Gene expression of granulosa and cumulus cells: The prospect in predicting the quality and developmental competence of oocytes in vitro maturation

BICHUN ZHAO¹; XUEQING WU²,*; YITONG YUAN¹; YUANTAO GAO¹; XIAO LI¹; ROCHEN DU¹; SUMING XU²; RUOXIN ZHANG¹; CHUNFANG WANG¹,*

¹ Laboratory Animal Center, Shanxi Medical University, Taiyuan, 030000, China
² Center of Reproductive Medicine, Children’s Hospital of Shanxi and Women Health Center of Shanxi, Taiyuan, 030000, China

Key words: Assisted reproductive technology, Development, In vitro fertilization

Abstract: In vitro maturation (IVM), a promising assisted reproductive technology (ART), has been evolving in clinical trials and applications. There is a huge potential demand for IVM in clinical practice because it reduces the stimulation of gonadotropins to patients and provides evidence for the safety of neonatal birth. Unfortunately, the maturation rate of oocytes in vitro is not as high as it is in vivo due to a different microenvironment. Moreover, there are still controversies in predicting the developmental capability of oocytes in IVM. The granulosa cells (GCs) and cumulus cells (CCs), closely surrounding the oocytes, play a critical role in oocytes development, while some studies have shown that they can reflect the quality of oocytes. Many studies have been conducted in terms of oocyte quality in transcriptional level in GCs and CCs of mice, Xenopus africanus, and Homo sapiens, which provides important enlightenment for the successful clinical application of IVM. However, no comprehensive reviews about how gene expression profiles affect oocytes quality have been reported. This review aimed to elucidate the gene expression profiles of GCs and CCs that have effects on the quality and developmental competence of oocytes maturation in vitro. And we also put forward a possible idea for ART in the future, integrating all gene expression profiles of GCs and CCs and predicting the quality of the oocytes.

Introduction

The developmental competence of oocytes refers to the ability of the oocyte to mature and then successfully fertilize, cleave, and enter the blastocyst stage to produce high-quality embryos. This sequence of events is continuous and precisely controlled in vitro, and the success of one does not guarantee the success of the next, making it difficult to cultivate in vitro. Experiments on cows have shown that 60% of oocytes in vitro reach the blastocyst stage, while less than 40% in vitro maturation (IVM) of oocytes reach the blastocyst stage (Marei et al., 2014). The success rate of IVM for human oocyte is lower. Oocyte IVM refers to the whole process in which immature oocytes (germinal vesicle, GV stage) recover from small-sized follicles, mature, fertilize, and develop into embryo under the laboratory-culture conditions. The clinical definition of IVM of oocyte has been proposed in recent years, that is, oocyte is extracted from small or medium-sized follicles for culture before the average diameter of the largest ovarian follicle reaches 13 mm (Dahan et al., 2016). IVM of the human oocytes has had profound clinical implications since the first live birth after the IVM procedure was reported in 1991 (Cha et al., 1991). However, the success rate of IVM of human oocytes is not as high as it is in animals. Additionally, selecting embryos with high transplantation potential is the crux of the IVM. Traditional selection methods are based on morphological assessment, which includes growth rate, developmental diameter, early cleavage, and degree of division (Ebner et al., 2003). Obviously, these assessments are invasive and imprecise. In recent years, quantitative methods such as time-lapse imaging of embryonic cell division dynamics and genetic screening of blastocysts have been developed. However, further studies are needed to evaluate the predictive value of other markers for human oocyte quality. Since a crucial role of granulosa cells (GCs) and cumulus cells (CCs) have on the development of

*Address correspondence to: Chunfang Wang, wangchunfang@sxmu.edu.cn; Xueqing Wu, xueqingwu416@126.com
Received: 21 May 2020; Accepted: 12 August 2020

Doi: 10.32604/biocell.2020.011638 www.techscience.com/journal/biocell

This work is licensed under a Creative Commons Attribution 4.0 International License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Oocytes in vitro, many researchers concentrate on these clusters of cells and apply them to IVM. Furthermore, there is a growing awareness that the gene expression profile of GCs and CCs are extremely critical in predicting the quality of oocytes in vitro maturation (Tabibnejad et al., 2019).

Oocytes arrested at metaphase-I stage are surrounded by GCs during follicular development and formation. The CCs involved in oocyte proliferation and development originate from GCs, which differentiate into mural granulosa cells (MGCs) and CCs during the development of antral follicles. One of the major functions of CCs is transmitting nutrients and metabolites to the oocytes to maintain the various stages of oocyte growth and development (Lonergan and Fair, 2016). Gene expression analysis of GCs and CCs as predictors of oocyte quality and developmental potential has received more and more attention in recent years. Brown et al. (2017) proposed that the matured CCs in vitro exhibited differential gene expression patterns compared with in vitro derived cumulus-oocyte complexes (COCs). This might be the primary reason for partial function defects of CCs in IVM, resulting in reduced oocyte quality. Previous articles explored the oocyte capacity by investigating the gene expression of CCs. This paper advanced a possible idea for assisted reproductive technology, updating all gene expression profiles of GCs and CCs that may be used to better predict oocyte quality. Combining one or more of these factors into a usable culture medium may increase the success rate of human clinical IVM therapy. Immediately afterward, IVM therapy was combined with standard IVF to promote embryo maturation and increase the live birth rate. Here, we attempted to expound the gene expression profiles of GCs and CCs at IVM system from a new perspective, to explore the most favorable genes for oocyte maturation, which could provide new ideas for solving clinical difficulties.

Cellular growth and proliferation

Just before ovulation, human oocytes will grow to approximately 100 µm in diameter (Mehlmann, 2005). The first thing that happens when a woman enters puberty is a surge in luteinizing hormone (LH), which affects oocyte meiosis and cytoplasmic maturation, leading to ovulation (Pan and Li, 2019). In the process of growth and proliferation, oocytes acquire meiotic ability (nuclear maturation) first and then developmental ability (cytoplasmatic maturation), both of which depend on the surrounding CCs (Tanghe et al., 2002). Initially, the transcripts of gremlin1 (GREM1), hyaluronic acid synthase 2 (HAS2), and cyclooxygenase 2 (COX2/PTGS2) in CCs were confirmed to be related to the growth and development of oocyte (Dunning et al., 2015). HAS2 is responsible for the formation of extracellular matrix (ECM) during ovulation. Meanwhile, HAS2 is specifically expressed by CCs under the joint action of growth differentiation factor-9 (GDF-9), and oocytes secreted follicle-stimulating hormone (FSH) (Dragovic et al., 2005), suggesting that its gene expression is associated with oocytes quality. Subsequently, the expression of various target genes in human CCs was studied (Feuerstein et al., 2007), such as Steroidogenic Acute Regulatory protein (STAR), COX2 or PTGS2, Amphiregulin (AREG), Stearoyl-Coenzyme A Desaturase 1 and 5 (SCD1 and SCD5). It has been observed that the nuclear maturation of oocytes accompanies the active gene expressions mentioned above. Then, it was reported that Tumor Necrosis Factor α-induced protein 6 (TNFAIP6), Pentraxin-3 (PTX3), and several EGF-like growth factors (epiregulin, amphiregulin, and betacellulin) were inhibited in vitro, which did not occur in mature oocytes in vivo (Ouandaogo et al., 2012). This might explain the low IVM rate of oocytes. More and more evidence showed that AREG likely played an important role in oocyte growth (Brown et al., 2017). AREG protein was revealed to be more abundant than other EGF-like factors in mature human follicular fluid (Peluffo et al., 2012; Zamah et al., 2010). Maternal Antigen That Embryos Require (MATER) in human CCs also has been reported that can affect follicular and oocyte growth (Sena et al., 2009). The expression of Versican in human CCs has been detected to be related to successful ART outcomes (Ekart et al., 2013; Gebhardt et al., 2011; Hammond et al., 2015; Watle et al., 2011). The ability of Versican to activate the EGF receptor (EGFR) in CCs and to stimulate COCs maturation was also found recently (Dunning et al., 2007; Dunning et al., 2015). All of these indicated that the Versican gene in the CCs has a role in promoting oocyte growth, proliferation, and maturation.

In the process of follicular development and formation, oocytes are initially surrounded by GCs, subsequently some of which form CCs. GCs discarded at IVF provide a good tool for studying follicle microenvironment. Changes in gene expression patterns of GCs can reflect the stress response of cells to the follicle microenvironment at a specific moment and further trace the relevant information of oocyte development. Some studies indicated that granulosa derived factors may activate COCs maturation and promote the growth and proliferation of oocytes under the induction of CCs (Huang et al., 2015; Shimada et al., 2006), so their low expression in IVM may be another key factor leading to poor quality results. Neuregulin 1 (NRG1) in GCs has been shown to inhibit the growth and proliferation of oocytes, indicating that it can be acted as an important granulosa derived factor (Kawashima et al., 2014). Since NRG1 is not a product of the cumulus gene, no NRG1 was found in the differential expression gene of IVM COCs, but the deficiency of NRG1 was caused by the lack of GCs, which may be the reason for the abnormal proliferation of IVM oocytes (Kawashima et al., 2014). In recent years, it has been found that the activation of EGFR in CCs contributes to the stimulation of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (PKB, AKT) signaling pathway in oocytes. This pathway governs oocyte growth, survival, and proliferation by maintaining genomic integrity (Chen et al., 2013; Franciosi et al., 2016; Maitardi et al., 2020). The maturation of the oocyte is finally triggered by the maturation promoting factor (MPF) (Gerhart et al., 1984), whose activity is regulated by mitogen-activated protein kinase (MAPK).

