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Mini Review

Energy requirements in mammalian oogenesis

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Abstract: Oogenesis is a lengthy, multi-step process occurring in mammals yielding single or multiple oocytes capable of being fertilized upon interaction with male gametes. The overall process is highly complex in nature, starting in the primordial follicles, and its ultimate completion is preceded by the meiotic cycle. There are two major phases in oogenesis: the growth phase, followed by a maturation phase that requires relatively less time. Both phases require energy for the various metabolic processes of the oocytes. The energy requirements and the timing of maturation vary significantly among mammalian species. This review describes the variations in the mammalian oocytes development and their energy requirements. It covers the types of mitochondria, the distribution of their changes, and the metabolic processes occurring during the oogenesis in different mammalian species. Oocyte abnormalities associated with glucose deficiency in mammals are discussed, along with the role of fat and protein as alternative energy substrates. The review concludes with recommendations for future studies on oogenesis in mammalian species in the context of energy requirements.

Key words: Oogenesis; Oocyte metabolism; Energy requirements; Glucose transporters.

Introduction

There are several steps involved in the development of mature female gametes. Though the overall gametogenesis process spans over weeks to months, the final phase of female gamete (oocyte) maturation is shorter, and varies among mammalian species. Studies have reported different time spans for the final oocyte maturation period, including 10-13 hours in murine (1, 2), 16-24 hours in bovine (1), and 48-72 hours in canine species (3-5). To attain complete physiological functionality, i.e. capability of being fertilized and becoming an embryo, the overall cellular organelles also need to mature. This involves various organelles in the cytoplasmic region, as well as nuclear processes. The nuclear maturation is preceded by two meiotic steps. In the first phase of meiosis, the homologous chromosomes separate; the second phase involves the movement of sister chromatids in opposite directions (6). An in-depth analysis further reveals that female gamete maturation is accomplished through changes in the nuclear membrane, rearrangement of cytoskeletal structures, and associated meiotic processes. In order to further define the maturation of organelles existing in the cytoplasm, it is important that an oocyte is able to perform various vital functions, including messenger ribonucleic acid (mRNA) synthesis and associated post-transcriptional changes, demonstrating readiness of protein synthesis as post-translational modifications cellular machinery. All of these processes are essential for a fully functional oocyte, as well as the nascent synthesis of the glutathione required for the fertilization processes and subsequent stability of the embryos (7-9). The above described nuclear and cytoplasmic maturation steps require an enormous amount of energy to accomplish the tasks described. This energy is mainly provided by the universal energy substrate glucose, and sometimes by the cellular metabolism of lipids and protein (10). A series of oxidative and reductive processes lead to the generation of reactive oxygen species (ROS), which must be properly handled. Calcium also contributes to all these processes. This review provides an extensive overview of recent advances in female gametogenesis (oogenesis) studies and the role of mitochondria, the energy-producing organelle. Overall, highly integrated metabolic and synthetic processes lead to functional oocyte ready to be fertilized and proceed with further embryogenesis.

Oocyte development in mammals

In describing the female gamete (oocyte) development, it is important to first understand the earlier developmental processes. The primordial germ cells (PGCs) region is the area in which oocyte development is initiated during embryogenesis (11, 12). Further development is associated with morphological changes in the PGCs and movement of oocytes towards the gonadal region, which ultimately becomes the ovary. In the gonadal region, the PGCs are subjected to mitosis characterized by non-cytokinesis, thus leading to interconnected germ

cell bunches (13). The overall cellular mass is covered with a layer of protective cells known as pre-granulosa and stromal mesenchymal cells of the ovary. There is a difference in the formation of oocytes among mammals; some exhibit *in utero* synthesis, for instance rats, and ruminants, including primates, whereas in certain other species, such as felines and canines, this process is completed post-birth (14).

