

—Mini Review—

## **Role of Oocyte-derived Factors in Ovarian Follicular Development and Ovulation**

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**Abstract:** Coordination of extra- and intrafollicular signals is required for the development of ovarian follicles and for the production of functional oocytes. Oocytes play an active role in this coordination. Oocytes produce two families of growth factors: members of the transforming growth factor beta (TGF $\beta$ ) superfamily, including bone morphogenetic protein (BMP) 6, BMP15, and growth differentiation factor 9 (GDF9); and members of fibroblast growth factors (FGFs), including FGF8. These oocyte-derived paracrine factors, in coordination with the other intrafollicular signals, regulate the development and function of oocyte-associated cumulus cells. In this review, we first summarize the role of oocytes in follicular development and ovulation, focusing on the effects of oocyte-derived paracrine factors on the development and function of cumulus cells. In addition, we summarize recent findings on the coordination of oocyte-derived signals with other intrafollicular signals, such as estrogen and epidermal growth factor (EGF) receptor signals, in cumulus cell development and function, and discuss the potential mechanisms driving this coordination.

**Key words:** Oocyte, Cumulus cell, BMP15, GDF9, FGF8

### **Introduction**

The roles of oocytes in the early stage of follicular development are revealed by targeted deletion of a gene encoding an oocyte-specific transcription factor FIGLA (folliculogenesis specific basic helix-loop-helix) and oocyte-specific knockout of a gene encoding PTEN (phosphatase and tensin homolog, a major negative regulator of phosphatidylinositol 3-kinase), which result

in failure to form primordial follicles and the activation of the entire pool of primordial follicles, respectively [1, 2]. Although pituitary hormones, follicle stimulating hormone (FSH) and luteinizing hormone (LH), are necessary for the development of antral follicles and subsequent ovulation [3–6], it is now evident that oocytes also play an active role in these processes. Oocytes, in coordination with other intrafollicular signals, affect antral follicular development probably by communicating with surrounding granulosa cells through paracrine and juxtacrine signals. Paracrine signals produced by oocytes include several members of the transforming growth factor beta (TGF $\beta$ ) superfamily and fibroblast growth factors (FGFs). These oocyte-derived factors are essential for the development and function of cumulus cells, which are a granulosa cell sub-population specialized in supporting oocyte development. This bi-directional communication between oocytes and cumulus cells is essential for the normal development of follicles as well as the development of both cell types [7]. In addition to bi-directional communication, recent studies suggest that oocyte-derived signals play a critical role in coordinating other intrafollicular signals. This mini review focuses on the role of oocytes during antral follicular development and ovulation with special emphasis on the effects of oocyte-derived paracrine factors on the development and function of cumulus cells. In addition, we summarize the current state of knowledge of the mechanism by which oocyte-derived signals coordinate other intrafollicular signals.

### **Bi-directional Communication between Oocytes and Cumulus Cells**

The first step of folliculogenesis is formation of primordial follicles in which oocytes arrested in the prophase of meiosis I are surrounded by a single layer

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of flattened somatic cells, often called pre-granulosa cells. Once primordial follicles are activated to form primary follicles, the oocytes start to grow and the surrounding somatic cells, now called granulosa cells, become cuboidal and proliferative. When oocytes at mid-growth are surrounded by more than one layer of granulosa cells, follicles are considered to be secondary follicles. As follicles grow, fluid-filled cavities develop between the layers of granulosa cells. Follicles of this stage are called antral follicles. Formation of antrum divides granulosa cells into two functionally and spatially different sub-populations of granulosa cells: cumulus cells associated with oocytes and mural granulosa cells lining the follicular wall.

