The Comparison Effects of the Porcine Follicular Fluid to a Mixture of Follicle-Stimulating Hormone, Luteinizing Hormone and Estradiol Supplements on In vitro Porcine Oocyte Maturation

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Abstract

The aim of this study was to compare the effects of supplementing porcine follicular fluid (pFF) and a mixture of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2) on *in vitro* porcine oocytes development. The pFF were collected from the Large Write Pigs' medium-sized follicle of 4-6 mm in diameter. oocytes at concentrations of 200 and 500 µg protein/mL cultured in the 37 ^{o}C sterile-conditioned thermos incubator with 5% high humidity and 95% CO_2 in the atmosphere, supplemented by 2.2 mg/mL Medium 199, 0.25 mM $NaHCO_3$, 15 µg/mL and 50 µg/mL pyruvate as well as gentamicin sulfate. The experiment was divided into 4 groups: Group 1, oocytes cultured in Medium 199 as a control group; Group 2, oocytes cultured in Medium 199 supplemented with LH, FSH and E_2; Group 3, oocytes cultured in Medium 199 supplemented with LH, FSH, E_2 and 10% FBS; and Group 4, oocytes cultured in Medium 199 supplemented with pFF in 200 and 500 µm protein/mL. We cultured for 24 and 44 h and measured the diameter of oocyte which had sizing extension as growing. The results demonstrated that the pFF with a 200 µg/mL concentration did not have impact on the growth of surrounding cells (). while that with a 500 µg/mL concentration leaded to a significantly higher growth percentage than that of the positive control (supplemented by hormones and bovine serum), statistically ($P<0.05$). The surrounding cells extended and turned more fibroblastic than the positive control. Conclusively, the pFF from

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medium-sized oocytes with a 500 µg protein/mL was the more appropriate supplement in the culture medium than those of costly hormones and BPS for enhancing the oocyte development.

Keywords: Porcine follicular fluid; pig; *in vitro* oocyte maturation

1. Introduction

It revealed that porcine medium- and large-sized oocytes secrete the follicular fluid having similar components of proteins as in cell secreted from porcine granulosa cells. Protein bars of 5-6 mm-sized oocytes, 7-8 mm-sized oocytes, 8 mm-sized oocytes, and 10 mm-sized oocytes composed of various molecular weights of 50, 65, 75, 90, 95, 110, 120, 160, 190, and over 220 kDa; 50, 65, 90, 110, 120, 160, 180, over 220 kDa; 50, 65, 75, 120, 160, 180 and over 220 kDa; and 50, 65, 90, 110, 120, 160, 180 over 220 kDa, respectively (Youngsabanant and Mettasart, 2020). These proteins are beneficial for supporting decomposition of follicular nucleus envelopes and oocyte development, and stimulating oocyte maturation and ovulation (Youngsabanant et. al., 2019; Zhao et al., 2002; Ducolomb et al., 2013).

Protein molecular weight of 27 kDa of small-sized oocytes contains five classes of immunoglobulin (Ig), namely, IgG, IgA, IgM, IgD and IgE, all have responsibilities immunizing against diseases but each group performs different roles (Youngsabanant and Mettasart, 2020). The protein molecular weight of 62-65 kDa is keratin which decomposed follicular nucleus envelopes and oocyte development. That of 70 kDa is a coagulation factor, which acts as reducing the oocyte growth rate into metaphase I while that of 80 kDa is the porcine inhibitor of carbonic anhydrase that eliminates growth into metaphase I as well (Ducolomb et al., 2013). This follicular fluid contains glycosaminoglycans (GAGs) and hyaluronic acid which absorb water, thus makes gel-like cell coat. It also contains keratin. Moreover, albumin, immunoglobulin and ceruloplasmin were also found in the follicular fluid, similar to the study of Alberto et al. (2009) studying on the components in the human oocyte and unveiled that the follicular fluid contains a variety of such hormones as follicle-stimulating hormone (FSH), luteinizing hormone (LH) estrogen and progesterone, hyaluronic acid, transforming growth factor-beta (TGF-beta) superfamily, other growth factors and interleukin, reactive oxygen species (ROS), anti-apoptotic factors, anti-apoptotic factors, and prostanoids. Suchanek et al. (1944) also discovered that in the phase of the human multiplied oocyte-cumulus cell complexes (COCs), FSH stimulates follicular granulosa cells to produce an increase in the amount of hyaluronic acid and enhances estrogen in supporting the development of cytoplasm for completion of the oocyte growth. Additionally,Kimura et al. (2002) reported about transient

