Selective Regulation of Oocyte Meiotic Events Enhances Progress in Fertility Preservation Methods

Onder Celik[1], Nilufer Celik[2], Sami Gungor[3], Esra Tustas Haberal[4] and Suleyman Aydin[5]

[1]Private Clinic, Obstetrics and Gynecology, Usak, Turkey. [2]Behçet Uz Children's Hospital, Department of Biochemistry, İzmir, Turkey. [3]Private Medical Hospital, Obstetrics and Gynecology, Elazig, Turkey. [4]Umraniye Education and Research Hospital, Obstetrics and Gynecology, İstanbul, Turkey. [5]Department of Medical Biochemistry (Firat Hormone Research Group), School of Medicine, Firat University, Elazig, Turkey.

ABSTRACT: Following early embryonic germ cell migration, oocytes are surrounded by somatic cells and remain arrested at diplotene stage until luteinizing hormone (LH) surge. Strict regulation of both meiotic arrest and meiotic resumption during dormant stage are critical for future fertility. Intercellular signaling system between the somatic compartment and oocyte regulates these meiotic events and determines the follicle quality. As well as the collected number of eggs, their qualities are also important for in vitro fertilization (IVF) outcome. In spontaneous and IVF cycles, germinal vesicle (GV)–stage oocytes, premature GV breakdown, and persistence of first meiotic arrest limit the reproductive performance. Likewise, both women with premature ovarian aging and young cancer women are undergoing chemoradiotherapy under the risk of follicle loss because of unregulated meiotic events. Understanding of oocyte meiotic events is therefore critical for the prevention of functional ovarian reserve. High levels of cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP) and low phosphodiesterase (PDE) 3A enzyme activity inside the oocyte are responsible for maintaining of meiotic arrest before the LH surge. cGMP is produced in the somatic compartment, and natriuretic peptide precursor C (Nppc) and natriuretic peptide receptor 2 (Npr2) regulate its production. cGMP diffuses into the oocyte and reduces the PDE3A activity, which inhibits the conversion of cAMP to the 5′AMP, and cAMP levels are enhanced. In addition, oocyte itself has the ability to produce cAMP. Taken together, accumulation of cAMP inside the oocyte induces protein kinase activity, which leads to the inhibition of maturation-promoting factor and meiotic arrest also continues. By stimulating the expression of epidermal growth factor, LH inhibits the Nppc/Npr2 system, blocks cGMP synthesis, and initiates meiotic resumption. Oocytes lacking the functional of this pathway may lead to persistence of the GV oocyte, which reduces the number of good quality eggs. Selective regulation of somatic cell signals and oocyte meiotic events enhance progress in fertility preservation methods, which may give us the opportunity to prevent follicle loss in prematurely aging women and young women with cancer are undergoing chemoradiotherapy.

KEYWORDS: oocyte meiosis, fertility preservation, cGMP, cAMP, PDEs, Nppc/Npr2

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CORRESPONDENCE: celiksudenaz@gmail.com; saydin1@hotmail.com

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Introduction

Oocyte–somatic cell complex is not only an extraordinary cell in the female body but also it has the ability to maintain meiotic arrest and initiates meiotic resumption. From an evolutionary perspective, prophase I arrest is preserved in many animal species including human. Following primordial germ cell (PGC) migration from yolk sack to the genital ridge, oocytes go into first meiotic division and remain arrested at the dictyate/diplotene stage of prophase I until luteinizing hormone (LH) surge. Duration of dormant stage in human and animals is different. In human beings, completion of the primary arrest period takes place within the years. In contrast, the length of this period in others animals has been reported as months, days, and minutes.[1–3] During the diplotene-stage arrest, oocyte accumulates a stockpile of organic and inorganic molecules, which are necessary for successful fertilization and implantation. Moreover, diplotene-stage oocytes are surrounded by pregranulosa cells, which are necessary for the initiation of meiotic division and prevent apoptotic follicle loss. Following LH surge, growing oocytes complete first meiotic division and undergo second meiotic arrest in metaphase stage until fertilization. The stage of second meiotic arrest varies between the species.[4] Interestingly, the secondary meiotic arrest does not take place in some animals.[4] After the fertilization, oocytes are released from second arrest and embryonic development begins.

Female infertility is not exclusively because of reduced oocyte number but is also the result of defective oocyte–somatic cells interaction, which reduces the fertilization and implantation capacity and is the reason for poor pregnancy outcome. Generation of new oocytes from embryonic stem cells has not been reported yet in humans. Hence, we are not able to prevent most of the infertility problems secondary to poor follicular development. Progress in the knowledge of the

somatic cells–oocyte interaction has improved the in vitro fertilization embryo transfer (IVF-ET) outcome. By selectively regulating somatic cell signaling, it is possible to solve some problems related to female infertility. Previous studies and reviews have discussed in detail the molecular and hormonal control of meiotic arrest and meiotic resumption.[5–14] In this review, we will try to find logical replies for three questions: (i) Which signaling molecules are responsible for maintaining first meiotic arrest? (ii) How does LH surge initiate meiotic resumption? (iii) Does the establishment of selective regulation methods to prevent follicle loss increase progress in fertility preservation technologies in prematurely aging women and young cancer patients who are undergoing chemoradiotherapy? Before start answering the questions, we will make a brief description about the oocyte–somatic cell interactions.

Insight into the Oocyte–somatic Cell Interactions

The strong relations between the granulosa cells and oocytes begin in the early embryogenesis and continue until fertilization. Following arrival in the genital ridge, PGCs are surrounded by the pregranulosa cells. These somatic cells prevent apoptosis of PGCs, and first meiotic division begins. Moreover, somatic pregranulosa cells protect PGCs from ovarian somatic signals, which disturb the proper establishment of the PGCs. Mural granulosa cells are the main sources of inhibitory signals responsible for first meiotic arrest,[15,16] but orchestration by the oocyte is also required.[17,18] If mural granulosa cells are removed from the cumulus granulosa cells, oocytes undergo meiotic resumption,[19,20] suggesting the inhibitory action of mural granulosa cells. The size of antral space determines the size and volume of growing follicle in many species. While physically large animals have larger follicles, physically small animals have smaller follicles.[21] Following antrum expansion, the follicle gradually reaches its full size and volume and somatic cells divide into mural and cumulus cells.[22,23] After the establishment of the mural and somatic cells compartments, the oocyte acquires the ability to release from dormant stage and undergoes meiotic resumption.[17,22,23] When preovulatory LH surge occurs, germinal vesicle (GV) of the dormant oocyte is broken, which leads to the second stage of first meiotic division.[24] Germinal vesicle breakdown (GVBD) can be detected microscopically,[24] and it is used for evaluating the follicle stage and quality in embryology laboratories. Following ovulation, while mural granulosa cells remain within the follicle wall, cumulus granulosa cells continue to surround oocyte. Until fertilization, cumulus granulosa cells provide energy for oocyte and maintain second meiotic arrest within the ampullary part of the tubes.

