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Rearrangements of Oocyte Cytoskeleton during Mammalian Oogenesis: Review

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Primary oocytes lose their centrosomes at pachytene stage. Chromosome movements during oocyte meiotic maturation are mediated by a barrel-shaped acentrosomal spindle, a unique situation for animal cells. Spindle poles are assembled from numerous small microtubule organizing centers. Microfilaments initially accumulate in the subplasmalemmal and perinuclear regions, then surround the spindle and form an actin cap. They mediate spindle migration and anchoring to the cell cortex, spindle rotation and the highly asymmetric cytokinesis. Cytokeratins and vimentin have also been shown to be present in mammalian oocytes and to undergo redistribution during oogenesis, though their precise functions are still to be clarified.

Key words: meiosis, oocyte maturation, microtubules, microfilaments, intermediate filaments.

Mammalian germ cells differentiate early in prenatal development in extraembryonic tissues and then migrate to the embryo proper, settle in gonadal ridges and proliferate by mitosis. During this process, they resemble undifferentiated somatic cells [15]. In female embryos, with the development of gonadal ridges into ovaries, primordial germ cells differentiate to oogonia. At this stage, their tubulin cytoskeleton is characterized by a typical juxtanuclear centrosome with a pair of centrioles and dense pericentriolar material rich at gamma tubulin which nucleates microtubules [11]. Microtubules are needed for the formation of Balbiani body, a transient complex of organelles that forms in oogonia and is the first lineage-specific morphological feature of female germ cells [5]. Microfilaments are located in the peripheral layer of cytoplasm, beneath the plasma membrane [11]. This cortex of fibrillar actin will persist throughout oogenesis and after fertilization [14].

During later prenatal development, oogonia differentiate into primary oocytes and start meiosis. It is arrested upon reaching diplotene. At the same stage, centrosomes degenerate, presumably to prevent parthenogenetic development. Centrioles are disassembled. The gamma tubulin from the pericentriolar material is included into two multivesicular aggregates. These structures, however, do not take over the function of centrosomes as microtubule organizing centers and do not nucleate microtubules, suggesting that gamma tubulin in them is inactive [6].

The third cytoskeletal system composed of cytoplasmic intermediate filaments is still poorly studied in female germ cells. Some authors, however, report the presence of keratins in fetal oocytes in the form of a perinuclear aggregate, showing that intermediate filaments are already present in early prophase I [10].

After the onset of puberty, with each estrous/menstrual cycle a group of oocytes resume meiosis. One or more of them, depending on the species, completes the first meiotic division, extrudes the first polar body, starts the second meiotic division and reaches its metaphase, where it is arrested until fertilization. This sequence from arrested prophase I to metaphase II, called oocyte meiotic maturation, requires major rearrangements of cytoskeletal structures. The tubulin cytoskeleton is organized without centrosomes, a situation quite atypical for animal cells. With the resumption of meiosis, the oocyte in prophase I, now often referred to as germinal vesicle stage, disassembles its multivesicular aggregates. Its gamma tubulin is transferred to multiple small microtubule organizing centers located in the vicinity of the nucleus [6]. Actually, researchers of oogenesis often use the term "microtubule organizing center" specifically to designate these small accumulations of gamma tubulin, excluding the centrosome. Microfilaments at germinal vesicle stage are found in subplasmalemmal and perinuclear position [1, 7]. The same regions are positive for cytokeratins and vimentin [7, 8] (**Fig. 1A**). The role of cytoplasmic intermediate filament proteins in oocytes is still to be clarified. Vimentin has been reported to facilitate pronuclear positioning in the zygote [9]. The distribution of keratins shows very similar patterns in maturing oocytes of different vertebrate classes, suggesting some important though yet unknown function [8].

