Mitochondria and reproductive diseases of the female tract

Carola Maria Conca Dioguardi1
Lucia De Santis 2
Joshua Johnson 3

1 Department of Gynecology and Reproductive Medicine, Humanitas Fertility Center, IRCCS Istituto Clinico Humanitas, Rozzano (MI), Italy
2 Department of Obstetrics & Gynecology, IVF Unit, Vita-Salute San Raffaele University/IRCCS San Raffaele Hospital, Milan, Italy
3 Assistant Professor, Division of Reproductive Sciences Secondary Appointment, Division of Reproductive Endocrinology and Infertility, University of Colorado, Denver, USA

Address for correspondence:
Carola Maria Conca Dioguardi
Department of Gynecology and Reproductive Medicine, Humanitas Fertility Center, IRCCS Istituto Clinico Humanitas
Via Manzoni 56
20089 Rozzano (MI), Italy
E-mail: carolaconcadioguardi@yahoo.it

Summary

Fertility has always played a crucial role in human’s life. Every oocyte requires a high amount of energy to mature and to undergo embryo development. If the organelles whose role is to produce energy do not function as they should, it is not difficult to imagine a potential dysregulation of the folliculogenesis and, therefore, of the whole process to get to a healthy pregnancy. In this review we want to emphasize the importance that mitochondria have in the fields of reproduction and fertility, focusing on how these small organelles can influence reproductive potential.

KEY WORDS: female tract diseases, mitochondria, oocyte quality, fragile X.

Introduction

In modern society, the problem of infertility is increasing, together with the will of women to postpone the time of maternity. Reproductive specialists are facing the problem of ovarian aging and dysfunction and are trying to overcome these conditions by using assisted reproductive technologies (1). New technologies are being used to better examine the mechanisms underlying ovarian factor infertility, but these are still far from understood. Below, we briefly review ovarian function and the development of oocytes within follicles, and consider how mitochondrial dysfunction can contribute to sub- and infertility.

Ovarian follicles and folliculogenesis

The ovarian follicle is a defined developmental unit consisting of a single oocyte surrounded by layers of granulosa cells (GC) and, later in development, theca cells (2). GC play an essential role in the process of follicular differentiation and development (3), leading to the optimal conditions for oocyte development and maturation (4-6) ovulation, fertilization (7), and subsequent implantation (8).

The first follicular stage is the primordial stage, which in humans occurs early in the second trimester of fetal development. Follicle growth activation starts when the oocyte grows in size and the GC transition from their flat morphology to a cuboidal, proliferative state (9). The transition from squamous to a single layer of cuboidal GC marks the transition from the primordial to the primary follicle stage (10-12). In humans, before puberty, echography shows the presence of only primary follicles: among them, the majority will disappear, undergoing a process called atresia (13). During adult life, a small fraction of follicles survive and complete development as follows (14).

Follicles that survive to develop two layers of...
GC are classified as secondary stage. If they have more than two layers of GC with no visible antrum, they are called pre-antral follicles (15); progression from the pre-antral stage to the antral stage is characterized by the formation of multiple fluid-filled (“antral”) cavities between GC. The follicle completes its development to the peri-ovulatory stage when these cavities coalesce into a single large antrum, with the oocyte held within a few layers of GC along the interior. Ovulation consists of the completion of oocyte maturation to produce a fertilization competent meiotic metaphase II egg, and the expulsion of the egg within adjacent cumulus GC into the extra-ovarian space/oviduct where fertilization may occur. Follicle development and maturation are dynamic processes that require energy; indicative of that, mitochondria, endoplasmic reticulum, and Golgi complexes in the ooplasm become more abundant and experience dynamic changes with oocyte growth (16). We now summarize our current understanding of how the energetic control of follicle development occurs, with emphasis on the mitochondria.

Mitochondria, oogenesis and oocyte quality

Mitochondria are described as “the powerhouses of the cells” because they generate most of the cell’s supply of adenosine triphosphate (ATP), which is used as a source of chemical energy (17). This process is carried out by the respiratory chain, which is composed of aggregates of proteins called mitochondrial complexes (complexes I-IV) and ATP synthetases (complex V) (18). The respiratory chain uses oxygen (O2) to convert chemical energy into ATP. In addition to supplying cellular energy, mitochondria are involved in other tasks, such as signaling, cellular differentiation, and cell death, as well as maintaining control of the cell cycle and cell growth (19).

