


Potassium bromate disrupts mitochondrial distribution within murine oocytes during in vitro maturation

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Abstract

Purpose: As disturbed mitochondrial distribution is thought to be a cause of the aging of oocytes, it was investigated whether oxidizing agents exert harmful effects on nuclear maturation and mitochondrial cluster formation in murine oocytes and whether antioxidants could rescue such harmful effects in vitro.

Methods: Oocytes were obtained from female Institute of Cancer Research mice 48 h after an intraperitoneal injection of 7.5 IU pregnant mare serum gonadotropin. The oocytes were cultured with potassium bromate, an oxidizing agent, in the presence or absence of the antioxidant, resveratrol. After 12 h, the nuclear phases and mitochondrial distribution were observed.

Results: Significantly decreased rates of metaphase II (MII) oocytes were observed with 750 μ M and 1000 μ M of potassium bromate, while a significant increase in abnormal mitochondrial clusters was induced at 500 μ M, 750 μ M, and 1,000 μ M. The addition of 10 μ M or 20 μ M resveratrol improved both MII maturity and the cluster formation rates in the presence of potassium bromate.

Conclusions: The addition of potassium bromate reduced MII maturity rates and induced abnormal mitochondrial cluster formation. This effect was alleviated by the antioxidant, resveratrol. The in vitro model used herein is useful for investigating the functions of antioxidants in the aging of oocytes.

KEYWORDS

aged oocytes, antioxidant, in vitro maturation, mitochondria, oxidizing agents

1 | INTRODUCTION

In vitro maturation (IVM) is an assisted reproductive technique to obtain mature mammalian oocytes involving a culture of immature oocytes that were harvested at the germinal vesicle (GV) stage from ovarian follicles. In vitro maturation technology has led to the mass production of livestock because it allows mature oocytes to be obtained in large quantities from immature oocytes.

For human-assisted reproductive technology, the first pregnancy and delivery with IVM was reported in 1991.¹ Using an IVM technique to induce mature oocytes with gonadotropin helps to avoid the risk of some of the harmful effects of ovarian hyperstimulation syndrome.^{2,3} In vitro maturation is also useful in conserving the fertile ovaries of patients who require chemotherapy or radiotherapy.⁴ However, the embryonic development rates using IVM remain quite low in both animals and humans.^{5–12} This is thought to be one of the causes of insufficient

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[Correction added on 14 Feb 2018, after first online publication: All instances of "mol" as a unit of measure have been corrected to " μ M" in the text, in Figures 1 to 4, and in Table 1.]

cytosolic maturation in IVM.¹³ Intracellular glutathione concentrations,^{14,15} as well as the distribution patterns of free calcium ions¹⁶ and cortical granules,¹⁷ are factors that are reportedly related to cytoplasmic maturation. Notably, it has been observed in recent years that a decline in mitochondrial function is related to poor oocyte quality.¹⁸

Aged murine oocytes often show an abnormal mitochondrial distribution.¹⁹ It has been reported that abnormalities in the mitochondrial distribution of aged oocytes are associated with decreased mitochondrial function. Oxidative stress is considered to be a primary cause of this mitochondrial abnormality. Therefore, it was investigated whether the disruption of oocyte nuclear maturation and abnormal mitochondrial distribution could be induced artificially by using compounds with an oxidizing effect to create an aged oocyte model. Potassium bromate, which is used as a food additive, reportedly acts as an oxidizing agent to increase the amount of 8-hydroxydeoxyguanosine, a marker for DNA oxidative damage.²⁰

In this study, the effects of potassium bromate as an oxidizing agent on oocyte nuclear maturation and mitochondrial distribution patterns were investigated. It was revealed that high doses of potassium bromate decreased the percentage of oocytes at the metaphase II (MII) stage and increased abnormal mitochondrial distribution. Furthermore, as the efficacy of resveratrol as an antioxidant has been reported,^{21,22} it was investigated whether resveratrol could improve the oxidizing actions that had been elicited by potassium bromate.

