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NEW RESEARCH HORIZON

Revisiting oocyte–somatic cell interactions: in search of novel intrafollicular predictors and regulators of oocyte developmental competence

Qinglei Li[1], Laurie J. McKenzie[1,2] and Martin M. Matzuk[1,3,4,5]

[1]Department of Pathology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA; [2]Houston IVF, Memorial Hermann Memorial City Hospital, Houston, TX 77024, USA; [3]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA; [4]Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

[5]Correspondence address: Tel: +1 713-798-6451; Fax: +1 713-798-5833; E-mail: mmatzuk@bcm.tmc.edu

Prediction and improvement of oocyte competence are two critical issues in assisted reproductive technology to improve infertility therapy. The lack of reliable and objective predictors of oocyte developmental competence for oocyte/embryo selection during *in vitro* fertilization hampers the effectiveness of this technology. Likewise, the low pregnancy rate resulting from *in vitro* maturation of human oocytes represents a major obstacle for its clinical application. Oocyte competence is progressively acquired during follicular development, and the oocyte plays a dominant role in regulating granulosa cell functions and maintaining the microenvironment appropriate for the development of its competence. Hence, granulosa cell functions are reflective of oocyte competence, and molecular markers of granulosa cells are potentially reliable predictors of oocyte quality. With the advent of the functional genomics era, the transcriptome of granulosa cells has been extensively characterized. Experimental data supporting granulosa cell markers as predictors of oocyte competence are now emerging in both animal models and humans. Future efforts should focus on integrating granulosa cell genetic markers as parameters for oocyte/embryo selection. Moreover, novel *in vitro* evidence highlights the effectiveness of exogenous oocyte-secreted factors in promoting oocyte developmental competence in animal models. The challenge in evaluating the effect of oocyte-secreted factors on oocyte quality in a clinical setting is to standardize the various preparations of these recombinant proteins and decipher their complex interactions/cooperativity within the germline-somatic cell regulatory loop.

Introduction

Two key technical issues to be resolved in assisted reproductive technology (ART) are the lack of objective and reliable predictors of oocyte developmental competence and the low successful rate of *in vitro* maturation (IVM). Oocyte competence is defined as the intrinsic ability of oocytes to undergo meiotic maturation, fertilization, embryonic development and successful pregnancy. Hence, utilization of the most competent oocytes during *in vitro* fertilization (IVF) is crucial to ensure the derivation of high-quality embryos and successful pregnancy. The morphological parameters of the cytoplasm, polar body and cumulus cells are routinely used for oocyte selection (Coticchio *et al*., 2004; Wang and Sun, 2007). However, the morphological criteria for grading and screening of oocytes are subjective and controversial (Serhal *et al*., 1997; Balaban *et al*., 1998). Thus, defining objective and noninvasive molecular markers predictive of oocyte competence is of critical importance. Moreover, since single-embryo transfer (SET), which has the key advantage of preventing multiple pregnancies (Gerris, 2005; Karlstrom and Bergh, 2007; Khalaf *et al*., 2008), will tend to be a norm in the future (Nygren, 2007), it is urgent to develop such a reliable diagnostic approach to identify the best quality embryo among those available for transfer. Notably, acquisition of oocyte competence is closely associated with normal follicular development, whereby the oocyte plays an active role in regulating the functions of surrounding somatic cells (i.e. cumulus cells adjacent to the oocyte and mural granulosa cells lining the follicle wall) (Eppig *et al*., 1997, 2002; Eppig, 2001; Matzuk *et al*., 2002). Therefore, identification of key molecules and signaling pathways within the oocyte–cumulus cell regulatory loop will be instrumental in gaining deep insights into the intricate mechanisms underlying the development of oocyte competence and uncovering novel regulators and reliable molecular predictors of oocyte quality. These efforts will ultimately lead to improved efficiency and health outcomes (i.e. reduced prematurity/perinatal mortality rate and maternal and pediatric complications) of ART.

