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Regulation of oocyte mitochondrial DNA copy number by follicular fluid, EGF, and neuregulin 1 during in vitro maturation affects embryo development in pigs

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Abstract

Little is known about mitochondrial DNA (mtDNA) replication during oocyte maturation and its regulation by extracellular factors. The present study determined the effects of supplementation of maturation medium with porcine follicular fluid (pFF; 0, 10%, 20%, and 30%) on mtDNA copy number and oocyte maturation in experiment 1; the effects on epidermal growth factor (EGF; 10 ng/mL), neuregulin 1 (NRG1; 20 ng/mL), and NRG1 + insulin-like growth factor 1 (IGF1; 100 ng/mL + NRG1 20 ng/mL), on mtDNA copy number, oocyte maturation, and embryo development after parthenogenic activation in experiment 2; and effects on embryo development after in vitro fertilization in experiment 3. Overall, mtDNA copy number increased from germinal vesicle (GV) to metaphase II (MII) stage oocytes after in vitro maturation (GV: 167 634.6 ± 20 740.4 vs. MII: 275 131.9 ± 9 758.4 in experiment 1; $P < 0.05$; GV: 185 004.7 ± 20 089.3 vs. MII: 239 392.8 ± 10 345.3 in experiment 2; $P < 0.05$; Least Squares Means ± SEM). Supplementation of IVM medium with pFF inhibited mtDNA replication (266 789.9 ± 11 790.4 vs. 318 510.1 ± 20 377.4; $P < 0.05$) and oocyte meiotic maturation (67.3 ± 0.7% vs. 73.2 ± 1.2%, for the pFF supplemented and zero pFF control, respectively; $P < 0.01$). Compared with the control, addition of growth factors enhanced oocyte maturation. Furthermore, supplementation of NRG1 stimulated mitochondrial replication, increased mtDNA copies in MII oocytes than in GV oocytes, and increased percentage of blastocysts in both parthenogenetic and in vitro fertilized embryos. In this study, mitochondrial biogenesis in oocytes was stimulated during in vitro maturation. Oocyte mtDNA copy number was associated with developmental competence. Supplementation of maturation medium with NRG1 increased mtDNA copy number, and thus provides a means to improve oocyte quality and developmental competence in pigs.

Keywords: Oocyte; Mitochondrial DNA; Follicular fluid; EGF; Neuregulin 1

1. Introduction

In the pig, the mitochondrial DNA (mtDNA) is 16.6 kilobase (kb) in size [1] and encodes 13 subunits of the electron transfer chain that are essential for cellular energy production [2], with the number of copies directly proportional to the amount of adenosine-5′-triphosphate (ATP) synthesized [2,3]. The mammalian oocyte typically con-

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tains approximately 100 000 mitochondria that can occupy up to 30% of the cytoplasmic space [4]. During oocyte growth within follicles and the early stages of maturation, oocytes are coupled to cumulus cells which provide metabolic support through the provision of ATP and pyruvate [5,6]. However, after ovulation, mammalian oocytes become uncoupled from cumulus cells and they rely on their own mitochondria to generate ATP [5,7]. From ovulation to the morula stage, the embryo relies on mitochondrial oxidative phosphorylation to supply most of the ATP demands for development [7,8].

However, during preimplantation development, mtDNA replication is limited, which on a per cell basis results in a progressive reduction in mtDNA content in cleaving blastomeres. Individual blastomeres in later-staged embryos, therefore, contain fewer mtDNA genomes than in earlier stages. The lack of mtDNA replication during early embryo development suggests that mtDNA copy number needs to be amplified to sufficient levels before fertilization. There was an increase in ATP during maturation in mouse [9], cattle [10], and pig [11]; furthermore, this ATP increase was correlated with embryo development in cattle [10]. A decrease in ATP content during maturation has been suggested to lead to developmental arrest at cleavage stages in human [12]. These data suggest that the status and activity of mitochondria in the mammalian oocyte is a determining factor of oocyte quality and viability, leading to the speculation that mtDNA copy number could be used as a marker of viability. A critical threshold of approximately 100 000 copies in the metaphase II (MII) oocyte has been proposed for the mouse [13], human [14], and pig [15].

In a specific cell type, mtDNA copy number can change in response to environmental signals, including temperature, energy deprivation, and availability of nutrients and growth factors [16]. In Schwann cells, neuregulin and insulin-like growth factor 1 (IGF1) synergize to drive mitochondrial biogenesis, resulting in an increase of mtDNA copy number [17]. Furthermore, it has been suggested that low mtDNA content and oocyte viability may result from deficient oocyte maturation, because immature oocytes have considerably fewer numbers of mtDNA as compared with mature oocytes [13,18].

Follicular fluid within the ovarian follicle provides a unique microenvironment necessary for oocyte growth and maturation. Follicular fluid is composed of transudates from the serum through the blood-follicle barrier, but it also contains locally produced molecules, e.g., estrogens and IGF1 [19,20]. Reports on the effects of porcine follicular fluid (pFF) supplementation in IVM medium are controversial. Some reported that pFF enhances meiotic maturation and blastocyst formation [21], decreases polyspermic fertilization [22], and has an antioxidative effect that may result in improved oocyte developmental competence [23]. Conversely, some reports found no effects [24] or negative effects [25,26] on oocyte maturation and embryo development. However, it is not known if pFF supplementation during maturation has any effects on mtDNA copy number in oocytes.

Epidermal growth factor (EGF) has been used in IVM systems for decades and has beneficial effects on oocyte maturation and cumulus expansion [24,27]. However, recent studies using mouse genetic models indicate that EGF-like growth factors, rather than EGF itself, accumulate in the follicular fluid at the time of ovulation. The EGF-like growth factor mRNA is regulated by luteinizing hormone, and its genes and proteins are detected in the follicle [28,29]. The EGF-like growth factors include several members, such as neuregulin (NRG). They play essential roles in oocyte meiotic maturation in primates [30] and rodents [31]. However, their roles in regulation of mtDNA copy number and embryonic development in pigs are not known. In the present study, we determined mtDNA copy number changes during oocyte maturation from germinal vesicle (GV) to MII stage and examined the effects of supplementation of maturation medium with various concentrations of pFF (0, 10%, 20%, and 30%), EGF (10 ng/mL), NRG1 (20 ng/mL), and NRG1 (20 ng/mL) + IGF1 (100 ng/mL) on mtDNA copy number, oocyte meiotic maturation, and subsequent embryo developmental competence.

2. Materials and methods

2.1. Experimental design

The overall objective was to determine the regulation of mtDNA copy number in the oocyte by porcine follicular fluid, EGF, neuregulin 1, and IGF1 and the importance of its regulation in embryo development. A series of three experiments were conducted.

2.1.1. Experiment 1: effects of pFF supplementation on mtDNA copy number and oocyte maturation

To study the effects of various doses of pFF supplementation in IVM medium on mtDNA copy number and oocyte meiotic maturation, four concentrations of pFF (0, 10%, 20%, and 30%) were added in IVM medium. The IVM medium was TCM 199 (Gibco BRL, Grand Island, NY) supplemented with 0.1%