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REVIEW ARTICLE

Cellular and molecular mechanisms of various types of oocyte aging

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Abstract It is well established that age-related decline of a woman's fertility is related to the poor developmental potential of her gametes. The age-associated decline in female fertility is largely attributable to the oocyte aging caused by ovarian aging. Age-associated oocyte aging results in a decrease in oocyte quality. In contrast to ovarian aging, there is a concept of postovulatory oocyte aging. Postovulatory aging of oocytes, not being fertilized for a prolonged time after ovulation, is known to significantly affect the development of oocytes. Both categories of oocyte aging have similar phenotypes of reproductive failure. However, the mechanisms of the decline in oocyte quality are not necessarily equivalent. An age-dependent increase in aneuploidy is a key determinant of oocyte quality. The reduced expression of molecules regulating cell cycle control during meiosis might be involved in the age-dependent increase in aneuploidy. The mechanism of age-associated oocyte aging might be involved in mitochondrial dysfunction, whose etiologies are still unknown. Alternatively, the mechanism of postovulatory oocyte aging might be involved in reactive oxygen species-induced mitochondrial injury pathways followed by abnormal intracellular Ca^{2+} regulation of the endoplasmic reticulum. We suggest that future research into the mechanism of oocyte aging will be necessary to develop a method to rescue the poor developmental potential of aged oocytes.

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Introduction

The problem of infertility in the developed countries has increased in the past 30 years [1]. It is difficult to accurately calculate the number of infertility patients. To date, the numbers of treatment cycles in assisted reproductive technology (ART) have steadily increased [2]. There are several factors involved in the increase in infertility patients. The most important factor is that the average age of patients seeking infertility treatment has increased [3]. Since the development of effective contraception in the 1960s, women have been able to delay childbearing at their own discretion, and the average maternal age has increased by approximately 5 years during the last 30 years [4, 5]. Decreased fecundity with increasing female age has been recognized from demographic and epidemiological studies [6]. Thus, delayed childbearing reduces the chance of achieving spontaneous pregnancy [7]. The age-associated decline in female fertility is largely attributable to the decrease in oocyte quality due to ovarian aging [8, 9]. Because the mechanisms of decline in oocyte quality remain unknown, there are no treatments available for patients whose infertility arises from this cause, except for oocyte donation programs [10, 11].

In contrast to ovarian aging, there is a concept of postovulatory oocyte aging. In mammals, ovulated oocytes are arrested at the metaphase stage of the second meiotic division until they are fertilized. The optimal period for oocyte fertilization lasts less than 10 h [12–14]. Fertilization within this narrow window of opportunity results in

normal embryo development. Postovulatory aging of oocytes, not being fertilized for a prolonged time after ovulation, is known to significantly affect the development of oocytes [15, 16]. Postovulatory oocyte aging also impairs oocyte quality and results in reproductive failures [15, 16]. However, it is not well known whether the mechanism for the impairment of oocyte quality by these two categories of oocyte aging is similar. The aim of the present paper is to review in detail the mechanisms of these two categories of oocyte aging.

Defining oocyte aging

Terminology and definition of "oocyte aging" are very confusing. There are two categories of oocyte aging; one is preovulatory and the other is postovulatory oocyte aging [15]. Classification of oocyte aging is shown in Table 1. Preovulatory oocyte aging can be further differentiated into two different types. One of the two types of preovulatory oocyte aging is a consequence of ovarian aging. Ovarian aging is seen in reproductive aged women over 35 years old [17]. The other type of preovulatory oocyte aging is seen when ovulation does not occur in timely fashion, and the oocytes stay in the ovarian follicles after LH or hCG stimulation [15]. In other words, it represents an "intrafollicular oocyte aging" after ovulation stimulation. This type of preovulatory oocyte aging can be induced by treatment of females with a sodium pentobarbital [18], a gonadotropin-releasing hormone antagonist [19], some progestins [20], or inosine monophosphate dehydrogenase inhibitors [21]. It is quite different from the concept of preovulatory oocyte aging with maternal aging, and is rather similar to postovulatory oocyte aging.