Crosstalk between oocytes and somatic cells: old story conveys new messages

Folliculogenesis is coordinately regulated by both endocrine and intraovarian factors; the fundamental roles of gonadotrophins and gonadotrophin-mediated signaling in multiple reproductive events including cumulus expansion and acquisition of oocyte competence have been well characterized and documented (Kumar *et al*., 1997; Thomas *et al*., 2003; Ma *et al*., 2004; Park *et al*., 2004; Conti *et al*., 2006; Sirard *et al*., 2007). This article mainly focuses on the recent advances in understanding the contribution of intraovarian factors, especially oocyte-produced factors, to oocyte developmental competence.

It is known that bidirectional interactions between the oocytes and surrounding somatic cells through gap junctions and paracrine signaling are pivotal in maintaining the growth and development of both cell types during folliculogenesis (Eppig, 1991, 2001; Eppig *et al*., 1997, 2002; Matzuk *et al*., 2002). Instead of being a passive recipient of nutritional support and regulatory signals from its companion granulosa cells, the oocyte plays an active role throughout folliculogenesis via secretion of paracrine factors that maintain an appropriate microenvironment for the acquisition of its developmental competence (Dong *et al*., 1996; Eppig *et al*., 1997, 2002; Eppig, 2001; Gilchrist *et al*., 2004). Importantly, oocytes specify the phenotype of adjacent cumulus cells, which is essential for oocyte development and cumulus cell function (Eppig *et al*., 1997; Li *et al*., 2000; Diaz *et al*., 2007). Furthermore, the SMAD2/3 pathway, one of the pathways downstream of transforming growth factor β (TGFβ) superfamily ligands in mammals, mediates oocyte signals that contribute to the specification of mouse cumulus cell lineage and cumulus expansion (Diaz *et al*., 2007; Dragovic *et al*., 2007). Studies on *Smad2/3* conditional knockout mice in our group indicate that ovarian SMAD2 and SMAD3 are indispensable for normal cumulus expansion in mouse (Li *et al*., 2008). The requirement of SMAD2/3 signaling in the initiation of cumulus expansion and induction of cumulus expansion-related genes (Diaz *et al*., 2007; Dragovic *et al*., 2007) as well as the defective cumulus phenotype manifested in *Smad4* conditional knockout model (Pangas *et al*., 2006) reemphasize the fundamental role of oocyte-secreted factors of the TGFβ superfamily in promoting cumulus cell function. Although the nature of the cumulus expansion-enabling factors is still unclear, it is highly possible that growth differentiation factor 9 (GDF9) and other oocyte-secreted factors are the candidates [e.g. bone morphogenetic protein 15 (BMP15)]. GDF9 can signal through SMAD2/3 to regulate granulosa cell function (Kaivo-Oja *et al*., 2003; Gilchrist *et al*., 2006). GDF9 induces the expression of expansion-related transcripts [pentraxin 3 (*Ptx3*), hyaluronan synthase 2 (*Has2*), tumor necrosis factor alpha-induced protein 6 (*Tnfaip6*) and prostaglandin-endoperoxide synthase 2 (*Ptgs2*)] in mouse granulosa cells *in vitro* (Elvin *et al*., 1999a; Varani *et al*., 2002). Another attractive candidate is BMP15, which can also regulate the function of murine granulosa cells (Otsuka *et al*., 2000, 2001; Otsuka and Shimasaki, 2002). Cumulus expansion and subsequent ovulation are the result of a coordinated bidirectional communication between oocytes and their companion somatic cells (Russell and Robker, 2007). Thus, the granulosa cell pathways are regulated by the oocyte, and the functional properties of the granulosa cells, especially cumulus cells, are reflective of oocyte quality and the integrity of signaling machinery in the granulosa cell compartment. On the basis of this concept, gene expression profiling of granulosa cells may indirectly provide novel and reliable parameters to assess oocyte competence.

