Review

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Ovarian aging: mechanisms and intervention strategies

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Abstract: Ovarian reserve is essential for fertility and influences healthy aging in women. Advanced maternal age correlates with the progressive loss of both the quantity and quality of oocytes. The molecular mechanisms and various contributing factors underlying ovarian aging have been uncovered. In this review, we highlight some of critical factors that impact oocyte quantity and quality during aging. Germ cell and follicle reserve at birth determines reproductive lifespan and timing the menopause in female mammals. Accelerated diminishing ovarian reserve leads to premature ovarian aging or insufficiency. Poor oocyte quality with increasing age could result from chromosomal cohesion deterioration and misaligned chromosomes, telomere shortening, DNA damage and associated genetic mutations, oxidative stress, mitochondrial dysfunction and epigenetic alteration. We also discuss the intervention strategies to delay ovarian aging. Both the efficacy of senotherapies by antioxidants against reproductive aging and mitochondrial therapy are discussed. Functional oocytes and ovarioids could be rejuvenated from pluripotent stem cells or somatic cells. We propose directions for future interventions. As couples increasingly begin delaying parenthood in life worldwide, understanding the molecular mechanisms during female reproductive aging and potential intervention strategies could benefit women in making earlier choices about their reproductive health.

Keywords: mitochondrial therapy; oocyte quality; oocyte quantity; ovarian aging; ovarioids; senotherapy.

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Introduction

Life expectancy of human beings has greatly increased from 45 to 85 years over the past 150 years [1], but the age at natural menopause (ANM) has remained around the age of 50 years [2]. According to cohort studies, the decline in female fertility begins around the late 20s to early 30s and accelerates after the age of 35 years, especially among nulliparous women [3–6], thus, fecundity decreases as age increases. Ovarian aging can be part of normal biological aging, where genetic as well as environmental factors influence its onset and determine the age of menopause [7–10]. The genetic integrity of oocytes decreased with advancing age [11] and almost half of women are infertile by the age of 40 years (Figure 1) [6, 12, 13]. More women are choosing to delay childbearing to more advanced age worldwide, resulting in more frequent use of assisted reproductive technology (ART) [14, 15]. Also, there is only an approximately 6.5% chance of achieving pregnancy with each mature oocyte thawed from the preservation of oocytes or ovarian tissue, which decreases with age [16, 17]. Therefore, prevention of age-associated infertility becomes extremely important to the woman as well as to the family and society.

In addition to reproductive functions, the ovarian also plays an important role in maintaining female hormone secretion. Fertility loss during female aging is associated with increasing basal follicle stimulating hormone (FSH) and decreasing anti-Mullerian hormone (AMH) concentrations, together with compromised oocyte quality, presumably due to increased oxidative stress (OS) and DNA damage, as well as reduced metabolic and meiotic competences [22, 23]. Excessive serum FSH also acts

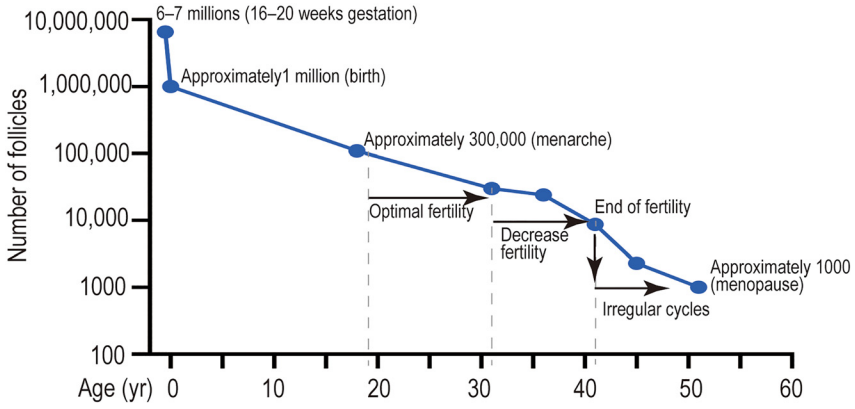

Figure 1: The dynamics of human follicular development and reserve during fetal development and in adult. Oocyte and follicle reserve decline in the ovary during aging. Follicular quantity reaches maximum at 16/20 weeks of fetal development [18, 19]. Approximately one million viable follicles remain at birth, the mechanism of primordial follicles loss remains to be fully determined [20]. At the time of menarche, women have 300,000 to 400,000 follicles, which are recruited cyclically until menopause (approximately 1000 follicles) [21]. yr, year.

directly on hippocampal and cortical neurons to accelerate amyloid-β and tau deposition and impair cognition in female mice displaying features of Alzheimer's disease [24]. Estrogen is produced in the ovaries by the follicles that house the oocytes. Physiological functional endocrine endows women their feminine characteristics and reproductive abilities, and is also key for maintaining healthy lifespan including heart health, bone density and mood. With a depleting ovarian reserve, both physiological fluctuation of estrogen and progesterone production will decline unpredictably and, eventually, halt [25]. Ovarian aging and disrupted endocrine function increases risk of reproductive aging-associated chronic diseases such as heart disease, incontinence, and osteoporosis.

Multiple factors, including non-germ-line stem cells in adulthood female mammals, chromosomal, genetic, mitochondrial, reactive oxygen species (ROS) and environmental factors, impact the quantity and quality of oocytes in the ovarian reserve (Figure 2). Elucidating the molecular mechanisms underlying the decline in ovarian reserve and oocyte function would facilitate achieving targeted intervention in postponing ovarian aging and improving oocyte quality. Although achievements have been gained in diagnostic and intervention field, once the ovarian reserve has declined markedly, few treatments other than ART are effective in treating infertility. Understanding of gonadal development and ovarian physiology may allow reconstituting the ovarioids to provide an alternative source of gametes for reproduction and rebuilding physiological endocrine function. Thus, there is requirement for developing better diagnostic tools and improving treatments in the fields of reproductive medicine. We here broadly review molecular mechanisms of ovarian aging, recent developed intervention strategies in the reproductive field, and prospective directions.

Main mechanisms of ovarian aging

Oocyte quantity and ovarian aging

Oocyte quantity

Women are born with a limited number of oocytes. During the fifth week of human pregnancy, female fetal ovaries contain 500 to 1300 primordial germ cells (PGCs) that undergo mitosis to generate approximately 6–7 million germ cells at the 20th gestation week [18]. Following the completion of mitosis, female germ cells undergo prophase of meiosis I, beginning at the time of E12.5 to E13.5 in mice and approximately E10 weeks to E11 weeks in human [19], exhibiting typical stages including leptotene, zygotene, pachytene and diplotene stage when the meiocytes arrest. This phase may last for months for mouse oocytes or years for human oocytes. The meiotic prophase resumes the diakinesis phase when the puberty is reached. The meiotic germ cells form cysts and generate primordial follicles that each oocyte is surrounded by a single layer of granulosa cells. Only 1–2 million viable follicles remain at birth, the mechanism of primordial follicles loss remains to be fully determined [20]. The number of primordial follicles is down to approximately 400,000 at puberty, of which about 350 oocytes ultimately are ovulated during reproductive life [21]. Follicle depletion is associated with reduced production of the hormones estrogen and inhibin by the ovary, disrupting the hypothalamic-pituitary-gonadal hormonal axis and eventually leading to menopause [26]. When reaching ANM at the mean age of about 51 years old, fewer than 1000 primordial follicles are left, which no longer sustain ovulation [27]. Mathematical models show that ovarian reserve depletion during the fertile years appears to accelerate with age, mainly because of

