PI3K/PTEN/Akt and TSC/mTOR signaling pathways, ovarian dysfunction, and infertility: an update

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Abstract

Abnormalities in ovarian function, including defective oogenesis and folliculogenesis, represent a key female reproductive deficiency. Accumulating evidence in the literature has shown that the PI3K/PTEN/Akt and TSC/mTOR signaling pathways are critical regulators of ovarian function including quiescence, activation, and survival of primordial follicles, granulosa cell proliferation and differentiation, and meiotic maturation of oocytes. Dysregulation of these signaling pathways may contribute to infertility caused by impaired follicular development, intrafollicular oocyte development, and ovulation. This article reviews the current state of knowledge of the functional role of the PI3K/PTEN/Akt and TSC/mTOR pathways during mammalian oogenesis and folliculogenesis and their association with female infertility.

Key Words

- ▶ PI3K/PTEN/Akt and TSC/mTOR pathways
- ▶ ovarian function
- ▶ oogenesis
- ▶ folliculogenesis
- ▶ infertility

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Introduction

Infertility is a global health issue, affecting an estimated 48.5 million couples worldwide; of this, the female factors are entirely responsible for one-third of cases (Jumayev *et al.* 2012, Mascarenhas *et al.* 2012). Ovarian dysfunction, tubal obstruction or adhesions, uterine malformations, and implantation failure are some of the well-known mechanisms underlying compromised fertility in mammalian species (Cahill & Wardle 2002). Abnormalities in ovarian function, including defective oogenesis and folliculogenesis, represent a key female reproductive deficiency. Advanced age, genetic factors, environmental toxins, autoimmune conditions, and exposure to chemotherapy or radiotherapy may cause perturbed follicular development or abnormalities in primordial follicle (PF) activation resulting in early depletion of ovarian follicular

reserve or premature ovarian failure (POF) (Hillier *et al.* 2010). This translates into poor oocyte quality and interferes with the orderly and intricate process of oocyte maturation, ovulation, fertilization, implantation, and early embryonic development, thus culminating in poor reproductive health outcomes and infertility (Fassnacht *et al.* 2006).

Ovarian functions are controlled by cyclic pituitary gonadotropic hormones (mainly follicle-stimulating hormone (FSH) and luteinizing hormone (LH)), which in turn are subject to stimulation by the hypothalamic peptide gonadotropin-releasing hormone and modulated by other ovarian factors such as activin and inhibin. While, FSH is mainly involved in follicular growth, cellular proliferation, and estrogen production (aromatase activity),

LH induces androgen biosynthesis, final maturation of the oocytes, ovulation, and terminal differentiation of ovulated follicles into the corpora lutea (CL) (Bhartiya *et al.* 2012). To accomplish their functions, FSH and LH trigger multiple downstream cascades of intraovarian pathways that are essential for maintaining folliculogenesis and oogenesis. Accumulating lines of evidence described in the literature have shown that the phosphoinositide 3-kinase (PI3K)/PTEN/Akt and TSC/mTOR signaling pathways are critical regulators of ovarian function including dormancy and activation of PFs, oocyte maintenance and activation, and granulosa cell (GC) proliferation and differentiation. Abnormalities in these signaling pathways may contribute to certain forms of female infertility due to PF depletion, such as POF and primary amenorrhea (Adhikari *et al.* 2010, 2012). Recently, genetically engineered mouse models with reproductive phenotypes have provided important insights into the role of signaling elements of this pathway as ovarian regulatory factors (Jagarlamudi *et al.* 2010). This review assesses the current state of knowledge of the functional role of the PI3K/PTEN/Akt and TSC/mTOR pathways during mammalian oogenesis and folliculogenesis and their association with female infertility.

Oogenesis and folliculogenesis

The mammalian ovary is a heterogeneous organ containing follicles at various stages of development, and CL, either active or at various stages of involution. Each follicle is composed of an oocyte arrested in prophase of the first meiotic cell division, enclosed by one or more layers of specialized somatic cells that support the oocyte during its growth. Follicular growth can be classified into three phases: i) the gonadotropin-independent phase that involves follicular growth through primordial, primary, and secondary stages; ii) the gonadotropin-responsive phase that involves transition of preantral follicles to early antral stage, and iii) the gonadotropin-dependent phase that involves continual growth beyond the early antral stage and includes follicle recruitment, selection, and ovulation (McNatty *et al.* 2007).

Oocytes are derived from primordial germ cells (PGCs), which expand through mitosis in the fetal ovary. The germ cells become oocytes once they enter meiosis at birth. Oocytes initially develop in clusters termed germ cell syncytia or nests. At least some nests contain germline cysts that arise by synchronous, incomplete mitotic division, but others may form by aggregation (Mork *et al.* 2012, Lei & Spradling 2013). PF formation occurs during gestation in humans and after birth in rodents and involves dissolution

of germ cell nests. Germ cell nest breakdown is accompanied by migration of pregranulosa cells together with disruption of interocyte bridges through apoptosis of individual oocytes. The surviving oocytes get surrounded by a single layer of flattened pregranulosa cells covered by a basal membrane (membrane of Slavjanski) to form PFs. Improper germ cell syncytia breakdown can lead to the generation of multiovular follicles (MOFs). Each female cyst gives rise to single oocyte along with sister cells that serve as nurse cells. Mitochondria and other organelles move through the intercellular bridges to form the Balbiani body or mitochondrial cloud of the oocyte (Pepling *et al.* 2007). The ovary may rely on alternative pathways for cell survival and cell death apart from apoptosis. Autophagy has been demonstrated to be an important regulator of germ cell survival before formation of the primordial follicular pool in murine ovaries (Gawriluk *et al.* 2011).

While the majority of PFs remain in a dormant state, a limited number of PFs are recruited from the resting follicle reservoir into the growing follicle pool. The controlled recruitment of PFs is a prerequisite for the development of mature oocytes and is a critical determinant of reproductive lifespan in all mammalian species. Recently, Zheng *et al.* (2014) have studied the developmental dynamics of PFs under physiological conditions and traced the *in vivo* development of two classes of PFs in knock-in mouse models. They found that a first wave of follicles exists in the ovaries for approximately 3 months and contributes to the onset of puberty and to early fertility. The PFs at the ovarian cortex gradually replace the first wave of follicles and dominate the ovary after 3 months of age, providing fertility until the end of reproductive life. The growth pattern of the first wave of activated follicles is conserved among mammals (including humans) (Peters *et al.* 1975). Fertility in women from onset of puberty to young adulthood may thus depend on the first wave of follicles that are already activated at the fetal stage.