The transcriptional activity of the oocyte genome and the maturation of oocytes are modulated through the dialog between oocytes and somatic cells during follicular development (Eppig, 1991; Goud *et al*., 1998; De La Fuente and Eppig, 2001; Luciano *et al*., 2005). The oocytes are supported and nurtured by the closely associated somatic cells in ovarian follicles, and oocyte growth, meiotic resumption and function are regulated by granulosa cells (Eppig, 1991). For example, it has been well established that granulosa cell-secreted KIT ligand (KITL) can bind to its receptor, KIT, which is localized on the oocyte surface, to stimulate oocyte growth (Packer *et al*., 1994). Indeed, KITL is regulated by oocyte-secreted factors (Elvin *et al*., 1999b; Joyce *et al*., 2000; Otsuka and Shimasaki, 2002), and oocytes from *Gdf9* null mice have increased growth rate owing to the loss of inhibitory effects of GDF9 on *Kitl* expression (Carabatsos *et al*., 1998; Elvin *et al*., 1999b). A recent study from the Gilchrist group (Hussein *et al*., 2005) demonstrated that oocyte-secreted factors, especially BMP15 and BMP6, protect the cumulus cells from undergoing apoptosis by establishing a morphogenic paracrine gradient of BMPs. Undoubtedly, the oocyte will benefit from the microenvironment where cumulus cells can appropriately maintain their viability. These results provide additional compelling evidence that an oocyte creates a favorable microenvironment by utilization of its own surrounding somatic cells (Hussein *et al*., 2005). An interesting question is thereby posed: can oocyte-secreted factors be applied *in vitro* to enhance oocyte quality (Hussein *et al*., 2006; Gilchrist and Thompson, 2007; Yeo *et al*., 2008)? The following sections will briefly review recent progress in this field, with a focus on the potential of cumulus genes and oocyte-secreted factors as the respective predictors and regulators of oocyte competence.

Can granulosa cell-expressed markers become molecular predictors of oocyte competence?

Cumulus/mural granulosa cells are typically discarded during IVF and intracytoplasmic sperm injection. These cells are easily accessible and plentiful, which makes them an ideal material to utilize for the potential assessment of oocyte quality and embryo development potential. With the increasing desire to implement more objective and reliable criteria for oocyte/embryo selection, a significant amount of research has been recently conducted using both animal models and clinical patients to evaluate the granulosa cell gene signature(s) as molecular predictors of oocyte competence (McKenzie *et al*., 2004; Zhang *et al*., 2005; Cillo *et al*., 2007; Feuerstein *et al*., 2007; Assidi *et al*., 2008; Bettegowda *et al*., 2008; Hamel *et al*., 2008). Oocyte gene expression profiles have also been investigated in correlation with oocyte competence (Patel *et al*., 2007; Hamatani *et al*., 2008), which is beyond the focus of this review.

With the advent of the functional genomics era, it has become possible to identify the transcriptome of granulosa cells using high throughput technology such as microarray. Evidence supporting granulosa cell gene markers as predictors of oocyte competence is now emerging. By using suppressive subtractive cDNA hybridization and microarray technologies, the Sirard group (Assidi *et al*., 2008) identified several potential cumulus cell markers of bovine oocyte competence including several GDF9 target genes [i.e. *HAS2*, *TNFAIP6*, *PTGS2* and gremlin 1 (*GREM1*)] (Elvin *et al*., 1999a; Varani *et al*., 2002; Pangas *et al*., 2004). Other candidates identified are inhibin βA (*INHBA*), epidermal growth factor receptor (*EGFR*), betacellulin (*BTC*) and *CD44* molecule (CD44) (Assidi *et al*., 2008). Another recent study using a bovine 'poor oocyte competence' model (prepubertal calf model) and microarray analysis found that the transcript abundance of genes encoding the cathepsin family of cysteine proteinases (*CTSB*, *CTSS* and *CTSZ*) is negatively associated with bovine oocyte competence (Bettegowda *et al*., 2008). The potential role of these genes in apoptosis has been proposed by the authors as treatment

Table I. Granulosa cell markers potentially associated with oocyte competence.