It is well established that cumulus cells play a critical role in the development of oocytes. Cumulus cells play a nurturing role in support of oocyte growth [8], promote acquisition of the competence to undergo fertilization and preimplantation development [8], participate in the global transcriptional suppression of oocytes before the resumption of meiotic progression [9], regulate the intracellular pH of growing oocytes [10], and participate in the meiotic arrest of oocytes by providing oocytes with cGMP [11]. On the other hand, oocytes affect proliferation, steroidogenesis, and in mice, expansion of cumulus cells (reviewed in [7]). In addition, a recent study has shown that production of cGMP by cumulus cells requires cumulus cell expression of *Npr2* transcripts encoding natriuretic peptide receptor 2, a guanylyl cyclase, and that oocyte-derived factors promote expression of *Npr2* mRNA by cumulus cells [12]. This indicates that oocytes participate in the maintenance of their own meiotic arrest, at least in mice.

The roles of specific growth factors produced by oocytes in regulating the development and function of cumulus cells as well as folliculogenesis are discussed in more detail below.

### Oocyte-derived Factors

Mammalian oocytes produce at least two families of growth factors: members of the TGF $\beta$  superfamily, including bone morphogenetic protein (BMP) 6, BMP15, and growth differentiation factor 9 (GDF9); and members of FGFs, including FGF8.

#### TGF $\beta$ superfamily

Many studies have investigated the function of oocyte-derived members of the TGF $\beta$  superfamily *in vivo* and *in vitro* [13–15]. Female mice deficient in *Gdf9*

are infertile due to a block in folliculogenesis at the primary stage [16, 17]. Consistent with this, recombinant GDF9 promotes primary to early antral follicular development in rat and human ovaries [18–20]. In the hamster, however, recombinant GDF9 promotes, whereas RNA interference-mediated silencing of *Gdf9* mRNA prevents, formation of primordial follicles [21, 22]. This indicates that GDF9 is critical for the formation of primordial follicles in the hamster, a characteristic that has not been reported in mice, rats and humans. This difference in requirement of GDF9 in primordial follicular formation may be explained by differential expression of GDF9 during oocyte development among species. In mice and rats, GDF9 is first expressed in the oocytes of primary follicles [23–26], whereas in the hamster, GDF9 protein is detected in the oocytes of primordial follicles [21].

Because ovarian follicles of *Gdf9* deficient animals do not develop beyond the primary stage, the function of GDF9 during antral follicular development has mostly been assessed using recombinant proteins. Recombinant GDF9 suppresses FSH-stimulated expression of *Lhcgr* mRNA, encoding luteinizing hormone/choriogonadotropin receptors, in granulosa cells, and promotes expansion of cumulus cells in mice [25]. Furthermore, recombinant GDF9 promotes proliferation of granulosa cells in mice and rats [27–29]; however, the proliferative effect of recombinant GDF9 was not detected in some studies [19, 30, 31]. It needs to be noted that different preparations of recombinant GDF9 have been used by different labs in the past, and the variation in the quality of those preparations may account for the diverse effects of GDF9 observed by different research groups [32].

The other members of the oocyte-derived TGF $\beta$  superfamily are BMP6 and BMP15 [24, 33–38]. Similar to *Gdf9* null mice, naturally occurring point mutations in sheep *BMP15* profoundly affect female fertility. Ewes carrying a homozygous mutation of *BMP15* are infertile, whereas heterozygous mutants exhibit an increase in ovulation rate [39–42]. Also, ewes carrying a mutation in a gene encoding a potential receptor for BMP6/15, BMPR1B (also known as ALK6), exhibit increased fertility [43–46]. In contrast to sheep, targeted deletion of the *Bmp15* or *Bmp6* gene does not affect the early stages of follicular development in mice [47–49]. Female mice deficient in *Bmp15* and/or *Bmp6* are subfertile with minimal effects on follicular development [47–49]. These species-specific differences in requirement of the BMP signal for female fertility have been implicated in the differences in ovulation rate

among species [50]. However, conditional deletion of genes encoding SMAD1/5/9 (formerly known as SMAD1/5/8), mediators of BMP signaling, in granulosa cells results in development of metastatic granulosa cell tumors and female mutant mice become infertile [51, 52]. Likewise, female mice deficient in *Bmpr1b* are infertile due to impaired cumulus cell function [53]. Therefore, it is important to note that BMP signaling is required for follicular development and female fertility in mice as in sheep. Oocyte-derived and the other BMP ligands produced within the follicles may constitute a total BMP signaling system in follicles, and this entire BMP signal is probably essential for normal follicular development and fertility in female mice.

