*, 57: 1765-1779.
16. Choi Y.H., M. Takagi, H. Kamishita, M.P.B. Wijayagunawadane, T.J. Acosta, K. Miyaawa, K. Sato, 1998. Developmental capacity of bovine oocyte matured in two kinds of follicular fluid and fertilized in vitro. *Anim. Reprod. Sci.*, 50: 27-33.
17. Avery, B., L. Strøbech, T. Jacobsen, I.B. Bøgh, T. Greve, 2003. In vitro maturation of bovine cumulus–oocyte complexes in undiluted follicular fluid: effect on nuclear maturation, pronucleus formation and embryo development. *Theriogenology*, 59: 987-999.
18. Abrieu, A., M. Doree And D. Fishter, 2002. The interplay between cyclin B-Cdc2 kinase [MPF] and MAP Kinase during maturation of oocytes. *J. Cell Science*, 114: 257-267.
19. Klumpp, A.M., 2004. The effect of holding bovine acolytes in follicular fluid on subsequent fertilization and embryonic development. Thesis. Lousiana State University.
20. Chian, R.C., K. Okuda and K. Niwa, 1995. Influence of cumulus cells on in vitro fertilization of bovine oocytes derived from in vitro maturation. *Anim. Reprod. Sci*., 38: 37-48.