Mitochondria are essential during the process of formation and maturation of the oocytes: at the beginning they are necessary for the establishment of the initial follicular pool (20); then, they give the energy to create a mature and competent oocyte. To that extent, during oogenesis is possible to observe an increase in the mitochondrial mass (21) which depends on the stage of maturation of the oocytes: approximately 100 copies of mtDNA were shown to be present in the primordial germ cells (22), while several hundred thousand copies where found in mature oocytes (23). For example, as soon as ovulation occurs, there is a drop in mtDNA copy number (24); this event is known as the “bottleneck theory” (25), and the reasons why it occurs are now being investigated (24).

After ovulation, if the oocyte is fertilized by a spermatozoa, they are the ones to sustain the initial development of the embryo (26, 27), since the contribution to the mitochondrial pool is only maternal (28).

Mitochondria are considered major determinants of oocyte quality. Ovarian ageing is characterized by alteration of the ovarian oocyte reserve, which results in infertility. Frequently, ovarian ageing is due to a decreased oocyte quality, and thus to mitochondrial dysfunction (29).

The relationship between ovarian ageing and mitochondria has been widely investigated: altered mitochondrial biogenesis, altered metabolic pathway and a reduced mitochondrial content in oocytes are just few of the features that have been associated with ovarian ageing (30).

Mitochondria, ROS and diseases

The electron transport chain usually is an efficient process, but a small percentage of electrons may prematurely reduce oxygen, forming reactive oxygen species (ROS) (31). In normal metabolism, low concentrations of ROS may be beneficial or even indispensable in processes such as intracellular signaling, homeostasis and defense against micro-organisms (32,33). Under environmental stress, however, ROS levels can increase dramatically (32) overwhelming protective cellular mechanisms. For example, cells can fail to inactivate ROS due to deficiencies in inactivating molecules referred to as antioxidants (34). The results are harmful effects collectively called oxidative stress. The most common consequences are DNA damage, oxidation of fatty acids and proteins, deactivation of specific enzymes, and damage to cell structures, each of which can result in cell death, often via apoptosis (35, 36).

The relationship between mitochondria and human diseases has become clearer over the past
Few years. Different studies have shown that mitochondrial dysfunction and ROS production are involved in the pathogenesis of many diseases. The link between mitochondria and neurodegenerative diseases such as multiple sclerosis (37), Alzheimer’s disease (38, 39), and other neurodegenerative diseases (40) including fragile X premutation (FXPM) (41, 42), is well known. More recently, reproductive investigators have started to ask whether reproductive tract diseases might also be related to compromised mitochondrial function (43-45).

Reproductive-tract disease and mitochondria

One example of the requirement for modulating energy use during reproduction is the formation of placenta. The differentiation of the trophoblast, and the invasion of the maternal endometrium are highly active processes; thus, it is reasonable that defects in the supply of energy or in its production can alter placental development and physiology. Within the ovary, it might predict that follicle development and the production of healthy eggs is energy-dependent. If the organelles whose role is to produce energy do not function as they should, is not difficult to imagine a potential dysregulation of folliculogenesis and the meiotic progression of oocytes. This conjecture is sustained by different groups, who for example have proposed a link between insufficient ATP availability in eggs and defective chromosomal segregation (46-48), or the reduced capacity for successful fertilization due to defective spindle formation (49). Consistent with this idea, disruption of mitochondrial oxidative phosphorylation in mouse oocytes results in reduced potential for meiotic maturation and fertilization, as well as decreased pre-implantation embryonic developmental potential (50).

Studies on mitochondrial defects and on trophoblasts impairment in placenta have been associated with the development placetas disorders, such as pre-eclampsia (PE) (51-53). A series of studies has evidenced an oxygen-sensitive accumulation and degradation, and alterations of mitochondrial proteins in the placentae of PE patients (54).