High proportions of abnormal nongrowing follicles, characterized by indistinct germinal vesicle membranes and absent nucleoli, have been reported to be present in prepubertal human ovaries (Anderson *et al.* 2014). However, the prevalence of these follicles declined with age to being absent in adult ovaries, indicating that they are preferentially lost, perhaps through unknown quality control mechanisms. Tingen *et al.* (2009) investigated the mechanism responsible for cell death and follicular atresia in prepubertal mouse ovary. Neither GCs nor oocytes of PFs underwent apoptosis and a nonapoptotic pathway has been suggested to be responsible for small follicle death.

Primary follicles are formed from PFs as the oocytes start to grow and the surrounding flattened pregranulosa cells become cuboidal and proliferative (Da Silva-Buttkus *et al.* 2008). The follicle is called secondary when GCs multiply to form a second layer around the oocyte. During the progression from the primary to the secondary follicle stage, gap junctions are formed between the surface membranes of the oocyte and the neighboring GCs. Other changes occurring at the secondary stage include deposition of zona pellucida (ZP) material around the oocyte, synthesis of cortical granules within the oocyte cytoplasm, nucleolus reorganization, and activation (Fair *et al.* 1997a,b). *FSHR* mRNA was first detected at this stage in cattle and sheep, indicating the establishment of responsiveness to gonadotropins (Xu *et al.* 1995, Bao & Garverick 1998). During the final stages of development, the secondary follicle appears to be surrounded by irregularly spaced islets of differentiated epitheloid cells, which comprise the theca interna, and is now called the preantral follicle. The theca externa is made up of a thick layer of collagen fibers, traversed by numerous blood capillaries; it contains myofibroblasts differentiated from fibroblasts of the stroma (Fakuda *et al.* 2009).

Growth and development of mammalian oocytes is critically dependent on a bidirectional communication between the oocyte and its companion somatic cells (Banerjee *et al.* 2014). The viability of primordial and primary follicles is determined mainly by survival factors derived from the oocyte, whereas the relative expression level of tumor suppressors, apoptotic proteins, and survival factors in GCs determines whether an ovarian follicle will grow or undergo atresia in the late preantral stage. PI3K acting via Akt has been suggested to be an indispensable factor in regulating follicular fate (Reddy *et al.* 2005).

The transition from the preantral to early antral stage is the last but one stage of development in relation to dependence on gonadotropins as well as follicle destiny. Although the development of preantral follicles is not gonadotropin-dependent, FSH treatment has been reported to promote early follicle development (Allan *et al.* 2006, Bhartiya *et al.* 2012). FSH drives the proliferation, growth, and differentiation of GCs characterized by increased vascularization of the theca interna layer peripheral to the basal lamina, formation of a fluid-filled antrum within the maturing follicle, and development of two anatomically and functionally distinct classes of GCs (the mural GCs (MGCs), which line the wall of the follicle and have a steroidogenic role, and the cumulus cells (CCs), which form an intimate association with the oocyte).

CCs have highly specialized transzonal cytoplasmic projections that penetrate through the ZP and form gap junctions at their tips with the oocyte, forming the cumulus–oocyte complex (COC) (Albertini & Rider 1994). FSH induces LH receptor expression in MGCs, necessary for follicles to respond to the LH surge. LH initiates ovulation, terminates GC proliferation, and mediates the genetic transition of GCs to luteal cells. It regulates production of androgen by the theca-interstitial (TI) cells of the ovary, which serves as a substrate for estrogen synthesis in GCs (Palaniappan & Menon 2012). Sufficient exposure of antral follicles to FSH is essential for the follicles to escape from atresia and reach the preovulatory stage. Available results indicate that the PI3K pathway can be activated by FSH signals and subsequently provide a strong survival signal to GCs by partially regulating the activities of the BCL2 family of proteins (Alam *et al.* 2004).

Besides the gonadotropins, several growth factors and intraovarian regulators including insulin-like growth factor (IGF) and members of the TGF β superfamily (GDF9, BMP15, activin, inhibin, etc.) are crucially involved in follicle development and function (Mani *et al.* 2010, Piotrowska *et al.* 2013). IGF is known to stimulate proliferation of granulosa and theca cells and enhances the ability of gonadotropins to stimulate steroidogenesis in these cell populations. Furthermore, IGF has a direct antiapoptotic effect and is selectively expressed in healthy follicles compared with atretic follicles. The Akt and ERK pathways are the key signaling pathways that mediate the effects of IGF (Ryan *et al.* 2008).

Ovulation is characterized by rupture of the follicle wall and release of the COC. The oocyte at this point has already resumed meiosis and progressed to the metaphase II (MII) stage. Meiotically competent oocytes acquire cytoplasmic maturation or developmental competence. Studies on oocyte–GC interactions and of oocyte regulation of GC function have shown that, before the LH surge, oocytes regulate metabolic activity of CCs within the COC (Sugiura *et al.* 2005). After the LH surge, oocytes control the expression of cumulus genes responsible for the mucification/expansion process (Su *et al.* 2004).

After expulsion of the oocyte, the follicle presents a pleated feature and is called a dehiscent follicle. The membrane of Slavjanski fully disappears, and the blood capillaries of the theca invade the granulosa, thereby inducing luteinization of these cells to form the corpus luteum. While GCs are converted into large luteal cells, cells of theca interna constitute small luteal or paraluteal cells situated at the periphery of the CL (Smith *et al.* 1994).

PI3K/PTEN/Akt and TSC/mTOR pathways

The PI3K/PTEN/Akt pathway is a key regulator of many normal cellular processes, including cell proliferation, survival, growth, motility, cytoskeletal rearrangement, and metabolism, through multiple downstream targets. Activation of PI3K, a heterodimer of the p85 regulatory subunit and p110 catalytic subunit, may take place in response to growth factor receptor tyrosine kinases (RTKs), G protein-coupled receptors (GPCRs), small GTPase Ras, or through the nongenomic actions of steroid hormones (SHs) either by direct interaction or through adaptor molecules. Although several RTKs have been proposed to activate PI3K, the KIT receptor is the most widely accepted candidate (Thomas & Vanderhyden 2006). KIT activates PI3K through the direct interaction with an SH2 domain on the p85 regulatory subunit of PI3K.