synthesis and the accumulation of hyaluronic acid, a component of the matrix outside cumulus cells, cause an extension of oocyte-cumulus cell complexes in the preovulatory phase of mammal oocytes, and also studied the expression of mRNA in hyaluronan synthase 2 (has2), hyaluronan synthase 3 (has3), cluster differentiation of 44 ZCD44) and ECG responses and secretion in porcine oocytes. These genes in COCs and oocytectomized complexes (OXCs) were found that cumulus cells possess mRNA expression of both has2 and CD44, and oocytes also express mRAN of has3 in harmony with other research studies which reported that crucial substances are present in oocytes, such as hyaluronic, collagen, hormones, proteins, and growth factors, helping encourage development of cells.

As aforementioned, it illuminated that porcine follicular secretion contains hormones, hyaluronic, collagen, proteins, growth factors and antibodies, and that mammal secretion contains FSH, LH, estrogen, progesterone, sugars, hyaluronic, growth factors of transforming growth factors, proteins, peptides, and amino acids. Here, FSH assumed a role in heightening the amount of hyaluronic by stimulating granulosa cells in oocytes and supporting estrogen in order to boost the development of cell cytoplasm. As a result, the porcine follicular secretion can probably effectively support the growth of oocytes cultured in in vitro as well. Thereby, in this study, we emphasized on testing the porcine follicular secretion in terms of supporting the development of the oocytes cultured in the culture medium compared to that cultured and supplemented using the substitute for hormones due to costly expenditure and time needed to import. We conducted the examination during the estrus to attain new experience and knowledge for the biotechnological field in use of the porcine follicular secretion in the timeliest phase during estrus in the future.

2. Methods

2.1 Sample collection and preparation

Porcine ovaries were harvested from Large White Pig aged approximately 210-250 days at local slaughterhouses in Nakhon Pathom Province, Thailand. The ovaries from the leftover carcasses were excised using a scissor, rinsed 2-3 times with 0.9 % normal saline mixed with an antibiotics composing of 100 international units/mL penicillin G, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B, placed in aseptic sampling bags, and then kept in thermos containing 30-35°C purified water. After that, transported to the laboratory within 3 hours. Ovaries were rinsed by normal saline mixed with antibiotics and the uterosacral ligament was removed using a pair of sharp-headed scissors and re-rinsed with 0.9 % normal saline mixed with antibiotics, and then dried with a sterile filter cloth. The follicles with various sizes of 1-8 mm in diameter were pierced using

a 5-mL syringe with an 18-gauge needle to obtain the porcine follicular fluid (pFF) (Areekijseree and Vejaratpimol, 2006) containing the follicular cell layers and granulosa cells live in the oocytes. The pFF was transferred to a petri dish and the oocytes with cell layers called cumulus oocyte complexes (COCs) were aspirated under a stereoscope using a 180–200 μm pipette and the pFF was finally centrifuged at 1,500 ×g for 15 min to collect the supernatant of pFF for further usage as medium for testing its effect on oocyte maturation.

2.2 The experimental protocols

The study of the optimum volume ratio of pFF on the oocyte growth rate during the estrous period was allocated into 4 groups as following: Group I, oocytes cultured in Medium 199 as a controlled group; Group II, oocytes cultured in Medium 199 supplemented with a mixture of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and estradiol (E_2); Group III, oocytes cultured in Medium 199 supplemented with the mixture of FSH, LH, E_2 plus 10% fetal bovine serum (FBS); and Group IV, oocytes cultured in Medium 199 supplemented with porcine follicular fluid from medium-sized porcine oocytes with 200 and 500 μm/mL protein concentrations (Youngsabanant and Rabiab, 2020). This study rested on the viability and growth of oocytes to the estrous cycle based on morphological studies investigated under an inverted microscope and SEM. Inspection of cell development on the porcine follicular fluid secretion**:** The investigation of cell growth was performed compared to the control group set 100%. If the outcome is more than 100%, it shows that cells develop; on the contrary, if less than 100%, it means that porcine follicular secretion negatively affects the cell culture.

