—Mini Review—

Cumulus Cells are an Essential Mediator of Ovulation Stimuli from Granulosa Cells to Oocyte

Masayuki Shimada[1] and JoAnne S. Richards[2]*

[1]Laboratory of Reproductive Endocrinology, Graduate School of Biosphere Science, Hiroshima University, Hiroshima 739-8528, Japan
[2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, 77030, USA

Abstract: *In preovulatory follicles, oocytes are surrounded by numerous layers of cumulus cells in a known as the cumulus cell-oocyte complex (COC). After stimulation of ovulation by the LH surge, the morphology of COC is dramatically changed as a hyaluronan rich matrix accumulates within cumulus cell layer, and the oocyte resumes meiosis by progressing to the metaphase II. Although both changes induced by LH surge are essential for successful fertilization in vivo, the expression of LH receptors is not detected in the oocyte and is minimal (negligible) in cumulus cells compared with granulosa cells. However, cumulus cells express members of the EGF receptor family (ErbB family), prostaglandin receptors (EP2 and EP4) and cytokine family receptors that respond to specific ligands secreted by granulosa cells during the ovulation process. By these intermediary steps, the cumulus cells mediate LH signaling from granulosa cells to induce oocyte maturation. This minireview focuses on the role of cumulus cells in oocyte maturation at the physiological and molecular levels.*

Key words: *EGF like factor, Progesterone, PGE2, ERK1/2, PI 3-kinase*

Introduction

Mammalian oocytes arrested at the diplotene stage of the first meiotic prophase are closely surrounded by somatic cells (designated pre-granulosa cells in primordial follicles and cumulus cells in growing follicles). During early stages of follicle growth, granulosa cells and cumulus cells transport energy sources and other factors into oocytes via numerous gap junctions, promoting oocyte growth to full development size [1–3]. After the preovulatory LH surge, the oocytes resume meiotic maturation, complete germinal vesicle breakdown (GVBD), and progress to metaphase II (MII) before ovulation. Because LH receptors (LHCGR) are not present on the surface of oocytes and because the expression in cumulus cells is minimal compared with that in granulosa cells [4], the LH surge dramatically and directly changes the pattern of gene expression and protein synthesis in granulosa cells [5]. Factors induced and secreted rapidly from granulosa cells then act on cumulus cells to induce hyaluronan synthesis and its accumulation within cumulus cell layers during the ovulation process [6]. Concomitantly with these differentiation-dependent changes in cumulus cell function, meiotic nuclear maturation and cytoplasmic maturation are also induced, thereby supporting the formation of the pronucleus and development of the early embryo [7]. These data suggest that cumulus cells play a critical role in oocyte maturation, however there is less information about the mechanisms by which cumulus cells differentiate and how these changes in cumulus cells impact oocyte maturation at the molecular level. The specific gene expression profiles, protein synthesis patterns and other modifications in cumulus cells of COCs during the ovulation process are the focus of this review.

The Function of EGF Like Factor on Cumulus Cells

Because cumulus cells of mouse and rat possess few

Received: November 26, 2009
Accepted: December 8, 2009
*To whom correspondence should be addressed.
e-mail: mashimad@hiroshima-u.ac.jp

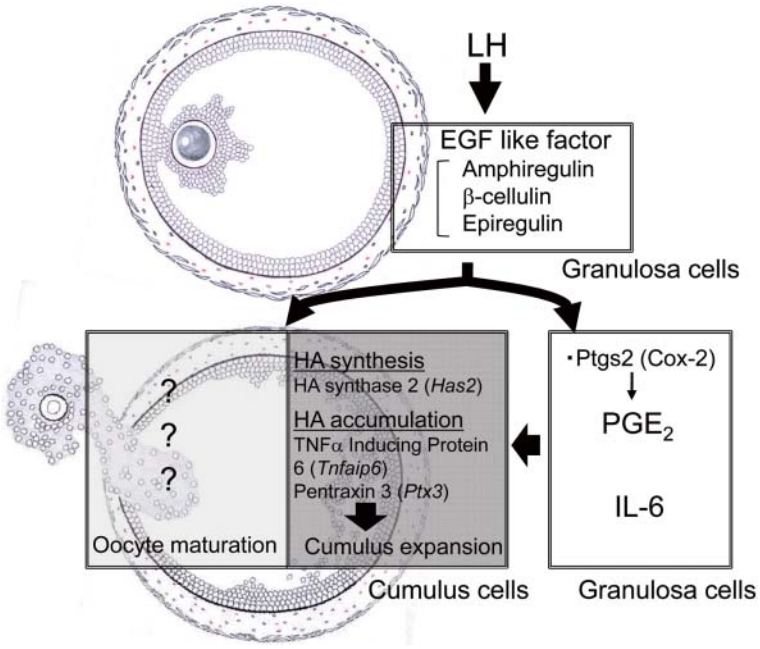

Fig. 1. The function of EGF like factor in cumulus cells.

if any LH receptors, the molecular and biochemical mechanisms by which LH impacts COC expansion have remained unclear. However, earlier studies have indicated that factors other than LH can induce cumulus expansion and oocyte maturation in culture, including growth factors [8].

