HUMAN OVUM ACTIVATION FOR BAD QUALITY OOCYTES BY EXTRACELLULAR ZINC EXTRACTION

Mohammed Abd Elhakim Quodi Ahmed; Essam Ebrahim Aly; Ahmed Samer Abd El Malek; Amer Ahmed Abd Allah; Abd Elrahman Hegazy Abd Elwahab

Article History: Received: 23.03.2023 **Revised:** 21.04.2023 **Accepted:** 27.04.2023

Abstract

Zinc is a critical component in a number of conserved processes that regulate female germ cell growth, fertility, and pregnancy. During follicle development, a sufficient intracellular concentration of zinc in the oocyte maintains meiotic arrest at prophase I until the germ cell is ready to undergo maturation. An adequate supply of zinc is necessary for the oocyte to form a fertilization-competent egg as dietary zinc deficiency or chelation of zinc disrupts maturation and reduces the oocyte quality. Following sperm fusion to the egg to initiate the acrosomal reaction, a quick release of zinc, known as the zinc spark, induces egg activation in addition to facilitating zona pellucida hardening and reducing sperm motility to prevent polyspermy. Symmetric division, proliferation, and differentiation of the preimplantation embryo rely on zinc availability, both during the oocyte development and post-fertilization. Further, the fetal contribution to the placenta, fetal limb growth, and neural tube development are hindered in females challenged with zinc deficiency during pregnancy. In this review, we discuss the role of zinc in germ cell development, fertilization, and pregnancy with a focus on recent studies in mammalian females.

Keywords: zinc, mammal, female

Obstetrics & Gynecology Department, Faculty of medicine, Minia University
Corresponding Email: Anjaz3036@gmail.com

1. INTRODUCTION

Despite its singular unique ability to give rise to an entirely new creature, the oocyte shares many of the signalling mechanisms and biochemical environment as the somatic cells. Till now, transition metal physiology within the oocyte has been studied exclusively using non-mammalian model systems where the cell is larger and easily isolated in significant quantities, such as Xenopus laevis and Caenorhabditis elegans [1-3]. Zinc is accumulated during oocyte growth in these systems and is thought to be stored in lipoproteins in preparation for later stages such as embryonic development [4,5]. Additionally, zinc-dependent kinases have been implicated in the control of cell cycle progression in maturing X. laevis oocytes [3].

The bulk of the embryo's cytoplasm originates from the oocyte -in fact-,it is the oocyte that provides the necessary components to support development (such as mRNA and proteins) until the embryo's own genome is activated and it is able to sustain its own growth [6], therefore, the fate of the embryo relies heavily on the integrity of its oocyte predecessor [7,8] . An understanding of the biological processes that create a “good egg” in vitro is really important.

Under physiological conditions, fusion of the sperm and oocyte plasma membranes leads to repetitive increases in the intracellular free calcium (Ca2+) concentration in the oocyte cytoplasm [5, 6]. These transient increases (termed oscillations) result from the release of phospholipase C-ζ (PLC-zeta) from the sperm head [7]. The Ca2+ oscillations activate Ca2+/calmodulin-dependent protein kinase II (CaMKII) [8] and the CaMKII phosphorylates early mitotic inhibitor 2 (EMI2, a.k.a. FBXO43) thus relieving the anaphase-promoting complex/cyclosome (APC/C) from FBXO43-mediated inhibition [9-11]. These events lead to the degradation of cyclin B [12, 13], a subunit of the M-phase promoting factor (MPF). These signalling pathways demonstrate that Ca2+ signalling is essential in the process of oocyte activation and in fact, disruption of Ca2+ signalling during oocyte activation can cause developmental defects [14].