2.3 Statistical analysis

Statistical analysis was done by SPSS for windows package, the result of percentage diameter thickness of each sample was analyzed and compare with SPSS for Windows (significant value = 0.05).

3. Results and discussion

3.1 Effect of porcine follicular fluid from medium-sized oocytes on oocyte morphology

As cultured at hour 0, porcine oocytes were round in shape and surrounded by zona pellucida and cumulus granulosa cells. Oocytes could be classified into 2 groups; one, intact-cumulus cell layers defined as oocytes tightly embraced with 3-5 cell layers and the other, multi-cumulus cell layers defined oocytes tightly embraced with more than 5 cell layers (Figure 1**)**.

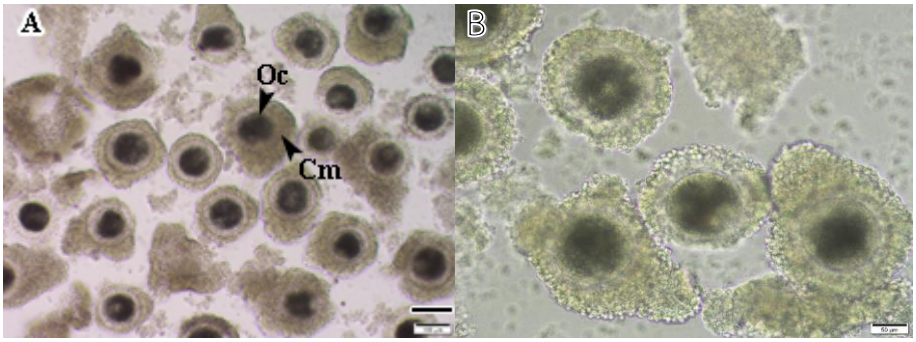

Figure 1. Micrographs showed oocytes from medium-sized porcine ovaries cultured in Medium 199. The oocytes were appeared round-shape firmly surrounded by cumulus cells. (A) X100× and (B) X400. Oc, oocyte and Cm, cumulus cell.

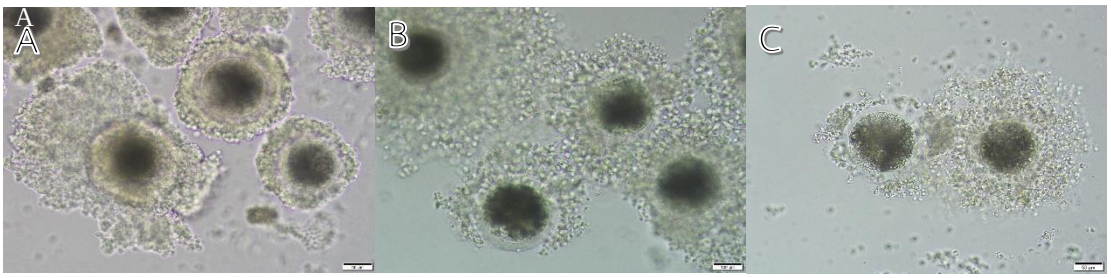

Figure 2. Micrographs showed oocytes from medium-sized porcine ovaries in group I cultured in M199 in a 37°C incubator with high humidity and 5% CO_2 at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400×.

With comparison to the supplemented with follicular fluid from medium-sized porcine oocytes with a 200 µm/mL protein concentration, the oocytes in Group 1, as cultured for 24 hours, appeared round shaped, spread, and detached from the zona pellucida and the cytoplasm of oocytes were initially decomposeded. After 44 h, the oocytes in Group 1 were found their cell layers appeared round shaped, expanding, and separating from the zona pellucida layer and the cytoplasm of oocytes decomposed **(**Figure 2**)**. The oocytes in Group 2, as cultured for 24 h, were spreaded and separated from the zona pellucida and their cell layers formed in circular shape; while some cells slightly were radiated and the cytoplasm of certain oocytes decomposed. After 44 h, the oocytes in Group 2 were found that their cell layers were spreaded and detached from the zona pellucida layer; whereas cell layers still attaching to oocytes kept slightly expanding out only at the top of them; some cells little radiated and the cytoplasm of certain oocytes remained like that at hour

24 as in Figure 3. The oocytes in Group 3, as cultured for 24 h, morphologically expanded, and radiated out to the cells nearby, appearing water like. After 44 h, the oocytes in Group 3 were found that their cell layers remained more water-shaped and radiated along to the adjacent cells than 24 h. as exhibited in Figure 4. The oocytes in Group 4, as cultured in medium-sized porcine follicular fluid secretion with a 200 µm/mL protein concentration for 24 h, were physically found that their cell layers spread out and radiated to the nearby cells, becoming fastigiated; and the cytoplasm of certain oocytes decomposed similar to Group 2. After 44 h, their cell layers equally extended and the cytoplasm of certain oocytes remained like Group 2 as in Figure 5.