Granulosa cell markers	Subject	Outcome/animal model	Sampling	Reference
Candidate gene experiment				
[b]*HAS2, PTGS2, GREM1*	Human	Day 3 embryo	CCs from individual COC	(McKenzie *et al*., 2004)
[b]*HAS2, GREM1*	Human	Day 3 embryo	CCs from individual COC	(Cillo *et al*., 2007)
[a]*STAR, PTGS2, AREG, CX43, SCD1, SCD5*	Human	Blastocyst	CCs from individual COC	(Feuerstein *et al*., 2007)
Microarray experiment				
[b]*PTX3*	Human	Day 3 embryo	Pooled CCs from COCs (array); CCs from individual COC (PCR)	(Zhang *et al*., 2005)
[b]*HSD3B1, FDX1, CYP19A1, SERPINE2, CDC42*	Human	Confirmed pregnancy	Mural GCs and CCs (pooled)	(Hamel *et al*., 2008)
[a]*GPX3, CXCR4, HSPB1, CCND2, DHCR7, DVL3, TRIM28, CTNND1*	Human	Early cleavage	CCs from individual COCs	(van Montfoort *et al*., 2008)
[b]*HAS2, TNFAIP6, PTGS2, GREM1, INHBA, EGFR, BTC, CD44*	Cattle	IVM-blastocyst rate	CCs from pooled COCs	(Assidi *et al*., 2008)
[a]*CTSB, CTSS, CTSZ*	Cattle	Poor oocyte quality model	CCs from pooled COCs	(Bettegowda *et al*., 2008)

Expression of markers is negatively (a) or positively (b) associated with oocyte competence. Only confirmed candidate genes are listed for the microarray experiment. CCs, cumulus cells; GCs, granulosa cells; COC, cumulus oocyte complex.

of cumulus–oocyte complexes (COCs) with cysteine proteinase inhibitor during IVM can reduce apoptotic cumulus cells and enhance the embryonic development potential of the oocytes (Bettegowda *et al*., 2008).

In search of potential human granulosa cell markers to complement the morphological criteria toward oocyte/embryo selection, our group found that the quality of human oocytes is correlated with transcript abundance for specific GDF9 targets (i.e. *HAS2*, *PTGS2* and *GREM1*) in the cumulus cell compartment (McKenzie *et al*., 2004). A subsequent study supported that *HAS2* and *GREM1* are candidate cumulus markers predictive of oocyte competence (Cillo *et al*., 2007). However, there is some controversy regarding the association of cumulus *PTX3* abundance with oocyte quality (McKenzie *et al*., 2004; Zhang *et al*., 2005; Cillo *et al*., 2007). Recently, Feuerstein *et al*. (2007) reported that a number of genes including *PTGS2*, steroidogenic acute protein (*STAR*), amphiregulin (*AREG*), stearoyl-co-enzyme A desaturase 1 and 5 (*SCD1* and *SCD5*) are associated with oocyte nuclear maturation and their transcript levels are elevated after meiosis resumption. Interestingly, lower cumulus mRNA abundance of the aforementioned genes as well as connexin 43 (*CX43*) is present in MII oocytes that develop to blastocysts (Feuerstein *et al*., 2007).