Cell cycle

One indispensable step that occurs before ovulation is the restoration of meiosis I (MI), the completion of the cell cycle. We attempt to illuminate the effect of differential gene
expression in CCs on it. Meiosis begins between 11 and 12 weeks of gestation in the ovary of a human embryo (Gondos et al., 1986). There are approximately 2 million primary oocytes in the two ovaries of newborn babies, all of which enter the MI without developmental abilities at this stage. Under the influence of pituitary gonadotropin, FSH and LH, these primary oocytes further develop and ovulate during sexual maturation. In most cases, oocytes maturation takes about one-month, during which CCs gene expression remains active (Hunt and Hassold, 2008; Tatone et al., 2008). Metaphase of meiosis II (MII) recovers after oocytes are fertilized by sperm. However, meiosis is spontaneously resumed when oocytes are removed from the follicular environment (Pincus and Enzmann, 1935). Therefore, the cell cycle of oocytes in IVM is totally different from that in vivo. Another study confirmed that cyclic adenosine monophosphate (cAMP) generated by CCs might be a pivotal factor controlling this process, and negatively regulates the germinal vesicles breakdown (GVBD) by activating cAMP-dependent protein kinase (PKA) (Eppig and Downs, 1984). Low cAMP concentration during IVM improves oocyte quality and subsequent embryonic development (Shu et al., 2008).

It has been revealed that CCs of IVM are higher mitotic than CCs in vivo, whose specific gene expression had different effects on the oocytes cycle (Ouandaogo et al., 2011). With the development and application of biotechnology, microarray and real-time PCR have been widely used in the field of gene research (van Montfoort et al., 2008). It was found that the expression of some genes can negatively regulate the maturation of oocytes, including cyclin D2 (CCND2), tripartite motif-containing 28 (TRIM28), 7 dehydrocholesterol reductase (DHCR7), catenin, cadherin associated protein, delta 1 (CTNND1). To further determine which genes are necessary for maintaining the normal cell cycle of the oocyte in CCs, Hamel et al. (2008) reported a set of oocyte markers. Finally, cell division cycle 42 (CDC42) was identified (Hamel et al., 2008). Activation of CDC42 results in the polarity establishment and meiotic division of oocytes (Zhang et al., 2017). Subsequently, a lot of genes are detected with an up-regulation in IVM oocyte by microarray technique, which includes cyclinV2 (CCNV2), cyclinB1 (CCNB1), cyclinE2 (CCNE2), and other cyclin and cyclin-dependent kinase (CDK) genes (Ouandaogo et al., 2012). Specifically, PKA is reduced right after the cAMP decrease in oocytes in vitro, resulting in the dephosphorylation of CDC2. CDK1 and CCNB are also activated subsequently, and then MI is completed.

In addition, some important genes were found to be involved in the cell cycle of human oocytes and CCs, including breast cancer 1 & 2 (BRCA1 & 2), breast cancer1-associated RING domain1 (BARD1), retinoblastoma-like 2 (RBL2), retinoblastoma binding protein 7 (RBBP7), budding uninhibited by benzimidazoles 3 (BUB3) and spindle checkpoint protein (BUB1B), mitotic arrest deficient protein2 (MAD2) (Gasca et al., 2007).

**Extracellular matrix**

With a surge in LH, ovulation is not the only event. The ECM, which is activated in COCs, also known as cumulus expansion (or mucification), relies on a cascade of specific signals within the cell to induce expression of related genes (Russell and Salustri, 2006). Successful expression of ECM has a positive effect on oocytes maturation rate, especially in IVM. Proper composition and assembly of the ECM are also crucial for the developmental competence of oocytes. Its active components come from a variety of sources, such as direct synthesis by CCs under the control of endocrine and oocyte-derived factors, or entry into follicles through plasma (Chang et al., 2002; Eppig, 1982; Eppig, 1991; Vanderhyden et al., 2003). The ECM mainly consists of hyaluronic acid (HA), PTX3, TNFAIP6, and the heavy chains (HCs) of serum-derived inter-a-inhibitor proteins. The expansion of the cumulus is accomplished by the synthesis of HA and the assembly of actin microfilaments to induce the comprehensive rearrangement of the cytoskeleton so that tightly packed CCs are transformed into a greater mass of mucous cells (Kidder and Vanderhyden, 2010). Ovulation is the extrusion of one or two of the most optimal oocytes from the follicular fluid when cumulus expansion and ECM formation are of the essence in this condition. This expanded process would be completed by two methods, such as oocyte secretion of paracrine factor and gonadotropin or EGF-like peptide stimulation (Fig. 1).

As an oocyte secretion factor, GDF-9 is extremely significant in the formation of ECM (Elvin et al., 1999a; Sutton et al., 2003). This secretory factor works associated...
with its receptor Bone Morphogenetic Protein Receptor Type 2 (BMPR2), which is highly expressed in CCs (Assou et al., 2006). It induces a cascade of reactions involving downstream genes (Sanfins et al., 2018). This process initiates the secretion of ECM substances, such as HA, TNFAIP6, and PTX3 (Hussein et al., 2006). Therefore, we concluded that the upregulation of the GDF9 receptor in CCs is related to quality, especially during in vitro maturation. In addition, the role of granulosa-derived Versican has been revealed in the cumulus expansion (Dunning et al., 2015). A surge of LH induced a rapid response of Versican in GCs, which was transmitted to CCs. At the same time, EGF-like factors were found to promote oocyte growth and maturation by inducing the expression of COX-2. The most critical role of EGF-like factors is to promote the cumulus expansion. Reported EGF-like factors include EREG, AREG, BTC (Liu et al., 2010; Park et al., 2004), HAS2, and TNFAIP6 (Fülöp et al., 2003; Park et al., 2004). These factors work via up-regulation of prostaglandin E2 (PGE2) receptor in the CCs, this process is essential for ECM formation and oocytes maturation (Ben-Ami et al., 2006; Liu et al., 2010). Moreover, these factors also play a direct role in cumulus expansion through PGE2 (Niringiyumukiza et al., 2018). EGF-L factors and CCs respond to LH-surge and gonadotropin /FSH signals, respectively, resulting in increased oocyte developmental competence (Diaz et al., 2006; Dragovic et al., 2005; Dragovic et al., 2007). Although the mechanisms of LH and FSH remain unknown, both tyrosine kinases or cAMP-dependent tyrosine kinases activated MAPK and extracellular regulated protein kinases (ERK) in CCs have been reported (Su et al., 2002). EGFR, FSH, or cAMP-induced cumulus amplification and ECM gene expression were restrained by Erks inhibitors (Ochsner et al., 2003; Su et al., 2002). Cumulus expansion and oocytes maturation could be inhibited by loss of function of conditional erk1/2. ECM formation is consequently inhibited, further supporting the physiological relevance of these factors. Progesterone induced TNFα-converting enzyme/A disintegrin and metalloproteinase domain 17 (TACE/ADAM17) can produce EGF domain in CCs, thus enhancing the functional changes of the CCs (Yamashita et al., 2016; Yamashita et al., 2014). In addition, a key enzyme in the process of ECM formation is PTX3 produced by human GCs, and PTX3 is required for the production of oocytes with high development potential and quality (Huang et al., 2013). Research indicated that TGF-β1 regulates the expression of PTX3. What is noteworthy is that some changes in ECM components did not adversely affect oocyte growth, maturation, or ovulation (Ploutarchou et al., 2015).

**Metabolism**

The development and maturation of oocyte are dependent on ATP which comes from highly active energy metabolism. It is the main energy procedure that oocytes produce ATP by the mitochondria oxidative phosphorylation with the glucose and/or its intermediate product, acetylformic acid, as substrate. In addition, fatty acids and amino acids, steroids, hemoglobin, glutathione peroxidase are also metabolic substrates in the oocyte (Thompson et al., 2007). Besides providing energy indirectly, those metabolites and intermediates also play roles in signal transduction, osmoregulation, and so on, guaranteeing the maturation of the oocyte nucleus and cytoplasm. Oocytes are precisely controlled by CCs and present different metabolic requirements (Assou et al., 2010). The metabolites of CCs are essential stimuli for bi-directional communication among the oocytes and cumulus vestment (Albertini et al., 2001; Eppig, 1991; Matzuk et al., 2002). CCs are taken more and more consideration by researchers in providing nutrients and substrates for oocytes maturation over the past two decades (Dumesic et al., 2015). Studying the metabolism of oocyte and CCs is helpful to improve the oocyte quality and IVM efficiency (Eppig, 2005; Preis et al., 2005; Su et al., 2009; Sutton-McDowall et al., 2004). Oocyte quality is also affected by phosphoglycerate kinase1 (PGK1), which encodes a transferase that plays an important role in the glycolytic pathway. Glycolysis is critical to the maturation and ability of oocytes, but in the final stage of follicular development, oocytes do not oxidize glucose during glycolysis and are highly dependent on the glycolysis products provided by GCs for energy (Gu et al., 2015). Therefore, upregulating the expression of PGK1 in GCs during IVM condition can improve glycogenesis and affect the maturation and oocyte ability.