Mutations in *BMP15* and *GDF9* have been reported in women with premature ovarian failure and polycystic ovary syndrome [54–60]. In addition, some human variants of *GDF9* are implicated in the likelihood of spontaneous dizygotic twinning [61, 62]. Therefore, it is likely that BMP15 and GDF9 also play critical roles in the fertility of women.

It is evident that there are synergistic interactions between GDF9 and BMP15 with respect to the development of granulosa cells and follicles. As mentioned above, *Bmp15* null (*Bmp15*<sup>-/-</sup>) mice are subfertile with relatively mild ovarian phenotypes [47]. Interestingly, although ovarian defects are not observed in *Gdf9* heterozygous mutant (*Gdf9*<sup>+/-</sup>) mice, *Bmp15*<sup>-/-</sup>/*Gdf9*<sup>+/-</sup> female mice show more severe ovarian defects than those observed in *Bmp15*<sup>-/-</sup> mice [47, 63]. In fact, a recent microarray study has identified that expression levels of more than 2,600 transcripts are significantly different between cumulus cells of *Bmp15*<sup>-/-</sup> and *Bmp15*<sup>-/-</sup>/*Gdf9*<sup>+/-</sup> mice [64]. The underlying mechanism of this cooperative interaction between BMP15 and GDF9 is not well understood, however, a recent study has suggested the involvement of a proregion of BMP15 in this cooperation [65].

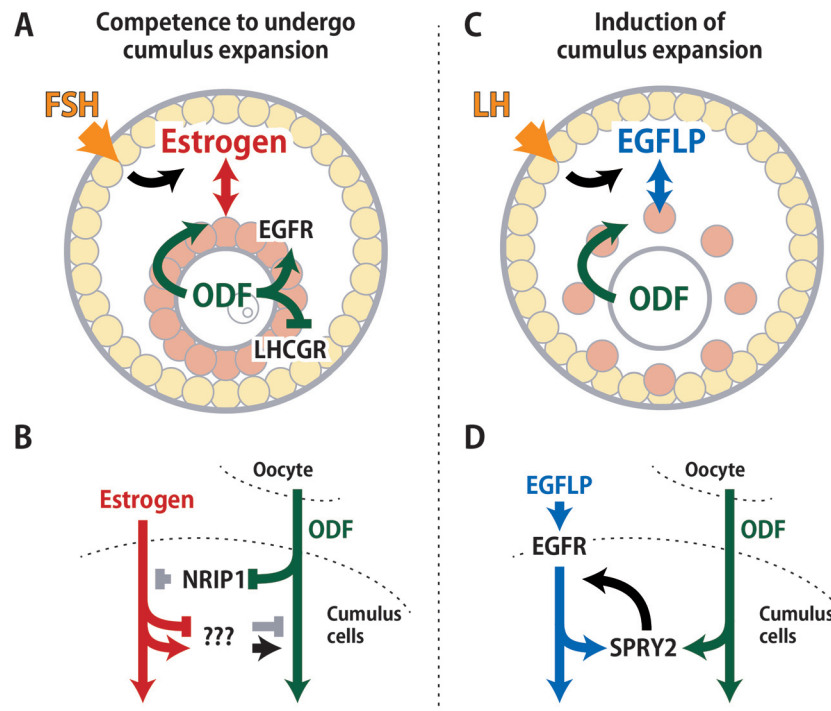
#### FGFs

The other growth factor family produced by mammalian oocytes is the FGF family, including FGF8 [66–68]. Expression of FGF receptors has been reported in granulosa cells of many species including the human, mouse, rat and bovine [69–72]. In addition, several mutant mouse models with attenuated FGF receptor signaling exhibit fertility defects in females [73–76]. Although these reports suggest oocyte-derived FGF signals may be important for the development and

