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Review

Nppc/Npr2/cGMP signaling cascade maintains oocyte developmental capacity

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Abstract: The follicle must fulfill the following criteria if it is to survive the period between early embryonic life and the luteinizing hormone (LH) peak. It should (i) be surrounded by pregranulosa cells; (ii) complete the first meiotic division and become dormant; and (iii) continue metabolism during the dormant stage. Interaction between the natriuretic peptide precursor type C (Nppc) and its receptor, natriuretic peptide receptor 2 (Npr2), affects female fertility through the production of oocytes with developmental capacity and maintain oocyte meiotic arrest. While Nppc is expressed in mural cells, cumulus cells express Npr2. Nppc/Npr2 system exerts its biological function on developing follicles by increasing the production of intracellular cyclic guanosine monophosphate (cGMP). This pathway not only contributes to the development of ovary and the uterus, but aids the formation of healthy eggs in terms of their morphological and genetic aspects. A defect in this pathway leads to asmall ovarian size, string-like uterine horns, and thin endometrium and myometrium. Disorganized chromosomes, abnormal cumulus expansion and early meiotic resumption occur in animals with defective Nppc/Npr2 signaling. The types and number of oocytes also decrease when there is incompetent Nppc/Npr2 signaling. This paper extends on most recent and relevant experimental evidence regarding Nppc/Npr2/cGMP signaling with regard to its crucial role in maintaining oocyte meiotic arrest and the production of oocytes with developmental capacity. We further discuss whether the agonist or antagonist forms of the members of this exciting pathway can be usedfor triggering final oocyte maturation.

Key words: Natriuretic peptide precursor type C (Nppc, CNP); Natriuretic peptide receptor 2 (Npr2, guanylyl cyclase-B); Granulosa cells; Follicle; Oocyte; Meiotic arrest; LH surge; Ovulation trigger.

Introduction

The natriuretic peptide precursor type C (Nppc, also known as CNP), the gene encoding C type natriuretic peptide and its receptor, natriuretic peptide receptor 2 (Npr2, also called guanylyl cyclase-B), are known to affect female fertility through the production of oocytes with developmental capacity in animals and humans. Nppc/Npr2 signaling is associated with several physiological functions, including maintaining oocyte meiotic arrest (1), follicle survival (2), and formation of a functioning cumulus oophorus. For instance, cumulus cells surrounding oocytes in mice with defective Nppc/Npr2/cGMP signaling were found to be significantly decreased in antral follicles, and absent in periovulatory follicles (3). Another important role of the Nppc/Npr2 systemis in the regulation of oocyte meiotic arrest (1).

The follicle must fulfill the following criteria if it is to survive the period between early embryonic life and the luteinizing hormone (LH) peak. Briefly, diplotene-stage oocytes are surrounded by pregranulosa cells necessary for the initiation of meiotic division and to prevent apoptotic follicle loss. Thereafter, as soon as they reach the genital ridge, primordial germ cells have to enter the first meiotic division and remain arrested at the dictyate/diplotene stage of prophase I until the LH surge. Following this surge, growing oocytes complete their first meiotic division and undergo a second meiotic arrest in metaphase stage until fertilization occurs. A visible germinal vesicle (GV) indicates prophase-stage meiotic arrest. GV breakdown is the first visible sign of meiotic resumption. Either absence of a GV or the presence of condensed chromosomes are considered as indications that meiosis hasresumed. Nppc/Npr2 signaling plays a crucial role in maintaining oocyte meiotic arrest in mammals since mutations in either Npr2 or Nppc results in premature meiotic resumption. When Npr2 is activated by Nppc, intracellular oocyte cGMP increases. This rise in cGMP level maintains meiotic arrest in both early and the late antral follicles by inhibiting oocyte PDE3A activity (4,5).

