—Mini Review—

Cytoskeletal Dynamics during Oocyte Maturation and Fertilization in Primates with Comparison to Rodents

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Abstract: Microtubules and microfilaments are elements of the cytoskeleton that are involved in cell motility. Dynamic and proper organization of the cytoskeleton is crucial for completion of oocyte maturation and fertilization. When performing mammalian developmental and reproductive techniques, information concerning the dynamism of the cytoskeleton is necessary and indispensable. Although rodents are widely used for developmental and reproductive technology, there are numerous differences between the cytoskeletal organization of rodent gametogenesis / fertilization and that of primates, including humans. Herein, a review of cytoskeletal organization during oocyte maturation and fertilization involving the differences between rodents, primates and other mammalian species is presented. Furthermore, we also review the function of the centrosome as a microtubule organizing center (MTOC). Proper information on the kinetics of the cytoskeleton is crucial for further expansion of developmental biology.

Key words: Cytoskeleton, Gametogenesis, Fertilization, Oocyte maturation

Introduction

Assisted reproductive technology (ART) that includes micromanipulation techniques is widely employed as a tool for treating human infertility and for developmental engineering. Production of offspring derived from immature spermatid injection and/or cloned animals is routinely successful in mice, but only controversial results have been obtained in humans and other mammals. While some investigators have claimed success, many others have reported complete failure.

The cytoskeleton plays a role in cell motility similar to a field worker within the cell. When using gametes for developmental and reproductive technology, including nuclear transfer, knowledge concerning the cytoskeleton is important for their management. In this article, the dynamic motility of cytoskeletons (microtubules and microfilaments) during oocyte maturation and fertilization is reviewed. There are numerous differences in cytoskeletal organization between the species. Furthermore, proper knowledge of centrosomal inheritance is crucial for developmental engineering that uses gametes.

Cytoskeletal Dynamics during Oocyte Maturation

Rodents

In the germinal vesicle period, mouse oocytes have a huge germinal vesicle (GV), and microfilaments and myosin II (IIA and IIB) are uniformly localized in the egg cortex [1]. The mouse GV is located at the center of the cell, and the first meiotic spindle is organized after germinal vesicle break down (GVBD) takes place in the center. The spindle, then migrates towards the cortex, and the first polar body is finally extruded. Following these events is the organization of the second meiotic spindle which is rotated 90° and is fixed vertically to the cytoplasmic membrane. Microfilaments are crucial for spindle movement because microfilament inhibitors (cytochalasin B, Jasplakinolide) inhibit mouse oocyte maturation by arresting the first meiotic spindle migration [2, 3]. The surface of the mouse matured M2 oocyte is covered with microvilli, except for the region (microvillus-free area) adjacent to the meiotic spindle

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[4]. Microfilaments accumulate beneath the cytoplasmic membrane at the microvillus-free area in mouse and hamster oocytes [4, 5]. However, this polarity is not recognized in the rat oocyte [6]. An important role of microfilaments during oocyte maturation is to sort out various kinds of organelles. For example, cortical granules localize homogenously in the GV oocyte and migrate to the egg cortex (microvillus area) during maturation. The type of migration is inhibited by microfilament inhibitors [7]. Furthermore, recent reports indicate that mitochondrial distribution, where movement is coordinated by microfilaments, might be an interesting indicator of oocyte quality [8].

The meiotic spindles of unfertilized oocytes are anastral, barrel-shaped, and attached to the oocyte cortex [9]. On the other hand, microtubules form numerous astral microtubules (cytoplasmic asters) in the mature M2 oocyte cytoplasm. An astral microtubule aids in the male and female genome union during fertilization. In mouse oocytes, several pericentriolar material foci have been found in acentriolar astral microtubules (cytoplasmic asters) [10]. It is thought that the MTOCs of cytoplasmic asters are complexes of functional centrosomal proteins (e.g., γ-tubulin).