2 | MATERIALS AND METHODS

2.1 | Collection of immature oocytes

Pregnant mare serum gonadotropin (7.5 IU; Teikokuzohki, Tokyo, Japan) was administrated intraperitoneally to 3 week old female Institute of Cancer Research mice (Japan SLC, Shizuoka, Japan). After 48 h, their ovaries were collected after euthanasia by cervical spine fracture dislocation. The follicles were punctured by needle in Leibovitz's L-15 culture medium (Invitrogen, Carlsbad, CA, USA), containing 5% fetal bovine serum (FBS) (Invitrogen). Oocytes with several layers of cumulus cells were used for the experiments.

2.2 | *In vitro* maturation and mitochondrial distribution

2.2.1 | Experiments 1 and 2

In Experiment 1, the oocytes were cultured in maturation medium (Waymouth MB 752/1, containing 5% FBS and 0.23 mM pyruvic acid) with 0, 10, 100, 500, 750, or 1000 μ M potassium bromate (Nacalai Tesque, Kyoto, Japan). In Experiment 2, the oocytes were cultured in maturation medium with 0, 10, or 20 μ M resveratrol (Wako Pure Chemical Industries, Osaka, Japan) in the presence of 500 μ M potassium bromate.

2.2.2 | Culture method

The culture was maintained by using the hanging-drop method. This single-oocyte culture system provides high through-put and excludes

the paracrine effects that are elicited by the presence of other oocytes.²³ Ninety-six-well plates were used for the culture of the oocytes in 10 μ L of culture medium and 10 μ L of mineral oil was used to cover each well. The culture was performed with the plate upside down at 37°C in 5% CO₂ and 95% air.

2.2.3 | Assessment of nuclear maturation and mitochondrial distribution

Twelve hours later, the cumulus cells were removed and the number of oocytes that released the first polar bodies was assessed under the microscope to calculate the MII maturation rate. For the observation of the mitochondrial distribution patterns, 250 nM of MitoTracker® Red CMXRos (Invitrogen) was added to the medium and the oocytes were incubated for 30 min. The oocytes then were washed with Leibovitz's L-15-containing culture medium with 5% FBS and mounted on glass slides that were covered with cover glass. The observations were made by using a confocal laser microscope (LSM-710; Carl Zeiss, Jena, Germany). The mitochondrial distribution patterns were determined by the presence or absence of cluster formations (Figure 1). The mitochondria that aggregated to a diameter of $\geq 5 \mu$ m were judged as a cluster formation.

2.3 | Statistical analysis

For the obtained MII maturation rate and mitochondrial cluster formation rate, a significant difference test between a control and the chemically treated group was conducted by the chi-square test. *P*-values < 0.05 were considered to be statistically significant.

2.4 | Ethical considerations

All the experimental procedures conformed to the "Regulations for Animal Experiments and Related Activities at Tohoku University," were reviewed by the Institutional Laboratory Animal Care and Use Committee of Tohoku University, and finally approved (2016 AgA-048) by the President of the University.

3 | RESULTS

3.1 | Experiment 1

On adding 0, 10, 100, 500, 750, or 1000 μ M of potassium bromate to the maturation medium, the observed rates of MII maturation were 95.6% (43/45), 87.9% (29/33), 86.1% (31/36), 86.3% (88/102), 65.3% (32/49), and 18.8% (24/128), respectively. The maturation rates were significantly decreased by the addition of 750 and 1000 μ M potassium bromate, with <18.8% of the oocytes maturing in the latter condition (Figure 2).

