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—Original Article—

The autophagic inducer and inhibitor display different activities on the meiotic and developmental competencies of porcine oocytes derived from small and medium follicles

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Abstract. This study aimed to examine the effect of rapamycin (autophagy inducer) and 3-methyladenine (3-MA, autophagy inhibitor) on the meiotic and developmental competencies of porcine oocytes derived from medium follicles (MF, 3–6 mm in diameter) and small follicles (SF, 1–2 mm in diameter) during *in vitro* maturation (IVM) process. The presence of 1 nM but not 10 nM rapamycin significantly increased the maturation rate of MF-derived oocytes ($P < 0.05$). However, the maturation rate of SF-derived oocytes was not affected by rapamycin at both concentrations (1 nM and 10 nM). The maturation rate of MF-derived oocytes decreased significantly ($P < 0.05$) in the presence of 0.2 mM but not 2 mM 3-MA than non-supplemented control. In contrast, in SF-derived oocytes, 3-MA at both 0.2 and 2 mM concentrations did not affect the maturation rates. The presence of 1 nM rapamycin significantly increased the blastocyst formation rate of MF-derived mature oocytes following parthenogenetic activation ($P < 0.05$). However, the blastocyst formation rate of SF-derived mature oocytes was not affected by the presence of rapamycin. The presence of 3-MA significantly reduced the blastocyst formation rate of MF-derived mature oocytes but did not change that of SF-derived oocytes. In conclusion, our study results show differences in activity of the autophagy inducer and inhibitor on the meiotic and developmental competencies of MF- and SF-derived porcine oocytes.
Key words: Autophagy, Developmental competence, *In vitro* maturation, Oocytes, Porcine

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For the *in vitro* production of porcine embryos, cumulus-oocyte complexes (COCs) from medium-sized antral follicles with 3–6 mm in diameter (medium follicles; MF) of prepubertal gilts are used routinely [1–3], since they are slaughtered around six months in developed countries. However, the number of MF on the surface of porcine ovaries is significantly lower than that of small-sized antral follicles with a diameter of less than 2 mm (small follicles; SF) [4]. In addition, the diameter of oocytes [5], as well as the number of surrounding cumulus cells [6], is significantly smaller in SF-derived oocytes than in MF-derived oocytes. Furthermore, it is well known that the meiotic and developmental competencies are lower in oocytes derived from SF than MF [7–9], whereas the *in vitro* ability of sperm to penetrate MF- and SF-derived oocytes to form male and female pronuclei were similar when mature oocytes with the first polar body were inseminated following *in vitro* maturation (IVM) [9]. Levels of cytoplasmic factors, such as transcript abundance of the *MOS* gene [9], cAMP and cGMP [10], or glutathione and metaphase-promoting factor [11] were shown to differ between MF- and SF-derived oocytes during IVM culture. However, the differences in meiotic and developmental competencies of MF- and SF-derived oocytes are nor clearly understood.

Autophagy plays an essential role in removing the intracellular proteins and organelles in cooperation with lysosomes [12]. In mammals, autophagic activity is vital not only during the early development of embryos [13–15] but also in the meiotic maturation of oocytes [16, 17]. The autophagic activity was determined by the level of microtubule-associated protein light chain 3 (LC-3) II in immature porcine oocytes, which was significantly higher than in mature oocytes [16]. Also, rapamycin is a well-known inducer of autophagosomal membrane formation with subsequent autophagy activation, by suppressing the mammalian target of rapamycin (mTOR), a serine/threonine-protein kinase, which plays an essential role in pathways that regulate the balance between cell growth and autophagy [18]. It has been reported that during the IVM process, the presence of 1 nM rapamycin improved the maturation rate of MF-derived porcine oocytes and their developmental competencies to the blastocyst stage [17].

Furthermore, 3-methyladenine (3-MA, an autophagy inhibitor, plays a vital role in controlling the activation of mTOR signaling pathway) effectively inhibits the autophagic activity of porcine and bovine embryos at 2.5 [14] and 0.02–2 mM [19] concentrations, respectively. Although the interaction of MF-derived oocytes with the autophagy inducer and inhibitor has been reported extensively, it is still unclear whether similarities exist between SF- and MF-derived oocytes. This study aimed to improve embryo production efficiency by elucidating the mechanism of oocyte maturation. Towards this, we examined the effects of an autophagy inducer and inhibitor rapamycin, and 3-MA, respectively, during the first phase of IVM

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culture, when LC-3 II level in MF-derived porcine oocytes was higher [16], on meiotic and developmental competences of MF- and SF-derived porcine oocytes.

