Regulation of Mitochondrial DNA Replication during Oocyte Maturation by Follicular Fluid, EGF, and Neuregulin 1 affects Oocyte Maturation and Development  

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INTRODUCTION
Mitochondria supply the majority of ATP in a cell. Mitochondrial DNA (mtDNA) copy number in oocytes might be used as a marker of viability and might be a key determinant of pre-implantation embryo development. However, its regulation by extracellular growth factors in oocyte meiotic maturation was not known.

OBJECTIVE
A series of experiments were carried out to determine:
1) the effects of various concentrations of porcine follicular fluid during in vitro maturation on mtDNA copy number and oocyte meiotic maturation;
2) the effects of EGF, neuregulin 1 and NRG1+IGF1 during in vitro maturation on mtDNA copy number, oocyte maturation and embryo development;
3) the effects of reduced mtDNA copy number by mechanical cytoplasm removal on embryo development.

MATERIALS AND METHODS
Experiment 1: Effects of porcine follicular fluid (pFF) supplementation on mtDNA copy number and oocyte maturation
Four concentrations of pFF at 0, 10, 20, and 30% was added in oocyte maturation medium. In vitro maturation medium was medium 199 supplemented with 0.1% polyvinylalcohol (w/v), 3.05 mM D-glucose, 0.91 mM sodium pyruvate, 0.57 mM cysteine, 0.5µg/ml luteinizing hormone, 0.5µg/ml follicle-stimuulating hormone, and 10 ng/ml EGF. The cumulus-oocyte complexes (COCs) were collected and cultured 50-60 per well in 4-well plate for 40-44 h. Sixteen GV stage oocytes before IVM and 16 MII oocytes with an extruded polar body after IVM from each pFF group were collected for mtDNA copy number assay. The number of MII oocytes was also recorded to determine meiotic maturation.

Experiment 2: Effects of EGF, neuregulin 1(NRG1), and NRG1+IGF1 supplementation on mtDNA copy number, oocyte maturation, and parthenogenetic embryo development
The basic maturation medium was the same as described in experiment 1 but without EGF addition. For treatment groups, 10 ng/ml EGF, 20 ng/ml neuregulin-1 (NRG1); or NRG1+IGF1 (20 ng/ml NRG1 + 100 ng/ml insulin-like growth factor 1), were added into basic maturation medium, with no growth factor supplementation as the control. For mtDNA analysis, 16 GV oocytes before IVM and 16 MII oocytes from each group after IVM were sampled. To study the effects of growth factor on embryo development, the oocytes were also activated electrically and cultured in porcine zygote medium 3 (PZM3). All embryos were evaluated for cleavage on Day 2, blastocyst formation and cell number in blastocyst-stage embryos on Day 7 (day of activation = 0).

Experiment 3: Effects of oocyte cytoplasm reduction on embryo development
In this experiment, a 0, 10, 20, and 30% cytoplasm was removed mechanically from MII oocytes. Fifteen oocytes were assayed by real time PCR to confirm that the expected number of mtDNA genomes was removed from each group. To avoid introducing extra variables such as sperm, MII oocytes were activated electrically as in experiment 2, and cultured in PZM3. Percentage of cleaved embryos was determined at day 2, percent blastocyst formation, and the number of nuclei in blastocyst stage embryos at day 7. All dependent variables were analyzed by the MIXED procedure of SAS ® software (v9.2), with treatment and aspiration day in class as the main effects. Mean differences were determined by using the Fisher Least Significant Difference. A probability of ≤0.05 was considered to be statistically significant.

RESULTS
mtDNA copy number change during in vitro maturation
Sixty nine GV-stage and 314 MII-stage oocytes from 5 replicates were assayed in experiment 1; 105 GV- and 394 MII-stage oocytes from 7 replicates assayed in experiment 2. The average mtDNA copy number was 167,634.6±220,740.4, and 275,131.9±7,585.4 for GV and MII oocytes, respectively in experiment 1; 185,004.7±20,089.3 and 239,392.8±10,345.3 for GV and MII oocytes in experiment 2. In both experiments, the resulting probability values were less than 0.05 (as indicated by a,b on graph), indicating higher mtDNA copy numbers in MII oocytes than the GV oocytes.

Experiment 1: Effects of porcine follicular fluid (pFF) supplementation on mtDNA copy number and oocyte maturation
mtDNA copy number in both 10% and 20% pFF groups was lower than that in the 0% control (P<0.05). But there was no difference between 30% pFF and the control or between the 3 pFF supplemented groups. The average mtDNA copy number in the pooled pFF-supplemented group was lower than that in the control (266,789.9±11,790.4 vs. 318,510.1±20,377.4, a,b: P<0.05).

Experiment 2: Effects of EGF, NRG1, and NRG1+IGF1 supplementation on mtDNA copy number, oocyte maturation, and parthenogenetic embryo development
There was no difference in mtDNA copy number in MII oocyte among the EGF, NRG1, NRG1+IGF1, and control groups. However, compared to the GV oocytes, mtDNA copy number in NRG1-supplemented group was higher (281,293±22,893.5 vs 192,288.7±21,675.4, P<0.05). Oocyte maturation rate in the EGF, NRG1, and NRG1+IGF1 groups was higher than the control (P<0.01), though there was no difference among the EGF, NRG1, and NRG1+IGF1 groups. The percentage of cleaved embryos in the EGF and NRG1+IGF1 groups was higher than the control and NRG1 (P<0.05). Blastocyst formation was determined on day 7 of culture (day of activation = 0). Only embryos with 16 or more nuclei were considered to be true blastocysts and were included in the analysis. Percentage of blastocyst formation in the NRG1 treatment group was higher than the control (P<0.05). There was no difference in percent blastocyst among EGF, NRG1, and NRG1+IGF1 groups.

Experiment 3: Effects of oocyte cytoplasm reduction on embryo development
There was no difference in the percentage of cleaved embryos among the 4 volume groups. When the oocyte cytoplasm was reduced by 10 and 20%, the percentage of blastocyst formation did not differ from 0% control. However, 30% volume reduction significantly decreased the blastocyst formation (a,b: P=0.05). Besides, there were significant negative linear correlations between oocyte cytoplasm reduction and percentage of blastocyst formation (r = -0.62, P=0.03), which means the more the cytoplasm removed, the lower the blastocyst formation would be.

CONCLUSION
This study shows that there was significant mtDNA copy number increase during oocyte maturation from GV to MII oocytes. Supplementation of IVM medium with follicular fluid suppressed mtDNA copy number and oocyte meiotic maturation. However, supplementation of EGF-like growth factor (NRG1) enhanced mtDNA replication, oocyte maturation, and blastocyst formation. Supplementation of maturation medium with NRG1 provides means to increase mtDNA copy number, and then improves oocyte viability and developmental competence.

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