Microarray technology is now being applied to define the gene expression profiles of human ovarian somatic cells in correlation with oocyte developmental competence (Hamel *et al*., 2008; van Montfoort *et al*., 2008). One study compared granulosa cell (mainly mural granulosa cell) gene expression profiles between follicles associated with successful pregnancy and those associated with arrested embryo development during IVF (Hamel *et al*., 2008). Identified candidates that positively correlate with oocyte development potential include, but not limited to, genes associated with steroidogenesis [hydroxy-delta-5-steroid dehydrogenase 3 beta- and steroid delta-isomerase 1 (*HSD3B1*), ferredoxin 1 (*FDX1*) and cytochrome P450 (*CYP19A1*)] and genes with potential involvement in apoptosis [serpin peptidase inhibitor clade E member 2 (*SERPINE2*) and cell division cycle 42 (*CDC42*)] (Hamel *et al*., 2008). Another study attempted to identify differentially expressed genes between cumulus cells derived from oocytes that develop to early cleavage (EC) embryos and cumulus cells from oocytes that fail to develop into EC (NEC) embryos (van Montfoort *et al*., 2008). The transcripts of genes increased in NEC samples [glutathione peroxidase 3 (*GPX3*), chemokine receptor 4 (*CXCR4*), stress-induced apoptosis inhibitor (*HSPB1*), cyclin D2 (*CCND2*), 7-dehydrocholesterol reductase (*DHCR7*), etc.] are reflective of a potentially hypoxic state of the cumulus cell microenvironment or delayed maturation of the oocytes (van Montfoort *et al*., 2008). Surprisingly, none of the GDF9 target genes (*HAS2*, *PTGS2, PTX3* and *GREM1*) identified by other studies (McKenzie *et al*., 2004; Zhang *et al*., 2005) is overrepresented in the EC samples (van Montfoort *et al*., 2008).

The aforementioned studies have generated valuable information on granulosa cell gene expression profiling associated with oocyte competence in animal models and humans. However, consistent markers predictive of oocyte competence are lacking from these studies, although *HAS2* and *GREM1* emerge in three different studies (Table I). The inconsistency of molecular markers identified by these studies may result from the lack of a common standard used for embryo viability/competence (e.g. EC embryo versus confirmed pregnancy), differences in sampling (e.g. cumulus cells from individual COC versus pooled cumulus cells or mural granulosa cells) or distinct platforms utilized for genome-wide analyses. It should also be noted that the diagnostic power will be substantially lessened if embryos are grouped or multiple embryos are transferred in such experiments. Therefore, more comprehensive studies which potentially include SET to assess the pregnancy outcome are needed in a clinical setting to establish/standardize objective molecular markers.

Oocyte-secreted factors: key local regulators of oocyte competence?

Oocyte competence is profoundly affected by multiple endocrine, paracrine and autocrine factors during oogenesis and follicular development, the importance of which has been highlighted in culture systems (Eppig *et al*., 1996; Eppig *et al*., 2000, 2002; Thomas *et al*., 2003; Sirard *et al*., 2006). Herein, we focus on the perspective of oocyte-secreted factors because of their unique roles in coordinating folliculogenesis (Eppig, 2001; Matzuk *et al*., 2002) and the relative paucity of knowledge on these factors. Oocyte-secreted factors, especially GDF9 and BMP15, are principal regulators of follicular development and fertility (Moore *et al*., 2004; Juengel and McNatty, 2005; McNatty *et al*., 2005). *Gdf9* null mice are infertile with follicles arrested at the one-layer primary follicle stage, indicating the essential role of GDF9 in early folliculogenesis (Dong *et al*., 1996). In contrast, targeted disruption of mouse *Bmp15* results in subfertile animals with minimal histopathological alterations in the ovary except the defective cumulus phenotype (Yan *et al*., 2001). Recently, transgenic mice with oocyte overexpression of BMP15 (a chimeric protein of human BMP15 proregion–mouse BMP15 mature region) reveals the growth-

