

—Review—

Growth of Primordial Oocytes in Neonatal and Adult Mammals

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Abstract. Mammalian ovaries are endowed with a huge number of small oocytes (primordial oocytes) in primordial follicles. A small number of primordial oocytes start to grow, while others remain quiescent. Little is known about the mechanism regulating the activation of primordial oocytes. Recently, we found that primordial follicles in mature cows and prepubertal pigs took longer to initiate growth in xenografts compared with those in neonatal animals. We think that primordial oocytes in adult mammals are different from those in neonatal mammals. In this review, we summarize the results regarding the activation of primordial oocytes in neonatal and adult ovaries of different species and propose a model in which ovaries of neonatal mammals contain a mixed population of both quiescent and activated primordial oocytes, while almost all primordial oocytes are quiescent in adult females. The dormancy of primordial oocytes may be required to reserve the non-growing oocyte pool for the long reproductive life in mammals. FOXO3 is considered one of the molecules responsible for the dormancy of primordial oocytes in adult ovaries. These quiescent primordial oocytes are activated, perhaps by certain mechanisms involving the interaction between stimulatory and inhibitory factors, to enter the growth phase.

Key words: Adult, Neonatal, Oocyte growth, Ovary, Primordial follicle

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In fetal ovaries, germ cells known as oogonia proliferate mitotically and become oocytes that enter the meiotic cell cycle, and the oocytes then become arrested at prophase I. At around birth in rodents and during mid-gestation in humans and pigs, oocytes (15–20 μm in diameter in rodents and 30 μm in diameter in humans and pigs) are enclosed by a single layer of flattened follicular epithelial cells (granulosa cells; Fig. 1). This unit consisting of an oocyte and granulosa cells is called the primordial follicle. The oocytes in primordial follicles are called “primordial oocytes” [1]. After formation of primordial follicles, some of the primordial oocytes begin to grow, although most spend months or years in the quiescent state. Activation of primordial oocytes causes the transformation of their surrounding granulosa cells to a cuboidal shape [2]. The follicles at this stage are called primary follicles that contain growing oocytes. The granulosa cells proliferate and become multilayered to form secondary follicles. As follicles develop through the primary, secondary and tertiary (antral) stages, they gain successive layers of granulosa cells, oocytes increase in size towards 70–75 μm in rodents and 120–125 μm in humans, cows and pigs and theca cell layers surround the follicles. Finally, a large fluid-filled antral cavity (follicular antrum) is formed, and the follicle is called a tertiary or antral follicle.

The time point at which first activation of primordial oocytes occurs depends on the species. In mice, growth of a portion of oocytes begins within several days after birth [3, 4], whereas in ruminants and primates, growth begins before birth [5, 6]. The ovaries of neonatal piglets contain a large number of primordial follicles and also a small population of developing follicles that contain growing oocytes. Primordial follicles represent more than

95% of the total follicular proportion in porcine ovaries at 2 weeks after birth [7, 8]. The ovaries of prepubertal and adult females possess numerous growing and fully-grown oocytes in the various-sized follicles as well as a number of non-growing primordial oocytes as the future stock. These non-growing oocytes are thought to be activated for growth throughout the female reproductive lifespan. We consider the mechanism regulating the later activation of primordial oocytes in adults to be different from that in fetal or neonatal mammals (the first activation of primordial oocytes). Here, we summarize our findings on the activation of primordial oocytes in neonatal and adult animals, mainly pigs, and review the findings in the literature.

Growth of Primordial Oocytes from Neonatal Females

Primordial follicles start to develop at the time of birth in mice [3] and at 3–4 days after birth in rats [9]. Since the ovaries at this time contain only primordial follicles, those ovaries have often been used to study the initiation of oocyte growth. Martinovich did the first successful culture of rodent ovaries in 1937. He observed that oocytes grew to 50–55 μm in diameter after 18 days of culture [10, 11]. Since then, efforts have been made to improve the culture media. Finally, Eppig and O'Brien [1] reported the first successful production of a baby mouse, “Eggbert,” derived from cultured primordial follicles. In their experiment, primordial oocytes were grown to full size by two sequential methods: first, the ovaries from newborn mice were grown in organ culture for 8 days, and then the developing oocyte-granulosa cell complexes were isolated and cultured for an additional 14 days.

