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Short Title: Metabolic Cooperation in the Ovarian Follicle

Metabolic Cooperation in the Ovarian Follicle

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Summary

Granulosa cells (GCs) are somatic cells essential for establishing and maintaining bi-directional communication with the oocytes. This connection has a profound importance for the delivery of energy substrates, structural components and ions to the maturing oocyte through gap junctions. Cumulus cells, group of closely associated GCs, surround the oocyte and can diminished the effect of harmful environmental insults. Both GCs and oocytes prefer different energy substrates in their cellular metabolism: GCs are more glycolytic, whereas oocytes rely more on oxidative phosphorylation pathway. The interconnection of these cells is emphasized by the fact that GCs supply oocytes with intermediates produced in glycolysis. The number of GCs surrounding the oocyte and their age affect the energy status of oocytes. This review summarises available studies collaboration of cellular types in the ovarian follicle from the point of view of energy metabolism, signalling and protection of toxic insults. A deeper knowledge of the underlying mechanisms is crucial for better methods to prevent and treat infertility and to improve the technology of *in vitro* fertilisation.

Key words: cumulus cells · granulosa cells · oocytes · energy metabolism · mitochondria

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List of abbreviations:

18-GA – 18α-glycyrrhetinic acid

Akt - protein kinase B

ATP - adenosine triphosphate

BA - bromopyruvic acid

BMP-6 - bone morphogenetic protein 6

BMP-15 - bone morphogenetic protein 15

BPA - bisphenol A

BPS - bisphenol S

cAMP - cyclic adenosine monophosphate

CCCP - carbonyl cyanide *m*-chlorophenyl hydrazone

CCs - cumulus granulosa cell

COCs - cumulus-oocyte complexes

ER - endoplasmic reticulum

ETC - electron transport chain

FF-MAS - follicular fluid meiosis-activating sterol

FSH - follicle stimulating hormone

GCs - granulosa cells

GDF-9 - growth-differentiation factor 9

GDF-9B - growth-differentiation factor 9B

GLUT - glucose transporter

GSH - glutathione

GV - germinal vesicle

GVBD - germinal vesicle breakdown

HA - hyaluronic acid

HAS-2 - hyaluronan synthase 2

HIF-1α - hypoxia-inducible factor 1-alpha

HClO - hypochlorus acid

IVM - in vitro maturation

LH - luteinizing hormone

MI - metaphase I stage oocyte

MII - metaphase II stage oocyte

MCT - monocarboxylic acid transporters

MGCs - mural granulosa cells

ROS - reactive oxygen species

SER - smooth endoplasmic reticulum

SIRT-1 - sirtuin (silent mating type information regulation 2 homolog) 1

TFAM - transcription factor A

uPA - urokinase-type plasminogen activator

VEGF - vascular endothelial growth factor

Introduction

Human oocytes develop in ovarian follicles surrounded by granulosa cells (GC). The coordinated development of all cell types in a follicle is crucial for the possibility of ovulating a fertile egg and therefore for normal or assisted reproduction. The details of the metabolic and signalling interplay within the follicle are therefore useful for the prevention and treatment of infertility and for the improvement of methods of assisted reproduction.

Granulosa cells create a kind of protective barrier and communication platform between the oocyte and extraovarian microenvironment. They deliver nutrients and signal molecules to the oocyte and they play an essential role during sperm penetration (Tanghe *et al.* 2002). These facts make granulosa cells attractive for research because the knowledge of their essential function in reproduction and of their metabolism can give us better view how GCs protect oocyte during maturation and how they contribute to the normal development of a human embryo. In this review we focus on the metabolic role of GCs and their participation in the oocyte developmental competence and protection.

Follicle growth and development

Ovarian primordial follicles are present at birth as a reserve of primary oocytes for the female reproductive life cycle. Oocytes in the primordial follicular stage are arrested in the prophase of the first meiotic division and they are surrounded by a single layer of squamous granulosa cells (De La Fuente and Eppig 2001). The maturation of ovarian follicles (folliculogenesis) continues by the development of primary primordial follicles into large antral secondary follicles. The follicles start as spheroidal sacs filled with the follicular fluid and containing oocytes. Oocytes in the preantral primary follicles are surrounded by a single layer of cuboidal cells called as preantral granulosa cells (Diaz *et al.* 2006). The primary follicles gradually develop into secondary follicles by forming the antrum, while at the same time the oocytes grow in size. During this process granulosa cells increase their mitotic activity. They divide into two subpopulations: (1) mural granulosa cells (MGCs) lining the

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wall of antrum and (2) cumulus granulosa cells (CCs) population surrounding the oocyte (Diaz *et al.* 2007).