Nppc/Npr2/cGMP signaling cascade is first descri-

bed in endochondral ossification having a role in the longitudinal growth of long bones in the limbs and vertebrae (6). Concordantly, expression of *Nppc/Npr2* has been reported in the chondrocytes of murine tibia (6). Ten years later, Zhang et al. (2010) showed that Nppc was not only expressed in long bones, but also in granulosa cells of the oocyte. Although oocytes do not express Npr2 strongly,it contributes to the maintenance of meiotic arrest by promoting cAMP production from the oocyte itself and Npr2 expression from cumulus cells (1). While Nppc mRNA is expressed predominantly in mural granulosa cells, cumulus cells have a higher Npr2 expression capacity (7,8). Some periantral mural granulosa cells show weak expression of Npr2 (1). Compared to cumulus cells, Nppc mRNA expression in mural granulosa cellsis ~10-fold higher. On the other hand, Npr2 mRNA expression capacity of cumulus cells is twice that in mural (1). Nppc/Npr2 mRNA are also expressed in the cal interstitial cells of the growing follicles (9). Although administration of Nppc to acumulus cell-enclosed oocyte culture inhibits spontaneous resumption of meiosis, this effect does not occur in denuded oocytes (1). Our paper is based on experimental observations regarding Nppc/Npr2 signaling with regard to its crucial role in maintaining oocyte meiotic arrest and the production of oocytes with developmental capacity. We furhter discuss whether the agonist or antagonist forms of the members of this exciting pathway can be used for triggering final oocyte maturation.

Relationship between cGMP, cAMP and Nppc/Npr2 signaling

Oocyte meiotic arrest and resumption are maintained by very well-coordinated communication between somatic cells and the oocyte. cAMP concentration in the cytoplasm of dormant and developing follicles is regulated by the balance between its production and degradation. Two enzymes, adenylyl cyclase (AC) and phosphodiesterase (PDE), regulate the intracytoplasmic concentration. Albeit oocytes produce sufficient cAMP, somatic cell derived cGMP contributes to oocyte meiotic arrest by maintaining high intracytoplasmic cAMP levels (10,11). cGMP produced from cumulus cells diffuses into the oocyte cytoplasm and maintains high cAMP concentration byinhibiting oocyte cAMP phosphodiesterase 3 (PDE3A) activity. If isolated oocyte-cumulus complexes or denuded oocytes are exposed to cGMP, meiotic resumption is blocked (12,13). But then, follicular cGMP levels decrease in response to LH or amphiregulin stimulation (10,11). Similarly, lower levels of cGMP in cumulus cells decrease oocyte cAMP levels after hCG administration or LH surge (10,11).

Are there any interactions between Nppc/Npr2 signaling cascade and estradiol, FSH, ODPF, and hypoxanthine?

Apart from LH/hCG, Nppc/Npr2 signaling is also regulated by factors like estradiol, FSH, oocyte-derived paracrine factors (ODPFs) and hypoxanthine in humans (Figure 1) Alone or co-administration of ODPFs, such as bone morphogenetic protein 15, growth differentiation factor 9 or fibroblast growth factor 8, induce the expression of Npr2 mRNA (1). Although high levels of hypoxanthine help maintain meiotic arrest (14), inhibition of inosine monophosphate dehydrogenase enzyme reverses meiotic arrest in hypoxanthine-arrested follicles (15). On the other hand, effectof FSH on Npr2 expression is unclear. If equine chorionic gonadotropin (eCG) primed mice are exposed to FSH, Npr2 mRNA expression increases. However, FSH administration does not increase Npr2 mRNA expression in cultured COCs, suggesting increased levels of Npr2 are not a direct effect of FSH (16). This increase in Npr2 mRNA expression in cumulus cells is probably an indirect effect of the FSH on estradiol production (17). Consistent with this finding is that administration of synthetic estrogen torat granulosa cells induces both Nppc/Npr2 mRNA expression and cGMP production in vivo (9). Likewise, estradiol also induces expression of Npr2 receptors on cumulus granulosa cells and participates in the maintenance of meiotic arrest (16,18). Moreover, the latter authorsshowedthe ability of Nppc to maintain meiotic arrest in cultured COCs was only transient unless the culture was kept in estradiol-containing medium. They also reported that cGMP synthesis is blocked in the absence of or, at low levels of estrogen. Huang et al. (2015) showed that cumulus cell Npr2 expression is significantly associated with both estrogen receptor alpha and estrogen receptor beta (19). Relatedly, Richard and Baltz (2014) found that both Nppc and estradiol should be added to culture medium to maintain meiotic arrest in punctured follicles indicating that estradiol is required to maintain function of Npr2 (20).