The second category, postovulatory oocyte aging, can be classified into two types: in vivo- and in vitro-postovulatory oocyte aging. If fertilization does not occur during an optimal period after ovulation, an unfertilized oocyte that remains in the oviduct (in vivo-postovulatory oocyte aging) or in in vitro culture (in vitro-postovulatory oocyte aging) goes through a time-dependent aging process [15, 22–24]. There is no consensus on the duration of postovulatory oocyte aging. In this review, we discuss these types of oocyte aging individually.

Table 1 Classification of oocyte aging mechanisms

A. Preovulatory oocyte aging
Oocyte aging caused by ovarian aging (maternal aging)
Intrafollicular oocyte aging[a]
B. Postovulatory oocyte aging
In vivo oocyte aging
In vitro oocyte aging

[a] See details in the text

Effects of oocyte aging on female reproduction

Preovulatory oocyte aging

Ovarian aging

Preovulatory oocyte aging has been considered to be equivalent to ovarian aging [25]. In general, ovarian aging is associated with chronological aging (maternal aging), except in cases of premature ovarian insufficiency (POI) [17]. It has been shown that female fertility declines in mammals including humans with increasing maternal age [1, 7, 17]. In humans, it is well established from demographic and epidemiological studies that female fertility begins to decline many years prior to the onset of menopause, despite continued regular ovulatory cycles [1, 4, 6]. Although there is no strict definition, advanced reproductive age in women is generally accepted as over 35 years [8, 9]. Under natural conditions, 75% of women who first try to conceive at age 30 years will have a conception ending in a live birth within 1 year, 66% at age 35 years, and 44% at aged 40 years. Within 4 years the success rates will be 91, 84, and 64%, respectively [26]. The age associated decline in female fertility and increased risk of spontaneous abortion are largely due to oocyte aging [8]. This is demonstrated by the fact that the age-associated decline in female fertility can be overcome by oocyte donation from younger women [10, 11].

Intrafollicular oocyte aging

The other type of preovulatory oocyte aging, an intrafollicular aging of the oocytes, also impairs female reproduction in experimental and domestic animals [15]. The female rats treated with sodium pentobarbital results in delayed ovulation and the oocytes exhibit decreased fertilization, polyspermy, chromosomal anomalies, abnormal embryo development, and increased fetal mortality [27, 28]. These detrimental effects by intrafollicular aging of oocytes are very similar to those caused by postovulatory aging of oocytes (discussed later).

Postovulatory oocyte aging

Numerous investigators have reported that in vivo- and in vitro-aged oocytes frequently exhibit lower fertilization rates, polyspermy, digyny, chromosomal anomalies, and abnormal embryo development [15]. These abnormalities of early embryo development result in decreased litter size in animals, lower pregnancy rate and an increased spontaneous miscarriage in humans [24, 29]. As well as abnormalities of early embryo development, postovulatory aging of oocytes is associated with retarded sensorimotor

integration during pre-weaning development, higher spontaneous motor activity, and higher emotionality in adulthood in the mouse [30]. A recent study demonstrated that postovulatory aging affects epigenetic changes in the mouse oocytes [31].

Cellular and molecular changes in aged oocytes

Preovulatory oocyte aging

Ovarian aging

The morphological changes in the ovary associated with aging have been extensively analyzed [32]. It is well known that ovarian aging results in ovarian follicle loss. Although the mechanism of ovarian follicle loss is still elusive, it is widely accepted that apoptosis is a driving force underlying age-related ovarian follicle loss [33–38]. However, little is known about the morphological changes in the oocyte caused by ovarian aging. There are several reports about aging-related morphological changes in the oocytes, such as cellular fragmentation, milky or dark cytoplasm, and presence of cellular remains enclosed by the zona pellucida [39, 40]. Oocytes with cellular fragmentation in aged mice indicate apoptosis of oocytes [39, 40]. In contrast with the results of previous reports, we cannot distinguish between the oocytes collected from aged mice and those from young mice under a microscope. While ovulation number of oocytes decreases in aged mice compared to young mice, these oocytes from both mice are apparently similar in terms of morphology (Takahashi and Kurachi, unpublished data).