The mitochondria clustering rates were 7.0% (3/43), 13.8% (4/29), 6.5% (2/31), 59.1% (52/88), 59.4% (19/32), and 70.8% (17/24), respectively. Although clustering within the MII oocytes was observed in all conditions, it was especially evident with the

FIGURE 1 Fluorescent images of mitochondrial distribution and transmission images of metaphase II-stage murine oocytes during *in vitro* maturation. The mitochondrial distribution patterns were determined by the presence or absence of cluster formation (stained with MitoTracker® Red CMXRos; Invitrogen, Carlsbad, CA, USA). A, Without potassium bromate treatment, no cluster formation was observed. B, Potassium bromate treatment resulted in cluster formations. Scale bar, 50 μ m

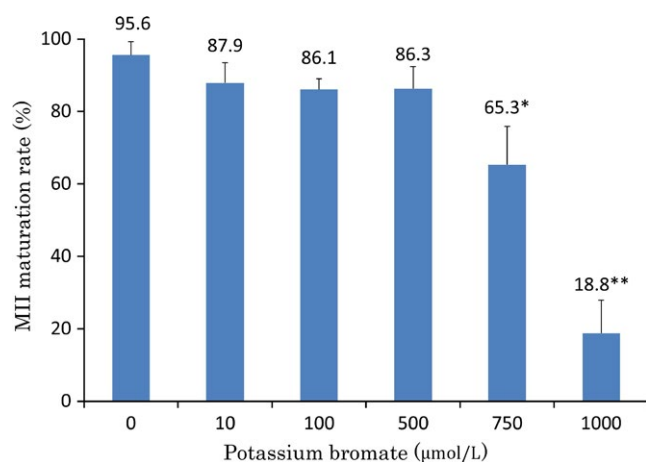
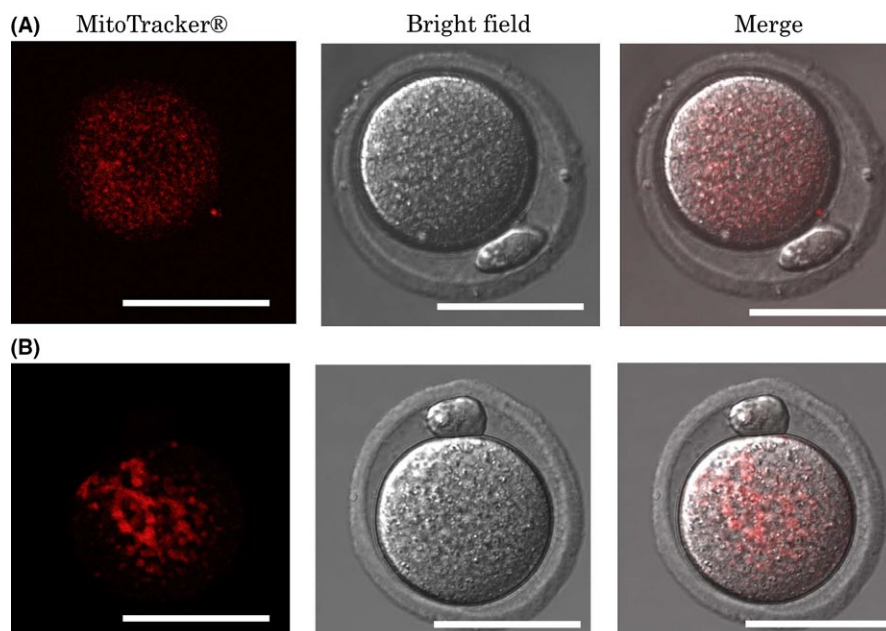


FIGURE 2 Effects of potassium bromate on the metaphase II (MII) oocyte maturation rate. The values represent the mean \pm standard error of the mean. * $P < .05$ and ** $P < .01$

addition of $>500 \mu$ M potassium bromate (almost 60% of the oocytes exhibited clustering), indicating a remarkable increase, compared with the medium to which $<100 \mu$ M had been added (Figures 3 and 4).