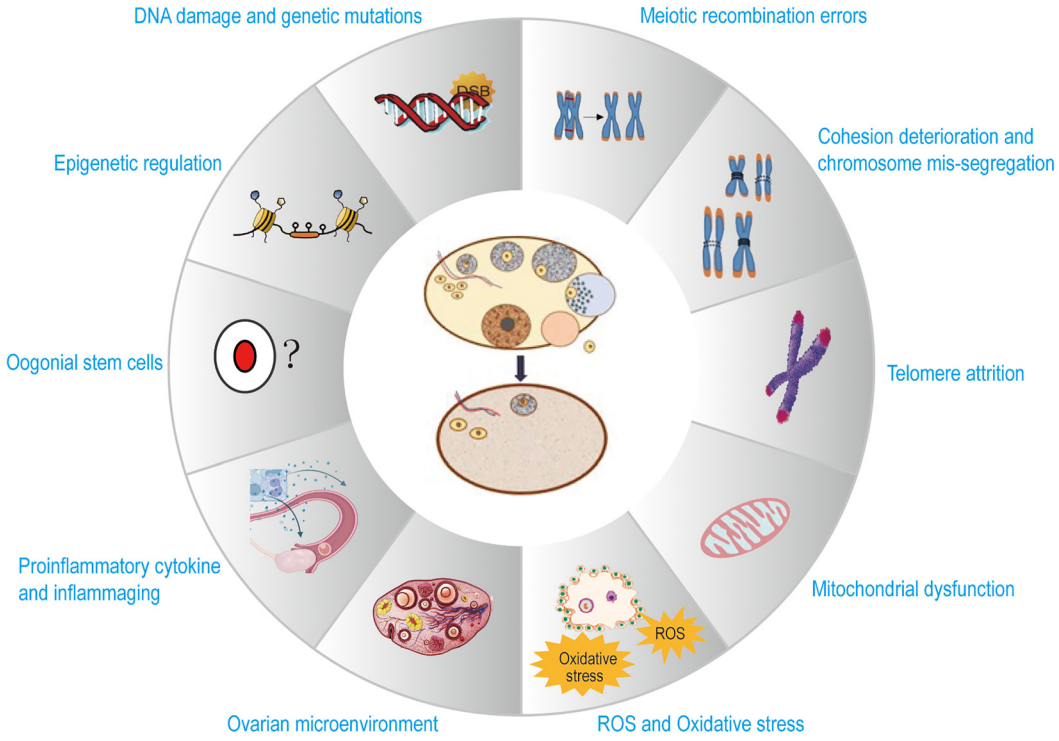

Figure 2: Current understanding of molecular mechanisms underlying ovarian aging. A multitude of physiological factors and molecular changes that can diminish the quantity and quality of ovarian reserve lead to ovarian aging. Most factors are explained further under subheadings in the text. DSBs, double-strand breaks; ROS, reactive oxygen species.

increased oocyte atresia [18]. Therefore, ANM is determined by the non-renewable functional ovarian reserve, which is established during fetal development and continuously depleted until ovarian senescence. ANM can vary significantly between individuals depending on genetic and environmental factors. Of the non-genetic risk factors, cigarette smoking is the main factor proven to accelerate the age of menopause by about three years [28], limiting reproductive potential. Primary ovarian insufficiency (POI), is determined by exhaustion of functional ovarian reserve in the individuals suffered certain genetic disorders, autoimmune diseases or after chemo- or radiotherapy for cancer treatment, leading to infertility before the age of 40 years [29]. Moreover, maternal age reduces pregnancy outcomes [30]. Therefore, follicle depletion serves as a timer for the major landmarks of female reproduction and ovarian aging.

Dispute over germ-line stem cells in female mammals

Stem cells are featured with the capacity of self-renewal as well as lineage differentiation. Germ-line stem cells share common features of self-renewal and differentiation specifically into meiotic cells or meiocytes that undergo homologous pairing and recombination. Germ-line stem cells are present and functional in many non-mammalian organisms and male mammals. Traditionally, they have been thought not to exist in female mammals. However, this traditional view had been challenged according to the expression of germ-cell markers by extra-follicular cells, arguing that female mammals possess oogonial stem cells (OSCs) that replenish their supply of oocytes in a physiological context. It was reported that ovarian surface epithelium of adult mice contained OSCs that sustained postnatal follicular renewal [31]. Moreover, using anti-Ddx4 antibody coupled to fluorescence activated cell sorting, up to 1000 Ddx4-positive cells were isolated per young adult mouse ovary [32]. The Vasa/Mvh/Ddx4 positive germ cells existed in the postnatal and adult ovaries were claimed as germline stem cells [31, 33]. It remains to be tested whether these Vasa/Mvh/Ddx4 positive germ cells isolated from adult ovaries can undergo self-renewal as well as are capable of differentiation into meiotic cells featured with homologous pairing shown by synaptonemal complex protein 1/synaptonemal complex protein 3 (SCP1/SCP3) filaments and recombination (e.g. presence of specific MutL homolog 1 [MLH1] foci). Interestingly, by labeling the Oct4-expressing small germ cells and tracing their

fates for up to 4 months in mouse ovary, Guo et al. observed that $Oct4^{+}Dazl^{-}$, but not $Oct4^{+}Dazl^{+}$ or $Oct4^{+}Ddx4^{+}$ cells, contain a population of germ stem cells in extra-follicular ovarian cells [34]. It remains to be investigated whether the marked cells can undergo meiotic homologous pairing and recombination and differentiate into oocytes. Thus far, existence of typical meiotic cells at prophase I stage that exhibit homologous pairing and recombination in the adult ovaries under physiological conditions has not been rigorously demonstrated.

Additionally, it was reported that bone marrow transplantation restored oocyte production in wild-type mice sterilized by chemotherapy, as well as in ataxia telangiectasia-mutated gene-deficient mice, which were otherwise incapable of making oocytes, and the bone marrow was considered to be a potential source of germ cells that could sustain oocyte production in adulthood [35]. However, Eggan et al. [36] established transplantation and parabiotic mouse models to assess the capacity of circulating bone marrow cells to generate ovulated oocytes, both in the steady state and after induced damage and showed that ovulated oocytes in adult mice derive from non-circulating germ cells [36].

The straightforward way to detect whether OSCs exist *in vivo* in the adult is to identify the extra-follicular ovarian cells that express the germ-line stem cell markers, which are expressed prior to differentiation into meiocytes in fetal ovaries. Interferon-induced transmembrane protein 3 (IFITM3) is expressed in the PGCs [37]. Using the anti-IFITM3 antibody, we isolated cells with germ-line characteristics from E12.5 embryos, which contain mitotically active germ cells, but not from adult mice ovaries [38]. Moreover, Ddx4, Dppa3 and POU class 5 homeobox 1 (POU5F1) were detected by immunoblotting in ovarian cells isolated using anti-IFITM3 from E12.5 female mouse embryos, which contained mitotically active germ cells. However, only Dppa3 was detected in the IFITM3-positive cells that were isolated from the adult ovaries [38]. These results are consistent with the traditional view that no OSCs remain in the adult ovary.

