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Laser Microtomography for IVF Oocyte of Human

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Abstract—For in vitro fertilization technology, the quality of oocytes has a direct impact on the egg fertilization and developmental competence of early embryo. The morphological criteria are used for the estimation of oocyte quality before its fertilization in vitro. To date, only one method is known to determine the maturity of oocyte. This is the routine observation with a light microscope. The aim of this article was to adapt the noninvasive quantitative laser scanning microtomography (QLSM) for the investigation of morphological features of a human oocyte in vitro. This approach was used to accumulate the Z-stack gallery of optical sections of IVF oocyte. The layer-by-layer acquisition allows the fine cytoplasmic structure imaging. Applying the QLSM Z-stack of optical sections, the cellular volume was calculated with quantitative 3D reconstruction of a human oocyte. The volume value and intracellular structure were used as novel criteria to assess the oocyte state after the stress evoked by cryopreservation procedure.

Keywords: human mature oocyte, quantitative laser microtomography (QLSM), in vitro fertilization technology (IVF), cryopreservation

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Mature oocytes represent a heterogeneous population of which only half is able to complete meiosis. Therefore, the efficiency of fertilization depends on the quality of the egg [1, 2]. In view of this, the diagnostic description of the structure of the female germ cell is a relevant task in IVF technology [3–7]. Currently, the set of tools that do not damage the egg is limited only to visual observation [5, 8, 9]. To characterize the full-value maturation of the oocyte, morphological signs are used: cell size, quality of the extracellular space, appearance of the polar body and perivitelline space, as well as the presence of various types of cytoplasmic inclusions. Additional data on the structure of a living human egg can be obtained or refined using quantitative laser microtomography (QLSM), a method of experimental biophysics developed for non-invasive testing of a microobject [10].

QLSM is based on layer-by-layer microtomography, which makes it possible to obtain a Z-stack galley of successive optical sections along the depth of a micron-sized object. Initially, this method of correlation microscopy was proposed for studying specimens, the preparation procedure of which causes cell death [11]. However, there are no resons that limit the possibility to study the native oocyte by QLSM. The aim of this work was to adapt this method for diagnosing a female germ cell incubated in a physiological solution. As a result, the main advantage of layer-by-layer tomography should be realized—noninvasive visualization of the internal structure of a living egg, as well as quantitative assessment of important spatial characteristics (for instance, cell volume).

The use of female germ cells for biomedical purposes is permitted by the ethics committee of the Kulakov Research Center for Obstetrics, Gynecology, and Perinatology of the Ministry of Health of the Russian Federation. Frozen donor oocytes were taken for scientific research due to the availability of such clinical material, approved by the ethical committee. The material was obtained from a cryobank of the Research Center. After thawing and washing, a single oocyte was examined using a Leica TCS SPE confocal microscope in the non-contact laser microtomography mode (red laser 635 nm, in transmitted light). The principles and technical features of the QLSM method were described in detail earlier [10, 12, 13]. An important detail is worth noting: the interval of laser action on the oocyte in obtaining a stack of slices (50 pieces) is no more than 60 s. To prevent drying of the analyzed sample, oocytes in a drop of buffer solution were placed into a microscope chamber, where an atmosphere of saturated water vapor was maintained. Figure 1 shows micrographs of a female germ cell

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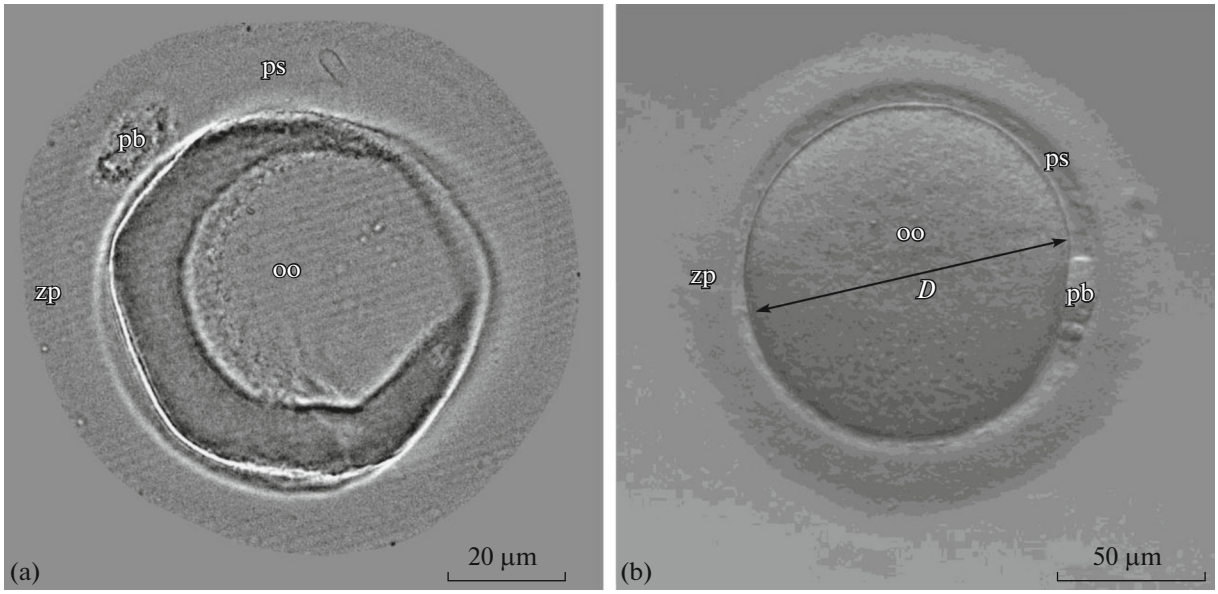

Fig. 1. Typical image of the human oocyte at the final stages of the cryopreservation protocol: (a) oocyte immediately after thawing, (b) oocyte washed from the cryoprotectant (DMSO) and ready for fertilization. Designations: zp—zona pellucida (shell), oo—ooplasm, pb—polar body, ps—space between the cell and oocyte membrane; *D*—egg effective diameter in the projection of the microscope field of view.

obtained before and after thawing and subsequent washing from a cryoprotectant (DMSO).