A possible role of EGF or EGF like factors in the ovulation process was strengthened by recent observations showing that LH induces the expression of mRNAs encoding the EGF-like factors amphiregulin (AREG), epiregulin (EREG) and betacellulin (BTC) in mouse and rat preovulatory follicles [9, 10]. Furthermore, an EGF receptor (EGFR) tyrosine kinase inhibitor (AG1478) blocked LH-mediated COC expansion and oocyte maturation in explanted whole follicles in culture [9–11]. Additionally, mutant mice null for both *Areg* and homozygous for *Egfr wa2* (*Areg-/-Egfrwa2/wa2*), exhibited significant reduction of EGFR phosphorylation in cumulus cells, impaired expansion of cumulus oocyte complexes and arrest of meiosis at the germinal vesicle (GV) stage in oocytes [12]. Thus, LH induction of EGF like factors in granulosa cells plays a critical role in oocyte maturation via an EGFR-dependent mechanism operative in cumulus cells (Fig. 1).

The expression of EGF like factors is induced rapidly (within 1 hr) by the LH surge, and reaches a maximum level at 2 hr post-LH treatment [9]. Factors downstream of EGF receptor activation are also transiently activated in both granulosa cells and cumulus cells within 2 hr after LH [13, 14], however the expression level of genes involved in cumulus expansion, such as *Has 2*, *Tnfaip6* or *Ptx3* are also increased within 2 hr and are maintained at high levels for at least 4 hr after the LH surge [15], suggesting that the cumulus cell expansion is achieved by additional factors. In our microarray database derived from analyses of gene expression profiles in cultured granulosa cells, AREG upregulates *Ptgs2* and *Il6* mRNA expression (Shimada *et al.*, unpublished data). *Ptgs2* encodes prostaglandin synthase 2, the rate-limiting enzyme in the synthesis of PGE2 [16, 17]. When mouse COCs are cultured with PGE2, cumulus expansion is observed [18]. In mice null for the prostaglandin E receptor 2 (*Ptger2*) or *Ptgs2*, the expression levels of *Tnfaip6* mRNA and protein in cumulus cells are significantly reduced compared to wild type mice [18] and the degree of cumulus expansion is impaired in both mutant mouse models [19, 20]. Thus, PGE2 is a secondary mediator of LH-EGF-like factor induction of cumulus expansion during the ovulation process (Fig. 1).

Recently, we have shown that the cytokine family, including IL-6, is expressed in granulosa cells and secreted by the Ca^{2+}-regulated exocytosis system [21]. The exocytosis process is dependent on the binding of

SNAP 25 and synaptotagmin 1 (SYT1) that are also induced in granulosa cells during ovulation process [22]. The secreted IL-6 can activate the co-receptors, IL6R and GP130, that are expressed on cumulus cells and thereby induce cumulus expansion, and oocyte cytoplasmic maturation [23]. Because AREG induces *Il6*, *Snap25* and *Syt 1* gene expression in cultured granulosa cells, the cytokine pathway might be required for the progression of cumulus expansion and oocyte maturation.

Signal Transduction in Cumulus Cells during Ovulation Process

During the ovulation process, multiple signaling pathways are activated in cumulus cells and granulosa cells. The activated EGFR, in turn, activates the RAS-cRAF-MEK1-ERK1/2 pathway in cumulus cells [14, 24]. When COCs are cultured with the MEK1 inhibitor (PD98059 or U0126), cumulus expansion is blocked dramatically [25, 26]. In ERK1/2 mutant mice in which both kinases are depleted in granulosa cells and cumulus cells, not only cumulus cell expansion but also oocyte meiotic resumption are completely suppressed [14], suggesting that the ERK1/2 pathway in cumulus cells plays an important role in oocyte maturation. ERK1/2 can regulate numerous genes via the phosphorylation and activation of key transcription factors, such as members of the AP-1 family (Fra2 and C-Jun) that are present in granulosa cells [27, 28]. The promoter of the *Tnfaip6* gene has an AP-1 site, and Fra2 and JunD present in nuclear extracts purified from bovine granulosa cells and bind the AP-1 site of *Tnfaip6* promoter region [29]. Other ERK1/2 target transcription factors are the CCAAT enhancing binding proteins (C/EBPα/β) [30, 31] that are expressed during ovulation and C/EBPβ can mediate induction of selected genes [14]. In C/EBPβ null mice, *Ptgs2* expression level was significantly lower than that in wild type mice [14, 32]. In *Ptgs2* KO mice, *Tnfaip6* expression was significantly decreased [18], suggesting that ERK1/2 regulates *Tnfaip6* gene expression by both direct and indirect mechanisms via PGE2 production to induce cumulus cell expansion.

The cAMP-PKA pathway is also essential for the induction of ovulation and oocyte maturation. A cAMP analog that activated PKA strongly induced COC expansion [33], whereas a PKA inhibitor suppressed the cumulus cell differentiation and oocyte maturation in *in vitro* culture [34, 35]. In granulosa cells, the LH receptor (LHCGR) directly induced cAMP production via G protein α subunit type S (Gαs)-induced adenylcyclase activation [36], whereas in cumulus cells the expression level of the receptor is much lower or even absent [4]. However, in cumulus cells, PGE2 rather than LH increases the level of cAMP [18, 37]. cAMP binds to the regulatory subunit of PKA and releases the catalytic (C) subunit [38], thereby allowing the free catalytically active C subunit to phosphorylate targeted proteins [39]. One such target is the cAMP response element binding protein (CREB). When CREB is phosphorylated by PKA, it binds to CRE sites of the promoter regions of specific genes [40] including the LH targets Areg, *Ptgs2* and *Tnfaip6* [41–43]. However, of these genes, only *Areg* and *Ereg* are induced by LH in ERK1/2 null mice indicating that ERK1/2 transcription factor targets are most potent in the control of *Ptgs2* and *Tnfaip6* [14]. Thus, the cAMP-PKA-CREB pathway is essential for signaling in cumulus cells during ovulation process, but its targets are more restricted than those of AREG/ERK1/2 mediated events.