3.2 | Experiment 2

Zero, 10, or 20 μ M of resveratrol was added to the culture medium in order to examine the antioxidant effects in the presence of potassium bromate. As 500 μ M potassium bromate did not affect the MII rate, but increased the cluster formation rate (as shown in Experiment 1), this concentration was chosen for this experiment. The MII maturation rates at 0, 10, and 20 μ M were 86.3% (88/102), 83.3% (85/102), and 74.5% (79/106), respectively. The addition of 20 μ M resveratrol significantly decreased the MII rate. The mitochondrial cluster formation rates were 59.1% (52/88), 11.8% (10/85), and 12.7% (10/79),

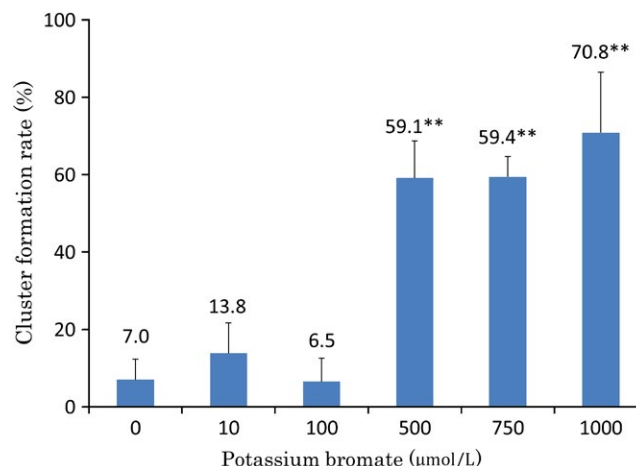


FIGURE 3 Effects of potassium bromate on the mitochondrial cluster formation rate. The values represent the mean \pm standard error of the mean. ** $P < .01$

respectively. Both 10 μ M and 20 μ M of resveratrol significantly decreased the cluster formation rate (Table 1).

4 | DISCUSSION

For patients with ovulatory disorders, such as polycystic ovary syndrome, the IVM technique is important in making effective use of rare oocytes, although further improvements are required. Immediately after starting IVM, the mitochondria aggregate just below the cell membrane and around the GV, then distribute throughout the cytoplasm at the MII stage. It is important that the mitochondria are properly distributed within the oocytes.^{24,25} In addition, it has been concluded that the appropriate distribution of the mitochondria is necessary for proper cytoplasmic maturation of the oocytes.¹⁹ In this way, it is suggested that the distribution of the mitochondria can be an index of oocyte quality.

