Journal of Reproduction and Development, Vol. 63, No 6, 2017

—Original Article—

Optimum culture duration for growing oocytes to attain meiotic and fertilization competence

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Abstract. To determine the optimum culture duration for porcine growing oocytes (GOs) to attain maturation competence, we examined the meiotic competence, chromatin configuration, and fertilization ability of porcine oocytes obtained from early antral follicles and cultured for 10–16 days. The survival rate of oocytes after 10 days of culture (62.8%) was similar to that of oocytes after 12 days of culture (55%) and significantly higher than that of oocytes cultured for 14 and 16 days (52.9 and 24.3%, respectively). No significant difference was observed in the diameter of ooplasm from oocytes cultured for different durations (117.4–118.3 μm). The maturation rates of surviving oocytes after 10 and 16 days of culture (38.3 and 22.7%, respectively) were significantly lower than those of oocytes cultured for 12 and 14 days, and their *in vivo* counterparts (52.8–62.4%). The number of oocytes with surrounded-nucleolus chromatin was significantly lower in the 10-day culture group (78.4%) as compared with 14-day culture and *in vivo* counterpart groups (93.6 and 95.1%, respectively). After *in vitro* maturation and intracytoplasmic sperm injection, no significant difference was observed in the rate of fertilization among oocytes cultured for 12 and 14 days, and their *in vivo* counterparts (40.5–47.2%). Thus, porcine GOs required at least 12 days to acquire meiotic and fertilization competence, and the culture duration to maximize the number of mature oocytes ranged from 12 to 14 days.
Key words: Chromatin configuration, Fertilization, *In vitro* growth of oocyte, Meiotic competence, Porcine oocyte

(**J. Reprod. Dev. 63:** 591–595, 2017)

The mammalian ovary contains a great number of oocytes at various growth stages. *In vitro* growth of growing oocytes (GOs) provides meiotic and developmental competence and displays the potential to supply mature oocytes for reproductive medicine and reproduction of livestock and endangered animals. Although fully-grown oocytes (FGOs) isolated from large antral follicles possess meiotic, fertilization, and full-term developmental competence, GOs in small follicles are insufficiently equipped with these potentials [1–4]. Thus, it is important to create a favorable culture environment for GOs.

Several groups have conducted *in vitro* growth experiments with porcine oocytes obtained from preantral [1, 5–11] and early antral follicles [9, 12–17]. In many cases, the meiotic competence of oocytes following *in vitro* growth was extremely low. To create favorable culture conditions for growing mammalian oocytes, the culture environment, including the medium [8], hormones [10–13, 16], serum [8, 18], macromolecules [9, 19], culture substratum [9, 17], oxygen concentration [20, 21], and medium volume [22, 23] have been assessed. Culture duration has been proposed to be one of the important factors to obtain oocytes with meiotic competence [4]. Both insufficient and excessive culture duration may cause a decrease in meiotic competence [24–26]. However, no reports have described the proper duration of culture for porcine GOs obtained from early antral follicles to attain meiotic competence.

In mammalian oocytes, the diameter of ooplasm correlates with meiotic competence [1–4]. In pigs, an immature oocyte with a diameter of more than 115 μm is considered as a marker for the attainment of meiotic competence [4]. To attain meiotic competence *in vitro*, porcine GOs are required to grow up to 115 μm. During oocyte growth, chromatin configuration changes from a dispersed chromatin state throughout the nucleoplasm (non-surrounded nucleolus; NSN) to a highly condensed chromatin surrounding the nucleolus (surrounded-nucleolus; SN) [27–29]. Moreover, germinal vesicle (GV) oocytes with SN chromatin have been shown to exhibit higher meiotic and developmental competence as compared to GV oocytes with NSN chromatin [30, 31]. Therefore, chromatin configuration may also act as an indicator to predict the meiotic competence of oocytes grown under *in vitro* conditions.

In the present study, the effects of various culture durations of porcine GOs on the meiotic competence of oocytes were assessed. Furthermore, we examined the chromatin configuration of oocytes and fertilization competence of mature oocytes following *in vitro* growth.