The germ-line cells also can be marked in the unperturbed ovary and traced their fate. Lei and Spradling generated *CAG-CreER/Esr1TM;ROSA-EGFP* mouse model [39], and Zhang et al. generated *Sohlh1-CreER^{T2};R26R* mouse model [40], the brief tamoxifen-based treatment strategy was taken to mark a subset of follicles. The marked primordial follicles were subsequently lost at the same rate as the unmarked follicles. Instead of monitoring the loss of marked follicles, Zhang et al. generated tamoxifen-induced *Forkhead box L2 (Foxl2)-CreER^{T2};mT/mG* mouse model to search for evidence that new follicles are occurred [40]. Foxl2 is expressed in granulosa cells but not in the pre-granulosa cells of the ovarian epithelium [41]. The authors triggered a switch from tdTomato to green fluorescent protein (GFP) expression in cells expressing Foxl2 during the brief tamoxifen treatment. Thus, if new follicles were assembled, their granulosa cells would be red. Yet, all ovaries at 2 weeks and at 2, 6, and 12 months contain only follicles with green fluorescent somatic cells. This implies that adult mice do not generate new follicles. Furthermore, Zhang et al. discovered that no adult oogenesis occurred after ablation of all oocytes in *Gdf9-Cre;iDTR* ovaries [40].

Another key criterion for identifying the presence of a newborn egg or neo-oogenesis is whether the germ cell undergo normal meiosis, where homologous chromosome pairing and recombination occur [42]. During meiosis, homologous chromosome pairing and recombination take place in the prophase of meiosis I, and the appearance of synaptonemal complexes provides the structural basis for the recombination between chromosomes. SCP3 is the key to the formation of synaptonemal complex, and the joint detection of the distribution of proteins such as SCP1, SCP3 and MLH1 can be used as an important marker to measure the normality of homologous chromosome synapsis and homologous recombination. In primate monkeys, the number of eggs decreases markedly with age. In monkey ovaries of different ages (3–18 years old), no meiotic cells were found to undergo SCP1/SCP3 homologous chromosome pairing and homologous recombination (MLH1 junctions), which indicated that no new eggs occurred in adult ovaries [43]. Also, neo-oogenesis and meiocytes were not found in adult human ovaries [44]. Thus far, no studies have reported neo-oogenesis and meiocytes in mice.

A more complete map of cell types in the human ovarian cortex was characterized by single-cell RNA-seq recently [45]. The important finding in the study was to determine that DDX4-positive cells, isolated by an DDX4 antibody used in previous study [32, 46], from ovarian cortex are perivascular cells but not oogonial stem cells. This result was verified using a different analysis, including immunostaining validation, which showed DDX4 antibody localization at perivascular sites in human ovarian tissue. This result provides strong evidence opposing the controversial studies arguing for the existence of oogonial stem cells in mammalian adult ovaries [47]. By careful comparison of the published results and analysis of the data, Hainaut and Clarke conclude that the weight of evidence strongly supports the traditional interpretation that germ-line stem cells do not exist postnatally in female mammals [48]. This concept is consistent with the fact that the continuously depleted non-renewable ovarian reserve with age is the key limiting factor in ovarian aging.

Oocyte quality and ovarian aging

Oocyte quality

In addition to the diminishing follicle reserve, oocyte quality also is declined with increasing maternal age. At birth, the finite ovarian reserve comprises non-growing primordial follicles arrested in the dictyate stage and do not complete their first meiotic division before puberty is reached. Some oocytes and their surrounding cells remain arrest stage for 40 years or more. ROS causes OS and damage to cells, which, over time, contributes to structural and functional damages that are distinctive for cellular senescence and aging [49]. An increase in ROS levels in ovaries has been suggested to play a key role in the pathogenesis of age-related infertility in female mammals [50, 51]. The gradual deterioration in oocyte quality begins at least after the age of 31 years. This coincides with a decrease in oocyte quantity and partly explains the decline in fertility long before the onset of menopause, along with the abnormal endocrine profile found in reproductively older women [52–54]. On cellular levels, the oocyte aging phenomenon are mainly manifested by cohesin dysfunction [55], crossover maturation inefficiency (CMI) [56], chromosome misalignments and meiotic spindle disruption [57], telomere attrition [58], DNA damage [59] and mitochondrial dysfunction [60]. CMI is a major contributing factor to increase the incidence of both chromosome mis-segregation, especially with increased age. The loss of oocyte quality is believed to be due to an increase in meiotic nondisjunction, resulting in aneuploidies and unsuccessful pregnancy outcomes at higher female ages [61, 62].

Main molecular mechanisms for the deterioration of oocyte quality

(1) Chromosomal cohesion deterioration

Aneuploidy in eggs is a leading cause of infertility, miscarriage and congenital syndromes. Aneuploidy and maternal aging follow a U-shaped relationship [11]. More than 20% of eggs are aneuploidy from young women 20–32 years of age, however, women in both very young (< 20 years) and advanced age group (> 35 years) can be greatly more at risk of generating aneuploid eggs than women 20–32 years of age. Notably, aneuploidy occurs in more than 50% of eggs from women aged 35 years and above [11, 63]. Data from mice suggest that cohesion levels on chromosomes could determine the U-shaped curve [64–68]. Sister chromatids are bound together by many ring-like protein structures called cohesion complexes that are installed during DNA replication [66]. Cohesion complexes are essential for the correct segregation of chromosomes during the divisions of meiosis in each ovulation cycle of adult female mammals. Importantly, evidence from mouse oocytes indicated that chromosomes might initially be overloaded with cohesin to ensure that adequate cohesin levels are maintained for the entire reproductive life span. This overloading may cause higher rates of chromosome mis-segregation during the first cohorts of oocytes to be ovulated in young women [11]. With increasing female age, chromosomes cohesion in oocytes naturally deteriorates, leading to premature chromosome separation [69–71]. Therefore, in the 20s and early 30s, cohesin levels might be at an ideal intermediate level, which is not too high to prevent chromosomes from segregating at anaphase but also not too low to maintain the integrity of the chromosomes. In contrast, cohesin in old oocytes are susceptible to removal by separase, a cysteine protease that cleaves the cohesin subunit REC8 and then destroy the cohesin ring. Importantly, separase is restricted from cleaving most cohesin that holds sister chromatids together as these links need to be maintained for sister chromatid alignment and separation during the second meiotic division. This function is provided by Shugoshin-like 2 (SGO2) in complex with PP2A that locally dephosphorylate REC8 [72, 73]. Notably, SGO2 expression decreases in oocytes with advancing age [74, 75]. Depletion of SGO2 in mouse oocytes causes loss of centromeric cohesion during anaphase I [75]. Decreased cohesion results in a greater probability of chromosome mis-segregation, premature chromatid separation, and aneuploid [69, 70, 76].

(2) Telomere shortening

Telomeres are composed of repetitive nucleotide sequences that form a “cap structure” which functions to maintain the integrity of chromosomes. Telomere length is known to directly correlated with both life expectancy and reproductive life span [77–79]. Comparison of telomere lengths in the leukocytes of women revealed that the average telomere length in the extended fertility group is significantly longer than in the normal fertility group [80, 81]. Telomeres are shorter in the leukocytes of postmenopausal women than those of a similar age but still menstruating [82]. Moreover, women with longer telomeres entered menopause up to 3 years later than in those with shorter telomeres [82]. These results suggest that menopause may occur after telomere shortening reaches a specific length. Moreover, patients

with polycystic ovary syndrome with low telomerase activity and short telomeres in granulosa cells, which support oocyte maturation, show an earlier onset of infertility [83].

A significant cause of telomere attrition in oocytes is the accumulation of ROS [84]. Telomere length is pivotal for accurate chromosomal alignment as well as adequate function of the spindle assembly checkpoint during meiosis, both of which prevent aneuploidy in embryos [79]. Shorter telomere is associated with higher rates of aneuploidy among infertile women undergoing *in vitro* fertilization [58, 85, 86]. Short telomeres impede germ cell specification by upregulating mitogen-activated protein kinase (MAPK) and transforming growth factor beta (TGF-β) signaling [87]. Therefore, oocytes and early embryos with telomere attrition may be an indicator of improper chromosome segregation, leading to aneuploidy, moreover, telomere shortening has been suggested to be involved in embryonic fragmentation and developmental arrest [79, 88].