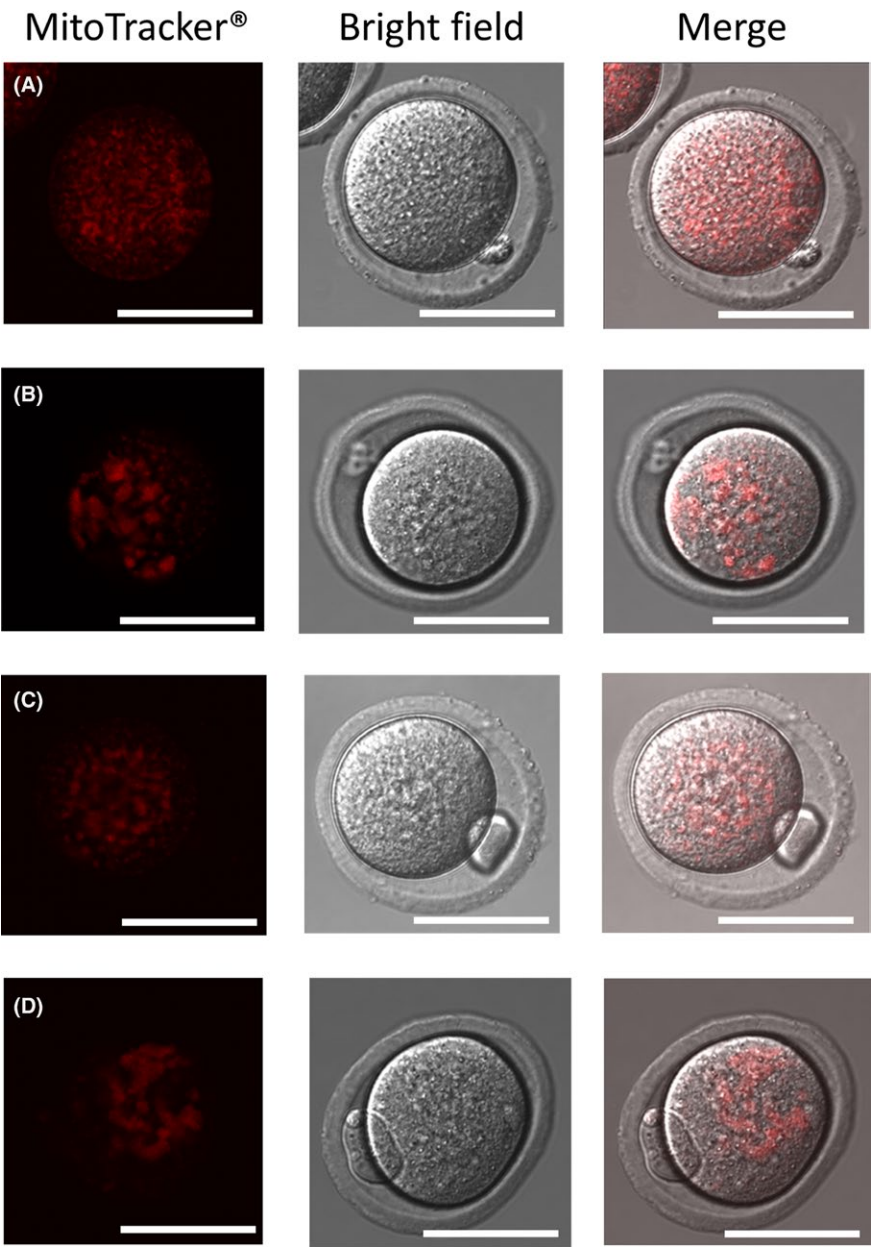


FIGURE 4 Changes in the intracellular distribution of mitochondria on the addition of varying concentrations of potassium bromate, which were (A) 0 μ M, (B) 500 μ M, (C) 750 μ M, and (D) 1000 μ M. Scale bar, 50 μ m

TABLE 1 Effects of resveratrol on metaphase II (MII) maturation and mitochondrial cluster formation rates in the presence of potassium bromate

| Concentration of resveratrol (μ M) | MI maturation rate (%) | Cluster formation rate (%) |
|---|------------------------|----------------------------|
| 0 | 86.3 (88/102) | 59.1 (52/88) |
| 10 | 83.3 (85/102) | 11.8** (10/85) |
| 20 | 74.5* (79/106) | 12.7** (10/79) |

* $P < .05$ and ** $P < .01$ indicate a significant difference between the control and the resveratrol-treated group.

Potassium bromate, which is used as a food additive, reportedly acts as an oxidizing agent to increase the amount of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker for DNA oxidative

damage,²⁰ and the increase in the levels of 8-OHdG in the granulosa cells during the ovulatory process has been shown to lead to a lowering of the fertilization rate and a decrease in the good-quality embryo rate in in vitro fertilization programs. As a marker of oxidative stress in the granulosa cells during the ovulatory process, 8-OHdG is one of the factors that adversely affects not only fertilization, but the growth of embryos.²⁶ Therefore, this study's result suggests that potassium bromate causes oxidative stress and reduces oocyte quality, although it needs to be addressed whether this drug increases reactive oxygen stress in the oocytes and/or granulosa cells in future studies.

In the present study, the addition of potassium bromate resulted in the inhibition of mitochondrial spreading throughout the cytoplasm and abnormal mitochondrial aggregation. It has been reported that the prominent aggregation of mitochondria is observed in aged oocytes and it is considered as one of the phenotypes.^{19,27}

Therefore, this study's model seems to be useful for partially mimicking the abnormal mitochondrial aggregation that is seen in aged oocytes.