On the other hand, numerous data support that oocyte aneuploidy increases with ovarian aging in mammals, including humans [41–43]. Increasing evidence shows that aneuploidy by ovarian aging results from a defective cell cycle control during meiosis I, especially metaphase I to anaphase I [25]. Ovarian aging affects some key molecules for cell cycle control in oocytes: there is a reduced number of transcripts for 'cohesion proteins' such as SMCβ1 and for 'spindle check point proteins' such as MAD2 [44–46].

Intrafollicular oocyte aging

Intrafollicular oocyte aging affects morphological and cellular changes in oocytes treated with sodium pentobarbital, resulting in irregularities in the oolemma, decreased number of cortical granules, disruption of cytoskeleton arrangement, and changes in mitochondrial structure [18, 27, 47–49]. These morphological changes in oocytes by intrafollicular oocyte aging are very similar to those caused by postovulatory oocyte aging [15].

Postovulatory oocyte aging

It is well established that in vivo- and in vitro-postovulatory aging of oocytes is associated with changes in various morphological, biochemical, and molecular pathways involved in intracellular signaling [15, 16]. In vivo- and in vitro-postovulatory aging of oocytes share many common properties.

Morphological and cellular changes

Postovulatory oocyte aging affects numerous morphological and cellular changes: changes in structure of oolemma, zona pellucida, cortical granules, mitochondria, cytoskeleton, meiotic spindle, and chromosome alignment [15, 16]. The lining of the oocyte cortex in the fresh oocytes is composed of thick and thin microfilament domains. Aged oocytes show disruption or loss of the thick microfilament domain beneath the oolemma [50–54]. The zona pellucida in fresh oocytes appears as a granulofibrillar, interconnected reticulum with pores, while the zona pellucida in aged oocytes shows a 'cobblestone' appearance [55]. Chymotrypsin-mediated dissolution of the zona pellucida takes more time in aged oocytes compared to fresh oocytes [16, 56, 57]. This indicates that zona pellucida hardening occurs naturally in aged oocytes. The reason for zona hardening in aged oocytes is that cortical reaction, exocytosis of cortical granules, is easily triggered spontaneously without fertilization [47, 56, 58]. Aged oocytes show an increase in the number of phosphatase positive organelles (lysosomes), aggregation of tubuli of smooth endoplasmic reticulum, and aggregation of small mitochondria-vesicle complexes [51, 59]. Meiotic spindle assembly is a cellular structure important for accurate chromosomal distribution [60]. The meiotic spindle consists of a central region of chromosomes and of microtubules radiating from the chromosomes to the two opposite spindle poles, which consist of foci of pericentriolar material [61]. The pericentriolar material consists of a network of 12–15 nm filaments with which the other components associate [62]. One well-characterized component of the pericentriolar material is the γ-tubulin ring complex [63]. There are many reports that postovulatory aging of oocytes results in disruption and loss of the meiotic spindle assembly in experimental animals and humans [50, 64–68]. In mouse studies, although the meiotic spindle is barrel-shaped and microtubules are clearly detected in fresh oocytes, microtubules become gradually lost from the spindle in aged oocytes [16]. In vitro-aged human oocytes, like those of experimental animals, show aberrant expression of γ-tubulin, which indicates disruption of centrosome structure at the meiotic poles [60, 69]. These changes in aged oocytes lead to

premature chromosomal separation, which is strongly associated with aneuploidy [44, 67].