Interactions betweenNppc/Npr2/cGMP cascade, gap junctions and oocyte

Nppc produced by mural granulosa cells diffuses through the antral fluid and binds to its Npr2 receptors on the cumulus granulosa cells (1,16). The meiotically

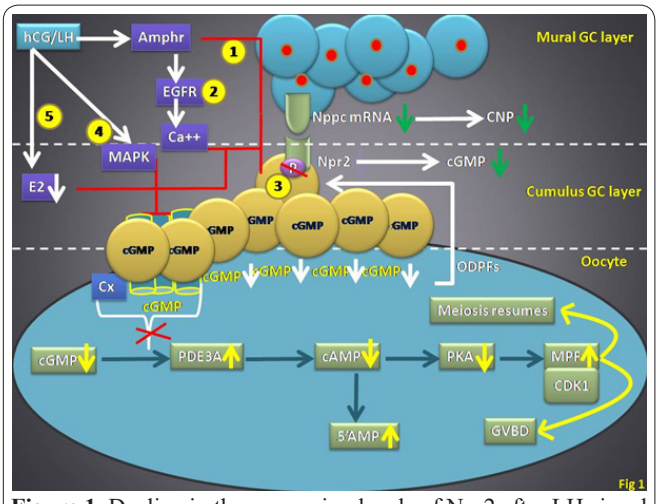

Figure 1. Decline in the expression levels of Npr2 after LH signal involves 5 possible mechanisms. First, by activating EGF-R, LH increases the secretion of amphiregulin, which leads to down regulation of Nppc expression ((1)). Second, activation of EGF-R increases the calcium levels inside the cumulus cells and reduces Npr2 activity. Third, induction of EGF-R activity decreases Npr2 expression in the cumulus cells by means of dephosphorylation. Fourth, LH activates MAPK, which phosphorylates the gap-junction proteins, leading to their closure. Fifth, by decreasing estradiol levels LH also inhibits Nppc and Npr2 expression (50).

competent GV oocyte is maintained arrested by Nppc and the Npr2 receptor. Gap junction isoforms, such as Cx37 and Cx43, are required to maintain meiotic arrest by the Nppc/Npr2 system. cGMP produced in the cumulus cells diffuses between granulosa cell layers primarily via Cx43. cGMP from the cumulus cells then enters the oocyte through Cx37 (20,21). It should be noted that Nppc/Npr2 signaling can maintain the somatic cell and oocyte cGMP concentrations independently from gap junctions (Figure 1).

Nppc/Npr2 signaling and cumulus formation

Cumulus cells multiply through controlled mitotic division. When they reach a certain number, contact inhibition stops celldivision. Cell division is regulated by systemic and local factors. The two-way communication between the oocyte and the granulosa cells is of critical importance in division. Secretory molecules from the granulosa cells and the oocyte initiate division in a definite order; disorder in any compartment prevents the formation of the cumulus layer. Hence, an impaired Nppc/Npr2 signal cascade can prevent formation of a healthy and sufficiently thick cumulus.

Presence of insufficient Nppc/Npr2 signal can negatively affect cumulus cell formation through different mechanisms. We accept that early meiotic activation is the first and the most important factor. In strong agreement with this, precocious meiotic resumption due to defective Nppc/Npr2 signal leads to abnormality in the release of oocyte-derived factors that induce granulosa cell proliferation (1,3,22). Hence, partial or complete absence of cumulus cells might be caused by a deficiency in oocyte-derived growth factors in the presence of incompetent Nppc/Npr2 signaling. On the other hand, despite precocious meiotic resumption, formation of the cumulus layer canbe normal in mice with GPR3-mutation (23). This led us to think that the underlying mechanisms of precocious meiotic resumption are more important than mitotic division itself in the formation of cumulus layer. Another explanation for failed cumulus formation in Npcc/Npr2 disrupted cases is the inhibition of the proliferative impact of Nppc on granulosa cells. Indeed, directNppc/Npr2 signaling induces granulosa cell proliferation and maintains cumulus cell survival. Likewise, a stimulatory effect of Nppc on chondrocyte proliferation has been reported (24,25). Another possibility is that Nppc/Npr2 signaling could affect granulosa cell apoptosis. Correspondingly, the anti-apoptotic properties of Nppc/Npr2/cGMP system on granulosa cellsmight allow the formation of a healthy cumulus layer. By disrupting the apoptotic process and Npr2 mRNA expression in the granulosa cells, incompetent Nppc/Npr2 signaling may be implicated in abnormal cumulus cell formation (1,2).