At the membrane, PI3K catalyzes the phosphorylation of phosphatidylinositol-4,5-bisphosphate (PIP₂) and converts it to phosphatidylinositol-3,4,5-trisphosphate (PIP₃), which recruits downstream molecules with pleckstrin homology domains particularly the serine-threonine kinases Akt (also known as protein kinase B) and phosphatidylinositol-dependent kinase 1 (PDK1) to the membrane. Phosphatase and tensin homolog ten (PTEN) is a negative regulator of PI3K and converts PIP₃ back to PIP₂. PDK1 phosphorylates and activates Akt, which in turn regulates a number of downstream targets. Akt promotes cell survival by inhibiting proapoptotic proteins such as BAX, BAD, forkhead, and p53 (TRP53), and activating prosurvival proteins such as BCL2 (Brown *et al.* 2010, McLaughlin *et al.* 2014). Cell cycle progression is regulated by Akt through indirect stabilization of cyclin D1 and Myc. Akt also stimulates protein synthesis and cell growth by activating the mammalian target of rapamycin (mTOR) pathway through the inhibition of the heterotrimeric complex consisting of tuberous sclerosis 1 or hamartin (TSC1) and tuberous sclerosis 2 or tuberin (TSC2) (Manning & Cantley 2007, Vadlakonda *et al.* 2013).

mTOR is an atypical serine/threonine protein kinase that interacts with several proteins to form two distinct complexes, namely mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). The mTORC1 pathway integrates inputs from growth factors, stress, energy status, oxygen, and amino acids to control processes including protein and lipid synthesis and autophagy (Wullschlegel *et al.* 2006). The TSC1 and TSC2 complex is a key upstream regulator of mTORC1 and functions as a GTPase-activating protein (GAP) for the Ras homolog enriched in brain

(Rheb). Phosphorylation of the TSC1/TSC2 complex and mTORC1 by the effector kinases of the PI3K and Ras pathways leads to inactivation of the former and activation of the latter. mTORC1 controls protein synthesis through phosphorylation of eukaryotic translation initiation factor 4E (4E-BP1) and S6 kinase 1 (S6K1).

When compared with mTORC1, the mTORC2 pathway is insensitive to nutrients but responds to growth factors such as insulin through poorly defined mechanisms that require PI3K. mTORC2 directly activates Akt by phosphorylating its Ser473 motif. PKCa is another kinase activated by mTORC2, which along with effectors such as paxillin and Rho GTPases regulates cell shape by affecting the actin cytoskeleton (Laplanche & Sabatini 2012; Fig. 1).

PI3K/PTEN/Akt and TSC/mTOR pathways in oocyte development and the quiescence, activation, survival, and growth of PFs

PGCs are potential targets for estrogens and estrogenic compounds during the early stages of embryonic development in mammals (Iguchi *et al.* 1986). ER α (ESR1) expression was observed in mouse PGCs, and 17 β -estradiol through this receptor rapidly stimulated Akt, ERK2, and SRC phosphorylation. In addition, the E₂ stimulatory effects were associated with increased phosphorylation of the KIT receptor (La Sala *et al.* 2010). Studies on mice carrying mutations at the steel and white spotting loci, encoding Kit ligand (KitL) and c-kit, respectively, have indicated a role for the receptor-ligand pair in PGC survival, migration, and proliferation (McCoshen & McCallion 1975). Mice homozygous for the viable allele steel dickie were infertile, possibly due to the failure of PGCs to migrate successfully to the fetal genital ridge (Flanagan *et al.* 1991). A role for KitL as a PGC chemoattractant and for the PI3K/Akt and Src kinase as players involved in the activation of the PGC migratory machinery has been suggested (Farini *et al.* 2007). Gu *et al.* (2011) found that the membrane-bound form of Steel factor (KitL) maintains a high local concentration for mouse PGC motility and defines their region of migration. In a previous study, Akt signaling has been reported to promote derivation of embryonic germ (EG) cells from PGCs in transgenic mice expressing an AKT-MER fusion protein. The kinase activity of this fusion protein has been shown to be regulated by 4-hydroxytamoxifen, a ligand-modified estrogen receptor. PGC-specific inactivation of PTEN enhanced both EG cell production and testicular teratoma formation (Kimura *et al.* 2008).

Figure 1

Schematic illustration of the PI3K/PTEN/Akt and TSC/mTOR pathways and their signaling crosstalk with other pathways in mammalian ovaries. Binding of extracellular ligands to cognate transmembrane RTKs such as IGF1R, insulin receptor or Kit, among others, results in activation of the PI3K pathway. An additional docking protein (IRS1/IRS2) is phosphorylated by insulin receptor and serves to activate PI3K. PI3K phosphorylation of PIP2 to PIP3 allows PIP3 to act as a secondary messenger, propagating intracellular signaling by directly binding PH domains of PDK1 and Akt. The tumor suppressor PTEN is a negative regulator of PI3K and dephosphorylates PIP3 to PIP2. Activated Akt phosphorylates a variety of effector molecules including BAD, Foxo1, GSK3 β , and p27, which then control cell survival and growth. Akt phosphorylates and inactivates Foxo3a by promoting translocation to the cytoplasm, which promotes proliferation. Akt regulates cell cycle progression by indirectly stabilizing cyclin D1 and Myc. Activated Akt phosphorylates TSC2, which, in association with TSC1, controls mTOR. mTOR exists as two distinct complexes: mTORC1, inhibited by rapamycin, controls translation, suppresses autophagy, and regulates transcription factors HIF1 α and VEGF expression. mTORC2 regulates the actin cytoskeleton and activates Akt through phosphorylation of ser473.

Signaling through RTKs activates the adaptor proteins growth factor receptor-bound receptor 2/Son of sevenless (GRB2/SOS), leading to activation of the ERK pathway. Phospholipase C/protein kinase C cascade is also involved in the downstream signaling of RTKs. PI3K can be stimulated by activated Ras, GPCRs, or through the nongenomic action of steroid hormones (SHs). GAB2 coordinates signals from the FSH-stimulated rise in cAMP that leads to activation of PI3K/Akt. ERK phosphorylates TSC2, thus inactivating its suppressive effect on mTORC1. RSK directly affects the mTORC1 complex by phosphorylation of Raptor. Members of TGF β family (BMP/GDF9) activate the PI3K/Akt pathway in a cell-type-specific manner. The downstream signaling initiated by TGF β binding to its serine/threonine kinase receptor is carried out by members of the Smad (Mothers against decapentaplegic homolog) family. TGF β signaling may function through activation of S6K1 and rpS6. The family of hairy enhancer of split (Hes) transcription factors are important mediators of Notch signaling and regulate the Kit receptor that stimulates the PI3K signaling pathway. A full colour version of this figure available via <http://dx.doi.org/10.1530/JME-14-0220>.