Biochemical and molecular changes

Postovulatory aging of oocytes affects various biochemical and molecular changes in mammalian oocytes [15, 16]. After fertilization, recruitment of maternal mRNAs occurs and results in changes in the spectrum of polypeptides synthesized in oocytes [70]. The newly synthesized protein expression patterns change in fertilized aged oocytes [56]. The intracytoplasmic level of glutathione (GSH), which plays a major role in protection against reactive oxygen species (ROS), decreases in aged oocytes [71]. The level of lipid peroxidation, which indicates the degree of oxidative stress, increases in in vivo-aged oocytes [72]. Moreover, the amount of ROS increases in aged oocytes with increasing time of in vitro culture [73]. The intracytoplasmic level of ATP decreases in aged oocytes [74, 75]. The inactivation of MPF, which consists of two subunits of $p34^{cdc2}$ and cyclin B, and MAPK occurs earlier in aged oocytes [56, 76–78]. The expression of BCL2, anti-apoptotic protein, is decreased in in vivo-and in vitro-aged oocytes [73, 78, 79]. Postovulatory aging of oocytes impairs intracellular Ca^{2+} regulation, which is most important for early events after fertilization and also for subsequent embryo development [80–83]. Abnormal intracellular Ca^{2+} regulation in aged oocytes will be discussed later in the section regarding mechanisms of oocytes aging.

Mechanisms of oocyte aging

We describe the mechanisms of two categories of oocyte aging: oocyte aging caused by ovarian aging and postovulatory oocyte aging.

Ovarian aging

Ovarian aging impairs both the quantity and the quality of oocytes [3, 5, 8]. The decrease in oocyte quantity is a part of ovarian follicle loss. As mentioned above, although the mechanism of ovarian follicle loss remains unknown, it is widely accepted that the age-dependent decline of ovarian follicles is involved in apoptotic pathways [33, 36, 38]. Activation of apoptotic pathways results in ovarian follicle atresia. Although every primordial follicle has the potential to grow, mature, and ovulate, this is not the case in reality [84]. The mechanism of follicle recruitment from the primordial follicle pool is still unknown. A recent study using genetically targeted mice reveals that PTEN/PI3K signaling pathways within oocytes are important for follicle recruitment [85–88]. The balance between follicle recruitment and follicle atresia might be important for ovarian follicle loss. Premature ovarian insufficiency is defined as cessation of ovarian function before the age of 40 years and affects about 1% of women in the general population [89]. POI cases without an obvious cause provide a model for the study of genetic mechanisms of ovarian aging [17, 90]. Many candidate genes have been reported to be involved in POI (see review in [17, 90]). A recent genome-wide association study reveals new loci of single-nucleotide polymorphisms in natural menopause cohorts [91, 92].

However, the mechanism of age-dependent decline of oocyte quality remains unknown [25]. Age-dependent increase in aneuploidy is a key determinant of oocyte quality. Little is known of how aneuploidy originates by increase in maternal age [3, 93]. As mentioned above, ovarian aging affects expression of some key molecules involved in cell cycle control, such as SMC1β and MAD2 [45, 46, 94]. The impairment of cell cycle control during meiosis might be involved in the age-dependent increase in aneuploidy.

Microarray methods reveal that ovarian aging changes the expression patterns of accumulation of maternal RNAs required for oocyte-specific processes and metabolism in mouse and human oocytes [95–97]. Ovarian aging negatively affects the expression of oocyte genes involved in mitochondrial functions, oxidative stress, cell cycle regulation, and DNA and chromosome stability [95–97]. Because mitochondria are organelles producing ATP via oxidative phosphorylation, they are most important for maintenance of oocyte quality. Ovarian aging changes mitochondrial morphology and functions in oocytes. Aberrant mitochondrial arrangement has been observed in aged-mouse oocytes [39]. Ovarian aging results in abnormal mitochondrial ultrastructure with high density of the matrix, vacuolization, and swelling in aged-human oocytes [98]. Wilding et al. [99] reported that the mitochondrial membrane potential, which indicates mitochondrial function of oocytes from reproductive-aged women is decreased compared to that of oocytes from young women. Moreover, oocytes from reproductive-aged women present an accumulation of the mitochondrial DNA point mutations and higher levels of mitochondrial DNA deletions [100, 101]. These results suggest that age-dependent impairment of mitochondrial function might be a cause for a decline of oocyte quality. A question that arises from these results is what mechanism of impairment of mitochondrial functions is involved? One relevant idea about aging involves the accumulation of damage exerted by increased levels of ROS, a condition known as oxidative stress [15, 102]. In fact, the levels of GSH and GSH-transferase activity, which play an important role in cellular defense against ROS, decreased in oocytes from aged

