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CORTICAL ACTIN CYTOSKELETON IN HUMAN OOCYTES: A COMPARISON WITH MOUSE OOCYTES

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A b s t r a c t

The arrangement of the cortical actin cytoskeleton was studied, using fluorescence microscopy, in human and mouse oocytes. The study was focused on the distribution of filamentous actin (F-actin) in the cortex region because our previous experiments have shown that the actin cytoskeleton has a high sensitivity to mechanical deformation of the cell during micromanipulation treatment. We found several similarities in morphology between the human and the mouse actin cytoskeleton, namely, a fine meshwork composed of short, thin actin fibres organised in clusters of varying density, formation of protrusions on the cell surface, etc. The participation of actin structures in the processes of oocyte maturation, known to exist in non-mammalian organisms, was also demonstrated. The observations presented in this study are preliminary results of a detail investigation into the actin cytoskeleton response to micromanipulation treatment which is related to assisted reproduction.

K e y w o r d s

F-actin, Microfilaments, Cortical cytoskeleton, Human and mouse oocytes

A b b r e v i a t i o n s

BSA, bovine serum albumin; DAPI, 4,6-diamidino-2-phenylindol; DMEM, Dulbecco's modified Eagle's medium; FCS, foetal calf serum; FSH, follicle-stimulating hormonn; PBS, phosphate buffer saline

INTRODUCTION

The actin cytoskeleton is a highly dynamic structure that takes part in the maintenance of cell integrity and shape, in the movement of cells as well as in many other cellular processes. In human or animal cells, polymerised actin (F-actin) is found in three main types of cytoskeletal structures: (i) a fine meshwork of microfilaments distributed throughout the cytoplasm, (ii) bundles of stress fibres usually arranged in the direction of cell movement, (iii) specific structures (microspikes or microvilli on the cell surface or a contractile ring formed in the process of cell division).

The cytoskeleton, and microtubules and microfilaments especially, is known to be involved in the processes of egg maturation and fertilisation. This role has been reported for different cytoskeletal structures in various kinds of non-mammalian

and mammalian oocytes in the last decade. The detail knowledge of cytoskeleton morphology and the functions of individual cytoskeletal structures in an oocyte may be important for the development of both human assisted reproduction techniques and animal biotechnology.

The oocytes of starfish, as an example of an invertebrate organism, have been used in investigations of the actin cytoskeleton and its changes during maturation. *Heil-Chapdelaine and Otto* distinguished two populations of cortical actin: F-actin in spikes and non-spike, cortical F-actin, both regulated in time and space (*1*).

The amphibian oocyte represents one of the most frequently used model objects for the study of maturation, fertilisation and embryo development in vertebrates. In fertilisation-competent oocytes, an actin-myosin network that facilitates cortical contraction accompanying fertilisation has been described (*2*). Co-localisation of F-actin and spectrin was found in *Xenopus* oocytes (*3*) as well as in *Discoglossus pictus* oocytes and eggs. In previtellogenic oocytes, both F-actin and spectrin persisted in the nucleus until germinal vesicles broke down; in eggs, they were found in the surface dimple (*4*). In *Xenopus* oocytes and eggs, interactions between individual cytoskeletal components and internal membranes are important for organisation of the cortex (*5*) and cortical F-actin is required for anchoring and rotation of the meiotic spindle (*6*). The fact that the organisation of the cytokeratin network depends on an intact actin meshwork was demonstrated in *Xenopus* oocytes, by using inhibitors that selectively disrupted actin microfilaments and cytokeratin filaments (*7, 8*).

On the other hand, in hamster oocytes, the reorganisation of cytokeratin filaments during the maturation process has been shown to be independent of both microfilaments and microtubules (*9*). In a previous study, it was reported that, if meiotic maturation was completed in the presence of FSH, it correlated with an enhanced polymerisation of cortical actin (*10*).

It has also been suggested that the first contact of mammalian sperm with an oocyte may involve an early reorganisation of the oocyte cytoskeleton (*11*). Clusters of mouse oocyte chromosomes are redistributed around the cortex in a microfilament-dependent process after dissolution of the metaphase spindle with nocodazole; in addition, oocyte chromosome clusters and sperm chromosomes induce a focal accumulation of subcortical actin (*12*).

In porcine oocytes, F-actin is found in the cortex, while G-actin is distributed throughout the whole cytoplasm (*13*). It has also been confirmed by the investigation of maturation dynamics that the migration of cortical granules is driven by microfilaments and not by microtubules. Anchorage of cortical granules to the cortex is independent of these cytoskeletal structures. Neither microtubules nor microfilaments are involved in exocytosis of cortical granules while sperm incorporation is mediated only by microfilaments. These findings are generally similar to those made in rodents (*14*).

