REPRODUCTION
REVIEW

Disorganization of the germ cell pool leads to primary ovarian insufficiency

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Abstract

The mammalian ovary is an organ that controls female germ cell development, storing them and releasing mature oocytes for transporting to the oviduct. During the fetal stage, female germ cells change from a proliferative state to meiosis before forming follicles with the potential for the growth of surrounding somatic cells. Understanding of molecular and physiological bases of germ cell development in the fetal ovary contributed not only to the elucidation of genetic disorders in primary ovarian insufficiency (POI), but also to the advancement of novel treatments for patients with POI. Accumulating evidence indicates that mutations in *NOBOX*, *DAZL* and *FIGLA* genes are associated with POI. In addition, cell biology studies revealed the important roles of these genes as essential translational factors for germ cell development. Recent insights into the role of the PI3K (phosphatidylinositol 3-kinase)-AKT signaling pathway in primordial follicle activation allowed the development of a new infertility treatment, IVA (*in vitro* activation), leading to successful pregnancy/delivery in POI patients. Furthermore, elucidation of genetic dynamics underlying female germ cell development could allow regeneration of oocytes from ES (embryonic stem)/iPS (induced pluripotent stem) cells in mammals. The purpose of this review is to summarize basic findings related to female germ cell development and potential clinical implications, especially focusing on POI etiologies. We also summarize evolving new POI therapies based on IVA as well as oocyte regeneration.
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Introduction

During the fetal stage, primordial germ cells (PGC) migrate into the fetal ovary. At 5 weeks after fertilization, the human ovary contains an estimated amount of 700–1300 germ cells (Witschi 1948). The number of germ cell reaches an estimated amount of 600,000 cells between 16 and 20 weeks of pregnancy (Barker 1963). In mouse ovaries, there are around 50 primordial germ cells at 7.5-day post coitus (dpc) (Ginsburg *et al*. 1990, MacGregor *et al*. 1995) and germ cell number peaks at 13.5 dpc, reaching about 20,000 cells (Tam & Snow 1981). For both species, variation patterns are the same, although cell number and timing are different. After increases in numbers, PGCs form colonies in the ovary. Once the PGCs form cysts, they initiate meiotic division and cell growth at 11–12 weeks post-fertilization in women (Gondos *et al*. 1986, Motta *et al*. 1997) and 12.5 dpc in mice (Tingen *et al*. 2009, Jameson *et al*. 2012). Meiotic cells were arrested in the diplotene stage of prophase I during germ cell cyst breakdown accompanied by reduction in cell numbers. Cyst breakdown is associated with the individualization of germ cells to form primordial oocytes, around 16 weeks of pregnancy in humans and at the time of birth in mice (Grive & Freiman 2015). Then, oocyte meiosis arrests in the dictyate stage of prophase I. Individual oocyte interacts with ovarian somatic cells to form primordial follicles in the ovary. Primordial follicles remain dormant for months and years and only a small fraction of them become activated under regulation of the kit ligand (Zhang *et al*. 2014). Once primordial follicle is activated, they grow into primary, secondary and antral follicle stages, accompanied by growth and maturation of the oocyte before ovulation of the mature oocyte (Richards & Pangas 2010).

Disorganization of germ cell development and primordial follicle formation likely cause a decline in the germ cell pool, resulting in decreases of follicles in women with the most severe cases of primary ovarian insufficiency (POI). POI, also known as premature ovarian failure (POF), is defined as menopause before 40 years of age associated with elevated serum gonadotropin levels (Coulam *et al*. 1986, Simpson & Rajkovic 1999). POI patients show symptoms of estrogen deficiency and infertility due to lack of follicle growth and ovulation. These patients are resistant to traditional gonadotropin treatment, and thus, oocyte donation

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is the only established treatment option. In terms of etiology, POI affects 1% of women and several different mechanisms can lead to ovarian dysfunction including genetic mutations, autoimmune diseases, iatrogenesis and other abnormalities.