Presence of the cell-to-cell connections between the granulosa cells and oocyte (mural to mural, cumulus to cumulus, mural to cumulus, cumulus to oocyte) is crucial for transporting the somatic signals and maintaining first meiotic arrest. These cytoplasmic connections are referred to as a gap junctions. Communication between two different cell types (cumulus to oocyte) is realized by heterologous gap junctions. On the other hand, homologous gap junctions are present between two similar cell types such as cumulus cells to mural cells. They are the most important intercellular communication routes between the oocyte and somatic cells. Each channel comprises connexin proteins and lets the diffusion of many organic and inorganic regulatory substances. Accordingly, mice lacking connexin protein are infertile.[25]

In the next section, we will review the function and possible roles of several key factors in maintaining oocyte meiotic arrest, such as cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP), oocyte maturation inhibitor (OMI), phosphodiesterase enzymes (PDEs), maturation-promoting factor (MPF), protein phosphatases (PPs), and natriuretic peptide precursor C (Nppc), and natriuretic peptide receptor 2 (Npr2) system, which are expressed or regulated by somatic cells and/or oocyte (Table 1).

(i) Which signaling molecules are responsible for maintaining first meiotic arrest?

Somatic Cell cGMP Production and its Transport into the Oocyte

Although oocyte has the ability to synthesize endogenous cAMP from its sources, maintenance of successful meiotic arrest is required in addition to receiving inhibitory signals from the somatic compartment. One of the main inhibitory products of somatic cells is cGMP, which maintains cAMP levels within the oocyte that is responsible for prophase I arrest.[26] When somatic cell-free oocytes or cumulus cell-enclosed oocytes were cultured with 8-Br-cGMP, a cGMP analog, inhibition of GVBD occurred, supporting the inhibitory role of cGMP on meiotic events.[27]

cGMP is generated by molecular interaction between the cumulus and mural granulosa cells.[28,29] Oocyte-derived paracrine factors (ODPFs) regulate the production of cGMP by somatic cells. There is a close relationship between the somatic cell-derived cGMP and intra-oocyte cAMP levels. Inhibitory cGMP signals from the somatic compartment reach the oocyte via gap junctions.[16,30] A relatively hypoxic environment within the cumulus cell-enclosed oocytes induces the cGMP synthesis by increasing the production of hypoxanthine and inosine substrates.[31]

Before the LH surge, accumulation of the cGMP within oocyte inhibits the enzymatic activity of PDE3A[26,30] and conversion of the cAMP into the 5′AMP is blocked. As a result, high cAMP concentration within the oocyte inhibits nuclear membrane dissolution.[26,30,32] Following LH surge, phosphorylation of the heterologous gap junctions inhibits the diffusion of cGMP into the oocyte.[26,30] Moreover, LH peak decreases the cGMP synthesis in the granulosa cells, suggesting dual effect of the LH peak on cGMP regulation.[26,30] Both suppression of the somatic production of cGMP and inhibition of its transport into the oocyte lead to an increase in oocyte-specific PDE3A activity.[30] Release of the PDE3A enzyme from the inhibitory effect of cGMP leads to an increase in its enzymatic

Table 1. Observations supporting the role of somatic signaling pathways responsible for prophase I arrest and resumption of meiosis in oocytes.

STUDY OBSERVATIONS	INTERPRETATION OF DATA	PUBLISHED STUDIES
Prophase I arrested oocyte contains a large nucleus covered by a nuclear envelope called as the germinal vesicle (GV).	First detectable sign of meiotic resumption is GV breakdown (GVBD). First meiotic division is completed by extruding a first polar body.	20
High levels of cAMP inside the oocyte are essential for first meiotic arrest. Somatic cell-derived cGMP contributes to the maintenance of elevated intra-oocyte cAMP levels.	Sufficient amount of cGMP synthesis by somatic cells are essential for maintaining prophase I arrest in fully-grown oocytes.	26,29
Regulation of meiotic events in oocytes are strictly controlled by changes in the activity of Cdk1–cyclin B complex also known as maturation or meiosis promoting factor (MPF).	An increase in Cdk1–cyclin B activity stimulates to progress into metaphase of meiosis I.	56,59
Prophase I arrested oocytes do not resume meiosis until LH surge.	LH surge initiates the meiotic resumption by blocking somatic cell derived inhibitory signals.	112
Fully-grown oocytes resume meiosis spontaneously when exit from the follicle and cultured in vitro.	Mural and cumulus granulosa cells prevent the meiotic resumption.	20
Functional LH receptors on mural cells make them main target for LH action. LH suppresses somatic cells-derived inhibitory signals.	Due to absence of functional LH receptors on the cumulus cells and oocyte LH does not have a direct action on them. LH action on cumulus cells and oocyte are indirect rather than direct.	99,113,114
Following the preovulatory LH surge catalytic activity of oocyte-specific PDE3A increases.	PDE3A hydrolyses cAMP into the 5′AMP inside the oocyte and initiates meiotic resumption.	92
LH surge mediates the phosphorylation of Cx43 and Cx37 leading to the disruption of the gap junctional communication. LH surge increases the expression of MAPK and phosphorylates gap junctions. Carbenoxolone, a gap junction blocker, reduces cGMP concentrations inside the oocyte.	Granulosa cells derived cGMP are transmitted into the oocyte via dephosphorylated gap junctions.	15,42,106,107,110
Mural granulosa cells express Nppc and cumulus granulosa cells express Nppc receptor 2 (Npr2). cGMP production in somatic cells is regulated by Nppc/Npr2 system.	Nppc/Npr2 system is crucial for maintaining oocyte meiotic arrest.	29,30,82
Nppc/Npr2 system responsible for maintaining the high cGMP and cAMP concentration inside the oocyte during prophase I arrest.	Oocytes having Nppc/Npr2 mutation undergo early meiotic resumption.	29,91
Both OMI and Nppc originated from outer somatic cells and exert their effects on cumulus cells. The molecular weight of the Nppc and OMI are ~2000 dalton.	Nppc and OMI may be the same molecule responsible for prophase I arrest.	29,89,97
Ovulatory dose of hCG in patient undergoing IVF leads to decline in follicular fluid CNP concentration.	hCG suppresses follicular fluid CNP secretion.	11

activity and a decrease in cAMP levels within the oocyte and initiates meiotic resumption.