Materials and Methods

Collection of oocyte-granulosa cell complexes (OGCs) and FGOs

The porcine ovaries were freshly obtained from prepubertal gilts, approximately 180 days old, at a local slaughterhouse. OGCs were

Received: August 21, 2017
Accepted: September 25, 2017
Published online in J-STAGE: October 12, 2017
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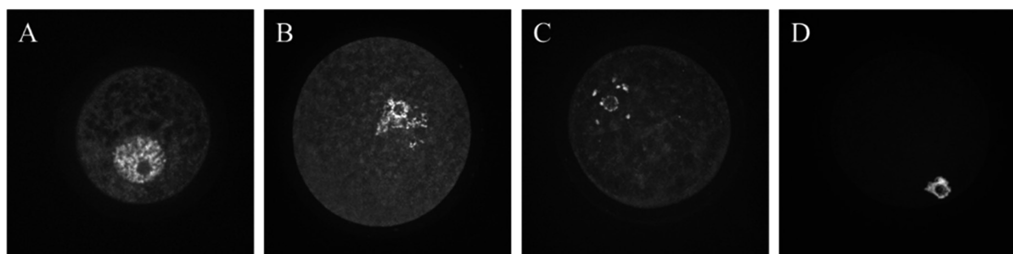

Fig. 1. Chromatin configuration of porcine oocytes. Representative images of chromatin configuration of porcine oocytes with NSN (A) and SN (B–D) chromatin are shown. (A) Porcine oocytes with NSN chromatin had diffused filamentous chromatin distributed over the nuclear area. (B–D) Porcine oocytes with SN chromatin had a condensed chromatin rim around the nucleolus with (B, C) or without (D) many condensed chromatin clumps in the nucleoplasm.

obtained from early antral follicles with diameters of 300–900 µm using scalpels. The diameter of ooplasm from GOs in OGCs was measured. GOs with ooplasm diameters ranging from 95–105 µm and surrounded by multiple layers of granulosa cells were selected. FGOs with ooplasm diameters between 115–125 µm were obtained by aspiration of large antral follicles (3–6 mm diameter) using a 21-gauge needle and 10 ml syringe. FGOs with multiple layers of compacted cumulus cells were selected. Ooplasm diameter was measured using an ocular micrometer under an inverted microscope (× 40; IX-71; Olympus, Tokyo, Japan).

In vitro culture of OGCs

OGCs were cultured as described by Hashimoto *et al.* [9, 32] with minor modifications. The culture media was composed of TCM199 Earle's salt (12340-030; Life Technologies, Carlsbad, CA, USA), 2% (w/v) polyvinylpyrrolidone (PVP; PVP360; Sigma-Aldrich, St. Louis, MO, USA), 0.05 mg/ml gentamycin (G1397; Sigma-Aldrich), 0.02% L-carnitine (Lonza, Tokyo, Japan) [32], 1 µg/ml estradiol (E-8875; Sigma-Aldrich), 50 µg/ml ascorbic acid (A4544; Sigma-Aldrich), 1% (v/v) insulin-transferrin-selenium-A supplement (51300-044; Life Technologies), and 3 mg/mL bovine serum albumin (BSA; A7638; Sigma-Aldrich). Each OGC was cultured individually in 25 µl droplets in cell culture dishes (353002; Life Technologies) at 38.5°C under 5% CO_2 with high humidity for 10, 12, 14, or 16 days. To evaluate the growth of oocytes, their ooplasm diameter was measured and one-half volume of the culture medium was exchanged with fresh medium every 2 days. Oocytes having at least firmly attached granulosa cells and showing no signs of degeneration were considered to be surviving oocytes.

In vitro maturation (IVM)

After 10, 12, 14, and 16 days of culture, GOs and FGOs were cultured individually in a 25 µl drop of porcine oocyte medium [33] containing 0.02 AU/ml follicle-stimulating hormone (Kyoritsu Seiyaku, Tokyo, Japan), 10 IU/ml human chorionic gonadotropin (Aska Pharmaceutical, Tokyo, Japan), 25 mmol/l 2-mercaptoethanol (M3148; Sigma-Aldrich), 1 µg/ml β-estradiol, 1 mM N^6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate sodium salt (D0627; Sigma Aldrich), and 3 mg/ml BSA for 20 h, followed by incubation in medium containing 25 mmol/l 2-mercaptoethanol and 3 mg/ml BSA for 24 h at 38.5°C under 5% CO_2 in air with high humidity. After IVM, the oocytes were treated with 1 mg/ml hyaluronidase (H3757; Sigma-Aldrich) in HEPES-buffered porcine X medium (PXM-Hepes) [33] for 1 min, and the surrounding cells were removed by gentle pipetting. Mature oocytes were determined by extrusion of the first polar body. To assess fertilization, mature oocytes were inseminated by intracytoplasmic sperm injection (ICSI), as described later. To examine meiotic maturation, oocytes after being cultured for maturation were mounted on glass slides, fixed in Farmer's solution (ethanol:acetic acid = 3:1), and stained with 1% orcein (157-00943; Wako Pure Chemical Industries, Osaka, Japan).