Loss of telomerase activity also leads to telomere shortening in ovaries. Telomerase activity presents in adult granulosa cells, but almost unmeasurable in mature oocytes [89, 90]. In mouse models, telomerase components (TERT and Terc) and telomere-associated proteins (TRF1, TRF2, and POT1a) have been shown to gradually decrease in the ovary from young to aged group as well as in the follicles from primordial to antral stages and their oocytes and granulosa cells, contributing to telomere shortening [90]. In women at 37 years old or less, a lack of telomerase activity or aberrant telomere homeostasis in human granulosa is associated with occult ovarian insufficiency [91]. Shortened telomere length and diminished telomerase activity were shown to be associated with biochemical POI [81, 92, 93]. Additionally, a woman with dyskeratosis congenita, a telomeropathy associated with a reduced ovarian reserve, responded poorly to hormonal treatment before *in vitro* fertilization and her oocytes contained critically short telomeres [94]. Therefore, telomere length and telomerase activity have a great impact on ovarian aging, and a mathematic model of telomere shortening in leukocytes could potentially be used to evaluate the reproductive aging status or ovarian aging.

(3) DNA damage response associated genetic mutations

The ANM varies between individuals. Genetic factors play an important role in ANM. Ovarian oocyte reserve is required to generate eggs and to response to gonadotropic hormones. POI is determined by exhaustion of follicles in the ovaries, which leads to infertility prior to the age of 40s. It is characterized by a strong familial and heterogeneous. Over the last years, several cohort studies have elucidated a likely oligogenic inheritance of POI [95–99], 107 genes related to POI etiology were identified. Of the 107 genes, the most genes linked to syndromic POI are mainly implicated in meiosis/DNA repair and metabolism function. Additionally, the majority of genes associated with non-syndromic POI are also mainly implicated in meiosis/DNA repair pathways [59, 100]. A recent genome-wide association study of approximately 200,000 women of European ancestry identified 290 genetic determinants of ovarian aging [10], highlighting a much broader involvement of DNA damage response (DDR) and of metabolic signaling networks such as the phosphatidylinositol 3-kinase pathway in the regulation of ovarian aging. Intact DNA repair mechanisms are essential to maintain metabolic balance and delay aging phenotypes [101, 102]. Cellular mechanisms that repair DNA damage become less effective during aging. In oocytes, deceased efficacy of DNA repair mechanisms could result in poor quality, apoptosis, and ultimately menopause, infertility and miscarriage [10, 103]. Activation of the DDR during meiosis can result in the elimination of oocytes with unrepaired meiotic DNA double-strand breaks (DSBs) above a threshold level via the TRP53 and TAp63 pathways in mice [104, 105]. DNA damage or the failure to mount DNA damage response promotes cell death, resulting in depleted oocyte reserve that induces POF.

Breast cancer-associated 1 (BRCA1) and BRCA2, with functions in homologous recombination repair, are associated with depleted oocyte reserve [106]. Both BRCA1 and BRCA2 are involved in the repair of ataxia telangiectasia mutated (ATM) and RAD3-related (ATR) protein kinase-mediated DNA DSBs in oocyte [107, 108]. The expression of ATM in ovaries and oocytes is dramatically reduced after mid-thirties, which is in line with the lower fecundity at the women from aged 35 years and above. BRCA1 expression declines around a decade earlier [107]. In *Brca1*-deficient mice, reproductive capacity is impaired, primordial follicle counts are lower, and DSBs are increased in remaining follicles with age relative to wild-type mice [107]. AMH is one of the best predictors known to evaluate ovarian reserve [109]. Women with *BRCA* mutations, particularly of the *BRCA1* gene, have lower serum AMH levels, consistent with diminished ovarian reserve [107, 110–115]. Also, women with *BRCA* mutations appear to experience menopause at earlier age [116–118]. Furthermore, women carrying loss-of-function variants in *BRCA1* and *BRCA2*

reported ANM 2.63 years earlier and 1.54 years earlier [10]. In general, women with *BRCA* mutations have lower ovarian reserve and experience earlier menopause.

Another component essential to the repair of DSBs is the cell cycle checkpoint kinase 2 (CHK2) [10, 119]. Women carrying loss-of-function variants in *CHK2* manifested ANM 3.49 years later [10]. CHK2 is a downstream effector of the ATM kinase that responds primarily to DSBs, and can also be activated by the ATR kinase that responds primarily to single stranded DNA [86, 120]. CHK2 has important functions in checkpoint signaling during the progression of the cell cycle in mitosis and meiosis [10, 104, 105, 119, 121]. CHK2 is essential for culling mouse oocytes bearing unrepaired meiotic or induced DNA DSBs. Both meiotically programmed and induced DSBs detected by CHK2 trigger the CHK2-dependent activation of TRP53 and TRP63 pathway to eliminate the defective oocytes [119].

(4) ROS and OS

ROS are the by-products of mitochondrial oxidative phosphorylation and include superoxide, hydroxyl, and hydrogen peroxide. ROS react with the surrounding DNA, proteins and lipids, leading to mutations and macromolecular damage. Under physiological conditions, the production and neutralization of ROS are balanced. Early oocytes have an apparent lack of ROS and balance essential mitochondrial activity by suppressing complex I [122].

Excessive ROS generation overwhelms the cellular anti-oxidant defenses, resulting in OS, mitochondrial and nuclear DNA damage, and premature aging of ovaries [123]. In mice, long-term OS caused by ROS has been associated with decreased follicle and oocyte quality without affecting oocyte quantity [124]. Ovaries of ozone-exposed mice show high levels of ROS as well as the decreased fertility [124]. Mice fed with the antioxidant N-acetyl-L-cysteine (NAC) have better quality oocytes and longer reproductive lifespan [125]. Moreover, NAC treatment also maintains oocyte telomere length and telomerase activity [125].

Humans and non-human primates have similar age-related ovarian alterations. Wang et al. [126] used single-cell RNA-seq analysis and found the down-regulation of antioxidant genes, including *GPX1*, *GSR*, *GPX4* and *PON1*, in aged early-stage monkey oocytes, and increased apoptosis plus decreased reductase activity-related genes, including *IDH1*, *PRDX4* and *NDUFB10*, in aged monkey granulosa cells, suggesting oxidative damage as an essential factor in ovarian aging. Furthermore, human granulosa cells isolated from the follicular fluid also exhibited aging-associated down-regulation of *IDH1*, *PRDX4* and *NDUFB10* [126], indicating that these genes can be biomarkers and targets for diagnosis and treatment of human age-related ovarian diseases, such as POI, and decreased ovarian reserve. In a study of physiologic aging in C57BL/6 wild type mice, ovarian *peroxiredoxin 3* and *thioredoxin 2* mRNA expression decreased significantly with age [127]. In human, high levels of OS within the ovary, follicular fluid, granulosa, and cumulus cells correlate with follicular atresia and poor oocyte quality, as well as decreased oocyte fertilization, embryo development and fertility [128]. Collectively, oxidative damage caused by ROS impacts reproductive potential by decreasing the quality of ovarian follicles and oocytes [129].