The ovaries of domestic mammals and humans are too large for organ culture, and thus ovarian cortical slices that contain primordial follicles are cultured instead of whole ovaries. Wandji *et al.* [12, 13] collected small pieces of cortical slices from fetal bovine

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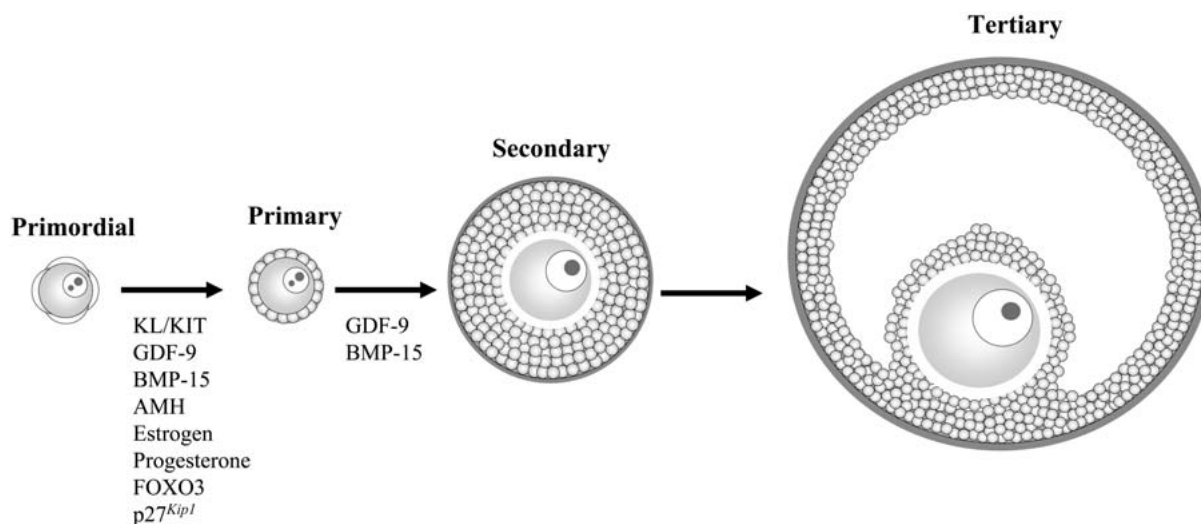


Fig. 1. Growth of oocytes and development of follicles in the mammalian ovary. After growth initiation of oocytes in primordial follicles, the shapes of the surrounding flattened epithelial cells (granulosa cells) change to a cuboidal form. The follicles are called primary follicles. The granulosa cells start to proliferate and become multilayered, and the follicles are designated as secondary follicles at this stage. When a fluid-filled antrum is formed, they are called tertiary follicles (antral follicles). Several factors (described in the text) are involved in the activation or quiescence of primordial oocytes.

and baboon ovaries and cultured them on culture plate inserts. After 2 days of culture, the number of primordial follicles was reduced, concomitant with an increase in the number of primary follicles. In the case of baboon ovarian culture, follicles developed to the secondary stage. We cultured small pieces of cortical tissues from 10-day-old piglets, which contained mainly primordial follicles, in tissue culture medium-199 (TCM-199) supplemented with 10% fetal calf serum (FCS) on Transwell membrane inserts (Cat. no. 3414, Corning, Corning, NY, USA). After 7 days, a number of primordial oocytes started to grow, and some of them reached around 50 μm in diameter, although the follicles remained in the primordial or primary stage (Fig. 2A). Primordial follicles collected from neonatal lambs started to develop at 2 days *in vitro* [14]. These results suggest that primordial oocytes from neonatal and fetal ovaries grow *in vitro*.

Xenotransplantation (transplantation to different species) of small oocytes to nude or severe combined immune deficiency (SCID) mice can be a substitute for an effective long-term culture system [15]. The nude mouse, a hairless mutant discovered in 1966 [16], is immunodeficient and does not reject grafts from other species. Mice homozygous for the *scid* mutation lack both humoral and cell-mediated immunity due to the absence of mature T and B lymphocytes [17, 18]. In addition to having a healthy lifespan and tolerating surgery well in a sterile environment, SCID mice readily accept ovarian tissues from other mammalian species. Gosden *et al.* [19] have developed a method of xenografting mammalian follicles into SCID mice as a model for investigating the early stages of follicular development.