In humans when a follicle reaches the size of a Graafian follicle and the oocyte grows to about 100 µm in diameter (Cavilla *et al.* 2008), ovulation is initiated after a preovulatory surge of luteinizing hormone (LH). Oocytes are arrested in the second meiotic metaphase (Madgwick and Jones 2007). The ovulation is coordinated by physiological and biochemical processes with an active participation of the cumulus granulosa cells. A few hours before ovulation the cumulus-oocyte complex initiates the synthesis and assembly of a highly viscoelastic extracellular matrix rich in proteins and proteoglycans such as hyaluronan, a non-sulfated glycosaminoglycan important for sperm penetration (Ikawa *et al.* 2010). Its main component, hyaluronic acid (HA), a large polyanionic glycosaminoglycan, is synthesized by both MGCs and CCs and is responsible for the expansion of the cumulus oophorus (Eppig 1979, Salustri *et al.* 1992, Salustri *et al.* 2004). The expanded oocyte complex is ovulated from the Graafian follicle and then fertilized by a sperm in the oviduct. Not only the mature oocyte but also the surrounding cumulus cells secrete sperm chemoattractants, to which human sperm cells are sensitive (Sun *et al.* 2005). One example is progesterone, which acts as a chemoattractant for mammalian spermatozoa at picomolar concentrations (Teves *et al.* 2006).

Oocyte maturation – role of the granulosa cells

Oocyte maturation is a short and dynamic process, which occurs with a synchronous nuclear and cytoplasmic maturation and many biochemical changes (Eppig 1996). During hormone-regulated transition from a primary into a mature secondary follicle, the oocyte has to increase its RNA synthesis and accumulation of RNAs and proteins (Picton *et al.* 1998). The first meiotic division includes the formation of the metaphase I spindle and its migration to the cortex in an actin-dependent manner with subsequent polar body extrusion (FitzHarris *et al.* 2007, Maro *et al.* 1984, Maro and Verlhac 2002). A dramatic reorganization of the endomembrane system occurs; similarly the Golgi apparatus undergoes a substantial re-organization between the germinal vesicle (GV) stage and the

metaphase II (MII) stage (Moreno *et al.* 2002, Racedo *et al.* 2012). During the oocyte maturation a reorganization of the endoplasmic reticulum (ER) occurs including a remodelling of the ER into the characteristic cortical clusters. This process is among the essential modifications in the ooplasm prior to fertilization (FitzHarris *et al.* 2007).

Cumulus granulosa cells play a crucial role in environmental support of the maturing oocytes. Virant-Klun *et al.* (2018) showed an improved maturation of human oocytes *in vitro* when co-cultured with cumulus cells isolated from mature oocytes. In growing oocytes there is a decondensed chromatin configuration (non-surrounded nucleolus, NSN), while in a fully grown oocyte before oocyte maturation the transcriptional activity winds down, the chromatin in the germinal vesicle (GV) surrounds the nucleolus (surrounded nucleolus – SN) and becomes progressively condensed (Debey *et al.* 1993, Inoue *et al.* 2008, De La Fuente *et al.* 2004). During this transcriptionally quiescent period CCs provide the oocyte with nutrients and regulatory signals molecules, CCs contribute to the promotion of nuclear and cytoplasmic oocyte maturation (Kolesarova *et al*. 2015, Sanchez and Smitz 2012). Metabolites of glucose (e.g. pyruvate), lipids, nucleotides and cholesterol provided by granulosa cells are important for the successful transition from prophase-I to germinal vesicle breakdown (GVBD), to undergo metaphase-I, and to progress through anaphase-I/telophase-I to metaphase-II (MII) (Eppig 1996, Xie *et al.* 2016). A scheme of the oocyte/follicle development is shown in Figure 1.
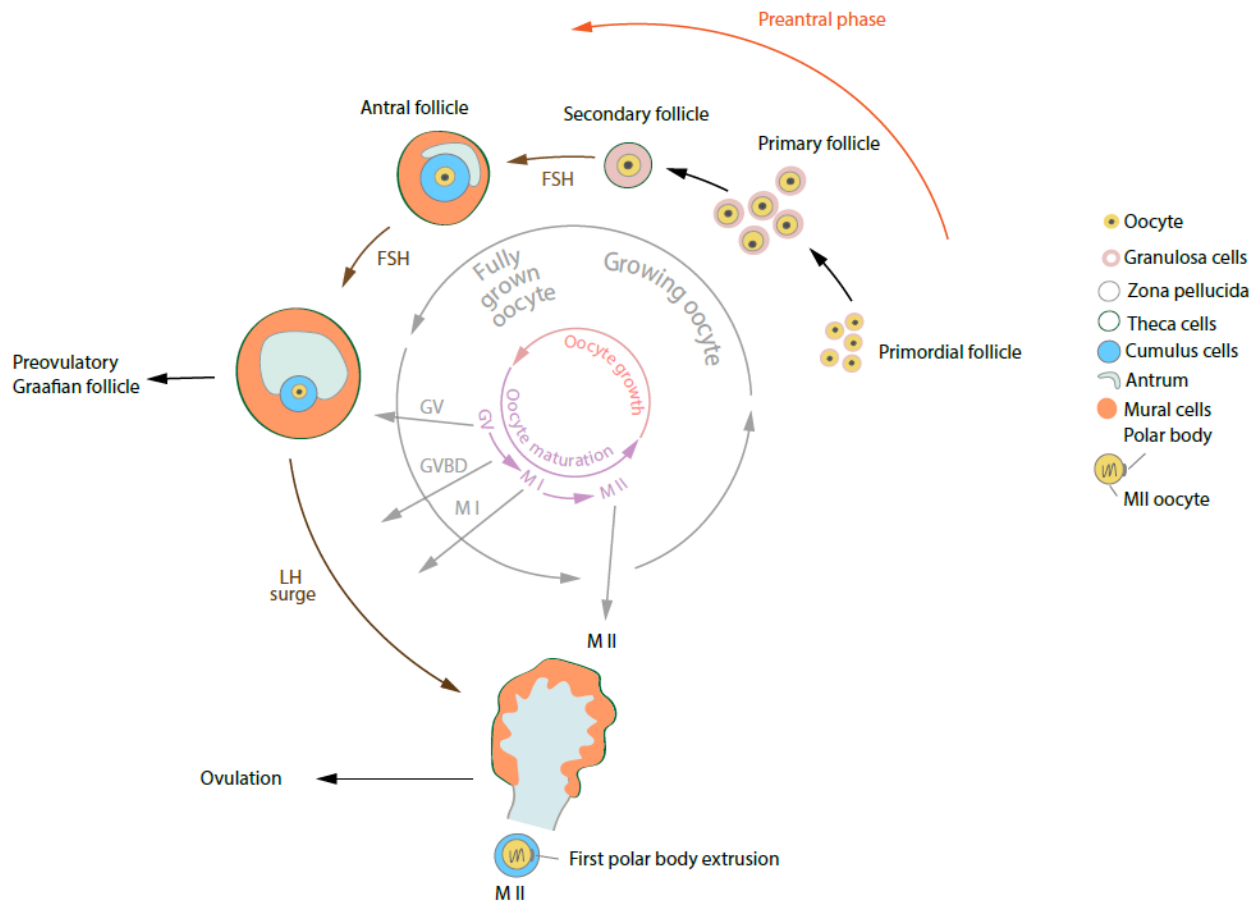
Fig. 1: Processes and signalling molecules in oocyte/follicle development.