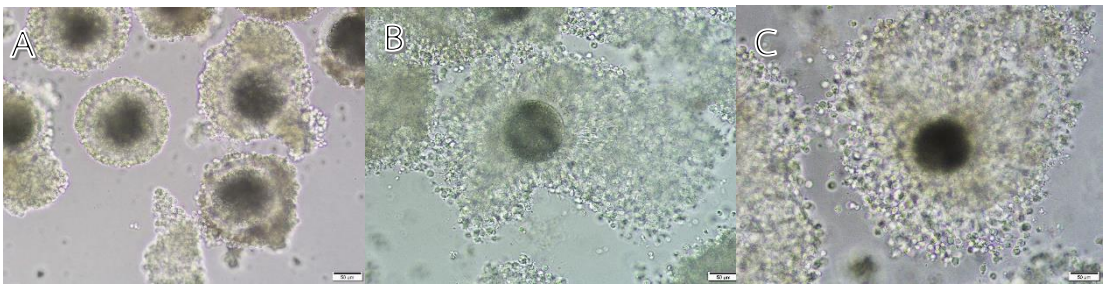

Figure 3. Micrographs showed oocytes from medium-sized porcine ovaries in Group II cultured in Medium 199 supplemented with LH, FSH, and E2 at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor x400.

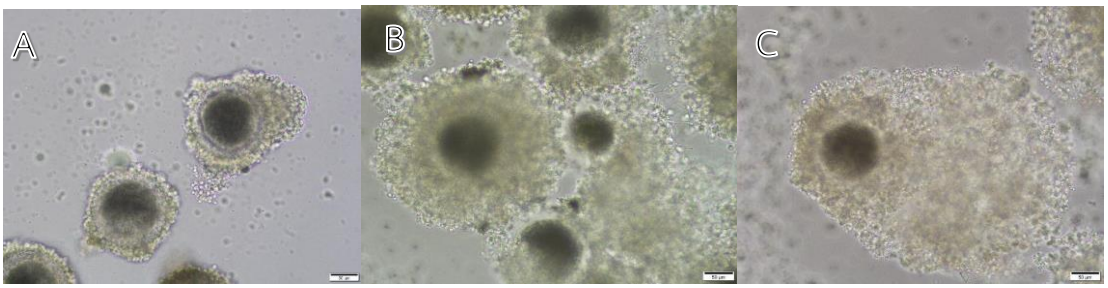

Figure 4. Micrographs showed oocytes from medium-sized porcine ovaries in Group III cultured in Medium 199 supplemented with LH, FSH, E_2, plus 10% FBS at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400.

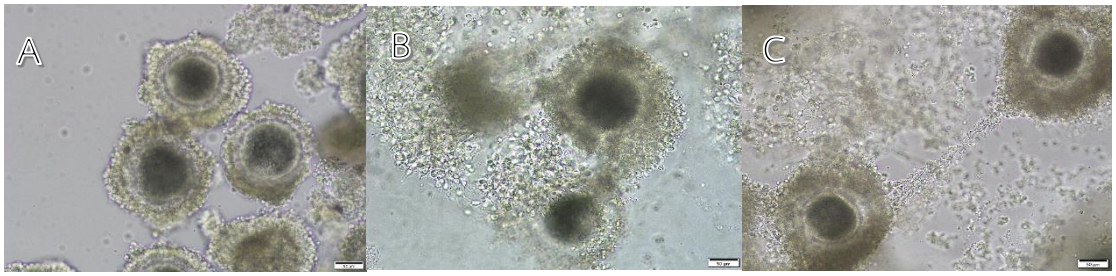

Figure 5. Micrographs showed oocytes from medium-sized porcine ovaries in Group IV cultured in Medium 199 supplemented with pFF at a 200 µm/mL protein concentration at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400.