The first morphological study on human oocytes was concerned more with microtubules than with actin. In unfertilised oocytes at metaphase of the second meiotic division, microtubules were detected only in association with the spindle and F-actin was described only in the form of a dense filamentous layer localised in the cortex (*15*). Disruption of the meiotic spindle due to fluctuation in temperature has also been described (*16*).

Our recent experiments on human diploid cells have shown that, of all cytoskeletal structures, actin filaments are most sensitive to mechanical micromanipulation (*17*). This paper was designed to study the cortical actin cytoskeleton in human oocytes and the observations presented here are the preliminary results of a through investigation into actin cytoskeleton reactions to the micromanipulation treatment used in assisted reproduction programmes. Because human oocytes are difficult to obtain for experimental purposes, our study also included mouse oocytes in which detailed information on cytoskeletal structures is available.

MATERIAL AND METHODS

Mouse oocytes were obtained from the Laboratory of Molecular Embryology (Brno, Czech Republic); human oocytes were obtained from Laurea sanatorium (Brno, Czech Republic). Both types of cells were transported in the complete DMEM and immediately treated for cytoskeleton labelling.

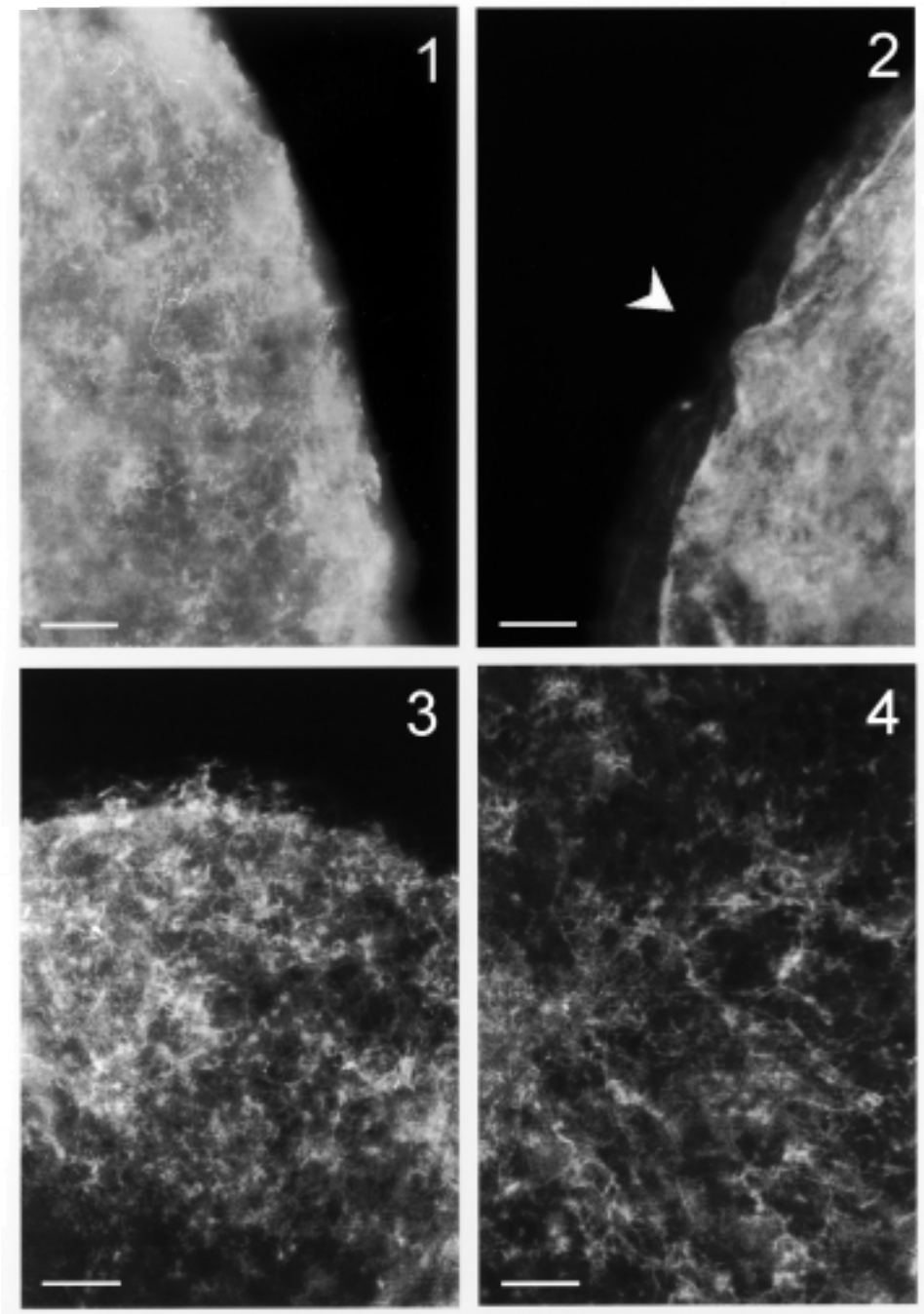
To visualize the actin cytoskeleton, both the human or mouse oocytes were rinsed in PBS (pH, 7.4) for 5 min and fixed in 3.75 % para-formaldehyde (Sigma) in PBS for 30 min at room temperature. After three changes of the buffer for 5 min each, cells were permeabilised with 1% Triton X-100 (Sigma) or Tween 20 (Sigma) in PBS for 10 min at room temperature. After permeabilisation, oocytes were washed in PBS with 1% BSA for 10 min. The actin cytoskeleton was labelled directly with phalloidin conjugated to TRITC (Sigma) for 45 min at 37°C; phalloidin bound specifically to F-actin. Finally, the cells were washed with 1% Triton X-100 (Sigma) or Tween 20 (Sigma) in PBS for 10 min and mounted onto glass slides in Vectashield mounting medium (Vector) with an addition of DAPI fluorescent dye (Sigma) specifically staining DNA molecules.