There are also differences in the initiation steps leading to the formation of primordial follicles, which in several mammals is established during the development of the fetus, including humans, monkeys, horses, cows, and pigs. In murine species this happens in the early days of life, and activation of this process in felines, canines, rabbits, and minks occurs in the second or third week post-birth (14). Initial phases of primordial follicle development are associated with degradation of a layer covering germ cells, followed by the release of a vast number of oocytes that invade pre-granulosa cells in the germ cell mass (13, 15). The breakdown of the sheath encasing oocytes is considered a physiological measure ensuring that healthy female gametes are left over in the follicles (13, 15). Certain mutations lead to germ cells having less mitochondria, or mitochondria unable to proliferate further, which ultimately results in the loss of oocytes (13).

As the fertilization involves a single female gamete, the majority of the primordial follicles must be eliminated, either through programmed cell death processes, several undefined processes, or developing in stages from primary, secondary to antral (16-19). In its early life, an increase in the size of nascent female gamete is observed. However, subsequent folliculogenesis leads to diminished growth, and the eventual attainment of the mature size, ready to proceed to the antral stage (20-22). Oocyte growth is also associated with the development of other organelles like mitochondria, endoplasmic reticulum, and Golgi apparatus (14), preparing the cells for protein synthesis and energy production requirements. Mature oocytes in mammals such as the canine species are resume the meiotic process, but only after their release from the follicular region (23). The meiotic resumption is associated with several changes: the movement of the nucleus towards the peripheral area, and the disappearance of the nuclear membrane, including nucleoli, followed by chromosomal condensation. This stage, known as germinal vesicle breakdown (GVBD), prepares the oocyte for the metaphase I stage, as the chromosomes are already positioned at the meiotic spindle and ready. This is followed by homologous chromosomes separating into two sets. Out of these, one set is retained in the secondary oocyte, and the other in the cytoplasmic second polar body. The chromosomes in the secondary oocyte are further subjected to metaphase II, until fertilization occurs.

During fertilization, the entry of spermatozoa into the oocytes is associated with completion of necessary meiotic processes. This is followed by the extrusion of gametes from the polar body, followed by their fusion with the male gamete leading to a zygote structure. Throughout all these processes, the oocyte is linked with the granulosa cells via trans-zonal procedures (24).

Cell functions are mainly regulated by mitochondria

The mitochondria are an essential organelle in every eukaryotic cell, fulfilling the energy needs of the cells via various ongoing metabolic pathways. An important characterizing feature of mitochondria is that they contain their own genomic material, namely mitochondrial deoxyribonucleic acid (mtDNA), within a membranous structure that is primarily inherited maternally (25). Among the several functions mitochondria perform within the cell, energy generation for various cellular processes and cellular death mediating through Ca^{++} involvement has received more scholarly attention (25, 26). As each and every cell type in mammals requires energy provided by mitochondria, any deformity and/or abnormality of this organelle is linked to pathological conditions in the heart (27, 28), brain (27), and sexual reproduction (26).

Provision of energy for various biological processes through mitochondria in the form of ATP involves a series of oxidative phosphorylation steps and generation of NADH2/FADH2 within the inner membranous structures of mitochondria (26, 29). The programmed cell death pathways are also regulated by the mitochondria and these processes are tightly regulated and monitored. Cytosolic cytochrome B activates various enzymes involved in cell death pathways and the most prominent of these enzymes caspases 3, 6 and 7 (30). In the wake of continued production of free radicals within mitochondria, it is important to have a tightly controlled redox balance inside the mitochondria to protect from harmful impacts. Importantly, several biological moieties, including glutathione (GSH) and GSH-linked antioxidant enzymes such as Gpx1 and 4 are involved in regulating mitochondrial internal environments with appropriate redox balance (30). Two mitochondrial proteins also play important roles in neutralizing the deleterious impacts of free radicals; glutathione accomplishes this task by providing the electrons necessary for the generation of H_2O from the peroxides produced, and thioredoxin helps in maintaining mitochondrial integrity in an environment that is rich in reactive oxygen species (30).

