1

# Molecular Alterations During Female Reproductive Aging: Can Aged Oocytes Remind Youth?

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## 1. Introduction

Aging is a multifunctional disorder that leads to cell death, tumors and the other diseases. Accumulation of improper molecular information during aging results in loss of functions in cells and tissues. The ovary is one of the first organs to age; women lose their fertility in their middle age (around 35 years old) and their fecundity expires soon thereafter (at more than 40 years old) (Baird *et al.* 2005; Alviggi *et al.* 2009). Although the exact mechanism underlying female reproductive aging remains unclear, common features among species, including loss of the ovarian follicle pool, disability of chromosome segregation leading to aneuploidy, and increasing mitochondrial dysfunctions, have been reported (Djahanbakhch *et al.* 2007). These changes are largely associated with the unique mechanism of oogenesis. Oocytes that mitotically proliferate during fetal development are stored in the ovaries without further proliferation and are repeatedly ovulated after they enter meiosis. Accordingly, oocytes that are stored for a longer duration gradually lose their functions because the ovarian microenvironment changes with aging.

Ovulation is known to produce reactive oxygen species (ROS) in the ovaries. Although ROS are toxic and sometimes lethal for any cell types, they are even necessary for proper ovulation because direct administration of ROS scavengers, N-acetylcysteine and dibutylhydroxytoluene, into mouse bursa blocked ovulation and hydrogen peroxide-assisted ovulation by functioning like luteinizing hormone (LH) (Shkolnik *et al.* 2011). Nevertheless, repeated exposure of stored oocytes to ROS at each ovulation results in loss of the integrity of these stored oocytes (Chao *et al.* 2005; Miyamoto *et al.* 2010). Oxidative stress is well known to damage macromolecules and cellular components, e.g., mitochondrial

desensitization, mitochondrial DNA mutation, irregular DNA methylation, and improper chromosome segregation. In addition, these changes affect the hormonal regulation; losing ovarian endocrine cells by both ovulation and oxidative damages alters the hormonal feedback system in the pituitary-gonad axis.

From a clinical viewpoint, age-associated infertility is not a small part in all the infertile patients. However, lack of knowledge regarding the aging mechanism hampers clinical approaches for treatment of aged women. Here we review recent findings on female reproductive aging and propose possible treatment options for age-associated infertility.

## 2. The prenatal and postnatal pathways of oogenesis

The debate on the duration of oogenesis in the whole life of females had been sealed for decades. However, recent reports on postnatal oogenesis and germline stem cells have resumed this debate. Herein we describe the prenatal and postnatal pathways of oogenesis from the viewpoint of reproductive aging.

### 2.1 The prenatal pathway of oogenesis

In the early stage of embryogenesis, primordial germ cells (PGCs) – from which oocytes originate – migrate from the dorsal yolk sac into the genital ridge where gonads would be formed (De Felici & Siracusa 1985). In mice, the germ cells originated from the proximal epiblast of the egg cylinder at embryonic day 5.5 to 6 in response to Bmp4 and Bmp8b signaling (Ying *et al.* 2001). In humans, this process occurs during the first month of gestation (Djahanbakhch *et al.* 2007). Then, the cells undergo mitosis; however the number of PGCs is highly limited at this time point. The PGCs proliferate rapidly, and approximately 7 × $10^6$ oogonia are eventually formed at 6–8 weeks of gestation in humans. During this process, transforming growth factor-β (TGF-β) family members, including activins, BMPs, and TGF-β1, support the proliferation of PGCs (Godin & Wylie 1991; Richards *et al.* 1999; Farini *et al.* 2005; Childs *et al.* 2010). Activins and their receptors are highly expressed in human oogonia at later stages of gestation and activin A supports the proliferation of oogonia *in vitro* (Martins da Silva *et al.* 2004). The oogonia then enter meiosis at 11–12 weeks of gestation in humans (Gondos *et al.* 1986).

After oogonia are enclosed by the granulosa cells and primordial follicles are formed, a number of oocytes are destined to die without contributing to reproduction during meiotic prophase I (Hussein 2005; Ghafari *et al.* 2007). More than one-third of all pachytene oocytes are proapoptotic, and a high frequency of atresia is observed between midterm and birth in the human ovaries (Speed 1988; De Pol *et al.* 1997). The large-scale loss of the ovarian follicle pool has been estimated to be more than 80% in humans (Martins da Silva *et al.* 2004).