Fluorescence was observed in a Leitz Laborux epifluorescence microscope. Photographs were recorded by a Wild Leitz microphotography camera on Fujicolor Super G 400 negative film.

RESULTS

The first part of our investigation was focused on an impact of the agent used for permeabilisation on actin cytoskeleton morphology. The results showed that the use of different permeabilisation agents in the same application mode provided an identical appearance of microfilament structures in both human and mouse oocytes.

The microfilaments of human oocytes formed a very fine meshwork localised particularly in the cortical region. This meshwork consisted of a high number of short thin, actin filaments accumulated into clusters of different density (*Fig. 1*). Individual fibres either extended into remnants of the zona pellucida or, occasionally, formed cell surface protrusions called microspikes or microvilli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(*Fig. 2*). In comparison with the fine meshwork of actin filaments in human oocytes, the bundles of microfilaments in a mouse oocyte were thicker and it was possible to detect individual filaments by using the highest magnification (*Fig. 3 4*). Some larger actin aggregates were detected near germinative vesicles.

An accumulation of labelled F-actin under the plasma membrane was usually observed in association with changes in the nucleus. Actin structures were involved in the process of meiotic division I, during which aggregates of F-actin were detected near the dividing nucleus (*Fig. 5*). Also the process of first polar body extrusion was accompanied by changes in actin distribution in the cortical region. A strong fluorescent signal was seen at the anterior side of the releasing polar body; similarly, a network of actin fibres was visible at the base of the polar body (*Fig. 6*).

DISCUSSION

Our results confirmed the general knowledge of the distribution and organisation of F-actin in the cortical region of mammalian oocytes and provided a partial description of the cytoskeleton in human oocytes. Our findings of F-actin localisation and actin filament arrangement agree with those described in oocytes of other mammalian species. It was also possible to find many similarities to the actin cytoskeleton of the non-mammalian oocyte.

The accumulation of large pools of non-filamentous, „soluble“ G-actin has been described in oocytes of the amphibian *Xenopus* (*18*) as well as in porcine, i.e., mammalian, oocytes (*13*). Filamentous, polymerised F-actin is localised in the cortex where it forms a fine, dense meshwork of short actin microfilaments. The cortical actin cytoskeleton in the late stages of human oocyte maturation has a similar appearance *(15)*. The fine meshwork of the cortical actin cytoskeleton has also been found in oocytes of other animal species such as starfish (*1*), *Xenopus* (*18, 5*), mouse (*19*), hamster (*10*), pig (*13*), etc.

A local redistribution of cortical F-actin usually occurs in association with other cellular processes during maturation and fertilisation. Our findings of large

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Fig.1.:
Human oocyte. The actin meshwork in the cortical region consists from a high number of short thin actin filaments, accumulated in clusters with different density. Bar, 10 mm.

Fig. 2.:
Human oocyte. The protrusion (arrow) formed from F-actin filaments on the cell surface. Bar, 10 mm.

Figs 3., 4.:
Mouse oocytes. The microfilaments forming the cortical actin cytoskeleton are thicker in comparison with the similar structures in human oocytes. Bar, 10 mm.