Chromatin configuration

After 10 and 14 days of culture, GOs and FGOs were fixed with 2% (v/v) paraformaldehyde (167-25981; Wako Pure Chemical Industries) at 37°C for 1 h. After washing, oocytes were stained with 10 µg/ml Hoechst 33342 (346-07951; Dojindo, Kumamoto, Japan) at room temperature (25°C) for 10 min. The oocytes were placed on glass slides, mounted with a small drop of Vectashield mounting medium (H-1000; Vector Laboratories, CA, USA), and observed using a laser scanning confocal microscope (CV1000; Yokogawa Electric, Tokyo, Japan). Chromatin configuration was classified into various categories (Fig. 1) based on previous reports [27]. GV oocytes had a nucleolus and diffused filamentous chromatin distributed throughout the nucleoplasm in GV0. In GV1, GV oocytes had a nucleolus that was surrounded by condensed chromatin. In GV2, GV oocytes showed a nucleolus surrounded by condensed chromatin and a few chromatin clumps near the nuclear membrane. In GV3, GV oocytes had a condensed chromatin surrounding the nucleolus with many chromatin clumps or strands distributed throughout the nucleoplasm. Oocytes with GV0 and GV1–3 were categorized into NSN and SN configuration, respectively.

ICSI

Frozen semen and sperm-thawing buffer were purchased from Hiroshima cryopreservation services (Hiroshima, Japan). Frozen semen was thawed in a water bath at 37°C for 30 min prior to ICSI. After thawing, spermatozoa were suspended in sperm-thawing buffer at 37°C for 15 min, followed by centrifugation at 700 × *g* at 37°C for 10 min. After centrifugation, spermatozoa were resuspended in 1 mL sperm-thawing buffer and stored at 37°C. The sperm suspension was placed on a manipulation chamber and covered with mineral oil.

Table 1. Meiotic competence of porcine oocytes after *in vitro* growth

Culture duration	Numbers of		Ooplasm diameter (μm ± SD)	Numbers of		The overall maturation rate per cultured GO
	Oocytes examined	Oocytes survived after *in vitro* growth (%)		GVBD oocytes after *in vitro* maturation (%)	mature oocytes after *in vitro* maturation (%)	
Non-cultured GO	96		101 ± 2.4	30 (31.2) [a]	0 (0) [a]	
10-day	366	230 (62.8) [a]	117.5 ± 4.2	153 (66.5) [b]	88 (38.3) [b]	24% [a]
12-day	358	197 (55) [ab]	117.4 ± 4.4	164 (83.2) [c]	104 (52.8) [c]	29.1% [ab]
14-day	397	210 (52.9) [b]	118.3 ± 4	176 (83.8) [c]	131 (62.4) [c]	33% [b]
16-day	181	44 (24.3) [c]	117.6 ± 2.7	35 (79.5) [c]	10 (22.7) [b]	5.5% [c]
FGO	229		117.9 ± 2.4	200 (87.3) [c]	142 (62) [c]	

The ooplasm diameters of surviving oocytes at each culture duration are shown as means ± SD. Non-cultured GOs are non-cultured growing oocytes obtained from small antral follicles (300–900 μm in diameter). FGOs are *in vivo* fully-grown oocytes obtained from large antral follicles (3–6 mm in diameter). [a–c] Different letters indicate statistically significant differences (P < 0.05).

