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The fertilization-induced Ca^{2+} oscillation in mouse oocytes is cytoplasmic maturation dependent[1]

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ABSTRACT

Mature eggs (at metaphase II stage) produce a series of Ca^{2+} oscillation at fertilization. To define whether the fertilization-induced Ca^{2+} oscillation is restrict to the metaphase II eggs and cell cycle dependent, mouse oocytes at prophase I (arrested at germinal vesicle stage), metaphase I, metaphase II, as well as the pronuclear embryos at interphase of the first mitotic division derived from fertilization or parthenogenetic activation were inseminated after removal of zona pellucida. The results show that the fertilization-induced Ca^{2+} oscillation is not specific to metaphase II eggs. This is supported by the fact that immature oocytes generated the Ca^{2+} oscillations at fertilization regardless of their nuclear progression from prophase I to metaphase I (*in vitro* matured) stage. More interestingly, it was first found that pronuclear embryos at interphase derived from parthenogenetic activation showed Ca^{2+} oscillations in response to fertilization while the zygotes at interphase did not after reinsemination or intracytoplasmic injection of sperm extracts which induce Ca^{2+} oscillations in MII eggs. This suggests that the ability of oocytes to generate Ca^{2+} oscillation in response to sperm penetration is not regulated in a cell cycle dependent manner but dependent on the cytoplasmic maturation.

Key words: *Fertilization-induced Ca^{2+} oscillation, mouse oocyte, pronuclear embryo, parthenogenetic activation, cell cycle.*

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INTRODUCTION

It has been widely reported that fertilization triggers Ca^{2+} oscillation in mature eggs arrested at metaphase II stage(see Whitaker and Swann 1993, for review)[1]. Previous studies showed that the fertilization-induced Ca^{2+} oscillation plays an essential role in initiation of egg activation[2-4]. The fertilization-induced Ca^{2+} oscillation in MII eggs usually ceased at the time of pronuclear formation[5, 6] and the Ca^{2+} oscillation became endless if the fertilized eggs were arrested at the metaphase using colcemid[5]. A conclusion is drawn by the author that the ability to generate Ca^{2+} oscillations is restricted to the metaphase oocytes and the oocytes lose the ability to produce Ca^{2+} oscillation after entry into interphase of the first mitotic division[5]. Our previous studies have shown that the fertilization-induced Ca^{2+} oscillation in mouse immature oocytes is dependent on the cytoplasmic maturation but unrelated to the nuclear progression[7]. To further define if the fertilization-induced Ca^{2+} oscillation is modulated in a cell cycle dependent manner, we compared the fertilization-induced Ca^{2+} responses of oocytes at different stage of cell cycle including prophase I, metaphase I, metaphase II and interphase pronuclear embryos derived from fertilization and parthenogenetic activation.

MATERIALS AND METHODS

Kunming albino mice were used in this experiment.

Collection of prophase I (GV) stage oocytes

GV oocytes were collected from ovaries by puncturing antral follicles. To prevent spontaneous maturation during manipulation, oocytes were maintained in the medium containing 0.1 mg/ml dbcAMP.

Collection of oocytes at metaphase I stage (MI)

In vivo matured MI oocytes were retrieved directly from PMSG (pregnant mare's serum gonadotrophin) primed mice 5-6 h after injection of hCG (human chorionic gonadotrophin) by puncturing the fully grown follicles on ovaries. *In vitro* matured MI oocytes were obtained by culturing the GV intact oocytes in M2 medium for 3-4 h[8]. The MI oocytes were judged by the occurrence of GVBD and disappearance of nucleoli but without extrusion of first polar body.

Collection of mature eggs arrested at metaphase H stage (MII)

Mature MII eggs were retrieved from superovulated mice 15-16 h post hCG injection by flushing the oviducts in M2.

Collection of pronuclear zygotes at the interphase of first mitotic division

Pronuclear zygotes were collected from superovulated and mated mice (with visible vaginal plug) 22-23 h after hCG injection and only those with intact nuclear envelope were selected for experiment.