Nppc/Npr2 signaling affects both the number andthe type of follicles

The literature contains conflicting results regarding the types of follicles in which Nppc/Npr2 signalingis incompetent. While some reported no remarkable changes in follicle types and number (1), others showed the presence of only primordial, primary, and secondary stage of follicles in Npr2-deficient mice (22). In contrast, Kiyosu et al. (2012) showed the existence of all follicle types in mutant mice for the Nppc/Npr2 system (3). The possible reason for the differences in follicle types may be the differences in the methods used to mutate any allele. Correspondingly, residual Npr2 activity caused by failed, or an inadequate mutation technique in the Npr2 or Nppc genes, may lead to the continued cGMP synthesis, which allows the survival and development of new follicles. Another possibility for the differences in follicle types may be individual differences in ovarian reserves of mutant animals. Similar to follicle phenotypes, the number of follicles in mutant animals is different between natural and stimulated cycles. Incompetent Nppc/Npr2 signaling decreases the number of oocytes in natural cycles. On the other hand, ovarian stimulation of mutant mice with PMSG leads to comparable number of ovulated oocytes (3). Considering ovarian reserve is different even among individuals of the same species, the different number and phenotypes of follicular development in mutant animals may be attributed to differences in ovarian reserve.

Uterine development and Nppc/Npr2 signaling

The female genitalia are derived from the urogenital ridge, which gives rise to paramesonephic ducts (Müllerian ducts) formed from longitudinal invaginations of the coelomic epithelium (26,27). In addition to AMH and nuclear factor kappa beta, both homeobox (HOX) genes and Wnt signalinig maintain the embryonic steps of uterine development (28,29). IGF-I is another critical regulator of uterine growth, which mediates the effects of 17-β-estradiol on the developing uterus. Both locally produced and circulating IGF-I is essential for the growth of the uterus (30). In another words, it was shown that systemic IGF-I maintained normal uterine growth and estradiol response. *Npr2* gene expression was noted in the murine uterus (31). Low serum IGF-I levels in female *Npr2*-/- mice lead to failed uterine development (22). Npr2 null mice also show string-like uterine horns, with a thin endometrium and myometrium. Moreover, the uterus of null mice does not contain glandular structures. Ovarian size of null animals is also smaller compared to healthy controls (22). Together, along with AMH, HOX genes and IGF-1, Nppc/Npr2 signaling appears to be essential for the growth and maturation of uterus and its structures.

Central effect of Nppc/Npr2 signaling

In addition to its impact on somatic cell cGMP production, Nppc/Npr2 signaling may also contribute to the regulation of FSH or LH secretion. Both gonadotroph cells of the anterior hypophysis and GnRH secreting cells of the arcuate nucleus express Nppc and its receptor (32,33). Although GnRH induces Nppc expression from the hypothalamus (34), the precise role of Nppc/Npr2 signaling on gonadotroph cells of hypophysis is unclear. Further investigations using Nppc analogues or antagonists would help to clarify whether Nppc/Npr2 signaling is actually associated with the regulation of FSH or LH secretion.

Reproductive consequences of Nppc/Npr2 mutation

Mutations of Npr2 and Nppc impair the functioning of the Nppc/Npr2/cGMP cascade (35,36). In order to understand the precise role of Nppc/Npr2 signaling in follicle development, female mice with mutant alleles for Nppc ($Nppc^{lbab}$) or Npr2 ($Npr2^{cn}$) were created by Kiyosu et al. (2012) (3). Morphologically the ovaries in mutant mice were smaller than those of fertile controls. Although $Npr2^{cn}/Npr2^{cn}$ female mice ovulated just as normal mice, they never produced a litter. Moreover, ovulated oocytes of $Npr2^{cn}/Npr2^{cn}$ and $Nppc^{lbab}/Nppc^{lbab}$ mice had fragmented ooplasm and disorganized chromosomes. Likewise, precocious resumption of meiosis occured in $Npr2^{cn}/Npr2^{cn}$ and $Nppc^{lbab}/Nppc^{lbab}$ mutant mice. Concordantly, premature resumption of meiosis caused by defective Nppc/Npr2/cGMP signaling cascade has also been reported by Zhang et al. (2010), however, unlike Kiyosu et al. (2012), they used $Npr2^{cn-2j}/Npr2^{cn-2j}$ and $Nppc^{lbab}/Nppc^{lbab}$ mutant mice (Tables 1 and 2) (1,3).