FSH – follicle-stimulating hormone, GV - germinal vesicle, GVBD - germinal vesicle breakdown, LH - luteinizing hormone, MI - metaphase I oocyte, MII- metaphase II oocyte

There is a strong evidence that during the oocyte maturation cumulus cells secrete factors important for oocyte development to the medium (Gilchrist *et al.* 2008). Uhde *et al.* (2018) described detailed metabolomic profiles of bovine CCs and the cumulus-oocyte complex conditioned medium during a 23-hour maturation period *in vitro* (IVM). Researchers have detected 369 different biochemical components in the CCs, of which 173 biochemical components were detected in the maturation medium. Significant changes were described in both the maturation medium and CCs during maturation with most of the changes related to amino acid, saccharide and lipid metabolism. In the CCs an increase of most of amino acids (esp. glutamine, leucine and isoleucine during the first 8 h), UDP-glucose and UDP-galactose (esp. during the first 8 h), lactate and pyruvate (during the final 15 h) and sphingomyelin, glycerophosphorylcholine and N-palmitoyl-sphinganine was

described. On the contrary, a decrease of levels in CCs was detected for betaine, taurine and ophthalmate, for intermediates of pathways of saccharide metabolism (e.g. phosphoenolpyruvate and 3-phosphoglycerate) and for the majority of intermediates in fatty acid/phospholipid metabolic pathways (the lowest for acetylcarnitine, octanoylcarnitine and carnitine). In the maturation medium the most important increases were detected for creatinine (increased 427-fold after 8 h and 516-fold after 23 h), various amino acids (incl. hypotaurine and ornithine) and urea (10-fold rise), for lactate (with 667-fold increase after 8 h and 1227-fold after 23 h), mannitol/sorbitol, ribitol and pyruvate and for cholinephosphate, myo-inositol, glycerol and carnitine. A decline in the medium was detected for glucose (indicating that it was consumed by the COCs) (Uhde *et al.* 2018). In a further investigation it was found that during oocyte *in vitro* maturation a supplementation of a fresh maturation medium with carnitine (concentration 2.5, 5 or 10 mmol/l), but not with creatine (concentration 5, 10 or 20 mmol/l) improves the developmental competence of denuded oocytes (Uhde *et al.* 2018).