Spermatozoa with normal morphology and motility were captured by an injection pipette, washed, and immobilized with piezo-pulses in 10% PVP. The immobilized spermatozoa were injected into mature oocytes in PXM-Hepes containing 0.1% (w/v) Poly(vinyl alcohol) (PVA; P8136; Sigma-Aldrich). After 1 h, the oocytes were transferred to an electrical activation buffer containing 0.28 M D(–)-mannitol solution supplemented with 0.05 mM calcium chloride ($CaCl_2$) dihydrate (039-00431; Wako Pure Chemical Industries), 0.1 mM magnesium sulfate ($MgSO_4$) heptahydrate (137-00402; Wako Pure Chemical Industries), and 0.01% (w/v) BSA. The oocytes were then activated with three DC pulses of 1.5 kv/cm for 50 μsec using the Electro Cell Fusion Generator (LF201; Nepa Gene, Chiba, Japan) and 1 mm gap electrode (CUY5000P1; Napa Gene). After activation, presumptive zygotes were cultured at 38.5°C under an atmosphere of 5% O_2, 5% CO_2, and 90% N_2 in porcine zygote medium-5 [33] containing 0.3% BSA. After 18-h activation, zygotes were mounted on glass slides, fixed in Farmer's solution, and stained with 1% orcein (157-00943; Wako Pure Chemical Industries). Normal fertilization was determined by the detection of two pronuclei and two polar bodies.

Statistical analysis

The survival rates of OGCs, meiotic maturation, number of oocytes with SN chromatin, and fertilization of oocytes were compared for different culture durations using Bonferroni-corrected chi-squared analysis. The ooplasm diameter at different culture durations was compared with the Tukey-Kramer test. A P value less than 0.05 was considered to be statistically significant.

Results

Effect of culture duration on ooplasm diameter and meiotic competence of oocytes

The survival rate of oocytes decreased with an increase in culture duration (Table 1). The survival rate of oocytes cultured for 10 days (62.8%) was significantly higher (P < 0.05) than that of oocytes cultured for 14 and 16 days (52.9 and 24.3%, respectively). Furthermore, the survival rate was significantly lower (P < 0.05) in oocytes cultured for 16 days as compared to other groups. No significant difference was observed in the diameter of the surviving oocytes between the 10-, 12-, 14- and 16-day culture groups (117.5 ± 4.2, 117.4 ± 4.4, 118.3 ± 4, and 117.6 ± 2.7 μm, respectively; Fig. 2).

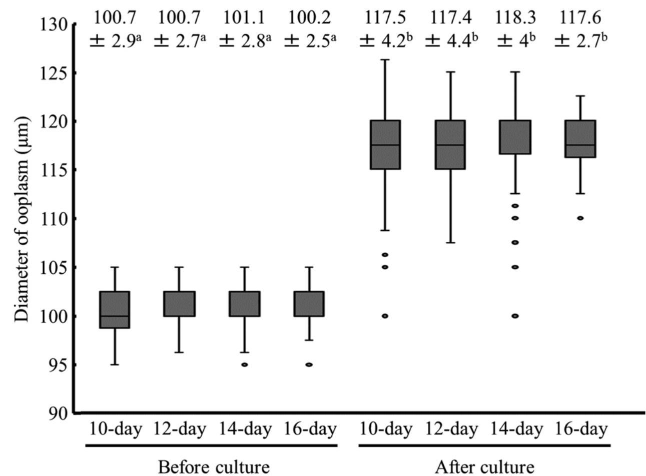

Fig. 2. Mean diameter of ooplasm and diameter distribution of oocytes before and after *in vitro* growth. The diameters of ooplasm before and after *in vitro* growth were measured using the surviving oocytes in each group. Data are shown as box plots, wherein the hash mark represents the median, the top and bottom of the box represent 25th and 75th percentiles, respectively, the upper and lower whiskers represent maximum and minimum, respectively, and the circles represent outlier. Mean ooplasm diameters at each culture duration are indicated at the top. [ab] Different letters indicate statistically significant differences (P < 0.05).

To assess their meiotic competence, the oocytes were cultured for maturation. The break down (BD) rate of GV in GOs (31.2%) was significantly lower (P < 0.05) than that in oocytes cultured *in vitro* (Table 1). The BD rate of GV in oocytes cultured for 10 days (66.5%) was significantly lower (P < 0.05) than that in oocytes cultured for 12 and 14 days, and FGOs (83.2, 83.8 and 87.3%, respectively). The maturation rate of oocytes cultured for 10 and 16 days (38.3 and 22.7%, respectively) was significantly lower (P < 0.05) than that of oocytes cultured for 12 and 14 days, and FGOs (52.8, 62.4 and 62%, respectively). No mature oocytes were obtained from GOs without *in vitro* growth.