Most artificial activation methods induce an increase in the intracellular free Ca2+ levels in the oocyte that mimic sperm-induced Ca2+ signalling. However, most methods are able to induce only a single Ca2+ rise in the ooplasm [15]. Compared to the repetitive Ca2+ increases observed after sperm-induced oocytes activation, a single Ca2+ spike is a relatively poor activator of oocytes. Different approaches have been attempted to increase the efficiency of oocyte activation. For example, it is possible to incubate activated oocytes with inhibitors in order to reduce the level of MPF [16, 17]; however, these inhibitors are not very specific toward MPF, and while they do degrade MPF, they also have a number of side effects.

Zinc (Zn2+) is important to maintain oocytes arrested at the MII stage. In mice there is a 50% increase in the intracellular amount of Zn2+ as oocytes develop from the germinal vesicle to MII stage [18]. Zn2+ is necessary in activating MPF as Zn2+ regulates the activity of CDC25 [19], a phosphatase that can dephosphorylate and thus activate cyclin-dependent kinase 1, a component of MPF. In addition, EMI2 (official gene symbol, FBXO43), a zinc-binding protein, is required to maintain high MPF activity during the MII arrest [12], and the increase in total intracellular Zn2+ during oocyte maturation directly controls FBXO43 activity [20]. A previous report demonstrated that removal of Zn2+ from MII stage oocytes can successfully induce oocyte activation, and thus permit oocytes to exit the MII stage [21].

Zn2+ is released from oocytes after fertilization indicating that removal of Zn2+ is a natural part of oocyte activation [22]. TPEN (N, N, N′, N′-tetrakis (2-pyridylmethyl) ethane-1,2-diamine), known to have a high specificity toward Zn2+, was used to lower the level of available Zn2+ to activate mouse oocytes [21]. In this study, we developed an efficient method to activate pig oocytes by reducing the intracellular level of Zn2+ using TPEN. We found that a combination of proper Ca2+ signal and TPEN treatment can increase the developmental potential of activated oocytes.

2. REFERENCES

1. **Bruinsma JJ, Jirakulaporn T, Muslin AJ, Kornfeld K.** Zinc ions and cation diffusion facilitator proteins regulate Ras-mediated signalling. Dev Cell. 2002; 2:567–78. [PubMed: 12015965]
2. **Nomizu T, Falchuk KH, Vallee BL.** Zinc, iron, and copper contents of Xenopus laevis oocytes and embryos. Mol Reprod Dev. 1993; 36:419–23. [PubMed: 8305203]
3. **Sun L, Chai Y, Hannigan R, Bhogaraju VK, Machaca K.** Zinc regulates the ability of Cdc25C to activate MPF/cdk1. J Cell Physiol. 2007; 213:98–104. [PubMed: 17443687]
4. **Falchuk KH, Montorzi M.** Zinc physiology and biochemistry in oocytes and embryos. Biometals. 2001; 14:385–95. [PubMed: 11831467]
5. **Falchuk KH, Montorzi M, Vallee BL.** Zinc uptake and distribution in Xenopus laevis oocytes and embryos. Biochemistry. 1995; 34:16524–31. [PubMed: 8845382]
6. **Stitzel ML, Seydoux G.** Regulation of the oocyte-to-zygote transition. Science. 2007; 316:407–8. [PubMed: 17446393]
7. **Gosden RG.** Oogenesis as a foundation for embryogenesis. Mol Cell Endocrinol. 2002; 186:149–53. [PubMed: 11900888]
8. **Gandolfi TA, Gandolfi F.** The maternal legacy to the embryo: cytoplasmic components and their effects on early development. Theriogenology. 2001; 55:1255–76. [PubMed: 11327683]
9. **Kline D, Kline JT.** Repetitive calcium transients and the role of calcium in exocytosis and cell cycle activation in the mouse egg. Developmental biology. 1992; 149:80–9. [PubMed: 1728596]
10. **Swann K, Ozil JP.** Dynamics of the calcium signal that triggers mammalian egg activation. International review of cytology. 1994; 152:183–222. [PubMed: 8206704]
11. **Saunders CM, Larman MG, Parrington J, Cox LJ, Royse J, Blayney LM, et al.** PLC zeta: a sperm-specific trigger of Ca (2+) oscillations in eggs and embryo development. Development. 2002; 129:3533–44. [PubMed: 12117804]
12. **Markoulaki S, Matson S, Ducibella T.** Fertilization stimulates long-lasting oscillations of CaMKII activity in mouse eggs. Developmental biology. 2004; 272:15–25. [PubMed: 15242787]
13. **Liu J, Maller JL.** Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytostatic factor. Current biology: CB. 2005; 15:1458–68. [PubMed: 16040245]
14. **Rauh NR, Schmidt A, Bormann J, Nigg EA, Mayer TU.** Calcium triggers exit from meiosis II by targeting the APC/C inhibitor XErp1 for degradation. Nature. 2005; 437:1048–52. [PubMed: 16127448]
15. **Hansen DV, Tung JJ, Jackson PK.** CaMKII and polo-like kinase 1 sequentially phosphorylate the cytostatic factor Emi2/XErp1 to trigger its destruction and meiotic exit. Proceedings of the National Academy of Sciences of the United States of America. 2006; 103:608–13. [PubMed: 16407128]
16. **Madgwick S, Hansen DV, Levasseur M, Jackson PK, Jones KT.** Mouse Emi2 is required to enter meiosis II by reestablishing cyclin B1 during interkinesis. The Journal of cell biology. 2006; 174:791–801. [PubMed: 16966421]