More glycolytic enzymes are active in CCs than oocytes, while the deficiency of glycolysis in the IVM system led to the lack of oocyte development ability, suggesting that the glycolytic level of CCs was associated with the quality of oocytes (Brown et al., 2017). Glycolysis has been reported to involve the expression of glucose transporter 1 (SLC2A1), D—lactate dehydrogenase (LDHD), Enolase 2 (ENO2), hexokinase (HK2) (Kind et al., 2013), and PGK1 (Hamel et al., 2010). Glucose– and glutamine–dependent HA matrix is largely inhibited by down-regulating glutamine-fructose-6-phosphate transaminase (GFTP1) and HAS2 in IVM CCs (Caixeta et al., 2013). Since FSH is a potent stimulator of glucose metabolism and nuclear maturation of CCs within COCs, so it is routinely added to the IVM system (Downs et al., 1996; Sutton-McDowall et al., 2004). Another important glycogen metabolism pathway is gluconeogenesis, in which the phosphoenolpyruvate carboxykinase 1 (PCK1) expressed in CCs participates. The cytoplasmic enzyme encoded by this gene, together with GTP, catalyzes oxaloacetic acid to form phosphoenolpyruvate to produce high-quality oocytes (Assou et al., 2008). An emerging interest is that the reduction of carbonyl reductase 3 (CBR3) in CCs negatively regulate oocytes maturation (Tatone et al., 2010; Tatone et al., 2011).

β-oxidation is another crucial energy source for oocytes maturation. A high-quality oocyte is involved in the resumption of MI and the completion of MII, which include the proliferation of GCs, the formation of ECM, and the rearrangement of chromatin, with a large amount of ATP to consume (Dunning et al., 2014). 106 ATPs are produced from the oxidation of a fatty acid compared with about 30 ATPs from glucose. Therefore, the significance of β-oxidation in oocyte maturation is becoming increasingly recognized (Brown et al., 2017; Zarezadeh et al., 2019).
β-oxidation mainly occurs in CCs (Dunning et al., 2014), which inspired us to explore the differential expression of genes involved in CC fatty acid metabolism in vitro and in vivo maturation. There were microarray evidences that the expression of Long-chain acyl-CoA synthetase1 (ACSL1), Long-chain acyl-CoA synthetase4 (ACSL4), arachidonate 5-lipoxygenase activating protein (ALOX5AP), and lysophosphatidylglycerol acyltransferase 1 (LPGAT1) in human CCs are reduced at IVM system (Kind et al., 2013; Ouandaogo et al., 2012), leading the poor oocyte quality. ACSL is a family of lipid metabolism-related enzymes. In Xenopus, suppression of ACSL1 by knocking out its transcripts leads to abnormal acceleration of oocyte maturation. Interestingly, ACSL1 activates the G protein-coupled receptor3 (GPR3)-G-protein alpha S subunit (Gαs)-adenyl cyclase (AC) signaling pathway, which maintains a high level of cAMP that is essential for arresting oocytes (Wang et al., 2012). This finding is supported by the fact that a surge in LH in vivo causes a significant increase in the expression of the beta oxidation-related genes in COCs (Dunning et al., 2010). Another research demonstrated that SCD1 and SCD5 also play a pivotal role in the lipid metabolism of oocytes (Feuerstein et al., 2007).

Amino acids also play an important role in oocytes through a succession of specific transport systems. However, genes involved in the regulation of amino acid metabolism in CCs remain largely unknown. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) also play different roles in regulating amino acid metabolism in CCs in vivo and cytokines. Steroid-related genes are highly expressed in oocyte through blood circulation, or work by affecting CCs during IVM (Dunning et al., 2012). This inspired us to attempt to illustrate the differential expression of steroid-related genes of CCs in vivo and in vitro maturation of oocytes. Estrogen is one of the most important steroids, whose synthesis is a clear sign of healthy communication between oocytes and CCs (Kidder and Vanderhyden, 2010). Therefore, it is necessary to further clarify the regulation mechanism of estrogen acting on oocyte matured in vitro to establish an excellent oocyte IVM system. Cholesterol side-chain cleavage-enzyme (CYP19A1) metabolizes androgens into estradiol-17β (Hamel et al., 2008), and high levels of estradiol promote the synthesis of c-type natriuretic peptide (NPPC) and natriuretic peptide receptor 2 (NPR2), which is an important factor controlling the arrest and recovery of meiosis of oocytes (Lee et al., 2013). It has been confirmed that the low expression of CYP19A1 during IVM condition will result in the reduction of progesterone and estradiol-17β levels in GCs conditioned medium, as well as low-quality oocytes (Nandi et al., 2018). Microarray analysis confirmed that DHC7R is involved in the synthesis of estrogen, and heat shock protein beta-1 (HSPB1) acts as a corepressor for estrogen signaling (van Montfoort et al., 2008). In addition, estrogen receptor also plays an important role in mediating the genomic and non-genomic effects of estrogen. There were two types of estrogen nuclear receptors, estrogen receptor-α (ERα) and estrogen receptor-β (ERβ), both of which bound to estrogen and acted as its transcription factor (Hewitt et al., 2016). G protein-coupled receptor 30 (GPR30, has been named G-protein coupled estrogen receptor, GPER) locates on the oocyte membrane and expresses during oocyte maturation, suggesting that GPR30 may play an important role in regulating oocyte maturation (Li et al., 2013).

Cholesterol, a precursor of steroid hormone synthesis, can also regulate the content and biological activity of steroid hormones in vivo. The transport of cholesterol was mainly mediated by protein in the process of follicular steroid synthesis. Previous studies have reported that intracytoplasmic cholesterol can be transported by Sterol regulatory element-binding protein-2 (SREBP-2) and cholesterol-specific START domain containing 4 (STARD4) to the outer membrane of mitochondria, and then transported by STAR to the inner membrane of mitochondria, further converted into progesterone (Feuerstein et al., 2007; Rimon et al., 2004). Thereby increasing the progesterone content in the follicular fluid can regulate oocyte maturation through affecting mitochondria development. Interestingly, the expression of some genes remains unaffected by IVM during the conversion of cholesterol to progesterone (Coticchio et al., 2017). Moreover, ferredoxin 1 (FDX1) and hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 1 (3βHSD) have been found to be responsible for progesterone synthesis (Hamel et al., 2008).

We still have little information about the function of hemoglobin in the COCs (Thompson et al., 2015). It mainly plays a role during ovulation, involved in oxygen binding and dissociation (Brown et al., 2017). It is also thought to act as an antioxidant (Thompson et al., 2015). Microarray analysis showed that hemoglobin A1 (HBA-c1) is significantly differentially expressed between in vivo and IVM (Brown et al., 2015; Kind et al., 2013; Brown et al., 2017). In addition, genes involved in oxygen transport in CCs include endothelin 2 (EDN2), Bnip3 (NIP3), B-cell lymphoma-2 (BCL2), LDHD, N-myc downstream-regulated gene 1 (NDRG1), vascular endothelial growth factor A (VEGFA), and HK2 are down-regulated during IVM (Kind et al., 2013).

The expression of glutathione peroxidase-1 (GPX1) in human oocytes has been reported to be related to the gamete quality (Ceko et al., 2015). Therefore, amino acids, especially cysteine, should be added into IVM medium, in clinical application, to increase the level of glutathione in oocytes.

Cell-to-cell signaling and interaction
The critical of immature oocyte IVM is to simulate the microendocrine environment of follicular development in the human body, which consists of follicular fluid, CCs, GCs, and theca cells. And in this microenvironment, cell-to-cell signaling, and interaction is indispensable (Anderson et al., 2018; Cui et al., 2018; Buratini and Comizzoli, 2019; Dumesic et al., 2015; Russell et al., 2016). Oocyte development is a highly coordinated and interdependent event. Hence, investigating differential gene expression during CCs development and intercellular various modes of action is vital for inspecting oocytes developmental potential.
synergistically recruit the coactivator p300 on the Anti-
found through the PI3K/AKT and Smad2/3 pathways
(Baris, 1998; Laitinen, 2004). Recently, GDF9 and BMP15 were
interactions in turn act on GCs and CCs to play a regulatory
molecules such as pyruvate, cholesterol, and alanine to
BMP15 can regulate the signaling pathway by
impacting the pivotal genes of CCs (Gilchrist et al., 2008).
Additionally, they also boost the proliferation and expansion
CCs proliferation is promoted by OSF through direct
protein 15 (BMP15) can regulate the signaling pathway by
transmitting of small molecules between cells (Kidder and
port the development of oocytes and synthesise EGF-
like peptide to reduce connexins 43(Cx43), also referred to
at Gap Junction alpha1 (GJA1), thereby reducing
intracellular cAMP and promoting oocyte maturation (Fig. 2).