Although female mice lacking Nppc/Npr2/cGMP signaling have normal menstrual cycle, mating ability, normal follicular growth, and ovulation, they are infertile due to abnormal meiotic resumption (3). Actually, the precocious resumption of meiosis in the antral follicles of $Npr2^{cn}/Npr2^{cn}$ mutant mice leads to ovulation of fragmented or degenerated oocytes. Both ovulated and unovulated oocytes of the two types of mutant mice were devoid of cumulus cells (3). This data indicates that oocytes with no developmental potential were ovulated in the $Npr2^{cn}/Npr2^{cn}$mutant mice. In addition to condensed chromatin content, antral follicles of mice with $Npr2^{cn}/Npr2^{cn}$ mutation did not show clear germinal vesicle formation.

By initiating metaphase spindle, chromosome alignment and cumulus expansion, LH or hCG induces resumption of meiosis in the oocytes of periovulatory follicles. However, similar changes did not occur in the oocytes of periovulatory follicles in $Npr2^{cn}/Npr2^{cn}$ mutant mice. A dispersed configuration of chromosomes and fragmented ooplasm did not allow normal

Table 1. Follicle types and morphology obtained from different Nppc/Npr2 mutation studies.

Study	Follicle development	Follicle morphology	Mutant allel
Tamura *et al*., (2004)	Only primordial to secondary follicle No corpora lutea	Normal	$Npr2^{-/-}$
Zhang *et al*., (2010)	All stage of follicle	Normal Early meiotic resumption in 50% of oocytes	$Npr2^{cn-2j}/Npr2^{cn-2j}$ and $Nppc^{lbab}/Nppc^{lbab}$
Kiyosu *et al*., (2012)	All follicular stage	Abnormal morphology and lack of COCs	$Npr2^{cn}/Npr2^{cn}$ and $Nppc^{lbab}/Nppc^{lbab}$
Tsuji et al. (2012)	All follicular stage	Early meiotic resumption in 58% of oocytes	$Npr2^{cn}/Npr2^{cn}$

Table 2. Mice with mutant allels for Nppc or Npr2 and reproductive consequences (Adapted from; Zhang et al. [2010] (1), Kiyosu et al. [2012] (3), Tsuji et al. [2012] (5), Tamura et al. [2004] (22)).

Mutant allels	Oocyte morphology	Cumulus cells
$Npr2^{cn-2j}/Npr2^{cn-2j}$	Early meiotic resumption in 50% of oocytes No major morphological abnormality	Tightly packed cumulus cells around maturing oocytes
$Npr2^{cn}/Npr2^{cn}$	comparable number of ovuated oocytes in stimulated cycles decreased number of ovuated oocytes in unstimulated cycles thin layers of cumulus cells failed to produce a litter small ovary	
$Npr2^{cn}/Npr2^{cn}$	fragmented or degenerated ooplasm failed to reach two-cell stage no clear germinal vesicle	
$Nppc^{lbab}/Nppc^{lbab}$	fragmented or degenerated ooplasm failed to reach two-cell stage abnormal cumulus expansion	devoid of cumulus cells
$Npr2^{cn}/Npr2^{cn}$	prematurely resumed meiosis in antral stage disorganized chromosomes fragmented ooplasm abnormal cumulus expansion	
$Nppc^{lbab}/Nppc^{lbab}$	prematurely resumed meiosis in antral stage disorganized chromosomes fragmented ooplasm thin cumulus layer	
Guanylyl cyclase B (Npr2-/-)	Female sterility Small ovarian size Failed uterine devolopment String-like uterine horn, Very thin endometrium and myometrium Lack of secondary glandular structures	Normal testes and epididymus Spermatid development in tubules of testes and epididymus

spindle formation, chromosome alignment and cumulus expansion. Likewise, a scattered configuration of chromosomes or fragmented ooplasm in the periovulatory follicles in $Nppc^{lbab}/Nppc^{lbab}$ mutant mice prevented a typical cumulus expansion. Likewise, in the antral follicles of $Npr2^{cn}/Npr2^{cn}$ mutant micecumulus oophorus which is a thin layer of cumulus cells surrounding the ovulated oocyte, was lacking (3).