Materials and Methods

Chemicals and culture media

Sodium chloride (NaCl), KCl, KH_2PO_4, $CaCl_2$-$2H_2O$, HCl, NaOH, gentamicin-sulfate, and liquid paraffin were purchased from Nacalai Tesque (Kyoto, Japan). Equine chorionic gonadotropin (eCG; Serotropin) and human chorionic gonadotropin (hCG; Gonadotropin) were obtained from ASKA Pharmaceutical (Tokyo, Japan). Unless specified, all other chemicals were purchased from Sigma Aldrich (St. Louis, MO, USA).

The medium used for collecting and washing cumulus-oocyte complexes (COCs) was modified HEPES-buffered Tyrode's lactate containing polyvinyl alcohol (TL-HEPES-PVA) [20]. The basic IVM medium was BSA-free chemically defined medium, porcine oocyte medium (POM, Research Institute for the Functional Peptides, Yamagata, Japan) modified with 50 μM beta-mercaptoethanol (mPOM) [20]. The medium for the transit culture following parthenogenetic activation was, Medium-199 (Invitrogen, Carlsbad, CA, USA) modified with 3.05 mM glucose, 2.92 mM Hemi-calcium lactate, 0.91 mM Na-pyruvate, 12 mM sorbitol, 75 μg/ml potassium penicillin G, and 25 μg/ml gentamicin (mM199) [21]. The chemically defined medium for *in vitro* development to the blastocyst stage was porcine zygote medium (PZM-5; Research Institute for the Functional Peptides) [22]. All media, except TL-HEPES-PVA, were equilibrated overnight at 39ºC in an atmosphere of 5% CO_2 in the air before use.

Preparation and IVM of COCs

Ovaries were collected from slaughtered pre-pubertal gilts at a local abattoir and transported to the laboratory in 0.9% NaCl containing 75 μg/ml potassium penicillin G and 50 μg/ml streptomycin sulfate. Within 3 h after ovary collection, COCs were aspirated from SF and MF on the surface of ovaries using an 18-gauge needle and a disposable 10-ml syringe and washed three times with modified TL-HEPES-PVA medium at room temperature (25ºC) [23]. Only COCs with uniform ooplasm and surrounding compact cumulus cell mass with more than three layers were washed three times with IVM medium. Fifty of the MF- and SF-derived COCs were cultured separately in the absence or presence of rapamycin (at 1 or 10 nM; Santa Cruz Biotechnology) or 3-methyladenine (at 0.2 or 2 mM; Santa Cruz Biotechnology) in 500 μl of the same medium supplemented with gonadotropins (10 iu eCG/ml and 10 iu hCG/ml) and 1 mM dibutyryl cyclic adenosine 3', 5'-monophosphate (dbcAMP) in a four-well culture plate (Thermo Fisher Scientifi, Roskilde, Denmark) for 20 h at 39ºC in an atmosphere of 5% CO_2 in air. The COCs were then transferred to 500 μl of the IVM medium without gonadotropins and dbcAMP (also in the absence of rapamycin and 3-MA), after being washed three times with the same medium and cultured for an additional 24 h [23, 24]. After IVM, cumulus cells surrounding the oocytes were removed by the addition of modified TL-HEPES-PVA medium containing 0.1% (w/v) hyaluronidase, and then the denuded oocytes were used to determine the meiotic progression or to assess the developmental ability to the blastocyst stage following parthenogenetic activation.

Evaluation of the meiotic stage of oocytes

After IVM culture, the oocytes were mounted on glass slides and fixed in acetic alcohol (25% (v/v) acetic acid in ethanol) for 2–4 days. The specimens were then stained with 1% (w/v) orcein in 45% (v/v) acetic acid for 5 min, and the meiotic progression of oocytes was evaluated under a phase-contrast microscope at a magnification of × 200 and × 400.