One example of the bidirectional communication
methods is that GCs and CCs promote the secretion of
bioactive molecules of oocytes by transmitting signals. These
factors in turn act on GCs and CCs to play a regulatory
role, thus completing the signal circuit (Cakmak et al., 2016). Two essential OSFs, GDF9 and bone morphogenetic
protein 15 (BMP15) can regulate the signaling pathway by
impacting the pivotal genes of CCs (Gilchrist et al., 2008).
Additionally, they also boost the proliferation and expansion
of CCs, regulate CCs metabolism, prevent CCs apoptosis,
and regulate the function of GCs (Gilchrist et al., 2008; Vanderhyden et al., 2003). GDF9 induces expression of
multiple genes that are required for mouse cumulus expansion, including Has2, Ptegr2, smad2/3, Cyp19a1, Prdx2, Tnfaip6, Ptx3, and Ptgs2 (Elvin et al., 1999b; Varani et al., 2002). Moreover, this factor appeared to be localized only to oocytes (Aaltonen et al., 1999). Detection of the expression of GDF9 downstream target genes in CCs was in favor of predicting the development quality of oocytes (McKenzie et al., 2004). A second oocyte-specific factor, BMP15 (also known as GDF9b), has also been reported (Dube et al., 1998; Laitinen et al., 1998). Expectedly, it has been tested that BMP15 and GDF9 are closely interrelated (Persani et al., 2014). Recently, GDF9 and BMP15 were found through the PI3K/AKT and Smad2/3 pathways synergistically recruit the coactivator p300 on the Anti-
Müllerian hormone (AMH) promoter region that facilitates
acetylation of histone 3 lysine 27 (H3K27ac), promoting the
secretion of AMH from GCs (Roy et al., 2018). This process
can promote the communication between oocytes, CCs, and
GCs in vitro, improving the developmental competence of
oocytes under IVM conditions. BMP15 not only promotes
the secretion of estrogen and FSH (Stephens and Johnson,
2016; Sutton-McDowall et al., 2015) but also plays an
important role in regulating the signaling of GCs (Persani
et al., 2014). Furthermore, BMP15 can prevent GCs
apoptosis and facilitate the maturation of oocytes (Belli and
Shimasaki, 2018; Persani et al., 2014). And the functions of
BMP15 in humans have been well-reviewed (Elisa
et al., 2004).

Another way of signaling and interaction between cells is
gap junctions. It has been reported that gap junctions existed in
CCs-oocytes (Amsterdam et al., 1976; Anderson and
Albertini, 1976; Hyttel et al., 1989) and GCs-oocytes (Edry
et al., 2006). They are specialized structures that occur at
 spots of extra tight intercellular connection, and they are
 composed of cell-cell channels that permit the direct
transmitting of small molecules between cells (Kidder and
Vanderhyden, 2010). Small molecules transferred from CCs
or GCs to oocytes included Na+, Cl−, cAMP, cGMP,
various ribonucleosides, etc. (Arellano et al., 2002;
Bornslaeger and Schultz, 1985; Brower and Schultz, 1982;
Colonna and Mangia, 1983; Heller and Schultz, 1980; Moor
et al., 1980; Norris et al., 2009). Fully developed GCs use
gap junctions to adjust the pH of oocytes via ion
transporters (Fitzbarris and Balz, 2006). Cx43, a major
component of human gap junctions, affects the development
ability of human oocytes (Hasegawa et al., 2007). Assou
et al. (2010) identified Cx37 (GJA4) and Cx40 (GJA5)
participated in gap junction using microarrays.

Apoptosis
Oocyte will undergo apoptosis programmed cell death (PCD),
if it does not fertilize in time in the IVM system (Haozui et al.,
2008; Miao et al., 2005). Early apoptotic signals are vital for
predicting the quality of oocyte development (Wu et al.,
2017). The control of oocyte apoptosis in vitro is important
for ART. Previous studies have shown that CCs accelerated

![FIGURE 2. Oocytes and cumulus cells regulate each other's development and function by bidirectional communication.](image-url)
the process of oocytes apoptosis (Miao et al., 2005; Qiao et al., 2008). Strong evidence has supported them via the expression of soluble TNFα (sTNFα), soluble Fas ligand (sFASL), BCL2L11, and BCL10 in CCs increased, thus induced the apoptosis of oocytes (Assou et al., 2008; Haouzi et al., 2008; Kong et al., 2018). In addition, TNFSF13 encodes a protein in the tumor necrosis factor (TNF) ligand family and up-regulates the BCL2L11 (Assou et al., 2006). In recent years, more and more researches have been done on neurotropins (NTs) in the field of female reproduction. Among them, brain-derived neurotrophic factor (BDNF) as well as its receptor, neurotrophic tyrosine kinase receptor, type2 (NTRK2), are mainly expressed in human GCs and CCs (Kawamura et al., 2005; Zhao et al., 2019). Interestingly, BDNF could down-regulate the expression of apoptosis-related genes Caspase 9 and TNF receptor superfamily, member 6 (FAS) in oocytes, while up-regulating the expression of NTRK2, thereby inhibiting the apoptosis of oocytes and promoting their development (Zhao et al., 2019). Fas system is a cell membrane glycoprotein that belongs to the tumor necrosis factor receptor family. FasL is the only natural ligand and homologous type II membrane protein in the body. Fas exists on target cells and FasL binds to it in the form of trimer, resulting in activation of the fas-related death domain (FADD). Correspondingly, FADD stimulates the self-activation of caspase 8, which is the initiator of the caspase family. Once activated, caspase 8 can activate other downstream caspase proteases. Among them, caspase-3 is the “core” protease in the FAS-mediated apoptosis signaling pathway and the final executor of apoptosis death. Therefore, we believe that the activation of the Fas-Fasl-FADD-caspase-8-caspase-3 pathway may eventually lead to irreversible apoptosis of oocytes. Furthermore, the presence of superoxide dismutase (SOD) in CCs can inhibit the death of oocytes (Matos et al., 2009). Another experiment revealed that oocytes prevented the death of CCs by promoting the expression of anti-apoptotic gene BCL-2 and inhibiting the expression of pro-apoptotic gene BAX (Gilchrist et al., 2008). However, this effect is limited in that the incidence of apoptosis of CCs cells around the oocyte is lower than that of CCs cells outside the COCs (Hussein et al., 2005). In addition, serpin peptidase inhibitor, clade E (SERPINE2), and CDC42 genes are also involved in oocyte apoptosis (Hamel et al., 2008). However, some studies have shown that Serpine2 does not play a key role in the process of follicular atresia, and compensatory effect may occur after the knockout of SerpinE2 (Cao et al., 2006).

Conclusion

The analysis of GCs and CCs gene expression may act as an indicator for revealing the physiological mechanism of oocyte maturation (Liu et al., 2018). The prediction and evaluation of oocyte development ability could improve the efficiency of IVM technology and increase the survival rate of transplanted embryos. The fact that CCS closely surrounds the oocyte, constantly responds to the follicular environment, and plays a central role in oocyte maturation. This led different research groups to focus on CCs to look for new markers that predict oocyte competence. Indeed, CCs analysis has exclusive advantages over direct assessment of oocytes. We did this review focused on the GCs and CCs gene expression analysis and tried to figure out their intrinsic links to oocyte maturation (Tab. 1). We found that microarray technology is a useful tool for finding new candidate genes among the literature (Assou et al., 2010).