Recently, a novel clinical approach, *in vitro* activation (IVA) of primordial follicle (Kawamura 2013), has been developed and succeeded in allowing POI patients to have their own genetic children. However, IVA method only activates dormant follicles and it cannot be applied to POI patients without primordial follicles. In order to treat POI patient lacking follicles, it is necessary to derive oocytes from stem cells. Remarkably, investigators attempted to derive oocytes as models for future POI therapy using murine iPS/ES cells (Hayashi *et al*. 2012, Hikabe *et al*. 2016) or human female germ stem cells (White *et al*. 2012). The present review focuses on the recent knowledge of germ cell development to facilitate future design of POI therapies.

Regulatory mechanisms underlying oocyte development and follicle formation

Germ cell proliferation and cyst formation

In human, germ cells migrate into the fetal ovary at 3 weeks after fertilization. These cells initiate proliferation and cyst formation before reaching maximum numbers in the ovary at 20 weeks of pregnancy. Germ cell growth and cyst formation are dependent upon extracellular matrixes in the basement membrane secreted by ovarian somatic cells (Heeren *et al*. 2015). Indeed, immunohistochemical studies show that dividing germ cells with Ki-67 positive signals are present in the ovary until postpartum (Stoop *et al*. 2005). Activin is known as a factor capable of inducing proliferation of these cells (Martins da Silva *et al*. 2004) (Fig. 1). As part of extracellular matrixes, collagen type IV, fibronectin and laminin have been found in germ cell cysts (Heeren *et al*. 2015).

In mice, germ cells mainly proliferate in the fetal ovary. Cell division and cyst formation of germ cells last only a few days from 7.5 dpc to 13.5 dpc (Tam & Snow 1981). Bone morphogenetic proteins (BMPs) of the transforming growth factor b (TGFb) family, including BMP2, 4 and 7, are important triggers for germ cell proliferation based on cell culture experiments (Pesce *et al*. 2002, Puglisi *et al*. 2004) (Fig. 1). In *in vivo* studies, *Bmp7* null mice showed a reduction of germ cells at 11.5 dpc (Ross *et al*. 2007). RNA-seq studies showed that pre-granulosa cells express Bmp2 and Bmp4 during 11.5–13.5 dpc (Jameson *et al*. 2012) (Fig. 1). These results suggest that BMPs probably induce proliferation of germ cell *in vivo*. Moreover, *in vitro* culture experiments showed that Kit Ligand (KITL) (Dolci *et al*. 1993), basic fibroblast growth factor (bFGF) (Matsui *et al*. 1992), tumor necrosis factor-a (TNFa) (Kawase *et al*. 1994) and pituitary adenylate cyclase activating peptide (PACAP) (Pesce *et al*. 1996) induce germ cell division. These reports indicate that germ cell proliferation may be regulated by multiple factors during fetal ovary development. Follistatin (FST), an antagonist of activin, was shown to maintain germ cell number and cyst (Fig. 1). Although mice lacking *Fst* have comparable number of germ cells in fetal ovary at 15.5 dpc as wild mice, germ cells decreased at 16.5 dpc and are completely depleted at birth (Yao *et al*. 2004). The treatment of mouse fetal ovaries with FST288, as activin-neutralizing isoform of FST, led to the inhibition