Several paracrine factors that affect oocyte survival have been reported (Fig. 1). For example, growth factors including KIT ligand, leukocyte inhibitory factor (LIF), BMP-4, SDF-1, and basic FGF have been shown to be able to sustain the survival and proliferation of PGCs in the absence of somatic cell support (Farini *et al.* 2005). In addition, SCF, insulin-like growth factor I (IGF-I), and LIF have been found to assist the survival of germ cells in mice (Morita *et al.* 1999; Gu *et al.* 2009). In contrast, tumor necrosis factor-α (TNF-α) promotes apoptosis at the neonatal stage in rats (Marcinkiewicz *et al.* 2002; Morrison & Marcinkiewicz 2002). In

addition, intracellular factors determine the fate of oocytes. Members of the B cell lymphoma/leukemia (BCL) protein family, including BCL-2 and BAX, have been suggested to be involved in this process (Felici *et al.* 1999); BCL-2 is expressed in oocytes undergoing meiosis, and its expression is stable during meiotic prophase I, whereas upregulation of BAX is observed in oocytes undergoing apoptosis. Genetic inactivation (knockout) of BAX in mice resulted in higher number of germ cells in peri-natal ovaries compared with wild-type or heterozygous mice (Alton & Taketo 2007). Moreover, NANOS3 and DND1 protect PGCs from apoptosis (Tsuda *et al.* 2003; Youngren *et al.* 2005). Although the biological basis of the oocyte selection has not been completely understood, prenatal loss of oocytes may occur because of exclusion of accumulated mutations in mitochondria, clearance of lethal errors arising during the mitosis or meiotic prophase, or increased survival of some oocytes within a particular sibling "nest" (Ghafari *et al.* 2007). An imbalance between cell death and survival signaling would result in an abnormal number of follicles that would be stored in the ovaries at this stage; higher frequency of oocyte death that is a result of atresia eventually leads to irreversible premature ovarian failure (Krysko *et al.* 2008). Therefore, the number of oocytes that are stored prenatally must be extremely important for the subsequent reproductive period.

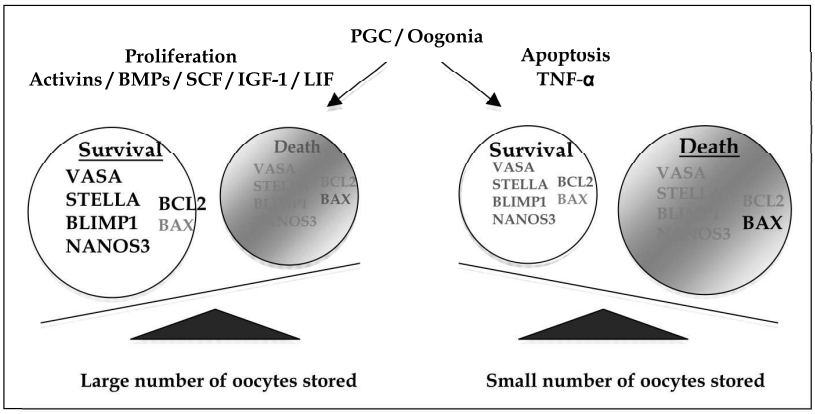


Fig. 1. Determinants of the maximum number of oocytes in the entire life of an organism. Several growth factors, including activins, BMPs, SCF, IGF-1, and LIF, promote proliferation of PGCs and oocyte growth, whereas TNF-α induces oocyte death. The number of follicles at this stage is determined by the balance between survival and death signaling. In addition, the intracellular balance between BCL-2 and BAX determines oocyte survival and death. NANOS3 is another anti-apoptotic molecule found in PGCs.

### 2.2 The postnatal pathway of oogenesis

Can oocytes be newly produced in adult ovaries? This ancient question arises with the observation of adult mice whose ovaries contain mitotically active germ cells (Johnson *et al.* 2004). This finding led to the hypothesis that oocytes can be generated from sources other than those prenatally stored in the ovaries. A possible origin of postnatal oocytes has been reported to be a specific set of bone marrow cells or peripheral blood cells expressing germline markers (Johnson *et al.* 2005). Johnson *et al.* reported that both bone marrow and

peripheral blood transplantations restored oocytes in mice that lost all oocytes by chemotherapy. However, another study claimed that fresh mature oocytes could not be obtained when wild-type and GFP-transgenic mice were parabiotically jointed to establish blood crossover (Eggan *et al.* 2006). Later, this report was supported when transplanted bone marrow cells could be transformed only into immature oocytes (Lee *et al.* 2007; Tilly *et al.* 2009). Moreover, other reports emphasized the possibility that putative germline stem cells exist inside and outside the ovaries in some species. In pigs, fetal skin cells have been reported to contribute to the generation of oocytes (Dyce *et al.* 2006). The ovarian surface epithelium (OSE) cells are another candidate for the origin of oocyte in adult human and rat ovaries (Bukovsky *et al.* 2008; Parte *et al.* 2011), although the candidate cells in OSE may originate from bone marrow cells. In addition, a pancreatic stem cell line seems to differentiate into oocyte-like cells in rats (Danner *et al.* 2007). Unfortunately, none of these germline stem cells have contributed to the production of the next generation. Furthermore, the molecular mechanisms underlying postnatal oogenesis of putative germline stem cells continue to be a black box. Even so, these cells might be useful for clinical applications if a specific condition in which mature fertile oocytes are postnatally generated is elucidated. Although these findings are fascinating, the following questions are arising.