This paper first classified the correlation between GCs/CCs gene expression profile and oocyte developmental potential. We also put forward a method to monitor the expression level of GCs/CCs related genes and factors under the condition of in vitro maturation to judge the development ability of oocytes. Additionally, an optimized

### Table 1

| GCs/CCs Genes | Effects on oocytes | References |
|---------------|--------------------|------------|
| GREM1         | Cellular growth and proliferation | (Dunning et al., 2015) |
| HAS2          | Cellular growth and proliferation | (Dunning et al., 2015) |
| COX2/PTGS2    | Extracellular matrix | (Fülöp et al., 2003; Park et al., 2004) |
| EGFR          | Metabolism | (Caixeta et al., 2013) |
| cAMP          | Cellular growth and proliferation | (Dunning et al., 2015) |
| CDC42         | Extracellular matrix | (Fülöp et al., 2003; Park et al., 2004) |
| GDF-9         | Cellular growth and proliferation | (Chen et al., 2013; Franciosi et al., 2016; Maidarti et al., 2020) |
| PTX3          | Cell cycle | (Shu et al., 2008) |
| ACSL          | Cell cycle | (Zhang et al., 2017) |
| CYP19A1       | Extracellular matrix | (Elvin et al., 1999a; Sutton et al., 2003) |
| BMP15         | Cell-to-cell signaling and interaction | (Gilchrist et al., 2008; Roy et al., 2018) |
| Caspase 9     | Extracellular matrix | (Huang et al., 2013) |
| FAS           | Metabolism | (Kind et al., 2013; Ouandaogo et al., 2012; Wang et al., 2012) |
| FAS           | Metabolism | (Hamel et al., 2008) |
|              | Cell-to-cell signaling and interaction | (Nandi et al., 2018) |
| Apoptosis     | Apoptosis | (Gilchrist et al., 2008; Roy et al., 2018) |
|              | Apoptosis | (Zhao et al., 2019) |

TABLE 1

Key genes that usually used to predict the quality of IVM oocytes
culture medium to maximize the maturation rate of oocytes is expected to be developed in the future supporting subsequent embryo development, clinical pregnancy, and live birth. Oocytes at the IVM system could be studied in a range of measures, nevertheless, many of which were invasive (Coticchio et al., 2015). Gene expression profile monitor of CCs is a commendable alternative method to predict the quality of oocytes in vitro, and it is completely non-invasive (Uyar et al., 2013). Under this circumstance, this review provides meritorious information for clinical IVM techniques. The study of the gene expression profile of the CCs will yield more thrilling data, which may shed light on basic theories of obstetrics and physiology, research and application of embryo engineering, and treatment of reproductive diseases related to oocyte abnormalities in the foreseeable future. Finally, more predictors need to be explored further before IVM progresses from a research technique to a treatment method.

**Conflict of Interest:** The authors declare that they have no conflicts of interest to report regarding the present study.

**Funding Statement:** This research was supported by the Shanxi Province key research and development projects (No. 201803D31068), Applied Basic Research Project of Shanxi Province (Nos. 201801D121212; 201901D211319; 201901D111384) and Science and Technology Innovation Project of Colleges and Universities in Shanxi Province (Nos. 2019L0445; 2019L0418).

**References**

Aaltonen J, Laitinen MP, Vuojolainen K, Jaatinen R, Horelli-Kuitunen N, Seppä L, Louhio H, Tuuri T, Sjöberg J, Bützow R (1999). Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. *Journal of Clinical Endocrinology and Metabolism* **84**: 2744–2750. DOI doi 10.1210/jc.84.8.2744.

Albertini DF, Combelles CM, Benecci E, Carabatsos MJ (2001). Cellular basis for paracrine regulation of ovarian follicle development. *Reproduction* **121**: 647–653. DOI 10.1530/rep.1210647.

Amsterdam A, Josephs R, Lieberman ME, Lindner HR (1976). Organization of intramembrane particles in freeze-cleaved gap junctions of rat graafian follicles: Optical-diffraction analysis. *Journal of Cell Science* **21**: 93–105.

Anderson A, Albertini DF (1976). Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. *Journal of Cell Biology* **71**: 680–686. DOI 10.1083/jcb.71.2.680.

Anderson SH, Glassner MJ, Melnikov A, Friedman G, Orynbayeva Z (2018). Respiriometric reserve capacity of cumulus cell mitochondria correlates with oocyte maturity. *Journal of Assisted Reproduction and Genetics* **35**: 1821–1830. DOI 10.1007/s10815-018-1271-9.

Arellano R O, Martinez-Torres A, Garay E (2002). Ionic currents activated via purinergic receptors in the cumulus cell-enclosed mouse oocyte. *Biology of Reproduction* **67**: 837–846. DOI 10.1095/biolreprod.102.003889.

Assou S, Anahory T, Pantesco V, Le Carrou T, Pellester F, Klein B, Reytmann L, Dechaud H, De Vos J, Hamamah S (2006). The human cumulus–oocyte complex gene-expression profile. *Human Reproduction* **21**: 1705–1719. DOI 10.1093/humrep/dei065.

Assou S, Haouzi D, De Vos J, Hamamah S (2010). Human cumulus cells as biomarkers for embryo and pregnancy outcomes. *Molecular Human Reproduction* **16**: 531–538. DOI 10.1093/molhr/gaq332.

Assou S, Haouzi D, Mahmoud K, Aouacheria A, Guillemin Y, Pantesco V, Remé T, Dechaud H, De Vos J, Hamamah S (2009). A non-invasive test for assessing embryo potential by gene expression profiles of human cumulus cells: a proof of concept study. *Molecular Human Reproduction* **14**: 711–719. DOI 10.1093/molhr/gan067.

Barrett SL, Albertini DF (2010). Cumulus cell contact during oocyte maturation in mice regulates meiotic spindle positioning and enhances developmental competence. *Journal of Assisted Reproduction and Genetics* **27**: 29–39. DOI 10.1007/s10815-009-9376-9.

Belli M, Shimasaki S (2018). Molecular aspects and clinical relevance of GDF9 and BMP15 in ovarian function. *Vitamins and Hormones* **107**: 317–348. DOI 10.1016/bs.vh.2017.12.003.

Ben-Ami I, Freimann S, Armon L, Dantes A, Strasserburg D, Friedler S, Raziel A, Seger R, Ron-El R, Amsterdam A (2006). PGE2 up-regulates EGF-like growth factor biosynthesis in human granulosa cells: new insights into the coordination between PGE2 and LH in ovulation. *MHR: Basic science of reproductive medicine* **12**: 593–599. DOI 10.1093/molhr/gal068.

Bornslaeger EA, Schultz RM (1985). Regulation of mouse oocyte maturation: Effect of elevating cumulus cell cAMP on oocyte cAMP levels. *Biology of Reproduction* **33**: 698–704. DOI 10.1095/biolreprod53.3.698.

Brower PT, Schultz RM (1982). Intercellular communication between granulosa cells and mouse oocytes: Existence and possible nutritional role during oocyte growth. *Developmental Biology* **90**: 144–153. DOI 10.1016/0012-1606(82)90219-6.

Brown HM, Anastasi MR, Frank LA, Kind KL, Richani D, Robker RL, Russell DL, Gilchrist RB, Thompson JG (2015). Hemoglobin: A gas transport molecule that is hormonally regulated in the ovarian follicle in mice and humans. *Biology of Reproduction* **92**: 707. DOI 10.1095/biolreprod.114.124594.

Brown HM, Dunning KR, Sutton-McDowall M, Gilchrist RB, Thompson JG, Russell DL (2017). Failure to launch: Aberrant cumulus gene expression during oocyte in vitro maturation. *Reproduction* **153**: R109–R120. DOI 10.1530/REP-16-0426.

Buratini J, Comizzoli P (2019). Unlocking the mysteries of the cumulus-oocyte complex—A critical cellular partnership for developmental competence. *Journal of Assisted Reproduction and Genetics* **36**: 411–412. DOI 10.1007/s10815-019-01421-0.

Caixeta ES, Sutton-McDowall ML, Gilchrist RB, Thompson JG, Price CA, Machado MF, Lima PF, Buratini J (2013). Bone morphogenetic protein 15 and fibroblast growth factor 10 enhance cumulus expansion, glucose uptake, and expression of genes in the ovulatory cascade during in vitro maturation of bovine cumulus–oocyte complexes. *Reproduction* **146**: 27–35. DOI 10.1530/REP-13-0079.

Cakmak H, Franciosi F, Zamah AM, Cedars MI, Conti M (2016). Dynamic secretion during meiotic reentry integrates the function of the oocyte and cumulus cells. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 2424–2429. DOI 10.1073/pnas.1519990113.
Cao M, Nicola E, Portela VM, Price CA (2006). Regulation of serine protease inhibitor-E2 and plasminogen activator expression and secretion by follicle stimulating hormone and growth factors in non-luteinizing bovine granulosa cells in vitro. Matrix Biology 25: 342–354. DOI 10.1016/j.matbio.2006.05.005.

Ceko MJ, Hummitzsch K, Hatzirodon N, Bonner WM, Aitken JB, Russell DL, Lane M, Rodgers RJ, Harris HH (2015). Correction: X-Ray fluorescence imaging and other analyses identify selenium and GPX1 as important in female reproductive function. Metallomics 7: 188–188. DOI 10.1039/C4MT00494A.

Cetica P, Pintos L, Dalvit G, Beconi M (2003). Involvement of enzymes of amino acid metabolism and tricarboxylic acid cycle in bovine oocyte maturation in vitro. Reproduction 126: 753–763. DOI 10.1530/rep.0.1260705.

Cha KY, Koj J, Choi DH, Han SY, Yoon TK (1991). Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program**Prize paper, presented at the 45th Annual Meeting Of The American Fertility Society, San Francisco, California, November 11 to 16, 1989.. Fertility and Sterility 55: 109–113. DOI 10.1016/S0015-0282(15)45006-0.