Parthenogenetic activation of oocytes

Following a total 44 h of IVM culture, mature oocytes with an extruded first polar body were selected and transferred to the electroporation medium, consisting of 0.25 M mannitol containing 0.01% (w/v) PVA, 0.5 mM HEPES, 100 μM $CaCl_2$-$2H_2O$, and 100 μM $MgCl_2$-$6H_2O$ (pH 7.2) at 39ºC in an activation chamber, followed by washing the same medium thrice. Mature oocytes were exposed to a single electrical pulse with a DC strength of 1.2 kV/cm for 30 μsec using BTX-Electro-Cell Manipulator 2001M (BTX, San Diego, CA, USA). The stimulated oocytes were transferred to mM199 supplemented with 0.4% (w/v) BSA and 5 μM cytochalasin B at 39ºC in an atmosphere of 5% CO_2 in the air for 4 h followed by washing with the same medium. The oocytes were washed three times with PZM-5 and cultured in 500 μl PZM-5 under paraffin oil in a four-well culture plate (Thermo Fisher Scientific) at 39ºC in an atmosphere of 5% CO_2 in the air for 5 days. To determine the developmental competence of oocytes, cleavage, and blastocyst formation rates were determined on days-2 and -5 after initiating culture. At the end of culture time, the number of blastomeres per blastocyst was assessed under a fluorescence microscope following staining with Hoechst33342.

Statistical analyses

Data from four or five replicates were analyzed for the variables (the meiotic stage of oocytes, blastocyst formation, and the number of blastomeres per blastocyst) by ANOVA using GraphPad Prism 6 software (GraphPad Software, San Diego, CA, USA). Percentage data were subjected to arc-sine transformation before statistical analysis, if the data contained percentages > 90 or < 10%. All data are expressed as mean ± SEM. Findings were considered significantly different at $P < 0.05$, and where a significant effect was present, values were compared with a Bonferroni/Dun ad-hoc test.

Results

When MF-derived oocytes were cultured for IVM in the absence or presence of 1 or 10 nM rapamycin during the first 20 h period, the maturation rate of the oocytes was significantly higher in the presence of 1 nM rapamycin than in controls (Table 1). However, the maturation rate in the presence of 10 nM rapamycin was not different compared to control (Table 1). In SF-derived oocytes, on the contrary, rapamycin at 1 and 10 nM did not affect the maturation rates (Table 2) and were significantly lower than MF-derived control.

On examining the effect of 3-MA on the meiosis of MF-derived oocytes during the first 20 h of IVM culture showed a significant reduction in the maturation rate in the presence of 0.2 mM 3-MA

compared to control, although the maturation rates in the presence of 2 mM 3-MA did not differ significantly compared to those percentages (Table 3). However, in SF-derived oocytes, the presence of 0.2 and 2 mM 3-MA did not affect the maturation rates and were significantly lower than those of MF-derived control oocytes cultured in the absence of 3-MA (Table 4).

In MF-derived oocytes, the presence of 1 nM rapamycin during the first 20 h period of IVM significantly increased the developmental competence to the blastocyst stage following parthenogenetic activation, compared to rapamycin-free control, whereas the blastocyst formation rate did not differ significantly from control in the presence of 10 nM rapamycin (Table 5). In contrast, in SF-derived oocytes, rapamycin did not affect the rate of blastocyst formation following electrical activation, and the rates were significantly lower than that of MF-derived oocytes (Table 6). Regardless of the origin of COCs, the presence of rapamycin did not affect both the cleavage rate and the

Table 1. Effect of rapamycin during the first 20 h of culture on the meiotic maturation of medium follicle (MF)-derived porcine oocytes *in vitro*

Conc. (nM) of rapamycin	No. of oocytes examined	No. (%) of oocytes degenerated	No. (%) of oocytes proceeded to the stage of			
			GV	M-I	A-I/T-I	M-II
0	173	1 (0.6 ± 0.6)	5 (2.9 ± 2.7)	40 (23.1 ± 2.4)	2 (1.2 ± 0.7)	125 (72.3 ± 1.4 [a])
1	195	1 (0.5 ± 0.5)	10 (5.1 ± 1.8)	27 (13.8 ± 2.4)	1 (0.5 ± 0.5)	156 (80.0 ± 1.5 [b])
10	170	2 (1.2 ± 0.7)	7 (4.1 ± 2.1)	28 (16.5 ± 2.2)	1 (0.6 ± 0.6)	132 (77.6 ± 1.5 [ab])

Percentage data in parentheses are shown as mean ± SEM from four replicates. GV, germinal vesicle; M-I, metaphase-I; A-I/T-I, anaphase-I/telophase-I; M-II, metaphase-II. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Table 2. Effect of rapamycin during the first 20 h of culture on the meiotic maturation of small follicle (SF)-derived porcine oocytes *in vitro*