The establishment of the PF pool is important for female fertility. Estradiol signaling plays a critical role in inhibiting oocyte nest breakdown and PF assembly (Chen *et al.* 2007). Exposure of mice to estrogenic compounds such as diethylstilbestrol, genistein, or bisphenol A during the neonatal period resulted in MOFs (Jefferson *et al.* 2006). Both ER α and ER β (ESR2) are expressed in neonatal

mouse ovaries, supporting their important role in cyst breakdown. E $_2$ has also been proposed to regulate cyst breakdown through a membrane-bound ER (Chen *et al.* 2009). Cyst breakdown is accompanied by oocyte death, pertinently, mutants lacking BAX protein were found to have more oocytes and delayed cyst breakdown (Greenfeld *et al.* 2007). Furthermore, the gene encoding the BCL2

protein has been shown to possess an ERE promoter region, indicating that BCL2 could be the link between the estrogen and apoptosis pathways (Klinge 2001). Brown *et al.* (2010) studied the effect of the null mutation of *Akt* (*Akt1*) on follicle development in mice. At postnatal day 25, MOFs were observed in *Akt1*^{-/-} ovaries, indicating a defect in cyst breakdown. Higher estradiol levels observed in *Akt1*^{-/-} ovaries signified the role of estrogen in regulating cyst breakdown. Pertinently, MOFs have also been reported in ovaries lacking GDF9 or BMP15 and in mice that overexpress the *Inha* gene (Yan *et al.* 2001). Huansheng *et al.* (2011) analyzed the effect of estrogen on PF assembly using an *in vitro* newborn mouse ovarian culture system and in neonatal mice treated with injections of estrogen. Unassembled follicles were more in estrogen-treated groups and estrogen inhibited development of follicles by regulating expression of KitL.

PI3K/PTEN/Akt signaling is of importance in determining the developmental course of the PFs, including their activation, survival, and death (Markholt *et al.* 2012). KIT receptor has been suggested to be the critical upstream regulator of PF activation via PI3K/Akt. However, employing a knock-in mutation (KitY719F) that completely abrogates signaling via PI3K, John *et al.* (2009) observed that although KIT has a specific role in the maintenance of PF reserve and in the transition of primary to secondary follicles, it is dispensable in PF activation in mice.

Overactivation of PI3K signaling by an oocyte-specific deletion of the *Pten* gene (using transgenic mice expressing Cre recombinase under the control of the *Gdf9* promoter) was observed to cause premature activation of the entire pool of PFs, followed by their depletion in early adulthood resulting in POF (Reddy *et al.* 2008). However, when the *Pten* gene was conditionally deleted from oocytes of primary and further developed follicles in transgenic mice expressing *Zp3* promoter-mediated Cre recombinase (Jagarlamudi *et al.* 2009), follicular development was found to be unaltered and oocyte maturation was also normal, which led to normal fertility and unaltered litter size. It was suggested that overactivation of PI3K signaling in oocytes does not affect the development of growing follicles and that there is a stage-specific function of PTEN/PI3K signaling in mouse oocytes that controls follicular activation.

PTEN inhibitors were found to effectively activate PFs both in neonatal mouse ovaries and in human cortical tissues (Li *et al.* 2010). Transient inhibition of PTEN triggered activation of PFs in neonatal mouse ovaries, which, under favorable growth conditions, developed into mature, fertilizable oocytes and could be used to obtain

fertile and healthy progeny mice (Adhikari *et al.* 2012). Recently, a live human birth has been reported following treatment of ovarian tissue with an Akt stimulant, followed by replacement, and IVF (Kawamura *et al.* 2013). Unlike the results of these studies, deleterious effects of PTEN inhibition on growth and survival of follicles have also been reported (McLaughlin *et al.* 2014).

PDK1 signaling in oocytes appears to be crucial for maintaining the survival of PFs, which in turn is essential for determining the duration of female fertility (Reddy *et al.* 2009). When compared with PTEN loss that resulted in POF due to excessive follicular activation followed by follicular atresia, *Pdk1* deficiency in mouse oocytes led to POF due to accelerated clearance of PFs directly from their dormant state. Deletion of *Pdk1* caused suppressed PDK1/Akt/S6K1/ribosomal protein S6 (rpS6) signaling in oocytes (Reddy *et al.* 2008). A twofold increase in the number of atretic follicles with degenerate oocytes in *Akt1*^{-/-} mice was observed by Brown *et al.* (2010) at postnatal day 25, when compared with *Akt*^{+/+} controls. At postnatal day 90, the ovaries of *Akt1*^{-/-} mice contained significantly fewer PFs. The KITL survival factor was significantly reduced in the *Akt1*^{-/-} ovary, and a decreased expression of the antiapoptotic factor, *Bcl2l1*, was also observed. Recently, a role of epidermal growth factor (EGF) in maintaining viability (but not promoting activation) of PFs by stimulating Akt phosphorylation has been demonstrated in prepubertal domestic cats (Fujihara *et al.* 2014).

Foxo3 transcription factor has been reported to be the major effector of the PI3K/Akt pathway in context of PF activation and an essential role of *Pten* in regulation of Foxo3 within the oocyte was demonstrated (John *et al.* 2008). In *Foxo3* knockout mice, PFs are assembled normally and subsequently undergo global activation, resulting in a distinctive syndrome of ovarian hyperplasia, follicle depletion, POF, and infertility (John *et al.* 2007). Expression of a constitutively active form of Foxo3a in mouse oocytes led not only to retardation of oocyte growth and follicular development, but also to anovulation and luteinization of unruptured follicles, causing infertility in the transgenic mice (Liu *et al.* 2007). However, in contrast to these findings, Pelosi *et al.* (2013) have shown that overexpression of constitutively active Foxo3 in mouse oocytes maintained ovarian reserve and increased reproductive capacity in mice. The *Foxo3* transgene was placed under the control of a *Kit* promoter for comparison with the *Zp3* promoter in the study conducted by Liu *et al.* (2007). It was presumed that *Zp3-Foxo3* transgene incorrectly expresses FOXO3 at a stage of ongoing oocyte growth, which may disrupt

folliculogenesis. Li *et al.* (2010) performed short-term, ovary-specific treatment of rodent and human ovaries with a PTEN inhibitor and/or a PI3K activator to increase nuclear extrusion of Foxo3 in primordial oocytes. After transplantation under kidney capsules of ovariectomized hosts, treated follicles developed to the preovulatory stage with oocytes capable of undergoing nuclear maturation. From activated murine follicles, mature oocytes could be retrieved for IVF and embryo transfer, followed by delivery of healthy pups with proven fertility. Recently, Zama & Uzumcu (2013) have demonstrated an age-dependent effect on epigenetic alterations in key molecules of specific signaling pathways such as PTEN, and IGF1, or rapid estrogen signaling on developmental exposure of rats to methoxychlor, an environmental endocrine-disrupting chemical. Hypermethylation of Igf1r, Pi3kr, and Foxo3 had an effect on follicular maturation supported by reduced ovulations observed in treated ovaries. In contrast to the results from the above studies, Tarnawa *et al.* (2013) noticed that Foxo3 is not uniquely required for maintenance of PFs in nonrodent species including humans. However, other Foxos, particularly Foxo1 might contribute to oocyte maintenance in a functionally redundant manner.