When COCs are cultured with IL-6, the transcription factors STAT3 and STAT5 are also phosphorylated and activated in cumulus cells [23] via the IL6R and GP130-activated JAK pathway, and presumably bind to the promoter regions of target genes [44, 45]. In support of this, a JAK inhibitor suppressed the phosphorylation of STAT(s), the expression of *Has2*, *Tnfaip6* and *Ptx3*, and impaired cumulus expansion [23]. Additionally, because IL-6 induces higher levels of *Runx1*, *Runx2*, and *Rip140/Nrip1*, it is possible that these transcriptional regulatory factors are regulated, in part, by the JAK/STAT signaling cascade. Because the EGF-like factors (AREG/EREG/BTC) peak 2 hr after hCG injection *in vivo*, whereas IL-6 increases dramatically 4 or 8 hr after hCG, it is tempting to speculate that the IL-6/JAK/STAT pathway as well as IL-6 activation of ERK1/2 act to help maintain the levels of key matrix genes as well as *Runx1*, *Runx2*, *Cepbb*, and *Nrip1* when levels of the EGF like factor-EGFR-ERK1/2 pathway decline [23].

The Role of Cumulus Cells in Oocyte Maturation

Although the ERK1/2 pathway in oocytes is not essential for the resumption of meiosis, the MEK inhibitor significantly suppresses this process [25, 46] presumably by blocking the activity or ERK1/2 in cumulus cell and granulosa cells. Moreover, EGF like factors can induce oocytes to resume meiosis and reach metaphase II stage, indicating that the activated

EGF receptor pathway in cumulus cells is required for the resumption of meiosis [9, 47]. Additionally, in *Ptgs2* knockout mice, in which the expression of EGF like factors is reduced [10], oocyte meiotic resumption and progression to the MII stage are delayed as compared with those in wild type mice [37]. Collectively, these results indicate that the EGF like factor/EGFR/ERK1/2 pathway is critical for oocyte maturation. This conception was supported recently by the total block of oocyte maturation in mutant mice lacking ERK1/2 in somatic cumulus/granulosa cells but not in the oocyte [14].

When cumulus cells are stripped from the oocytes and then the denuded oocytes are cultured, oocyte meiosis progresses to the MII stage. However, the matured oocytes exhibit less fertilization and developmental competence than those in matured in intact COCs [7]. The data indicate that cumulus cells provide specific support for oocytes to acquire competence (cytoplasmic maturation). IL-6 may be one, but not the only factor needed to enhance cytoplasmic maturation [23].

In amphibians, sea urchins and fish, the follicle cell-secreted factors that directly act on oocyte to induce oocyte maturation have already been defined; however, the precise factors controlling mammalian oocyte maturation remain to be defined clearly. Bayskov *et al.* [48] reported that meiosis-activating sterols (FF-MAS) can induce meiotic resumption of mouse oocytes. FF-MAS (4,4-dimethyl-5α-cholesta-8,14,24-trien-3β-ol) was first purified from human follicular fluid and has been shown to be synthesized by follicular cells in response to LH (hCG) stimulation [49, 50]. This type of sterol is an intermediate in the cholesterol biosynthetic pathway where it is produced by 14 alpha-demethylase and is metabolized by delta 14-reductase [51, 52]. Because *de-novo* cholesterol synthesis and enzymes of the cholesterol biosynthetic pathway are induced and activated in granulosa cells and cumulus cells to produce progesterone [53–55], and because a receptor for FF-MAS has not been defined in the oocyte [56], the physiological function(s) of FF-MAS remain unclear. It has been reported that Leydig insulin-like 3 (INSL3) is expressed in theca cells after the LH surge and acts on LGR8, a G protein coupling receptor expressed in oocytes [57]. LGR8 activates Gαi that in turn can activate the PI 3-kinase pathway that is presumed to be involved in cAMP degradation via phosphodiesterase type III [57–59]. Thus, INSL3 can induce meiotic resumption of oocytes. However, the physiological significance of this is unclear because the meiotic resumption of oocytes is completely suppressed in mutant mice lacking ERK1/2 in granulosa/cumulus cells but not theca cells. Although in this context, INSL3 does not appear to be the necessary and sufficient factor, INSL3 expression in theca cells of ERK1/2 mutant granulosa cells and cumulus cells remains to be determined.