1. Can the germline stem cells participate in oogenesis over the entire life of females? 2. Do other germline stem cells exist? 3. Is there a specific condition in which the germline stem cells participate in oogenesis? 4. How many oocytes are generated in the ovaries through postnatal oogenesis? 5. Are the factors that assist postnatal oogenesis the same as those that assist in prenatal oogenesis? 6. What is the exact role of postnatal oogenesis? Unfortunately, we still have to wait many years to obtain sufficient data to answer these questions.

## 3. Modification of oocyte quantities and qualities during aging

The common physiology of the ovaries during aging among species includes loss of the ovarian follicle pool, chromosomal abnormalities and cytoplasmic abnormalities (Fig. 2). All these changes may be inevitable and are associated with declining oocyte quality. Here recent findings regarding the alterations in oocyte quantities and qualities are discussed.

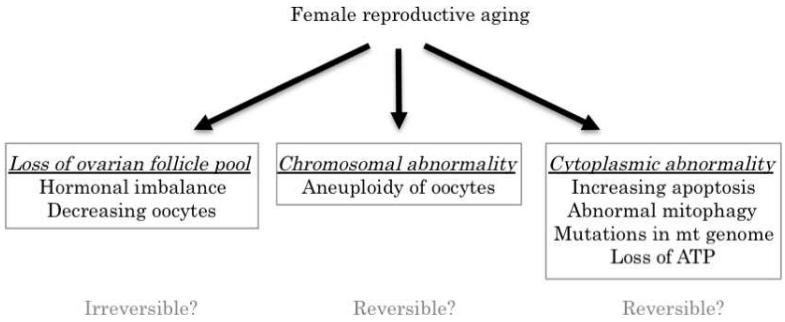


Fig. 2. Common features of female reproductive aging in mammals. Loss of the ovarian follicle pool is caused partly by characteristic oogenesis and partly by ovulation, thereby leading to hormonal imbalance. Chromosomal aberrancy and cytoplasmic abnormalities are possibly induced by longer exposures of stored oocytes to oxidative stress. Recently, these abnormalities have been shown to be reversible by calorie restriction (Selesniemi *et al.* 2011).

### 3.1 Age-associated decrease of the ovarian follicle pool

After puberty, the number of oocytes steadily decreases due to repeated ovulation. A lot of oocytes are consumed in 1 estrous cycle, but only a few oocytes are eventually ovulated. In humans, only 400 oocytes are dedicated for ovulation throughout the lifespan. During aging, the ovarian follicle pool declines continuously, and this is partly because of atresia. Resting follicles in humans enter atresia through a necrotic process during the initial recruitment phase of folliculogenesis because the ooplasm in those follicles contains increasing numbers of multivesicular bodies and lipid droplets, dilation of the smooth endoplasmic reticulum and the Golgi apparatus, and irregular mitochondria with changed matrix densities (de Bruin *et al.* 2002; de Bruin *et al.* 2004). The atresia of ovarian follicles during aging may be induced by dysfunctions of proteosomes and the endoplasmic reticulum (Matsumine *et al.* 2008).

Although most primordial follicles that are initiated to grow are destined to cell death, the recruitment of follicles from the resting ovarian follicle pool is the sole method to salvage the follicles. FSH is a strong trophic factor that supports both the cyclic recruitment of antral follicles and the growth of follicular somatic cells (Chun *et al.* 1996).

### 3.2 Age-associated aberrancies of oocyte chromosomes

The most deleterious damage in oocytes is often observed in chromosomes. The relationship between maternal age and the increased incidence of oocyte aneuploidies has been studied in several epidemiological studies (Hassold & Jacobs 1984; McFadden & Friedman 1997; Pellestor *et al.* 2003). In women in their early 20's, the risk of trisomy in a clinically recognized pregnancy is only approximately 2%, whereas it increases up to 35% in women in their 40's (Hassold & Chiu 1985). Supportively, more than half of oocytes from patients of advanced age exhibit aneuploidy (Kuliev & Verlinsky 2004; Pellestor *et al.* 2005). This abnormality is believed to occur because of chromosomal nondisjunction during either meiosis I or II (Nicolaidis & Petersen 1998; Hassold *et al.* 2007). The incidence of aneuploidy is not random; abnormalities of chromosomes 16 and 22 originate more frequently in meiosis II than in meiosis I, and those of chromosomes 13, 18, and 21 occur more frequently in meiosis I than in meiosis II (Kuliev *et al.* 2005). In addition to this nondisjunction theory, the premature separation of chromatids during meiosis is suggested to be responsible for aneuploidy; the age-associated degradation of cohesins or the other molecules sustaining chromatids during metaphase I may contribute to the age-related increase in aneuploidy (Watanabe & Nurse 1999).