17. **Jones KT.** Intracellular calcium in the fertilization and development of mammalian eggs. Clinical and experimental pharmacology & physiology. 2007; 34:1084–9. [PubMed: 17714098]
18. **Vitullo AD, Ozil JP.** Repetitive calcium stimuli drive meiotic resumption and pronuclear development during mouse oocyte activation. Developmental biology. 1992; 151:128–36. [PubMed: 1577185]
19. **Machaty Z, Wang WH, Day BN, Prather RS.** Complete activation of porcine oocytes induced by the sulfhydryl reagent, thimerosal. Biology of reproduction. 1997; 57:1123–7. [PubMed: 9369179]
20. **Nanassy L, Lee K, Javor A, Machaty Z.** Effects of activation methods and culture conditions on development of parthenogenetic porcine embryos. Animal reproduction science. 2008; 104:264–74. [PubMed: 17320316]
21. **Nanassy L, Lee K, Javor A, Machaty Z.** Changes in MPF and MAPK activities in porcine oocytes activated by different methods. Theriogenology. 2007; 68:146–52. [PubMed: 17524467]
22. **Kim AM, Vogt S, O'Halloran TV, Woodruff TK**. Zinc availability regulates exit from meiosis in maturing mammalian oocytes. Nature chemical biology. 2010; 6:674–81. [PubMed: 20693991]
23. **Sun L, Chai Y, Hannigan R, Bhogaraju VK, Machaca K.** Zinc regulates the ability of Cdc25C to activate MPF/cdk1. Journal of cellular physiology. 2007; 213:98–104. [PubMed: 17443687]
24. **Bernhardt ML, Kong BY, Kim AM, O'Halloran TV, Woodruff TK.** A zinc-dependent mechanism regulates meiotic progression in mammalian oocytes. Biology of reproduction. 2012; 86:114. [PubMed: 22302686]
25. **Suzuki T, Yoshida N, Suzuki E, Okuda E, Perry AC.** Full-term mouse development by abolishing Zn2+-dependent metaphase II arrest without Ca2+ release. Development. 2010; 137:2659–69. [PubMed: 20591924]
26. **Kim AM, Bernhardt ML, Kong BY, Ahn RW, Vogt S, Woodruff TK, et al.** Zinc sparks are triggered by fertilization and facilitate cell cycle resumption in mammalian eggs. ACS chemical biology. 2011; 6:716–23. [PubMed: 21526836]