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

Takayuki YAMOCHI1), Shu HASHIMOTO1), Masaya YAMANAKA1), Yoshiharu NAKAOKA1) and Yoshiharu MORIMOTO1, 2)

1)IVF Namba Clinic, Osaka 550-0015, Japan
2)HORAC Grand Front Osaka Clinic, Osaka 530-0011, Japan

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

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
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-8877; 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), and 3 mg/mL bovine serum albumin (BSA; A7638; Sigma-Aldrich) (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 nucleolus 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 showed a nucleolus surrounded by condensed chromatin and a few chromatin clumps in air with high humidity. 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 nucleolus 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 showed a condensed chromatin surrounding the nucleolus with many chromatin clumps or strands distributed throughout the nucleolasm. 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.
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 (–)-mannitol solution supplemented with 0.05 mM calcium chloride (CaCl₂) dihydrate (039-00431; Wako Pure Chemical Industries), 0.1 mM magnesium sulfate (MgSO₄ 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) (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% O₂, 5% CO₂ and 90% N₂ in porcine zygote medium-5 [33] containing 0.3% 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% O₂, 5% CO₂, and 90% N₂ 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 containing 0.3% BSA. After 18-h activation, zygotes were mounted on glass slides, fixed in Farmer’s solution, and stained with 1% orcein containing 0.3% BSA.

**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, 117.5 ± 4.2, and 117.4 ± 4.4, respectively; Fig. 2). 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

---

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

| Culture duration | Numbers of Oocytes examined | Numbers of Oocytes survived after in vitro growth (%) | Ooplasm diameter (µm ± SD) | Numbers of GVBD oocytes after in vitro maturation (%) | Numbers of mature oocytes after in vitro maturation (%) | The overall maturation rate per cultured GO |
|------------------|-----------------------------|-----------------------------------------------------|-----------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------|
| Non-cultured GO  | 96                          | 101 ± 2.4                                           | 30 (31.2) a                  | 0 (0) a                                       | 24% a                                        |
| 10-day           | 366                         | 230 (62.8) a                                        | 117.5 ± 4.2                 | 153 (66.5) b                                  | 88 (38.3) b                                   | 29.1% ab                                    |
| 12-day           | 358                         | 197 (55) ab                                         | 117.4 ± 4.4                 | 164 (83.2) c                                  | 104 (52.8) c                                  | 33% b                                       |
| 14-day           | 397                         | 210 (52.9) b                                        | 118.3 ± 4                   | 176 (83.8) c                                  | 131 (62.4) c                                  | 5.5% c                                       |
| 16-day           | 181                         | 44 (24.3) c                                         | 117.6 ± 2.7                 | 35 (79.5) c                                   | 10 (22.7) b                                   |                                             |
| FGO              | 229                         | 44 (24.3) c                                         | 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 vitro fully-grown oocytes obtained from large antral follicles (3–6 mm in diameter). ab Different letters indicate statistically significant differences (P < 0.05).

---

**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).
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%).

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, 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 2. Number of oocytes with condensed chromatin surrounding the nucleus 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). * Different letters indicate statistically significant differences (P < 0.05).

### 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                       |
| 10-day           | 56                    | 117.3 ± 2                 | 37 (66.1)                              | 15 (40.5) a               |
| 12-day           | 48                    | 116.7 ± 3.5               | 36 (75)                                | 17 (47.2) ab              |
| 14-day           | 56                    | 116.7 ± 4.1               | 47 (83.9)                              | 21 (44.7) ab              |
| 16-day           | 10                    | 118.9 ± 3                 | 10 (100)                               | 1 (10)                    |
| FGO              | 82                    | 117.8 ± 3                 | 68 (82.9)                              | 32 (47.1) ab              |