Chang H, Brown CW, Matzuk MM (2002). Genetic analysis of the mammalian transforming growth factor-beta superfamily. Endocrine Reviews 23: 787–823. DOI 10.1210/er.2002-0003.

Chen J, Torcia S, Xie F, Lin CJ, Cakmak H, Franciosi F, Horner K, Chang H, Brown CW, Matzuk MM (2002). Genetic analysis of the mammalian transforming growth factor-beta superfamily. 55 California, November 11 to 16, 1989.. Fertility and Sterility 55: 109–113. DOI 10.1016/S0015-0282(15)45006-0.

Cetica P, Pintos L, Dalvit G, Beconi M (2003). Involvement of enzymes of amino acid metabolism and tricarboxylic acid cycle in bovine oocyte maturation in vitro. Reproduction 126: 753–763. DOI 10.1530/rep.0.1260705.

Dahlan MH, Tan SL, Chung J, Son WY (2016). Clinical definition paper on in vitro maturation of human oocytes. Human Reproduction 31: 1383–1386. DOI 10.1093/humrep/dew109.

Diaz FJ, O’Brien MJ, Wigglesworth K, Eppig JJ (2006). The preantral granulosa cell to cumulus cell transition in the mouse ovary: Development of competence to undergo expansion. Developmental Biology 299: 91–104. DOI 10.1016/j.ydbio.2006.07.012.

Downs SM, Humpherson PG, Martin KL, Leese HJ (1996). Glucose utilization during gonadotropin-induced meiotic maturation in cumulus cell-enclosed mouse oocytes. Molecular Reproduction and Development 44: 121–131. DOI 10.1002/ (SIC)1098-2795(199605)44:1<121::AID-MRD143>3.0.CO;2-7.

Dragovic RA, Ritter LJ, Schulz SJ, Amato F, Armstrong DT, Gilchrist RB (2005). Role of oocyte-secreted growth differentiation factor 9 in the regulation of mouse cumulus expansion. Endocrinology 146: 2798–2806. DOI 10.1210/endo.2005-0098.

Dragovic RA, Ritter LJ, Schulz SJ, Amato F, Thompson JG, Armstrong DT, Gilchrist RB (2007). Oocyte-secreted factor activation of SMAD 2/3 signaling enables initiation of mouse cumulus cell expansion. Biology of Reproduction 76: 848–857. DOI 10.1095/bioreprod.106.057471.

Dube JL, Wang P, Elvin J, Lyons KM, Celeste AJ, Matzuk MM (1998). The bone morphogenetic protein 15 gene is X-linked and expressed in oocytes. Molecular Endocrinology 12: 1809–1817. DOI 10.1210/mend.12.12.0206.

Dumesic DA, Meldrum DR, Katz-Jaffe MG, Krisher RL, Schoolcraft WB (2015). Oocyte environment: Follicular fluid and cumulus cells are critical for oocyte health. Fertility and Sterility 103: 303–316. DOI 10.1016/j.fertnstert.2014.11.015.

Dunning KR, Cashman K, Russell DL, Thompson JG, Norman Rj, Robker RL (2010). Beta-oxidation is essential for mouse oocyte developmental competence and early embryo development. Biology of Reproduction 83: 909–918. DOI 10.1095/bioreprod.110.084145.

Dunning KR, Lane M, Brown HM, Yeo C, Robker RL, Russell DL (2007). Altered composition of the cumulus-oocyte complex matrix during in vitro maturation of oocytes. Human Reproduction 22: 2842–2850. DOI 10.1093/humrep/den277.

Dunning KR, Russell DL, Robker RL (2014). Lipids and oocyte developmental competence: The role of fatty acids and beta-oxidation. Reproduction 148: R15–R27. DOI 10.1530/ REP-13-0251.

Dunning KR, Watson LN, Zhang VJ, Brown HM, Kaczmarek AK, Robker RL, Russell DL (2015). Activation of mouse cumulus-oocyte complex maturation in vitro through EGF-like activity of Versican. Biology of Reproduction 92: 289. DOI 10.1095/bioreprod.114.127274.

Ebner T, Moser M, Sommergruber M, Tews G (2003). Selection based on morphological assessment of oocytes and embryos at different stages of preimplantation development: A review. Human Reproduction Update 9: 251–262. DOI 10.1093/humupd/dmg021.

Edry I, Sela-Abrahamovich S, Dekel N (2006). Meiotic arrest of oocytes depends on cell-to-cell communication in the ovarian follicle. Molecular and Cellular Endocrinology 252: 102–106. DOI 10.1016/j.mce.2006.03.009.

Ekart J, McNatty K, Hutton J, Pittman J (2013). Ranking and selection of MII oocytes in human ICSI cycles using gene expression levels from associated cumulus cells. Human Reproduction 28: 2930–2942. DOI 10.1093/humrep/det357.

Di Pasquale E, Beck-Peccoz P, Persiani L (2004). Hypergonadotrophic ovarian failure associated with an inherited mutation of human bone morphogenetic protein-15 (BMP15) gene. American Journal of Human Genetics 75: 106–111. DOI 10.1086/422103.
factor (BDNF) promotes the development of oocytes into preimplantation embryos. *Proceedings of the National Academy of Sciences of the United States of America* **102**: 9206–9211. DOI 10.1073/pnas.0502442102.

Kawashima I, Umehara T, Noma N, Kawai T, Shtanaka M, Richards JS, Shimada M (2014). Targeted disruption of Nrg1 in granulosa cells alters the temporal progression of oocyte maturation. *Molecular Endocrinology* **28**: 706–721. DOI 10.1210/me.2013-1316.

Kidder GM, Vanderhyden BC (2010). Bidirectional communication between oocytes and follicle cells: Ensuring oocyte developmental competence. *Canadian Journal of Physiology and Pharmacology* **88**: 399–413. DOI 10.1139/Y10-009.

Kind KL, Banwell KM, Gebhardt KM, Macpherson A, Gauld A, Russell DL, Thompson JG (2013). Microarray analysis of mRNA from cumulus cells following *in vivo* or *in vitro* maturation of mouse cumulus-oocyte complexes. *Reproduction, Fertility and Development* **25**: 426–438. DOI 10.1071/RD11305.

Kong QW, Wang J, Xiao B, Lin FH, Zhu J, Sun HY, Wang Y, Richards JS (2010). Cyclic GMP from the surrounding somatic cells regulates cumulus cell-growth and cell functions. *Development* **137**: 511–521. DOI 10.1210/me.2010-0086.

Lee KB, Zhang M, Sugura K, Wigglesworth K, Uliasz T, Jaffe LA, Eppig JJ (2013). Hormonal coordination of natriuretic peptide type C and natriuretic peptide receptor 3 expression in mouse granulosa cells. *Biology of Reproduction* **88**: 682. DOI 10.1095/biolreprod.112.104810.

Li YR, Ren CE, Zhang Q, Li JC, Chian RC (2013). Expression of G protein estrogen receptor (GPER) on membrane of mouse oocytes during folliculogenesis. *Mechanisms of Development* **179**: 135–140. DOI 10.1016/S0925-4773(98)00161-0.

Laitinen M, Vuojokainen K, Jaatinen R, Ketola I, Aaltojän J, Lehtonen E, Heikinheimo M, Ritvos O (1998). A novel growth differentiation factor-9 (GDF-9) related factor is co-expressed with GDF-9 in mouse oocytes during folliculogenesis. *Mechanisms of Development* **78**: 135–140. DOI 10.1016/S0925-4773(98)00161-0.

Lee KB, Zhang M, Sugura K, Wigglesworth K, Uliasz T, Jaffe LA, Eppig JJ (2013). Hormonal coordination of natriuretic peptide type C and natriuretic peptide receptor 3 expression in mouse granulosa cells. *Biology of Reproduction* **88**: 682. DOI 10.1095/biolreprod.112.104810.

Li YR, Ren CE, Zhang Q, Li JC, Chian RC (2013). Expression of G protein estrogen receptor (GPER) on membrane of mouse oocytes during folliculogenesis. *Journal of Assisted Reproduction and Genetics* **30**: 227–232. DOI 10.1007/s10815-013-9942-z.

Liu Q, Zhang J, Wen H, Feng Y, Zhang X, Xiang H, Cao Y, Tong X (2018). Analyzing the transcriptome profile of human cumulus cells related to embryo quality via RNA sequencing. *BioMed Research International* **18**: 1–8. DOI 10.1155/2018/9846274.

Liu Z, Fan HY, Wang Y, Richards JS (2010). Targeted disruption of Mapk14 (p38MAPKα) in granulosa cells and cumulus cells causes cell-specific changes in gene expression profiles that rescue COC expansion and maintain fertility. *Molecular Endocrinology* **24**: 1794–1804. DOI 10.1210/me.2010-0086.

Lonergan P, Fair T (2016). Maturation of oocytes *in vitro*. *Annual Review of Animal Biosciences* **4**: 225–268. DOI 10.1146/annurev-animal-022114-110822.

