Effects of Zinc on Mammalian Oocyte Quality and Its Mechanism of Action (Postprint)

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Abstract

Oocyte quality directly affects fertilization rates, early embryonic survival, and even disease susceptibility in adulthood. Zinc is an essential trace element for animals and, to date, the one with the most diverse functions among all essential trace elements discovered. In recent years, scientists have found that it plays a crucial role in regulating oocyte quality. Research indicates that zinc influences oocyte meiosis by affecting maturation-promoting factor (MPF) activity and the mitogen-activated protein kinase (MAPK) signaling pathway, modulates oocyte antioxidant function via glutathione, and impacts animal oocyte quality through mechanisms such as altering histone and DNA methylation levels. This review summarizes the effects of zinc on mammalian oocyte quality and its underlying mechanisms.

Full Text

Effects and Action Pathway of Zinc on Mammalian Oocyte Quality

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Abstract: Oocyte quality directly affects fertilization rates, early embryo survival, and even adult-onset diseases. Zinc, one of the essential trace elements in animals, is the most functionally diverse trace element discovered to date. Recent research has revealed that zinc plays crucial roles in regulating oocyte quality. Studies have demonstrated that zinc influences oocyte meiosis through its effects on maturation promoting factor (MPF) activity and the mitogen-activated protein kinase (MAPK) signaling pathway, regulates oocyte antioxidant function via glutathione, and affects oocyte quality by influencing histone

and DNA methylation levels. This review summarizes the effects of zinc on mammalian oocyte quality and its underlying mechanisms.

Keywords: zinc; mammalian; oocyte; quality

Both sperm and oocytes provide genetic material during life formation, while oocytes also supply essential nutrients for early embryonic development. Consequently, as the foundation of life, oocyte quality directly influences fertilization rates, cleavage rates, early embryo survival, pregnancy establishment and maintenance, fetal development, and even the lifelong health of offspring. Oocyte quality is primarily evaluated by analyzing morphological structure and maturation status, based on structural and biochemical changes that occur during oocyte growth and development. Zinc (Zn) is an essential trace element for animals. Although the requirement is minimal, its physiological and biochemical functions are critical, as zinc is indispensable for normal gene transcription, translation, cell apoptosis, proliferation, differentiation, and signal transduction. Zinc deficiency in reproduction often manifests as abnormal ovarian development, impaired luteinizing hormone/follicle-stimulating hormone (LH/FSH) synthesis and secretion, disordered estrous cycles, and fetal congenital malformations. Recent research has made significant progress in understanding zinc' s effects on oocyte quality. This review summarizes the influence of zinc on oocyte quality in female animals and its potential mechanisms.

Zinc and Its Homeostatic Regulation

Zinc is an essential trace element for animals. The element was discovered by Klaproth in 1789, and Todd et al. first demonstrated its nutritional essentiality in 1934. In 1955, Tuker and Salmem identified and confirmed that zinc deficiency causes parakeratosis in pigs. Since then, numerous studies have verified that zinc participates in all aspects of life activities. First, zinc is associated with the structure and activity regulation of more than 300 enzymes, including DNA polymerase, alcohol dehydrogenase, and alkaline phosphatase. Second, zinc serves as a structural ion essential for biological membranes and is closely related to protein synthesis. Third, zinc participates in forming zinc finger structures, playing special roles in endocrine function regulation and gene expression, while also significantly influencing cell division. Finally, zinc is associated with many important hormones, such as thyroid hormone, testosterone, and insulin, thereby promoting the development of sexual organs and functions. Recent studies have found that zinc homeostasis is primarily regulated by metallothioneins and zinc transporters.

Regulation of Zinc Homeostasis by Metallothioneins

Metallothioneins are a class of small molecular weight metal-binding proteins rich in cysteine, containing over 60 amino acids and capable of binding seven zinc ions. Metallothioneins can function as both zinc receptors and zinc donors

to regulate zinc ions when their concentration exceeds certain limits [Figure 1: see original paper]. The different states of metallothioneins in cells depend on the degree of zinc utilization and redox balance. The chemical characteristics of thiol ligands centered in metallothioneins regulate zinc ion binding capacity. Through this mechanism, metallothioneins become redox-active proteins that can convert redox signals into zinc signals. Strong oxidizing conditions increase the likelihood of metallothioneins acting as zinc donors, whereas under strongly reducing conditions, they serve as potential zinc receptors. To date, no other proteins for temporary zinc storage have been identified in cells besides metallothioneins.