We transplanted ovaries from fetal (13-day post coitum: dpc) and neonatal (2-day-old) mice (C57BL/6, SLC, Shizuoka, Japan) under the kidney capsule of SCID mice. After 2–3 weeks, the fol-

licles developed to the preantral stage along with oocyte growth [20]. Liu *et al.* [21] transplanted frozen-thawed newborn mouse ovaries under the kidney capsule of inbred recipients. After 2 weeks, primordial follicles developed to preantral follicles, which were then recovered and cultured for 12 days. After *in vitro* maturation (IVM) and *in vitro* fertilization (IVF), they transferred late morulae-early blastocysts, which resulted in the production of live offspring. In large animals, Hosoe *et al.* [22] showed that primordial follicles from bovine fetuses developed to the antral stage at 15 weeks after xenografting to nude mice. Kaneko *et al.* [23] reported the growth of oocytes from 20-day-old piglets in ovariectomized nude mice. They xenografted ovarian tissues containing mainly primordial follicles and a few primary follicles and found antral follicles at 45–75 days after xenografting. Similarly, we found that primordial follicles from neonatal (10- to 20-day-old) piglets developed to the antral stage along with enlargement of oocytes at 2 months after xenografting in SCID mice [24].

Growth of Primordial Oocytes from Adult Females

Although primordial oocytes from fetal and neonatal rodents initiate growth *in vitro*, we have observed that similar culture systems are hardly applicable for adult domestic animals. The oocytes are comparatively large in size and have a long growth phase. For example, a follicle takes several months to complete development from the primordial to the final preovulatory stage in pigs. When porcine primordial follicles were isolated and cultured in a Petri dish, the granulosa cells were detached within several days and the oocytes then started to degenerate [25]. We cultured small pieces of ovarian cortical tissues from prepubertal pigs, which contained mainly primordial follicles, in TCM-199 supplemented with 10%

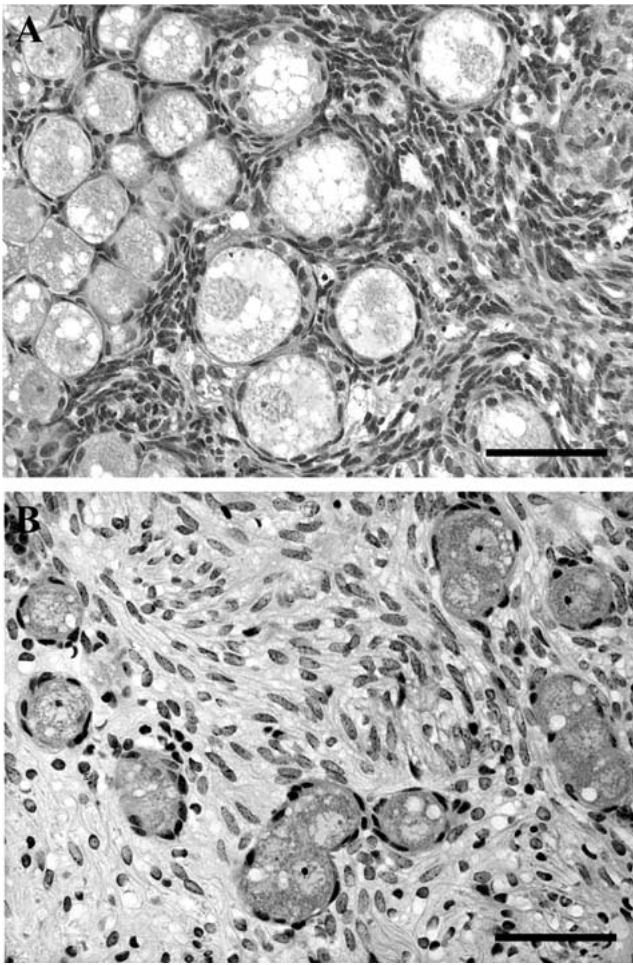


Fig. 2. Histological sections of ovarian tissues from neonatal and prepubertal pigs after culture. Ovarian tissues containing primordial follicles were cultured in TCM-199 supplemented with 10% fetal calf serum on Transwell membrane inserts. After 7 days, follicles remained at a similar stage without changes in oocyte diameter in prepubertal pigs (B), whereas in neonatal pigs, the diameters of some oocytes increased remarkably, although the follicles remained in the primordial or primary stage (A). Scale bars represent 60 μm .

FCS on Transwell membrane inserts. After 7 days, the primordial oocytes survived but none of them initiated growth, and the follicles remained in the primordial stage (Fig. 2B). It is thought that these follicles will require more time to initiate growth.