function of granulosa cells, no evidence for this has been presented until recently. Recently, it was shown that oocyte-derived FGFs, mainly FGF8, cooperate with BMP15 to promote glycolysis in cumulus cells in mice [68]. It has long been recognized that oocytes are deficient in carrying out glycolysis, and depend on the product of glycolysis provided from cumulus cells for their own development [77–79]. Therefore, it is now apparent that oocytes regulate the metabolic cooperativity between oocytes and cumulus cells [64, 80–83].

Another example of cooperative interaction of FGF8 and BMP signals was recently provided in rat granulosa cells [84]. BMP signals stimulate phosphorylation of SMAD1/5/9 as well as promoter activity of a BMP target gene in rat granulosa cells, and these effects of BMP signals are further enhanced by addition of FGF8. Moreover, while FGF8 alone has little effect on FSH-induced cAMP production by rat granulosa cells, it augments the effects of BMP signals on the suppression of FSH-induced cAMP production.

Mechanisms involved in the crosstalk between ovarian follicular BMP and FGF signals in cumulus cells have not been determined. A recent study showed the potential involvement of a FGF antagonist, sprouty 2 (SPRY2), in this cooperation [85]. Expression of *Spry2* transcripts is promoted in isolated cumulus cells by FGFs, including FGF8, suggesting the presence of a negative-feedback system that suppresses FGF signaling in cumulus cells. However, BMP signals, including BMP6 and BMP15, suppress FGF-induced *Spry2* expression in cumulus cells, therefore BMP signals may promote the FGF signaling by suppressing the negative-feedback system. Expression of transcripts encoding sprouty family proteins has been also reported in human granulosa-lutein cells [86] and bovine granulosa cells [87]; thus, it is possible that a similar mechanism is involved in the signal crosstalk in these species. Also, in contrast to the FGF-induced *Spry2* expression in cumulus cells, which is suppressed by oocytes, epidermal growth factor (EGF)-induced *Spry2* expression is promoted by oocytes [85]. Since mammalian SPRY proteins appear to potentiate EGF receptor signals by preventing ubiquitylation and degradation of activated EGF receptors [88–91], it is possible that oocytes potentiate EGF receptor signals in cumulus cells by promoting *Spry2* expression in cumulus cells (see below).



**Fig. 1.** Coordination of intrafollicular signals in the maintenance of cumulus expansion competence (A) and the induction of cumulus expansion (C). Hypothetic models of the coordination of oocyte-derived paracrine factors (ODF) with an estrogen signal (B) or an EGF-like peptide (EGFLP) signal (D) are presented (see text for details).

### Crosstalk of Oocyte Signaling with Other Intrafollicular Signals in Cumulus Cells: Facts and Potential Mechanisms

Although it is evident that oocytes play a dominant role in determining the rate of follicular development [92], participation of other follicular signals, such as FSH, LH and steroids, is also critical. Emerging evidence suggests that oocytes play an active role in the coordination of these intrafollicular signals during follicular development and ovulation. Expansion or mucification of cumulus cells, that is a prerequisite process for normal ovulation [93], requires signal coordination by oocytes (Fig. 1). Here, we summarize current knowledge of the coordination of intrafollicular signals by oocytes using mouse cumulus expansion as an example.