From the initiation of female gamete formation there is a continued increase in the number as well as volume of mitochondria. For example, during folliculogenesis and oogenesis, an increase in the existing cell mitochondria has been reported. Importantly pre-migratory primordial germ (PGC) cells contain less than ten mitochondria (25). Upon their arrival in the ovary, the PGCs are subject to a ten-fold increase in the number of mitochondria, with an additional two-fold increase upon attaining the oocyte morphology. This figure continues to rise, and follicular oocytes have 10,000 mitochondria, reaching 0.1 million upon maturation (26). This continued mitochondrial increase is evidenced by changes in their distribution within this energy producing organelle (26). It is worth mentioning that histological changes are in the primary follicle, and the mitochondria are in the nuclear periphery, distributed all across the cytoplasm on maturation (14). A fully grown oocyte has homogeneity in the cytoplasmic allocation of mitochondria (31). The changes observed in the localization of mitochondria during meiotic maturation coincide with the cellular energy requirements (32-34) at various

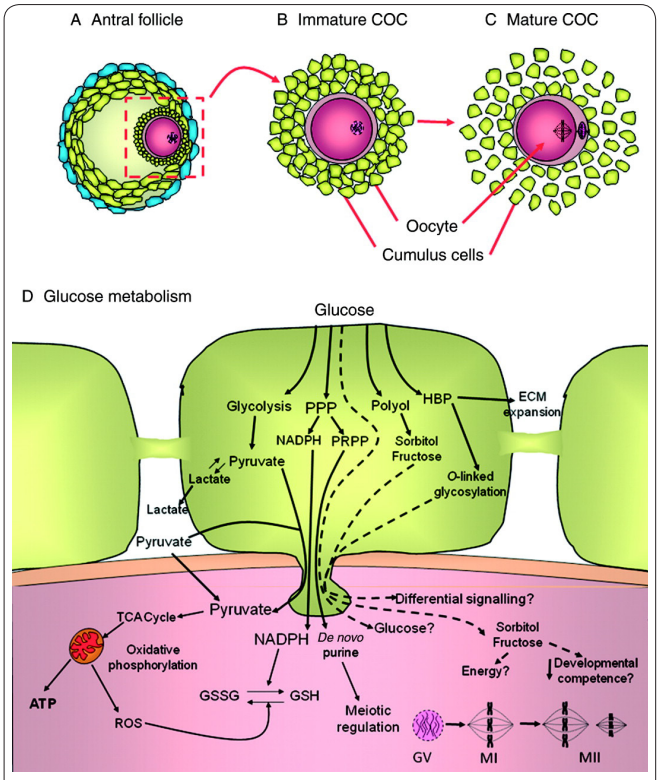

Figure 1. (A) Immature cumulus oocyte complexes (COCs; within red square) within antral follicles are characterized as having compact cumulus vestments and are arrested at prophase I (germinal vesicle stage, GV) of meiosis (B). Maturation occurs in response to gonadotrophin surges *in vivo* or release of the COC *in vitro,* and is characterized by (C) expansion of the cumulus vestment and extrusion of the first polar body (metaphase II; MII). (D) Within the COC, glucose can be metabolized via four pathways. Glycolysis results in the production of pyruvate, which can be further metabolized via the tricarboxylic acid (TCA) cycle, followed by oxidative phosphorylation for energy production (ATP). The pentose phosphate pathway (PPP) produces NADPH for the reduction of the anti-oxidant glutathione (GSSG, oxidize glutathione; GSH, reduced glutathione). Phosphoribosylpyrophosphate (PRPP) is also produced by PPP and is a substrate for *de novo* purine synthesis, important for meiotic regulation within the oocyte. Products of the polyol pathway (polyol) include fructose and sorbitol. The hexosamine biosynthetic pathway (HBP) is important for producing substrates for extracellular matrices (ECM) for cumulus expansion and *O*-linked glycosylation (cell signaling). MI, metaphase I; ROS, reactive oxygen species. Reproduced with permission from Sutton-McDowall et al. 2010 (39).

steps (32). As such, histological changes in mitochondrial redistribution should be examined from the perspective of energy requirements based on their primary functionality (26).

Mammalian oocytes are reported to have two types of mitochondria, which differ in their polarization state, with a smaller proportion of highly polarized and a higher number of less polarized mitochondria (26, 35, 36). In both the murine and human female gametes, the highly polarized mitochondria are mainly found in the peri-cortical areas of the cytoplasm. This is physiologically beneficial, as the major energy requirements during fertilization steps are primarily in the plasmalemma region; in addition, regulating the membranous transport of calcium plays a significant role during the activation of oocytes (26, 36, 37). Studies have also reported linkages between mitochondrial polarity with capacity for fertilization on the passage to embryo development (25, 35, 37, 38).