Origin of COCs	Conc. (nM) of rapamycin	No. of oocytes examined	No. (%) of oocytes degenerated	No. (%) of oocytes proceeded to the stage of			
				GV	M-I	A-I/T-I	M-II
MF	0	221	2 (0.9 ± 1.1 [a])	3 (1.4 ± 0.9 [a])	35 (15.8 ± 2.6 [a])	4 (1.8 ± 1.6)	177 (80.1 ± 2.3 [a])
SF	0	215	1 (0.5 ± 0.5 [a])	34 (15.8 ± 3.0 [b])	71 (33.0 ± 3.5 [b])	0 (0)	109 (50.7 ± 2.7 [b])
SF	1	224	12 (5.4 ± 1.0 [b])	14 (6.3 ± 1.5 [ab])	57 (25.4 ± 3.4 [ab])	1 (0.4 ± 0.4)	140 (62.5 ± 1.9 [b])
SF	10	169	3 (1.8 ± 1.2 [a])	17 (10.1 ± 2.8 [b])	47 (27.8 ± 4.9 [ab])	0 (0)	102 (60.4 ± 4.9 [b])

Percentage data in parentheses are shown as mean ± SEM from five replicates. COCs, cumulus-oocyte complexes; MF, medium follicles; GV, germinal vesicle; M-I, metaphase-I; A-I/T-I, anaphase-I/telophase-I; M-II, metaphase-II. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Table 3. Effect of 3-methyladenine (3-MA) during the first 20 h of culture on the meiotic maturation of medium follicle (MF)-derived porcine oocytes *in vitro*

Conc. (mM) of 3-MA	No. of oocytes examined	No. (%) of oocytes degenerated	No. (%) of oocytes proceeded to the stage of			
			GV	M-I	A-I/T-I	M-II
0	193	0 (0)	17 (8.8 ± 3.1)	31 (16.1 ± 2.2 [a])	0 (0)	145 (75.1 ± 3.1 [a])
0.2	192	2 (1.0 ± 0.6)	21 (10.9 ± 3.1)	48 (25.0 ± 2.2 [b])	0 (0)	121 (63.0 ± 1.6 [b])
2	192	0 (0)	12 (6.3 ± 1.3)	53 (27.6 ± 2.1 [b])	3 (1.6 ± 0.5)	124 (64.6 ± 3.0 [ab])

Percentage data in parentheses are shown as mean ± SEM from four replicates. GV, germinal vesicle; M-I, metaphase-I; A-I/T-I, anaphase-I/telophase-I; M-II, metaphase-II. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Table 4. Effect of 3-methyladenine (3-MA) during the first 20 h of culture on the meiotic maturation of small follicle (SF)-derived porcine oocytes *in vitro*

Origin of COCs	Conc. (mM) of 3-MA	No. of oocytes examined	No. (%) of oocytes degenerated	No. (%) of oocytes proceeded to the stage of			
				GV	M-I	A-I/T-I	M-II
MF	0	201	11 (5.5 ± 1.8)	16 (8.0 ± 1.6)	32 (15.9 ± 3.0 [a])	2 (1.0 ± 0.6)	140 (69.7 ± 2.8 [a])
SF	0	186	7 (3.8 ± 1.5)	36 (19.4 ± 2.9)	55 (29.6 ± 1.9 [b])	0 (0)	88 (47.3 ± 0.4 [b])
SF	0.2	213	8 (3.8 ± 1.7)	30 (14.1 ± 3.0)	66 (31.0 ± 3.0 [b])	1 (0.5 ± 0.4)	108 (50.7 ± 4.0 [b])
SF	2	224	2 (0.9 ± 1.0)	19 (8.5 ± 3.2)	97 (43.3 ± 2.1 [c])	1 (0.4 ± 0.4)	105 (46.9 ± 3.4 [b])

Percentage data in parentheses are shown as mean ± SEM from five replicates. COCs, cumulus-oocyte complexes; MF, medium follicles; GV, germinal vesicle; M-I, metaphase-I; A-I/T-I, anaphase-I/telophase-I; M-II, metaphase-II. [a–c] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

number of blastomeres per blastocyst (Tables 5 and 6) significantly.