promoting role of BMP15 in ovarian follicles (McMahon *et al*., 2008). Our genetic studies clearly illustrate the interaction between BMP15 and GDF9 in the mouse ovary (Yan *et al*., 2001). Moreover, species-specific roles of oocyte-secreted factors have also been demonstrated, and it has been well documented that ewes carrying heterozygous mutations of BMP15 or GDF9 have enhanced ovulation rates, whereas ewes that are homozygous for BMP15 or GDF9 mutations are infertile with defective follicular development (Galloway *et al*., 2000; Montgomery *et al*., 2001; Hanrahan *et al*., 2004; McNatty *et al*., 2005). Distinct and cooperative roles of BMP15 and GDF9 in regulating ovulation rate in sheep were highlighted by the evidence that carriers of single-copy mutations of both BMP15 and GDF9 have higher ovulation rates than those with single mutations of either gene (Hanrahan *et al*., 2004). In corroborating the significant roles of these oocyte-secreted factors in follicular development and ovarian function in humans, mutations of both BMP15 and GDF9 genes have been identified in patients with premature ovarian failure (Di Pasquale *et al*., 2004; Dixit *et al*., 2006; Laissue *et al*., 2006) or, more accurately, primary ovarian insufficiency (Welt, 2008), although the association of BMP15 mutation with this disorder warrants further investigation (Ledig *et al*., 2008).

In further support of the interaction between BMP15 and GDF9, a recent study by Su *et al*. (2008) demonstrates the cooperativity of BMP15 and GDF9 in regulating cumulus cell cholesterol biosynthesis. However, little was known about the potentially complex interactions of the oocyte-secreted factors except GDF9 and BMP15, until the description of the cooperativity between BMP15 and another oocyte-produced factor, fibroblast growth factor 8B (FGF8B), in promoting glycolysis (Sugiura *et al*., 2007). The above study revealed that oocytes from *Bmp15*$^{-/-}$ mice and *Gdf9*$^{+/-}$; *Bmp15*$^{-/-}$ double-mutant mice are deficient in promoting glycolysis and inducing gene expressions of the glycolytic enzymes, platelet phosphofructokinase (*Pfkp*) and lactate dehydrogenase A (*Ldha*). To further address the role of GDF9 and BMP15, recombinant BMP15, GDF9 and FGF8B proteins were tested in the cumulus cell cultures. Through examining the various combinations of treatment, the authors demonstrated that combination of BMP15 and FGF8B is capable of promoting glycolysis and gene expression of glycolytic enzymes (Sugiura *et al*., 2007). Since mammalian oocytes are deficient in the glycolysis pathway and dependent on the glycolytic products from cumulus cells, the BMP15 and FGF8B from the oocytes regulating this important function may be of particular significance for oocyte development.

Although one major advantage of IVM versus traditional IVF is to bypass the ovarian stimulation procedure that may cause ovarian stimulation syndrome (Rao and Tan, 2005), IVM often produces low-quality oocytes in contrast to *in vivo* matured oocytes (Dunning *et al*., 2007). Since oocyte-secreted factors are of paramount importance in regulating cumulus cell functions favorable to oocyte development, an interesting question is raised: can oocyte-secreted factors be applied to IVM to improve the microenvironment surrounding the oocyte and thus enhance the quality of the oocyte and embryo development (Hussein *et al*., 2006; Gilchrist and Thompson, 2007)? Hussein *et al*. (2006) treated bovine COCs with BMP15 and/or GDF9 and observed an increase in the oocyte developmental potential to blastocyst stage. Furthermore, application of antagonists of BMP15 (follistatin) or GDF9 (ALK4/5/7 inhibitor; SB431542) can reduce the oocyte developmental competence (Hussein *et al*., 2006). The same group subsequently demonstrated that addition of GDF9 to the IVM medium can promote mouse embryo development and increase fetal viability without affecting embryo implantation rate (Yeo *et al*., 2008). These data generate enthusiasm in the field of assisted reproduction, although they are derived from animal models. In the future, extensive research should be undertaken to evaluate the effectiveness of combinations of oocyte-secreted factors in promoting oocyte competence in both animal models and humans.