Increasing evidences supports the notion that lifestyle modification to minimize OS preconception can improve female fertility. Caloric restriction (CR) is known to delay the aging caused functional decline in tissue throughout the body [130–132]. CR has also been found to extend reproductive lifespan in female mice [133, 134]. Obesity is being considered as a state of OS and is known to reduce the live birth [135, 136]. Maternal obesity induces the defective telomeres in oocytes and embryos [137]. Of note, Children of mothers who had the metabolic syndrome (or obesity) in pregnancy had significant shorter telomeres than of normal [138, 139]. Additionally, several environmental factors such as cigarette smoke [140, 141], alcohol [142], bisphenol A [143, 144], exposure to chemicals and radiation have been linked OS with ovarian aging. However, a healthy balanced diet and regular exercise can reduce excess OS and correlates positively with clinical pregnancy rate in *in vitro* fertilization (IVF) [145–148].

(5) Mitochondrial dysfunction

Mitochondria in mammalian oocytes are spherical with a few cristae surrounding the electron-dense matrix [149, 150]. Mitochondria function requires coordination of both mitochondrial and nuclear genomes. Normally, mitochondria in the oocyte are generated during oogenesis, and then production ceases at the stage of the mature oocyte [151]. Mitochondria play a central role in follicular atresia and could be the main target of the ooplasmic factors determining oocyte quality adversely affected by aging. The aging effects on oocyte mitochondria have been seen through morphological and functional abnormalities. Mitochondrial swelling and vacuolization are associated with reduced adenosine-triphosphate (ATP) and mitochondrial DNA (mtDNA) content in aged mouse and hamster oocytes [152, 153]. Mitochondria are highly dynamic organelles responding to cellular stress through changes in

overall mass, interconnectedness, and sub-cellular localization [154, 155]. The multiple mechanisms through which mitochondria drive ovarian aging include mitochondrial dysfunction, impaired mitochondrial dynamics (biogenesis, fusion and fission, mitophagy, apoptosis, mitocytosis [156], and spatial dynamics), accumulation of mtDNA mutations, altered membrane potential, and defective electron transport chain function. mtDNA mutations exacerbate female reproductive aging by impairing nicotinamide adenine dinucleotide ($NADH/NAD^+$) redox [157], and increasing ovarian NAD^+ levels improve mitochondrial functions and reverse ovarian aging [158]. Mitochondrial biogenesis is required for cell growth to produce increased metabolites and energy and defects in biogenesis are frequently lethal to cells and organisms. Mitochondrial biogenesis might be initiated by the small, round-shaped vesicles in the nuclei, followed by their translocation into the cytoplasm and protein import to achieve their complete construction and biological functions [159–161]. Mitochondrial biogenesis depends upon the activity of a hierarchy of nuclear factors, including peroxisome proliferator activator receptor alpha (PPARα), PPARγ, nuclear respiratory factor-1 and -2, estrogen related receptor. All of these transcription factors are critical dependent for their activity on PPARγ coactivator 1 alpha (PGC-1α). Nutrient supply and energy balance in the cell modulates PGC-1α activity at both the transcriptional and post-translational level [162]. Deceased mitochondrial biogenesis, as indicated by lower mtDNA content, is routinely observed during POI, diminished ovarian reserve (DOR), and physiological ovarian aging. Compared with those of normal ovarian reserve, PGC-1α expression is lower in cumulus cells and accompanied by decreased mtDNA content in cumulus cells and oocytes in women with DOR [163].

Re-modeling of the mitochondrial network in cells is mechanically regulated by key dynamin-related fusion and fission gene products. Mitochondrial fusion is promoted by homotypic/heterotypic interaction of the Mitofusin 1 (Mfn1) and Mitofusion 2 (Mfn2) at the outer mitochondrial membrane (OMM) of adjacent mitochondria and by Opa-1 at the inner mitochondria membrane [154, 164]. Mitochondrial fission is promoted by the GTPase activity of the dynamin-related protein (DRP1) that is recruited to the OMM where it forms ring-like oligomers that pinch off mitochondria into small fragmented mitochondria in response to stresses [164]. Fusion and fission defects have significant implications in ovarian aging. Oocyte-specific deletion of *Mfn1* in mice leads to female sterility associated with the defective folliculogenesis and impaired oocyte quality [165, 166]. Targeted deletion of *Mfn2* in oocytes results in mitochondrial dysfunction and female subfertility associated with impaired oocyte maturation and follicle development [167]. Oocyte-specific depletion of *Drp1* leads to decreased oocyte quality due to maturation defects [168, 169]. Also, fission-deficient oocytes due to the oocyte-specific depletion of *Drp1* exhibit a high frequency of failure in peri- and post-implantation development [170].

Mitochondrial mass in cells is also regulated by the changes in mitophagy and mitocytosis, and these processes are tightly regulated in response to cellular stress. Phosphoglycerate translocase 5 (PGAM5), located in the mitochondrial membrane, is associated with mitophagy, necroptosis and apoptosis. PGAM5 activates DRP1 through dephosphorylation and induces mitochondrial fracture and necrosis [171]. Moreover, highly expressed PGAM5 is directly related to a significant reduction in cellular ATP production [172].

(6) Epigenetic influences on ovarian aging

Mature oocyte DNA has higher methylation levels, but with age, the levels of DNA methyltransferases (DNMTs) decrease in oocytes and DNA demethylases (TETs) increased, hence overall DNA methylation levels decreased [173]. Combined analysis of single oocyte transcriptomes and DNA methylomes in young and old mice showed that changes in cellular transcription of aged oocytes result from differential gene-methylation [174]. *Tet1*-deficiency in mouse model down-regulates the premature ovarian failure (POF) related gene *Fmr1*, reduces the number of follicles, causes POF, and thus reduces reproductive performance [175]. *Tet2* deletion increases DNA damage and impairs pathways such as the spindle checkpoint and the actin cytoskeleton, thereby reducing oocyte quality and reproductive performance [176].

During the maturation of oocytes, histone modifications show dynamic changes, and in the germinal vesicle (GV) phase, H4K12 and H4K16 are acetylated. Deacetylation by deacetylase occurs during MI and MII of oocytes [177]. With increasing age, the acetylation levels of H4K12 and H4K16 in aged GV oocytes decrease, but the deacetylation of H4K12 is impaired in MII oocytes. Excessive histone acetylation in MII oocytes can lead to increased aneuploidy and death of post-fertilization embryos [178–180]. Histone methylation modifications however are relatively stable during oocyte maturation [181]. Yet, the level of histone H3K4 trimethylation (H3K4me3) is high in fully grown oocytes derived from young mammalian females but decreased during aging due to the decreased expression of epigenetic factors responsible for H3K4me3 accumulation [182]. Oocyte-specific knockout of the gene encoding CxxC-finger

protein 1 (CXXC1), a DNA-binding subunit of SETD1 methyltransferase, causes ooplasm changes associated with accelerated aging and impaired maternal mRNA translation and degradation [182]. Therefore, a network of CXXC1-maintained H3K4me3, in association with mRNA decay competence, sets a timer for oocyte deterioration and plays a role in oocyte aging in both mouse and human oocytes.