Maintenance of Elevated cAMP Levels Inside the Oocyte

Several central and peripheral molecules exert their effects on reproductive tissue by triggering intracellular cAMP. It is exactly not known how this single molecule can regulate the activities of several enzymes and expression of some proteins within the oocyte. Intra-oocyte cAMP level is mainly responsible for maintaining prophase I meiotic arrest. Similarly, analogs of cAMP, such as dibutyryl-cAMP and 8-bromo-cAMP, increase the intra-oocyte cAMP levels and block follicle maturation.[6,33,34] Main production site of cAMP is the oocyte. As shown in Figure 1A and B, cAMP is generated by the oocyte adenylyl cyclase (AC). The G-protein-coupled receptors 3 and 12 are present in the oocyte plasma membrane and regulate the activity of AC enzyme.[17,18,35–37] GPR3 induces Gs expression and activates the AC enzyme inside the oocyte.[17] When somatic cell-free mouse oocytes were cultured with forskolin, the activity of AC enzyme increases. This leads to an elevation of cAMP synthesis, and the onset of nuclear envelope dissolution is delayed.[38] However, maintenance of meiotic arrest is required to sustain high levels of cAMP and cGMP inside the oocyte. Somatic compartment cells provide cGMP for oocyte.[39,40] By inhibiting PDE3A, cGMP increases intra-oocyte cAMP levels. In agreement, cAMP levels inside the oocyte decrease when oocytes exit from the follicle,[20] confirming somatic contribution.

Different molecular mechanisms may be responsible for maintaining of the first meiotic arrest in small and growing oocytes. Studies have demonstrated that increased levels of cAMP is obligatory for prophase I meiotic arrest in growing oocytes.[18,35] On the other hand, when the size of oocytes is under 60 µm, they fail to resume meiosis despite exiting from the follicles.[41] Thus, persistence of high intra-oocyte cAMP levels may not be obligatory for maintaining prophase I arrest in smaller oocytes. Taken together, maintaining elevated cAMP levels inside the oocytes is essential for prophase I arrest[15,42] in growing oocytes. Nevertheless, small oocytes that do not have the ability to complete meiotic division high levels of cAMP inside the oocyte may not be necessary for maintaining prophase I arrest. The oocytes

somehow produce sufficient amount of cAMP to maintain prophase I arrest.[17,18] However, cGMP transport from granulosa cells contributes to the maintenance of increased cAMP levels inside the oocyte.[15,42]

PDE3A, PDE5, PDE9A, and PDE4 Enzymes Hydrolyze Somatic Cell-derived cGMP

Differentiation and maturation of follicles following LH surge is a cAMP-mediated process. However, the sustained exposure of cAMP inhibits oocyte maturation.[43] This paradox might be explained by the compartmentalization hypothesis, which is very helpful for understanding the contribution of the different PDEs upon follicular maturation. According to this hypothesis, regulation of the cAMP concentration within the somatic compartment and oocyte is different because of the differential settlement of the PDE subtypes.[44] In agreement with our results, Thomas et al have showed the compartmentalization of PDE4 and PDE3 within the bovine follicle.[45]

The enzymes that metabolize somatic cell-derived cGMP in a growing oocyte are not known in detail. PDE enzymes have an important role in cell-to-cell signaling between somatic compartment and the oocyte. PDEs have the ability to degrade both cAMP and cGMP.[46] So far, more than 10 classes of PDE enzymes, such as PDE3A, PDE5, PDE9A, and PDE4, have been reported. PDE3A is the oocyte-specific enzyme that hydrolyzes cAMP into 5′AMP and leads to meiotic resumption.[47] Inhibition of PDE3A activity in humans prevents both spontaneous and gonadotropin-induced follicular growth.[48] Likewise, PDE3A mutant mice are infertile because of GV oocyte.[49] In a recent study, Hanna et al have shown that PDE9A is the only cGMP-specific phosphodiesterase present in the GV oocyte.[50] Moreover, they have detected 17 of the 19 PDE genes assayed in the somatic cells. They have concluded that cGMP residues inside the oocyte are hydrolyzed by the PDE9A after the LH surge. Similarly, previous studies have demonstrated the presence of PDE4 activity in the somatic cells but not within the oocyte.[44] While PDE4 enzyme hydrolyzes cAMP, it is not sensitive to cGMP. The oocyte is not responsive to the PDE4 inhibitors, suggesting that PDE4 enzyme is expressed only in the somatic cells.[45]

Although follicular somatic cells contain PDE5 enzyme antigen,[51] PDE5 enzyme activity is not detected within the oocytes.[26] Unlikely the oocyte PDE3A enzyme, PDE5 enzyme is not specific to somatic cells. Similarly, PDE5 enzyme activity is present in the different mammalian cell types and tissues in which hydrolysis of cGMP occurs.[52] Likewise, PDE5 enzyme in the somatic compartment metabolizes granulosa cell-derived cGMP. Hydrolysis of somatic cGMP by the PDE5 enzyme causes meiotic resumption by decreasing cGMP concentration within the oocyte cytoplasm.[26,51] Sildenafil and zaprinast are specific and potent PDE5 inhibitors.[53,54] Both these inhibit the cGMP degradation in the cumulus cells. Culture medium containing sildenafil inhibits LH-dependent meiotic resumption in the oocytes.[26] By using zaprinast, a recent in vitro study has investigated the possible roles of this drug on