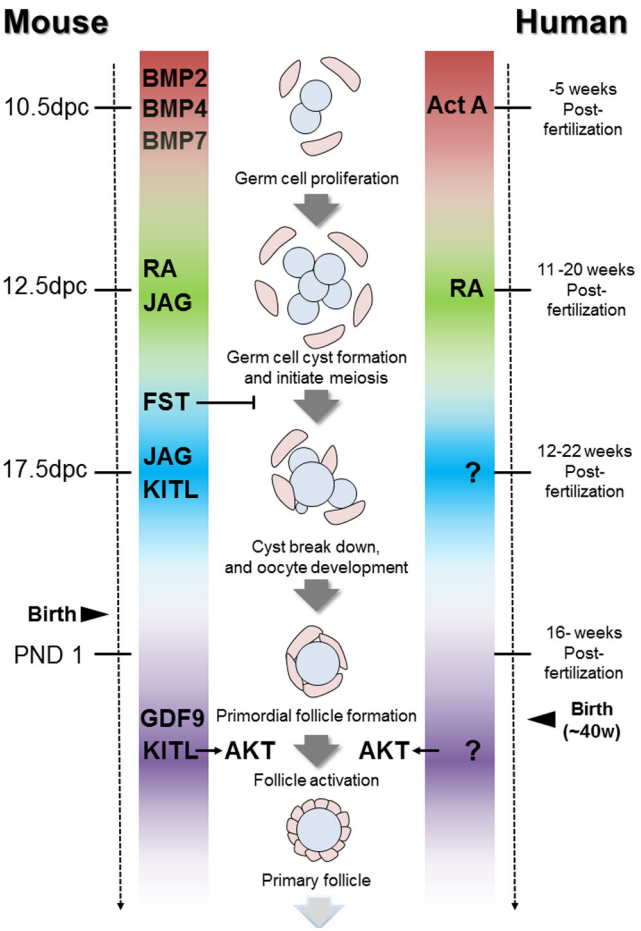

Figure 1 Temporal contribution of secretory factors during human and mouse germ cell development. Primordial germ cell proliferates in the fetal ovary at 10.5 dpc in mice and at approximately 5 weeks of gestation in human. These cell proliferations are induced by BMPs in mice, but Activin A in human. FST maintains germ cell proliferation and cyst formation. Proliferating cells colonize on the basement membrane shaped by somatic cells in fetal ovary at 12.5 dpc in mice and at 11–20 weeks of gestation in human. Colonized germ cells initiate meiotic division following RA and Jagged (JAG) stimulations in mouse and RA stimulation in human. During meiosis, germ cell cyst breaks down and oogonia grow at 17.5 dpc in mice and at 12–22 weeks of gestation in human. These processes are induced by Jagged and KITL in mouse, but by unknown factors in human. Primordial follicles form from post natal day 1 (PND1) in mice and from 16 weeks of gestation in human. Activation of primordial follicles to develop primary follicles is mediated by the PI3K-Akt signaling pathway.

of cyst breakdown and germ cell growth, together with the reduction of germ cell number (Wang *et al*. 2015). These results indicate that FST controls germ cell proliferation and germ cell cyst formation (Fig. 1).

In human, the relationship between these secreted factors inducing germ cell proliferation and POI has not been reported. It is well know that patients with Turner's syndrome (complete X (45X) or partial monosomy X (46X, i)) have small numbers of germ cells at 20 weeks of gestation (Modi & Bhartiya 2003). About 90% of patients with 45X and 46X, i show primary amenorrhea (Ogata & Matsuo 1995, James *et al*. 1998) due to a decline in germ cell proliferation in the fetal ovary. Therefore, these secreted factors are likely deleted or down regulated in patients with Turner's syndrome. However, *BMPs*, *FST* and other secreted factors involved in the induction of germ cell proliferation are not located in X chromosome. Mutations of X chromosome-linked gene in fragile X mental retardation 1 (FMR1), which acts to initiate DNA replication during meiotic division (Schmucker *et al*. 1996), exhibit the POI phenotype due to the expansion of a CGG trinucleotide repeat (Pietrobono *et al*. 2005, Sullivan *et al*. 2005, Terracciano *et al*. 2005). Because FMRI regulates mRNA trafficking from the nucleus to the cytoplasm, it is interesting to investigate changes in RNA trafficking during germ cell development.

