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ORIGINAL ARTICLE

Ovarian aging in humans: potential strategies for extending reproductive lifespan

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Abstract Ovarian reserve is a term used to estimate the total number of immature follicles present in the ovaries. Between birth and menopause, there is a progressive decrease in the number of ovarian follicles. Ovarian aging is a continuous physiological phenomenon, with menopause being the clinical mark of the end of ovarian function. Genetics, measured as family history for age at the onset of menopause, is the main determinant. However, physical activity, diet, and lifestyle are important factors that can influence the age of menopause. The low estrogen levels after natural or premature menopause increased the risk for several diseases, resulting in increased mortality risk. Besides that, the decreasing ovarian reserve is associated to reduced fertility. In women with infertility undergoing in vitro fertilization, reduced markers of ovarian reserve, including antral follicular count and anti-Mullerian hormone, are the main indicators of reduced chances of becoming pregnant. Therefore, it becomes clear that the ovarian reserve has a central role in women's life, affecting fertility early in life and overall health later in life. Based on this, the ideal strategy for delaying ovarian aging should have the following characteristics: (1) be initiated in the presence of good ovarian reserve; (2) maintained for a long period; (3) have an action on the dynamics of primordial follicles, controlling the rate of activation and atresia; and (4) safe use in pre-conception, pregnancy, and lactation. In this review, we therefore discuss some of these strategies and its feasibility for preventing a decline in the ovarian reserve.

Keywords Menopause · Fertility · Aging · Females · Ovarian reserve

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Introduction

The ovaries are glands of the female reproductive system responsible for the development of the gamete (oocyte) and production of female sex hormones, which regulate reproductive and non-reproductive functions [1]. Anatomically, the ovary can be divided into two compartments, medulla (inner layer, composed of loose vascularized connective tissue) and cortex (outer layer containing the ovarian follicles)

[1]. The functional unit of the ovary is the follicle, which is constituted by an oocyte surrounded, initially, only by granulosa cells and, later, also by theca cells. The ovarian follicle can be classified into different types (primordial, primary, secondary, antral, and pre-ovulatory) according to the degree of oocyte maturity and its histological structure [1, 2].

Ovarian reserve is a term used to estimate the total number of immature follicles present in the ovaries. In humans, the maximum number of ovarian follicles is reached during a woman's intrauterine life, containing about 7 million follicles around the 20th week of gestation. Between birth, menarche, and menopause, there is a progressive decrease in the number of ovarian follicles, estimated for approximately 1 to 2 million, 300–400,000, and 1000 follicles, respectively [3]. The primordial follicles remain in a state of quiescence and may enter directly into atresia or start the maturation process (folliculogenesis), culminating in ovulation or follicular atresia during any stage. During a woman's reproductive life, approximately 400 ovulations occur [1, 2, 4].

Folliculogenesis is a continuous process, divided into two stages, beginning with the activation of primordial follicles and ending with follicular rupture. Inhibitory and stimulatory intraovarian factors trigger the first stage (initial recruitment) of folliculogenesis, starting about 150 days before ovulation, independent of follicle-stimulating hormone (FSH) [2]. Activation of primordial follicles induces modifications of granulosa cells around the oocyte, which assume a cuboidal shape in a single layer (primary follicle) and later in multiple layers (secondary follicle). Then, granulosa cells continue proliferating and theca cells appear, forming the preantral and antral follicles, at which point the second stage begins (cyclic recruitment) of folliculogenesis, dependent on FSH [2].

Theca and granulosa cells are responsible for the synthesis and secretion of estrogen, the main ovarian hormone. The presence of estrogen receptors in other non-reproductive organs (brain, bone, liver, intestine, skin, and salivary glands) demonstrates the relevance of the ovary in the regulation of different body functions [1, 2, 5]. The duration of menacme, the period in which the ovary plays its physiological role in full, depends not only on the ovarian reserve, but also on the rate of activation of primordial follicles and follicular atresia. Numerous local or systemic mechanisms are responsible for ovarian aging, with reproductive and non-reproductive repercussions [6, 7]. Basically, ovarian aging is characterized by a decline in follicular quantity and quality, and changes in the synthesis of ovarian hormones, resulting in a drop in estrogen levels [2].

Progressive ovarian aging promotes the depletion of female gonadal function, initiating a period of transition from the reproductive to the non-reproductive phase in a woman's life, known as the climacteric [2, 8, 9]. Therefore, a good understanding of folliculogenesis and understanding of the ovarian aging process are fundamental for anti-aging interventions to be proposed.

Lessons from menopause studies

Ovarian aging is a continuous physiological phenomenon, with menopause being the clinical mark of the end of ovarian function. Usually, menopause occurs between the ages of 45 and 55 years [10–12]. Numerous epidemiological studies evaluate conditions that can influence the age of menopause. Genetics, measured as family history for age at the onset of menopause, is the main determinant. Ethnicity, physical activity, diet, and habits are other significant factors that influence the age of menopause [11].

Current smoking, low socioeconomic status, low education, unemployment, early menarche, nulliparity, unilateral oophorectomy, vigorous physical activity, severe weight loss, vegetarian or high-carbohydrate diet, and high consumption of polyunsaturated fats are conditions that accelerate the onset of menopause [11, 13]. On the other hand, multiparity, first pregnancy at an advanced age, use of oral contraceptive pills, Japanese ethnicity, higher body mass index, moderate physical activity, and moderate alcohol consumption are associated with late menopause [11, 13].