The overall maturation rate per cultured GO in oocytes cultured

for 14 days (33%) was similar to that in oocytes cultured for 12 days (29.1%), which was significantly higher ($P < 0.05$) than that in oocytes cultured for 10 days (24%). Furthermore, the maturation rate of oocytes after 16 days of culture (5.5%) was significantly lower than that of oocytes after 10, 12, and 14 days of culture.

Chromatin configuration

Oocytes grown in culture for 10 and 14 days, as well as FGOs were examined for chromatin configuration, and showed no significant difference in their ooplasm diameter (117 ± 5.1, 117 ± 3.4, and 118.4 ± 2.8 µm, respectively; Table 2). *In vitro* culture for oocyte growth increased ($P < 0.05$) the number of oocytes with SN chromatin (78.4–95.1%) as compared with GOs (5.6%). In comparison with oocytes in 14-day culture and FGOs, oocytes in 10-day culture showed significantly ($P < 0.05$) lower levels of SN chromatin (78.4, 93.6 and 95.1% for oocytes cultured for 10 and 14 days, and FGOs, respectively).

Fertilization competence of oocytes after in vitro growth and IVM

As shown in Table 3, no significant differences were observed in the diameter of mature oocytes, survival rates after ICSI, and normal fertilization rates among *in vitro* culture groups and FGOs (diameters: 116.7–118.9 µm; survival rates: 66.1–100%; fertilization rates: 10–47.2%). However, the activation failure rate after ICSI (metaphase II and sperm) of oocytes grown *in vitro* for 16 days (80%) significantly increased ($P = 0.00005$) as compared to FGOs (25%).

Table 2. Number of oocytes with condensed chromatin surrounding the nucleolus after *in vitro* growth

Culture duration	No. of OGCs	Ooplasm diameter (µm ± SD)	No. of oocytes with SN chromatin (%)
Non-cultured GO	72	102 ± 2.5 [a]	4 (5.6) [a]
10-day	88	117 ± 5.1 [b]	69 (78.4) [b]
14-day	94	117 ± 3.4 [b]	88 (93.6) [c]
FGO	61	118.4 ± 2.8 [b]	58 (95.1) [c]

The diameter of ooplasm is shown as means ± SD. Non-cultured GOs: growing oocytes examined immediately after collection from small antral follicles (300–900 µm in diameter). FGOs: fully grown oocytes obtained from large antral follicles (3–6 mm in diameter). [a–c] Different letters indicate statistically significant differences ($P < 0.05$).

Discussion

In this study, we showed that porcine oocytes cultured for 12 and 14 days exhibited meiotic competence similar to that of their *in vivo* counterparts.

Although oocyte diameter has been proposed to be one of the important indicators of meiotic competence [1–4], the meiotic competence of oocytes after 10 days of culture was lower than that of oocytes cultured for 12 and 14 days, and FGOs; however, no difference was observed in the oocyte diameter. Thus, oocyte diameter may not be the sole indicator of oocyte meiotic competence.

We assessed chromatin configuration to evaluate meiotic competence of *in vitro*-grown oocytes. The proportion of oocytes with SN chromatin was lower in 10-day culture group as compared to 14-day culture group and FGOs. Most of the FGOs derived from large antral follicles display SN chromatin [30], as observed in our study. GV oocytes with NSN chromatin are known to be transcriptionally active [34], and the transcriptional activity of GV oocytes is thought to be negatively correlated with their meiotic competence [35]. In particular, the expression of mRNAs [36, 37] and proteins, including cytoplasmic lattice, which stores maternally derived mRNA and ribosome [38] and acts as a marker of attainment of meiotic and developmental competence [37], differed between oocytes with SN and NSN chromatin. GV oocytes with SN chromatin have been shown to display high meiotic and developmental competence as compared to those with NSN chromatin [30, 31]. Taken together, SN chromatin may act as a marker of the final stage of oocyte growth [35]. Thus, a lower proportion of oocytes with SN chromatin is indicative of low oocyte meiotic competence after 10 days of culture. Our results showed that porcine GOs obtained from early antral follicles require at least 12 days of culture to attain meiotic competence.

Excessive culture duration has been shown to decrease the viability of both oocytes and granulosa cells in cattle [25]. The expansion of granulosa cells and meiotic competence of oocytes are known to reduce by extended culture duration in mice [26]. Consistent with these previous reports, the longer duration (16 days) of culture drastically decreased the viability of oocytes and meiotic competence of the surviving oocytes (Table 1). Our data showed that 16 days of culture is too long to maintain the *in vitro* viability of GOs obtained from early antral follicles.