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. * 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 (17gm01010404102 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. Otsi 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 Mammm 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, McCanley 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, Haruma N, Miyata Y, Fukuda A, Hossi Y, Iritani A, Marimoto V. 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 lateinizing 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, Koyama Y. Estradiol has a major role in antrum formation of porcine preantral follicles cultured in vitro. Theriogenology 2013; 79: 809–814. [Medline] [CrossRef]
12. Cayo-Colea IS, Yamagami Y, Phan TC, Miyano T. A combination of FSII 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-Colea 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 porcine oocytes grown in vitro. J Reprod Dev 2015; 61: 191–197. [Medline] [CrossRef]
15. Itami N, Shirasuna K, Kuwayama T, Iwata H. Reversal of the effects of preantral follicle growth on mature oocytes. Mol Reprod Dev 2017; 84: 44–54. [Medline] [CrossRef]
16. Sugimoto H, Kid a Y, Miyamoto Y, Kitada K, Matsumoto K, Sasaki K, Taniguchi T, Hossi 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]
17. Hirao Y, Ino T, Shimizu M, Iga K, Aoyagi K, Kuboyashi M, Kazuki 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]
18. Hiro Y, Shimizu M, Iga K, Takenouchi N. Optimization of oxygen concentration for growing bovine oocytes in vitro; constant low and high oxygen concentrations compromise competence during development of fully grown oocytes. J Reprod Dev 2012; 58: 204–211. [Medline] [CrossRef]
19. 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]
20. Huang W, Nagano M, Kang SS, Yanagawa Y, Takahas Y. Effects of in vitro growth culture duration and premature culture on maturation and developmental competence of bovine oocytes derived from early antral follicles. Theriogenology 2013; 80: 793–799. [Medline] [CrossRef]
21. Seger A, Adriaenssens T, Ozuruk E, Smitt 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]
22. Sun XS, Liu Y, Yue KZ, Ma SF, Tan JH. Changes in germinal vesicle (GV) chromatins configurations during growth and maturation of porcine oocytes. Mol Reprod Dev 2004; 69: 228–234. [Medline] [CrossRef]
23. Pesty A, Miyara F, Dehey P, Lefevre B, Poirier C. Multiparameter assessment of mouse oogenesis during follicular growth in vitro. Mol Hum Reprod 2007; 13: 3–9. [Medline] [CrossRef]
24. Tan JH, Wang HL, Sun XS, Liu Y, Sui HS, Zhang J. Chromatin configurations in the germinval vesicle of mammalian oocytes. Mol Hum Reprod 2009; 15: 1–19. [Medline] [CrossRef]
25. 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]
26. Inoue A, Nakajima R, Nagata M, Askii 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]
27. Hashimoto S. Application of in vitro maturation to assisted reproductive technology. J Reprod Dev 2009; 55: 1–16. [Medline] [CrossRef]
28. Yoshikai K, Suzuki C, Ogawa A. Defined system for in vitro production of porcine embryos using a single basic medium. J Reprod Dev 2008; 54: 208–213. [Medline] [CrossRef]
29. Lodde V, Modina S, Maddos-Hyttel F, 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; 78: 915–924. [Medline] [CrossRef]
30. Liu H, Askii F. Transcriptional activity associated with meiotic competence in fully grown mouse GV oocytes. Zygote 2002; 10: 327–332. [Medline] [CrossRef]
31. Labrecque R, Lodde V, Dieci C, Tessaro L, Luciano AM, Sirard MA. Chromatin remodelling and histone RNA accumulation in bovine germinal vesicle oocytes. Mol Reprod Dev 2015; 82: 450–462. [Medline] [CrossRef]
32. Monti M, Zanoni M, Calligaro A, Ko MS, Mauri P, Redi CA. Developmental arrest and mouse antral not-surrounded nuclei oocytes. Biol Reprod 2013; 88: 2. [Medline] [CrossRef]
33. Kim R, Kuo R, Angulah L, Nelson JM, Connard SA. Potential role of MATER in cytoplasmic lattice formation in murine oocytes. PLoS ONE 2010; 8:e25878. [Medline] [CrossRef]