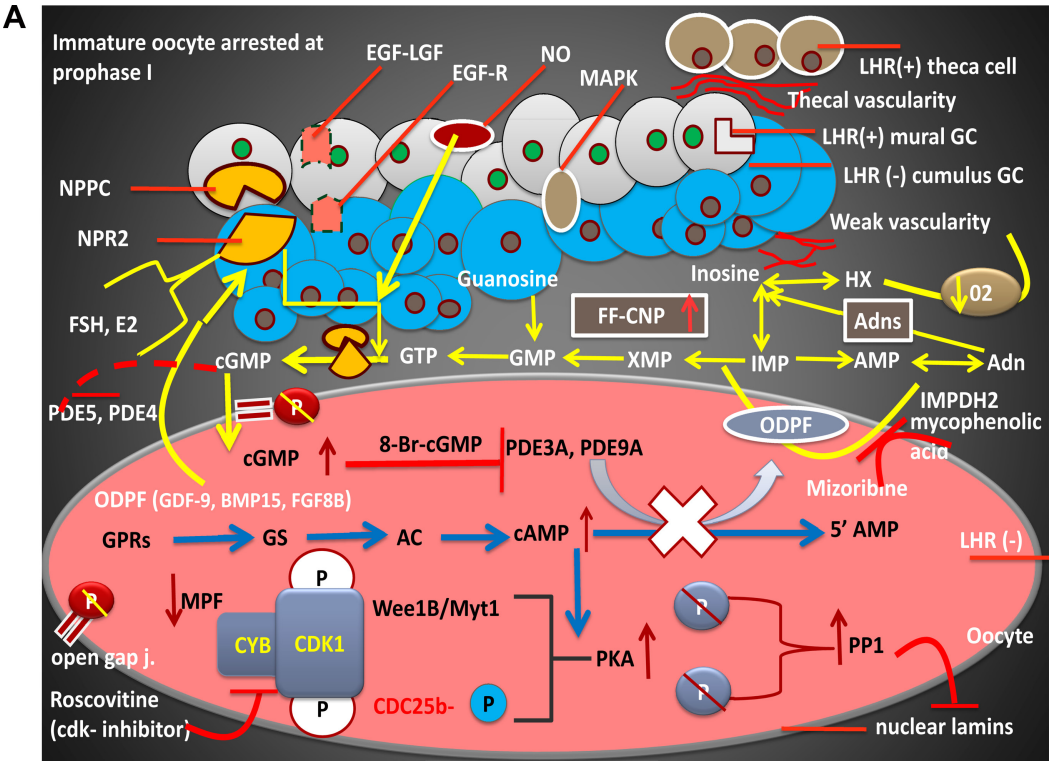

Figure 1. (*Continued*)

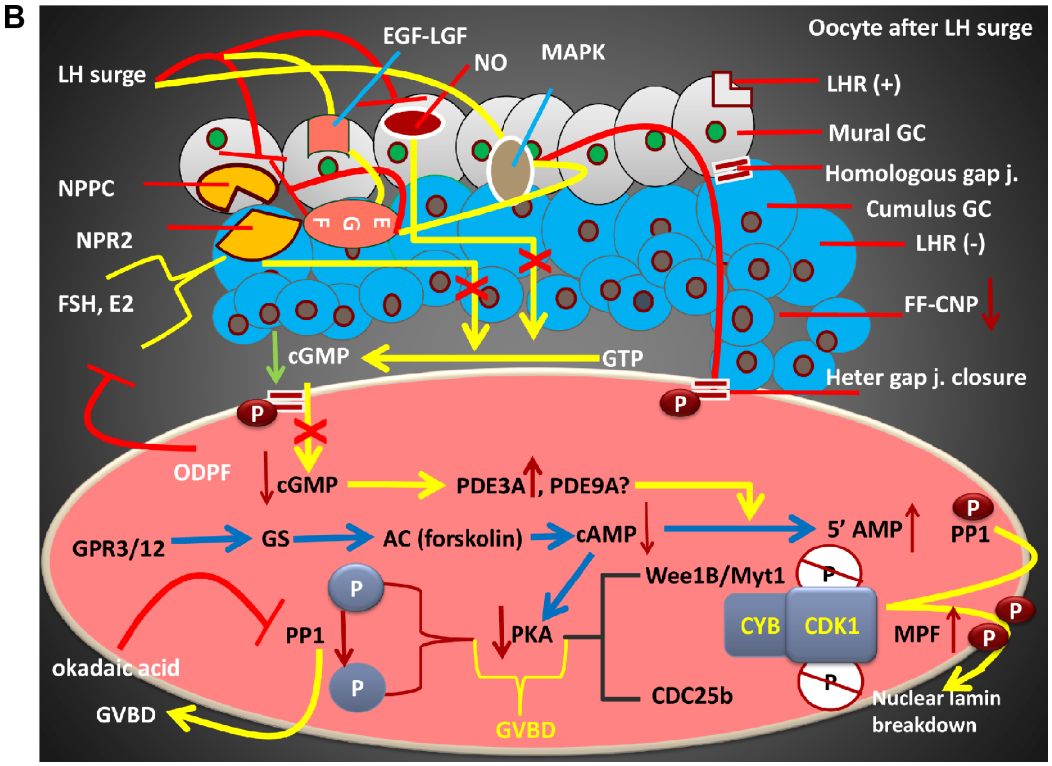

Figure 1. Abbreviated pathways to illustrate inhibitory signals maintaining meiotic arrest before the LH surge. (**A**) Mild hypoxia within the somatic cells induces hypoxanthine (HX) synthesis, which increases the synthesis of inosine, which is the responsible substrate for GTP and cGMP synthesis. Nppc/Npr2 system controls cGMP production in the somatic cells. Npr2 is activated by the Nppc and produces cGMP from GTP. cGMP diffuses into the oocyte via gap junctions (two thick red lines). In the absence of LH surge, gap junctions undergo dephosphorylation and become activate. cGMP inhibits the PDE3A, which inhibits the hydrolysis of cAMP into 5′AMP. 8-Br-cGMP, a cGMP analog, also blocks the PDE3A activity. cGMP synthesis in somatic cells may overcome the hydrolytic activity of PDE5 enzyme (dashed red line). Note the compartmentalization of the PDEs. GPRs induce Gs expression and activate AC, which stimulates cAMP synthesis. Accumulation of cAMP inside the oocyte mediates meiotic arrest by increasing PKA. Activation or inhibition of PKA by cAMP regulates the functions of the Cdc25 and the Wee1B/Myt1. While the Cdc25 dephosphorylates Cdk1, Wee1B/Myt1 kinase phosphorylates it. E2, FSH, and ODPFs (GDF-9, BMP-15) contribute to maintain first meiotic arrest by inducing Npr2 mRNA in cumulus cells. ODPFs also control the activity of IMPDH. The action of IMPDH, which converts IMP into GMP, is blocked by mizoribine and mycophenolic acid. High PP1 activities inside the oocyte prevent nuclear envelope dissolution. Pharmacological inhibition of MPF with the roscovitine blocks GVBD. High follicular fluid (FF) CNP levels were noted before ovulation. (**B**) Abbreviated pathways to illustrate the role of LH surge on meiotic resumption. LH acts on the mural cells because of the absence of functional LHRs in the cumulus cells and oocyte. LH surge leads to disruption of arresting mechanisms, which would result in the activation of PDE3A. Inhibition of the Nppc/Npr2 by LH surge leads to a reduction in cGMP synthesis. LH surge mediates the phosphorylation (P) of connexin 43 and 37, leading to the closure of the gap junctions (thick red lines). Phosphorylation of gap-junction is a MAPK-dependent process. LH decreases granulosa cell nitric oxide expression and activates cGMP synthesis. LH surge also initiates the dephosphorylation of Cdk1 and the MPF becomes active. MPF phosphorylates both PP1 and nuclear envelope, which causes GVBD. OA increases the phosphorylated PP1 (inactive PP1) levels and leads to GVBD. Taken together, both the synthesis and transfer of cGMP into the oocyte are blocked. Decline in the cGMP concentration inside the oocyte allows PDE3A to hydrolyze cAMP into 5′AMP, leading to meiotic resumption. Yellow and blue arrows indicate excitatory signals, and red lines indicate inhibitory signals. See text for details. (Adapted from Refs. 5–14.)