(7) The ovarian microenvironment affects ovarian aging

Ovarian tissue consists of two parts: the cortex and the medulla. The medulla is composed of connective tissue, fibrous tissue, and blood vessels. The ovarian microenvironment is composed of different types of cells within the ovary, and communication between oocytes and ovarian microenvironment is mediated by direct contact with surrounding cells, the extracellular matrix, and signaling molecules, including hormones, growth factors, and metabolites [183]. Therefore, the ovarian microenvironment can affect the quality of oocytes, and even accelerates the oocyte aging and leads to diseases such as infertility. Bidirectional communication between the oocyte and its associated somatic cells plays a key role in fertility and embryogenesis. Cumulus cells provide essential nutrients for oocyte maturation through different paracrine signaling pathways. In addition, cumulus cells are also involved in the regulation of oocyte aging [151]. For example, cumulus cells accelerate oocyte senescence by activating the Fas/FasL pathway [184, 185]. Ceramides can also be transferred from cumulus cells to oocytes through gap junctions, increasing age-related oocyte apoptosis [186, 187]. Altered homeostasis between synthesis and degradation of extracellular matrix (ECM) components affects tissue structure and function, and excessive accumulation of ECM contributes to tissue fibrosis. In addition to organs such as heart, lung, and kidney, fibrosis is also present in ovarian tissue, and fibrosis within the mouse ovarian stroma increases with reproductive age, so fibrosis is an early marker of ovarian stroma aging [188]. Ovarian fibrosis is reversible, and antifibrosis drugs (pirfenidone and BGP-15) is reported to eliminate fibrotic collagen and restore ovulation in reproductively old and obese mice, in association with dampened M2 macrophage polarization and up-regulated MMP13 protease [189]. On the other hand, the temporary suppression of angiogenesis by axitinib administration pauses ovarian development and keeps the ovarian reserve in the long term, leading to postponed ovarian senescence and an extension of the female reproductive life span [190].

miRNAs are small non-coding RNAs that bind to target messenger RNA (mRNA) to inhibit their expression. miRNAs, either freely present or enclosed in vesicles (exosomes) in human follicular fluid [191], are also involved in the regulation of oocyte senescence. For example, *miR-16-5p*, *miR214-3p*, and *miR-449a* are down-regulated and *miR-125b*, *miR-155-5p*, and *miR-372* up-regulated in the follicular fluid of older women, affecting vesicular release, oocyte maturation, and response to stress response [192]. Circular RNAs (circRNAs) are a unique class of endogenous RNAs that can be used as potential diagnostic and prognostic biomarkers for many diseases. Abnormal expression of circRNAs is found in aging ovaries, which are related to the metabolic processes, regulatory secretory pathways, redox processes, and steroid hormone biosynthesis associated with ovarian aging [193]. Specifically, the expression of *circRNA_103827* and *circRNA_104816* is increased in aged human granulosa cells, and the high expression is closely associated with decreased ovarian reserve and poor reproductive outcomes in women [194]. Therefore, the ovarian microenvironment modulates the process of ovarian aging.

Ovarian aging intervention strategy

To date, there are no clinically feasible techniques to either maintain or reverse ovarian dysfunction associated with advanced age [195]. However, important advances have been made in the field of senotherapy during the last few years, although the majority of the studies are performed in mouse models, these pre-clinical tests may show potential in improving female fertility.

Efficacy of antioxidants on reproductive aging

Even though there are no clinically practical less invasive therapeutic measures to reverse ovarian dysfunction associated with advanced age, several drugs are performed in organismal models. Important advances have been made in the field of senotherapy to extend the reproductive lifespan in female mammals [195, 196]. Oxidative damage is a crucial factor in ovarian functional decline with age, therefore, antioxidants, such as resveratrol, nicotinamide mononucleotide (NMN), NAC, melatonin and coenzyme Q10 (CoQ10), may prevent oxidative damage and delay ovarian aging.

Resveratrol (3,5,4′-trihydroxystilbene) is a polyphenolic compound found in the skin of red grapes with antioxidant, anti-inflammatory, cardioprotective and anti-neoplastic properties [197]. Resveratrol improves mitochondrial function and protects against metabolic disease by activating longevity-related protein Sirtuin 1 (SIRT1) and PGC-1a in different type of cells [198–200]. Several studies have established that resveratrol regulates animal reproduction. Resveratrol enhances maturation and quality of aged oocytes [201], and is an effective cryo-protectant with antioxidant and anti-apoptotic effects [202]. Excitingly, long-term-oral administration of resveratrol protects against the reduction of fertility with reproductive aging in mice by improving healthy follicle number, telomere length and telomerase activity, as well as oocyte quantity and quality [203]. Methylglyoxal affects the mouse oocyte quality by resulting in excessive ROS production, aberrant mitochondrial distribution and high level lipid peroxidation. Resveratrol protects the oocytes from methylglyoxal-induced cytotoxicity and this is mainly through the correction of the abnormity of cellular ROS metabolism [204]. Moreover, resveratrol significantly increases the weights of POF mice and their ovaries as well as the number of follicles, while decreasing the atresia rate of follicles [205]. Therefore, resveratrol has the potential in intervening the diminished ovarian reserve and function through its suppression of OS. But resveratrol also has adverse effects on implantation and endometrial decidualization [206]. Effects of resveratrol are time and dosage dependent. While low dose of resveratrol promotes oocyte quality and ovarian function, the high dose leads to embryo apoptosis [203]. Given that doses ≥ 1.0 g may produce side effects, including headache, dizziness, nausea, diarrhea, and liver dysfunction [207, 208], the optimal frequency, dosage for resveratrol treatment needs to be further explored.

NMN is an intermediate of the metabolic cofactor nicotinamide adenine dinucleotide (NAD^+/NADH), and decreased with advanced age. NAD^+ is a very important metabolic redox cofactor and enzyme substrate that mediates a variety of biological processes including cell death, aging, gene expression, neuro-inflammation and DNA repair [209]. As revealed by many recent studies, deficiency of NAD^+ can be compensated by the NMN supplementation that successfully prevent age-associated conditions from metabolic and neurodegenerative disease to cancer [210]. In aged mice ovaries, NMN supplementation improves the oocyte quality as evidenced by meiotic competency, increases ovulation and fertilisation capability through restoring NAD^+ levels [211]. Furthermore, NMN reinstates mitochondrial functions of aged oocytes to mitigate accumulation of both DNA damage and ROS. Moreover, long-term treatment of NMN improves age-related diminished ovary reserve through enhancing the mitophagy level of granulosa cells in mice [212]. To date, a number of NMN commercial products have been produced by various pharmaceutical, biotechnology and health food companies. The quantity of NMN in available products vary from 50 to 500 mg/capsule, whereas some consumers take two 150 mg capsules per day [213, 214]. Given NMN treatment (400 mg/kg) obstructed the exercise-induced benefits of a mouse model of diet-induced obesity such as reduced hepatic triglyceride accumulation, glucose stimulated insulin secretion from islets and glucose tolerance [215], the recommended safe dose levels for long term administration requires rigorous scientific preclinical, clinical and toxicological testing.

Melatonin (5-methoxy-N-acetyltryptamine) is an endogenous hormone that is primarily released by the pineal gland to modulate many important physiological reactions [216]. Additionally, melatonin can be synthesized in the follicular granulosa cells and oocytes. Melatonin and its metabolites efficiently decrease OS levels by directly scavenging ROS as well as enhancing antioxidant activity and modulation of mitochondrial and inflammatory activities [217–219]. Melatonin administration induced high melatonin concentration in the follicular fluid microenvironment improves oocyte quantity and quality in reproductive old females [220, 221]. Insufficient amounts of melatonin in follicular fluid are highly correlated with advanced maternal age-related meiotic defects [222, 223]. Melatonin can increase telomere length, upregulate the glutathione peroxidase expression and SIRT pathways, and decrease inflammation [224, 225]. Moreover, melatonin improves the quality of maternally aged oocytes by maintaining intercellular communication and antioxidant metabolite supply [221].