mice [103]. Moreover, the genes involved in protecting against oxidative stresses, such as *Sod1* and the thioredoxin family (*Txn1* and *Apacd*) are downregulated in aged-mouse oocytes [95, 96]. However, there is no evidence that ovarian aging directly affects oxidative stress to the oocytes. Further studies are needed to determine whether oxidative stress is involved in the age-dependent decline of oocyte quality.

Recent evidence suggests that epigenetic mechanisms in oocytes may be altered by ovarian aging. In mouse oocytes, several transcripts encoding proteins involved in epigenetic modifications, such as chromatin remodeling and DNA methylation, are affected by aging, including the DNA methyltransferases (*Dnmt*)-*1*, *1o*, *3a*, *3L* and 3b, and DNMT-associated protein-1 (*Dmap1*) [95, 104].

Postovulatory oocyte aging

As described in the section regarding cellular and molecular changes in postovulatory aged oocytes, these morphological and biochemical changes are translated into detrimental early and late phases of embryo development, such as lower fertilization rate, polyspermy, dingy, chromosomal anomalies, abnormal embryo development, and post-implantation mortality [15]. Although the mechanism of poor embryo development by postovulatory aging of oocytes remains unknown, there are some clues to help researchers puzzle out the mechanism. Unfertilized aged oocytes undergo spontaneous cytoplasmic fragmentation [105]. Like unfertilized oocytes, embryos derived from aged oocytes exhibit fragmentation after fertilization [73]. The fact that these fragmented oocytes and embryos show TUNEL-positive staining [40, 73, 105] suggests the activation of apoptosis pathways during the period of postovulatory aging. Mammalian oocytes express several caspases and anti- and pro-apoptotic members of the BCL2 gene family [106, 107]. Pro-apoptotic molecules such as Bax induce the release of cytochrome c, which activates caspases, while anti-apoptotic molecules such as BCL2 prevent it [108]. In mouse and pig oocytes, the expression of BCL2 is decreased and the percentage of TUNEL-positive unfertilized oocytes is increased with in vitro-aging [78, 79]. In addition, we and Gordo et al. have reported that the expression of BCL2 protein is decreased whereas that of BAX protein is unchanged in oocytes aged in vitro [73, 109]. We also confirmed that the expression of BCL2 was decreased in in vivo-aged mouse oocytes (Takahashi and Kurachi, unpublished data). These results suggest that the postovulatory aged oocytes are prone to undergo apoptosis due to the decreased BCL2 expression.

In mammalian oocytes at fertilization, sperm induces drastic changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which consists of a single long-lasting rise in $[Ca^{2+}]_i$, followed by short repetitive changes in $[Ca^{2+}]_i$ lasting for several hours. These temporal changes in $[Ca^{2+}]_i$ are termed as "Ca^{2+} oscillations" [110]. The increase in $[Ca^{2+}]_i$ plays important roles in fertilization, cortical granule exocytosis, resumption of meiosis, pronucleus formation, and subsequent embryo development [111–114]. In addition, the patterns of Ca^{2+} oscillations, such as amplitude and frequency of Ca^{2+} oscillations, affect early and post-implantation embryo development [115]. We have demonstrated that in vivo-and in vitro-postovulatory aging alter the patterns of Ca^{2+} oscillations at fertilization in mouse oocytes [73, 81]. Frequency of Ca^{2+} oscillations at fertilization in aged oocytes is higher than that in freshly ovulated oocytes, while the amplitude of individual Ca^{2+} oscillations is lower in the former than in the latter [73, 81]. Jones and Whittingham [83] also reported that both the amplitude and rate of rise of individual Ca^{2+} oscillations at fertilization are decreased in in vivo-aged mouse oocytes. We and other groups have reported the Ca^{2+} release from the inositol 1,4,5-triphosphate ($InsP_3$)-sensitive Ca^{2+} stores is decreased in in vivo-aged oocytes compared to that in fresh oocytes [82, 83]. Furthermore, we have reported that both the Ca^{2+} reuptake by Ca^{2+}-ATPases and Ca^{2+} stores in the endoplasmic reticulum (ER) in in vivo-aged oocytes are decreased compared to those in fresh oocytes [81, 82]. We have reported that Ca^{2+} stores in the ER in in vitro-aged oocytes are also decreased [73]. Collectively, these results indicate the impaired Ca^{2+} homeostasis in postovulatory aged oocytes.

