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Oocyte competence and cumulus cells energy Disfunction

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Abstract
Deterioration of oocyte quality occurring with age is the main factor limiting pregnancy possibility in women aged older than 35 years. During oocyte development by means of gap junctions, it is closely associated with cumulus cells. Metabolic state of the oocyte depends itself on their potential. Choice of cumulus cells for assessing reproductive potential of an oocyte is determined not only by their intimate functional relationship, but also by their convenience as a model: cumulus cells are extracted as a by-product in the process of oocyte collection during *in vitro* fertilization (IVF), after which they can be cultured in conditions typical for primary adherent cultures. In the process of maturation, oocyte needs constant energy support; it cannot independently synthesize the amount of ATP that it needs for normal development. Neither it can independently metabolize some other substances. Transport of ions and small molecules is carried out by means of gap junctions connecting the oocyte with cumulus cells. This relationship plays key role in normal functioning of the oocyte. Presence of such a close connection makes special demand on mitochondrial apparatus of cumulus cells. Accumulation of mutations in the mitochondrial DNA (mtDNA) of cumulus cells may serve as a cause of cumulus cells-oocyte system insufficiency, as mitochondria do not contain such effective repair systems as the nuclear genome has, so mutations and damage accumulate in mtDNA in the course of life. caused both by errors of the own replication system and by external damaging agents. Presence of basic excisional repair system in mitochondria is known fairly well, however the problem of presence of analogues of systems for repairing double-strand breaks, repairing interstrand cross-links, and repair coupled with transcription, remains open. Study of cumulus cells energy status helps to understand an important cause of female infertility, and to determine directions of its therapeutic correction. The present mini-review highlights the state of the problem in terms of quantity and of quality of mitochondria in cumulus cells.

Keywords: infertility, oocyte, cumulus cells, mitochondria, reactive oxygen species, mitochondrial networks, telomeres

Introduction

Over the past several decades, the problem of female infertility has become acutely aggravated in the societies of developed countries. Even despite the development of assisted reproductive technologies, it is still not always possible to achieve successful pregnancy. The main limiting factor is apparently the quality of oocytes, which tends to deteriorate with age [1]. Assessment of the reproductive potential of an oocyte is of great practical importance both in clinical medicine and in fundamental science, but it is fraught with a number of difficulties.

Working with animal oocytes is quite difficult, due to the increased sensitivity of oocytes to environmental factors, in particular to temperature and lighting, as well as to quite stringent requirements for their cultivation and visualization. Other limitations of working directly with oocytes are caused by complexity of their isolation and their small number, which significantly complicates obtaining statistically reliable samples. It is also important to note high price of the necessary components necessary for the cultivation of oocytes (special serum-free media, etc.) [2]. For ethical reasons, working directly with human oocytes is impossible in principle. Therefore, a different model is to be used.

Cumulus cells are a layer of follicular cells that surround an oocyte during its growth and development [3, 4]. Due to the close physiological relationship of the oocyte with cumulus cells, the study of various aspects of the state of the latter for the subsequent assessment of the oocyte's competence can be a very convenient source of information that does not require the use of oocytes themselves, which are valuable and extremely difficult for means of cell study [5].

Choice of cumulus cells as a model for assessing the reproductive potential of an oocyte is determined not only by their close functional relationship, but also by their convenience as a model: cumulus cells are extracted as a by-product in the process of oocyte collection during *in vitro* fertilization (IVF), after which they can be cultured in conditions typical for primary adherent cultures. The behavior of cumulus cells, both in isolated culture, and during co-cultivation with an oocyte is described well in the literature [4].

Metabolic interaction of the oocyte with cumulus cells occurs through gap junctions located both between the cumulus cells themselves, and at the ends of their processes penetrating the zona pellucida, and in contact with the oolemma [6]. Through

these compounds, ions and small molecules are transported. This relationship plays a key role in the normal functioning of the oocyte, due to the inability of the latter to independently metabolize some substances [5].

An especially important role in this process is played by glucose. It is unable to be either directly absorbed, due to the absence of glucose transporters [7], or to be efficiently metabolized on its own, due to the low activity of the phosphofructokinase enzyme [8]. Instead, most of the glucose is taken up by cumulus cells, where it is incorporated into appropriate biochemical pathways, after which metabolic intermediates are delivered to the oocyte [9].

The main function of glucose in the cumulus-oocyte complex is providing energy. The pyruvate formed in cumulus cells during glycolysis reactions, after entering the oocyte, is included into the tricarboxylic acid cycle, allowing the oocyte to carry out oxidative phosphorylation, with the formation of ATP, which is necessary for its further development. It is believed presently that cumulus cells directly affect the level of ATP in the oocyte. In experiments with *in vitro* oocyte maturation, it was demonstrated that oocytes surrounded by cumulus cells in this process, have a higher level of ATP than oocytes maturing alone. Blocking tight contacts between them also led to decrease in ATP content in the oocyte. It is assumed that, along these contacts, not only metabolites, but also ready-made ATP molecules are transported from cumulus cells. The oocyte, in its turn, is able to regulate the level of glucose metabolism in the surrounding cells, releasing paracrine factors, the most important of which are considered to be GDF9, BMP15, and FGF8, which increase transcriptional activity of genes encoding glycolysis enzymes [10].