Production of parthenogenetic pronuclear embryos

MII eggs collected 17-18 h post hCG were treated with 8% ethanol in M2 for 5-6 min and cultured in M16 medium for 5-6 h[8]. Pronuclear embryos at the interphase of first mitotic division

were selected as described above.

In vitro fertilization and measurement of intracellular Ca^{2+}

The collected oocytes and embryos at different stage were labeled with 4 μM Fura-2/AM (Molecular Probe Inc.) for 30-40 min and washed several times in M2. Zona pellucida were removed by repetitive aspirating the oocytes or embryos through a fine-bore-pipette in acidic Tyrode solution (PH 2.5)[8]. The zona free oocytes or embryos were transferred to a coverslip-bottom chamber containing IVF medium[8] covered with light mineral oil and allowed to attach tightly to the bottom coverslip. To prevent the movement of oocytes or embryos during insemination and $[Ca^{2+}]_i$ measurement, bovine serum albumin (BSA, fraction V) was not added to the medium until zona free oocytes or embryos sticked firmly to the coverslip. Sperm were obtained from the cauda epididymides and cultured in IVF +15 mg/ml BSA for 1.5 h for capacitation. The capacitated sperm suspension was added to IVF containing zona free oocytes or embryos and $[Ca^{2+}]_i$ was measured using Miracal Image system (from UK).

All chemicals used in this experiment were from Sigma unless stated otherwise.

RESULTS

Fertilization-induced Ca^{2+} oscillation in prophase I oocytes

Prophase I oocytes arrested at GV stage showed a spontaneous Ca^{2+}oscillation lasting for 3-4 h after release from follicles. Inhibition of GVBD using dbcAMP had no influence on the occurrence and timing of the spontaneous Ca^{2+} oscillation and fertilization[7]. To prevent the possible interference of the spontaneous Ca^{2+} oscillation on the fertilization-induced Ca^{2+} oscillation, the oocytes arrested at GV stage in dbcAMP containing medium were not inseminated until the the spontaneous Ca^{2+} oscillation had ceased completely. The prophase I oocytes arrested at GV stage produced a new series of Ca^{2+} oscillation lasting for 2-5 h after fertilization (n=30/30) (Fig 1).