Additionally, other small compounds could be used for delaying ovarian aging and improving fertility. For example, α-Ketoglutarate can delay ovarian aging and telomere shortening in mice by inhibiting metabolism and mammalian target of rapamycin (mTOR) pathway [226]. Some natural antioxidants such as quercetin and curcumin can also protect the ovaries [129]. More compounds are proving beneficial in improving oocyte and ovarian functions.

Mitochondrial therapy

Aged oocytes have significantly reduced amounts of mitochondria, leading to lower fertilization rates and poor embryonic development. Therefore, search for better

sources of mitochondria improvement by means of mitochondrial nutrient therapy or mitochondrial-transfer therapy to combat the stress caused by aging are among the most challenging approaches.

CoQ10 is an essential component for transporting electrons in the mitochondrial respiratory chain to produce cellular energy [227, 228]. Ubiquinol, the reduced form of CoQ10, acts as an antioxidant in cellular metabolism via inhibition of lipid peroxidation, protein, and DNA oxidation [229–231]. CoQ10 promotes repair of dysfunctional oocyte mitochondria and reduces the ovarian expression of 8-hydroxydeoxyguanosine [232]. CoQ10 synthesis decreases in the oocyte with age, coinciding with the decline in oocyte quality and general fertility [233]. CoQ10 supplementation in an aged animal model delayed depletion of ovarian reserve, restored oocyte mitochondrial gene expression, and improved mitochondrial activity. But CoQ10 supplementation had no impact on ovarian reserve or oocyte quality of young females in which mitochondrial function is intact, implying the effect of CoQ10 to specifically target age-associated mitochondrial dysfunction [233]. A randomized double-blind study conducted on a small group of IVF-ICSI (*in vitro* fertilization-intracytoplasmic sperm injection) women (35–43 years of age) found the rate of oocyte aneuploidy to be 46.5% with CoQ10 treatment (600 mg) vs. 62.8% in the control group. Clinical pregnancy rate was 33% for the CoQ10 group and 26.7% for the control group, neither of these results was statistically significant owing to the small scale of the study [234]. Another randomized controlled trial on low-prognosis young women with decreased ovarian reserve demonstrated pretreatment with CoQ10 to be effective in improving the ovarian response to stimulation and embryo quality during IVF-ICSI cycles [235]. These results are encouraging for the treatment of age-associated infertility using CoQ10, although further work is needed to determine the overall effect on pregnancy complications and live birth rates as well as the optimal timing and dosage of CoQ10 supplementation.

Due to the relevance of aging in infertility, mitochondrial enrichment has been proposed as a potential therapy option in infertile patients to improve oocyte quality. Various techniques have been attempted to use heterologous or autologous sources of mitochondria to rejuvenate and improve oocyte health by introducing new sources of energy [236, 237]. In the heterologous approach, mitochondrial enrichment can be performed by relocating a healthy cytoplasm into the patient's oocyte (partial cytoplasm transfer) or replacing the compromised cytoplasm with a competent one by means of nuclear transfer technology (total cytoplasm transfer). In 1982, Ooplasmic transfer (OT) in mice laid the foundations of oocyte rejuvenation treatments [238]. OT involves transferring a cytoplasm portion from a donor's oocyte to the patient's oocyte to introduce potentially beneficial components that might restore oocyte viability [239]. In 1997, Cohen and colleagues announced the first human pregnancy after OT [240]. Following this achievement, this method has been successfully used in low-prognosis patients [239, 241, 242]. But this approach entails many ethical and safety concerns that mainly arise from the uncertain degree of mitochondrial heteroplasmy deriving from it. Mitochondrial heteroplasmy in the offspring and its unknown consequences have led to alternative strategies being proposed to improve oocyte quality.

Germinal vesicle, spindle, pronuclear, polar body and blastomere transfer constitute different ways of relocating the genetic material from a patient's compromised oocyte or zygote to a healthy cytoplasm. GV transfer consists of relocating the GV from the compromised oocyte to an enucleated healthy oocyte at the same developmental stage by electrofusion, subsequently matured *in vitro* to the MII stage [243]. The GV transfer in humans was performed by Zhang's group and Takeuchi respectively [244, 245], but no live birth and healthy offspring have been described in humans yet, compared to what has been found in animal studies [246, 247].

Using germinal vesicle nuclear transfer, Liu and colleagues found that both nuclear and cytoplasmic factors contributed to the meiotic defects of the old oocytes of Senescence-accelerated mice and that the nuclear compartment played the predominant role in the etiology of aging-related meiotic defects [248]. To data, the main limitations of GV transfer include: high mitochondrial aggregation around the GV and the maturation process needed from the GV to the MII stage [244, 249]. Nuclear genetic materials assemble in a spindle structure at the metaphase of the second meiosis [236]. Given spindle structure removal is a common procedure employed in cloning [250], spindle transfer is less invasive than GV transfer, as condensed chromosomes can be easily aspirated with a smaller enucleation pipette and a minimal amount of cytoplasm due to its location at the periphery of the oocyte [243]. Tachibana and colleagues demonstrated that monkey reconstructed oocytes following spindle transfer were capable of supporting normal fertilization, embryo development and produced healthy offspring [251]. However, they failed to translate these successful results to humans [252]. In 2017, the first human live birth derived from oocyte spindle transfer to prevent Leigh syndrome. It was reported that transfer of the embryo resulted in a pregnancy with delivery of a boy

with neonatal mtDNA mutation load of 2.36%–9.23% in his tested tissues [253].

Yet, the heterologous approach entails many ethical and safety concerns that mainly arise from the uncertain degree of mitochondrial heteroplasmy deriving from it. Therefore, the autologous approach is proposed a suitable potential tool to improve oocyte quality by overcoming the heteroplasmy issue. Mitochondrial autologous sources include immature oocytes [254], granulosa cells [255], germline stem cells [256], and adipose-derived stem cells [257]. However, the autologous germline mitochondrial energy transfer (AUGMENT) has not yielded as many beneficial outcomes as initially expected [256]. The triple-blind, single-center, randomized, controlled experimental pilot study was conducted by Labarta and colleagues at IVI-RMA Valencia (Spain) from October 2015 to June 2017. A total of 57 poor-prognosis patients with previous IVF failures and well-documented poor embryo quality were included in this study, and an ovarian cortex biopsy was performed to isolate egg precursor cells to obtain their mitochondria. Sibling MIIs were randomly allocated to AUGMENT (experimental) or intracytoplasmic sperm injection (Control). In AUGMENT, mitochondrial suspension was injected along with the sperm. Eventually, AUGMENT did not seem to improve prognosis in this population [256]. Together, it seems that these autologous techniques do not improve clinical outcomes in human infertile patients presently. Search for better sources of mitochondria and organelle improvement by means of gene editing/engineering or culture media additives to combat the stress caused by aging are among the most challenging approaches.