A thawed oocyte is a deformed irregularly shaped disc (Fig. 1a). The oocyte takes this shape as a result of the replacement of cell water with the cryoprotectant (DMSO) at the stage of preparing for freezing in liquid nitrogen. After defrosting and subsequent washing, the oocyte takes the round shape again (Fig. 1b). Unfortunately, in the field of view of a light microscope, the result of superimposing images of the internal structures of the cell throughout its entire thickness is observed, which limits the possibility of studying the fine internal morphology of the oocyte. Another disadvantage is that the microobject is visualized in one projection, which does not allow assessing the spatial shape of the studied egg. For example, on the basis on the oocyte diameter (104 μm) visible in the microscope field of view (Fig. 1b), the cell volume (~590 pL) is calculated. This value will correspond to the true volume of the cell only if the oocyte has not spread out during incubation and retained a near-spherical shape. Non-invasive laser microtomography overcomes these limitations, supplementing a number of diagnostic features with details of the internal structure at different levels of the oocyte, its shape and volume (Fig. 2).

As can be seen in Fig. 2, layer-by-layer visualization of the internal structure of a living female germ cell is realized by means of QLSM. To illustrate this mode, the micrographs of the optical sections at the "top" (Fig. 2a) and in the equatorial planes (Fig. 2b) of the oocyte are shown. In both images, an accumulation of vesicles in the cortical region of the egg can be seen. This morphological feature may reflect the cell response to replacing the cryoprotectant with water coming from the extracellular space [14]. The observed dysmorphism is possibly due to an increase in the size of the oocyte as it is washed from DMSO, which causes the accumulation of lipid vesicles, which are the building material for the plasma membrane [15]. Thus, the image on optical sections contains the main structural characteristics of a mature oocyte, which make it possible to assess the presence of dysmorphism.

Figure 2c shows an image of an oocyte after its 3D reconstruction. It can be seen that, after thawing and washing, the egg cell regained its typical rounded shape, and the volume (600 pL) of the computer model does not significantly differ from the value calculated on the basis of spherical extrapolation and the magnitude of the effective diameter of the oocyte (Fig. 1b).

The following conclusions can be drawn. In IVF technology, non-contact laser microtomography is an effective approach for diagnostic description of the female germ cell morphology. Using this method, the main advantage of layer-by-layer tomography is realized—non-invasive visualization of the internal structure of the native human oocyte. The accumulation of small vesicles in the cortical region of the washed oocyte is shown. This dysmorphism may be due to the incomplete establishment of steady state after washing off the cryoprotectant. Subsequent computer 3D reconstruction made it possible to supplement the characteristic of the oocyte with the cell volume value,

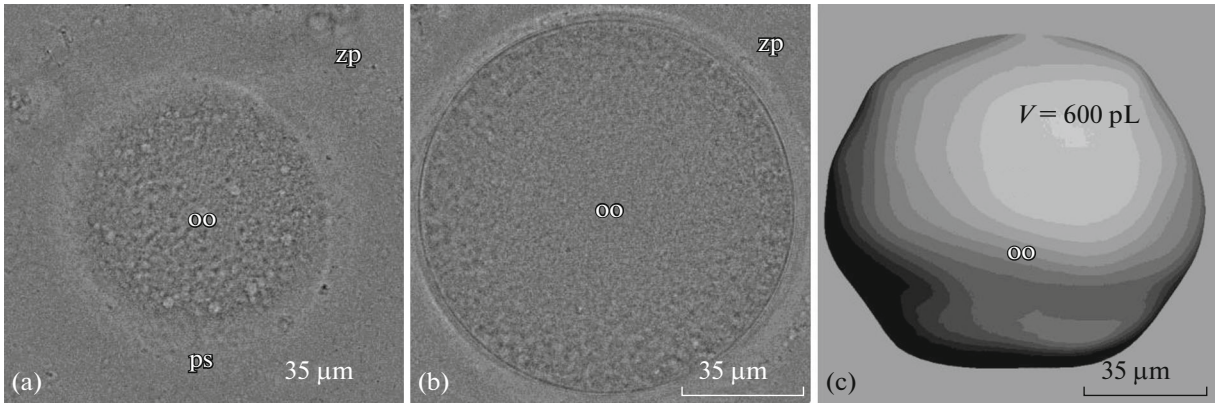

Fig. 2. Results of the non-invasive laser microtomography (QLSM) for an intact human oocyte incubated in the buffer solution: (a) the internal structure of the oocyte in the plane of the optical section no. 5, top view; (b) oocyte internal structure in the plane of the optical section no. 20, top view; (c) computer 3D model of a viable human oocyte, where *V* is the volume (pL, picoliters) of the studied oocyte. Designations: zp—zona pellucida (shell), oo—ooplasm, ps—space between oocyte and membrane.

which reflects an important functional character—the osmotic status of the egg.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. The use of female germ cells for biomedical purposes is permitted by the ethics committee of the Kulakov Research Center for Obstetrics, Gynecology, and Perinatology of the Ministry of Health of the Russian Federation.

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