The abnormal intracellular Ca^{2+} handling in aged oocytes leads to the apoptosis of oocytes [14]. Gordo et al. [116] reported that injection of Ca^{2+} oscillators, such as sperm cytosolic factor and adenophostin A, a potent agonist of $InsP_3$ receptor, into in vitro-aged oocytes causes increase in the fragmentation and caspase activity of oocytes. They also reported that injection of Ca^{2+} oscillators into in vitro-aged oocytes induces abnormal Ca^{2+} oscillations with low amplitude and abrupt cessation [116]. Moreover, we and other groups reported that decrease in the ER Ca^{2+} stores of fresh oocytes by thapsigargin, which is a specific inhibitor of smooth endoplasmic reticulum Ca^{2+}-ATPases (SERCA), results in abnormal Ca^{2+} oscillations, with low amplitude and high frequency at fertilization compared to those observed in the vehicle-treated fresh oocytes [73, 83, 117]. Moreover, we have reported that thapsigargin treatment of fresh oocytes causes lower fertilization rate, lower blastocyst formation rate, and higher rate of fragmented embryo after in vitro fertilization compared to those in the vehicle-treated fresh oocyte [73]. These results suggest that abnormal Ca^{2+} handling in the postovulatory aged oocytes might be related to poor embryo development after fertilization.

What mechanisms are involved in the impairment of Ca^{2+} homeostasis in postovulatory aged oocytes? In mammals, the ER is the major intracellular Ca^{2+} storage site and the type I $InsP_3$ receptor of the ER membrane mediates Ca^{2+} oscillations in oocytes [118, 119]. As mentioned above, we have reported that the activity of SERCA is decreased in in vivo-aged mouse oocytes [81] and the Ca^{2+} store of the ER is decreased in in vivo- and in vitro-aged mouse oocytes [73, 82]. The Ca^{2+} stores are maintained within the ER by the replenishment of Ca^{2+} from the cytosol through the activity of the SERCA [120, 121]. The activity of SERCA is highly dependent on availability of intracellular ATP [122]. In fact, Chi et al. [74] reported that in vitro culture decreases ATP content in unfertilized mouse oocytes. In addition, we have reported that the ATP content of fertilized in vivo aged oocytes is significantly decreased compared to that in fertilized fresh oocytes [75]. Mitochondrial ATP production is prerequisite for Ca^{2+} oscillations at fertilization and Ca^{2+} homeostasis in oocytes [123, 124]. Thus, impairment of fertilization-triggered mitochondrial ATP production possibly links to impairment of Ca^{2+} homeostasis and abnormal patterns of Ca^{2+} oscillations at fertilization in aged oocytes. On the other hand, reduced amount of BCL2 may negatively affect the function of SERCA. Overexpression of BCL2 maintains Ca^{2+} stores of the ER and prevents thapsigargin-induced apoptosis in lymphoma cells [125]. In addition, BCL2 prevents Ca^{2+} store of the ER by upregulating SERCA [126]. In fact, as mentioned above, the expression of BCL2 is decreased in aged oocytes [73, 109]. Taken together, these results suggest that reduction in both ATP production and BCL2 expression might be involved in the impairment of Ca^{2+} homeostasis in aged oocytes.