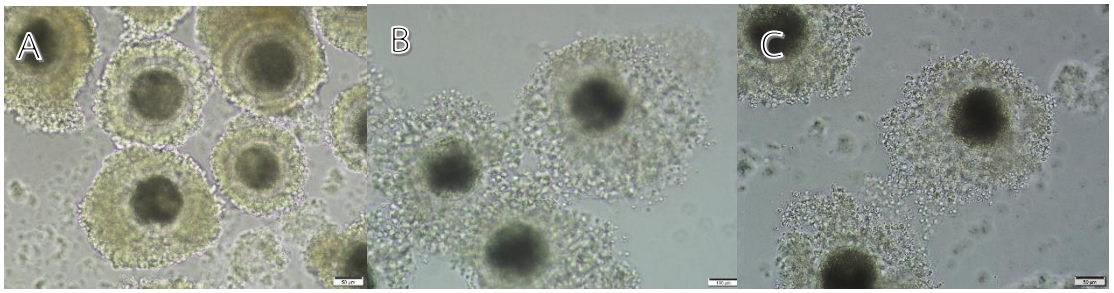

Figure 6. Micrographs showed oocytes from medium-sized porcine ovaries in Group I cultured in M199 in a 37°C incubator with high humidity and 5% CO_2 at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400.

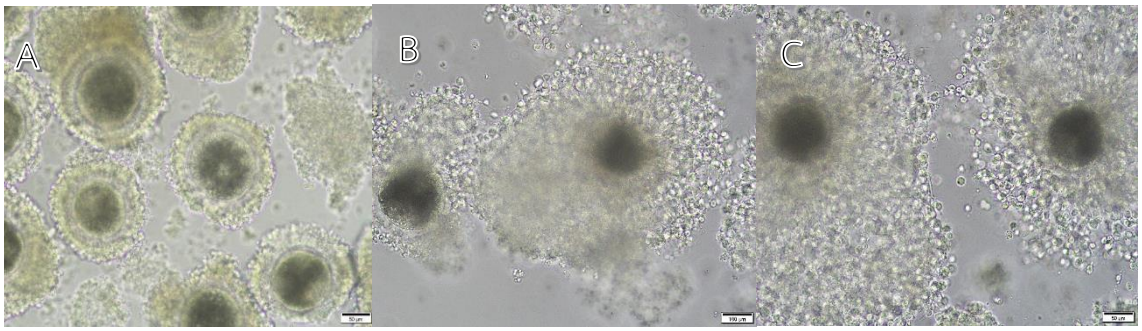

Figure 7. Micrographs showed the oocytes from medium-sized porcine ovaries in Group II cultured in M199 supplemented with LH, FSH, E_2 at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400 .

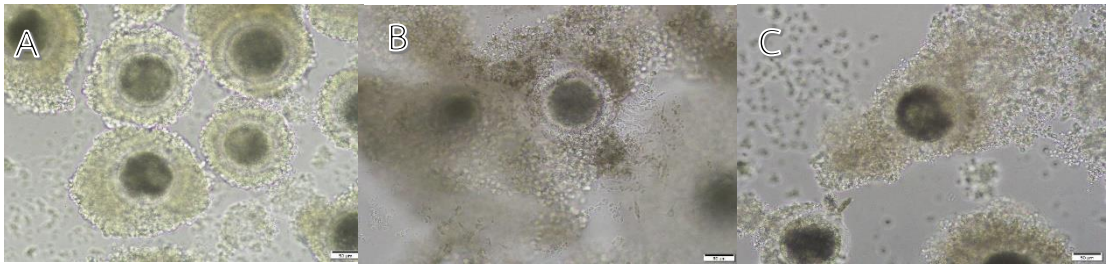

Figure 8. Micrographs showed oocytes from medium-sized porcine ovaries in Group III cultured in M199 supplemented with hormones and 10% FBS in a 37°C incubator with high humidity and 5% CO_2 at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, and (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400.