In order to determine whether Tsc/mTORC1 signaling in oocytes is involved in regulation of follicle activation, *Tsc2* (Adhikari *et al.* 2009) and *Tsc1* genes (Adhikari *et al.* 2010) were deleted from mouse oocytes in primordial and developing follicles. Deletion of both *Tsc2* and *Tsc1* caused premature activation of all PFs around the time of puberty due to elevated mTORC1 activity in oocytes, leading finally to POF. Using a mouse fetal ovary culture system, Wang *et al.* (2014) demonstrated the role of TGF β in maintenance of the PF pool and regulating oocyte growth through activation of rpS6, but not via activation of Akt or Foxo3a.

To further elucidate the functional and pathological roles of elevated mTORC1 signaling in oocytes, Adhikari *et al.* (2013) treated *Pten*-mutant mice with the specific mTORC1 inhibitor rapamycin before activation of the PFs. Rapamycin treatment prevented global follicular activation and preserved the ovarian reserve. Zhang *et al.* (2013b) studied the effects of rapamycin on PF development in rats. The treated group demonstrated decreased mTOR but increased sirtuin signaling, indicating that rapamycin might inhibit the transition from primordial to developing follicles and preserve the follicle pool reserve via modulation of the mTOR and sirtuin signaling (Table 1).

PI3K/PTEN/Akt and TSC/mTOR pathways and ovarian somatic cells

Following a gonadotropin-independent phase, the mammalian follicular growth first becomes FSH-dependent at the secondary stage and then LH-dependent before ovulation. FSH is a critical survival factor for GCs and directs them to proliferate and produce SHs and growth factors. Pertinently, GCs have been found to overexpress genes involved in steroid synthesis and regulation during preantral follicle stages (Bonnet *et al.* 2013). FSH-withdrawal-induced apoptosis in GCs was observed to be accompanied by the accumulation of proapoptotic protein B-cell lymphoma 2-interacting modulator of cell death-extra long (BimEL) (Wang *et al.* 2012). Results from a recent study have indicated that 3,5,3'-triiodothyronine (T₃) increases FSH-induced preantral follicle growth *in vitro* through inhibition of cell apoptosis and promotion of cell proliferation. The survival effects of hormones were mediated through activation of the Src/PI3K/Akt pathway (Zhang *et al.* 2013b).

FSH and LH exert their effect by binding to specific GPCRs, which in turn cause an increase in cAMP production and activation of the protein kinase A (PKA) pathway (Ryan *et al.* 2008). Hunzicker-Dunn *et al.* (2012) identified a pathway by which FSH-stimulated PKA appropriates growth factor receptor-bound protein 2-associated binding protein 2 (GAB2) and insulin receptor substrate 1 adapters to activate the PI3K pathway in GCs. Reports are available on the synergistic actions of IGF1 and FSH on GC function. FSH and IGF1 increased cell number and expression of *CYP19A1* mRNA in bovine GCs involving the PI3K signaling cascade (Mani *et al.* 2010). Expression of IGFR1 (FCGR1) and the overexpression of the downstream signaling molecules IRS1 and AKT2 in GCs at preantral stages in sheep have been reported (Bonnet *et al.* 2013). Inefficiencies in the intraovarian IGF system have been shown to contribute to steroidogenic incompetence and decreased reproductive capacity in women with diminished ovarian reserves (Greenseid *et al.* 2011). A synergistic effect of cAMP and IGF1 on expression of important ovulatory response genes in murine GCs has been reported. A cooperative increase in Akt phosphorylation was responsible for the effect (Mack *et al.* 2012).

PTEN has been suggested to be involved in the regulation of proliferation and differentiation of GCs in human ovaries via the PI3K/Akt pathway (Goto *et al.* 2007). Dephosphorylation of Akt with PTEN induced by LH/hCG abolished cell proliferation stimulated with IGF1 in luteinizing GCs. The results indicated that PTEN is a trigger for the proliferation/differentiation transition in

Table 1 PI3K/PTEN/Akt and TSC/mTOR pathways in oocyte development and the quiescence, activation, survival, and growth of primordial follicles

Stage	Function	Model	Reference
PGC migration/ embryonic germ cell derivation	KitL and c-kit play a crucial role in PGC survival, migration, and proliferation	Mouse	McCoshen & McCallion (1975) and Flanagan <i>et al.</i> (1991)
	KitL functions as a PGC chemoattractant; PI3K/Akt and Src kinase play a role in activation of PGC migration	Mouse	Farini <i>et al.</i> (2007)
	PTEN inactivation and Akt signaling promote derivation of embryonic germ cells from PGCs	Mouse	Kimura <i>et al.</i> (2008)
	17 β -E ₂ acting through ER α in PGCs stimulates Akt, ERK2, SRC, and KIT receptor phosphorylation		La Sala <i>et al.</i> (2010)
	Membrane-bound kitL maintains a high local concentration for PGC motility and defines their region of migration	Mouse	Gu <i>et al.</i> (2011)
Oocyte nest breakdown and PF assembly	Bcl2 could be the link between estrogen and apoptosis pathways; mutants lacking BAX protein have more oocytes and delayed cyst breakdown	Mouse	Klinge (2001) and Greenfeld <i>et al.</i> (2007)
	Estradiol signaling plays a critical role in inhibiting oocyte nest breakdown and PF assembly	Mouse	Chen <i>et al.</i> (2007, 2009) and Jefferson <i>et al.</i> (2006)
	Akt null mutation results in MOFs indicating a defect in cyst breakdown	Mouse	Brown <i>et al.</i> (2010)
	Estrogen controls PF assembly and development by regulating expression of KitL	Mouse	Huansheng <i>et al.</i> (2011)
PF activation, survival, and growth	Expression of a constitutively active form of Foxo3a not only led to retardation of oocyte growth and follicular development, but also to anovulation and luteinization of unruptured follicles and infertility	Mouse	Liu <i>et al.</i> (2007)
	<i>Pten</i> plays an essential role within the oocyte in regulation of Foxo3. Lack of Foxo3 expression resulted in POF due to global activation of the PF pool; overexpression of Foxo3 resulted in infertility due to lack of PF activation	Mouse	John <i>et al.</i> (2007, 2008)
	Oocyte-specific deletion of <i>Pten</i> results in premature activation of the entire pool of PFs followed by their depletion in early adulthood resulting in POF	Mouse	Reddy <i>et al.</i> (2008)
	Kit is dispensable in PF activation in mice; however, it has a specific role in the maintenance of PF reserve and in the primary to secondary follicle transition	Mouse	John <i>et al.</i> (2009)
	Elevated PI3K–Akt signaling in oocytes of primary and further developed follicles by conditional deletion of the <i>Pten</i> gene did not affect the pool of PFs	Mouse	Jagarlamudi <i>et al.</i> (2009)
	Oocyte-specific deletion of <i>Pdk1</i> results in POF due to accelerated clearance of PFs from their dormant state	Mouse	Reddy <i>et al.</i> (2009)
	<i>Tsc2</i> and <i>Tsc1</i> deletion caused premature activation of all PFs around the time of puberty due to elevated mTORC1 activity in oocytes, leading finally to POF	Mouse	Adhikari <i>et al.</i> (2009, 2010)
	<i>Akt1</i> ^{-/-} mice contain significantly fewer PFs at postnatal day 90, leading to infertility	Mouse	Brown <i>et al.</i> (2010)
	Short-term and ovary-specific treatment with a PTEN inhibitor and/or PI3K activator increases nuclear Foxo3 extrusion in primordial oocytes, leading to activation of dormant PFs	Mouse ovaries and human ovarian cortical fragments	Li <i>et al.</i> (2010)
	Transient inhibition of PTEN triggered activation of dormant PFs, which, under favorable growth conditions, developed into mature, fertilizable oocytes	Mouse	Adhikari <i>et al.</i> (2012)
	Hypermethylation of Igf1r, Pi3kr, and Foxo3 affects follicular maturation, resulting in reduced ovulations	Rat	Zama & Uzumcu (2013)
	Overexpression of constitutively active Foxo3 in oocytes maintained ovarian reserve and increased reproductive capacity	Mouse	Pelosi <i>et al.</i> (2013)
	Foxo3 is not uniquely required for PF maintenance in nonrodent species including humans		Tarnawa <i>et al.</i> (2013)
	Rapamycin treatment of <i>Pten</i> -mutant mice prevents global follicular activation and preserves ovarian reserve	Mouse	Adhikari <i>et al.</i> (2013)