Oocyte maturation is powered mainly by glucose

In several mammals, the energy requirements during the maturation process of oocytes and their further development to become an embryo are met by glucose (39, 40). Several *in vitro* studies utilizing lower levels of glucose have reported abnormalities and delay in oocyte maturation vital processes such as meiotic processes leading to haploid gametes, and their further physiological functioning (39, 41, 42). Enhancing glucose metabolic processes through experimental manipulation in laboratory environments increases the biological efficiency of oocytes in bovines (43) and swine (44), whereas glucose deficient environments are associated with delayed meiotic processes (45). Any type of abnormality in glucose metabolic processes negatively impacts upon oogenesis, as reported in the diabetic mouse model, in the form of complications in metabolic energy generation via mitochondria and meiotic processes (46, 47).

The primary energy-providing molecule glucose is taken up by the oocytes through glucose transporter (GLUT) via facilitative mechanisms in murine (48), bovine (49), ovine (50), human (51) and simian species (52). Despite the uptake processes, it is important to consider that oocytes in certain mammals do not efficiently use glucose as an energy source (39, 45, 48, 53), as lower levels of enzymes used in glucose metabolism the phosphofructokinase have been reported (54). However, it is also important to mention here that oogenesis in mammals, and glucose utilization as an energy-providing molecule, are aided by cumulus cells possessing all the enzymes and necessary glucose-metabolizing biological efficiency, thus generating energy-rich glucose biological moieties like NADPH and readily metabolizable pyruvate (39, 55). Importantly, utilization of glucose as a primary energy source in dog gametogenesis when compared with other mammals (56) suggests that these cells possess the necessary glucose transport and metabolic machinery. This finding indicates that dog gametes may contain additional GLUT or high levels of glycolytic enzyme compared to those in other species.

As described above, there is a close association between the cumulus cells and oocytes maturation, as the energy requirements are fulfilled through the action of these important cells. It has been reported that four major metabolic pathways – glycolytic, pentose phosphate (PPP), hexosamine (HBP), and polyol pathways - operate in the cumulus cells, ready to provide energy for oocyte developmental processes. (39). Importantly, the former two aid murine nuclear and cytoplasmic maturation (57), and porcine (40, 44), bovine (40, 45, 58) and cat (59) female gamete development.

The cumulus cells and oocytes are functionally integrated into the form of a cumulus-oocyte complex (COC), which mainly utilizes glucose to produce energy in the form of ATP and necessary pyruvate. Critically, the pyruvate generated can be further used for energy generation in the oocytes through Krebs's Cycle and oxidative phosphorylative pathways in the mitochondria, which are considered the energy powerhouse of a

cell. Several studies have supported the generation of energy through glycolytic pathways for mature functional oocytes, particularly in bovine (45, 58), feline (59) and porcine oocytes (44). In particular, glucose derivative pathways for the generation of energy in cat oogenesis induced several steps, including maturation in the meiotic process and GV-MII conversion process. (59). This, further illustrates the involvement of glycolytic pathways in embryogenesis, particularly in the formation of the blastocyst.

It is important to consider that PPP generates the energy molecules ATP through indirect mechanisms, i.e. the synthesis of NADPH, which is required for the stability of cytoplasm and necessary GSH synthesis (39, 60). The PPP generates an important molecule, ribose-5-phosphate, needed for the synthetic pathways of nucleic acids (39, 40, 61). In murine oocytes, PPP usually leads to glucose metabolism and stimulates germinal vesicle breakdown, particularly in environments favorable for nucleic acid synthesis, i.e. phosphoribosyl pyrophosphate mediated elevated purines synthesis (62). The PPP continues to play a role even when the oocyte is fertilized, through generating NADPH and inducing other signaling molecules (63). Particularly in porcines, the PPP-mediated induction of meiosis and germinal vesicle breakdown, followed by MII stages, is critical to oogenesis (41, 44, 64). However, studies have also shown that controlling PPP leads to reduced glycolytic pathways and lowered levels of GSH necessary for oocyte development (44).