Pentose phosphate pathway also plays an important role in the energetics of the maturing oocyte, although only a small part of the absorbed glucose is metabolized in this way. Formed in its oxidative phase, NADPH serves both in anabolic pathways, for example, for synthesis of fatty acids and nucleotides, and acts as one of the main reducing equivalents. In particular, it reduces the oxidized form of glutanion. Glutanion is the main intracellular antioxidant that is cyclically oxidized, protecting cells from interacting with reactive oxygen species, which is reduced by NADPH. NAPDH can also act as an antioxidant directly, being oxidized by free radical and restoring it to a harmless state. Ribose-5-phosphate, also formed in the course of the pentose phosphate pathway, is involved in the biosynthesis of nucleotides.

Another important source of ATP in the cumulus-oocyte complex is lipid beta-oxidation. Acetyl-CoA, formed during this process, can be then included in the tricarboxylic acid cycle, thus providing oxidative phosphorylation and the formation of ATP.

Despite the fact that the intracellular content of ATP varies significantly during the development of the oocyte, high level of ATP in the oocyte is most often considered to be a marker of its good quality. However there is evidence that a too high level of ATP can in some cases serve as negative sign, since excessive formation of ATP is most likely associated with increased formation of reactive oxygen species, which negatively affect general state of the cumulus-oocyte complex [10, 11, 12].

Thus energy potential of the oocyte depends on the normal functioning of bioenergetic pathways, both in itself, and in the surrounding cumulus cells. Mitochondria play key role in providing the oocyte with energy being necessary for its successful development. Mitochondria are the main source of ATP in most of the body cells, including the oocyte itself, and the surrounding cumulus cells. In addition, they are also involved in many metabolic processes, and into regulation of apoptosis [13]. It was shown that mitochondrial disfunction of cumulus cells negatively affects the oocyte [10, 12, 14, 15, 16].

Mitochondria have their own genome, represented by unmethylated circular DNA molecules. The human mitochondrial genome contains 16,569 bp and consists of 37 genes, 13 of which encode components of the mitochondrial electron transport chain. The rest of the genes of the mitochondrial genome encode transport and ribosomal RNAs necessary for the assembly of the mitochondrial translation apparatus. All the other mitochondrial proteins are encoded by the nuclear genome, translated in the cytoplasm, to further enter the mitochondria through carrier proteins. Individual mitochondria can contain multiple copies of the mytochondrial genome.

Mitochondria are maternally inherited organelles, and therefore the mitochondrial genome of each individual is normally identical in all of its cells [17]. However mitochondria seem not to contain such effective repair systems as the nuclear gene possesses, Thus mutations and damage accumulate over the course of life in mtDNA, caused both by errors in its own replication system, and by external damaging agents. Presence of basic excisional repair system in mitochondria is fairly well known, but the question of presence of analogues of double-strand break repair systems, interstrand cross-linking, and transcription-coupled repair, remains open, even despite the identification of some potential components of these systems [18].

One of the main sources of mtDNA damage is the active oxygen species (ROS). More than 95% of ROS is produced as a by-product of the electron transport chain. During the operation of Complex I, semiquinones are formed, which serve as a potential source of ROS. In Complex III, oxygen is reduced to water in a four-step process of adding electrons to it. During this process, superoxide anion is formed, which is then converted to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide, if it is not decomposed by catalase, when interacting with a metal ion in the Fenton reaction, can be converted into an extremely active hydroxyl radical. It is this free radical that causes most damage in the cell due to its high activity. In addition, when free radicals interact with a substance, formation of secondary radicals is possible, which occurs, e.g., during lipid peroxidation [19, 20].

Normally cells are able to neutralize ROS produced by endogenous antioxidant systems, such as catalase, superoxide dismutase, and glutanion peroxidase. However when a certain level of ROS is exceeded in the cell, these systems no longer cope with their neutralization, and ROS can begin to damage intracellular components, such as proteins, lipids, and DNA, due to their oxidation. This situation can occur when Complex I or III is damaged in the electron transport chain, chemical poisoning or ischemia. As a result, redox balance shift occurs in the cell, which is called oxidative stress [17, 20].

Mitochondrial DNA, due to the lack of effective repair systems, is particularly susceptible to oxidative damage. It has been shown that mitochondrial DNA is damaged by ROS much faster and stronger than genomic DNA. An important role in this process is

also played by absence of nucleosomal histone organization in mtDNA, which could protect it from the effects of free radicals, as well as by presence of metal ions in mitochondria that can catalyze the formation of secondary ROS. Oxidants also increase release of calcium ions from mitochondria into the cytoplasm, which leads to an increase in the activity of calcium-dependent enzymes, such as proteases, nucleases, and phospholipases, further disrupting normal cellular metabolism [19].