### 3.3 Age-associated decline of mitochondrial activities

The mitochondria alter their organization, shape, and size, depending on various signals (Bereiter-Hahn & Voth 1994). Mitochondrial turnover is the most important process to maintain a healthy state of the mitochondria. During this process, they undergo biogenesis and degradation (Seo *et al.* 2010). Mitochondrial biogenesis is enhanced in muscle cells under certain physiological conditions such as myogenesis, exercise, cold exposure, hypoxia, and calorie restriction (CR) (Freyssenet *et al.* 1996; Nisoli *et al.* 2003; Kraft *et al.* 2006; Civitarese *et al.* 2007; Zhu *et al.* 2010). Damaged or incompetent mitochondria are removed by macroautophagy (Wang & Klionsky 2011).

Although whether mitochondrial dynamics are important for the maintenance of oocyte integrity during aging remains unclear, the copy number of the mitochondria is one of the factors that affect the developmental capacity of oocytes after implantation in mice (Wai *et al.* 2010). In addition, mouse oocytes with artificial mitochondrial damages lost their ability to be matured *in vitro* (Takeuchi *et al.* 2005). Thus, the oocyte quality is largely dependent on mitochondrial health.

Progressive and accumulative damages to mitochondrial DNA (mtDNA) have been postulated to be responsible for the aging process. In aged human fibroblasts, point mutations are likely to occur at specific positions in the replication-controlling region (Michikawa *et al.* 1999). Although these specific mutations have never been reported in aged human oocytes, several mutations in mtDNA were responsible for the decreased ability of oocytes to develop (Barritt *et al.* 2000). In the report, ooplasmic transfer from young oocytes to aged oocytes improved the quality of aged oocytes, indicating that the decreased mitochondrial activities in aged oocytes were complemented.

## 4. Molecular events during aging

Oocyte quality declines during aging in a complicated process involving several events (Fig. 3). Oxidative stress affects both the size of the ovarian follicle pool and oocyte quality. The reduced follicle pool accelerates hormonal dysregulation. This, in turn, promotes the decrease in the size of the ovarian follicle pool and oocyte quality. In this section, the recent findings regarding the molecular events that occur during reproductive aging are discussed.

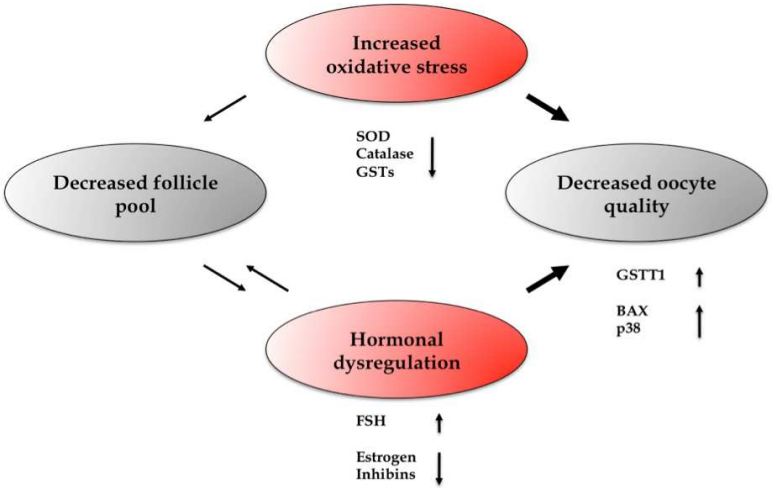


Fig. 3. A negative loop leading to the age-associated decline of oocyte quality. Because of repeated ovulation and the loss of antioxidants including SOD, catalase, glutathione S-transferases (GSTs) etc., excess oxidative stress accelerates the decrease of both oocyte quality and the size of the follicle pool. The decreased follicle pool results in the insufficient secretion of ovarian estrogens and inhibins and the rise of FSH. These changes accelerate the decrease of the follicle pool and directly affect oocyte quality. Both oxidative stress and aberrant hormones induce the molecular alterations (GSTT1, p-p38 etc.) in oocytes and granulosa cells.

### 4.1 Serum hormone levels

Full competence of oocytes to develop to term is acquired depending on the proper timing of hormonal activation. The decrease in follicle numbers because of aging ensures aberrant hormonal regulation as a result of incomplete feedback mechanisms. This hormonal dysregulation will, in turn, accelerate the loss of follicles at the advanced age (McTavish *et al.* 2007).