The communicative interconnection and metabolic cooperation between the oocyte and cumulus cells consist of interactions mediated by paracrine factors and by gap junctions through connexin 37 (Anderson and Albertini 1976, Kidder and Mhawi 2002, Gilchrist *et al.* 2008). First, there are homologous gap junctions between granulosa cells themselves. The second type of connection - heterologous junctions - is between granulosa cells and the oocyte (Larsen and Wert 1988, Downs 1995). Small molecules are moving directly through gap junctions between the cells leading to the development of a metabolic coupling (Gilula *et al.* 1972). A whole spectrum of nutrients e.g. energy substrates, nucleotides, and amino acids are taken up into the oocyte (Buccione *et al.* 1990). These gap junction channels seem also to have signalling functions. They enable the exchange of low molecular weight compounds such as cAMP through the plasma membrane between the oocyte and cumulus cells, and between cumulus cells themselves (Eppig 1991). There is a clear evidence in the literature that cAMP and other purine derivatives (e.g. guanyl compounds) play a crucial role in the maintenance of oocyte arrest in meiosis I prophase (Downs *et al.* 1988). The disassembly of gap junctions between the mural and cumulus cells results in a reduced supply of

meiosis-arresting substances to the oocyte leading to the occurrence of GVBD (Eppig 1991, Wert and Larsen 1990).

The regulatory interaction between granulosa cells and oocytes is bi-directional. Human oocytes express and secrete factors, which regulate MGCs and CCs function in vitro: namely GDF-9 (growth-differentiation factor 9) and GDF-9B (growth-differentiation factor 9B = BMP-15 - bone morphogenetic protein 15) (Dube *et al.* 1998, Aaltonen *et al.* 1999, Teixeira *et al.* 2002). These molecules activate signalling pathways important for CC differentiation (Gilchrist *et al.* 2008) and the presence of exogenous GDF-9 in media during *in vitro* maturation enhances embryo development (Yeo *et al*. 2008). Cumulus cells denuded from oocytes becomes unable to synthesize hyaluronic acid and hence fail to undergo expansion. The synthesis of hyaluronic acid by cumulus cells in response to the presence of follicle-stimulating hormone (FSH) is restored if they are co-cultured with isolated oocytes (Salustri *et al.* 1990, Buccione *et al*. 1990). A scheme of types of mutual interconnections and communication between oocytes and cumulus cells is shown in Figure 2. Figure 3 summarizes the processes related to the production of hyaluronic acid in cumulus cells.
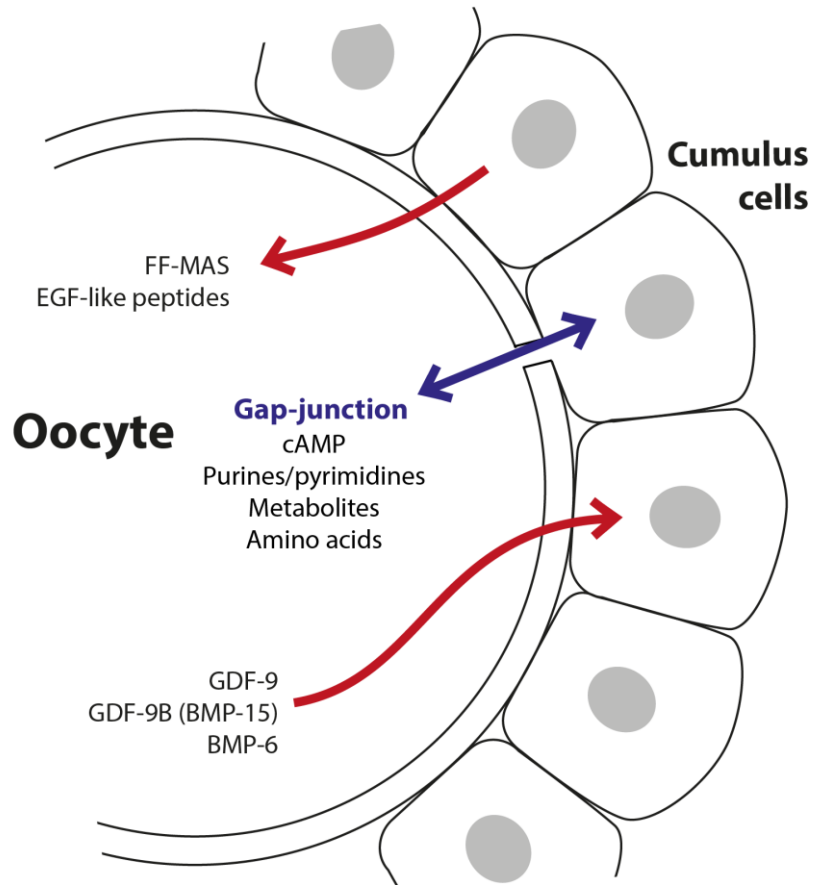
Fig. 2: Transfer of molecules between the oocyte and cumulus cells (modified from GILCHRIST *et al.* 2004).