It is obvious from xenografting experiments that primordial follicles from adult females take longer to initiate development compared with those from neonates. Primordial follicles from 6- to 12-month-old sheep require a period of 8 to 20 weeks to develop to the antral stage in xenografts [19]. In humans, the primordial follicles of a 17-year-old patient developed to the antral stage at 17 weeks after xenografting [26]. We dissected thin cortical slices of ovaries containing mainly primordial follicles and a small number of primary follicles from mature cows. At 6–8 weeks after xenografting, primordial follicles did not develop, and the propor-

tion of primary follicles remained similar to that before xenografting [27]. Next, we dissected cortical slices that contained mainly primordial follicles from the ovaries of prepubertal pigs (6-month-old). It is notable that unlike bovine ovaries, some of the outermost regions of the porcine ovarian cortex contain only primordial follicles. Histological examination confirmed that thin (about 0.5-mm thick) cortical tissues collected from the outer surface of the ovaries from prepubertal pigs did not contain primary follicles. At 2 months after xenografting, the follicles remained in the primordial stage without any change in oocyte diameter. After the same duration of xenografting, primordial follicles from neonatal (10- to 20-day-old) piglets developed to the antral stage along with enlargement of oocytes [24]. At 4 months after xenografting, a few primordial follicles started to develop, and at 6 months, the follicles developed to various stages, including antral follicles, along with oocyte growth [28]. With respect to growth initiation, the ability of primordial oocytes in adult ovaries might be different from that in fetal or neonatal females. The faster development of primordial follicles in younger mammals than in older ones is not limited to the xenograft situation. Faster development of follicles in younger rats has been reported in an autoradiographic study [29]. In mice and rats, follicle development begins within a few days of birth, and antral follicles appear during the third week post partum, whereas follicle development in adults is estimated to take around 2 months [4]. Primordial oocytes from adult ovaries take longer to initiate their growth compared with those in neonatal animals. Therefore, activation of primordial oocytes in neonatal ovaries (first activation) is considered to be different from activation (later activation) of primordial oocytes in prepubertal/adult females, although the reasons behind this difference are not clear. We consider the activation of primordial oocytes to be the result of a balanced interaction between stimulatory and inhibitory factors.

Stimulatory Factors of Primordial Oocytes

Several growth factors and cytokines have been thought to regulate the process of oocyte growth and follicular development [30]. Among them, KIT, a tyrosine kinase receptor, and its ligand, KIT ligand (KL, which is also known as steel factor, stem cell factor and mast cell growth factor), play a key role in oocyte growth and follicular development [31]. KIT and KL are reported to be expressed in the ovaries of several species. *KL* mRNAs for both soluble- and membrane-bound forms have been detected in juvenile and adult mouse ovaries and in granulosa cells isolated from mouse preantral follicles [32, 33]. The expression of *KIT* mRNA and KIT protein has been detected in primordial, growing and full-grown oocytes in the mouse [31]. The mRNA encoding *KIT* and *KL* was detected in the porcine ovary, in which *KL* mRNA was localized in granulosa cells and *KIT* mRNA was detected in oocytes [34, 35]. In our study [24], KIT was expressed in all primordial oocytes from both neonatal and prepubertal pigs. This result agreed with the expression pattern in sheep [36] and humans [37], in which KIT expression was similarly found in primordial oocytes from fetal and postnatal ovaries. To stimulate the activation of primordial oocytes from prepubertal pigs, ovarian tissues, which contained mainly primordial oocytes, were cultured in a medium supplemented with KL (50

and 100 ng/ml) for 1 and 3 days before xenografting. After 2 months, however, the oocytes did not grow, and the follicles remained at the same stage, although a higher number of primordial oocytes survived in the KL-treated tissues than in the untreated ones [24]. This result suggests that KIT-KL might not be associated with the activation of porcine primordial oocytes, although they are necessary for the survival of the oocytes. Similar results have been reported by Carlsson *et al.* [38], who employed organ culture for human ovarian tissues. They showed that KL did not affect follicular development, although the number of viable follicles was increased. Thus, questions on the role of KIT-KL in the activation of primordial oocytes have arisen.