Summary

Recent studies on the intercellular communication between germ cells and companion somatic cells reveal the potential of cumulus cell markers as reliable molecular predictors of oocyte developmental competence, as well as the oocyte-secreted factors as enhancers of oocyte quality (Table I and Fig. 1). Prior to the establishment of standardized criteria for oocyte selection utilizing cumulus cell gene markers as key parameters, well-controlled clinical studies are needed to evaluate the potential of various reported cumulus gene candidates as predictors of oocyte quality. The verified candidates from different functional categories may thus be included as markers for oocyte competence. One major challenge faced to evaluate the effectiveness of oocyte-secreted factors to improve oocyte competence in human IVM stems from our limited knowledge of the identity and/or cooperativity of these factors in the oocyte–somatic cell regulatory loop. Given the potentially complex interaction among oocyte-secreted factors (Su *et al*., 2004; Sugiura *et al*., 2007; Su *et al*., 2008) and their divergence among species (Juengel and McNatty, 2005; Bettegowda *et al*., 2007), the question is: what combination of the oocyte-produced factors may have the most potent synergistic effect in the human IVM system? Moreover, the hurdle of lack of standardized preparations of the recombinant proteins (Pangas and Matzuk, 2005) should be overcome before the clinical trials can be implemented. With the availability of a reliable approach capable of identifying the best quality oocytes/embryos, multiple embryo transfer

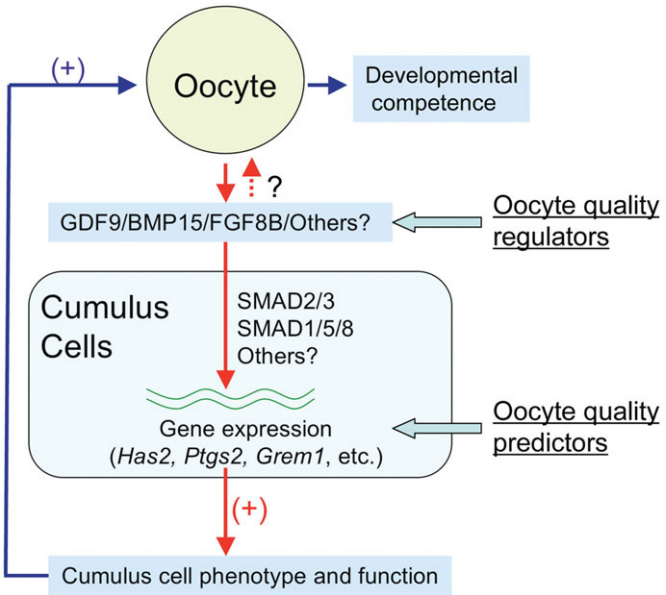

Figure 1: Potential intrafollicular predictors and regulators of oocyte quality within the oocyte–somatic cell regulatory loop. Oocyte-secreted factors (GDF9, BMP15, FGF8B and other unknown factors) can act on the adjacent cumulus cells via SMAD2/3, SMAD1/5/8 or other pathways to induce the expression of genes from a variety of categories (e.g. cumulus expansion-related genes *Has2* and *Ptgs2*). Some of the markers may be indirect and reliable parameters to assess oocyte competence. The oocyte-produced factors can regulate numerous cumulus cell functions such as cumulus expansion, apoptosis, metabolism (glycolysis and cholesterol synthesis), and these functions are critical in the development of oocyte competence. It is unclear whether oocyte-secreted factors can signal through autocrine pathway to regulate oocyte function. GDF9, growth differentiation factor 9; BMP15, bone morphogenetic protein 15; FGF8B, fibroblast growth factor 8B; *Has2*, hyaluronan synthase 2; *Ptgs2*, prostaglandin-endoperoxide synthase 2; *Grem1*, gremlin 1.

may no longer be necessary in the future. Undoubtedly, further studies delineating the nature of novel oocyte-secreted factors and deciphering the interaction/cooperativity of these factors will be informative and should shed new light on our understanding of the enigma of oocyte maturation and developmental competence.

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