The presence of 3-MA during the first 20 h period of IVM culture reduced the blastocyst formation rates of MF-derived oocytes (Table 7), but not those of SF-derived oocytes (Table 8), following parthenogenetic activation. Although the presence of 3-MA did not change the cleavage rate and the number of blastomeres per blastocyst of both MF- and SF-derived oocytes (Tables 7 and 8), the blastocyst formation rates of SF-derived oocytes was significantly lower than that of MF-derived control cultured in the absence of 3-MA (Table 8).

Table 5. Effect of rapamycin during the first 20 h of *in vitro* maturation on the early developmental competence following parthenogenetic activation of medium follicle (MF)-derived porcine oocytes

Conc. (nM) of rapamycin	No. of mature oocytes examined	No. (%) of oocytes		No. of blastomeres per blastocyst
		cleaved	formed blastocyst	
0	144	128 (88.9 ± 2.6)	34 (23.6 ± 2.3 [a])	30.1 ± 1.5
1	153	141 (92.2 ± 2.4)	54 (35.3 ± 2.4 [b])	28.3 ± 1.4
10	144	131 (91.0 ± 1.1)	41 (28.5 ± 2.7 [ab])	28.1 ± 1.6

Percentage data in parentheses are shown as mean ± SEM from five replicates. Only mature oocytes with an extruded first polar body were used following *in vitro* maturation culture. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Table 6. Effect of rapamycin during the first 20 h of *in vitro* maturation on the early developmental competence following parthenogenetic activation of small follicle (SF)-derived porcine oocytes

Origin of COCs	Conc. (nM) of rapamycin	No. of mature oocytes examined	No. (%) of oocytes		No. of blastomeres per blastocyst
			cleaved	formed blastocyst	
MF	0	121	106 (87.6 ± 2.7)	37 (30.6 ± 2.6 [a])	30.1 ± 2.2
SF	0	127	114 (89.8 ± 3.5)	23 (18.1 ± 3.4 [b])	26.2 ± 2.2
SF	1	122	114 (93.4 ± 3.4)	19 (15.6 ± 3.1 [b])	27.1 ± 2.9
SF	10	120	110 (91.7 ± 2.8)	28 (23.3 ± 2.9 [ab])	28.6 ± 2.4

Percentage data in parentheses are shown as mean ± SEM from five replicates. COCs, cumulus-oocyte complexes; MF, medium follicles. Only mature oocytes with an extruded first polar body were used following *in vitro* maturation culture. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Table 7. Effect of 3-methyladenine (3-MA) during the first 20 h of *in vitro* maturation on the early developmental competence following parthenogenetic activation of medium follicle (MF)-derived porcine oocytes

Conc. (mM) of 3-MA	No. of mature oocytes examined	No. (%) of oocytes		No. of blastomeres per blastocyst
		cleaved	formed blastocyst	
0	102	93 (91.2 ± 3.9)	50 (49.0 ± 5.8 [a])	31.6 ± 1.2
0.2	99	89 (89.9 ± 2.8)	22 (22.2 ± 5.7 [b])	27.4 ± 1.0
2	102	89 (87.3 ± 4.9)	23 (22.5 ± 6.3 [b])	28.0 ± 1.1

Percentage data in parentheses are shown as mean ± SEM from four replicates. Only mature oocytes with an extruded first polar body were used following *in vitro* maturation culture. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Table 8. Effect of 3-methyladenine (3-MA) during the first 20 h of *in vitro* maturation on the early developmental competence following parthenogenetic activation of small follicle (SF)-derived porcine oocytes

Origin of COCs	Conc. (mM) of 3-MA	No. of mature oocytes examined	No. (%) of oocytes		No. of blastomeres per blastocyst
			cleaved	formed blastocyst	
MF	0	141	134 (95.0 ± 1.4)	35 (24.8 ± 3.9 [a])	27.7 ± 1.2
SF	0	120	105 (87.5 ± 3.2)	11 (9.2 ± 3.1 [b])	25.2 ± 2.4
SF	0.2	105	84 (80.0 ± 3.0)	7 (6.7 ± 2.5 [b])	30.5 ± 2.0
SF	2	104	88 (84.6 ± 7.0)	6 (5.8 ± 2.7 [b])	27.8 ± 2.2

Percentage data in parentheses are shown as mean ± SEM from five replicates. COCs, cumulus-oocyte complexes; MF, medium follicles. Only mature oocytes with an extruded first polar body were used following *in vitro* maturation culture. [a, b] Within a column, percentage with different superscript differed significantly ($P < 0.05$).