Coordinating meiotic progression in the ovary

In female mammals, there is only one round of meiosis in germ cells during lifetime. At 11–12 weeks of gestation, germ cells in human fetal ovary initiate meiotic division, starting from the S-phase until the diplotene stage (Fig. 1). Many studies in mice revealed various intracellular factors regulating meiosis. Before cell-cycle changes, germ cells exhibit increases in RNA levels for deleted in Azoospermia-Like (*Dazl*) and DEAD-box helicase 4 (*Ddx4* (alias *Vasa*)) (Castrillon *et al*. 2000, Medrano *et al*. 2002). The DAZL protein mediates transcription of synaptonemal complex proteins (SCPs) including SCP3 and SCP1 (Reynolds *et al*. 2007). SCPs are meiosis-related proteins essential for chromosome segregation via chromosome pairing and meiotic recombination (Pittman *et al*. 1998). *Scp1* knock down in cultured fetal ovaries showed decreases in diplotene stage oogonium (Paredes *et al*. 2005), suggesting that diplotene stage progression is supported by SCP1. In contrast, studies in stimulated by retinoic acid 8 (*Stra8*) null mice showed no effect in meiotic arrest of oocytes and follicle development, but ovulated oocytes were abnormal (Doksin *et al*. 2013). Despite these studies, detailed mechanisms of these factors are not fully understood. For example, retinoic acid (RA) can induce meiosis genes expression, such as *Scp3* and *Stra8* (Oulad-Abdelghani *et al*. 1996, Menke *et al*. 2003, Tedesco *et al*. 2013) (Fig. 1). Moreover, a recent study showed that novel meiosis regulatory factors, *Jagged 1* and *2*, were expressed in fetal gonads, whereas their receptors NOTCH 1-3 were expressed in germ cells, during 11.5-14.5 dpc in mice and their inhibition led to a decline of *Stra8* gene expression and suppression of germ cell progression to the diplotene stage. These findings suggested the important roles of NOTCH receptor signaling in meiosis progression (Feng *et al*. 2014) (Fig. 1). Furthermore, meiotic progression also needs secreting factors from ovarian somatic cells based on the studies of oocyte regeneration from ES/iPS cells in mice. Further research will be required to fully understand the intraovarian mechanisms underlying meiotic division.

In contrast to accumulating knowledge on meiotic progression in mice, study in human is limited. Recent review implies the contribution of *DAZL* mutation in pathophysiology in POI (Rosario *et al*. 2016) based on a correlation between several common polymorphisms within the *DAZL* gene and onset age of POI (Tung *et al*. 2006*a*,*b*). Comprehensive studies focused on DAZL-induced meiotic cascade would open a new understanding on human meiotic progression and elucidate molecular mechanisms underlying human meiotic progression to allow the development of new methods to regenerate oocytes.

Germ cell attrition and continuing development

In human, germ cell death is observed from 8 weeks of gestation, and the number of germ cells begins to decline after a peak at 20 weeks in the fetal ovary. During this decline, the germ cell cyst breaks down, and germ cells are separated from the ovarian somatic cells followed by oogonia growth (Fig. 1). During oogonia growth, the newly formed oogonia increase their volume with the elevation of the numbers of mitochondria and ribosome from 14.5 dpc to 4 days after birth in mice (Fig. 1). The germ cell cyst breakdown is accompanied by apoptosis and autophagy resulting in rapid decreases in germ cell numbers (Rodrigues *et al*. 2009). In mice, Jagged 1/2 and KITL are known to stimulate these processes (Fig. 1). Ovarian somatic cell (pre-granulosa cell)-specific deletion of *Notch2*, the receptor for *Jagged 1/2*, in mice showed the maintenance of oocyte numbers and multi-oocyte follicles at 3 weeks of age caused by defects in cyst breakdown (Xu & Gridley 2013) (Fig. 1). Using 17.5 dpc ovaries, organ culture studies indicated that KITL enhances germ cell cyst breakdown and oogonia growth (Jones & Pepling 2013). Although molecular mechanism underlying germ cell attrition is unknown, a recent murine study indicated that dominant oogonia receive cytoplasm, mitochondria, microtubules and centrosome from neighboring oogonia via intercellular bridges and gaps (Lei & Spradling 2016). Neighboring germ cells are connected to each other via a channel protein, TEX14 (Greenbaum *et al*. 2009). In human, fetal ovary after 12 weeks of gestation expresses channel protein TEX14 (Hormard *et al*. 2009), suggesting that the growth of oogonia may be induced by material transport from neighbor oogonia via the channels.