A second question that arises from these results is what mechanisms are responsible for impairment of mitochondrial function and reduced expression of BCL2 in aged oocytes? Mitochondria are organelles that produce ATP by oxidative phosphorylation to supply energy for various cell functions. Mitochondrial dysfunction has been linked with pathological conditions, including various reproductive

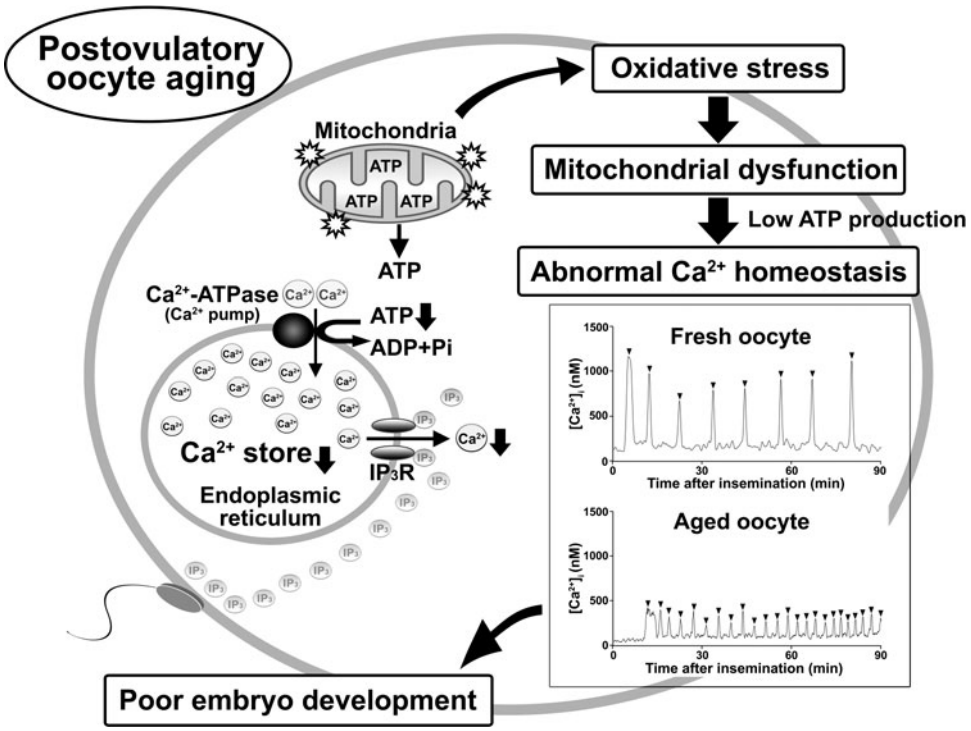

Fig. 1 Scheme of the mechanism of poor embryo development in postovulatory oocyte aging. We show the model of the mechanism of poor embryo development in postovulatory-aged oocytes. Postovulatory aging of oocytes results in increase in mitochondrial oxidative stress. Oxidative stress-induced mitochondrial dysfunction results in low ATP production followed by impairment of intracellular Ca^{2+} regulation, such as decrease in the Ca^{2+} stores of the endoplasmic reticulum (ER) and the Ca^{2+} release from the ER via inositol 1,4, 5-triphosphate ($InsP_3$) receptor. When postovulatory aged-oocytes, which are impaired in the intracellular Ca^{2+} regulations, are fertilized with sperm, the abnormal Ca^{2+} oscillations occur at fertilization. Representative data show that sperm triggers Ca^{2+} oscillations in the fresh (14 h after hCG treatment) and the aged (20 h after hCG treatment) mouse oocytes. The patterns of Ca^{2+} oscillations at fertilization are changed by postovulatory oocyte aging: In the aged oocytes, the abnormal Ca^{2+} oscillations with lower amplitude and high frequency are shown. The abnormal Ca^{2+} oscillations may result in poor embryo development in postovulatory-aged oocytes. *Arrowheads* indicate the individual Ca^{2+} oscillations. IP_3, $InsP_3$. IP_3R, $InsP_3$ receptor