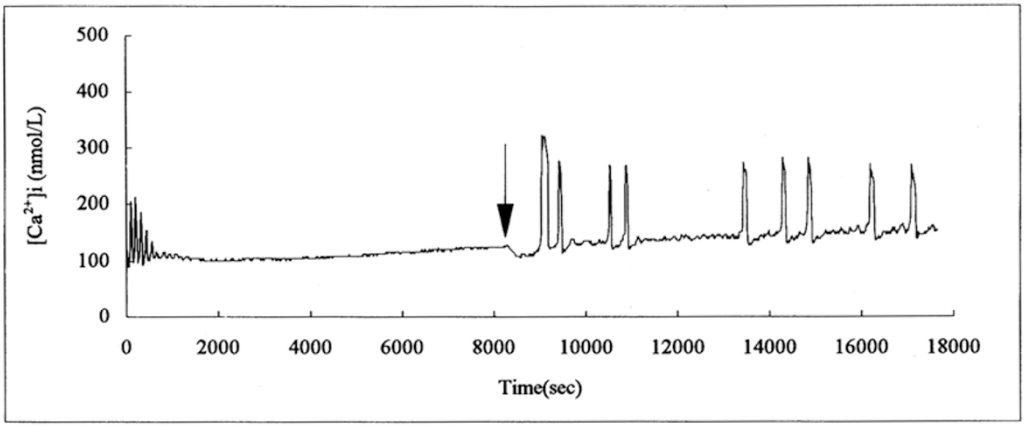
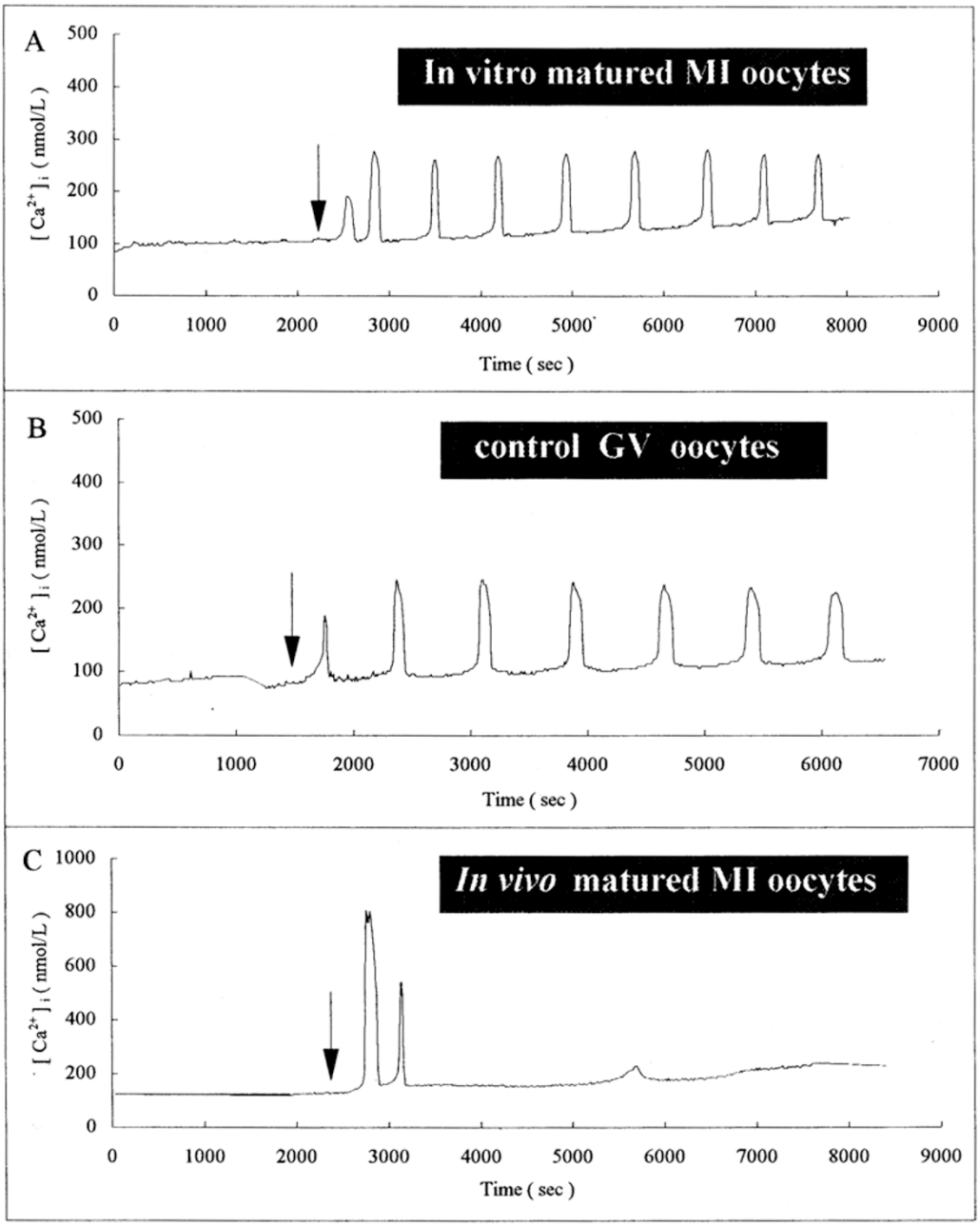


Fig 1. The fertihzation-induced Ca^{2+} oscillation in prophase I oocytes. Sperm were added after spontaneous Ca^{2+} oscillation had ceased for more than 1.5 h. The oocytes were blocked at GV stage using dbcAMP (0.1 mg/ml) during experiment. The arrow indicates the time of insemination.