To assess the fertilization ability of oocytes after *in vitro* growth and maturation, we performed ICSI. The rate of normal fertilization of mature oocytes after 10, 12, and 14 days of culture was similar to that of FGOs, indicating that *in vitro*-grown oocytes acquire meiotic

Table 3. Effect of culture duration on the fertilization competence of oocytes after *in vitro* growth

Culture duration	No. of mature oocytes	Ooplasm diameter (µm ± SD)	No.of surviving oocytes after ICSI (%)	From surviving oocytes (%)		
				2PN	FP + sperm	metaphase II + sperm
10-day	56	117.3 ± 2	37 (66.1)	15 (40.5)	4 (10.8)	18 (48.7) [ab]
12-day	48	116.7 ± 3.5	36 (75)	17 (47.2)	2 (5.6)	17 (47.2) [ab]
14-day	56	116.7 ± 4.1	47 (83.9)	21 (44.7)	6 (12.8)	20 (42.6) [ab]
16-day	10	118.9 ± 3	10 (100)	1 (10)	1 (10)	8 (80) [a]
FGO	82	117.8 ± 3	68 (82.9)	32 (47.1)	19 (27.9)	17 (25) [b]

The ooplasm diameters of mature oocytes after *in vitro* growth and maturation are shown as means ± SD. FGOs: fully-grown oocytes obtained from large antral follicles (3–6 mm in diameter). 2PN: two pronuclei. FP: female pronucleus. Normal fertilization was determined by extrusion of the second polar body and formation of two pronuclei. [ab] Different letters indicate statistically significant differences ($P < 0.05$).

as well as fertilization competence. The activation failure after ICSI was statistically higher in oocytes cultured for 16 days than FGOs. In addition, the fertilization rate of these oocytes was extremely low, although no statistical difference was observed (probably due to the small number). Excessive culture duration of GOs may decrease the oocyte viability.

Our results demonstrated that the proper duration for the overall maturation of cultured GOs ranged from 12 to 14 days (Table 1). Cell viability decreased with increase in culture duration, while shorter culture duration was insufficient to attain meiotic competence, as previously reported [24, 25]. Thus, it is important to establish proper culture duration to obtain mature oocytes with developmental competence.

Data of the present study show that porcine GOs obtained from early antral follicles require at least 12 days of culture to acquire meiotic and fertilization competence, and the culture duration to maximize the number of mature oocytes ranges from 12 to 14 days.

Acknowledgements

Part of this work was supported by a grant from the Japan Agency for Medical Research and Development (17gk0110014s0402 to SH and YM) and a grant from the Japan Society for the Promotion of Science (KAKENHI 17K08144 to SH). Authors thank Dr H Goto for his technical support.