The Positive Effects of Cumulus Cell Expansion on Fertilization

Our microarray analyses revealed that pattern recognition receptors (PRRs) of the Toll-like receptor (TLR) family and related molecules are expressed in cumulus cells of ovulated COCs and exhibite functions similar to those in macrophages [60, 61]. Specifically, we showed that when COCs were cultured with the TLR4 ligand bacterial lipopolysaccharide (LPS), the expression of *Il-6*, *Tnf alpha* and *Ptgs2* is induced [60]. However, the endogenous ligand for the TLR family and its physiological role in ovulated COCs is not known. Recently, Jiang *et al*. [62] reported that hyaluronan fragments, but not the high molecular weight hyaluronan polymers, can activate TLR2 and TLR4. Because cumulus cells produce a hyaluronan rich matrix during the ovulation process, and the matrix is broken down by sperm-secreted hyaluronidase during fertilization [6], this process might regulate TLRs in cumulus cells. In fact, high molecular weight hyaluronan did not stimulate cumulus cell TLR2 and TLR4, however, small hyaluronan fragments activated both receptors and increased mRNAs encoding specific cytokines/chemokines, *Il6*, *Mip2* and *Ccl5*, ~7-fold, ~10-fold and ~15-fold, respectively [63]. Anti-TLR2 and anti-TLR4 monoclonal antibodies significantly suppressed hyaluronan fragment- and hyaluronidase-induced expression of these genes in cumulus cells of ovulated COCs [63]. Thus, TLR2/TLR4 on cumulus cells of ovulated COCs are functional and activated by hyaluronan fragments. Furthermore, during the fertilization process, CCL-5, G-CSF, IL-1b, IL-6, IL-9, IL-12 IL-16, MCP-1, MIP-1b, and TNFα are secreted from cumulus cells of COCs, and anti-TLR2 and anti-TLR4 antibodies significantly suppress the secretion of CCL-5, G-CSF, MCP-1, MIP-1b, and IL-6. Concomitantly, fertilization rates decrease to 32 % from 75 % in oocytes without antibodies. Moreover, *Ccr1*, *Ccr2*, *Ccr3* and *Ccr5* mRNAs that encode CCL5, MCP-1, and MIP-1b receptors are expressed in spermatozoa (Fig. 2). Sperm motility was enhanced by CCL5 suggesting that the chemokines secreted from cumulus cells of ovulated

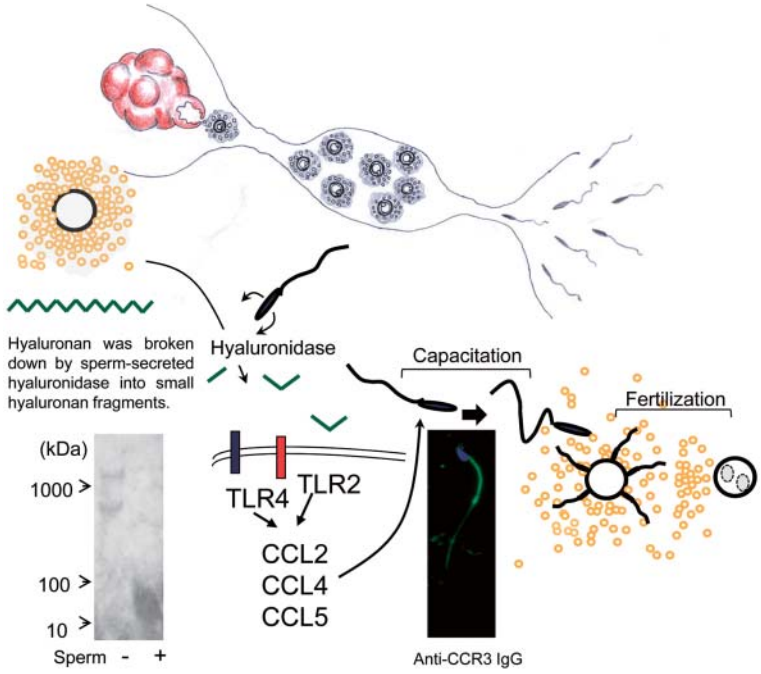

Fig. 2. The positive effects of cumulus cell expansion in fertilization.

COCs can induce sperm capacitation and subsequent oocyte penetration by mechanisms dependent on TLR2/4 activation (Fig. 2).

In Vitro Maturation (IVM) of COC

During the ovulation process, cumulus cells were stimulated by several kinds of granulosa cells-secreted factors, such as the EGF like factor, PGE2 and cytokines as described above. Some of these factors are expressed dominantly in granulosa cells, others are only expressed in granulosa cells but not in cumulus cells [61, 64]. Thus, it is possible that the supplementation of these factors to the maturation medium of cultured COCs will enhance oocyte maturation in vitro. Indeed, when pig COCs are cultured with EGF, the developmental rate of fertilized oocytes to the blastocyst stage is significantly higher than that in oocytes matured without EGF [65]. However, the positive effect of EGF is only observed when the COCs are pre-cultured with FSH [66]. Generally, porcine COCs are collected from small antral follicles (3–5 mm in diameter) for use in IVM, and the cumulus cells of these small follicles do not express a sufficient level of EGF receptor to respond efficiently to EGF [65]. Rather, during follicular development, the responsiveness of cumulus cells to EGF is enhanced by FSH stimulation [65], because when porcine COCs are recovered from medium- or large-sized follicles (more than 5 mm in diameter), EGF alone induces cumulus expansion [66]. More interestingly, the ERK1/2-targeted transcriptional factor, C/EBPβ is only expressed during the ovulation process but not follicular development stage [14]. Thus, the cAMP signaling pathway may be required to increase expression of specific transcription factors in cumulus cells in order to allow FSH pre-cultured COCs to respond fully to EGF or other factors enhancing oocyte competence and blastocyst development.

The Future of Human Infertility Care Derived from Basic Research of Cumulus Cell Functions

The positive roles of cumulus cells in oocyte maturation in vitro have been well known for the past four decades. Recent microarray studies have identified novel activators secreted from granulosa cells that induce the differentiation of cumulus cells. Additionally, gene targeting technology has shown clearly which signaling pathways are required in cumulus cells for oocyte maturation. The new information base should provide new methods for

improved in vitro oocyte maturation. We have already reported a new system of porcine COC maturation [65] which we briefly describe below.