Also examined was whether potassium bromate-induced mitochondrial aggregation could be rescued by the addition of the antioxidant, resveratrol, which has been suggested to be useful in IVM and embryo culture systems. Several studies have reported improvements with resveratrol: the fertilization rate was increased by a reduction of active oxygen,²⁸ the pregnancy rate was increased by improved mitochondrial function,²¹ and fertility and embryo developmental capacities were improved by an increased number of active mitochondria, leading to better-quality oocytes.²⁹ Collectively, these reports suggest that resveratrol improves mitochondrial function. This study also showed the utility of resveratrol as an antioxidant, as the appearance of mitochondrial aggregation within the oocyte cytoplasm was successfully avoided, even in the presence of the oxidizing agent. Resveratrol has been reported to have an antioxidant effect against cumulus cells.²² In this study, the culture occurred in the cumulus–oocyte complex, instead of the denuded oocyte. Therefore, cytoplasmic maturation of the cumulus cells could be promoted by a reduction in oxidative stress of the cumulus cells. However, it is still necessary to examine whether the addition of this antioxidant is useful for enhancing fertilization rates and early embryonic development under similar conditions. The authors believe that the current observations yield insight for the potential improvement of the IVM system by reducing oxidization of the immature oocytes in order to enhance mitochondrial function.

It was concluded that there was success in constructing an *in vitro* experimental system that can control the promotion and suppression of mitochondrial cluster formation in MII oocytes by using potassium bromate and resveratrol. This experimental model could help to evaluate oocyte cytoplasm maturation. It also would be useful to compare and analyze the effects of other antioxidants for oocyte maturation.

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DISCLOSURES

Conflict of interest: The authors declare no conflict of interest. **Human and Animal Rights:** All the experimental procedures conformed to the "Regulations for Animal Experiments and Related Activities at Tohoku University" and were reviewed by the Institutional Laboratory Animal Care and Use Committee of Tohoku University, finally being approved (2016 AgA-048) by the President of the University. This article does not contain any study with human participants that was performed by any of the authors.

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REFERENCES

1. Cha KY, Chian RC. Maturation *in vitro* of immature human oocytes for clinical use. *Hum Reprod Update*. 1998;4:103-120.
2. Trounson A, Wood C, Kausche A. *In vitro* maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril*. 1994;62:353-362.
3. Yoshida H, Aono N, Araki Y, Naganuma T. Delivery of a healthy newborn using vitrified zygotes that developed from *in vitro* matured oocytes retrieved from a patient with polycystic ovarian syndrome. *Reprod Med Biol*. 2003;2:87-90.
4. Uzelac PS, Delaney AA, Christensen GL, Bohler HC, Nakajima ST. Live birth following *in vitro* maturation of oocytes retrieved from extracorporeal ovarian tissue aspiration and embryo cryopreservation for 5 years. *Fertil Steril*. 2015;104:1258-1260.
5. Eppig JJ, O'Brien MJ. Comparison of preimplantation developmental competence after mouse oocyte growth and development *in vitro* and *in vivo*. *Theriogenology*. 1998;49:415-422.
6. Mermillod P, Oussaid B, Cognié Y. Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. *J Reprod Fertil Suppl*. 1999;54:449-460.
7. Moor R, Dai Y. Maturation of pig oocytes *in vivo* and *in vitro*. *Reprod Suppl*. 2001;58:91-104.
8. Trounson A, Anderiesz C, Jones G. Maturation of human oocytes *in vitro* and their developmental competence. *Reproduction*. 2001;121:51-75.
9. Cha KY, Han SY, Chung HM, et al. Pregnancies and deliveries after *in vitro* maturation culture followed by *in vitro* fertilization and embryo transfer without stimulation in women with polycystic ovary syndrome. *Fertil Steril*. 2000;73:978-983.
10. Child TJ, Abdul-Jalil AK, Gulekli B, Tan SL. *In vitro* maturation and fertilization of oocytes from unstimulated normal ovaries, polycystic ovaries, and women with polycystic ovary syndrome. *Fertil Steril*. 2001;76:936-942.
11. Piquette GN. The *in vitro* maturation (IVM) of human oocytes for *in vitro* fertilization (IVF): is it time yet to switch to IVM-IVF? *Fertil Steril*. 2006;85:833-835.
12. Lanzendorf SE. Developmental potential of *in vitro*- and *in vivo*-matured human oocytes collected from stimulated and unstimulated ovaries. *Fertil Steril*. 2006;85:836-837.
13. Combelles CM, Cekleniak NA, Racowsky C, Albertini DF. Assessment of nuclear and cytoplasmic maturation in *in-vitro* matured human oocytes. *Hum Reprod*. 2002;17:1006-1016.
14. Yoshida M, Ishigaki K, Nagai T, Chikyu M, Pursel VG. Glutathione concentration during maturation and after fertilization in pig oocytes: relevance to the ability of oocytes. *Biol Reprod*. 1993;49:89-94.
15. Funahashi H, Stumpf TT, Cantley TC, Kim NH, Day BN. Pronuclear formation and intracellular glutathione content of *in vitro*-matured porcine oocytes following *in vitro* fertilization and/or electrical activation. *Zygote*. 1995;3:273-281.
16. Carroll J, Depypere H, Matthews CD. Freeze-thaw-induced changes of the zona pellucida explain decreased rates of fertilization in frozen-thawed mouse oocytes. *J Reprod Fertil*. 1990;90:547-553.
17. Long CR, Damiani P, Pinto-Correia C, MacLean RA, Duby RT, Robl JM. Morphology and subsequent development in culture of bovine oocytes matured *in vitro* under various conditions of fertilization. *J Reprod Fertil*. 1994;102:361-369.
18. Tilly JL, Sinclair DA. Germline energetics, aging, and female infertility. *Cell Metab*. 2013;17:838-850.
19. Udagawa O, Ishihara T, Maeda M, et al. Mitochondrial fission factor Drp1 maintains oocyte quality via dynamic rearrangement of multiple organelles. *Curr Biol*. 2014;24:2451-2458.
20. Murata M, Bansho Y, Inoue S, et al. Requirement of glutathione and cysteine in guanine-specific oxidation of DNA by carcinogenic potassium bromate. *Chem Res Toxicol*. 2001;14:678-685.