BMP-6 - bone morphogenetic protein 6, cAMP - cyclic adenosine monophosphate, FF-MAS - follicular fluid meiosis-activating sterol, GDF-9 - growth-differentiation factor 9, GDF-9B - growth-differentiation factor 9B, BMP-15 - bone morphogenetic protein 15
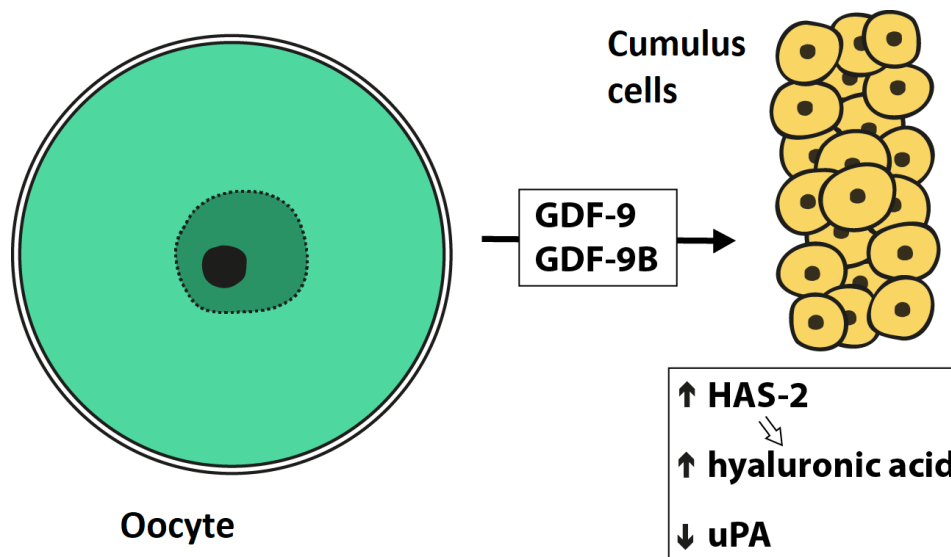
Fig. 3: Signalling processes related to the production of hyaluronic acid in cumulus cells (modified from GILCHRIST *et al.* 2004).

GDF-9 - growth-differentiation factor 9, GDF-9B - growth-differentiation factor 9B, HAS-2-hyaluronan synthase 2, uPA - urokinase-type plasminogen activator

Energy metabolism of granulosa cells and oocytes

The oocyte growth and development depend significantly on interconnections with GCs, which supply them with energy substrates via gap junctional communication (Orisaka *et al.* 2009). The two cells types show different preferences for energy substrates in order to produce ATP. GCs have been described to be more dependent on glycolysis, whereas oocytes rely more on mitochondrial oxidative phosphorylation (Kansaku *et al.* 2017a, Xie *et al.* 2016, Sutton-McDowall *et al.* 2010). It was shown that GC proliferation is dependent on an up-regulation of HIF1α-VEGF-Akt pathway and the activation of glycolysis (Shiratsuki *et al.* 2016). The importance of the mutual interconnection of these cells is emphasized by the fact that GCs supply oocytes with intermediates produced in the glycolytic pathway, e.g. pyruvate and lactate. Several studies have shown that the number of GCs surrounding the oocyte plays an important (and positive) role in its energy status (ATP levels)

(Munakata *et al.* 2016a, Sugiyama *et al.* 2016). Mitochondria are essential for the normal development and viability of oocytes. Their low number is associated with a significant negative impact (Wai *et al.* 2010, Stojkovic *et al.* 2001).

Ageing has a well described effect on mitochondrial quantity and quality (Pantos *et al.* 1999, Iwata *et al.* 2011, Bartmann *et al.* 2004). The oocyte quality decreases with maternal age and the age-associated mitochondrial deterioration is closely related to this decline in oocyte quality (Pantos *et al.* 1999, Tilly and Sinclair 2013, Kansaku *et al.* 2017b). Several studies described a decrease in the mitochondrial DNA copy number, decline in the ATP content and increased levels of reactive oxygen species (ROS) in oocytes in different animal species (Simsek-Duran *et al.* 2013, Rambags *et al.* 2014, Kansaku *et al.* 2017b). Kansaku *et al.* (2017b) described a lower level of mitochondrial biogenesis and an inadequate activation of mitochondrial biogenesis in response to uncoupler-induced mitochondrial dysfunction in oocytes of aged cows. They hypothesized that high ROS and SIRT1 (silent mating type information regulation 2 homolog 1) levels in aged bovine oocytes restrain the proper activation of the mitochondrial quality control system and might cause aged-associated decline in oocyte quality.

A proper coordination of metabolic processes is crucial for the correct pace of maturation of oocytes (Funahashi *et al.* 2008). Levels of ATP and the gene expression of key proteins of the electron transport chain (ETC) fluctuate during the maturation process in oocytes, with high levels of ATP at the GVBD stage and early MII stage (Yu *et al*. 2010, Yamamoto *et al.* 2010). Gap junctions between CCs and oocytes are open at the beginning of oocyte maturation, whereas in its later stages they close thus reducing the importance of the mutual interconnections and the delivery of substrates to oocytes (Appeltant *et al.* 2015, Ozawa *et al.* 2008). An RNA-sequencing analysis of oocytes and GCs revealed an increased expression of genes associated with glycolysis in GCs and genes associated with ETC in oocytes during the final stages of maturation (Munakata *et al*. 2016b, Kansaku *et al.* 2017a). These findings explain, why mitochondrial dysfunction affects differently the ATP content in oocytes depending on the stage of their maturation (Kansaku *et al.* 2017a).