1. COCs are recovered from small antral follicles (3–5 mm in diameter).
2. The recovered COCs are pre-cultured with 2 ng/ml FSH and 100 ng/ml estradiol 17β for 10 hr to induce cell proliferation.
3. At 10 hr, 20 ng/ml of progesterone is added to the FSH- and estradiol-containing medium to suppress cell proliferation and induce *Lhcgr* mRNA expression.
4. After 20 hr of culture, COCs are moved to fresh medium with 1 μg/ml LH, 1 ng/ml EGF and 100 ng/ml progesterone for an additional 24 hr.

Using this modified culture system based on *in vivo* changes in hormones and growth factor production, the matured porcine COCs exhibit full expansion, the cumulus cells remain healthy (low number of apoptotic cells) and when oocytes obtained from these COCs are used for *in vitro* fertilization, developmental competence to the blastocyst stage is significantly improved as compared with the conventional FSH+LH culture system.

In human COC maturation, the basic information about the changes of hormonal condition within follicles and the expression of genes involved in cumulus cell differentiation is limited. However, modification of human IVM conditions is required to get matured oocytes that have high developmental competence. The porcine culture system contributes to the development of an improved human IVM system.

Basic information about cumulus cells is also beneficial for judging whether a matured oocyte has developmental competence or not. We have shown that the high expression of *Sult1e1* in cumulus cells of matured human COCs collected from ovarian stimulation cycles can be used as a predictive measure of oocyte developmental competence to the blastocyst stage (Tabata *et al.*, unpublished data), other groups have reported significant correlations between oocyte developmental competence and the gene expression levels of *Gremlin*, *Pgr*, and *Cx43* in cumulus cells recovered from matured human oocytes [67–69]. However, the key factors transported or secreted by cumulus cells that control oocyte nuclear and cytoplasmic maturation remain unclear. Further study to clarify the unknown factors is required for *in vitro* maturation of oocytes.

Acknowledgement

Dr. Heng-Yu Fan, Dr. Zhilin Liu, Dr. Yasuhisa Yamashita, and Dr. Tetsuji Okazaki have provided outstanding assistance and good suggestions. This work was supported in part, by Grant-in-Aid for Scientific Research (No. 18688016, No. 21688019, No. 21028014, No. 21248032) from the Japan Society for the Promotion of Science (JSPS) (to M.S), and National Institute of Health (NIH)-HD-16229 and HD-07495 (Project II, Specialized Cooperative Program in Reproductive Research, SCPRR) (to J.S.R).

References

1) Downs, S.M. and Eppig, J.J. (1984): Cyclic adenosine monophosphate and ovarian follicular fluid act synergistically to inhibit mouse oocyte maturation. Endocrinology, 114, 418–427.
2) Downs, S.M., Coleman, D.L. and Eppig, J.J. (1986): Maintenance of murine oocyte meiotic arrest: uptake and metabolism of hypoxanthine and adenosine by cumulus cell-enclosed and denuded oocytes. Dev. Biol., 117, 174–183.
3) Simon, A.M., Goodenough, D.A., Li, E. and Paul, D.L. (1997): Female infertility in mice lacking connexin 37. Nature, 385, 525–529.
4) Peng, X.R., Hsueh, A.J., LaPolt, P.S., Bjersing, L. and Ny, T. (1991): Localization of luteinizing hormone receptor messenger ribonucleic acid expression in ovarian cell types during follicle development and ovulation. Endocrinology, 129, 3200–3207.
5) Richards, J.S. (1994): Hormonal control of gene expression in the ovary. Endocr. Rev., 15, 725–751.
6) Richards, J.S. (2005): Ovulation: new factors that prepare the oocyte for fertilization. Mol. Cell. Endocrinol., 234, 75–79.
7) Cross, P.C. and Brinster, R.L. (1970): In vitro development of mouse oocytes. Biol. Reprod., 3, 298–307.
8) Downs, S.M. (1989): Specificity of epidermal growth factor action on maturation of the murine oocyte and cumulus oophorus in vitro. Biol. Reprod., 41, 371–379.
9) Park, J.Y., Su, Y.Q., Ariga, M., Law, E., Jin, S.L. and Conti, M. (2004): EGF-like growth factors as mediators of LH action in the ovulatory follicle. Science, 303, 682–684.
10) Shimada, M., Hernandez-Gonzalez, I., Gonzalez-Robayna, I. and Richards, J.S. (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. Mol. Endocrinol., 1352–1365.
11) Ashkenazi, H., Cao, X., Motola, S., Popliker, M., Conti, M. and Tsafriri, A. (2005): Epidermal growth factor family members: endogenous mediators of the ovulatory response. Endocrinology, 146, 77–84.
12) Hsieh, M., Lee, D., Panigone, S., Horner, K., Chen, R.,

Theologis, A., Lee, D.C., Threadgill, D.W. and Conti, M. (2007): Luteinizing hormone-dependent activation of the epidermal growth factor network is essential for ovulation. Mol. Cell. Biol., 27, 1914–1924.