The most well-known hormones that affect aging are FSH and LH. Under the normal conditions, relatively high levels of FSH promote the synthesis of estradiol, inhibin A, and inhibin B in granulosa cells (Tonetta & diZerega 1989). On the other hand, LH regulates the production of androgens in theca cells of small antral follicles and promotes the conversion of androgens to estradiol by aromatization (Erickson *et al.* 1985). These factors, in turn, decrease the serum levels of FSH and LH. With luteal regression, the downregulation of estradiol and inhibin A in luteinized granulosa cells allows the rise of FSH again at the onset of the subsequent menstrual cycle (Broekmans *et al.* 2009). Therefore, the negative feedback system between the pituitary and the ovary enables follicles to grow properly.

The dysfunction of the hypothalamic GnRH pulse generators results in an abnormal release of FSH and LH from the pituitary around menopause (Wise *et al.* 1996). However, the factor that is critical to induce improper hormonal regulation is the loss of the follicle pool (Fig. 3). Decreasing numbers of follicles in the ovaries result in decreasing concentrations of circulating estrogens and inhibins during aging (Broekmans *et al.* 2009). Accordingly, the serum concentration of FSH is elevated because of aging (Klein *et al.* 1996). The hormonal changes, especially the decrease in the levels of inhibins, are highly associated with oocyte quality (Chang *et al.* 2002).

Anti-Mullerian inhibitory hormone (AMH) is expressed in granulosa cells of nonatretic preantral and small antral follicles (Baarends *et al.* 1995). AMH has been postulated to regulate the entry of primordial follicles into the growing pool (Durlinger *et al.* 2002). As the number of antral follicles decreases with age, the serum amount of AMH diminishes (van Rooij *et al.* 2004).

Because these changes are largely associated with the unique features of oogenesis, age-associated hormonal changes are inevitable. Abnormal levels of hormones can be a risk factor for certain diseases other than infertility. For example, elevated FSH levels stimulate TNF-α synthesis directly from bone marrow granulocytes and macrophages and promote osteoporosis in mice (Iqbal *et al.* 2006; Sun *et al.* 2006). In addition, the single nucleotide polymorphism (SNP) rs6166 of the FSHR gene significantly influences bone mineral density in postmenopausal women (Rendina *et al.* 2010).

### 4.2 Oxidative stress and cellular scavengers

Oxidative stress is generally accepted as the major cause of aging. The major source of ROS is believed to be the mitochondria, because ROS are generated as byproducts of electron transport during respiration. Although about 1 – 2 % of oxygen in the heart is converted into ROS under physiological conditions, ROS generation increases under pathological conditions (O'Rourke *et al.* 2005; Valko *et al.* 2007). ROS are removed rapidly through multiple pathways to protect cells and tissues in normal young individuals.

A similar system must be present in the ovaries. Free radical activities in human follicular fluid have been shown to be increased during aging (Wiener-Megnazi *et al.* 2004). However, the levels of free radical scavengers, including SOD1, SOD2, and catalase, were significantly decreased in the granulosa cells from older women compared with those in the granulosa cells from younger women (Tatone *et al.* 2006). In addition, oxidative damages measured by the expression of 8-hydroxydeoxyguanosine were observed in oocytes after ovulation (Chao *et al.* 2005; Miyamoto *et al.* 2010). Although the ovarian levels of oxidative stress during aging remain unclear, excess ROS induced by ovulation may affect the quality of oocytes that are stored in the ovaries (Fig. 3). In fact, ovulation induced ROS generation in the ovaries, resulting in oxidative damage of genomic DNA and mitochondrial DNA mutations (Agarwal *et al.* 2005; Chao *et al.* 2005).

Glutathione (GSH) – a direct ROS scavenger – protects cells from deleterious attacks of ROS. Accordingly, it is highly correlated with oocyte quality in terms of viability (Zuelke *et al.* 2003; Luberda 2005). However, whether the level of GSH in oocytes from aged females is decreased compared with that from young females is unclear.

GSTs are well-known detoxification factors that excrete genotoxins by conjugation of GSH directly to the genotoxins (Sheehan *et al.* 2001). In addition to this characteristic, some GSTs have been shown to play important roles in ROS scavenging by affecting JNK stress signaling (Adler *et al.* 1999; Cheng *et al.* 2001). As expected from the known functions, GST activities in aged oocytes were lower than those in young oocytes (Tarin *et al.* 2004). We previously reported that GSTT1 was upregulated in aged granulosa cells, although the other isoforms of GSTs were downregulated (Ito *et al.* 2008). GSTT1 is known to have bilateral features in that it removes toxins and oxidative stress from cells and tissues and it produces harmful formaldehyde using halogenated substrates (Sherratt *et al.* 1998; Landi 2000). Although it remains uncertain whether GSTT1 is positively or negatively involved in reproductive aging, GSTT1-depleted granulosa cells exhibit mitochondrial hyperpolarization, suggesting that GSTT1 plays a role in controlling mitochondrial activities (Ito *et al.*, 2011).