#### Competence to undergo cumulus expansion

Oocyte-associated granulosa cells of late secondary follicles differentiate into cumulus cells and acquire the competence to undergo expansion during antral follicle

development [94]. Oocyte-derived BMP15 and GDF9 are required for developing the competence as demonstrated by the fact that cumulus cells of *Bmp15*<sup>-/-</sup> or *Bmp15*<sup>-/-</sup>/*Gdf9*<sup>+/-</sup> mice do not undergo normal cumulus expansion [47, 63]. In addition to oocyte-derived factors, other factors such as estrogen are also required for acquisition and/or maintenance of competence [95–99]. A very recent study revealed interactions of estrogen and oocyte signals in the acquisition and maintenance of competence to undergo cumulus expansion in mice (Fig. 1A) [100]. The study showed that estrogen can maintain the competence to undergo expansion of cumulus cell-oocyte complexes (COCs) isolated from antral follicles, but this effect of estrogen requires oocyte-derived BMP15 and/or GDF9. Furthermore, expression levels of *Nrip1* transcripts encoding nuclear receptor interacting protein 1, a potential inhibitor of estrogen receptor signals, are suppressed in cumulus cells by oocytes or recombinant GDF9 [100]. This suggests that, in addition to the direct effect of oocytes on estrogen production by cumulus cells [101], oocytes may also amplify the estrogen

signal in cumulus cells by suppressing expression of the estrogen signal antagonist, NRIP1 (Fig. 1B). In addition to NRIP1, other unidentified factors (indicated as “???” in Fig. 1B) that are regulated by estrogen and are capable of affecting oocyte signals positively or negatively are likely to participate in the estrogen-oocytes cooperation (Fig. 1B). Therefore, it is likely that oocytes and estrogen signals are mutually dependent on each other. Identifying factors that participate in this estrogen-oocyte crosstalk will be an important step toward understanding the mechanism of follicular development.

#### Induction of cumulus expansion

Cumulus expansion *in vivo* is induced by the LH surge [102], and requires expression of transcripts encoding HAS2, PTGS2, PTX3 and TNFAIP6 [103–107]. Since expression of LH receptor (*Lhcgr*) in cumulus cells is suppressed by oocyte-derived GDF9 [25], the LH signal is not directly transduced to cumulus cells (Fig. 1C). LH induces production of EGF-like peptides by mural granulosa cells, and these peptides then bind to EGF receptor (EGFR, also known as ERBB1) expressed by cumulus cells to induce cumulus expansion and maturation of oocytes [108–110]. Oocyte-derived BMP15 and GDF9 enable this process by promoting expression of *Egfr* transcripts in cumulus cells (Fig. 1C) [111].

The EGFR signal is mediated by MAPK3/1 (also known as ERK1/2), and cumulus expansion requires phosphorylation of MAPK3/1 in cumulus cells [112, 113]. In addition, a recent study has shown that sustained activity of the EGFR-MAPK3/1 system in cumulus cells is required for cumulus expansion [114]. Importantly, oocyte-derived paracrine signals, probably GDF9 and/or BMP15, are required for cumulus expansion [25, 115, 116], and for MAPK3/1 activation in cumulus cells [117]. The mechanisms driving the oocyte-derived signals participating in the sustained activity of the EGFR signal have yet to be discovered. As mentioned above, mouse oocytes promote EGF-induced *Spry2* mRNA expression in cumulus cells [85], probably preventing inactivation of activated EGFR signal by preventing the degradation of EGFR [88–91]. Therefore, it is possible that oocytes may sustain the EGFR signal in cumulus cells by promoting *Spry2* expression in cumulus cells (Fig. 1D).

#### Conclusion

Over the past two decades, significant new insights

into the communication between oocytes and cumulus cells have accumulated. Now it is apparent that oocytes are not merely passive recipients of support from associated cumulus cells, but rather play an active role in regulating the development and function of cumulus cells. In addition, the rate of follicular development is determined by the stage of oocyte development [92]. Oocytes may use their abilities to regulate granulosa cell metabolism [82, 83] and to coordinate intrafollicular signals to orchestrate the rate of follicular development. The current challenges are to discover the underlying mechanism of the coordination and the interaction of oocyte and other intrafollicular signals. Better understanding of the mechanisms governing follicular development would facilitate the development of new approaches for assessing oocyte quality and improving the fertility of women.

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