Discussion

The autophagic activity, as assessed by the level of LC3-II protein has been reported to increase significantly in porcine oocytes, after exposure of the COCs to gonadotropins and dibutyryl cAMP, and then decreased until the end of IVM culture [16]. It was demonstrated earlier that rapamycin at 1 nM significantly increased both LC3-II level and meiotic and developmental competencies of porcine oocytes; however, the size of the aspirated follicles was not apparent [17]. In the current study, we confirmed the stimulating effects of rapamycin on the meiotic and developmental competencies of MF-derived oocytes. Our results showed significantly increased maturation rate and blastocyst formation following parthenogenetic activation when 1 nM rapamycin was present in the medium during the first 20 h of IVM culture. These results suggest that MF-derived oocytes respond to rapamycin, increase their autophagic activity, which is negatively regulated by the mTOR signaling pathway [18], and consequently improve the meiotic and developmental competencies of the oocytes. Furthermore, the presence of 3-MA significantly decreased the meiotic and developmental competencies of MF-derived porcine oocytes. This result was consistent with the results of a recent study using another selective autophagy inhibitor, LY294002 [25]. The effective concentration of 3-MA in the current study was also similar to that in a previous report on porcine parthenotes [14]. Therefore, these results suggest that MF-derived oocytes are sensitive to 3-MA, an inhibitor of autophagy through the activation of the mTOR signaling pathway, consequently decreasing the meiotic and developmental competences of the oocytes. Thus, MF-oocytes appear to have the ability to regulate autophagic activity by removing factors that should be degraded through the mTOR signaling pathway to achieve meiosis and early development.

In SF-derived oocytes, however, we noticed that the presence of 1 and 10 nM rapamycin during the first 20 h of IVM culture did not affect either the number of oocytes proceeding to the metaphase-II stage or the percentage of mature oocytes developing to the blastocyst stage following parthenogenetic activation. This result was in sharp contrast to the responses of MF-derived oocytes in the present and previous studies [17]. This result suggests that porcine SF-derived oocytes have a reduced or deficient response to rapamycin, and that rapamycin has no effect on the autophagic activity and resulting meiotic and developmental competencies of the oocytes. Furthermore, we showed that the meiotic and developmental competencies of SF-derived oocytes were unaffected by supplementation with an autophagy inhibitor, 3-MA, during the first 20 h period of IVM culture, showing that SF-oocytes seem to have no or very low reactivity to 3-MA. This result was different from the result with the MF-derived oocytes in the present study. Therefore, this evidence suggests that SF-derived oocytes may be deficient or have a low ability to regulate autophagic activity through the mTOR signaling pathway.

In the present study, although a difference in rapamycin and 3-MA activity was noticed between MF- and SF-oocytes, the underlying mechanism of how MF-derived oocytes acquire the ability for follicular development remains unclear. The diameter of oocytes [5], as well as the meiotic and developmental competencies, are known to be significantly lower in SF-derived oocytes than in MF-derived oocytes [7–9]. The ability to regulate autophagic activity through the mTOR signaling pathway, which affects the meiotic and developmental competences, was observed only in MF-derived oocytes, but not in SF-derived oocytes, in this study. Our finding suggests that differences in response to regulate autophagic activity in MF- and SF-derived oocytes could be a contributing factor to the differences in the meiotic and developmental competencies. The meiotic and developmental competencies of oocytes are acquired gradually through communication between oocytes and surrounding somatic cells [26]. Factors secreted from oocytes, such as bone morphogenetic protein 15 and growth and differentiation factor 9 [26–28], and surrounding cumulus cells, such as vascular endothelial growth factor [6, 29], have been known to promote the meiotic and developmental competencies. Since the presence of rapamycin during IVM culture improved the developmental competence of MF-derived oocytes surrounded by poor cumulus cells [30], the ability of oocytes to regulate autophagic activity through mTOR signaling pathway would readily reflect the competence. Further experiments are required to understand the mechanism underlying the ability of MF-derived oocytes to regulate autophagic activity via the mTOR signaling pathway, during the communication between oocyte and the surrounding somatic cells.

In conclusion, our current results demonstrate that response to the autophagy inducer (rapamycin) and inhibitor (3-MA), and the ability to regulate autophagic activity through the mTOR signaling pathway, might differ between MF- and SF-derived porcine oocytes, thus affecting the meiotic and developmental competencies.

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