### 4.3 Genes-related to apoptosis during reproductive aging

BCL family members are closely related to apoptosis in ovarian cells as well as in other cell types (Tilly *et al.* 1997). Overexpression of BCL-2 in mouse ovaries leads to decreased follicular apoptosis (Hsu *et al.* 1996). A prominent decrease of BCL-2 was also observed in eggs aged *in vitro* (Gordo *et al.* 2002). In addition, the upregulation of BIM in cumulus cells seems to accelerate oocyte aging (Wu *et al.* 2011). More impressively, ovarian functions in mice with genetically engineered BAX were prolonged (Perez *et al.* 1999). Supporting this report, damaged oocytes in mice exhibited higher expression level of BAX (Kujjo *et al.* 2010). Hence, BAX may be a therapeutic target for oocyte rejuvenation.

### 4.4 Cellular signaling involved in oocyte survival and death

The crosstalk of the signal kinases is important for oocyte survival. For the survival of primordial follicles, 3-phosphoinositide-dependent protein kinase-1 (PDK1) in oocytes preserves the lifespan by maintaining the ovarian follicle pool (Reddy *et al.* 2009). PDK1 and PTEN have been reported to be critical regulators in the phosphatidylinositol 3-kinase (PI3K)

signaling pathway (Iwanami *et al.* 2009). Therefore, the loss of PTEN results in the activation and depletion of the primordial follicle pool in early adulthood (Reddy *et al.* 2008).

Higher levels of M-phase promoting factors and mitogen-activated protein kinases (MAPKs) have been observed in ovulated oocytes from aged mice (Tarin *et al.* 2004). Although how these signaling molecules are involved in oocyte activities remains uncertain, JNK, but not p38 MAPK, was found to participate in oocyte fragmentation and parthenogenetic activation in aged oocytes (Petrova *et al.* 2009).

In our previous report, p38 MAPK in human granulosa cells showed a unique pattern of activation and localization during aging (Ito *et al.* 2010). Because p38 has been shown to translocate between the nucleus and cytoplasm upon stimulation (Gong *et al.* 2010), the changes in the subcellular localization of active p38 during aging reflect the microenvironmental status around oocytes and granulosa cells. The nuclear localization of p38 in young granulosa cells may be due to the proper transactivation of genes in response to hormones. On the other hand, the cytoplasmic localization of p38 in aged granulosa cells may be resulted from exposure to oxidative stress. Although it is unclear whether or not such kind of age-associated changes occurs in oocytes, regulation of activation and localization of p38 may contribute to oocyte juvenescence.

## 5. Is it possible to rescue age-related infertility?

### 5.1 Anti-aging effects of calorie restriction

Although aged oocytes adapt to the stressful environment and endure multiple disorders that occur inside and outside oocytes, they eventually lose their ability to develop to term. Is it possible to rejuvenate aged oocytes? Although oocyte rejuvenation should be considerably attractive for treatment of age-related infertility, it has not succeeded so far. However, it may be possible to prolong ovarian functions (or to delay ovarian aging). Physical juvenescence can be achieved with several methods, including calorie restriction (CR), moderate fitness and nutritional supply (Prokopov & Reinmuth 2010). These treatments are believed to maximize mitochondrial performance and lower the incidence of mitochondrial dysfunction (Lopez-Lluch *et al.* 2006; McKiernan *et al.* 2007).

CR has been suggested to elongate the lifespan of many organisms (Wolf 2006). CR influences energy metabolism, oxidative damage, inflammation, and insulin sensitivity. CR is reported to activate SIRT1, a key factor that regulates longevity (Allard *et al.* 2009). An important role of SIRT1 may be refreshment of damaged mitochondria by inducing macroautophagy (Kume *et al.* 2010). Therefore, CR enhances cellular homeostasis. Apart from the beneficial effects of CR on longevity, it was believed to be the cost for reproductive capacities (Holliday 1989). Harsh CR (30% or more) is unable to maintain stored oocytes enough for their subsequent development, whereas milder CR (20%) resulted in the loss of the negative effects on fecundity (Rocha *et al.* 2007). Recently, CR has been shown to be beneficial for maintaining the integrity of oocyte chromosomes in mice (Selesniemi *et al.* 2011). These effects were mimicked by the genetic loss of the metabolic regulator, peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1$\alpha$). Our preliminary data also reveal that CR (approximately 15% reduction of body mass after one year of treatment) did not reduce fertility in mice. Rather, the CR-treated mice bore more offspring. These data indicate that CR supports the oocyte quality in aged females.