failures [127, 128]. ROS, such as superoxide anion radical, hydrogen peroxide (H_2O_2), and hydroxyl radical, are produced endogenously by proton electrochemical gradient during mitochondrial respiration. Because the mitochondria are a major source of ROS, mitochondria need continuous protection from free radical attack by ROS scavenger systems [129]. Tarin et al. [15] proposed a mechanism based on the "the oxygen radical mitochondrial injury hypothesis of aging" to explain the effects of postovulatory aging on impairment of early and embryo and fetal development. This mechanism is based on the idea that ROS harm mitochondrial DNA, proteins, and lipids [15]. In fact, we have reported that the magnitude of lipid membrane peroxidation in in vivo-aged oocytes is increased compared to that in fresh oocytes [72]. And we have also reported that the levels of ROS in in vitro-aged oocytes compare to those in fresh oocytes [73]. Boerjan and de Boer [71] reported that the amount of GSH, which is a ROS scavenger, is decreased in in vivo-aged mouse oocytes. These results suggest that aged oocytes are prone to oxidative stresses by decrease in ROS scavengers. Furthermore, we have reported that exposure of fresh oocytes to 100 μM H_2O_2 results in abnormal patterns of Ca^{2+} oscillations with low amplitude and high frequency, which are similar to those in postovulatory aged oocytes [72]. We have also reported that the H_2O_2-pretreated fresh oocytes results in poor embryo development after fertilization [73]. In somatic cells, ROS are important mediators of intracellular signaling for numerous cell functions, including Ca^{2+} homeostasis through modulating SERCA and $InsP_3$ receptor functions [130, 131]. The increase in ROS production in aged oocytes might directly affect Ca^{2+} homeostasis and/or impair mitochondrial function followed by ATP depletion. On the other hand, the mechanism for the decrease in BCL2 expression in aged oocytes remains unknown. Although transcriptional control of BCL2 has been reported, increasing evidence suggests that an important component of BCL2 regulation is post-transcriptional, such as micro RNAs (miR15A and miR16-1) [132]. There are very few reports about the mechanism of BCL2 regulation in oocytes. We have reported that treatments of H_2O_2 and thapsigargin to fresh oocytes result in decreased expression of BCL2 [73]. As mentioned above, BCL2 expression is closely related to the Ca^{2+} store of the ER [125, 126]. Alternatively, ROS control the expression of BCL2 by regulating its phosphorylation and ubiquitination in cancer cells [133]. In addition, ROS downregulate the expression of BCL2 in T cells [134]. These results suggest that the increase in ROS levels in postovulatory aged oocytes might result in both mitochondrial dysfunction and reduced expression of BCL2. We show a model of mechanism of poor embryo development in post-ovulatory aging of oocytes (Fig. 1).

Conclusion

We here review that there are two categories of oocyte aging: oocyte aging caused by ovarian aging and postovulatory oocyte aging. Both categories of oocyte aging have similar phenotypes of reproductive failure. However, the mechanisms for the impairment in oocyte quality are not necessarily equivalent. The mechanism of oocyte aging caused by ovarian aging might be a chronic process of damage to the oocytes and/or ovarian follicle cells, such as thecal and granulosa cells. There are several problems with studying the oocyte aging caused by ovarian aging; it takes over 1 year for experimental animals to grow older and the numbers of oocytes available are very small. Moreover, there is no animal model anaologous to the ovarian aging study. In contrast to the limitations of the data in the ovarian aging study, a model to study the postovulatory aging of oocytes is more easily accessible and more data are available. ROS-induced mitochondrial injury pathways followed by abnormal intracellular Ca^{2+} regulation of the ER may be involved in the mechanism of postovulatory oocyte aging. According to this scenario, the antioxidant treatment in vivo and in vitro might prevent the oocyte damage by postovulatory aging. We suggest that future research into the mechanism of oocyte aging will be necessary in order to develop a method to rescue the poor developmental potential of aged oocytes.

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