KIT and KL are encoded at white spotting (*W*) and steel (*Sl*) loci in the mouse, respectively [39–43]. The mutant mice of *W* and *Sl* loci show similar phenotypes such as anemia, a decrease in the number of mast cells, lack of pigmentation and infertility [44]. Many alleles of variable severity at both the *W* and *Sl* loci have been identified [45]. *W^v* mutant mice with the C57BL/6 background have a single point mutation of 2007 (C–T) on the *Kit* sequence, which is the change of threonine at position 660 to methionine in the cytoplasmic region of KIT resulting in a deficient signal of KIT. Lack of pigmentation, macrocytic anemia, deficiency of mast cells, premature death, poor body conditions and infertility induced by severe death of germ cells are observed in homozygotes (*W^v/W^v*) [44, 45].

Sakata *et al.* [46] found that Fas-deficiency rescued germ cells in KIT-deficient *W^v/W^v:Fas^{-/-}* mice. Fas is a member of the death receptor family. It induces programmed cell death in various organs including the testis and ovary. It is expressed in granulosa cells and oocytes during folliculogenesis and in luteal cells [47]. Its expression in granulosa and luteal cells suggests that Fas is involved in follicular atresia and luteolysis. Defective KIT signaling leads to the activation of a Fas-mediated apoptotic signal in germ cells [46]. We investigated germ cell survival, oocyte growth and follicular development in KIT-deficient (*W^v/W^v:Fas^{+/+}*), Fas-deficient (*+/+:Fas^{-/-}*) and both KIT- and Fas-deficient (*W^v/W^v:Fas^{-/-}*) mice during the fetal and postnatal periods. KIT-deficient *W^v/W^v:Fas^{+/+}* mouse ovaries contained no oocytes after birth, although they contained germ cells in the fetal stage. KIT- and Fas-double deficient (*W^v/W^v:Fas^{-/-}*) mice, however, contained oocytes even after birth, and some of the follicles developed to the secondary stage or beyond, although the number of oocytes was decreased compared with that in age-matched wild-type (*+/+:Fas^{+/+}*) mice [20]. These results suggest that KIT is involved in the survival, rather than the growth, of oocytes in pigs and mice.

It has been demonstrated that oocytes coordinate the development of ovarian follicles beyond the primary stage in mice [30]. Some factors secreted by oocytes have been identified as regulating follicular development and oocyte growth. Growth differentiation factor 9 (GDF-9) is an oocyte-specific member of the transforming growth factor β (TGF- β) family produced by oocytes [48]. In GDF-9 knockout mice, oocyte diameters were increased, although follicular development was blocked at the primary stage [49]. GDF-9 is expressed in growing oocytes but not in primordial oocytes in rodents [50, 51] and humans [52]. However, it stimulates the development of primordial follicles in human [53], goat

[54] and hamster ovaries [55]. Bone morphogenetic protein 15 (BMP-15), another member of the TGF- β family, is expressed in growing oocytes but not in primordial oocytes [56–58]. Homozygosity of BMP-15 mutation in sheep results in sterility due to failure of the follicles to develop beyond the primary stage [59], similar to that observed in GDF-9 null mice [49]. These results suggest that GDF-9 and BMP-15 regulate follicular development after the primary stage but are not involved in the activation of primordial oocytes.

Hormonal Influence on Primordial Oocytes

We found that primordial oocytes from prepubertal pigs took longer before initiation of their growth compared with those from neonatal pigs. It has been reported that the LH concentration is significantly higher in pigs during a period from 90 days post coitum to 10 days after birth than at a later age [60]. This indicates the possible role of LH in the activation of primordial follicles in pigs. In contrast, it has also been reported that ovarian follicles do not develop remarkably during the period of elevated LH [7]. Thus, the role of LH in the activation of primordial follicles is unclear. Peters *et al.* [61] revealed that the initiation of primordial follicle development is independent of pituitary gonadotropins. They found that equine chorionic gonadotropin (eCG) injection did not enhance the development of primordial follicles in 3- to 6-day-old mice. In addition, the injection of antigonadotropin antisera for 5 days during the neonatal period did not prevent the development of primordial follicles [62], which suggested that gonadotropins did not stimulate the primordial follicles to start developing. Furthermore, the addition of FSH has no effect on the development of primordial follicles in pieces of bovine cortex [63] because the FSH receptor is not expressed in primordial follicles [64]. Follicles develop to the secondary stage along with oocyte growth in the ovaries of mice lacking expression of the FSH β -subunit [65] or lacking expression of the FSH receptor, although folliculogenesis is blocked before antrum formation [66, 67]. These results further support the gonadotropin-independent activation of primordial oocytes.