Maidarti M, Anderson RA, Telfer EE (2020). Crosstalk between PTEN/PI3K/Akt signalling and DNA damage in the oocyte: implications for primordial follicle activation, oocyte quality and ageing. *Cells* **9**: 200. DOI 10.3390/cells9010200.

Marei WF, Ayabaysekara DR, Wathes DC, Fouladi-Nashta AA (2014). Role of PTGS2-generated PGE2 during gonadotrophin-induced bovine oocyte maturation and cumulus cell expansion. *Reproductive BioMedicine Online* **28**: 388–400. DOI 10.1016/j.rbmo.2013.11.005.

Matos L, Stevenson D, Gomes F, Silva-Carvalho JL, Almeida H (2009). Superoxide dismutase expression in human cumulus oophorus cells. *Molecular Human Reproduction* **15**: 411–419. DOI 10.1093/molehr/gap034.

Matzuk MM, Burns KH, Viveiros MM, Eppig JJ (2002). Intercellular communication in the mammalian ovary: Oocytes carry the conversation. *Science* **296**: 2178–2180. DOI 10.1126/science.1071965.

McKenzie LJ, Pangas SA, Carson SA, Kovanci E, Cisneros P, Buster JE, Amato P, Matzuk MM (2004). Human cumulus granulosa cell gene expression: A predictor of fertilization and embryo selection in women undergoing IVF. *Human Reproduction* **19**: 2869–2874. DOI 10.1093/humrep/deh535.

Mehlmann LM (2005). Stops and starts in mammalian oocytes: recent advances in understanding the regulation of meiotic arrest and oocyte maturation. *Reproduction* **130**: 791–799. DOI 10.1530/repro.1.00793.

Miao YL, Liu XY, Qiao TW, Miao DQ, Luo MJ, Tan JH (2005). Cumulus cells accelerate aging of mouse oocytes. *Biologia of Reproduction* **73**: 1025–1031. DOI 10.1095/biobreprod.105.043703.

Moor RM, Smith MW, Dawson RM (1980). Measurement of intracellular coupling between oocytes and cumulus cells using intracellular markers. *Experimental Cell Research* **126**: 15–29. DOI 10.1016/0014-4827(80)90466-8.

Nandi S, Tripathi SK, Gupta P, Mondal S (2018). Nutritional and metabolic stressors on ovine oocyte development and granulosa cell functions *in vitro*. *Cell Stress and Chaperones* **23**: 357–371. DOI 10.1007/s12192-017-0846-1.

Niringiyumukiza JD, Cai H, Xiang W (2018). Prostaglandin E2 involvement in mammalian female fertility: Ovulation, fertilization, embryo development and early implantation. *Reproductive Biology and Endocrinology* **16**: 398. DOI 10.1186/s12958-018-0359-5.

Norris RP, Ratzan WJ, Freudzon M, Mehlmann LM, Kral J, Movsesian MA, Wang H, Ke H, Nikolaev VO, Jaffe LA (2009). Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. *Development* **136**: 1869–1878. DOI 10.1242/dev.035238.

Ochsner SA, Day AJ, Rugg MS, Breyer RM, Gomer RH, Richards JS (2003). Disrupted function of tumor necrosis factor-α-stimulated gene 6 blocks cumulus cell-oocyte complex expansion. *Endocrinology* **144**: 4376–4384. DOI 10.1210/en.2003-0487.

Ouandaogo ZG, Frydman N, Hesters L, Assou S, Haouzi D, Dechaud H, Kadoch IJ, De Vos J, Ouandaogo ZG, Haouzi D, Assou S, Dechaud H, Kadoch IJ, De Vos J, Peluffo MC, Ting AY, Zamah AM, Conti M, Stouffer RL, Zelinski MB, Hennebold JD (2012). Differences in transcriptional profiles of human cumulus cells isolated from oocytes at GV, MI and MII stages after *in vivo* and *in vitro* oocyte maturation. *Human Reproduction* **27**: 2438–2447. DOI 10.1093/humrep/des172.

Ouandaogo ZG, Haouzi D, Assou S, Dechaud H, Kadoch IJ, De Vos J, Hamamah S (2011). Human cumulus cells molecular signature in relation to oocyte nuclear maturity stage. *PLoS One* **6**: e27179. DOI 10.1371/journal.pone.0027179.

Pan B, Li J (2019). The art of oocyte meiotic arrest regulation. *Reproductive Biology and Endocrinology* **17**: 1. DOI 10.1186/s12958-018-0445-8.

Park JY, Su YQ, Ariga M, Law E, Jin SL, Conti M (2004). EGF-like growth factors as mediators of LH action in the ovariule follicle. *Science* **303**: 682–684. DOI 10.1126/science.1092463.

Peluffo MC, Ting AY, Zamah AM, Conti M, Stouffer RL, Zelinski MB, Hennebold JD (2012). Amphiregulin promotes the rescue COC expansion and maintain fertility. *Reproductive Biology and Endocrinology* **16**: 398. DOI 10.1186/s12958-018-0359-5.
Persani L, Rossetti R, Di Pasquale E, Cacciatore C, Fabre S (2014). The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. Human Reproduction Update 20: 869–883. DOI 10.1093/humupd/dmu036.

Pincus G, Enzmann EV (1935). The comparative behavior of mammalian eggs in vivo and in vitro: I. The activation of ovarian eggs. Journal of Experimental Medicine 62: 665–675. DOI 10.1084/jem.62.5.665.

Ploutarchou P, Melo P, Day AJ, Milner CM, Williams SA (2015). Molecular analysis of the cumulus matrix: Insights from mice with O-glycan-deficient oocytes. Reproduction 149: 533–543. DOI 10.1530/REP-14-0503.

Preis KA, Seidel G, Gardner DK (2005). Metabolic markers of developmental competence for in vitro-matured mouse oocytes. Reproduction 130: 475–483. DOI 10.1530/rep.1.00831.

Qiao TW, Liu N, Miao DQ, Zhang X, Han D, Ge L, Tan JH (2008). Cumulus cells accelerate aging of mouse oocytes by secreting a soluble factor(s). Molecular Reproduction and Development 75: 521–528. DOI 10.1002/mrd.20779.

Rimon E, Sasson R, Dantes A, Land-Bracha A, Amsterdam A (2004). Gonadotropin-induced gene regulation in human granulosa cells obtained from IVF patients: Modulation of genes coding for growth factors and their receptors and genes involved in cancer and other diseases. International Journal of Oncology 24: 1325–1338. DOI doi 10.3892/ijo.24.5.1325.

Roy S, Gandra D, Seger C, Biswas A, Nguyen V, Gleicher N, Kumar TR, Sen A (2018). Oocyte-derived factors (GDF9 and BMP15) and FSH regulate AMH expression via modulation of H3K27AC in granulosa cells. Endocrinology 159: 3433–3445. DOI 10.1210/endo.2018-00609.

Russell DL, Gilchrist RB, Thompson JG (2016). Bidirectional communication between cumulus cells and the oocyte: Old hands and new players? Theriogenology 86: 62–68. DOI 10.1016/j.theriogenology.2016.04.019.

Russell DL, Salustri A (2006). Extracellular matrix of the cumulus-oocyte complex. Seminars in Reproductive Medicine 24: 217–227. DOI 10.1055/s-2006-948551.

Sanfins A, Rodrigues P, Albertini DF (2018). GDF-9 and BMP-15 direct the follicle symphony. Journal of Assisted Reproduction and Genetics 35: 1741–1750. DOI 10.1007/s10815-018-1268-4.

Sen A, Riccio M, Marzona L, Nicoli A, Mansela T, Marmiroli S, Bertacchini J, Fano RA, La Sala GB, De Pol A (2009). Human MATER localization in specific cell domains of oocytes and follicular cells. Reproductive BioMedicine Online 18: 226–234. DOI 10.1016/S1472-6483(10)60260-x.

Shimada M, Hernandez-Gonzalez I, Gonzalez-Robayna I, Richards JS (2006). Paracrine and autocrine regulation of epidermal growth factor-like factors in cumulus oocyte complexes and granulosa cells: Key roles for prostaglandin synthase 2 and progesterone receptor. Molecular Endocrinology 20: 1352–1365. DOI 10.1210/me.2005-0504.

Shu YM, Zeng HT, Ren Z, Zhuang GL, Liang XY, Shen HW, Yao SZ, Ke PQ, Wang NN (2008). Effects of cilostamide and forskolin on the meiotic resumption and embryonic development of immature human oocytes. Human Reproduction 23: 504–513. DOI 10.1093/humrep/dem344.

Stephens CS, Johnson PA (2016). Bone morphogenetic protein 15 may promote follicle selection in the hen. General and Comparative Endocrinology 235: 170–176. DOI 10.1016/j.ygcen.2016.06.027.