There are several signaling molecules involved in folliculogenesis and among these, follicle stimulating hormone (FSH) plays a significant role (16). It has been reported that FSH induces meiosis by enhancing glucose utilization in murine oocytes. Besides augmenting this vital process of female gamete development and maturation in mice, the FSH increases cellular absorption of glucose (65), which is then utilized through its metabolism via glycolytic and PPP pathways (66). Another report suggests that in cows the FSH stimulates the tricarboxylic acid cycle (67). Besides FSH, the other signaling molecule associated with maturation of haploid gametes is luteinizing hormone (LH) (16, 68, 69). An elevated level of LH separates granulosa cells from the gamete by degrading proteins and establishing a gap junction between the two, concomitantly inducing the growth of cumulus cells providing glucose-metabolizing support. The overall LH signaling processes are mediated through the G-protein-coupled receptors, and the second messenger, cAMP, affects several meiotic and maturation steps involved in ovulation (69, 70). In bovine oocyte development in particular, LH induces glucose metabolism through glycolytic and Krebs's Cycle pathways, thus generating an energy-rich environment (67).

Glucose transporters in oocyte metabolism

As glucose is a highly hydrophilic molecule, its transport across the membrane cannot occur to the necessary extent passively. Glucose is actively transported across membranes by sodium-coupled glucose transporters (SGLTs) or through glucose transporters (GLUTs) via a facilitative process. Both occur in oocytes, but the role of SGLTs has been found to be minimal (71). Humans express 14 types of GLUTs, including GLUT1-12, 14 and H+ coupled myo-inositol-transporter (72). These variants show a high degree of sequence-homology, but remarkable differences in kinetic, distribution, and substrate specificity characteristics (72).

GLUT 1, 3 and 8 are expressed in murine, sheep, rhesus monkey, human, and bovine oocytes (Table-1) (73-76). While oocytes show expression of the GLUT subtypes solute carrier family 2, facilitated glucose transporter member 1, 2 and 3 (SLC2A1, SLC2A3 and SLC2A8), an additional transporter, SLC2A4, is expressed in the cumulus cells (77, 78). SLC2A4 is an insulin-sensitive transporter and has a much higher affinity for glucose, which facilitates cumulus activity even in a low glucose concentration environment (79).

The oocytes undergo significant changes during maturation, which alter their metabolic requirements and membrane characteristics. An ideally mixed distribution of GLUTs in the oocyte ensures that glucose supply is maintained during these transformations. Abnormal environmental conditions for oocytes, like hyperglycemia, hyperinsulinemia, and insulin resistance down-regulates the expression of GLUTs, leading to compromised fertility (83).

As pyruvate is the major source of energy in oocytes, glucose consumption is lower (84). Pyruvate, produced by the cumulus cells, accumulates in the oviduct and follicular fluid (85). The glycolytic metabolism pathway is well-established even though pyruvate is the chief substrate (86). Both the glucose and pyruvate are transported by a carrier-mediated mechanism in human oocytes (86).

Proton-linked monocarboxylate (MCT) carriers transport lactate and pyruvate. Though 14 MCTs have been identified, only MCTs 1-4 are linked with lactate and pyruvate transport (87, 88). While MCTs 1-4 are confirmed in murine unfertilized oocytes, human unfertilized oocytes express MCTs 1-2. The mRNA has been identified, however, the functional distribution of the proteins in oocytes remains unconfirmed (89-91).

While the role of pyruvate in oocytes is established, the transport mechanism is less understood. However, normal glucose transport is essential for fertility, and dysfunction is associated with infertility (92). For instance, diabetic mouse produce significantly smaller oocytes, though this can be reversed by insulin administration (93). Genetic studies have showed that GLUT

Table 1. A summary of the distribution of GLUTs in unfertilized oocytes

GLUT	mRNA/Protein	Distribution	Species	Reference
1	Y/Y	Plasma membrane, cytoplasm	Murine, monkey, humans	(75, 80, 81)
3-6, 8, 12	Y/N	Not known	Monkey	(73)
7, 9, HMIT	N/Y	Cytoplasm	Murine	(82)
1-12 and HMIT	Y/Y	Not known	Murine	(82)

expression is upregulated by insulin growth factor (IGF-1) and estradiol, which indicates a likely steroidal regulation of GLUT expression in oocytes (94, 95).