Regulation of Zinc Homeostasis by Zinc Transporters

Zinc transporters maintain zinc homeostasis by controlling zinc influx and efflux to coordinate zinc demands. Mammalian zinc transporters comprise two families: the SLC30 (solute-linked carrier 30, ZnT) family and the SLC39 (solute-linked carrier 39, ZIP) family. The ZnT family facilitates zinc ion efflux from the cytoplasm to outside the cell or into organelles. The ZnT family can be divided into three subfamilies (I, II, and III), with members widely distributed in prokaryotic and eukaryotic organisms. To date, ten ZnT family members (ZnT1–10) have been identified in mammals. In pigs, ZnT1–9 have been cloned, and ZnT1–7 and ZnT9 are expressed in porcine ovaries at the mRNA level. In contrast, the ZIP family promotes zinc entry from outside the cell or from organelles into the cytoplasm. The mammalian ZIP family is divided into four subfamilies: I, II, LIV-1, and gufA. Currently, fourteen ZIP members (ZIP1–14) have been discovered in humans, and eleven ZIP sequences (ZIP1–4, 6–8, 11–14) have been identified in pigs. The organism relies on these two zinc transport systems to maintain intracellular zinc homeostasis.

Effects of Zinc on Oocyte Quality

In vitro maturation (IVM) of porcine oocytes followed by somatic cell nuclear transfer embryo transplantation revealed that adding zinc (0.8 μg/mL) to the IVM medium significantly increased the number of cloned pigs delivered. When equine oocytes were cultured in vitro with zinc supplementation, no significant effect was observed on cleavage rates, but blastocyst rates at day 7 of culture were improved (45% vs. 8%). Under zinc-deficient conditions, oocytes exhibited abnormal division during IVM, a finding confirmed by in vivo studies. Zinc-deficient porcine oocytes showed abnormal microfilament formation and significantly reduced rates of reaching metaphase II (MII). These findings demonstrate that zinc is crucial for oocyte quality.

Effects of Zinc on Oocyte Meiotic Maturation

Research has shown that zinc content increases significantly from the germinal vesicle (GV) stage to the MII stage, then begins to decline at the zygote

and 2-cell embryo stages, suggesting that zinc may play an important role during oocyte maturation and early embryonic development. Adding N,N,N' ,N' -tetrakis(2-pyridylmethyl)ethylenediamine (TPEN), a high-affinity zinc chelator, to mouse oocyte IVM systems resulted in symmetric division in 32% of oocytes, indicating that TPEN disrupted intracellular zinc homeostasis and interfered with asymmetric division. Other studies found that zinc deficiency during IVM produced larger polar bodies, suggesting defects at the spindle level during meiosis. Analysis of spindle morphology during meiosis revealed that control oocytes normally progressed through metaphase I (MI), underwent chromosome segregation, and arrested at MII. In contrast, zinc-deficient oocytes passed through MI and chromosome segregation but failed to develop to MII, exhibiting telophase spindles with degraded chromosomes at both poles. These studies indicate that zinc disruption causes spindle abnormalities, preventing normal meiotic progression and leading to premature arrest at telophase I.

Further research found that zinc inhibition (TPEN treatment) during in vitro oocyte culture caused premature resumption of meiosis I arrest, but immunofluorescence analysis of spindle morphology revealed that despite zinc deficiency inducing germinal vesicle breakdown (GVBD), chromosome condensation occurred without completion of the first meiotic division. In vivo studies showed that feeding mice a zinc-deficient diet resulted in 42.5% of oocytes undergoing GVBD without ovulation signals, whereas control oocytes remained entirely in the GV stage. Confocal immunofluorescence of oocytes from zinc-deficient mice revealed abnormal spindle structures, including arrest at MI and telophase. These in vitro and in vivo studies demonstrate that zinc is essential for normal meiotic progression, and deficiency impairs oocyte meiotic maturation.