Su YQ, Sugiura K, Eppig JJ (2009). Mouse oocyte control of granulosa cell development and function: Paracrine regulation of cumulus cell metabolism. Seminars in Reproductive Medicine 27: 032–042. DOI 10.1055/s-0028-1108008.

Su YQ, Sugiura K, Wigglesworth K, O’Brien MJ, Affourtit JP, Pangas SA, Matzuk MM, Eppig JJ (2007). Oocyte regulation of metabolic cooperativity between mouse cumulus cells and oocytes: BMP15 and GDPF9 control cholesterol biosynthesis in cumulus cells. Development 134: 111–121. DOI 10.1242/dev.009068.

Su YQ, Wigglesworth K, Pendola FL, O’Brien MJ, Eppig JJ (2002). Mitogen-activated protein kinase activity in cumulus cells is essential for gonadotropin-induced oocyte meiotic resumption and cumulus expansion in the mouse. Endocrinology 143: 2221–2232. DOI 10.1210/endo.143.6.8845.

Sugiura K, Su YQ, Dazl FJ, Pangas SA, Sharma S, Wigglesworth K, O’Brien MJ, Matzuk MM, Shimasaki S, Eppig JJ (2007). Oocyte-derived BMP15 and FGfs cooperate to promote glycolysis in cumulus cells. Development 134: 2593–2603. DOI 10.1242/dev.006882.

Sutton ML, Gilchrist RB, Thompson JG (2003). Effects of in-vivo and in-vitro environments on the metabolism of the cumulus-oocyte complex and its influence on oocyte developmental capacity. Human Reproduction Update 9: 35–48. DOI 10.1093/humupd/dmg009.

Sutton-McDowall ML, Gilchrist RB, Thompson JG (2004). Cumulus expansion and glucose utilisation by bovine cumulus–oocyte complexes during in vitro maturation: The influence of glucosamine and follicle-stimulating hormone. Reproduction 128: 313–319. DOI 10.1530/rep.1.00225.

Tatone C, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Tatone C, Carbone MC, Campanella G, Festuccia C, Artini PG, Marci R, Artini PG, Piomboni P, Focarelli R (2008). Female syndromes women. Molecular Reproduction and Development 82: 281–294. DOI 10.1002/mrd.22470.

Tabibnejad N, Sheikhha MH, Ghasemi F, Fesahat F, Soleimani M, Aflatoonian A (2019). Association between early embryo morphokinetics plus cumulus cell gene expression and assisted reproduction outcomes in polycystic ovary syndrome women. Reproductive BioMedicine Online 38: 139–151. DOI 10.1016/j.rbmo.2018.10.010.

Tanghe S, Van Soom A, Nauwynck H, Coryn M, de Kruif A (2002). Minireview: Functions of the cumulus oophoros during oocyte maturation, ovulation, and fertilization. Molecular Reproduction and Development 61: 414–424. DOI 10.1002/mrd.10102.

Tatone C, Amicarelli F, Carbone MC, Monteleone P, Caserta D, Marci R, Artini PG, Piomboni P, Focarelli R (2008). Cellular and molecular aspects of ovarian follicle ageing. Human Reproduction Update 14: 131–142. DOI 10.1093/humupd/dnn048.

Tatone C, Carbone MC, Campanella G, Festuccia C, Artini PG, Talesa V, Focarelli R, Amicarelli F (2010). Female reproductive dysfunction during ageing: role of methyglyoxal in the formation of advanced glycation endproducts in ovaries of reproducitively-aged mice. Journal of Biological Regulators and Homeostatic Agents 24: 63–72.

Tatone C, Heizenrieder T, Di Edmido G, Treffon P, Amicarelli F, Seidel T, Eichenlaub-Ritter U (2011). Evidence that carbonyl stress by methyglyoxal exposure induces DNA damage and spindle aberrations, affects mitochondrial...
integrity in mammalian oocytes and contributes to oocyte ageing. Human Reproduction 26: 1843–1859. DOI 10.1093/humrep/der140.

Thompson JG, Brown HM, Kind KL, Russell DL (2015). The ovarian antral follicle: Living on the edge of hypoxia or not? Biology of Reproduction 92: 673. DOI 10.1095/bioreprod.115.128660.

Thompson JG, Lane M, Gilchrist RB (2007). Metabolism of the bovine cumulus-oocyte complex and influence on subsequent developmental competence. Reproduction in Domestic Ruminants 64: 179–190. DOI 10.5661/RDR-V1-179.

Uyar A, Torrealday S, Seli E (2013). Cumulus and granulosa cell markers of oocyte and embryo quality. Fertility and Sterility 99: 979–997. DOI 10.1016/j.fertstert.2013.01.129.

van Montfoort AP, Geraedts JP, Dumoulin JC, Stassen AP, Evers JL, Ayoubi TA (2008). Differential gene expression in cumulus cells as a prognostic indicator of embryo viability: a microarray analysis. Molecular Human Reproduction 14: 157–168. DOI 10.1093/molehr/gam088.

Vanderhyden BC, Macdonald EA, Nagyova E, Dhawan A (2003). Evaluation of members of the TGFβ superfamily as candidates for the oocyte factors that control mouse cumulus expansion and steroidogenesis. Reproduction 61: 55–70.

Varani S, Elvin JA, Yan C, DeMayo J, DeMayo FJ, Horton HF, Byrne MC, Matzuk MM (2002). Knockout of pentraxin 3, a downstream target of growth differentiation factor-9, causes female subfertility. Molecular Endocrinology 16: 1154–1167. DOI 10.1210/mend.16.6.0859.

Wang HW, Fang JS, Kuang X, Miao LY, Wang C, Xia GL, King ML, Zhang J (2012). Activity of long-chain acyl-CoA synthetase is required for maintaining meiotic arrest in Xenopus laevis. Biology of Reproduction 87: 74–74. DOI 10.1093/bioreprod/87.s1.74.

Wathlet S, Adriaenssens T, Segers I, Verheyen G, Van de Velde H, Coucke W, Ron El R, Devroey P, Smits J (2011). Cumulus cell gene expression predicts better cleavage-stage embryo or blastocyst development and pregnancy for ICSI patients. Human Reproduction 26: 1035–1051. DOI 10.1093/humrep/der036.

Wigglesworth K, Lee KB, O’Brien MJ, Peng J, Matzuk MM, Eppig JJ (2013). Bidirectional communication between oocytes and ovarian follicular somatic cells is required for meiotic arrest of mammalian oocytes. Proceedings of the National Academy of Sciences of the United States of America 110: E3723–E3729. DOI 10.1073/pnas.1314829110.

Wu Y, Zhang N, Li YH, Zhao L, Yang M, Jin Y, Xu YN, Guo H (2017). Citrinin exposure affects oocyte maturation and embryo development by inducing oxidative stress-mediated apoptosis. Oncotarget 8: 34525–34533. DOI 10.18632/oncotarget.15776.

Yamashita Y, Kawashima I, Gunji Y, Hishinuma M, Shimada M (2010). Progesterone is essential for maintenance of Tace/Adam17 mRNA expression, but not EGF-like factor, in cumulus cells, which enhances the EGF receptor signaling pathway during in vitro maturation of porcine COCs. Journal of Reproduction and Development 56: 315–323. DOI 10.1262/jrd.09-199H.

Yamashita Y, Okamoto M, Ikeda M, Okamoto A, Sakai M, Gunji Y, Nishimura R, Hishinuma M, Shimada M (2014). Protein kinase C (PKC) increases TACE/ADAM17 enzyme activity in porcine ovarian somatic cells, which is essential for granulosa cell luteinization and oocyte maturation. Endocrinology 155: 1080–1090. DOI 10.1210/en.2013-1655.

Zamah AM, Hsieh M, Chen J, Vigne JL, Rosen MP, Cedars MI, Conti M (2010). Human oocyte maturation is dependent on LH-stimulated accumulation of the epidermal growth factor-like growth factor, amphiregulin†. Human Reproduction 25: 2569–2578. DOI 10.1093/humrep/deq212.

Zarezadeh R, Meh dizadeh A, Leroy J, Nouri M, Fayazi S, Darabí M (2019). Action mechanisms of n-3 polysaturated fatty acids on the oocyte maturation and developmental competence: Potential advantages and disadvantages. Journal of Cellular Physiology 234: 1016–1029. DOI 10.1002/jcp.27101.

Zhang J, Ma R, Li L, Wang L, Hou X, Han L, Ge J, Li M, Wang Q (2017). Intersectin 2 controls actin cap formation and meiotic division in mouse oocytes through the Cdc42 pathway. FASEB Journal 31: 4277–4285. DOI 10.1096/fj.201700179R.

Zhao X, Du F, Liu X, Ruan Q, Wu Z, Lei C, Deng Y, Luo C, Jiang J, Shi D, Lu F (2019). Brain-derived neurotrophic factor (BDNF) is expressed in buffalo (Bubalus bubalis) ovarian follicles and promotes oocyte maturation and early embryonic development. Theriogenology 130: 79–88. DOI 10.1016/j.theriogenology.2019.02.020.