Oxygen is the most important and essential nutrient for the cellular growth and energy metabolism and the number of granulosa cells and their metabolism determine the follicular oxygen concentration (Li *et al*. 2013, Clark *et al.* 2006). Oxygen levels in human follicular fluid increase rapidly during the early antral stages of follicle growth, then decline to low levels in the late antral and pre-ovulatory phases (Redding *et al.* 2008). Hypoxia activates glycolysis and the gene analysis revealed that HIF1-associated genes are upregulated in the granulosa cells during the normal follicle development. HIF1 is the key regulator of glycolysis and oxidative phosphorylation in granulosa cells and oocytes (Takahashi *et al.* 2016, Shiratsuki *et al.* 2016).

Metabolism of saccharides and lipids

Mitochondria of female mammalian germ cells undergo important changes during the oogenesis, maturation and fertilization. They show dynamic morphological changes as they increase in number and populate different cell domains within the oocyte. At the same time mitochondria form complex relationships with other cellular organelles (e.g. smooth endoplasmic reticulum and vesicles), according to different energetic/metabolic needs of the cell. At the end they are inherited by the developing embryo, where they assume a more typical somatic cell form. (Motta *et al.* 2000). Their main role is in providing ATP molecules through the metabolism of saccharides and lipids contained within the cell cytoplasm and in the medium (Wilding *et al.* 2001). Embryos generate a large amount of lactate during *in vitro* culture with a very low intensity of glucose oxidation, perhaps only 10 % of total glucose utilization (Wales *et al.* 1987, Thompson *et al.* 1991, Bavister and Squirrell 2000). These data might indicate that the mitochondrial metabolic activity is low during early embryo development, or may reflect a restricted metabolism of glucose via glycolysis (Wales *et al.* 1987) or a competition for mitochondrial metabolism by other oxidizable substrates (glutamine, pyruvate) provided in the culture medium (Thompson *et al.* 1991, Bavister and Squirrell 2000).

Glucose is taken up by cumulus cells via the GLUT transporters and converted to pyruvate. It can be then directly transferred into the oocyte through gap junctions, or it can also be secreted by

the cumulus cells and taken up from the surrounding medium by the oocyte (Wang *et al.* 2012, Su *et al.* 2009). Observations showed that the maturation of oocytes is possible in a glucose-containing medium but only in the presence of follicle granulosa cells producing pyruvate and/or oxaloacetate (Donahue and Stern 1968, Leese and Barton 1985, Downs and Hudson 2000). On the other hand, there are studies describing the presence of hexokinase, glucose-6-phosphate dehydrogenase, enzymes of the pentose phosphate pathway, as well as GLUTs, in oocytes suggests a possible involvement of glucose metabolism in some oocyte functions (Wang *et al.* 2012). Pyruvate transferred from CCs together with fatty acids are the most important sources of substrates for ATP production in the mitochondria of maturing oocytes (Simerman *et al.* 2015). Uhde *et al.* (2018) describe that during the maturation of cumulus-oocyte complexes (COCs) for 23h there was a significant increase of concentrations of both pyruvate and lactate in the medium, while glucose concentrations decreased. In particular the lactate concentration in the maturation medium increased 667-fold after an 8-hour maturation *in vitro* (Uhde *et al.* 2018). Oocytes are rich in members of the monocarboxylic acid transporters (MCT) family that enable the transport of lactate (Uhde *et al.* 2018, Herubel *et al.* 2002).

Lipids are important in terms of energy production during the oocyte maturation and the establishment of a developmentally competent oocyte (Uhde *et al.* 2018). The molar yield of ATP is much higher for β-oxidation than for glycolysis. Lipid droplets have been found in CCs and β-oxidation takes place predominantly in them (Dunning *et al.* 2014b, Aardema *et al.* 2013). Fatty acid oxidation (assessed by the production ^3H_2O formed via oxidation of 9,10-[^{3}H]palmitate) in CCs is increased in cumulus-oocyte complexes matured *in vivo* and conversely deficient during *in vitro* maturation (Dunning *et al.* 2014a). *In vitro*, the supplementation of maturation medium with palmitic and stearic acids resulted in a detrimental effect on oocyte maturation, whereas linolenic acid was described to have a positive effect on oocyte maturation and embryo development (Marei *et al.* 2009, Aardema *et al.* 2011, Uhde *et al.* 2018). An optimisation of oocyte maturation *in vitro* still has a way to go in order to improve reproductive technology.