Effects of Zinc on Oocyte Ovulation Rate, Fertilization Rate, and Early Embryonic Development

Cumulus expansion is essential for oocyte maturation and ovulation. TPEN treatment of cumulus-oocyte complexes (COCs) during IVM blocked epidermal growth factor (EGF)-induced cumulus expansion, while zinc supplementation restored EGF-induced expansion. In vivo studies feeding mice a zinc-deficient diet for 10 days found no oocytes in the oviducts of superovulated mice, indicating that zinc deficiency caused ovulation disorders. In vitro assessment of fertilization capacity in zinc-deficient mouse oocytes revealed reduced fertilization rates after 3 or 5 days of treatment. These results demonstrate that zinc plays an irreplaceable role in mammalian ovulation and fertilization.

Treatment of porcine IVM oocytes with different zinc concentrations showed that 1.2 μg/mL zinc increased blastocyst formation rates after parthenogenetic activation, while 0.8 μg/mL zinc improved blastocyst formation rates after in vitro fertilization, indicating that zinc treatment enhances oocyte quality and promotes blastocyst development. Studies on pre-implantation embryos also found that the initial mitotic divisions require strict zinc balance and regulation. Adding TPEN to oocyte IVM medium to induce zinc deficiency revealed

that nearly all oocytes in both TPEN and TPEN+Zn groups remained at the 1-cell stage with no blastocyst formation. Vitrification of mouse ovaries with zinc supplementation in the cryopreservation solution improved the viability and in vitro maturation-fertilization rates of oocytes derived from thawed ovaries. Feeding mice a zinc-deficient diet for 5 days before mating increased the proportion of non-blastocysts (1-cell to morula) and decreased the blastocyst proportion. Another study found that 4–5 days of pre-mating zinc deficiency in mice significantly reduced embryonic crown-rump length at gestation day 10.5 and dramatically increased the proportion of implantation sites without embryos or with non-viable embryos (46% vs. 2%).

These in vitro and in vivo studies demonstrate that zinc affects mammalian oocyte ovulation rates, fertilization rates, and early embryonic development by influencing oocyte quality and viability. Zinc is indispensable for oocyte and early embryonic development, though the effective zinc concentration varies among mammalian species.

Zinc and Oocyte Meiosis and Maturation

Maturation promoting factor (MPF) and mitogen-activated protein kinases (MAPK) are key regulators of oocyte maturation. MPF consists of the catalytic subunit P34cdc2 and the regulatory subunit cyclin B, with its activity determining whether oocytes can transition from G2 phase to M phase and undergo GVBD. MAPK, also known as extracellular signal-regulated kinase (ERK), includes ERK1 and ERK2. The Mos-MEK-MAPK-RSK signaling pathway, composed of MAPK and its upstream signaling molecule proto-oncogene protein (Mos) and downstream signaling molecule ribosomal protein S6 kinase (RSK), can activate MPF and plays an extremely important role in oocyte maturation.

Disrupting cellular zinc homeostasis during oocyte IVM caused premature resumption of MI arrest (GVBD). Analysis of cyclic adenosine monophosphate (cAMP) content and MPF activity before GVBD (6 h) and during GVBD (10 h) revealed significantly increased cAMP content before GVBD, with no significant difference between control and treatment groups at 10 h. However, MPF activity was significantly elevated in the treatment group at 10–14 h, indicating that zinc deficiency induced GVBD by increasing MPF activity.

Zinc-deficient (TPEN-treated) oocytes showed significantly elevated protein levels of Mos and phosphorylated MAPK3/1 (pMAPK3/1) before MPF activation and GVBD. To verify whether inhibiting protein synthesis in the Mos-MAPK pathway prevents TPEN-induced GVBD, cycloheximide [which inhibits Mos, phosphorylated MAP2K1/2 (pMAP2K1/2), and pMAPK3/1 protein expression] was added to oocyte culture medium containing TPEN for 14 h. All cycloheximide concentrations significantly inhibited TPEN-induced GVBD. These findings indicate that all components of the Mos-MAPK pathway are activated during zinc deficiency-induced GVBD, and inhibiting related protein synthesis

in this pathway prevents TPEN-induced GVBD, demonstrating that premature activation of the Mos-MAPK pathway mediates the adverse effects of zinc deficiency on oocyte meiotic maturation.