Estrogen-deficient aromatase-knockout (ArKO) mice show reduced numbers of primordial and primary follicles than wild type mice [68], although they show normal growth of oocytes capable of maturation and fertilization [69]. It has been reported that ovaries from neonatal mice do not secrete detectable amount of steroids including progesterone, testosterone, androstenedione and estradiol-17 β , whereas the secretion of these steroids increases at the juvenile stage [70]. Higher levels of steroids in the ovary arrest the development of primordial follicles, and the decline in steroid levels during or after birth allows the initiation of primordial follicle development in rodents [71, 72] and monkeys [73]. These results indicate that the activation of primordial oocytes might be retarded by estrogen.

Inhibitory Factors of Primordial Oocytes

In adult ovaries, a large number of oocytes in the primordial follicles are quiescent, while a small number are recruited to the

growth phase. When whole ovaries from newborn rodents are cultured, a small number of follicles are activated, whereas many remain quiescent, suggesting that mammalian ovaries possess a certain inhibitory mechanism to maintain a pool of non-growing oocytes. Dormancy of primordial oocytes is required to reserve a non-growing oocyte pool for a long reproductive life because depletion of this pool causes premature ovarian failure. Activation of primordial oocytes might be prevented by some inhibitory factors in the ovary, and the later activation of those non-growing oocytes might involve their release from such inhibitory actions [74, 75].

Anti-Müllerian hormone (AMH, also called Müllerian inhibiting substance), a member of the TGF- β super family, is known as an inhibitory factor in the development of primordial follicles in mice. Durlinger *et al.* [76] reported that AMH prevents the development of primordial follicles. The number of developing follicles is elevated in *Amh*^{-/-} mice. These homozygous mutants are fertile, but primordial follicles were found to be depleted after 4 months of age, which was considered due to the excessive recruitment of primordial follicles to the growing pool. AMH caused a 40–50% reduction in the number of growing follicles after 2 and 4 days of culture [77] and suppressed the development of human primordial follicles when ovarian cortices were cultured in the presence of recombinant AMH [78]. AMH is expressed in secondary, preantral and small antral follicles but not in primordial follicles in humans [79] and rodents [80, 81]. In the adult ovary, secondary or antral follicles provide a source of AMH that may inhibit the development of primordial follicles.

It has been suggested that Foxo3 negatively regulates the activation of primordial follicles in the mouse ovary [75]. The forkhead transcription factors FOXO1 (FKHR), FOXO3 (FKHRL1) and FOXO4 (AFX) are characterized by the presence of a highly conserved, monomeric DNA-binding domain, also known as the forkhead box or FOX [82]. In mammalian somatic cells, FOXO factors induce cell cycle arrest (in G₁/S transition) and apoptosis [83–85]. FOXO3 expression has been observed in the nuclei of mouse and rat oocytes [86], and *Foxo3*^{-/-} mice show global follicular activation, which leads to the early depletion of ovarian follicles [87]. These studies suggest that Foxo3 inhibits primordial oocyte activation in newborn rodents.

Our recent report revealed that FOXO3 regulates the dormancy and later activation of primordial oocytes in adult ovaries [28]. In prepubertal pigs, FOXO3 was expressed in the nuclei of almost all primordial oocytes, and in infant pigs, a number of primordial oocytes did not exhibit FOXO3. The results of Western blots reflected the higher proportion of FOXO3-positive primordial oocytes from prepubertal pigs than from infants. In xenotransplantation experiments, we found that primordial follicles from neonatal pigs developed to the antral stage along with oocyte growth at 2 months after xenografting, whereas those from prepubertal pigs survived without initiation of development even after 4 months; thereafter, they started to develop and reached the antral stage after 6 months. At 4 months after xenografting, some oocytes from prepubertal pigs became FOXO3-negative, and some oocytes grew in the subsequent 2 months. Thus, FOXO3 expression was correlated with growth initiation of pig primordial oocytes in