Further studies are needed to elucidate the underlying intercellular mechanisms.

No evidence of POI associated with defects in this process has been reported. However, *Fst* mutant mice with FST288 skipping exon 6 showed POI phenotypes with increased germ cell attrition, leading to decreased follicle and pups numbers (Kimura *et al*. 2010). Thus, further research on the mutation of *Fst* and factors related to oogonia growth could elucidate molecular defects in POI patients.

Primordial follicle formation

In human, primordial follicles are found in the ovary from 16 weeks of gestation (Fig. 1). After cyst breakdown, oogonia are surrounded by somatic cells, named as pre-granulosa cells. The complex of oogonia and pre-granulosa cells form 1,000,000–2,000,000 primordial follicles immediately after birth (Strauss & William 2004). Morphologically, cyst breakdown and subsequent cooperation of germ cells and ovarian somatic cells form primordial follicles. Factor in the germline alpha (*Figla*) is essential for follicle formation. The expression of *Figla* is observed at 17.5–19.5 dpc when meiosis has been completed (Liang *et al*. 1997, Soya *et al*. 2000). Of note, a study using *Figla* null mice showed the absence of primordial follicle formation at birth (Soya *et al*. 2000).

In human, *FIGLA* is expressed in fetal ovary after 15 weeks of gestation (Bayne *et al*. 2004), and thus suggesting its involvement in follicle formation. Importantly, mutation in *FIGLA* gene at exon 1 or 3 is associated with POI (Zhao *et al*. 2008, Tosh *et al*. 2015). In Chinese population, *FIGLA* mutation is detected in 4% of POI patients (4 out of 100). The onset of POI in patients with *FIGLA* mutation is relatively young (22, 27, 32 and 36 years of age) and the abnormal phenotype is observed only in ovaries (Zhao *et al*. 2008).

Follicle development

Primordial follicles keep oocytes in a dormant condition for a long time (Franchi & Mandl 1962, Weakley 1967). Studies using genetically modified mice indicate that oocyte regulates follicle survival. For example, mice lacking genes encoding oocyte transcriptional factor *Sohlh1* (spermatogenesis and oogenesis specific basic helix-loop-helix 1), *Lhx8* (LIM Homeobox 8) or *Sohlh2* showed normal primordial development, but had no follicles left at 3 weeks of age (Pangas *et al*. 2006, Choi *et al*. 2008). Oocyte-specific deletion of the homeobox gene *Nobox* in mice showed follicle-like structure lacking oocytes in the ovary at 2 weeks of age (Rajkovic *et al*. 2004). However, it is unclear how these transcriptional factors support follicular survival. On the other hand, follicular granulosa cells are also known to regulate oocyte survival. FOXL2 (Forkhead Box L2) is a transcription factor acting in granulosa cells at primordial and primary stage. Follicles in *Foxl2* null mice showed growth arrest and all follicles were depleted at 16 weeks after birth (Schmidt *et al*. 2003).

Based on the analyses of 96 cases of POI patients, the relationship between *NOBOX* gene mutation and POI was reported (Qin *et al*. 2007), showing down regulation of follicle growth factors such as *GDF9* and *KITL* (Bouilly *et al*. 2015). Furthermore, *FOXL2* gene mutation is also found in patients with POI (Crisponi *et al*. 2001).