Although the hypothesis that physical juvenescence correlates with the maintenance of oocyte integrity must be explored further, other treatments that lead to anti-aging may support oocyte integrity during aging.

### 5.2 Nutritional supports of fertility

Since CR has been demonstrated to be beneficial for the juvenescence of cells and tissues including germ cells, as described above, the daily nutritional intake must be crucial for cellular juvenescence. One of the most successful nutrients that affect anti-aging may be polyphenols. For example, turmeric-derived tetrahydrocurcumin and green tea polyphenols promote longevity of mice (Kitani *et al.* 2007). Of those polyphenols, resveratrol has been a potent therapeutic target for age-related diseases. Resveratrol is found in eucalyptus, peanuts, and grapevines (Soleas *et al.* 1997), and its functional properties are versatile probably because of its divergent targets. It has beneficial effects in terms of prevention of various cancers, cardiovascular diseases, neurodegenerative diseases, and diabetes in animal models (Vang *et al.* 2011), because of its antioxidant and anti-inflammatory properties (Schmitt *et al.* 2010). Moreover, resveratrol has been demonstrated to prolong lifespan in short-lived vertebrates (Valenzano *et al.* 2006), because it greatly enhances the activity of SIRT1 (Howitz *et al.* 2003; Knutson & Leeuwenburgh 2008). In addition to its anti-aging properties, resveratrol has been reported to function as estrogen through direct association with estrogen-responsive element (ERE) (Klinge *et al.* 2003). Although the beneficial effects of resveratrol in humans remain to be determined, it is expected to be a mimetic of CR and have the potential to preserve oocyte quality in aged females. Supportively, resveratrol assisted the increase of the ovarian follicle pool in both neonatal and aged rat ovaries (Kong *et al.* 2011). Genistein, one of isoflavones, also seems to increase the ovarian follicle pool by inhibiting atresia in aged rats (Chen *et al.* 2010).

Royal jelly (RJ) was reported to contribute to the prolongation of longevity in mice (Inoue *et al.* 2003). RJ reduced the damages of DNA by acting as an antioxidant. Similar to resveratrol, RJ contains an estrogen-like component that associates with the ERE (Mishima *et al.* 2005). Traditionally, it has been used to treat menopausal symptoms, although the detailed mechanism by which RJ treats menopausal disorders is yet to be determined. RJ seems to rebalance the hormonal concentration in the blood; it decreased the FSH concentrations and increased the estrogen concentrations in aged mice (Fig. 4). These changes, in turn, increased the number of ovulated oocytes. This may improve the oocyte quality in aged body, although further investigation is required.

Recently, a probiotic strain, LKM512, present in yogurt was shown to prolong the longevity of mice (Matsumoto *et al.* 2011). LKM512 has been suggested to act on the polyamines circulating bodies and result in the unexpected prolongation of longevity. Although whether these beneficial effects on longevity can sustain the maintenance of oocyte quality remains unclear, some of these nutritional elements may alleviate female reproductive aging.

The exact mechanism by which some anti-aging treatments improve female reproductive capacity remains unknown. However, hormonal regulation in aged females becomes similar to that in young females with anti-aging treatments, and this is probably due to the prevention of follicle loss or the enhancement of hormonal secretion from aged ovaries (Fig. 5).

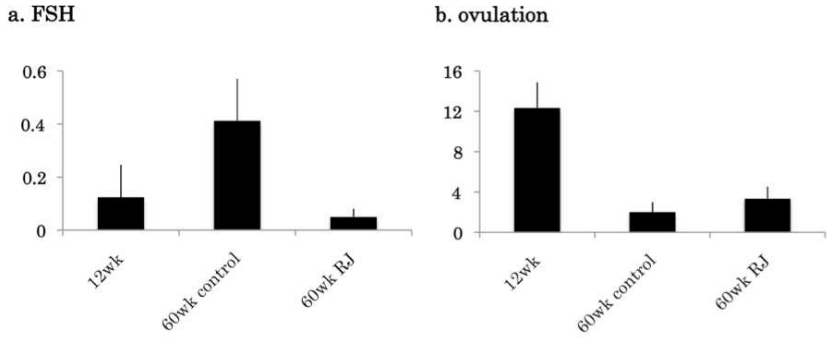


Fig. 4. Effects of RJ on female reproductive capacities. The administration of RJ in drinking water to aged female mice (60 weeks old) for 2 months markedly decreased the serum concentrations of FSH (a). RJ slightly increased the number of ovulated oocytes (b).