c-type natriuretic peptide (CNP)-induced inhibition of nuclear envelope dissolution in COCs. However, they did not find any significant effect on GVBD.[11] In light of the above findings, authors have suggested that CNP-induced cGMP synthesis in somatic cells may overcome the hydrolytic activity of PDE5 enzyme and may continue to transport cGMP into oocytes to inhibit nuclear envelope breakdown. Nevertheless, it should be remembered that zaprinast not only inhibits PDE5 enzyme but also inhibits PDE6, 9, 10, and 11 enzymes.[55] Therefore, the development of more specific PDE5 inhibitors will increase our knowledge of their functional role in GVBD.

Crosstalk between the MPF Complex and PPs

Sleep and vigilance of the oocyte is regulated by phosphorylation and dephosphorylation events during the reproductive period. Changes in the levels and activity of cyclin-dependent kinase-1 (Cdk1)–cyclin B protein complex (MPF) inside the oocyte are established as the first step of meiotic division.[56] While some denominates it as a MPF, others called it as a meiosis-promoting factor or M-phase-promoting factor.[56] In meiotically competent oocytes, MPF is localized inside the nucleus.[57] Oocytes undergo metaphase II when the concentration and activity of MPF increase inside the oocyte nucleus.[58–60]

Inhibition of MPF with the roscovitine prevents GVBD in the mouse oocytes.[61] The effects of intra-oocyte cAMP levels on phosphorylation/dephosphorylation reactions and the regulation of MPF are not well understood. Phosphorylation reactions and MPF activation during meiotic division are regulated by cAMP, protein kinase A (PKA), and PPs.[9,62–64] One of the main substance maintaining the first meiotic arrest is high PKA activity inside the oocyte.[62,63] cAMP mediates first meiotic arrest by increasing PKA levels. In contrast, decline in the PKA activity inside the oocyte leads to GVBD.[49] Before the LH surge, presence of high cAMP concentration inside the oocyte increases PKA activity and leads to the phosphorylation of Cdk1 and the inactive MPF complex.[63] After the LH surge, decline in the intra-oocyte cAMP concentration leads to dephosphorylation of Cdk1 and MPF becomes active. Nevertheless, the effect of PKA on the Cdk1 phosphorylation status is indirect rather than direct. Namely, activation or inhibition of PKA by cAMP inside the oocyte regulates the functions of the Cdc25 phosphatase and the Wee1B/Myt1 kinase.[62] While the Cdc25 dephosphorylates Cdk1, Wee1B/Myt1 kinase phosphorylates it. In good agreement, meiotic resumption does not take place in the Cdc25b knockout mice, supporting an indirect role of PKA.[65]

Another substance that has an important role in maintaining meiotic arrest is PPs. PP1 is localized both in the cytoplasm and the nucleus of oocytes, and PP2A is found within the cytoplasm.[9,66,67] However, PP1 shows diverse subcellular localization in okadaic acid (OA)-mediated GVBD with higher nuclear PP1 expression in meiotically competent oocytes.[9] OA is an inhibitor of PP1, PP2A, and Ser/Thr PPs and is generated from the marine black sponges and starfish.[68–71] Exposure of oocyte to OA leads to premature GVBD.[72] When incompetent mouse oocytes are given OA, their capacity for resuming meiosis increases, suggesting the high activity of PP1 in incompetent oocytes.[73] Nevertheless, possible effects of OA administration on human oocytes are unknown.

Little is known about the specific targets and molecular regulators of the PP1. MPF and oocyte nuclear envelope are possible targets of PP1. Moreover, differential localization of PP1 inside the cytoplasm and nucleus might regulate meiotic maturation.[9] There is a strong relationship between the Cdk1 and PPs. Cdk1 discomposes the structural stability of the oocyte nuclear membrane by transferring phosphates on lamins and causes the first morphological sign of nuclear membrane dissolution.[5,74,75] Similarly, by removing phosphate from phosphoproteins, PPs regulate both meiotic and mitotic division.[76,77] Moreover, phosphorylation of PP1 during meiotic maturation inhibits the PP1 activity and causes nuclear envelope dissolution.[67,76,77]

PP1 and PP2A have been detected in oocytes of some animals.[72,76] Similar to cAMP, PPs have an antagonistic effect against MPF.[64] If the activity of PP1 increases, the activity of MPF decreases. The opposite is also true. Both LH surge and OA administration inhibit (phosphorylated) the PP1 activity, and MPF is released from PP1-dependent inhibition. Moreover, inhibition of PP1 increases the phosphorylation of nuclear envelope and leads to GVBD.[5] It is well known that Cdk1 inhibits enzymatic function of PP1 by phosphorylating it at Thr320.[78,79] In good agreement, in the oocytes of Cdk1-null mice, Cdk1 phosphorylates the PP1, resulting in suppression of PP1 activity thus facilitating the activation of the main factors involved in GVBD.[5] Consistent with this, the concentration of phosphorylated nuclear PP1 (inactive PP1) is elevated in OA-mediated GVBD.[5,9] Taken together, the strong relationship between the MPF complex and PP1 seems to be regulating meiotic arrest and resumption of meisosis.[9]

Nppc/Npr2 System and its Regulators

The majority of the studies investigating the possible mechanisms regulating the first meiotic arrest and follicle maturation in mammals are based on the works conducted on model organisms such as rat and mouse. However, meiotic regulation sites and signals vary between two animals. Meiotic regulatory signals stem from outer somatic cells in rat, whereas in mice, regulatory signals stem from inner somatic cells.[80] Although rat genome shows close similarity to the human genome,[81] we should be careful while adapting the meiotic regulation findings obtained from this model organisms to humans.