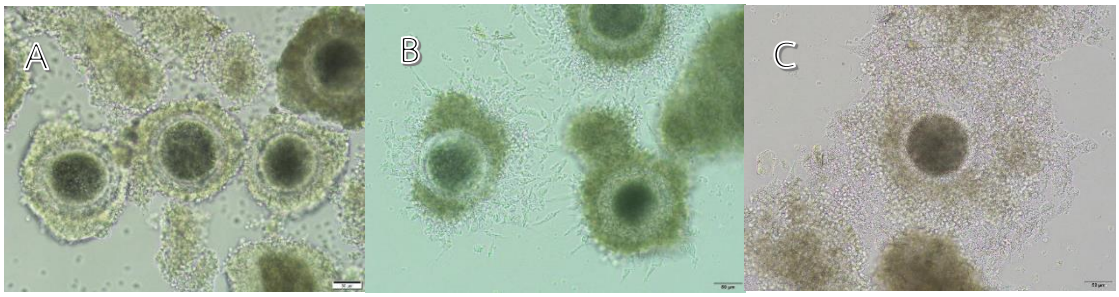

Figure 9. Micrographs showed oocytes from medium-sized porcine ovaries in Group IV cultured in Medium 199 supplemented with pFF at a 500 µm/mL protein concentration at (A) initially cultured oocytes, (B) 24-h-cultured oocytes with cumulus cells, nd (C) 44-h-cultured oocytes with cumulus cells. This micrograph was magnification factor X400.

With comparison to the supplemented with follicular fluid from medium-sized porcine oocytes with a 500 µm/ mL protein concentration. The morphological result based on an inverted microscope unveiled that the oocytes in Group 1, as cultured for 24 h, appeared round shaped, spread, and detached from the zona pellucida, and the cytoplasm of oocytes turned darker in color and initiated to decompose. After 44 h, the oocytes in Group 1 were found their cell layers appeared circular, expanding, and separating from the zona pellucida layer. The diameter of oocytes with spread cell layers compared with the initially cultured ones stayed quite similar and the cytoplasm of oocytes decomposed as shown in Figure 6**.** The oocytes in Group 2, as cultured for 24 h, were spreaded, and separated from the zona pellucida while some cells slightly radiated; and the cytoplasm of certain oocytes was disappeared. After 44 h, their cell layers in Group 2 were spreaded and detached from the zona pellucida layer whereas cell layers still attaching to oocytes

kept expanding out only at the top of them which some cells showed a little radiation and the cytoplasm of certain oocytes remained like that at hour 24 (Figure 7). The oocytes in Group 3, as cultured for 24 h, were morphologically expanded, and radiated out to the neighboring cells, appearing as water-like. After 44 h, the cell layers remained more water-shaped and radiated along to the adjacent cells than those of 24 culture (Figure 8). Their cell layers in Group 4, as cultured for 24 h, were physically of spreaded out and turned to be fastigiated, radiated to the nearby cells, similar to Group 2. After 44 h, their cell layers became fibroblastic, radiating to the nearby cells and more expanding than those couture at 24 hours and apparently considered as the highest cell growth rate of all groups. The expansion of cumulus cells was similar to that of Group 3 cultured in M199 supplemented with hormones and 10% FBS (Figure 9).

Table1. The percentages of the oocyte growth rate for 4 different groups comparing the with surrounding cumulus cells compared to the initially cultured oocytes in each group and in a culture, medium supplemented with follicular fluid from medium-sized porcine oocytes with a 200 µm/mL protein concentration.

Groups	Experimental protocol	Oocyte growth rate (%)	
		24 h	44 h
I	Medium 199	25.03 ± 0.03 [a] .	27.31± 0.14 [a] .
II	Medium 199 supplemented with FSH, LH, and E_2	35.09 ± 0.11 [b]	41.64± 0.13 [b]
III	Medium 199 supplemented with FSH, LH, and E_2 plus 10% FBS	35.01 ± 0.20 [b]	38.97± 0.09 [b]
IV	Medium 199 supplemented with pFF at 200 µm protein/mL	25.47± 0.12 [a]	26.59± 0.06 [a]

a, b Applied Duncan's test to categorize with $P \leq 0.05$

3.2 Effect of porcine follicular fluid from medium-sized oocytes on *in vitro* oocyte maturation

With comparison to the supplemented with follicular fluid from medium-sized porcine oocytes with a 200 µm/mL protein concentration, it was found that for cells cultured at 24 h, the means of cell growth rate in the Group 1, 2, 3, and 4 were 25.03 ± 0.03., 35.09± 0.11, 35.01± 0.20, and 25.47± 0.12percent, respectively while for cells cultured at 44 h, the means of cell growth rate in the Group 1, 2, 3, and 4 were 27.31± 0.14, 41.64± 0.13, 38.97± 0.09, and 26.59± 0.06. percent,

respectively. Consequently, it is explainable that Group 2 and Group 3 had no difference in their growth rate of the oocytes with surrounding cumulus cells, and Group 4 showed a lower rate than in Group 2 and 3, showing that a 200 µm/mL protein concentration of the medium-sized porcine follicular fluid did not provide an impact on the oocyte development due to having a similar rate to that cultured in M199 (Table 1).