Is there any relationship between Nppc/Npr2 expression and IVF outcome?

A mid-cycle LH surge induces a series of changes in developing follicle. Nppc/Npr2 system is involved in LH triggering events. Role of Nppc/Npr2 system in follicle maturation has been shown in various species (1,5,37). By decreasing Nppc/Npr2 expression in granulosa cells, LH surge leads to meiotic resumption and ovulation (1). Likewise, administration of hCG/LH decreases Nppc levels of follicular fluid in humans, suggesting that granulosa cell Nppc/Npr2 system can be critical infollicular maturation (5,38). However, Huang et al. (2015) found no obvious correlation between Nppc/Npr2 expression levels and IVF outcome in subjects with tubalor male factor infertility (19). Taken together, failed expression of this signaling system may cause different ovulatory dysfunctions, such as polycystic ovary syndrome, oocyte maturation defect or maturation arrest.

Modulators of Nppc/Npr2 system

Expression of Nppc/Npr2 signal in follicles is regulated by endogenous gonadotropins. Likewise, treatment with exogenous gonadotropins sufficient to induce folliculogenesis increases the expression of Nppc/Npr2 signal. Administration of pregnant mare's serum gonadotropin (PMSG) enhances the expression of mRNAs encoding Nppc/Npr2 from 27 to 50 nM, and stimulates the production of cGMP to maintain meiotic arrest (1,5). Conversely, injection of human chorionic gonadotropin (hCG) reduces Nppc/Npr2 expression and triggers oocyte maturation (1). In good agreement with this, Npcc level in follicular fluid decreases following administration of an ovulatory dose of hCG (38). LH has a triple inhibitory effect on Nppc/Npr2 signaling: (i) similar to hCG, LH surge or exogenous LH administration decreases Npr2 expression. Zhang et al. (2010) showed that LH relieves the inhibitory effect of Nppc on meiotic arrest by decreasing Nppc expression (1). They also noted a 10-fold decline in Nppc levels of porcine follicular fluid following LH injection. Likewise, the Nppc concentration of human follicular fluid decreases 20-fold following LH surge (39). (ii) Related to estradiol contribution to Nppc-associated meiotic arrest, reduced estradiol production following LH surge induces meiotic resumption. Similarly, administration of estrogen increases expression of Npr2 mRNA (9); however following LH surge, there is a decline in estradiol levels from 20 to 1 pg/ml leading to decreased Npr2 expression (40). (iii) Amphiregulin is a mediator of LH/hCG activity through EGFR and it is localized with Nppc in mural granulosa cells (41). Tsuji et al. (2012) showed an association between hCG/LH, amphiregulin/EGFR and Nppc/Npr2 signaling (5). Nppc mRNA expression in the follicle of equine chorionic gonadotropin primed mice following injection of human hCG was significantly lower than that in those without hCG administration. By activating both amphiregulin and EGFR production, LH surge or hCG administration inhibits Nppc expression in mural granulosa cells. This cascade leads to a decrease in cumulus cell cGMP levels and meiotic resumption begins (5).