A large number of peptides, including natriuretic peptides (NPs), are involved in maintaining reproductive processes. Evolutionary mechanisms controlling the oocyte meiotic arrest are preserved in the oocytes of many species.[82–84] The NP regulate fluid homeostasis in vertebrates.[85] They show varied effects in different tissues of the same and different species. Atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and c-type natriuretic peptide (CNP) have structural similarity. ANP and BNP use NPR-A, while the connection site of CNP is the NPR-B.[86] This pattern is different in fish, where CNP uses NPR-B and NPR-A receptors.[83] ANP is encoded by Nppa. Amino acid sequences of ANP and CNP in many species are similar. The possible roles of the ANP and BNP on meiotic division and oocyte maturation are controversial. Tornell et al have reported that ANP inhibits spontaneous maturation in rat oocytes.[87] In contrast, Kawamura et al have showed that the expression of Nppa in the oocytes of mice is not affected by hCG administration.[11] Further investigations are necessary to elucidate the possible role of ANP on meiotic maturation. In contrast, a recent in vitro study has shown that both BNP and CNP stimulate the generation of cGMP in porcine oocytes to maintain prophase I arrest.[88]

Nppc is a peptide that contains 22 amino acids with a molecular mass of ~2,000 dalton, and its sequence shows similarity between the species.[89] Mural members of the somatic compartment cells express Nppc. On the other hand, Nppc receptor (Npr2) is a guanylyl cyclase and is expressed by cumulus cells[28] and periantral mural granulosa cells. When the distance from the oocyte membrane increases, Npr2 expression on the periantral mural cells decreases. Nppc

induces Npr2 expression and encodes CNP. Somatic cells of secondary and antral follicles have the ability to express Nppa mRNA. However, expression of Nppc was noted only in the granulosa cells of antral follicles.[90] Interactions between the Nppc and Npr2 systems play an important role in controlling the prophase I arrest. Similarly, activation of Nppc/Npr2 system inhibits the nuclear membrane dissolution in cumulus cell-surrounded oocytes. Nevertheless, Nppc does not lead to GVBD in cumulus cell-free oocytes. Initiation of meiotic resumption in Npr2 or Nppc-null mice supports the inhibitory role of Nppc/Npr2 system.[29] Relevantly, premature meiotic resumption occurs in the early stage of antral follicles of Npr2 and Nppc mutant mice.[91]

By increasing the Npr2 activity in cumulus cells, Nppc enhances cGMP synthesis.[30,92] In a recent study, Zhang et al have demonstrated that outer somatic cells induce the synthesis of cGMP by secreting Nppc. Once released, Nppc binds and stimulates the Npr2 in the inner somatic cells and initiates cGMP synthesis.[29] GTP is the last substrate for cGMP synthesis in somatic cells. Conversion of the GTP into cGMP in somatic cells is catalyzed by Npr2.[29] Because somatic cells do not have sufficient vascular supply, they have to live in an relatively hypoxic milieu.[93] Hypoxic environment within the somatic cells induces the production of hypoxanthine and inosine. Both are required for GTP synthesis.[7,31,94]

Somatic cell-derived cGMP is transferred into the oocyte cytoplasm through heterologous gap-junctional communication.[29] Accumulation of cGMP inside the oocyte inhibits PDE3A activity and suppresses the conversion of cAMP into 5′AMP. Administration of the oocyte-specific PDE3A inhibitors increases the cAMP concentration inside the oocytes of many species,[95,96] supporting the indirect and critical role of somatic cGMP on this enzyme. Taken together, all these chain reactions lead to accumulation of cGMP and cAMP inside the oocyte that maintains prophase I arrest until the LH surge.[26,30,92] Nppc/Npr2 signaling pathway maintains the elevated cGMP concentrations both in the somatic compartment and inside the oocyte during the first meiotic arrest.

Activation of Nppc/Npr2 system in somatic compartment cells is modulated by various stimuli. In addition to local factors generated by the somatic compartment, paracrine factors generated by oocytes induce the expression of Npr2 in cumulus cells.[29] Some substances secreted by the oocytes, including growth differentiation factor-9 and bone morphogenetic protein-15, induce Npr2 activation and lead to an increase in cGMP synthesis.[7,29] Nppc/Npr2-mediated cGMP synthesis is also controlled by the inosine monophosphate dehydrogenase (IMPDH), which is the rate-limiting enzyme in cGMP synthesis.[7] The action of IMPDH, which converts inosine monophosphate (IMP) into the xanthosine monophosphate, is blocked by mizoribine, which is a purine synthesis inhibitor (4-carbamoyl-1-β-D-ribofuranosyl imidazololium-5-olate). IMPDH is also blocked by mycophenolic acid. Inhibition of IMPDH reduces the levels of intra-oocyte guanine. ODPFs regulate the activity of IMPDH enzyme and estradiol synthesis in somatic cells.[7] A recent study has reported that estradiol participates in the maintenance of meiotic arrest by increasing the expression of Nppc/Npr2 system.[82] Thus, the signals responsible for prophase I arrest are not exclusively from the somatic cells to oocytes but also from oocyte to the somatic cells supporting the bidirectional communication[7] (Figs. 1 and 2).

Are Nppc and OMI the Same Peptides?

Although Nppc demonstrates many properties similar to those reported for OMI, whether Nppc satisfies the criteria as an OMI remains to be determined. On the other hand, some similarities between the OMI and Nppc makes one think that OMI and Nppc are same the molecules. For example, (a) the molecular weight of the two substances are ~2,000 dalton;[89,97] (b) both OMI and Nppc originated from the outer somatic cells and exert their effects on cumulus cells;[29] (c) both of these inhibit the premature meiotic maturation;[29] (d) follicular fluid contains both Nppc and OMI;[11,98] and (e) cumulus cells do not show OMI expression, whereas weak Nppc activity is seen in the cumulus cells.[29,98]

Data supporting the similarity of OMI and Nppc come from two studies.[11,28] It is well known that the effect of OMI on cumulus cells was blocked by the LH surge.[19,97] Robinson et al have showed that Npr2 expression in mouse cumulus cells is dropped by LH.[28] Likewise, it has been reported that administration of LH/hCG suppresses the Nppc synthesis in mice follicles.[11] Moreover, Nppc administration inhibits both spontaneous and hCG-induced nuclear envelope dissolution in mice.[11] Taken together, Nppc and OMI may be the molecules responsible for prophase I meiotic arrest, but further studies are necessary to elucidate this close relation between the OMI and Nppc.