Activation of dormant primordial follicles to primary follicles is regulated by the PI3K-AKT pathway in the oocyte (Reddy *et al*. 2008) (Figs 1 and 2). Kit ligand binds its cognate tyrosine kinase receptor C-KIT and stimulates PI3K, leading to the conversion of the lipid second messenger PIP2 (phosphatidylinositol (4,5) bisphosphate) into PIP3 (phosphatidylinositol (3,4,5) triphosphate). Then, PIP3 activates AKT and promotes translocation of AKT to the nucleus to inhibit the activity of a transcriptional factor, FOXO3. PTEN negatively regulates this pathway by dephosphorylating PIP3 to PIP2 (Fig. 2) (Reddy *et al*. 2005).

Studies using ovarian cultures showed that KITL treatment dramatically increased the number of primary follicles (Parrot & Skinner 1999, Jones & Pepling 2013, Zhang *et al*. 2014) (Figs 1 and 2). Oocyte-specific deletion of *Pten*, a negative regulator of PI3K, in mice (GDF9-Cre mice) showed that all primordial follicles developed simultaneously, and no follicle in the ovary remained at 16 weeks of age (Reddy *et al*. 2008, Jagarlamudi *et al*.

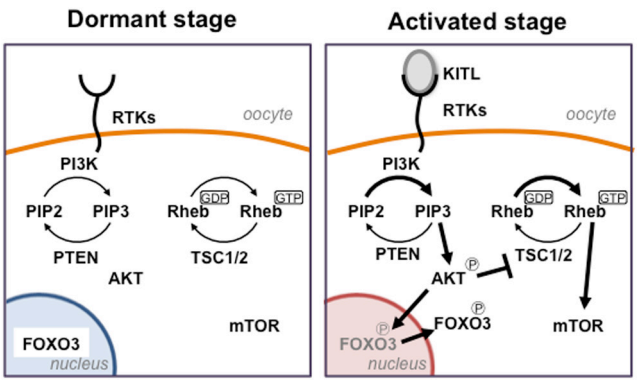

Figure 2 The PI3K-AKT pathway regulates primordial follicle activation. FOXO3 located in oocyte nucleus prevents the activation of dormant primordial follicles. The activation of upstream receptor tyrosine kinases (RTKs) by their cognate ligands (e.g. kit ligand (KITL), etc.) stimulates PI3K activity, resulting in increases in PIP3 levels followed by AKT stimulation. Then, activated AKT translocate to the nucleus and phosphorylate FOXO3 to promote nuclear export of FOXO3, leading to the suppression of FOXO3 activity to stimulate primordial follicle activation. The activated Akt also suppresses TSC1 and TSC2 complex to increase active GTP-bound form of Ras homolog enriched in brain (Rheb) and then activates mTOR, leading to activation of dormant follicles. PTEN converts PIP3 to PIP2 thus suppressing the actions of PI3K. Deletion of the *PTEN* and *FOXO3* genes exhibit activation of primordial follicles.

2009). Furthermore, deletion of the transcription factor FOXO3A, downstream of PI3K-Akt signaling, in mice showed the same phenotype as oocyte-specific *Pten* null mice (Castrillon *et al*. 2003). Therefore, activated Foxo3a is essential for the maintenance of the oocyte pool. Also, the action of PI3K-AKT signaling induces follicle development from the primordial stage (Brenkman *et al*. 2003) (Fig. 2). Mammalian target of rapamycin (mTOR) is the catalytic subunit of two structurally distinct complexes, mTORC1 and mTORC2. *Tsc1* or *Tsc2* null mice also showed spontaneous activation of dormant primordial follicles to primary follicles (Adhikari *et al*. 2009, 2010). Interestingly, double deletion of *Tsc1* and *Pten* leads to synergistically enhanced primordial follicle activation, demonstrating the essential and cooperative roles of PI3K-AKT and mTOR signaling pathways in regulating primordial follicle dormancy (Adhikari & Liu 2010) (Fig. 2).