Fertility preservation by ovarioids rejuvenated from pluripotent stem cells

Given the limited ovarian reserve at birth and no self-renewal germ cells in the adulthood, any intervention by anti-aging compounds or mimics of antioxidation cannot extend the reproductive lifespan for longer term. Reconstitution of ovarian functions emerge as a prosper strategy for extending reproductive lifespan in future. In early studies, transplantation of young ovaries restored cardio-protective influence and increased life span in post-reproductive-aged mice [258, 259]. Reconstruction of ovarian and reproductive endocrine functions is a new strategy for fertility preservation, which is of great significance for fertility protection and reducing reproductive aging-related chronic diseases. PGCs, which give rise to both oocytes and the spermatozoa, are specified at around E7.25 in the mouse. Spermatogonia and oogonia differentiate from PGCs and initiate meiosis after E12.5–13.5 via signaling from the somatic genital ridge [260, 261]. PGCs-like cells (PGCLCs) have been successfully induced from pluripotent stem cells (PSCs) [262, 263]. PGCs and PGCLCs complete meiosis *in vitro* and generate functional gametes that produce live offspring [264, 265]. Importantly, functional oocytes can be developed in the recipients following transplantation of PGCs aggregated with E12.5 fetal somatic pre-granulosa cells into kidney capsule, ovarian bursa or intra-ovarian injection [266–269]. Transplantation of PGCs aggregated with gonadal somatic cells effectively restores folliculogenesis and endocrine function (Figure 3A). However, the majority of PGCs enter meiosis and undergo folliculogenesis soon after transplantation, and proliferative PGCs and meiocytes disappear about six weeks following transplantation [270]. Encouragingly, mTOR inhibition by INK128 improves and extends the reconstituted ovarian and endocrine functions in reproductive aging and premature aging mice [271].

Follicular granulosa cells isolated from adult mouse ovaries can be robustly induced to generate germline-competent pluripotent stem cells (gPSCs) by a purely chemical approach, with additional Rock inhibition and critical reprogramming facilitated by crotonic sodium or acid. These gPSCs acquired high germline competency and could consistently be directed to differentiate into PGCLCs and form functional oocytes that produce fertile mice (Figure 3A) [272]. Also, parthenogenetic embryonic stem cells are able to differentiate into PGCLCs and form oocytes following *in vivo* transplantation into kidney capsule that produce fertile pups and reconstitute ovarian endocrine function (Figure 3A) [273]. Recently, culture conditions were developed to recreate the stepwise differentiation process from pluripotent cells to fetal ovarian somatic cell-like cells (FOSLCs). When FOSLCs were aggregated with PGCLCs derived from embryonic stem cells, the PGCLCs entered meiosis to generate functional oocytes capable of fertilization and development to live offspring (Figure 3B) [274]. This methodology opens the possibility for application in fertility preservation because it does not require embryonic gonads. Taken together, we can conclude that germ cells could be generated from mouse somatic cells without transfection of transcription factors [272, 275]. Human oogonia also could be generated from PSCs in culture [276, 277].

Conclusion and perspectives

Women increasingly delay childbearing later in their life worldwide [14, 15]. Though cryogenically stored eggs or

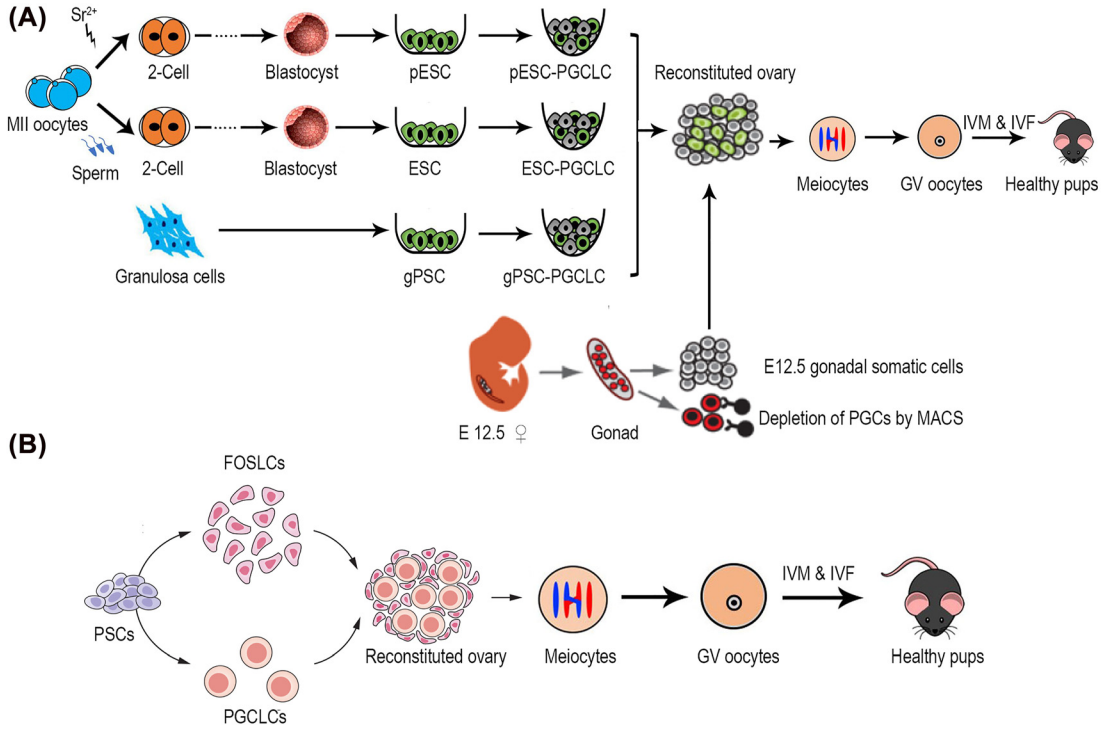

Figure 3: Current strategies for reconstitution of follicle structures. (A) Scheme for PGCLC induction from PSCs and ovary reconstitution. PGCLCs aggregated with gonadal somatic cells effectively restores folliculogenesis and endocrine function. (B) Scheme for reconstitution of both FOSLCs and PGCLCs from PSCs. Oocytes in the reconstituted environment gave rise to offspring after fertilization. PGCLCs, primordial germ cells-like cells; PSCs, pluripotent stem cells; FOSLCs, fetal ovarian somatic cells-like cells; ESC, embryonic stem cell; pESC, parthenogenetic embryonic stem cell; gPSC, granulosa cells-derived germline competent pluripotent stem cell; Sr, strontium; GV, germinal vesicle; IVM, *in vitro* maturation; IVF, *in vitro* fertilization.

ovarian tissues avoid exposure to the biological and environmental factors that compromise the quality of oocytes in the ovary, these procedures together with ART are costly and carry an approximately only 6.5% chance of achieving pregnancy with each mature oocyte thawed from the preservation of oocytes or ovarian tissue, which further decreases with age. Moreover, after ANM, ovaries are doomed to lose the function in maintaining female hormone secretion, leading to increased risk for certain chronic diseases associated with age. Thus, a better understanding of oocyte biology is essential to develop the strategies to prevent the decline in fertility as maternal age advances. This review provides a broad survey and insight into mechanisms of ovarian aging.

Recent studies have provided a deeper understanding of the mechanisms of ovarian aging and the major factors that impact it including non-OSCs in adulthood female mammals, chromosomal cohesion deterioration, telomere shortening, DNA damage and DDR-associated genetic mutations, mitochondrial dysfunction, ROS and OS, epigenetic alteration and aging ovarian microenvironment. These factors influence both the quantity and quality of the oocytes. A range of promising strategies have been developed to prolong fertility in women. Limiting exposure to oxidative damage may help preserve more oocytes with high quality within the ovaries during aging. Antioxidants, such as resveratrol, NMN, NAC, melatonin and CoQ10, may prevent oxidative damage and delay oocyte aging. If successful, delaying reproductive aging with the use of antioxidants could help lower the costs of infertility treatment when using ART. Currently, the main limitation for use the antioxidants in human reproductive aging is to establish the correct time, the optimal frequency and dosage, as well as the possible side effect of long-term administration. In future, it is possible to realize fertility preservation or reconstruct the ovarian function by ovarioids rejuvenated from pluripotent stem cells, however, this methodology may have the challenges with ethical and technical concerns to overcome.

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