(ii) How does LH surge initiate meiotic resumption?

The possible effects of LH surge on meiotic resumption are uncertain. The expression of LH receptor (LHR) within the COCs is site and cell specific. While some members of the COCs such as mural granulosa cells and theca cells express LHR, cumulus granulosa cells and oocyte do not contain functionally active LHR.[99,100] Hence, the presence of functional LHRs on mural granulosa cells make them the main target for LH action. In contrast, LH action on cumulus cells and oocytes is indirect rather than direct. LH-related mural cell signals reach the cumulus cells and oocytes via local factors and gap junctions.[101] Genome-wide analyses showed that LH peak stimulates the expression of endothelin-1, leptin,[102,103] epidermal growth factor-like ligands (EGF-LGF),[104] and insulin like-3 transcript[105] and initiates meiotic resumption. Before the puberty, because of immature LH pulse generator, LH-catalyzing reactions do not take place within the somatic cells and the follicle remains arrested in prophase. Until puberty, LH-dependent reactions within the somatic cells are either inhibited or functionally inactive. After the puberty, LH surge enables the oocyte to progress into the completion of first meiotic division.

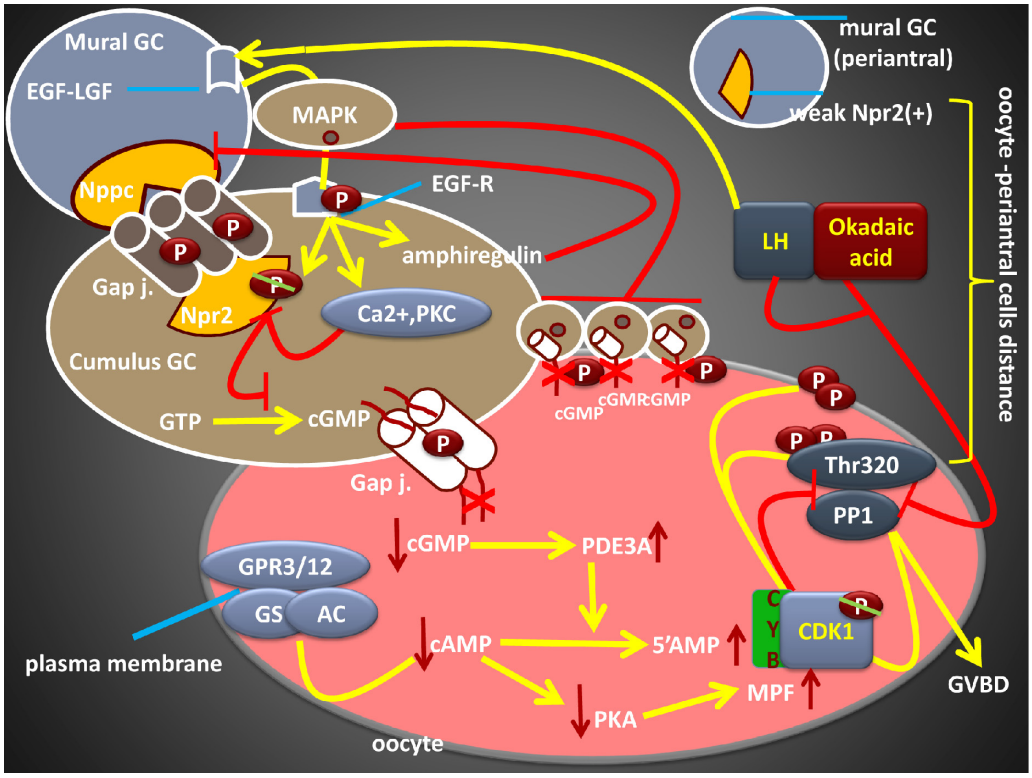

Figure 2. Abbreviated pathways to illustrate the LH effect on the Nppc/Npr2 system. EGF-LGF potentiates the effectiveness of LH signal, induces the expression of EGF-R, and inhibits the Npr2 activity. Likewise, after the LH surge, expression of mitogen-activated protein kinase (MAPK) increases and activates the EGF-R. Phosphorylation of EGF-R activates it. Decline in the expression levels of Npr2 after LH signal takes place by three possible mechanisms. First, activation of EGF-R increases the calcium levels inside the cumulus cells and reduces the Npr2 activity. Second, induction of EGF-R activity decreases Npr2 expression in the cumulus cells by means of dephosphorylation reactions. Third, by activating EGF-R, LH increases the secretion of amphiregulin, which leads to downregulation of the Nppc expression. Moreover, EGF-R activation inhibits the Nppc mRNA expression in the somatic cells. Taken together, EGF- and MAPK-mediated LH action in the Nppc/Npr2 system blocks the conversion of GTP to cGMP, and the oocyte undergoes meiotic resumption. Concentration of nuclear phosphorylated PP1 (inactive PP1) is elevated in OA-mediated GVBD. LH surge induces the activation of MAPK, which phosphorylates the gap-junction proteins and leads to their closure. Cdk1 inhibits PP1 by phosphorylating it at Thr320. Cdk1 also phosphorylates the nuclear envelope and initiates GVBD. Periantral mural cells signify weak Npr2 activity. When the distance from oocyte membrane increases, Npr2 activity in periantral mural cells decreases. (Adapted from Refs. 5–14.)

Both prophase I arrest and meiotic resumption are maintained by the cAMP levels inside the oocyte. Significant decline in cAMP concentrations inside the oocyte is required for the resumption of meiosis.[97] By inhibiting its synthesis and transport, LH surge decreases cGMP concentrations inside the oocyte and initiates the meiotic resumption. It has been reported that closure of gap junctions by LH surge inhibits cGMP transport from the somatic compartment into the oocyte.[106] In order to close gap junctions, LH phosphorylates the connexin 43 protein.[107] The gap junction–blocking effect of the LH can be mimicked by carbenoxolone, a gap junction blocker.[106] Administration of this agent leads to premature nuclear envelope dissolution. Paradoxically, LH induces cAMP synthesis in the somatic compartment.[97] However, closure of gap-junctional communication by the LH surge inhibits the transfer of granulosa cell-derived cAMP into the oocyte. Taken together, decline in the cGMP concentrations inside the oocyte alleviates the suppressive effect of cGMP on PDE3A,[26,30,92,108] which hydrolyzes cAMP inside the oocyte.