Effect of inhibitors of energy metabolism

Kansaku *et al.* (2017a) studied in detail the effects of different mitochondrial inhibitors, an inhibitor of glycolysis (bromopyruvic acid - BA) and a gap-junction inhibitor (18α-glycyrrhetinic acid – 18-GA) on the ATP content in GCs and oocytes (Kansaku *et al.* 2017a). Basic types of mitochondrial inhibitors/modulators (uncoupler CCCP, complex III inhibitor antimycin A and ATP-synthase inhibitor oligomycin) have profound negative effects on the ATP production of denuded oocytes, whereas BA caused no negative effect. GCs showed an opposite result: BA reduced the ATP content, whereas mitochondrial inhibitors did not change it. COCs treated with CCCP or 18-GA showed a reduced ATP content in oocytes, where 18-GA caused only slight decrease. A higher number of GCs was able partially to mitigate the negative effect of CCCP on the ATP production in oocytes (rich vs. poor COCs), a co-treatment with 18-GA abolished the effect. These results suggest that oocytes primarily depend on ATP production via the electron transport chain, whereas GCs depend mainly on glycolysis. A mitochondrial dysfunction therefore only affects the energy status of oocytes, allowing GCs to act as an ATP provider for oocytes (via working gap junctions), even following treatment with the uncoupler CCCP. Oocytes depend on both: self-production of ATP through mitochondria and ATP provided by GCs (Kansaku *et al.* 2017a). A mutual exchange of metabolic substrates is shown in Figure 4.
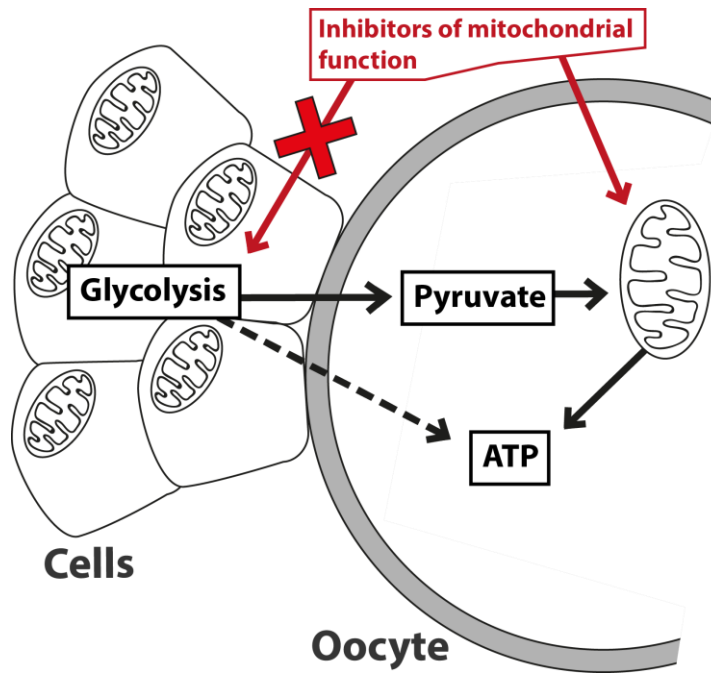
Fig. 4: Exchange of energy substrates between the oocyte and cumulus cells as described by Kansaku *et al.* 2017a.

Similar results were described by Dalton *et al.* (2014), who studied the effect of the ATP synthase inhibitor oligomycin and the inhibitor of glycolysis iodoacetic acid (inhibits glyceraldehyde 3-phosphate dehydrogenase) on ATP levels (Dalton *et al.* 2014) using a recombinant FRET-based probe. Oligomycin caused a significant decline of ATP content in oocytes, whereas iodoacetic acid caused no decrease. An inhibition of gap junction transport abolished the higher level of ATP in cumulus enclosed oocytes (Dalton *et al.* 2014). The uncoupler CCCP was shown to enhance mitochondrial biogenesis and degradation in oocytes, marked by an increased mitochondrial DNA copy number and an increased expression of mitochondrial transcription factor A (TFAM), a key regulator of mitochondrial biogenesis (Itami *et al.* 2015).

There are, however, some methodological limitations to these studies: (1) measurement of ATP content instead of ATP/ADP ratio is not an accurate parameter for the evaluation of the cellular energy state and (2) some inhibitors used in these studies are not specific enough for the processes that were assessed. Therefore, more detailed studies are necessary for a deeper understanding of the effects of the inhibition of the mitochondrial ETC and other metabolic pathways in COCs.

Reactive oxygen species and antioxidant mechanisms

The role of reactive oxygen species in the physiological and pathological processes in the human body has been well described (Dröge 2002, Valko *et al.* 2007). Together with antioxidants they play an important part in the regulation of reproductive processes and fertility in humans. The oxidant/antioxidant imbalance has been shown to be involved in the development of a number of female reproductive diseases, including the polycystic ovary syndrome, endometriosis and preeclampsia (Lu *et al.* 2018). Defects of the mitochondrial respiratory chain cause severe ATP deficiencies and increased generation of ROS (Schatten *et al.* 2014). The overproduction of ROS leads among other things to a damage of mtDNA with severe consequences on mitochondrial functions (Chappel 2013). Endometriosis is an illustrative example of a disease with a known pathogenesis involving a ROS overproduction. The follicular fluid from patients with endometriosis contains ROS and damages nuclear DNA in the oocyte (Hamdan *et al.* 2016). The disease can result in cumulus cell mitochondrial dysfunction and cause defective apoptosis and increased oxidative stress (Zhang *et al.* 2017). As a consequence, cumulus cells of women with endometriosis have a lower ATP production and a reduced ability to protect the developing oocyte with a negative impact on the fertility of the patients (Hsu *et al.* 2015). However, cumulus cells have a significant protective role against the oxidative damage caused by ROS. They show an ability to protect the oocyte against lower levels of H_2O_2 and $\cdot OH$ (Shaeib *et al.* 2016). When the concentration of H_2O_2 increases, it dramatically affects the cumulus cell number and this reduction in the antioxidant capacity makes the oocyte more susceptible to oxidative damage (Shaeib *et al.* 2016).