References

1. **Hirao Y, Nagai T, Kubo M, Miyano T, Miyake M, Kato S.** *In vitro* growth and maturation of pig oocytes. *J Reprod Fertil* 1994; **100**: 333–339. [Medline] [CrossRef]
2. **Otoi T, Yamamoto K, Koyama N, Tachikawa S, Suzuki T.** Bovine oocyte diameter in relation to developmental competence. *Theriogenology* 1997; **48**: 769–774. [Medline] [CrossRef]
3. **Miyano T, Hirao Y.** *In vitro* growth of oocytes from domestic species. *J Mamm Ova Res* 2003; **20**: 78–85. [CrossRef]
4. **Motlik J, Fulka J.** Factors affecting meiotic competence in pig oocyte. *Theriogenology* 1986; **25**: 87–96. [CrossRef]
5. **Telfer EE.** The development of methods for isolation and culture of preantral follicles from bovine and porcine ovaries. *Theriogenology* 1996; **45**: 101–110. [CrossRef]
6. **Telfer EE, Binnie JP, McCaffery FH, Campbell BK.** *In vitro* development of oocytes from porcine and bovine primary follicles. *Mol Cell Endocrinol* 2000; **163**: 117–123. [Medline] [CrossRef]
7. **Wu J, Emery BR, Carrell DT.** *In vitro* growth, maturation, fertilization, and embryonic development of oocytes from porcine preantral follicles. *Biol Reprod* 2001; **64**: 375–381. [Medline] [CrossRef]
8. **Mao J, Wu G, Smith MF, McCauley TC, Cantley TC, Prather RS, Didion BA, Day BN.** Effects of culture medium, serum type, and various concentrations of follicle-stimulating hormone on porcine preantral follicular development and antrum formation *in vitro*. *Biol Reprod* 2002; **67**: 1197–1203. [Medline] [CrossRef]
9. **Hashimoto S, Ohsumi K, Tsuji Y, Harauma N, Miyata Y, Fukuda A, Hosoi Y, Iritani A, Morimoto Y.** Growing porcine oocyte-granulosa cell complexes acquired meiotic competence during *in vitro* culture. *J Reprod Dev* 2007; **53**: 379–384. [Medline] [CrossRef]
10. **Wu J, Xu B, Wang W.** Effects of luteinizing hormone and follicle stimulating hormone on the developmental competence of porcine preantral follicle oocytes grown in vitro. *J Assist Reprod Genet* 2007; **24**: 419–424. [Medline] [CrossRef]
11. **Tasaki H, Iwata H, Sato D, Monji Y, Kuwayama T.** Estradiol has a major role in antrum formation of porcine preantral follicles cultured *in vitro*. *Theriogenology* 2013; **79**: 809–814. [Medline] [CrossRef]
12. **Cayo-Colca IS, Yamagami Y, Phan TC, Miyano T.** A combination of FSH and dibutyryl cyclic AMP promote growth and acquisition of meiotic competence of oocytes from early porcine antral follicles. *Theriogenology* 2011; **75**: 1602–1612. [Medline] [CrossRef]
13. **Kubo N, Cayo-Colca IS, Miyano T.** Effect of estradiol-17β during *in vitro* growth culture on the growth, maturation, cumulus expansion and development of porcine oocytes from early antral follicles. *Anim Sci J* 2015; **86**: 251–259. [Medline] [CrossRef]
14. **Oi A, Tasaki H, Munakata Y, Shirasuna K, Kuwayama T, Iwata H.** Effects of reaggregated granulosa cells and oocytes derived from early antral follicles on the properties of oocytes grown *in vitro*. *J Reprod Dev* 2015; **61**: 191–197. [Medline] [CrossRef]
15. **Itami N, Shirasuna K, Kuwayama T, Iwata H.** Resveratrol improves the quality of pig oocytes derived from early antral follicles through sirtuin 1 activation. *Theriogenology* 2015; **83**: 1360–1367. [Medline] [CrossRef]
16. **Itami N, Munakata Y, Shirasuna K, Kuwayama T, Iwata H.** Promotion of glucose utilization by insulin enhances granulosa cell proliferation and developmental competence of porcine oocyte grown *in vitro*. *Zygote* 2017; **25**: 65–74. [Medline] [CrossRef]
17. **Munakata Y, Kawahara-Miki R, Shirasuna K, Kuwayama T, Iwata H.** Polyacrylamide gel as a culture substrate improves in vitro oocyte growth from porcine early antral follicles. *Mol Reprod Dev* 2017; **84**: 44–54. [Medline] [CrossRef]
18. **Sugimoto H, Kida Y, Miyamoto Y, Kitada K, Matsumoto K, Saeki K, Taniguchi T, Hosoi Y.** Growth and development of rabbit oocytes *in vitro*: effect of fetal bovine serum concentration on culture medium. *Theriogenology* 2012; **78**: 1040–1047. [Medline] [CrossRef]
19. **Hirao Y, Itoh T, Shimizu M, Iga K, Aoyagi K, Kobayashi M, Kacchi M, Hoshi H, Takenouchi N.** *In vitro* growth and development of bovine oocyte-granulosa cell complexes on the flat substratum: effects of high polyvinylpyrrolidone concentration in culture medium. *Biol Reprod* 2004; **70**: 83–91. [Medline] [CrossRef]
