Ultrastructural aspects of human immature oocytes

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Abstract

In vitro fertilization, one of assisted reproduction's technique is, for some couples, the only possibility to conceive a child. One of the main condition that must be fulfilled in order to reach this goal is the quality of human oocytes.

The ovarian stimulation protocols used have as an important goal the oocytes quality. The maturity grade of the oocytes is a good indicator of their quality. The mature oocytes are preferred for fertilization, because the immature oocytes do not fertilize. The main objective of this study is to characterize the immature oocyte, in order to explain why they do not fertilize. For this study were used human oocytes obtained from the couples who underwent infertility treatment at „Bega” University Clinic of Obstetric and Gynecology Timisoara, IVF Department. The results obtained revealed new aspects of zona pellucida’s ultrastructure. These aspects can explain why the immature oocytes do not fertilize, compared with the mature oocytes. The conclusion which can be drawn is that in order to increase the pregnancy rate for the infertile couples which undergo IVF, the stimulation has to be made in such way that the number of immature oocytes obtained to be minimum.

Keywords: in vitro fertilization, maturity grade, zona pellucida

Introduction

Even if it seems surprising, in human reproduction, naturally are more failures than successes due to natural causes and exogenous influences.

In vitro fertilization has a modest success rate at the first glance (25-30%), but it still represents a way of solving infertility for a lot of couples.

For the embryologist the main goal is to obtain high quality embryos, with high implantation potential (CHECIU & al.[1])

Starting from this premise we considered that new investigations regarding oocytes ultrastructure, may bring new data about their quality.

The observations we have made taking in account the bibliographic data available and our own data led us to investigate the ultrastructure of human oocytes. At follicular punction, the oocytes can be: immature, mature and postmature RABE & all [2]. For fertilization are used mature oocytes, because the immature ones do not fertilize.

Material and methods

The biologic material was obtained after correct information of the patients about the objective of the study and risks involved and after we obtained written consent from each couple. The study was made between 2000 and 2009, and we used 280 immature oocytes during this period of time.
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The female biologic material is represented by oocytes, harvested by follicular puncture, under ultrasound guidance.

After the follicular puncture, the oocytes were assessed using optic microscope (40X and 125X magnification) (Leica inverted microscope) to establish their maturity grade. The most important argument to assess the oocyte maturity is to be able to determine the correct timing for insemination in order to obtain the highest percentage of fertilized oocytes. Due to the fact that the human oocytes are very sensitive at temperature and pH variations (PICKERING & al. [3] the evaluation of the oocytes must be made as quickly as possible in order to keep their integrity and functions. For this classification we took in consideration the optical aspect of the complex oocyte-cumulus oophorus/corona radiata (SATHANANTHAN & al [4]. After the assessment, the oocytes elected for the study were fixed using glutaraldehyde and phosphate buffered solution. The postfixation was made with OsO₄ in 1.5M phosphate buffered solution, followed by inclusion in Westopal. The contrastation was made with uranil acetate and Pb citrate.

The sections obtained were analyzed at Babes-Bolyai University Electronic microscopy Center.

The ultrastructural details found can allow us to establish a link between ultrastructural and optical aspect of the oocytes.

Results and discussion

Using optical microscopy the immature oocyte can be identified due to cumulus and corona radiata aspect. The cumulus has an irregular aspect. The corona radiata cells are tightly packed around the oocyte, making the identification of immature oocyte easy. (Fig.1)

![Immature human oocyte (original)](image)

*Ultrastructure of immature oocyte*

The ultrastructural aspect of immature oocytes fixed immediately after follicular puncture present aspects unquoted in literature.

*Corona radiata.*

The corona radiata cells are tightly packed which can be easy seen in electronic microscopy. (Fig.2,3)
The corona radiata cells have 2 reticulat nucleoli which proves the existence of an intense protein synthesis process. (Fig. 4,5).
Zona pellucida.
Zona pellucida has in genera the outer layer rare and the inner layer denser, with dense electronoptic granules.

The characteristic feature of immature oocytes zona pellucida is that the zona has not complete the structure. From the oocytes starts „bridges”, which have the same aspect and density as the material from which is made the zona pellucida (Fig. 5,6).

The perivitelin space has a large number of microvilli which start from the oocyte. (Fig. 6,7)
Cytoplasm
Oocyte cytoplasm contain:
- mitochondria in large number, round shaped with high electronoptical density
- RER and the ribosomes are not present.
- the REN vesicles contain an amorphous material which has the same density and aspect as the material from zona pellucida, and are present in large number.
- the Golgi complex is located in the central area of the oocyte, and is present in small number.
- are present a large number of secretion vesicles which contain a material with the same density and aspect as the material from zona pellucida. At high magnification can be seen these vesicles during the exocitosis process in the perivitelin space. During this process the material synthesised in cytoplasm is released in the perivitelin space and will became part of inner layer of zona pellucida. This process has not been quoted in literature.

In periferic cytoplasm of immature oocyte we observed only a reduced number of cortical vesicles, this can be considered another aspect characteristic to immature oocyte (Fig. 8,9).

During fertilization, when the sperm’s head touches the oocyte plasmalemma, the content of cortical vesicles is exocitosed in the perivitelin space and the zona pellucida becomes impenetrable for other sperms (blocking the polysperma)
Conclusions

The oocyte is a unique cell in animal kingdom. Generally it has the same structure as any other eukariot cell, but the number and the disposition of organelles are unique for this cell.

Our findings:
- a large number of REN vesicles present in oocyte cytoplasm.
- low number of cortical vesicles in oocyte cytoplasm.
- exocytosis processes are present.

These findings pleed for an immature structure of the zona pellucida, which explain why these oocytes are unable to fertilize.

This aspect proves the importance of oocyte itself in zona pellucida building, also shows that if zona pellucida is not complete, the fertilization cannot occur.

We support the necessity of an excellent ovarian stimulation protocols use, which can provide the embryologist only mature oocytes, taking in account that in some cases (polycystic ovarian syndrome) the oocytes are all immature.

The IVF teams can follow two important therapeutic lines. The majority of teams tend to stimulate the ovary aggressively in order to obtain a large number of oocyte and a large number of embryos to be transferred.

From our data, in these cases the number of immature oocytes obtained are also large, so we do not support this kind of attitude.

From our results obtained from ultrastructural investigation we performed upon oocytes we support the use of moderate dosage of stimulative medication, which can help us to obtain few but good quality oocytes.(mature oocytes).

References