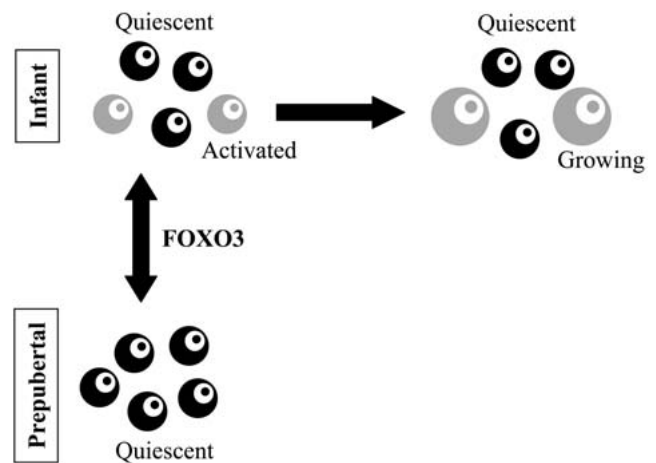


Fig. 3. In neonatal mammalian ovaries, some oocytes start to grow, while others remain or become quiescent. On the other hand, almost all of the primordial oocytes in adult ovaries are quiescent. After certain period, a few of them are reactivated and enter the growth phase. FOXO3 is thought to be responsible for the dormancy of primordial oocytes in adult/prepubertal ovaries.

xenografts. We speculate that in neonatal animals, some oocytes are activated that are directed to enter the growth phase, while others remain or become dormant to reserve a pool of non-growing oocytes for the future (Fig. 3). After a certain time, the quiescent oocytes are thought to be reactivated and initiate growth. It is likely that FOXO3 may be involved in the dormancy of primordial oocytes in prepubertal and perhaps adult porcine ovaries and that the oocytes start to grow when they are released from the inhibitory action of FOXO3. To clarify the involvement of FOXO3 in the suppression of oocyte growth in prepubertal pigs, we conducted a FOXO3 knockdown experiment using small interfering RNAs (siRNAs). After knockdown of FOXO3, a proportion of primordial oocytes became activated and entered the growth phase in the xenografts (Fig. 4). These results suggest that FOXO3 induces, at least in part, the dormancy of primordial oocytes in pig ovaries.

How the expression of FOXO3 is regulated in primordial oocytes is not clearly understood. The functions of FOXO molecules are regulated at multiple levels, which include phosphorylation, ubiquitylation and acetylation [88]. The mechanisms associated with the regulation of FOXO molecules have been characterized mainly in somatic cells in response to growth factor signaling *via* the phosphatidylinositol 3-kinase (PI3K)—protein kinase B (PKB) pathway [89]. PKB-induced phosphorylation inhibits transcriptional activity of the FOXO members [83, 90–92]. In mice, the oocyte-specific deletion of *Pten* (phosphatase and tensin homolog deleted on chromosome 10), which negatively regulates the action of PI3K, causes excessive activation of primordial follicles [93, 94]. Li *et al.* [95] treated human ovarian fragments and mouse ovaries with a PTEN inhibitor and transplanted them to SCID mice. After transplantation, increased numbers of antral follicles were obtained from inhibitor-treated groups than the control. Reddy *et al.* [94] suggest that the KIT-induced PI3K pathway regulates primordial follicle activation. However, the role of PI3K in