Fertilization-induced Ca^{2+} oscillation in oocytes at metaphase I

After 3-4 h culture in M2, the GV oocytes underwent GVBD and progressed to MI stage. The *in vitro* matured MI oocytes showed repetitive Ca^{2+} oscillation after fertilization (n=25/25) (Fig 2A). The MI oocytes shared much similarity with the GV intact oocytes in their Ca^{2+} response to fertilization (n=30/30) (Fig 2B). In contrast, The *in vivo* matured MI oocytes collected from mature follicles (5-6 h post hCG) ekhibited only 1-2 Ca^{2+} transients (n=18/30, Fig 2 C).



Fertilization-induced Ca^{2+} oscillation in the metaphase II eggs

MII eggs (15-16 h post hCG injection) usually generated a big Ca^{2+} spike at the begining and then followed by a series of regular Ca^{2+} oscillations which lasted for 3-6 h (Fig 3) and ceased prior to pronuclear formation.

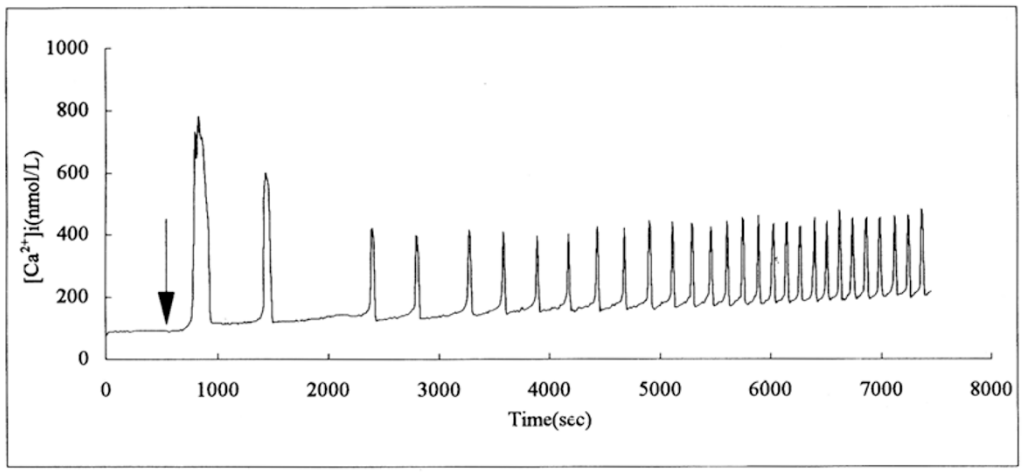


Fig 3. Typical pattern of fertilization-induced Ca^{2+} oscillation in mature MII eggs (15-16 h post hCG injection). Time of insemination is indicated by the arrow.

Intracellular Ca^{2+} response of fertilized zygotes at interphase to reinsemination or sperm extract injection

The pronuclear zygotes showed no Ca^{2+} change after reinsemination (n=50/50) (Fig 4). To preclude the possible unfertilizability of the pronuclear zygotes, sperm extracts[10] capable of causing Ca^{2+} oscillation in MII eggs were injected into the zygotes. No Ca^{2+} oscillation was observed after the injection (unpublished results), indicating that the pronuclear zygotes had lost their ability to generate Ca^{2+} oscillation in response to reinsemination.

◁ **Fig 2.** Effect of cytoplasmic maturation on the Ca^{2+} response of oocytes to fertilization. The *in vitro* matured MI oocytes behaved much like the GV oocytes in their Ca^{2+} responses to ferilization (A, B). The *in vivo* matured MI oocytes collected from follicles 5-6 h post hCG exhibited only 1-2 Ca^{2+} transients(C). The arrows indicate the time of insemination.