Primates and others

In human and other mammalian GV stage eggs, microfilaments are observed homogenously in both the cortex and around the GV [11]. After GVBD and following chromosomal condensation, the first meiotic spindle organized by microtubules is formed and located horizontally to the cortex of the egg. The drastic polarity found in mouse oocyte microfilaments is not observed in human and other domestic mammalian matured oocytes. Microfilaments are localized homogenously beneath the cytoplasmic membrane. Accumulation of microfilaments has only been seen at the time of the first polar body extrusion, whereas, the motility of rodent oocyte maturation is more drastic.

Microtubules in the mammalian meiotic spindle are highly sensitive to temperature. Some investigators have reported that transient cooling causes irreversible disruption of the meiotic spindle in the human oocyte [12, 13]. This may affect genetic balance due to faulty chromosomal interaction with microtubules during segregation. Moreover, the cytoplasmic aster is not observed in the primate, including human oocytes, because the centrosome (cytoplasmic MTOC) of the primate oocyte is reduced and inactivated during oogenesis. The metaphase 2 spindle, at either pole, has neither centrioles nor dense granulo-vesicular centrosomal material that nucleate MTs in mouse oocytes that have a functional maternal centrosome. In humans, the oogonia present a pair of well-defined centrioles that are involved in cell division. As in most mammals, they are lost during oogenesis, and the mature oocyte is devoid of centrioles, which are present only up to the pachytene stage [14]. The human oocyte also does not have granular centrosomal material at its meiotic spindle poles, although mouse oocytes do have a dominant maternal centrosome [10]. Thus, the oocyte centrosome is greatly reduced and inactivated during oogenesis in non-rodent mammals. The functional centrosomal structure is, however, restored in the zygote after fertilization with some maternal input around the sperm centriole that duplicates at the pronuclear stage, forms a sperm aster and proceeds to form the first mitotic spindle. This is the ancestor of centrosomes in embryonic, fetal and adult somatic cells.

Microtubule Organization during Fertilization

Rodents

In mice, the paternal centrosome completely disappears in spermiogenesis and therefore does not contribute to fertilization [15]. Cytoplasmic asters are recognized in mature M2 oocytes as described previously. After ovulation, and frequently at the time of sperm incorporation, multiple cytoplasmic asters assemble in association with the oocyte cortex. During sperm incorporation, these asters enlarge and are often found in association with the pronuclei; the meiotic spindle then rotates, which results in formation of a second polar body with persistence a midbody microtubules. As the pronuclei move to the egg center, these asters enlarge into a dense array. At the end of first interphase, the dense array disassembles adjacent to the pronuclei. Syngamy (pronuclear fusion) is not observed, and the mitotic apparatus emerges from these perinuclear microtubules as barrel-shaped and anastral, reminiscent of plant cell spindles; the sperm centriole does not nucleate mitotic microtubules. At prophase, the chromosomes condense separately, and an irregular sphere of microtubules assembles within a monoastral structure. Consequently, a spindle begins to emerge, and by metaphase (first mitotic spindle), it typically appears barrel-shaped and anastral [9]. This microtubule organization during fertilization is shown in Fig. 1.

Rabbit

Rabbits are lagomorphs, and their centrosome considerably follows a paternal pattern of inheritance due