Negative physiological effect of ROS on mitochondria manifests itself not only in damage to mtDNA, which disrupts its transcriptional activity, but also in direct damage to the components of the electron transport chain, which, in its turn, can cause an even greater increase in the formation of new ROS. One of the mechanisms of damage to these and other transmembrane proteins (in particular, characteristic of the hydroxyl radical) is the oxidation of sulfhydryl groups in the composition of cysteine residues. Oxidation of cysteines enables them to form disulfide bridges, which leads to the aggregation of proteins with each other and disruption of their functions. Lipid peroxidation is accompanied by the formation of a large number of free radical intermediates. All this leads to decrease in the functional activity of mitochondria and, consequently, to a their lesser production of ATP. Ultimately, a high content of ROS in mitochondria can lead to necrotic cell death due to irreparable damage to their components, or to the activation of apoptosis systems. The mechanism of ROS signaling in apoptosis is not fully understood, but, apparently, during this process, the activity of some key regulators of apoptosis, such as the Bcl-2 family of proteins, is modified [20].

In cumulus cells, these processes are extremely undesirable. Since they are physically connected to the oocyte through gap junctions, the ROS formed in cumulus cells can also pass into the oocyte, causing damage to occur already inside it. In addition, damaged components of metabolic pathways of cumulus cells will also influence the state of the oocyte, due to its dependence on metabolites supplied by cumulus cells. Reduced ATP levels are especially critical. Finally, toxic products of necrotic degradation of cumulus cells can spread through gap junctions into the oocyte. All this negatively affects its reproductive potential [12].

There are a number of predictors that make it possible to assess both presence and level of these pathological processes in cumulus cells. Thus it was shown that number of copies of mtDNA is a good indicator of the quality of the oocyte. Different cells of the body, depending on their energy needs, have a different number of mitochondria. Mitochondrial mass is controlled during the process of biogenesis and degradation of damaged mitochondria. There is evidence that more active biogenesis of mitochondria in cumulus cells correlates with similar processes in the oocyte, contributing to its normal development. It is rather difficult to assess this process visually, due to dynamic nature of structure of mitochondria in the cell. The method for estimating the amount of mtDNA through real-time PCR is in fact easier to interpret. Large number of mtDNA copies allows positive prediction concerning the quality of the oocyte [14].

Dynamism of cell mitochondrial structure is also an interesting marker. Unlike traditional ideas concerning the spatial organization of mitochondria as separate bean-like structures, probably due to the fact how the organelles are seen on ultrathin sections in a transmission electron microscope, real mitochondria are usually a dynamic structure resembling a network. Dynamics of shape of this network is provided by processes of fusion and division of mitochondria. Fusion of mitochondria serves both to ensure interactions between them, e.g., through the exchange of mtDNA transcription products, and to smooth out the consequences of the defects that have arisen, that would hinder the work of single mitochondria. In addition, the fusion of mitochondria is able to slow down apoptotic processes in the cell. Division, on the other hand, can help isolate particularly severely damaged organelles, including for their subsequent degradation by mitophagy. It is important for normal mitochondrial segregation during cell division, also playing a definite role in apoptosis, where fragmentation of the mitochondrial network immediately precedes caspase activation and leads to release of cytochrome c. Balance between mitochondrial fission and fusion, is a finely regulated process controlled by GTPases - mitofusins, which control the fusion process, and the DRP family, which provide division [21, 22].

It is rather difficult to assess the dynamics of fixed preparations, so the information most closely corresponding to reality can be obtained in the course of a very laborious intravital cell survey. Moreover, volume of mitochondrial network means that deconvolution techniques and confocal microscopy are to be applied for means of its accurate deinition, which imposes rather serious requirements on the qualifications of the researcher. Processing and interpretation of the obtained data is also quite difficult, so there are few works related to quantitative assessment of changes in mitochondrial dynamics in cumulus cells. Nevertheless, such data can provide valuable information on the physiological state of the cell [23].

Another marker that can be analyzed on cumulus cells, which has demonstrated its ability to predict the quality of the oocyte, is their telomere length [24]. Telomeres are specialized protective structures located at the ends of chromosomes. They are complexes of arrays of tandem repeats TTAGGG and associated proteins of the shelterin complex. There are several factors that affect telomere length, including cell division rate, telomerase activity, and oxidative stress. In particular, it has been shown that the latter has a significant effect on telomere contraction. Telomeres do not have the ability to effectively restore DNA single-strand breaks formed during oxidative stress, and they accumulate a large number of such damage over time. The GGG triplet is especially sensitive to oxidation. Single-stranded breaks in telomeric DNA lead to disturbances in the mechanism of telomere replication, which in its turn leads to their shortening [25]. Molecular mechanism that determines greater reproductive potential of oocytes, whose cumulus cells have longer telomeres, is not exactly known. However, it is assumed presently that long telomeres in cumulus cells directly correlate with the level of antioxidant activit, such as creatine kinase B and peroxiredoxin 2. Short telomere length also leads to increased incidence of apoptosis in cumulus cells [24]. These data allow to regard telomere length in cumulus cells as another marker of the functional state of the oocyte.

Thus the assessment of the bioenergetic state of cumulus cells, both biochemically and visually, forms currently a promising direction in reproductive biology, which can in future form

starting point for the elaboration of new methods both of diagnostics and therapy of female infertility.

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