Mediators of LH action on Nppc/Npr2

A well coordinated interaction between mural and cumulus granulosa cells produces inhibitory signalsthat are the basis of oocyte meiotic arrest until LH surge (1,38,42). While Nppc is mainly located in mural granulosa cells, Npr2 is expressed by cumulus granulosa cells (1,5). By binding to the Npr2 receptor, Nppc induces cGMP generation in cumulus granulosa cells, which is transferred to the oocyte cytoplasm. This oocyte cGMP inhibits phosphodiesterase 3A (PDE3A), there by sustaining increased levels of cyclic adenosine monophosphate (cAMP) required for the maintenance of meiotic arrest (43,44). Following LH surge, both the expression of Nppc mRNA and the guanylyl cyclase activity of the Npr2 receptor are reduced. LH also reduces Npr2 mRNA expression; this whole cascade of events initiates the resumption of oocyte meiosis (38,45). LH effects on oocyte maturation and cumulus expansion are mediated by various signaling pathways, including protein kinase A (PKA) (41,46) and epidermal growth factor receptor (EGFR) (47). Likewise, the inhibitory effect of LH on Npr2 expression is mediated by EGFR. In a few words, LH induces the release of EGF-like factors (EGF-LGF) including amphiregulin, epiregulin and betacellulin. By binding to the EGF-receptor, EGF-LGF amplifies the effect of LH signal, induces expression of EGF-R, and inhibits Npr2 activity. Likewise, after LH surge, expression of mitogen-activated protein kinase (MAPK) increases and activates EGF-R. Decline in the expression of Npr2 after LH surge involves four possible mechanisms. First, activation of EGF-R increases calcium levels inside the cumulus cells and reduces Npr2 activity. Second, induction of EGF-R activity decreases Npr2 expression in the cumulus cells by means of dephosphorylation. Third, by activating EGF-R, LH increases the secretion of amphiregulin, which leads to down regulation of Nppc expression. Fourth, LH activates mitogen-activated protein kinase (MAPK), which phosphorylates gap-junction proteins, leading to their closure. Moreover, EGF-R activation inhibits Nppc mRNA expression in somatic cells. Together, EGF- and MAPK-mediated LH action in the Nppc/Npr2 system blocks conversion of GTP to cGMP, and the oocyte undergoes meiotic resumption (41,48-50). In conclusion, LH-induced oocyte meiotic resumption appears to run either in an amphiregulin/EGFR-mediated gap junction closure-dependent or amphiregulin/EGFR-mediated but gap junction closure-independent manner.

Does Nppc/Npr2 antagonist more physiological for ovulation trigger than hCG/LH?

LH acts on the mural cells because of the absence of functional LH receptors on cumulus cells and oo-

cytes. Although the most important drugs that are available to trigger final oocyte maturation are hCG preparations,certainside effects limit their use, in particular ovarian hyperstimulation syndrome (OHSS). Moreover, some cases in which oocyte maturation is not achieved despite hCG administration present a major problem. Therefore, search for alternative drugs to trigger oocyte maturation is in progress. Gonadotropin releasing hormone agonist (GnRHa) is a conventional drug now being used for this purpose as it significantly reduces the risk of OHSS in IVF/ICSI cycles. On the other hand, compared to hCG administration, the use of GnRHa for oocyte maturation trigger has beenassociated with a lower live birth rate and a higher rate of early miscarriage, drawbacks that limit itsuse. Exogenous Nppc antagonist administration may be a novel alternative fortriggering oocyte maturation to currently used drugs, e.g. hCG or GnRHa. Furthermore, if oocyte meiotic arrest can be maintainedusing Nppc analogues, we can prevent the loss of follicles due to chemotherapy.

Conclusions

Since Nppc/Npr2 signaling is involved in oocyte meiotic resumption, ovulation, and the formation of the cumulus oophorus, failure of itsexpression may result in female infertility. Understanding the precise role of Nppc/Npr2 pathway might improve the oocyte quality of women suffering from infertility caused by failed follicular development. The design and development of Nppc/Npr2 antagonists might therefore provide infertility specialists with anopportunity of a more physiological approach in ovulation trigger than existing conventional methods.

There are two different signaling pathways that maintain LH-related meiotic resumption (10). In the first pathway, gap junction closure is required to decrease cGMP transport from somatic cells to oocytes. The reduction in oocyte cGMP or cAMP levels following LH/hCG administration is due to closure of gap junctions between granulosa-granulosa cell or granulosa cell-oocyte (10,51). In the second signaling pathway, thepresence of gap junction is not obligatory for LH action, and LH-induced EGFR inhibits Nppc/Npr2 expression, thereby decreasing cGMP levels independent of gap junction closure (10). As both direct or indirect suppression of Nppc/Npr2 signaling with or without gap junction closureareexpected to decrease the levels of oocyte cGMP provided from somatic cells, a new drug that allows oocyte meiotic resumption bylowering oocyte cAMP levels might be developed. Exogenous Nppc antagonists seems to be a promising method of triggering oocyte maturation following ovarian stimulation with gonadotropins in women undergoing IVF/ICSI. By means of newdrugs which directly suppress Nppc mRNA expression,both somatic cell and oocyte cGMP production can be decreased. Likewise, the design and development of drugs which activateamphiregulin/EGFR signaling may reduce CNP inhibition of meiotic resumption by down-regulating Nppc. Further studies are needed to establish the Nppc antagonist trigger of oocyte maturation, and thereby to improve the reproductive outcome for women undergoing IVF/ICSI treatment.

Conflict of Interest

There is no conflict of interest.

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