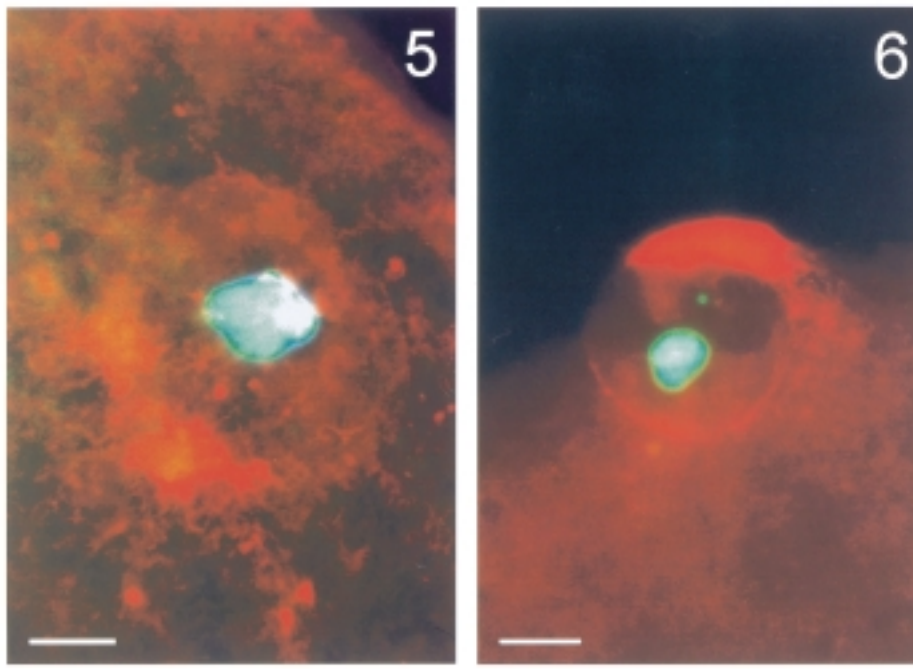

Fig. 5.:
Human oocyte. Double staining for F-actin (red) and DNA (blue). The accumulation of large actin aggregates near the cell nucleus. Bar, 10 mm.

Fig. 6.:
Human oocyte. Double staining for F-actin (red) and DNA (blue). The extrusion of the first polar body: strong fluorescent signal on the anterior side of the releasing polar body and actin fibres on the base of polar body. Bar, 10 mm.

actin aggregates near the cell nucleus and meiotic figures both in human and mouse oocytes are similar to the actin-rich domains overlaying meiotic spindles in rat oocytes arrested in metaphase II, as described by *Zernicka-Goetz et al.* (*20*). By using specific inhibitors of the cytoskeleton, it has been shown that the centrifugal migration of chromosomes in maturing mouse oocytes depends on a microfilament-mediated process (*12, 21*).

Participation of the cortical actin cytoskeleton in the process of first polar body extrusion and release observed in our study confirmed that actin filaments play a key role in morphological changes taking place on the cell surface. The accumulation of actin and non-erythroid spectrin has been described in the area of contact between the mouse oocyte and the polar body (*22*) and, similarly,

a concentrated ring of actin in the cleavage furrow between the human oocyte and the second polar body has been observed (*15*). Moreover, apart from the actin microfilaments concentrated at the base of the polar body, we found a strong fluorescent signal for F-actin at the anterior side of the releasing polar body. A similar situation has been observed in maturing oocytes of the stripe-faced dunnart, *Smithopsis macroura* in which formation of the first polar body is associated with a local cortical concentration of microfilaments that are extruded with the polar body; extrusion of the second polar body is not associated with changes in cortical actin and the body contains only little polymerised actin (*23*).

Our observations showed many similarities in actin filament organisation between human and mouse oocytes. They also suggested that the cortical actin cytoskeleton of human oocytes plays a key role in the morphology of the cell surface as well as in the processes associated with oocyte maturation. The detail knowledge of the cortical actin filament organisation will serve as a basis for our further studies on cell reactions to mechanical stress during micromanipulation treatment.

A c k n o w l e d g e m e n t s

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KORTIKÁLNÍ AKTINOVÝ CYTOSKELET LIDSKÝCH OOCYTŮ: POROVNÁNÍ S MYŠÍMI OOCYTY

S o u h r n

Organizace kortikálního aktinového cytoskeletu byla studována na modelu lidských a myších oocytů za využití fluorescenční mikroskopie. Práce byla zaměřena na sledování distribuce vláknitého aktinu, tzv. F-aktinu, v kortikální oblasti oocytu, neboť naše předchozí výsledky ukazují na vysokou citlivost aktinového cytoskeletu vůči mechanickému deformování buňky v průběhu mikromanipulačního zásahu. Práce vedla k závěru, že v oocytech lze detekovat mnoho podobností v uspořádání kortikálního aktinového cytoskeletu; zejména jde o jemnou síť krátkých tenkých aktinových vláken, která jsou uspořádána do shluků s rozdílnou densitou, vytváření protruzí na povrchu buňky atd. Rovněž byla prokázána účast aktinových struktur na jednotlivých procesech během maturace oocytu, která je již známa u jiných živočichů, ale nebyla dosud popsána na námi použitých modelových objektech. Předkládaná práce představuje úvodní studii k detailnímu výzkumu reakcí aktinového cytoskeletu na mikromanipulační zásahy během procesu asistované reprodukce.

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