It is well known that LH inhibits the production of cGMP synthesis in somatic cells. As described in detail above, cGMP production in somatic cells is regulated by the Nppc/Npr2 system.[30,92] On the other hand, little is known about the possible effects of LH surge on Nppc/Npr2 system. In goat, while FHS administration induces Nppc expression in somatic cells, LH reduces its expression.[90] In contrast, neither FSH nor LH administration leads to significant changes on Nppa expression.[90] In mouse, Npr2 expression in cumulus cells is blocked by LH.[28] Likewise, elevated levels of follicular fluid Nppc were detected in humans undergoing IVF-ET before ovulation.[11] Administration of ovulatory dose of hCG in patients undergoing IVF causes a decline in follicular fluid Nppc concentration, confirming its suppression by hCG.[11] Taken together, Nppc/Npr2 is a system that is responsive to LH, FSH, and hCG.[11,28,90]

A possible mediator of the LH effect on the Nppc/Npr2 system is the epidermal growth factor (EGF).[109] While EGF and EGF-like growth factors (EGF-LGF) are located on the

mural granulosa cells, EGF receptors (EGF-R) are located on the cumulus granulosa cells. EGF-LGF potentiates the effectiveness of LH signal, induces the expression of EGF-R,[91,108] and inhibits the Npr2 activity. Following LH surge, expression of cumulus cell-derived mitogen-activated protein kinase (MAPK) increases and activates the EGF-R.[110] Little is known about how EGF-LGF initiates the meiotic resumption. Decline in the expression levels of Npr2 after the LH signal takes place by three possible mechanisms. First, activation of EGF-R increases calcium levels inside the cumulus granulosa cells and reduces Npr2 activity.[109] Second, induction of EGF-R activity decreases Npr2 expression in the cumulus cells by means of dephosphorylation reactions.[111] Third, by activating EGF-R, LH increases the secretion of amphiregulin, which leads to downregulation in Nppc expression.[91] Moreover, EGF-R activation inhibits the Nppc mRNA expression in the somatic cells[91] and causes a decline in Nppc concentration.[28] To sum up, EGF-mediated LH action inhibits the expression of Nppc/Npr2 in the somatic cells and conversion of the GTP into the cGMP is blocked.[11] As a result, both cAMP and cGMP concentrations decrease inside the oocyte, which leads to meiotic resumption[29,108] (Fig. 2).

(iii) Does the establishment of selective regulation methods to prevent follicle loss increase progress in fertility preservation technologies in prematurely aging women and young cancer patients undergoing chemoradiotherapy?

Differentiation and maturation of follicles are regulated by the signaling pathways that present between the oocyte and granulosa cells. Each member of this complex needs the support of the others, during both the dormant stage and folliculogenesis. This cell complex is very sensitive to the harmful effects of senescence, genetic and metabolic factors, and chemoradiotherapy. Defect in a member adversely affects the other. For instance, apoptosis of somatic cells causes the death of the oocyte and vice versa.

The pool of dormant-stage follicles inside the ovarian cortex determines individual-functional ovarian reserve. Sufficient amount of inhibitory molecules such as cGMP and cAMP is obligatory to maintain this dormancy. In addition to the oocyte's own inhibitory molecules, somatic cell-derived inhibitory signals are also required to maintain meiotic arrest.[6,15,42] In agreement with previous studies, cGMP is produced by somatic cells and diffuses into the oocyte[28,29] and maintains sufficient amount of cAMP within the oocyte.[26] Defect in this signaling pathways leads to obtaining a lower quality follicle in IVF cycles. The best example of the disturbed somatic cell signal is the persistence of meiotic arrest after the oocyte pick-up (OPU). Actually, management of the oocytes having meiotic arrest after the OPU is a very important problem in many IVF laboratories. Closure of intercellular communication of the GV oocyte by using gap junction blockers results in the resumption of meiosis and improves the oocyte stage. Moreover, placing a GV-stage oocyte in the phosphate-containing medium may elevate the GV oocyte to a higher stage.

The risk of follicle loss increases in young women with cancer and undergoing chemoradiotherapy. Both radiotherapy and chemotherapy carry a great risk for fertility outcome. Following chemoradiotherapy, PGCs may undergo irreversible damage leading to premature ovarian aging. Ovarian tissue cryopreservation, oocyte vitrification, and slow freezing at the GV stage are promising methods to improve the success rate of IVF-ET in these women. Nevertheless, cryoprotectants, cooling rate, storage time, and thawing methods may have a detrimental effect upon the follicle. There is no effective preventive measure to protect chemoradiotherapy-induced follicle loss in young women with cancer, who do not accept oocyte or ovarian tissue cryopreservation. Reducing the radiation exposure to the gonads by shielding or removing them from the field of radiation may preserve follicle loss. However, these preventive measures are invasive, and escape from follicular apoptosis is not possible. Targeted inhibition of both PDE3A and PDE9A before chemoradiotherapy may prevent the follicle maturation and lead to a decline in the apoptotic follicle death. Similar effects can be obtained by the administration of cGMP or cAMP analogs. By using selective signal modulators, if we can regulate the phosphorylation reactions inside the oocyte, we may prevent the follicle loss and extend the reproductive life span for women under the risk of premature ovarian aging.

Conclusion

Disturbance in oocyte meiotic events can lead to subfertility or premature aging by reducing the functional ovarian reserve. Selective regulation of somatic signals may be used in the treatment for infertile patients who suffer from the lack of good quality eggs. Management of the intercellular communication via gap junction blockers increases our control upon oocyte meiotic events. Accordingly, by using PDE inhibitors and/or cGMP or cAMP analogs, we can keep the oocytes at the GV stage and slow down their metabolism. As a consequence, we may inhibit chemoradiotherapy-induced follicle loss and preserve the future fertility of young female cancer survivors.

Author Contributions

Conceived the study, wrote the first draft of manuscript, and drew the pictures: OC. Contributed to the writing of the manuscript: NC. Jointly developed the structure and arguments for the review and made critical revisions: SG, ETH, and SA. All authors contributed to the design and preparation of the manuscript, and reviewed and approved of the final manuscript.

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