Table 1 Continued

Stage	Function	Model	Reference
	Rapamycin might inhibit the transition from primordial to developing follicles and preserve the follicle pool reserve via modulation of mTOR and sirtuin signaling	Rat	Zhang <i>et al.</i> (2013b)
	PTEN inhibition with bpV (HOPic) increases activation of PFs but compromises development of growing follicles	Human ovarian tissue	McLaughlin <i>et al.</i> (2014)
	EGF plays a role in maintaining viability (but not promoting activation) of PFs by stimulating Akt phosphorylation	Prepubertal domestic cats	Fujihara <i>et al.</i> (2014)
	TGF β plays a role in maintenance of the PF pool and regulation of oocyte growth through activation of rpS6	Mouse	Wang <i>et al.</i> (2014)

human GCs (Goto *et al.* 2009). Fan *et al.* (2008) selectively disrupted *Pten* expression in GCs and mated *Pten*-deficient mice with transgenic mice expressing cAMP response element recombinase driven by the *Cyp19* promoter. The resultant *Pten* mutant mice were fertile, ovulated more oocytes, and produced moderately more pups than control mice, indicating that *Pten* has an effect on the survival/lifespan of granulosa/luteal cells. Decreased fertility was observed in *Akt*^{-/-} mice and was attributed in part to decreased GC proliferation/or an increase in atretic follicles. Furthermore, *Akt1*-depleted ovaries showed twofold reduction in levels of *Kitl* survival factor when compared with *Akt1*^{+/+} ovaries (Brown *et al.* 2010). *Kitl* gene expression in mouse and human GCs has recently been shown to be negatively regulated by GDF9 (Tuck *et al.* 2014).

The role of the Foxo class of transcription factors in human folliculogenesis and luteinization has been described. Although FOXO1, FOXO3, and FOXO4 expression was observed in human MGCs (Pisarska *et al.* 2009), mutations in the human *FOXO1A* and *FOXO3A* genes were identified in women with POF (Watkins *et al.* 2006). Results from a recent study have indicated that depletion of *Foxo1* and *Foxo3* in mouse GCs reduced expression of *Igf1* and *Irs2* and resulted in an infertile phenotype. Furthermore, FOXO1/FOXO3 was shown to regulate follicle growth by interacting with the activin or BMP pathways and by modulating FSH production in the pituitary (Liu *et al.* 2013).

FSH activates mTOR in GCs via the PI3K signaling pathway, which in turn leads to upregulation of hypoxia-inducible factor 1 α and VEGF expression. Inhibition of mTOR in primary mouse GCs and follicles reduced GC proliferation *in vitro*, each in a dose-dependent manner (Alam *et al.* 2004). A proliferative and survival role of VEGF in early antral follicles and in GCs isolated from DES-treated prepubertal rats has been demonstrated. The PI3K/Akt and ERK/MEK signaling pathways were suggested to be involved in these processes (Irusta *et al.* 2010).

The mTOR pathway activity is enhanced during the M phase of the cell cycle. p-mTOR (ser 2448) was found to be enriched on or near the mitotic spindle and also near the contractile ring during cytokinesis (Yaba *et al.* 2008). Treatment of spontaneously immortalized rat GCs with rapamycin caused a dose-responsive arrest of cells in the G1 cell cycle stage. The fraction of GCs that continued to divide in the presence of rapamycin exhibited a dose-dependent increase in aberrant mitotic figures known as anaphase bridges (Yu *et al.* 2011). McLaughlin *et al.* (2011) treated cultures of human ovarian cortical strips with rapamycin and observed oocyte loss characterized by empty follicles. mTOR inhibition resulted in a conserved destruction of the oocyte by adjacent GCs in the absence of activated apoptosis. An involvement of Akt through mTOR signaling has also been demonstrated in the regulation of GC autophagy in rats (Choi *et al.* 2013). Autophagy is known to induce apoptotic cell death by the accumulation of autophagosomes in ovarian cells, including GCs (Choi *et al.* 2010) and luteal cells (Choi *et al.* 2011).

Conditional deletion of *Tsc1* by a knock-in allele of the anti-Müllerian hormone type 2 receptor (*Amhr2*) driving Cre expression and the subsequent activation of mTOR in GCs and in oviductal and uterine stromal cells was found to affect fertility in mice. Deletion of *Tsc1* in GCs led to the detection of significantly fewer PFs in mutant mice at 12 weeks, indicating premature ovarian insufficiency. A significantly higher number of degenerated oocytes after normal ovulation and superovulation were also observed, indicating compromised oocyte quality as well (Tanaka *et al.* 2012). In contrast to this study, Huang *et al.* (2013) used *cyp19-cre* to specifically delete *Tsc1* in mouse GCs. Increased activity of mTORC1 as a result of *Tsc1* deletion did not cause sterility. In contrast, it improved the reproductive capacity, stimulated folliculogenesis, and led to the progressive accumulation of CL.