13) Panigone, S., Hsieh, M., Fu, M., Persani, L. and Conti, M. (2008): Luteinizing hormone signaling in preovulatory follicles involves early activation of the epidermal growth factor receptor pathway. Mol. Endocrinol., 22, 924–936.
14) Fan, H.Y., Liu, Z., Shimada, M., Sterneck, E., Johnson, P.F., Hedrick, S.M. and Richards, J.S. (2009): MAPK3/1 (ERK1/2) in ovarian granulosa cells are essential for female fertility. Science, 324, 938–941.
15) Salustri, A., Garlanda, C., Hirsch, E., De Acetis, M., Maccagno, A., Bottazzi, B., Doni, A., Bastone, A., Mantovani, G., Beck-Peccoz, P., Salvatori, G., Mahoney, D.J., Day, A.J., Siracusa, G., Romani, L. and Mantovani, A. (2004): PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in in vivo fertilization. Development, 131, 1577–1586.
16) Wong, W.Y. and Richards, J.S. (1991): Evidence for two antigenically distinct molecular weight variants of prostaglandin H synthase in the rat ovary. Mol. Endocrinol., 5, 1269–1279.
17) Sirois, J. and Richards, J.S. (1992): Purification and characterization of a novel, distinct isoform of prostaglandin endoperoxide synthase induced by human chorionic gonadotropin in granulosa cells of rat preovulatory follicles. J. Biol. Chem., 267, 6382–6388.
18) Ochsner, S.A., Russell, D.L., Day, A.J., Breyer, R.M. and Richards, J.S. (2003): Decreased expression of tumor necrosis factor-alpha-stimulated gene 6 in cumulus cells of the cyclooxygenase-2 and EP2 null mice. Endocrinology, 144, 1008–1019.
19) Davis, B.J., Lennard, D.E., Lee, C.A., Tiano, H.F., Morham, S.G., Wetsel, W.C. and Langenbach, R. (1999): Anovulation in cyclooxygenase-2-deficient mice is restored by prostaglandin E2 and interleukin-1beta. Endocrinology, 140, 2685–2695.
20) Matsumoto, H., Ma, W., Smalley, W., Trzaskos, J., Breyer, R.M. and Dey, S.K. (2001): Diversification of cyclooxygenase-2-derived prostaglandins in ovulation and implantation. Biol. Reprod., 64, 1557–1565.
21) Shimada, M., Yanai, Y., Okazaki, T., Yamashita, Y., Sriraman, V., Wilson, M.C. and Richards, J.S. (2007): Synaptosomal-associated protein 25 gene expression is hormonally regulated during ovulation and is involved in cytokine/chemokine exocytosis from granulosa cells. Mol. Endocrinol., 21, 2487–2502.
22) Noma, N., Nishimtsu, K., Enjoji, M., Tabata, K., Shitanaka, M. and Shimada, M. (2008): Induced synaptotagmin (SYT) protein family binds to membrane SNAP25 to facilitate vesicle secretion in murine granulosa/cumulus cells during ovulation. J. Mamm. Ova Res., 25, 193–200.
23) Liu, Z., de Matos, D.G., Fan, H.Y., Shimada, M., Palmer, S. and Richards, J.S. (2009): Interleukin-6: an autocrine regulator of the mouse cumulus cell-oocyte complex expansion process. Endocrinology, 150, 3360–3368.
24) Fan, H.Y., Shimada, M., Liu, Z., Cahill, N., Noma, N., Wu, Y., Gossen, J. and Richards, J.S. (2008): Selective expression of $Kras^{G12D}$ in granulosa cells of the mouse ovary causes defects in follicle development and ovulation. Development, 135, 2127–2137.
25) Su, Y.Q., Wigglesworth, K., Pendola, F.L., O'Brien, M.J. and Eppig, J.J. (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.
26) Yamashita, Y., Kawashima, I., Yanai, Y., Nishibori, M., Richards, J.S. and Shimada, M. (2007): Hormone-induced expression of tumor necrosis factor alpha-converting enzyme/a disintegrin and metalloprotease-17 impacts porcine cumulus cell oocyte complex expansion and meiotic maturation via ligand activation of the epidermal growth factor receptor. Endocrinology, 148, 6164–6175.
27) Sharma, S.C. and Richards, J.S. (2000): Regulation of AP1 (Jun/Fos) factor expression and activation in ovarian granulosa cells. Relation of JunD and Fra2 to terminal differentiation. J. Biol. Chem., 275, 33718–33728.
28) Cook, S.J., Aziz, N. and McMahon, M. (1999): The repertoire of fos and jun proteins expressed during the G1 phase of the cell cycle is determined by the duration of mitogen-activated protein kinase activation. Mol. Cell. Biol., 19, 330–341.
29) Sayasith, K., Bouchard, N., Doré, M. and Sirois, J. (2008): Regulation of bovine tumor necrosis factor-alpha-induced protein 6 in ovarian follicles during the ovulatory process and promoter activation in granulosa cells. Endocrinology, 149, 6213–6225.
30) Nakajima, T., Kinoshita, S., Sasagawa, T., Sasaki, K., Naruto, M., Kishimoto, T. and Akira, S. (1993): Phosphorylation at threonine-235 by a ras-dependent mitogen-activated protein kinase cascade is essential for transcription factor NF-IL6. Proc. Natl. Acad. Sci. USA., 90, 2207–2211.
31) Sebastian, T. and Johnson, P.F. (2006): Stop and go: anti-proliferative and mitogenic functions of the transcription factor C/EBPbeta. Cell Cycle, 5, 953–957.
32) Sterneck, E., Tessarollo, L. and Johnson, P.F. (1997): An essential role for C/EBPbeta in female reproduction. Genes Dev., 11, 2153–2162.
33) Downs, S.M. and Hunzicker-Dunn, M. (1995): Differential regulation of oocyte maturation and cumulus expansion in the mouse oocyte-cumulus cell complex by site-selective analogs of cyclic adenosine monophosphate. Dev. Biol., 172, 72–85.
34) Shimada, M., Samizo, N., Yamashita, Y., Matsuo, K. and Terada, T. (2002): Both Ca^{2+}-protein kinase C pathway and cAMP-protein kinase A pathway are involved in progesterone production in FSH- and LH-stimulated cumulus cells during in vitro maturation of porcine oocytes. J. Mamm. Ova Res., 19, 81–88.
35) Sasseville, M., Côté, N., Vigneault, C., Guillemette, C. and Richard, F.J. (2007): 3'5'-cyclic adenosine