After development to the primary stage of follicles, oocyte secreted GDF9 that is essential for the follicle growth (Eppig 2001) (Fig. 1). *Gdf9* null mice showed defects in growth after the secondary follicle stage (Dong *et al*. 1996). Moreover, sheep with mutation of the related *BMP15* gene also showed defective follicle development after the primary stage (Galloway *et al*. 2000), Because *Bmp15* null mice are fertile (Yan *et al*. 2001), these reports indicate divergent gene functions between species. After secondary follicle stage, follicle growth and ovulation are mainly regulated by the pituitary hormone follicle stimulating hormone (FSH) and luteinizing hormone (LH) (Richard *et al*. 2002).

In human, *GDF9* and *BMP15* are expressed in the oocyte of primordial follicles. From the analyses of 127 cases of POI patients, the relationship between *GDF9* gene mutation and POI has been reported (Dixit *et al*. 2005). Furthermore, two sisters with the *BMP15* mutation are associated with the POI phenotype (Di Pasquale *et al*. 2004). *GDF9* and *BMP15* mutations are also associated with deficiency in early follicle growth leading to their infertility (Di Pasquale *et al*. 2004, Chand *et al*. 2006). Also, FSH receptor (*FSHR*) and *INHA* (Inhibin a), mutations are associated with the POI phenotype in woman. From the analyses of 7 families of POI patients, mutation of A C566T transition in exon7 of the *FSHR* gene showed reduction of ligand binding capacity and signal transduction in receptor-transfected cells (Aittomaki *et al*. 1995). Likewise, *INHA* mutation in the *INHA* sequence occurs in ~7% of POI patients (Shelling *et al*. 2000).

Development of new therapy for POI based on PI3K-Akt signaling

Primordial follicle number decreases during aging. In adult female, approximately 1000 primordial follicles are activated every month. If the number of primordial follicle in the ovary is low (less than 1000), activation of primordial follicle is defective (Macklon *et al*. 1999), resulting in anovulation. POI shows menopause before 40 years of age with abnormally low residual follicles. Hence, it is extremely difficult to induce follicle growth in POI patients even under ovarian hyper-stimulation (Nelson 2009), and egg donation is the only established method for infertility treatment. Recently, we developed a novel method which enables POI patients to conceive their own genetic children by the activation of residual dormant follicles focusing on the PI3K-AKT-FOXO3 signaling pathway. Because of difficulty in performing gene therapy in human, we activated dormant follicles using a PTEN inhibitor and a PI3K activator based on a short-term (2 days) *in vitro* activation protocol. Previous studies showed that a PTEN inhibitor, bpV, indirectly activates the PI3K-AKT signaling pathway (Posner *et al*. 1994, Bavan *et al*. 1995, Schmid *et al*. 2004), whereas a cell-permeable phosphopeptide, 740Y-P, binds to the SH2 domain of p85 regulatory subunit of PI3K to activate its catalytic subunit (Derossi *et al*. 1998). Using both drugs, we demonstrated that short-term culture of neonatal mouse and human ovaries increased primary follicle numbers via nuclear exclusion of FOXO3 *in vitro* (Li *et al*. 2010). Also, we generated mature oocytes displaying normal epigenetic modification in imprinted genes after the transplantation of activated ovaries into the kidney capsule (Li *et al*. 2001). Based on these successes of basic findings, we named this approach as IVA (*in vitro* activation) and applied it for clinical studies to treat infertility in patients with POI. We reported two deliveries of healthy babies after IVA (Kawamura *et al*. 2012, Suzuki *et al*. 2015) (Fig. 3). After our publication of IVA, two separate centers have repeated the IVA approach and one successful delivery was reported (Zhai *et al*. 2016). Currently, the IVA treatment is expanding to more infertility centers.