To understand the molecular basis of follicular growth and GC maturation, Hatzirodos *et al.* (2014) undertook transcriptome profiling of GCs from small and large healthy bovine follicles. Substantial changes in gene expression were observed in GCs as follicles enlarged from small to large antral sizes. Some important networks were associated with NOTCH, SLIT/ROBO, and PI3K signaling and ITGB5 and extracellular matrix signaling through ERKs. Results from a previous study have revealed

expression of *Hes1*, an important downstream effector of the Notch pathway, in ovarian somatic cells and oocyte cytoplasm. Conventional *Hes1* knock-out (KO), *Hes1* conditional KO in the ovarian somatic tissue, and chemical inhibition of Notch signaling decreased the total number, size, and maturation of oocytes and increased the number of pregranulosa cells in mouse ovaries at postnatal day 0. An increase in expression of proapoptotic gene *Inhbb* and a decrease in levels of the antiapoptotic and oocyte

Table 2 PI3K/Pten/Akt and TSC/mTOR signaling pathways in ovarian somatic cells

Cell type	Function	Model	Reference
Granulosa cells	hCG-induced PTEN inactivates Akt, attenuates IGF1-induced cell proliferation, and may be a trigger for proliferation/differentiation transition	Human	Goto <i>et al.</i> (2007, 2009)
	FSH activates mTOR by the PI3K signaling pathway, which in turn leads to upregulation of VEGF expression	Mouse	Yaba <i>et al.</i> (2008)
	<i>Pten</i> has an effect on the survival/lifespan of granulosa/luteal cells	Mouse	Fan <i>et al.</i> (2008)
	FOXO1, FOXO3, and FOXO4 transcription factors are expressed in human luteinized mural granulosa cells	Human	Pisarska <i>et al.</i> (2009)
	FSH and IGF1 increase cell number and expression of <i>CYP19A1</i> mRNA involving the PI3K signaling cascade	Bovine	Mani <i>et al.</i> (2010)
	Decreased GC proliferation/or an increase in atretic follicles in <i>Akt</i> ^{-/-} mice results in reduced fertility	Mouse	Brown <i>et al.</i> (2010)
	VEGFA acting through the PI3K/Akt and ERK/MEK signaling pathways has a proliferative and survival role	Rat	Irusta <i>et al.</i> (2010)
	Increased intrafollicular concentrations of IGF1 cause changes in expression of genes associated with steroidogenesis and apoptosis in GCs of preovulatory follicles	Buffalo Cow	Rao <i>et al.</i> (2011)
	The mTOR pathway activity is enhanced during the M phase of the cell cycle. Rapamycin treatment arrests GCs in G1 cell cycle stage	Rat (SIGC)	Yu <i>et al.</i> (2011)
	mTOR inhibition results in a conserved destruction of the oocyte by adjacent GCs in the absence of activated apoptosis	Human ovarian cortical strips	McLaughlin <i>et al.</i> (2011)
	FSH may prevent GC apoptosis by downregulating expression of BimEL via the PI3K/Akt/FOXO3a pathway	Porcine	Wang <i>et al.</i> (2012)
	IGF1 amplifies cAMP-dependent expression of ovulatory response genes via cooperative increases in Akt phosphorylation	Murine	Mack <i>et al.</i> (2012)
	Conditional deletion of <i>Tsc1</i> and subsequent activation of mTOR in GCs resulted in significantly fewer PFs and compromised oocyte quality	Mouse	Tanaka <i>et al.</i> (2012)
	GCs at preantral stage express IGFR1 and downstream signaling molecules IRS1 and AKT2	Sheep	Bonnet <i>et al.</i> (2013)
	FOXO1/FOXO3 regulates follicle growth and death by interacting with the activin or BMP pathways and by modulating production of FSH by the pituitary	Mouse	Liu <i>et al.</i> (2013)
	Increased activity of mTORC1 as a result of specific <i>Tsc1</i> deletion improved reproductive capacity, stimulated folliculogenesis, and led to progressive accumulation of CL	Mouse	Huang <i>et al.</i> (2013)
	3,5,3'-triiodothyronine increases FSH-induced preantral follicle growth <i>in vitro</i> through activation of the Src/PI3K/Akt pathway	Rat	Zhang <i>et al.</i> (2013a)
	Developmental processes stimulated by KIT are active in small follicles; PI3K signaling is associated with large follicles	<i>Bos taurus</i> cows	Hatzirodos <i>et al.</i> (2014)
	The PI3K/Akt pathway through mTOR regulates GC autophagy	Rat	Choi <i>et al.</i> (2013)
Theca cells	LH-induced PI3K/Akt activation and androstenedione production are modulated by MAPK	Bovine	Fakuda <i>et al.</i> (2009)
	LH/hCG-mediated activation of the mTORC1 signaling cascade is involved in the regulation of steroidogenic enzymes in androgen biosynthesis	Rat	Palaniappan & Menon (2012)
	Insulin activates TI cell proliferation and expression of cell cycle regulatory components by triggering the mTORC1-dependent pathway	Rat	Palaniappan <i>et al.</i> (2013)

maturation marker Kit was observed in the *Hes1* KO ovaries (Manosalva *et al.* 2012).

The final phase of folliculogenesis is triggered by a surge in LH; it stimulates *CYP17A1* mRNA expression and androgen production in bovine theca cells via activation of the PI3K pathway (Fakuda *et al.* 2009). mTORC1 plays a significant role in androgen biosynthesis by regulating expression of genes encoding steroidogenic enzymes. Rapamycin or small interfering RNA-mediated knockdown of *MTOR* was shown to inhibit LH/hCG-induced induction of steroidogenic enzymes selectively (Palaniappan & Menon 2012). Recently, the authors have reported that insulin activates TI cell proliferation and the expression of cell-cycle-regulatory components (CDK4, CCND3, and PCNA) by triggering the mTORC1-dependent pathway (Palaniappan *et al.* 2013). Rao *et al.* (2011) analyzed changes in transcriptome of preovulatory follicles in response to the LH surge in buffalo cows. The global gene expression data suggested differential expression of many genes associated with the IGF1 system and its signaling (Table 2).