monophosphate-dependent up-regulation of phosphodiesterase type 3A in porcine cumulus cells. Endocrinology, 148, 1858–1867.
36) Ji, T.H., Ryu, K.S., Gilchrist, R. and Ji, I. (1997): Interaction, signal generation, signal divergence, and signal transduction of LH/CG and the receptor. Recent. Prog. Horm. Res., 52, 431–453.
37) Takahashi, T., Morrow, J.D., Wang, H. and Dey, S.K. (2006): Cyclooxygenase-2-derived prostaglandin E(2) directs oocyte maturation by differentially influencing multiple signaling pathways. J. Biol. Chem., 281, 37117–37129.
38) Jahnsen, T., Hedin, L., Lohmann, S.M., Walter, U. and Richards, J.S. (1986): The neural type II regulatory subunit of cAMP-dependent protein kinase is present and regulated by hormones in the rat ovary. J. Biol. Chem., 261, 6637–6639.
39) Conti, M. (2002): Specificity of the cyclic adenosine 3',5'-monophosphate signal in granulosa cell function. Biol Reprod., 67, 1653–1661.
40) Sassone-Corsi, P. (1998): Coupling gene expression to cAMP signalling: role of CREB and CREM. Int. J. Biochem. Cell. Biol., 30, 27–38.
41) Richards, J.S., Fitzpatrick, S.L., Clemens, J.W., Morris, J.K., Alliston, T. and Sirois, J. (1995): Ovarian cell differentiation: a cascade of multiple hormones, cellular signals, and regulated genes. Recent. Prog. Horm. Res., 50, 223–254.
42) Richards, J.S. (2001): New signaling pathways for hormones and cyclic adenosine 3',5'-monophosphate action in endocrine cells. Mol. Endocrinol., 2, 209–218.
43) Qin, L., Tamasi, J., Raggatt, L., Li, X., Feyen, J.H., Lee, D.C., Dicicco-Bloom, E. and Partridge, N.C. (2005): Amphiregulin is a novel growth factor involved in normal bone development and in the cellular response to parathyroid hormone stimulation. J. Biol. Chem., 280, 3974–3981.
44) Heim, M.H. (1996): The Jak-STAT pathway: specific signal transduction from the cell membrane to the nucleus. Eur. J. Clin. Invest., 26, 1–12.
45) Akira, S. (1997): IL-6-regulated transcription factors. Int. J. Biochem. Cell. Biol., 29, 1401–1418.
46) Su, Y.Q., Nyegaard, M., Overgaard, M.T., Qiao, J. and Giudice, L.C. (2006): Participation of mitogen-activated protein kinase in luteinizing hormone-induced differential regulation of steroidogenesis and steroidogenic gene expression in mural and cumulus granulosa cells of mouse preovulatory follicles. Biol. Reprod., 75, 859–867.
47) Conti, M., Hsieh, M., Park, J.Y. and Su, Y.Q. (2006): Role of the epidermal growth factor network in ovarian follicles. Mol. Endocrinol., 20, 715–723.
48) Byskov, A.G., Andersen, C.Y., Nordholm, L., Thøgersen, H., Xia, G., Wassmann, O., Andersen, J.V., Guddal, E. and Roed, T. (1995): Chemical structure of sterols that activate oocyte meiosis. Nature, 374, 559–562.
49) Baltsen, M. (2001): Gonadotropin-induced accumulation of 4,4-dimethylsterols in mouse ovaries and its temporal relation to meiosis. Biol. Reprod., 65, 1743–1750.
50) Grøndahl, C., Breinholt, J., Wahl, P., Murray, A., Hansen, T.H., Faerge, I., Stidsen, C.E., Raun, K. and Hegele-Hartung, C. (2003): Physiology of meiosis-activating sterol: endogenous formation and mode of action. Hum. Reprod., 18, 122–129.
51) Ruan, B., Watanabe, S., Eppig, J.J., Kwoh, C., Dzidic, N., Pang, J., Wilson, W.K. and Schroepfer, G.J. Jr. (1998): Sterols affecting meiosis: novel chemical syntheses and the biological activity and spectral properties of the synthetic sterols. J. Lipid. Res., 39, 2005–2020.
52) Shimada, M., Yamashita, Y. and Nishibori M. (2005): Transcripts encoding the enzymes that convert acetyl-coA to cholesterol are induced in cumulus cells and are essential for progesterone biosynthesis and meiotic resumption of porcine oocytes. Jap. J. Reprod. Endocrinol., 10, 15–20.
53) Yamashita, Y., Shimada, M., Okazaki, T., Maeda, T. and Terada, T. (2003): Production of progesterone from de novo-synthesized cholesterol in cumulus cells and its physiological role during meiotic resumption of porcine oocytes. Biol. Reprod., 68, 1193–1198.
54) Yamashita, Y., Nishibori, M., Terada, T., Isobe, N. and Shimada, M. (2005): Gonadotropin-induced delta 14-reductase and delta 7-reductase gene expression in cumulus cells during meiotic resumption of porcine oocytes. Endocrinology, 146, 186–194.
55) Liu, Z., Rudd, M.D., Hernandez-Gonzalez, I., Gonzalez-Robayna, I., Fan, H.Y., Zeleznik, A.J. and Richards, J.S. (2009): FSH and FOXO1 regulate genes in the sterol/steroid and lipid biosynthetic pathways in granulosa cells. Mol. Endocrinol., 23, 649–661.
56) Faerge, I., Grøndahl, C., Ottesen, J.L. and Hyttel, P. (2001): Autoradiographic localization of specific binding of meiosis-activating sterol to cumulus-oocyte complexes from marmoset, cow, and mouse. Biol. Reprod., 64, 527–536.
57) Kawamura, K., Kumagai, J., Sudo, S., Chun, S.Y., Pisarska, M., Morita, H., Toppari, J., Fu, P., Wade, J.D., Bathgate, R.A. and Hsueh, A.J. (2004): Paracrine regulation of mammalian oocyte maturation and male germ cell survival. Proc. Natl. Acad. Sci. USA., 101, 7323–7328.
58) Kumagai, J., Hsu, S.Y., Matsumi, H., Roh, J.S., Fu, P., Wade, J.D., Bathgate, R.A. and Hsueh, A.J. (2002): INSL3/Leydig insulin-like peptide activates the LGR8 receptor important in testis descent. J. Biol. Chem., 277, 31283–31286.
59) Han, S.J., Vaccari, S., Nedachi, T., Andersen, C.B., Kovacina, K.S., Roth, R.A. and Conti, M. (2006): Protein kinase B/Akt phosphorylation of PDE3A and its role in mammalian oocyte maturation. EMBO J., 25, 5716–5725.
60) Shimada, M., Hernandez-Gonzalez, I., Gonzalez-Robanya, I. and Richards, J.S. (2006): Induced expression of pattern recognition receptors in cumulus oocyte complexes: novel evidence for innate immune-like functions during ovulation. Mol. Endocrinol., 20, 3228–3239.
61) Hernandez-Gonzalez, I., Gonzalez-Robayna, I., Shimada, M., Wayne, C.M., Ochsner, S.A., White, L. and Richards,