These studies demonstrate that zinc deficiency causes premature GVBD, impairing oocyte maturation. Zinc primarily affects oocyte quality by regulating MPF activation and Mos-MAPK pathway activation, thereby influencing the maintenance of prophase I arrest and the resumption of meiosis.

Zinc and Oocyte Antioxidant Function

Glutathione (GSH) plays an important role in oocyte maturation, and intracellular GSH levels serve as an important indicator of oocyte cytoplasmic maturation. Adding antioxidants to mouse and bovine oocyte IVM media upregulated GSH levels and benefited embryonic development. Adding cysteine and -mercaptoethanol to IVM media increased intracellular GSH levels and improved bovine embryonic developmental capacity.

Zinc deficiency during IVM significantly reduced GSH levels in both bovine oocytes and cumulus cells. Adding different zinc concentrations to porcine oocyte IVM media revealed that 0.8 and 1.2 μg/mL zinc increased oocyte GSH levels and reduced reactive oxygen species (ROS) levels. Further examination of embryos derived from parthenogenetic activation and in vitro fertilization showed the highest blastocyst formation rates in these two groups.

Although these studies indicate that zinc can improve oocyte quality by enhancing antioxidant capacity, different zinc sources exert varying effects. Unlike conventional zinc, intact nanoparticles can be detected in ovarian tissue and cells after nano-zinc oxide treatment. Nano-zinc oxide treatment of oocytes at the maturation stage inhibited early embryonic development through -H2AX (a molecular marker of DNA double-strand breaks) and nuclear factor- B (NF- B) signaling pathways. Additionally, nanoparticles can increase ROS levels, thereby affecting oocyte quality. These findings demonstrate that while zinc is indispensable for oocyte development, nano-zinc oxide should be avoided in breeding animals.

Zinc and Oocyte Epigenetics

Maternal epigenetics significantly impacts embryonic developmental capacity and postnatal health. Oocyte epigenetics undergo substantial changes during oogenesis, with overall DNA methylation levels low during early oogenesis and peaking at full oocyte development. Oocyte epigenetics primarily includes histone modifications and cytosine methylation. DNA methylation is essential for imprinted gene expression and for suppressing excessive expression of repetitive sequences in oocytes. Failure to silence repetitive sequences can cause abnormal expression of neighboring genes or genomic instability, such as increased DNA double-strand breaks.

Feeding mice a zinc-deficient diet for 3 or 5 days before ovulation, followed by collection of GV-stage oocytes for immunofluorescence staining analysis of chromatin and DNA methylation levels, revealed impaired in vitro fertilization and pre-implantation developmental capacity in zinc-deficient oocytes. No trimethylated histone H3K4 was observed in zinc-deficient oocytes. Total DNA methylation analysis showed substantially decreased DNA methylation in zinc-deficient oocytes compared to controls. Analysis of repetitive sequence expression revealed significantly increased transcription levels of several repetitive sequences in the zinc-deficient group, with intracisternal A-particle (Iap) transcript levels increasing 20-fold (methylated Iap prevents inappropriate expression of the neighboring Agouti gene; hypomethylation increases Iap activity, causing ectopic Agouti expression and leading to obesity and other metabolic diseases in mice), while Line1, Sineb1, and Sineb2 transcript levels increased 2–3-fold. In vitro culture of oocytes from zinc-deficient mice with methyl donors restored trimethylated histone H3K4 content during IVM and doubled the in vitro fertilization capacity of matured oocytes, indicating that zinc deficiency-induced methylation abnormalities can be partially restored by methyl donor supplementation.

These findings demonstrate that zinc deficiency causes oocyte epigenetic defects, manifested by decreased histone and DNA methylation levels accompanied by increased repetitive sequence expression, thereby affecting oocyte quality.

Summary

Cellular zinc homeostasis is primarily regulated by metallothioneins and zinc transporters. Zinc affects oocyte meiosis by influencing MPF activity and the Mos-MAPK signaling pathway, modulates oocyte antioxidant capacity by affecting GSH levels, and alters oocyte epigenetics by influencing histone and DNA methylation levels, thereby affecting oocyte quality in female animals.

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Note: Figure translations are in progress. See original paper for figures.