Intrauterine growth restriction (IUGR) is a multifactorial disease of pregnancy characterized by defects in placental metabolism and transport, where the fetus fails to reach its growth potential (55-57). Different studies have evidenced an alteration in the mitochondrial copy number of placentas and maternal blood in IUGR and PE pregnancies (58); since mtDNA copy number is an index of mitochondrial content (59, 60), those data suggest that a critical regulation on mitochondria is fundamental for the establishment of a healthy pregnancy (58). Other alterations of the metabolic activity of the placenta, which could lead to its damage, has been shown also in other pregnancy-related pathologies: hypertensive disorders in pregnancy (HDP), gestational diabetes, and maternal obesity. The energy and oxygen supply through placenta is one of the key elements that determine the fetal growth. An altered diet or an inappropriate intake of calories can be associated with low birth weight and, consequently, to an increased risk for developing type 2 diabetes and other metabolic diseases (61). Moreover, it is well established that metabolic diseases, such as obesity or anorexy, can also alter the mechanisms that anticipate the pregnancy, and often such women have to undergo to assisted reproduction techniques (ART).

In science, an open mind and curiosity towards different field of investigation often lead to the discovery of potential new target. Mitochondrial abnormalities and poor mitochondrial function have previously been associated to the neurological consequences of FXPM. Strikingly, FXPM is also associated with poorer ovarian function in carriers, in a condition called FX primary ovarian insufficiency (FXPOI). Female carriers of FXPM alleles under 40 years of age often exhibit fertility problems, menstrual cycle irregularities and a 20-fold increased risk of premature ovarian failure. The FXPOI condition is definitive when amenorrhea is present for 4 or more months, with two serum FSH levels (obtained at least 1 month apart) in the menopausal range (62). In addition to those with a clinical definition of FXPOI, FXPM carriers enter menopause on average 5 years earlier than their siblings without the PM. Interestingly, FXPOI does not occur in all female FX PM carriers. Instead, its incidence is approximately 28% of carriers (63). All told, FXPOI is thought to constitute 11.5% of all familial cases of ovarian failure, and 3.5% of idiopathic cases (64, 65).
Applying what was already known in the neurological phenotype of FXPM, we found that mitochondrial dysfunction occurs in granulosa cells and oocytes in a mouse model of FXPOI; the analysis of the ovarian function in a knock-in FXPM mouse model carrying 130 CGG repeats shows a defect in the large antral stage in heterozygous and homozygous mice; moreover, the histomorphometric assessment of the ovary showed a reduced number of follicle and corpora lutea. Flow cytometric analysis revealed that PM/+ and PM/PM animals lack the cumulus GC that harbor the greatest mitochondrial content as found in wild-type animals. Electron microscopic evaluation of granulosa cells of small follicles revealed anomalies in the mitochondrial structure, in particular showing disorganized and vacuolar cristae. We performed flow cytometric analysis, which revealed lack of cumulus granulosa cells that harbor the greatest mitochondrial content in both type of mutants. Finally, aberrant mitochondrial gene expression was detected. We interpreted our data as suggesting that aberrant mitochondria are involved in the pathophysiology of FXPOI (66).

**Conclusion**

In the mid-to-late 1990s, to overcome the mitochondrial dysfunction, a trial of a new fertility protocol termed ooplasmic transfer was started (67-70). Under the assumption that women selected for the trial had recurrent failure to achieve pregnancies in part due to impairment in the quality of their eggs, their next cycle of IVF included transfer of a small amount of cytoplasm extracted from young donor oocytes into their oocytes (69). Despite the first successes, the aim for the adoption of ooplasmic transfer as a clinical protocol was short-lived, also because of concerns about mitochondrial heteroplasm (68, 70). From that finding, researchers have noticed that not only the quality, but also the quantity of mitochondria can have a pathological effect (71). Now, the world is starting to understand the interaction between mitochondria and fertility, and it is trying to understand if mitochondrial involvement is primary or secondary. In other words, they are trying to determine if mitochondrial defects are causes or the consequences of the diseases.

**References**

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60. Nisoli E, Clementi E, Paolucci C, Cozzi V, Tonello C,