J.S. (2006): Gene expression profiles of cumulus cell oocyte complexes during ovulation reveal cumulus cells express neuronal and immune-related genes: does this expand their role in the ovulation process? Mol. Endocrinol., 20, 1300–1321.

62) Shimada, M., Yanai, Y., Okazaki, T., Noma, N., Kawashima, I., Mori, T. and Richards, J.S. (2008): Hyaluronan fragments generated by sperm-secreted hyaluronidase stimulate cytokine/chemokine production via the TLR2 and TLR4 pathway in cumulus cells of ovulated COCs, which may enhance fertilization. Development, 135, 2001–2011.
63) Jiang, D., Liang, J., Fan, J., Yu, S., Chen, S., Luo, Y., Prestwich, G.D., Mascarenhas, M.M., Garg, H.G., Quinn, D.A., Homer, R.J., Goldstein, D.R., Bucala, R., Lee, P.J., Medzhitov, R. and Noble, P.W. (2005): Regulation of lung injury and repair by Toll-like receptors and hyaluronan. Nat. Med., 11, 1173–1179.
64) Sugiura, K., Su, Y.Q., Li, Q., Wigglesworth, K., Matzuk, M.M. and Eppig, J.J. (2009): Fibroblast growth factors and epidermal growth factor cooperate with oocyte-derived members of the TGFbeta superfamily to regulate Spry2 mRNA levels in mouse cumulus cells. Biol. Reprod., 81, 833–841.
65) Kawashima, I., Okazaki, T., Noma, N., Nishibori, M., Yamashita, Y. and Shimada, M. (2008): Sequential exposure of porcine cumulus cells to FSH and/or LH is critical for appropriate expression of steroidogenic and ovulation-related genes that impact oocyte maturation in vivo and in vitro. Reproduction, 136, 9–21.
66) Prochazka, R., Kalab, P. and Nagyova, E. (2003): Epidermal growth factor-receptor tyrosine kinase activity regulates expansion of porcine oocyte-cumulus cell complexes in vitro. Biol. Reprod., 68, 797–803.
67) McKenzie, L.J., Pangas, S.A., Carson, S.A., Kovanci, E., Cisneros, P., Buster, J.E., Amato, P. and Matzuk, M.M. (2004): Human cumulus granulosa cell gene expression: a predictor of fertilization and embryo selection in women undergoing IVF. Hum. Reprod., 19, 2869–2874.
68) Hasegawa, J., Yanaihara, A., Iwasaki, S., Otsuka, Y., Negishi, M., Akahane, T. and Okai, T. (2005): Reduction of progesterone receptor expression in human cumulus cells at the time of oocyte collection during IVF is associated with good embryo quality. Hum, Reprod., 20, 2194–2200.
69) Hasegawa, J., Yanaihara, A., Iwasaki, S., Mitsukawa, K., Negishi, M. and Okai, T. (2007): Reduction of connexin 43 in human cumulus cells yields good embryo competence during ICSI. J. Assist. Reprod. Genet., 24, 463–466.