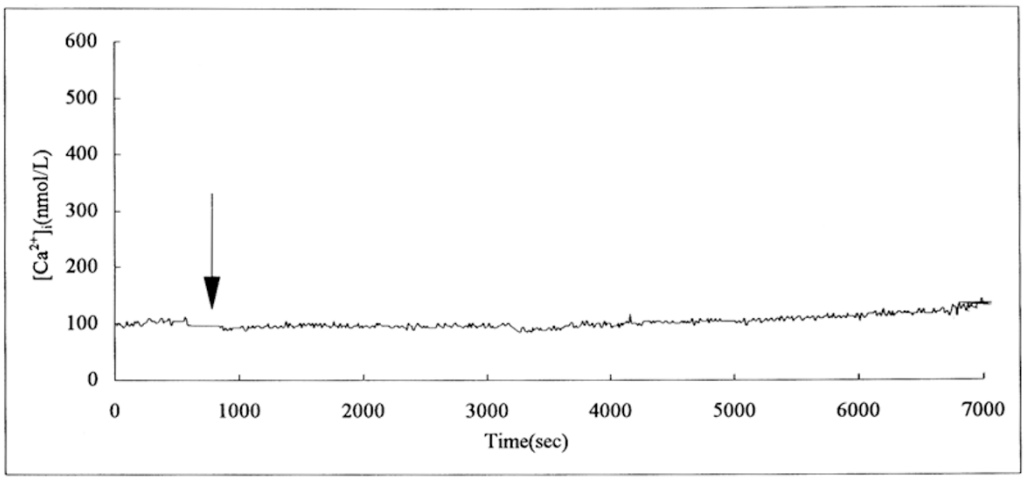


Fig 4. The fertilized zygotes at interphase of the first mitotic division failed to generate Ca^{2+} oscillation in response to reinsemination after removal of zona pellucida. Intracytoplasmic injection of sperm extract did not induce Ca^{2+} oscillation either. The arrow indicates the time of insemination or injection of the sperm extract.

Parthenogenetically-derived pronuclear embryos at interphase of the first mitotic division retained the ability to generate Ca^{2+} oscillation at fertilization

In contrast to the pronuclear zygotes, the interphase pronuclear embryos derived from parthenogenetic activation showed the repetitive Ca^{2+} oscillation after fertilization (n=42/45, Fig 5). The control uninseminated parthenogenetic pronuclear embryos exhibited no Ca^{2+} change during the measurement (n=40/40).

DISCUSSION

Fertilization-induced Ca^{2+} oscillation in mouse maturing oocytes is cytoplasmic maturation dependent but independent of nuclear maturation

GV oocytes not only show the spontaneous Ca^{2+} oscillation after release from follicles but also generate Ca^{2+} oscillation lasting for several hours in response to fertilization[9, 11, 12]. This indicates that the mechanism of Ca^{2+} oscillation in mouse oocytes is established early during meiotic maturation and oocytes acquire the ability to respond to fertilization in Ca^{2+} oscillation during the GV stage. The fertilization-induced Ca^{2+} oscillation can not be attributed to the temporal coincidence with the spontaneous Ca^{2+} oscillation in GV oocytes because the spontaneous Ca^{2+} oscillation usually ceased within 2-3 h and we monitored the fertilization-induced Ca^{2+}