Cumulus cells synthesize glutathione (GSH), a key antioxidant molecule of cells (Luberda 2005). It protects cells from oxidative damage and supports the maintenance of redox homeostasis (Meister 1983, Forman *et al.* 2009). Glutathione is a tripeptide thiol found in mammalian cells, where it plays major roles in the redox metabolism, detoxification of xenobiotics and amino acid transport. It has been shown that glutathione is an important cytoplasmic factor of the oocyte. A modulation of GSH levels during the oocyte maturation and fertilization is crucial for sperm chromatin

decondensation and male pronucleus formation after sperm penetration in some animal species (Calvin *et al.* 1986, Perreault *et al*. 1988, Yoshida 1993, Sutovsky and Schatten 1997). Human spermatozoa decondense also *in vitro* in the presence of physiological concentrations of heparin and GSH (Reyes *et al.* 1989, Caglar *et al.* 2005). When the maturation of the oocyte begins there is an evidence of an increasing content of GSH in cumulus cells (Zuelke *et al.* 2003). In an *in vitro* maturation medium supplemented with a higher concentration of cysteamine as a precursor for the GSH synthesis cumulus cells have higher levels of GSH (de Matos *et al.* 1997).

However, the protection of oocytes against ROS harmful effect provided by cumulus cells has some limitations. Lower concentrations of H_2O_2 and $\cdot OH$ can be effectively detoxified, whereas the effects of higher concentrations of these or other ROS such as HClO could not be prevented (Shaeib *et al*. 2016).

Granular cells as targets of environmental toxins

The human body is exposed to a variety of environmental insults e.g. air pollution, food additives, toxins and radiation leading to genotoxicity. These factors induce a damage at different levels: damage to oocyte DNA or to surrounding granulosa cells (Winship *et al.* 2018). The follicular fluid, which is the most important environment for a proper oocyte development, is also negatively affected by these factors (Younglai *et al.* 2002). With the increasing incidence of cancer during recent years and the corresponding increase in the use of effective non-surgical cancer therapy (chemotherapy and radiotherapy) the risk ovarian failure due to these treatments becomes a highly relevant problem (Molina *et al.* 2005, Roness *et al.* 2014, Warne *et al.* 1973). Growing cells in both mature and immature follicles are the initial targets of a long-term exposure to chemotherapeutic agents (Jeruss and Wooddruff 2009). Pyknosis indicating granulosa cells death is detectable within a few hours after irradiation (Stroud *et al.* 2009).

Environmental toxins known as endocrine-disrupting compounds such as bisphenol A (BPA), a component of plastics widely found in water, air and food, decrease the viability of GCs by inducing their apoptosis and a G2-to-M arrest (Xu *et al.* 2002). Similarly, bisphenol S (BPS) causes a failure

of microtubule formation and disrupts cumulus cells expansion (Zalmanova *et al.* 2017). The impact of endocrine disruptors on newborn steroidogenesis was shown in a study of Kolatorova *et al.* (2018), which confirmed accumulation of phthalate in the fetal area via measurements in maternal plasma and in the cord blood of their newborns. These facts taken together have a significant impact on quality of oocytes and success of human reproduction. Further studies should be made to elucidate the mechanism of action of these compounds and their effect on the collaborative follicular niche.

Conclusions

Follicle growth and the development and maturation of oocytes are highly dependent on and regulated by the metabolic and signalling coupling between oocytes and granulosa cells. The bi-directional communication is mediated by a wide spectrum of signalling molecules regulating the processes in the partner cells. This coordinated interconnection is crucial for the delivery of energy substrates and other important components. Both GCs and oocytes prefer different energy substrates in covering their needs: GCs are more glycolytic, whereas oocytes depend more on the oxidative phosphorylation pathway. At the same time, oocytes are supplied with intermediates produced in glycolysis by GCs. Environmental insults, genetic predisposition and some therapeutic methods can interrupt these processes leading to changes of the cellular metabolic and signalling profiles leading to a decreased quality of oocytes, development of diseases of the female reproductive tract and to lower fertility. Further investigations are necessary for a deeper understanding of these harmful mechanism in order to develop treatments to limit their effects and improve methods of *in vitro* fertilization.

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