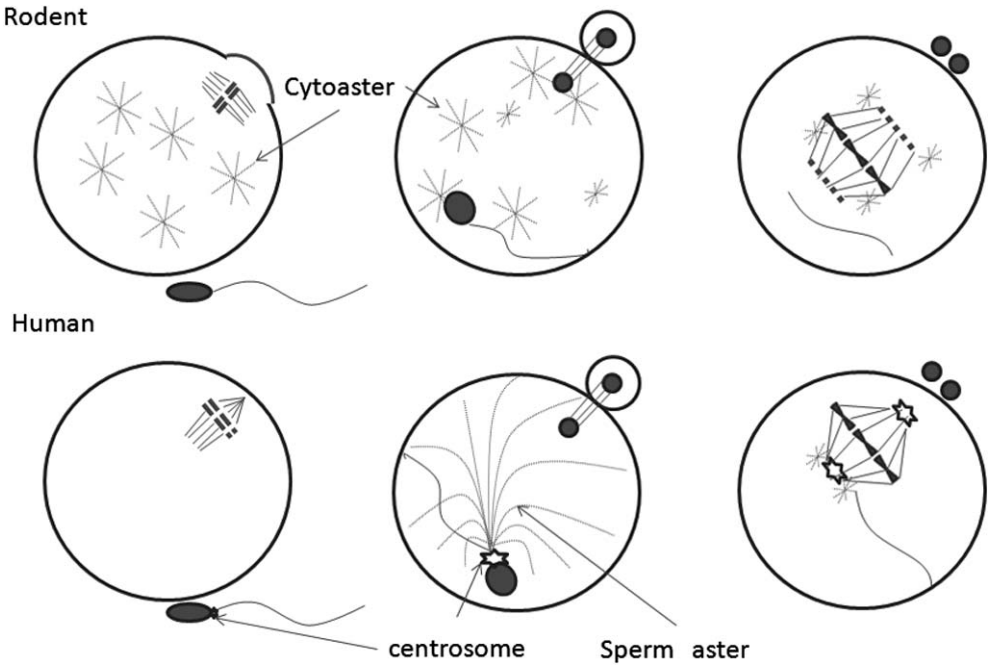

Fig. 1. Microtubule organization and centrosome inheritance are shown.
In mice, mature spermatozoa do not possess centrosomes, and sperm centrosomes do not contribute to fertilization. Multiple cytoplasmic asters are present in the mature M2 oocyte. During sperm incorporation, these asters enlarge and are often found in association with the pronuclei; the meiotic spindle rotates, which results in formation of the second polar body with persistence of a midbody microtubules. As the pronuclei move to the egg center, these asters enlarge into a dense array. After syngamy (pronuclear fusion), a spindle begins to emerge, and by metaphase (first mitotic spindle), it typically appears barrel-shaped and anastral.
In humans, the mature M2 oocyte has a second meiotic spindle located in the oocyte cortex, and the major axis of the spindle is parallel to the cell membrane. An astral microtubule, so-called 'sperm aster', forms from the sperm neck (sperm centrosome) after sperm incorporation, and microtubules are also present in the meiotic midbody. A sperm aster will then extend throughout the whole oocyte cytoplasm and reach the female pronuclei. Subsequently, the first mitotic spindle begins to emerge, and small astral microtubules can be observed at the spindle in the area of the sperm tail. The first mitotic spindle usually does not localize in the center of the oocyte cytoplasm at that time. These observations are considered to be paternal patterns of centrosome inheritance.

to the presence of a monastral sperm aster during fertilization [16]. In addition, isolated rabbit sperm heads without a midpiece (without centrosomes) fail to nucleate sperm asters in rabbit eggs [17]. However, after sperm aster formation, cytoplasmic microtubules form and surround male and female pronuclei without any distinct nucleation site, suggesting that centrosomal inheritance involves a blend of paternal and maternal patterns [18].

Mammals (humans, primates and others)

In fertilization for most mammals, a zygote requires one functional centrosome to organize its microtubules before the first cleavage of fertilization [19]. In humans [20], primates [21], cattle [22], pigs [23] and sheep [24], this centrosome is typically introduced by spermatozoa. Like most other somatic cells, a spermatid has two centrioles within its centrosome, while a mature spermatozoa has only one centriole. During spermiogenesis, a partial reduction of the male centrosome occurs. The proximal centriole (PC) remains intact, however, and the distal centriole (DC) that gave rise to the sperm flagellum is partially reduced [25]. The functional PC is located in a 'black box' within the neck that is composed of the capitulum beneath the basal plate and is flanked laterally by 9 segmented columns showing the typical '9 + 0' organization of MT triplets [26].