21. Takeo S, Sato D, Kimura K, et al. Resveratrol improves the mitochondrial function and fertilization outcome of bovine oocytes. *J Reprod Dev.* 2014;24:92-99.
22. Wang F, Tian X, Zhang L, et al. Beneficial effect of resveratrol on bovine oocyte maturation and subsequent embryonic development after *in vitro* fertilization. *Fertil Steril.* 2014;101:577-586.
23. Ishikawa S, Machida R, Hiraga K, Hiradate Y, Suda Y, Tanemura K. Hanging drop monoculture for selection of optimal antioxidants during *in vitro* maturation of porcine oocytes. *Reprod Domest Anim.* 2014;49:e26-e30.
24. Wakai T. Mitochondrial localization in mouse oocytes. *J Mamm Ova Res.* 2012;29:155-160.
25. Yu Y, Dumollard R, Rossbach A, Lai FA, Swann K. Redistribution of mitochondria leads to bursts of ATP production during spontaneous mouse oocyte maturation. *J Cell Physiol.* 2010;224:672-680.
26. Seino T, Saito H, Kaneko T, Takahashi T, Kawachiya S, Kurachi H. Eight-hydroxy-2'-deoxyguanosine in granulosa cells is correlated with the quality of oocytes and embryos in an *in vitro* fertilization-embryo transfer program. *Fertil Steril.* 2002;77:1184-1190.
27. Nagai S, Mabuchi T, Hirata S, et al. Correlation of abnormal mitochondrial distribution in mouse oocytes with reduced developmental competence. *Tohoku J Exp Med.* 2006;210:137-144.
28. Takeo S, Kawahara-Miki R, Goto H, et al. Age-associated changes in gene expression and developmental competence of bovine oocytes, and a possible countermeasure against age-associated events. *Mol Reprod Dev.* 2013;80:508-521.
29. Sato D, Itami N, Tasaki H, Takeo S, Kuwayama T, Iwata H. Relationship between mitochondrial DNA copy number and SIRT1 expression in porcine oocytes. *PLoS ONE.* 2014;9:e94488.

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