PI3K/PTEN/Akt and TSC/mTOR pathways, and regulation of oocyte meiosis

Oocytes in mammalian ovaries are naturally arrested in the prophase of the first meiotic division or the so-called GV

stage and resume meiosis after the LH surge. In mouse oocytes, suppression of Akt activity delayed resumption of meiosis and was accompanied by a decrease in cyclin-dependent kinase 1 (CDK1) activity. CDK1 or p34^{cdc2} kinase is the catalytic subunit of maturation-promoting factor (MPF), whose activity has been reported to be crucial for meiotic maturation of oocytes (Kalous *et al.* 2006). Increased cAMP levels have been reported to be necessary for maintaining oocytes under meiotic arrest. Cyclic guanosine monophosphate is fluxed from the CCs into the oocyte to inhibit the activation of cAMP-phosphodiesterase (PDE3A) that particularly degrades cAMP. PKA and Akt kinase phosphorylate PDE3A to enhance its enzymatic activity at the onset of oocyte maturation (Han *et al.* 2006, Vaccari *et al.* 2008). mRNAs for the Akt isoforms and the phosphorylated form of AKT protein in GV oocytes have been reported (Cecconi *et al.* 2010). During FSH-induced meiotic maturation, distribution of pAkt (Ser473) was found to be similar to the localization of microtubules, while pAkt (Thr 308) was present in the pericentriolar materials in MI and MII mouse oocytes (Hoshino *et al.* 2004). Akt inhibition *in vitro* suppressed GV breakdown (GVBD), polar body 1 (PB1) emission, and cumulus expansion. At the MII stage, Akt was reported to control PB2 emission and normal chromosomal alignment on microtubules (Hoshino & Sato 2008). Although Akt activity

Table 3 PI3K/PTEN/Akt and TSC/mTOR pathways and regulation of oocyte meiosis

Function	Model	Reference
Akt regulates meiotic MI/MII transition	Bovine oocytes	Tomek & Smiljakovic (2005)
PKB/Akt is involved in CDK1 activation and resumption of meiosis	Mouse oocytes	Kalous <i>et al.</i> (2006)
GV and metaphase oocytes express Akt isoforms and pAkt. Akt inhibition suppressed GVBD, PB1 emission, and cumulus expansion and decreased the amount of pAkt in MI and MII oocytes. pAkt ser473 is involved in PB2 emission, whereas pAkt Thr308 regulates the organization of microtubules	Mouse oocytes	Hoshino <i>et al.</i> (2004), Hoshino & Sato (2008) and Cecconi <i>et al.</i> (2010)
Akt activity is not essential for induction of GBVD; however, it plays a substantial role during progression of meiosis to the MI/MII-stage	Porcine oocytes	Kalous <i>et al.</i> (2009)
Increased cAMP levels are necessary to maintain oocytes under meiotic arrest. PKA and Akt kinase phosphorylate PDE3A to enhance its enzymatic activity at the onset of oocyte maturation	Mouse oocytes	Han <i>et al.</i> (2006) and Vaccari <i>et al.</i> (2008)
<i>mTor</i> mRNA is expressed in GV until MI and increases during MII. Rapamycin treatment inhibits spindle migration and asymmetric division during oocyte maturation via the mTOR-mediated small GTPase signaling pathways	Mouse oocytes	Lee <i>et al.</i> (2012)
mTORC1 is important for controlling spindle function during mitosis and meiosis. mTORC2 contributes to actin-dependent asymmetric division during meiotic maturation	Mouse oocytes	Kogasaka <i>et al.</i> (2013)
Maternal mRNA and phosphorylated EIF4EBP1 variants are associated with the oocyte spindle	Mouse oocytes	Romasko <i>et al.</i> (2013)
Blocking 4E-BP1 phosphorylation using Torin2, an active site mTOR inhibitor, during maturation of bovine oocytes results in arrest of up to 60% of oocytes in the MI stage	Bovine oocytes	Mayer <i>et al.</i> (2014)
Survivin, a downstream target protein of the PI3K/Akt and mTOR pathways, regulates proper meiotic spindle organization, spindle assembly checkpoint activity, timely metaphase-to-anaphase transition and cytokinesis	Mouse oocytes	Jiang <i>et al.</i> (2014)

has not been found to be essential for induction of GVBD in porcine oocytes, it plays a substantial role during progression of meiosis to MI/MII-stage (Kalous *et al.* 2009). Similar studies on bovine oocytes have demonstrated the involvement of Akt in the regulation of the meiotic MI/MII transition (Tomek & Smiljakovic 2005).

Lee *et al.* (2012) studied the effect of rapamycin treatment on cytoskeleton reorganization during meiotic maturation of mouse oocytes. *mTOR* mRNA was expressed in GV until MI and increased during MII. mTOR protein was localized around the spindle and in the cytoplasm of oocytes. Rapamycin treatment altered asymmetric division of oocytes and disrupted the formation of actin cap and the cortical-granule-free domain, thus indicating the failure of spindle migration. Additionally, reduction in mRNA expression of small GTPases (*Rhoa*, *Cdc42*, and *Rac1*), which are crucial regulatory factors for cytoskeleton reorganization, was observed. Results from a study conducted by Kogasaka *et al.* (2013) have indicated a role of mTORC1 in controlling spindle function during mitosis and meiosis and a contribution of mTORC2 to actin-dependent asymmetric division during meiotic maturation.

Maternal mRNA and phosphorylated EIF4EBP1 variants were shown to be associated with the mouse oocyte MII spindle (Romasko *et al.* 2013). The dynamic spatially restricted patterns of EIF4EBP1 were thought to promote localized mRNA translation to support spindle formation, maintenance, function, and other related processes. Mayer *et al.* (2014) blocked 4E-BP1 phosphorylation using Torin2, an inhibitor of the mTOR active site during maturation of bovine oocytes, and observed arrest of up to 60% of the oocytes at the MI stage.

Recently, an essential role for survivin, the smallest member of the inhibitor of apoptosis protein (IAP) family and the downstream target protein of the PI3K/Akt and mTOR pathways, for fertile egg production and female fertility in mice has been identified. Survivin was found to be important for regulating proper meiotic spindle organization, spindle assembly checkpoint activity, timely metaphase-to-anaphase transition, and cytokinesis (Jiang *et al.* 2014; Table 3).

Conclusion

In conclusion, the findings of studies summarized in the present review emphasize the importance of the PI3K/PTEN/Akt and TSC/mTOR signaling pathways as critical regulators of ovarian function including quiescence, activation and survival of PFs, proliferation and differentiation of granulosa and thecal cells, and oocyte meiotic

maturation. Important clues uncovered using animal models by deletion of specific genes support the essential role of elements of these signaling pathways in preserving the normal female reproductive lifespan, which, in turn, is crucial for determining the duration of female fertility. The summarized research data provide a rationale for exploring the possible use of targeted therapies for inhibition of the PI3K/PTEN/Akt and TSC/mTOR signaling cascades in pathological conditions of the ovary, including POF and infertility. Additionally, the results of studies presented herein will be a valuable resource for establishing conditions to improve current assisted reproductive technologies including *in vitro* maturation of immature oocytes, and also for preservation of fertility by *in vitro* activation of follicles in cryopreserved ovarian tissue obtained from cancer patients or from women with diminished ovarian reserves.

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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