Table 2. Percentage of the growth rate of oocytes with surrounding cumulus cells compared to the initially cultured oocytes in each group and in a culture, medium supplemented with fluid secretion from medium-sized porcine oocytes with a 500 µm/mL protein concentration.

Groups	Experimental protocol	Oocyte growth rate (%)	
		24 h	44 h
I	Medium 199	10.07 ± 0.02 [a]	14.03± 0.01 [a]
II	Medium 199 supplemented with FSH, LH, and E_2	35.24 ± 0.18 [c]	38.33±0.20 [b]
III	Medium 199 supplemented with FSH, LH, and E_2 plus 10% FBS	43.01 ±0.15 [c]	45.98±0.12 [b]
IV	Medium 199 supplemented with pFF at 500 µm protein/mL	24.00±0.01 [b]	49.20±0.14 [b]

a, b, c Applied Duncan's test to categorize with $P \leq 0.05$

With comparison to the supplemented with follicular fluid from medium-sized porcine oocytes with a 500 µm/mL protein concentration. It was found that for cells cultured at 24 h, the means of cell growth rate in the Group 1, 2, 3, and 4 were 10.07± 0.02, 35.24± 0.18, 43.01± 0.15, and 24.00± 0.01, respectively. As further cultured up to 44 h, it was found that the means of cell growth rate in the Group 1, 2, 3, and 4 were 14.03± 0.01, 38.33± 0.02, 45.98± 0.12, and 49.20± 0.14, respectively. Consequently, it was certain that Group 2 had the highest development rate; meanwhile, Group 1 demonstrated as the lowest development rate. After cultured for 44 h, Group 4 had the highest rate of cell growth rate of 49.20± 0.14, Conversely, Group 1 stayed at the lowest rate of the cell development. This can be summarized that a 500 µm/mL protein concentration of the medium-sized porcine follicular fluid provided the largest impact on oocyte development during at hour 44 culture. And its development percentage was higher than that of the Group 3 (Table 2).

3.3 DISCUSSION

In this study, we utilized the follicular fluid of oocytes collected from the medium-sized follicles which can be cultured to promote *in vitro* oocyte maturation as a study of Marchal et al. (2002). They reported the use of follicle from the small-sized of less than 3 mm. in diameter, medium-sized of between 3-5 mm in diameter and the large-sized of over 5 mm in diameter and found that the oocytes collected from the medium- and large-sized possess the higher maturation rates that those of the small-sized follicles. Moreover, the result made obviously that the oocytes from the medium- and large-sized could stimulate the fertilization with sperm, and the embryo had a higher tendency to be developed into the blastocyst than those of the small-sized follicle. As a result, we used the oocytes from the medium-sized porcine follicles to be considered very potentially to assist *in vitro* oocyte maturation and inspected the effectiveness of the use of the porcine follicular fluid.

The growth of the oocytes cultured *in vitro* can be performed based on the development of cumulus cells since during this development, there being a cellular interaction prior to estrus, the cumulus cells will tightly line up. Nonetheless, only after the estrus ends, cumulus cells will be spreaded and enlarged, called corona radiata, which connected to the cytoplasm of cumulus cells through zona pellucida and oolemma of oocytes to synthesize substances to intracellular matrix, affecting the oocyte growth in the future. (Motta et al., 1994; Yokoo and Sato, 2004). The studies disclosed that the development of porcine follicular cell layers is not reliant on the epidermal growth factor of the insulin-like growth factor I, but on other components existing in the follicular fluid secretion (Yokoo and Sato, 2004; Yoshida et al. (1992a). This study was inspected using the medium-sized porcine follicular fluid with 200 and 500 µg/mL protein concentrations, finding that the development rate based on a 200 µg/mL protein concentration was lower than those of the culture medium supplemented with FBS, but higher than those of the controlled group. In addition, once tested with medium-sized porcine follicular fluid at a 500 µg/mL protein concentration, finding that the development rate was the highest, which was higher than those cultured in medium supplemented with FBS, but higher than those of the controlled group. According to the results, it was apparently present that the medium-sized porcine follicular fluid mixed in the culture medium results in oocyte growth. Especially, the medium-sized porcine follicular fluid secretion at a 500 µg/mL protein concentration had affected on the oocytes to be developed higher than those the medium supplemented by hormones or serum, presumably resulting from internal components that supported growth (Ducolomb et al., 2013; Revelli et al., 2009). Additionally, the chemical components of oocytes can be identified as electrolytes, glucose, uric acid, lipid enzyme, cyclic