Limitation of IVA treatment and future studies: the prospect of oocyte regeneration

Ovaries from around half of the POI patients contain no residual follicles under histological analyses (Kawamura *et al*. 2012, Zhai *et al*. 2016). Although we grafted the activated ovarian tissues after IVA back into these patients, no follicle growth was found (Kawamura *et al*. 2012, Suzuki *et al*. 2015). Oocyte regeneration is a potential method to treat these patients so that they could conceive their own genetic children. Recently, a group reported the success of oocyte generation from murine ES/iPS cells (Hayashi *et al*. 2012, Hikabe *et al*. 2016). Researchers induced primordial germ cell-like cells (PGCLCs) from murine ES/iPS cells and then co-cultured PGCLCs with murine fetal ovarian somatic cells to form re-aggregated ovaries. In the aggregated ovaries, PGCLCs resumed meiosis and formed follicles, leading to the production of competent oocytes for fertilization and development into healthy

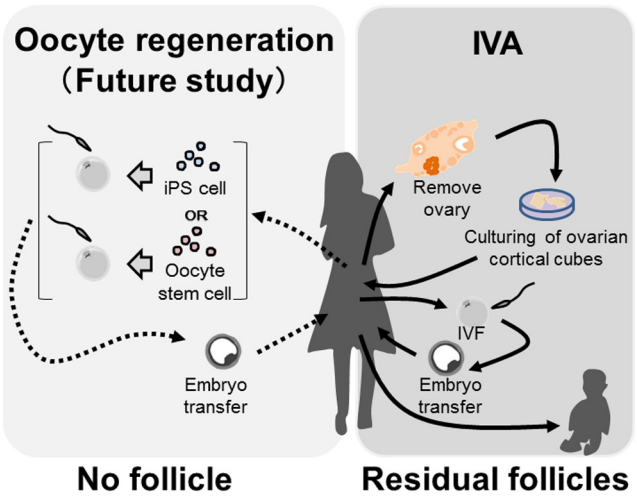

Figure 3 Potential infertility treatments for POI patients to conceive their own genetic children. The IVA approach is effective to treat patients with POI who have residual follicles. However, IVA cannot apply to the POI patients without residual follicles. Oocyte donation is the only infertility therapy to treat POI patients with no residual follicles. To establish the method to conceive their own genetic children in such patients, investigators attempt to regenerate mature oocytes from iPS or oocyte stem cells.

pups (Hayashi *et al*. 2012, Hayashi & Saito 2013, Hikabe *et al*. 2016). Although this method is attractive to regenerate oocytes from POI patients using iPS cells, there are several obstacles to overcome before applying this technique to humans. In particular, it is difficult to obtain ovarian somatic cells from human fetal ovaries. Furthermore, the efficacy of the differentiation of iPS cells into mature oocytes is extremely low under published procedures (Fig. 3).

To address these issues, researchers are trying to regenerate oocytes from oocyte stem cells presumably present in ovaries. A group showed regeneration of mature oocytes in mouse and human from oocyte stem cells isolated from ovarian cortex using DDX4 antibodies through fluorescence activated cell sorting (Johnson *et al*. 2004, 2005, White *et al*. 2012) (Fig. 3). However, many labs could not confirm the presence of oocyte stem cells in mouse (Eggan *et al*. 2006, Lei & Spradling 2013) and human (Byskov *et al*. 2011). Moreover, recent work showed uncertainty in the specificity of antibodies used for the isolation of murine and human oocyte stem cells (Albertini & Gleicher 2015), and the inability of follicle formation after grafting these oocyte stem cells into mouse postnatal ovaries (Zhang *et al*. 2015). Therefore, more studies will be required to establish the method for regeneration of oocytes from oocyte stem cells.

Conclusion

Most of the knowledge accumulated on germ cell development is based on murine studies. In contrast, knowledge on human germ cell development is limited. Although it is difficult to study human germ line cells due to ethical issues, more findings on human germ cell development could help us understand pathophysiology of POI. It could also contribute to the improvement of IVA treatment for POI patients as well as the development of new methods for oocyte regeneration from POI patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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