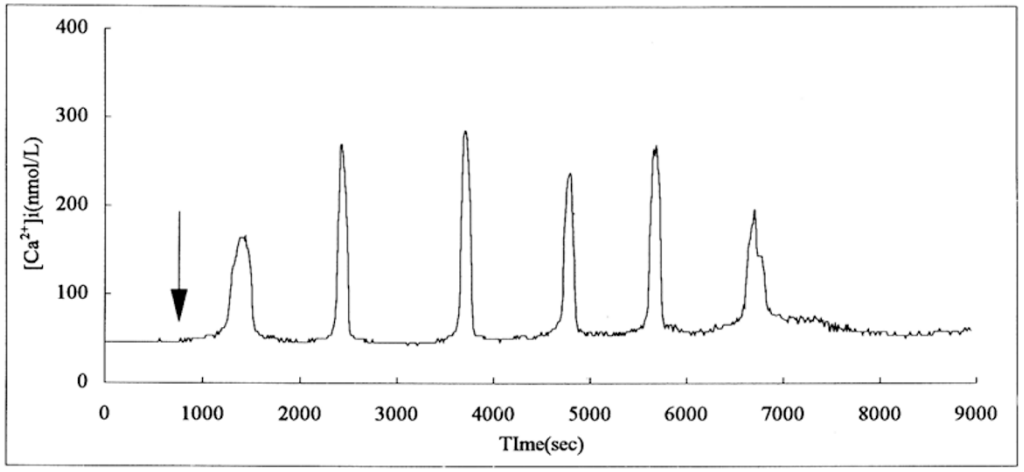


Fig 5. The fertilization-induced Ca^{2+} oscillation in the parthenogenetic pronucleax embryos at the interphase of the first mitotic division. The arrow indicates the time of insemination.

oscillation only after the complete cessation of the spontaneous Ca^{2+} oscillation for enough long time. Furthermore, the interphase pronuclear embryos derived from parthenogenetic activation exhibit Ca^{2+} oscillation at fertilization. This strongly suggests that the ability to generate Ca^{2+} oscillation in response to fertlization is not restricted to the metaphse II eggs as was reported before[5, 16]. What determines the intracellular Ca^{2+} excitability of oocytes in response to fertilization? Jones et al attributed this to the cell cycle progression[5]. Our experiments demonstrated that the fertilization-induced Ca^{2+} oscillation is more reliant on the cytoplasmic maturation in oocytes or eggs. First, nuclear maturation and cell cycle progression per se does not influence the Ca^{2+} response to fertilization, since the oocytes showed a similar pattern of Ca^{2+} oscillation regardless of the occurrence of GVBD and the progression from prophase I to MI *in vitro*. It is also known that nuclear maturation of oocytes progressed more rapidly than cytoplasmic maturation in vitro after their release from follicles and resulted in premature nuclear maturation[13]. Second, the *in vivo* matured MI oocytes (5-6 h post HCG) and early MII oocytes (11-12 h post HCG) showed low Ca^{2+} excitability in response to fertilization and regained high excitability after further maturation. The intracellular Ca^{2+} excitability shows a dual phase modification during meiotic maturation. The Ca^{2+} excitability reaches high at GV stage, and later declines at MI and early MII stage and then attains high again after further cytoplasmic maturation. Although the underlying mecha-

nism and biological significance of this modification of cytoplasm Ca^{2+} excitability during meiotic maturation is unknown at present, it clearly indicates that the ability to generate Ca^{2+} oscillation in oocytes is mainly dependent on cytoplasmic maturation but not nuclear maturation. In addition, the removal of intact GV had no effect on fertilization-induced Ca^{2+} oscillation (our unpublished results). This suggested that fertilization-induced Ca^{2+} oscillation in immature oocytes is independent to the nuclear progression or even the presence of nucleus, but can be modulated in a cytoplasmic maturation dependent manner.

Why interphase pronuclear embryos derived from fertilization and from parthenogenetic activation show different Ca^{2+} response to reinsemination and fertilization?

The generation of repetitive Ca^{2+} oscillation in interphase parthenogenetic pronuclear embryos at fertilization suggested that the failure of interphase pronuclear zygotes to exhibit Ca^{2+} oscillation in response to reinsemination can not be attributed to cell cycle progression. We propose that fertilization and artificial activation may impose different actions on the oocyte cytoplasm, because artificial stimulation activates eggs by inducing monotonic Ca^{2+} rise[4], while sperm induces the Ca^{2+} oscillation at fertilization. The Ca^{2+} oscillation but not the single Ca^{2+} rise may dismantle the reactory apparatus in the cytoplasm of oocytes and result in the differences in the Ca^{2+} respone to fertilization and other aspects of cytoplasmic activity compared with the fertilized eggs[14, 15]. It is proposed that although the parthenogenetically activated eggs progress to the interphase, the cytoplasm may retain to some extent the characteristics of MII eggs and maintain the ability to generate Ca^{2+} oscillation at fertilization. The mechanisms underlying the modulation of intracellular Ca^{2+} excitability during meiotic maturation and the biological significance of this modification in regulation of cell function require further investigation.

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