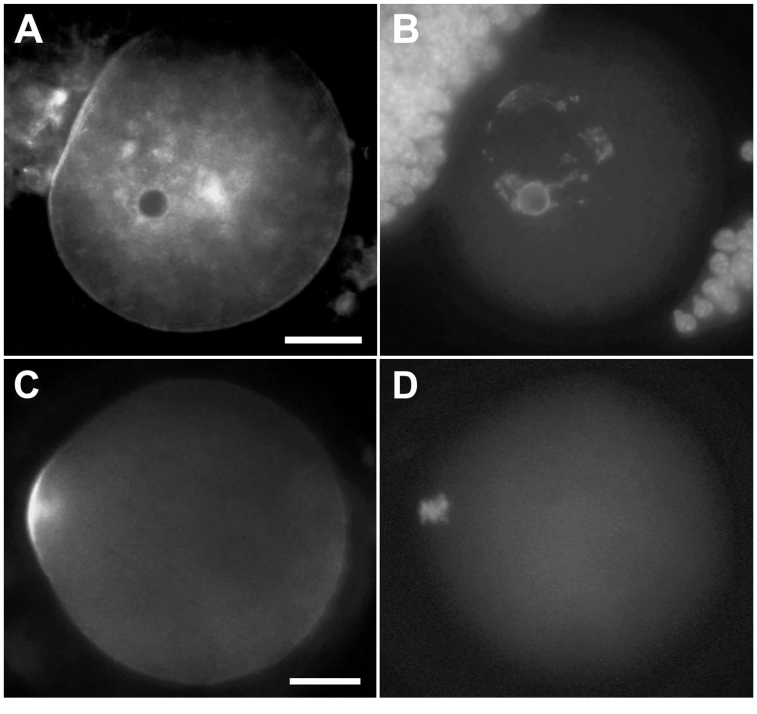

Fig. 1. Immunofluorescent localization of cytokeratins 1, 5, 6 and 8 in mouse oocytes, performed as described in [8]: **A** – germinal vesicle stage (surrounded by cumulus); **C** – metaphase I; **B** and **D** show DNA in the same cells, visualized by Hoechst 33258. Bars = 20 μm

Meiotic resumption is triggered by the cyclin-dependent kinase MPF. Its activity soon causes disassembly of the nuclear envelope during a brief stage called germinal vesicle breakdown. Chromosomes now have free contact with cytoplasmic components and this allows them to drive the meiotic spindle formation. A protein component of chromatin (particularly of centromeric chromatin) known as RCC1 activates a soluble GTPase called Ran, which in turn activates the microtubule organizing centers at close distance. This leads to formation of small asters in the vicinity of chromosomes and subsequent binding of the (+) ends of their microtubules to kinetochores. Microtubules stabilized this way elongate and eventually form a barrel-shaped bipolar spindle [12]. The process is mediated by microtubule associated proteins such as NuMA, kinesins (notably Kif11) and dyneins that "focus" the (–) ends of microtubules, binding them together into spindle poles [6]. Meanwhile, microfilaments that have been in perinuclear position now encircle the chromosomes and the microtubules, forming a larger spindle-shaped actin cage around the tubulin spindle [2].

Once formed, the meiotic spindle carries out the process of chromosome congression and arranges the bivalents at the equatorial plane. The oocyte is now in metaphase I. At this stage, fibrillar actin is localized in the cortex and around the meiotic spindle. Cytokeratins and vimentin are found in the same regions and also associated with the chromosomes [7, 8] (**Fig. 1C**).

Initially, the metaphase I spindle has central localization. Then microfilaments connect one of its poles to the cortex, pull it to peripheral position and anchor it under the cell membrane, a process called spindle migration [1]. This is a prerequisite for the subsequent asymmetric division. The migration is regulated by the microfilament nucleator protein formin-2 [2]. Upon reaching the cortex, the spindle causes its modification. Microfilaments accumulate above the spindle and form an actin cap devoid of microvilli. It will take part in anchoring the spindle to subplasmalemmal position and in cytokinesis. Cap formation is caused by Ran GTPase activated locally by the chromosomes [3]. The thickening of the cap is accompanied by reduction of other parts of actin cortex, most likely to make it more dynamic and to reflect the disconnection from cumulus cells. Similar changes affect the distribution of intermediate filament proteins that colocalize with actin [7] (**Fig. 1C**).

After the metaphase plate is properly arranged, anaphase I and telophase I follow quickly and chromosomes are segregated into two haploid sets. Microfilaments from the actin cap form a contractile ring and extrude the 1st polar body. The transition from meiosis I to II in animal oocytes is brief, with chromosomes remaining condensed [4]. It is sometimes referred to as "metaphase I – metaphase II transition". The actin cap and the meiotic spindle undergo fast reorganization and the oocyte reaches metaphase II. The spindle is parallel to the cell membrane and remains anchored beneath it until fertilization [13]. The distribution of actin and intermediate filament proteins in metaphase II is the same as in metaphase I [7, 8]. At this stage, the oocyte is ready to be fertilized and undergoes ovulation.

At the beginning of fertilization, the contact with the sperm cell activates the oocyte and triggers microfilament-mediated rotation of its spindle to make it perpendicular to the cell surface, a prerequisite for cytokinesis. Spindle rotation is followed by anaphase II, telophase II and second polar body extrusion. Experiments with cytoskeletal inhibitors show that damage to microtubules arrests meiosis in metaphase while damage to microfilaments prevents spindle migration, rotation and polar body extrusion [16]. Carrying out telophase II is the last function of the acentrosomal meiotic spindle of the oocyte. After gamete fusion, sperm centrioles organize a dominant microtubule organizing center by recruiting dispersed oocyte centrosomal proteins; this new centrosome forms an aster responsible for pronuclear migration. An exception are rodents, in which sperm centrioles degenerate and centrosomes are formed *de novo* during cleavage [6].

It can be concluded that the action of cytoskeletal structures mediates the mechanical aspects of oogenesis, with microtubules being responsible for chromosome congression and segregation and microfilaments for spindle positioning and cytokinesis. Knowledge of cytoskeletal reorganizations during oogenesis is crucial for understanding the physiology and pathology of the oocyte and for the success of assisted reproductive technologies.

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