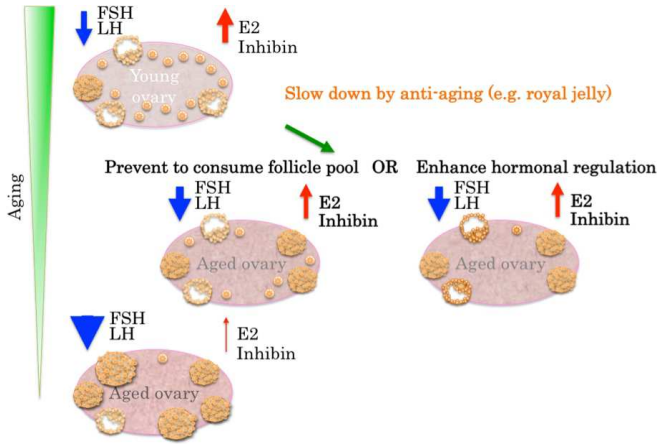


Fig. 5. Age-dependent hormonal regulation in the ovaries. In young females, the negative feedback system in the pituitary-gonadal axis is active, and the amount of FSH and LH is regulated by estrogens (E2) and inhibins secreted from the ovaries. However, inadequate amounts of E2 and inhibins synthesized from aged ovaries cannot decrease the serum levels of FSH and LH. These changes can be treated by anti-aging therapies, such as the supplemental administration of RJ that may prevent the waste of follicles during ovulation or enhance the synthesis of ovarian hormones.

### 5.3 Other compounds that affect oocyte aging

Because oxidative stress promotes reproductive aging, antioxidants can be effective in regaining reproductive juvenescence. In fact, oral administration of vitamins C and E could prevent age-related ovarian disorders in mice (Tarin *et al.* 1998a; Tarin *et al.* 2002). In addition, some antioxidants are used to treat infertility (Visioli & Hagen 2011).

L-cystine, a component of GSH, and β-mercaptoethanol decreases oocyte quality to develop to the blastocyst stage, whereas dithiothreitol (DTT) enhances the fertilization rate and the

developmental capacity of oocytes (Tarin *et al.* 1998b). Moreover, DTT supports embryonic integrity regarding cell number in inner cell mass cells (Rausell *et al.* 2007). Therefore, reagents that assist redox may be effective in enhancing oocyte quality. However, all these compounds were tested in oocytes that were aged *in vitro*, and thus, their reported effects may not be observed in oocytes from aged females.

On the other hand, nitric oxide (NO) seems to be a strong candidate for the treatment of the declining oocyte quality in aged females, because the exposure of aged oocytes to NO decreased the loss of cortical granules and the frequency of spindle abnormalities (Goud *et al.* 2005).

## 6. Future perspectives to achieve the juvenescence of female fertility

The impact of reproduction on the maternal longevity has been postulated by numerous epidemiological and historical studies (Westendorp & Kirkwood 1998; Le Bourg 2007; Mitteldorf 2010), and the tradeoff between fertility and longevity may occur through genetic or metabolic factors. However, some studies reported a positive correlation between maternal age at reproduction and female longevity (Muller *et al.* 2002; Helle *et al.* 2005). Although the outcomes of those surveys varied, maternal age at the time of the first childbirth seems to be positively correlated with female longevity.

Therefore, the most important factor affecting their fecundity must be physical juvenescence. Because the quality of oocytes from women who successfully give birth at advanced ages is somehow integral, physical juvenescence can increase oocyte quality (chromosomes and cytoplasm), although it may be difficult to increase the number of follicles stored in aged ovaries. Regarding longevity, fitness, or CR is successful in several species, including humans (Lahdenpera *et al.* 2004). In addition, some nutritional supplements could assist the longevity of animals, as described above. Although it remains uncertain whether or not those supplements can improve the integrity of oocytes even after aging, these kinds of treatment may help age-associated infertility in the future.

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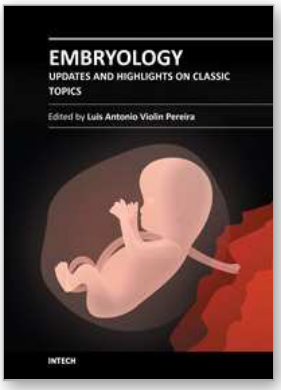


**Embryology - Updates and Highlights on Classic Topics**

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Embryology is a branch of science concerned with the morphological aspects of organismal development. The genomic and molecular revolution of the second half of the 20th century, together with the classic descriptive aspects of this science have allowed greater integration in our understanding of many developmental events. Through such integration, modern embryology seeks to provide practical knowledge that can be applied to assisted reproduction, stem cell therapy, birth defects, fetal surgery and other fields. This book focuses on human embryology and aims to provide an up-to-date source of information on a variety of selected topics. The book consists of nine chapters organized into three sections, namely: 1) gametes and infertility, 2) implantation, placentation and early development, and 3) perspectives in embryology. The contents of this book should be of interest to biology and medical students, clinical embryologists, laboratory researchers, obstetricians and urologists, developmental biologists, molecular geneticists and anyone who wishes to know more about recent advances in human development.



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