20. **Hirao Y, Shimizu M, Iga K, Takenouchi N.** Optimization of oxygen concentration for growing bovine oocytes *in vitro*: constant low and high oxygen concentrations compromise the yield of fully grown oocytes. *J Reprod Dev* 2012; **58**: 204–211. [Medline] [CrossRef]
21. **Eppig JJ, Wigglesworth K.** Factors affecting the developmental competence of mouse oocytes grown *in vitro*: oxygen concentration. *Mol Reprod Dev* 1995; **42**: 447–456. [Medline] [CrossRef]
22. **Hirao Y, Shimizu M, Iga K, Takenouchi N.** Growth of bovine oocyte-granulosa cell complexes cultured individually in microdrops of various sizes. *J Reprod Dev* 2009; **55**: 88–93. [Medline] [CrossRef]
23. **Magalhães DM, Fernandes DD, Mororó MB, Silva CM, Rodrigues GQ, Bruno JB, Matos MH, Campello CC, Figueiredo JR.** Effect of the medium replacement interval on the viability, growth and *in vitro* maturation of isolated caprine and ovine pre-antral follicles. *Reprod Domest Anim* 2011; **46**: 134–140. [Medline] [CrossRef]
24. **Hirao Y, Miyano T.** *In vitro* growth of mouse oocytes: Oocyte size at the beginning of culture influences the appropriate length of culture period. *J Mamm Ova Res* 2008; **25**: 56–62. [CrossRef]
25. **Huang W, Nagano M, Kang SS, Yanagawa Y, Takahashi Y.** Effects of *in vitro* growth culture duration and prematuration culture on maturational and developmental competences of bovine oocytes derived from early antral follicles. *Theriogenology* 2013; **80**: 793–799. [Medline] [CrossRef]
26. **Segers I, Adriaenssens T, Ozturk E, Smitz J.** Acquisition and loss of oocyte meiotic and developmental competence during in vitro antral follicle growth in mouse. *Fertil Steril* 2010; **93**: 2695–2700. [Medline] [CrossRef]
27. **Sun XS, Liu Y, Yue KZ, Ma SF, Tan JH.** Changes in germinal vesicle (GV) chromatin configurations during growth and maturation of porcine oocytes. *Mol Reprod Dev* 2004; **69**: 228–234. [Medline] [CrossRef]
28. **Pesty A, Miyara F, Debey P, Lefevre B, Poirot C.** Multiparameter assessment of mouse oogenesis during follicular growth *in vitro*. *Mol Hum Reprod* 2007; **13**: 3–9. [Medline] [CrossRef]
29. **Tan JH, Wang HL, Sun XS, Liu Y, Sui HS, Zhang J.** Chromatin configurations in the germinal vesicle of mammalian oocytes. *Mol Hum Reprod* 2009; **15**: 1–9. [Medline] [CrossRef]
30. **Bellone M, Zuccotti M, Redi CA, Garagna S.** The position of the germinal vesicle and the chromatin organization together provide a marker of the developmental competence of mouse antral oocytes. *Reproduction* 2009; **138**: 639–643. [Medline] [CrossRef]
31. **Inoue A, Nakajima R, Nagata M, Aoki F.** Contribution of the oocyte nucleus and cytoplasm to the determination of meiotic and developmental competence in mice. *Hum Reprod* 2008; **23**: 1377–1384. [Medline] [CrossRef]
32. **Hashimoto S.** Application of *in vitro* maturation to assisted reproductive technology. *J Reprod Dev* 2009; **55**: 1–10. [Medline] [CrossRef]
33. **Yoshioka K, Suzuki C, Onishi A.** Defined system for *in vitro* production of porcine embryos using a single basic medium. *J Reprod Dev* 2008; **54**: 208–213. [Medline] [CrossRef]
34. **Lodde V, Modina S, Maddox-Hyttel P, Franciosi F, Lauria A, Luciano AM.** Oocyte morphology and transcriptional silencing in relation to chromatin remodeling during the final phases of bovine oocyte growth. *Mol Reprod Dev* 2008; **75**: 915–924. [Medline] [CrossRef]
35. **Liu H, Aoki F.** Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes. *Zygote* 2002; **10**: 327–332. [Medline] [CrossRef]
36. **Labrecque R, Lodde V, Dieci C, Tessaro I, Luciano AM, Sirard MA.** Chromatin remodelling and histone m RNA accumulation in bovine germinal vesicle oocytes. *Mol Reprod Dev* 2015; **82**: 450–462. [Medline] [CrossRef]
37. **Monti M, Zanoni M, Calligaro A, Ko MS, Mauri P, Redi CA.** Developmental arrest and mouse antral not-surrounded nucleolus oocytes. *Biol Reprod* 2013; **88**: 2. [Medline] [CrossRef]
38. **Kim B, Kan R, Anguish L, Nelson LM, Coonrod SA.** Potential role for MATER in cytoplasmic lattice formation in murine oocytes. *PLoS ONE* 2010; **5**: e12587. [Medline] [CrossRef]