AMP, growth-supporting substances, amino acids, and hormones (Chang et al., 1976; Ito et al., 2008; Kor et al., 2014; Leibfried and First, 1980; Spitzer et al., 1996; Revelli et al., 2009). Yoshida et al. (1992a) also reported that follicular components can cause oocytes to be developed and mentioned that certain components within 10,000 - 200,000 MW porcine follicular fluid promoted oocyte growth, and large-sized oocytes in the same way nurture viability of cumulus cells and follicular estrus, in connection with this study in which 4-6 mm in diameter or medium-sized porcine follicular fluid was utilized to supplement the culture medium, resulting in oocyte development which was examined based on the expansion rate of cumulus cells, similar to a study by Ito et al. (2008). He researched the efficacy of porcine follicular fluid from the small-sized of 3-4 mm in diameter and large-sized of 5-6 mm in diameter mixed in NSCU-37 for the *in vitro* cell culture, assuring that the percentage of estrus enhanced by pFF in the laboratory was obviously proved higher than the oocytes cultured in small-sized pFF. Moreover, pFF consisting of significant components of both FSH and LH caused the better of development of granulosa cells than the medium supplemented with 10% fetal bovine serum (Lawrence et al., 1979) as our results of the use of the three sizes of pFF in the culture medium. Meanwhile, Lawrence et al. (1979), based on FSH and LH supplements, also discovered that granulosa cells changed their shape, and the percentage of cell development arise. Agung et al. (2013) also recorded that pFF stimulated statistically significantly higher development in cells to the estrous cycle in the laboratory. Having cultured oocytes with sperm, he found that pFF statistically significantly promoted faster development into the blastocyst stage. Furthermore, there was a report of the use of oocytes with cell layers collected from 2-5 mm medium-sized porcine follicular fluid, it unveiled that supplementing TCM 199 by pFF from 2-5 mm porcine follicular fluid with a 10% protein concentration and FSH delivered an impact on germinal vesicle breakdown (GVBD) and increasingly affected growth into metaphase II. This leaded to a conclusion that the development of oocytes is dependent upon both FSH and pFF (Rath et al., 1995). Huang et al. (2002) further conducted a comparison between the mediums supplemented with pFF at 40% and 100% protein concentrations and summarized that 40% was more effective in stimulating maturation than that of 100%. A record by Leibfried and First (1980) provided the details of the cell cultured in the bovine culture medium supplemented with follicular fluid from less than 5 mm in diameter and found that the maturation percentage of bovine oocytes was higher than that of the control group. Besides, a record regarding the effect of bovine follicular fluid on the *in vitro* sheep oocyte maturation based on 10, 15, 20, 25, 50, 100, 1,000, 2,000 and 5,000 ng/mL concentrations of peptides extracted from bovine ovarian follicular fluid secretion and supplemented with epidermal

growth factors (20 ng/mL EGF) were utilized, and oocyte growth was detected focusing on the expansion of cumulus cells and the incidence of the first polar body. Nevertheless, when compared to the positive control group (culture medium supplemented with 20% bovine serum (v/v) and 20 ng/mL EGF), the most suitable concentration was 100 ng/mL, resulting in the highest growth rate of oocytes.

4. Conclusion

They also concluded that the usage of porcine follicular fluid for *in vitro* oocyte maturation could effectively reduce the chemical cost. Similarly, in this study, the outcome lightened up that the follicular fluid could be substituted for maturation-supporting substances in order to markedly lessen the expenditure on chemicals as well, mainly serum for *in vitro* oocyte maturation.

5. Acknowledgments

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