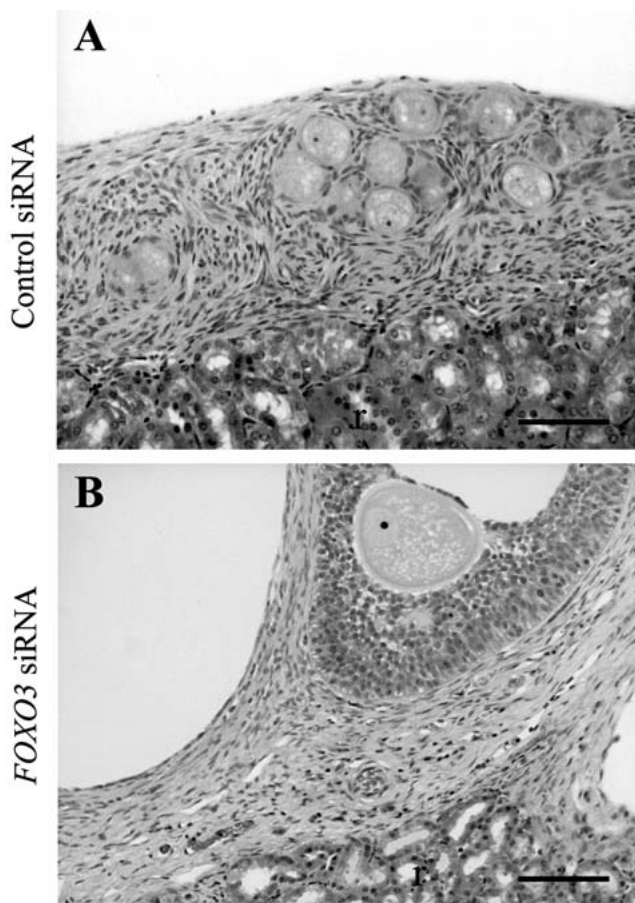


Fig. 4. Histological sections of *FOXO3* siRNA-treated ovarian tissues from prepubertal pigs after xenografting. Ovarian tissues containing primordial follicles were treated with control or *FOXO3* siRNAs and xenografted to SCID mice. At 2 months after xenografting, primordial follicles did not develop in control siRNA-treated tissues (A). Under similar conditions, follicles developed to the antral stage in *FOXO3* siRNA-treated tissues along with oocyte growth (B). The 'r' indicates mouse renal tissues. Scale bars represent 60 μm .

the activation of primordial follicles is still controversial, since it has been found that KIT-deficient mice possessed growing oocytes in developing follicles [20, 96].

How FOXO3 prevents primordial follicle activation is not clear. It has been reported that Foxo3 positively regulates p27^{Kip1} (Cdkn1b) expression in cultured cells [97]. p27^{Kip1} is a member of the Cip/Kip family of Cdk inhibitors. Overexpression of p27^{Kip1} leads to cell-cycle arrest in the G₁ phase in human cells [98, 99]. In quiescent cells (*i.e.*, in the G₀ phase), p27^{Kip1} levels are high with low Cdk activity. Mice deficient in p27^{Kip1} exhibit gonadal hyperplasia and are infertile due to defects in ovulation and corpus luteum formation [100]. Rajareddy *et al.* [101] reported that p27^{Kip1} expression was not detected in oogonia or oocytes by immunostaining at 14.5 and 18.5 dpc, even in 1-day-old mice, but

was found in somatic cells in ovaries. Its expression first appeared in the primordial oocytes of 4-day-old mice. They found excessive activation of primordial follicles in p27^{Kip1}-deficient mice, suggesting that p27^{Kip1} negatively regulated primordial follicle development in the mice. Unlike in somatic cells, p27^{Kip1} and Foxo3 do not function in an upstream/downstream fashion in oocytes. The expression/phosphorylation levels of Foxo3 in p27^{Kip1}-/- oocytes are similar compared with those in p27^{Kip1}+/+ oocytes, and the p27^{Kip1} expression in Foxo3^{-/-} oocytes is also at a similar level compared with Foxo3^{+/+} oocytes [101]. In double knockout mice that lack both p27^{Kip1} and Foxo3, synergistically accelerated follicle activation has been observed, indicating that the mechanisms of suppression of follicle activation by p27^{Kip1} and Foxo3 are independent of each other. It is possible that Foxo3 controls multiple molecules in primordial oocytes that contribute to maintaining the quiescent state until the appropriate signal induces activation of the primordial oocytes.

Perspectives and Conclusions

Usually, not all oocytes start to grow at the same time in an ovary. Mammals have a long reproductive lifespan, and the ovary is required to maintain a pool of non-growing oocytes throughout the female reproductive life. Primordial oocytes are thought to refrain from entering the growth phase under certain inhibitory mechanisms for future recruitment to produce eggs for fertilization. The ovaries of neonatal piglets contain a mixed population of both quiescent and activated primordial oocytes, and almost all the primordial oocytes are quiescent in prepubertal/adult pigs (Fig. 3).

In the ovaries, developing follicles are found in well-vascularized cortical-medullary borders, whereas primordial follicles are located in a relatively avascular region in the cortex [6] and might suffer from a shortage of nutrients and growth factors due to poor blood supply. In a condition of nutrient deprivation, the proliferation of somatic cells is arrested in G₁ or enters a quiescent state (G₀), and after exposure to nutrients for a prolonged period, the cells return to the G₁ stage [102]. We hypothesize that primordial oocytes in adult/prepubertal mammals enter such a quiescent state, although they are in the diplotene stage of meiosis I, which corresponds to the G₂ stage in somatic cells. They are thought to be activated later by certain mechanisms involving the interaction between stimulatory and inhibitory factors that are still unknown. Although several molecules have been reported to be involved in this process, we are still a long way from achieving *in vitro* growth of primordial oocytes from humans and large domestic animals. If we had a better understanding of the mechanism regulating their growth, these non-growing primordial oocytes could be utilized for farm animal production and for alleviating human infertility.

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