After sperm incorporation and soon after its release from the 'black box', the sperm centriole is activated and

duplicated and also acquires more and more dense pericentriolar material (PCM) that nucleates MT, thus becoming a functional centrosome. The sperm centrosome receives some modifications, such as phosphorylation, disulfide bond reduction and γ-tubulin recruited from oocytes. An extensive MT network is formed by the sperm aster, which reorganizes the whole cytoskeleton of the oocyte soon after sperm incorporation, as revealed by fluorescent microscopy [19, 20]. In human mature M2 oocytes, a second meiotic spindle is located in the oocyte cortex, with its major axis parallel to the cell membrane. This second meiotic spindle pole is notably narrow on the cell membrane side, while its opposite side is wide. Cytoplasmic asters are not present in mammalian mature oocytes. An astral microtubule, the so-called 'sperm aster', is organized from the sperm neck (sperm centrosome) after sperm incorporation, and microtubules are also present in the meiotic midbody. A sperm aster extends throughout the whole oocyte cytoplasm and reaches the female pronuclei. Fertilization is complete once both the paternal and maternal genomes unite, and this requires migration of the egg nucleus to the sperm nucleus on microtubules. Neither the molecular mechanism of the pronucleus binding to microtubules nor the role of motor proteins in regulating pronuclear motility has been fully elucidated. However, a model in which dynein accumulates and binds to the female pronucleus on sperm aster microtubules, where it interacts with dynactin, has been proposed [27]. When the female pronucleus moves closer to the male pronucleus, two astral microtubules are then present around both the male and female nuclei. This results in the emergence of the first mitotic spindle. Subsequently, small astral microtubules can be seen at the spindle pole where the sperm tail exists. The first mitotic spindle usually does not localize in the center of the oocyte cytoplasm at this time [20]. This microtubule organization during fertilization is shown in Fig. 1.

Microfilament Organization during Fertilization

Rodents

In the mature metaphase 2 oocyte, microfilaments are found mainly in the cortex but with a greater concentration in the area overlying the meiotic spindle located near and parallel to the cell membrane. This area of the cell surface is also devoid of microvilli as described previously [4]. During sperm-egg fusion, spermatozoa do not normally fertilize eggs in this microvilli-poor and microfilament-rich area located over the meiotic spindle [28]. After fertilization, the meiotic cleavage furrow begins to form in this actin-rich domain of the cortex at the equator of the spindle. Two actin-rich domains are thereby created on each side of the furrow. One of these subsequently shrinks, while the other expands causing rotation of the spindle and leading to formation of the second polar body [29]. Following fertilization, an area free of microvilli and rich in microfilaments develops near the decondensing sperm nucleus and forms the incorporation cone [29, 30]. These two actin-rich, microvilli-free domains disappear when the pronuclei form and migrate towards the egg center, and at the same time, numerous microfilaments can be observed around the pronuclei [29]. Experiments using Cytochalasin, a microfilament inhibitor, suggest that microfilaments are not required for sperm entry at fertilization, but are necessary for spindle rotation, polar body formation and migration of the pronuclei towards the center of the egg [29].

Mammals (humans, primates and others)

The drastic polarity seen in mouse oocyte microfilaments is not observed in human and other domestic mammalian matured oocytes. Furthermore, microfilaments are localized homogenously beneath the vitelline membrane in mature metaphase 2 oocytes, as described previously. The incorporation cone is not present during mammalian fertilization. However, accumulation of microfilaments can be observed at the site of sperm incorporation [31].

Conclusion

There are numerous differences in cytoskeletal organization and centrosomal inheritance between species. When using gametes experimentally, these differences must be taken into consideration. For example, there has been an increase in the number of reports on successful full-term births in cloned mammals, and these reports include parthenogenetic and immature germ cell techniques. These techniques enable production of mammals without the contribution of the sperm centrosome, whose function apparently contributes to appropriate chromosome segregation. In addition, there may be a likely association of this event with further embryonic development and pregnancy in mammals that demonstrate paternal patterns of inheritance.

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