Species specificity in energy preference

The existing scientific literature strongly suggests differential energy requirements for female gamete development and maturation. Murine species are mainly dependent on externally supplied energy in the form pyruvate for various nuclear and cytoplasmic organelles in the maturation of oocytes (96). This specific energy utilization from pyruvate is due to lower levels of lipids inside the cellular membranes of oocytes (97), which has been confirmed through *in vitro* studies showing that murine oocytes cannot progress to the MII stage in the absence of energy substrates like pyruvate and glucose (96). By contrast, bovine species oocytes are capable of progressing to maturation even if the external energy substrates are lacking (98). These differences between murine and bovine species arise because cow oocytes have higher contents of lipids, almost 20-fold higher that the mouse, which can be used as a potential energy source.

In terms of energy requirements in oocyte maturation, the majority of mammalian species prefer pyruvate over glucose. This has been reported for murine (55, 98, 100, 101), bovine (45) and feline species (59). The preferential usage of pyruvate as an energy substrate is potentially due to the quick generation of energy, as glycolytic steps are needed for the conversion of glucose to pyruvate, whereas pyruvate is quickly assimilated, thus supporting the overall process and viability of these delicate cells (55, 100, 101). Pyruvate energy substrate utilization is linked to various intermediary steps related to oocyte development, and a reduction in pyruvate utilization has been observed in arrested oocytes (GV or MII), suggesting a pivotal role for this energy providing molecule (100). Oogenesis in bovine species also needs pyruvate, which is provided by glycolytic pathways in the adjacent cumulus cells (54). Importantly, a higher concentration of glucose 6 phosphate dehydrogenase (G6PDH) further confirms that gametogenesis glucose utilization occurs through PPP pathways and not through glycolysis (54).

Differential use of energy-providing substrates is further confirmed through studies in swine and canines utilizing glucose for oocyte development (23, 40), although an earlier previous report suggests lipids as an energy substrate (102). Utilization of lipids in the oogenesis of swine makes more sense, due to higher lipid levels (102). The role of lipids in dog oogenesis is yet to be explored.

Summary and future perspectives

The female gamete maturation process is dependent on the energy provided by various metabolic processes. There is an urgent need to develop *in vitro* models for oocyte maturation as well as the preceding folliculogenesis, which could enhance understanding of various requirements for the overall process. As of now, studies have focused on glucose as the primary molecule providing energy for nuclear and cytoplasmic maturation. The role of other energy-rich components in oogenesis, mainly lipids, has not been explored in detail. It is important to keep in mind that various biological processes relevant to oogenesis occur in tightly controlled environments and biological molecules like fatty acids, the major components of lipids, can be utilized as a better source of energy. When comparing the production of energy in biological systems, one mole of a fatty acid like palmitic acid, a major component of several lipids, generates 130 moles of ATP, compared with 38 moles by the glucose. There is also involvement of different metabolic pathways, such as beta oxidation, in the production of energy from fatty acids. Thus, fatty acids might be a better source of energy for oogenesis when compared with glucose; however, this has never been explored.

Evidence has begun to emerge that physiological development of oocytes in mice is associated with elevated beta-oxidation and related involvement of mitochondria, despite the limited amount of lipids in these cells ((103). Similarly, the preceding folliculogenesis that demonstrates lipid utilization in these processes (104). Therefore, research is needed aimed at understanding the role of lipids in oogenesis and the biological competence of cells involved in the reproduction of life.

It is important to consider that there could be potential differences among different mammalian species in regard to oogenesis and associated metabolic processes. Future studies should be directed not only toward understanding the metabolic aspects of oocyte development, particularly in carnivorous species, but should also take into consideration inter-species differences. The establishment of *in vitro* models for such studies can provide a wealth of detailed information regarding these processes.

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