Menopause-induced hypoestrogenism has short- and long-term consequences for women's health. Hot flashes, night sweats, palpitations, headache, vaginal dryness, burning and genital irritation, dyspareunia, urinary urgency, dysuria, and recurrent urinary tract infection are short-term repercussions, while osteoporosis and cardiovascular and neurological diseases are long-term consequences [14]. The North American Menopause Society recommends hormone therapy as a supportive intervention to improve vasomotor

symptoms and relieve genitourinary menopausal syndrome, as well as prevent bone loss and fractures, especially in women under 60 years of age and less than 10 years after menopause onset [8].

Several studies have evaluated ovarian aging markers with the potential to predict the onset of menopause, such as antral follicle count (AFC) and blood levels of inhibins A and B, follicle-stimulating hormone (FSH), estradiol, and anti-Mullerian hormone (AMH) [15–20]. AMH is produced by the granulosa cells of growing follicles, from primary follicle to small antral follicles. AMH is currently the most promising marker for predicting age at natural menopause [15, 18].

Pathological ovarian aging can anticipate the occurrence of menopause. The term "early menopause" is used to refer to the onset of menopause before age 45, while premature ovarian insufficiency (POI) is when the last menstrual period occurs before age 40 [21]. POI is a gynecological condition that affects approximately 1% of women. In about 90% of cases, the cause of POI is unknown (idiopathic POI), and specific diagnoses are genetic abnormalities (such as Turner syndrome, Fragile X syndrome, and Autosomal gene mutations), exposure to gonadotoxic agents (chemotherapy and radiotherapy), autoimmune disorders (such as lymphocytic oophoritis, thyroid disease, Addison's disease, and celiac disease), viral infections, oophorectomy, and endometriosis [21–23].

The low estrogen levels detected in the POI have consequences for women's health, potentially more serious than those observed in physiological menopause [24]. Malek et al. observed a 15% increase in the risk of all-cause mortality among women with early age at menopause [25]. Mortality was also higher among women with POI (34.7% vs. 19.3%, $p < 0.001$) in a long-term cohort of Chilean women followed for three decades, especially deaths from cardiovascular disease (12.0% vs. 5.1%; OR 2.55, 95% CI 1.21–5.39) [26]. Other studies have confirmed that women with POI have an increased risk of death from all causes, as well as a higher prevalence of cardiovascular disease, autoimmune conditions, osteoporosis, cognitive dysfunction, mood, and sexual disorders [24, 27–31].

Currently, there is no reliable intervention to reverse ovarian aging in women diagnosed with POI. Estrogen hormone therapy, with or without progesterone, is recommended to improve quality of life and reduce comorbidities. However, regular practice of moderate physical activity, healthy diet, maintenance of healthy habits, and early diagnosis and control of acute or chronic diseases are good ovarian antiaging strategies.

Lessons from reproductive medicine

Infertility in women is defined as the inability to establish pregnancy spontaneously after a period of 12 months with regular sexual intercourse; the period is reduced to 6 months when the woman is over 35 years old [21]. Disorders such as polycystic ovary syndrome, low ovarian reserve, and POI are responsible for up to 25% of female infertility cases [19, 21]. Interestingly, epidemiological studies suggest that there is an association between female reproductive aspects and life expectancy and risk of chronic diseases [32, 33].

The live birth rates of women undergoing infertility treatment by in vitro fertilization (IVF) are directly related to the ovarian response to controlled stimulation. The Bologna and POSEIDON (Patient-Oriented Strategies Encompassing IndividualizeD Oocyte Number) criteria are the two most used ways to define poor ovarian response (POR). Both are based on the woman's age, ovarian reserve biomarkers, and number of oocytes recovered from previous ovarian stimulations [34–36]. According to the Bologna criteria, a woman with a POR must have at least two of the following conditions: advanced maternal age (> 40 years), a previous poor response to controlled stimulation (defined as ≤ 3 oocytes retrieved), or an abnormal ovarian reserve test (antral follicle count [AFC] < 5–7 follicles and/or AMH < 1.1 ng/mL) [34–36]. POSEIDON criteria divide patients into four groups based on a combination of factors: group 1—patients < 35 years old, presenting with adequate values of AFC (≥ 5 follicles) and/or AMH ≥ 1.2 ng/mL (subgroup 1a: < 4 oocytes retrieved and subgroup 1b: 4–9 oocytes retrieved, after standard ovarian stimulation); group 2—patients ≥ 35 years old, presenting with adequate values of AFC (≥ 5 follicles) and/or AMH ≥ 1.2 ng/mL (subgroup 2a: < 4 oocytes retrieved and subgroup 2b: 4–9 oocytes retrieved, after standard ovarian stimulation); group 3—patients < 35 years old, presenting with poor values of AFC (< 5 follicles) and/or AMH (< 1.2 ng/mL);

and group 4—POR patients ≥ 35 years old, presenting with poor values of AFC (< 5 follicles) and/or AMH (< 1.2 ng/mL) [34–36].

POR accounts for up to 20% of women undergoing to IVF [37, 38]. Some factors responsible for POR are already well recognized and associated with ovarian aging, such as endometriosis, prior ovarian surgery, chemotherapy, radiation therapy, smoking, infections, and autoimmune disorders [39, 40]. However, little is known about the mechanisms responsible for POR in women with normal or abnormal ovarian reserve biomarkers [39].

In order to increase the number and quality of aspirated oocytes and, consequently, increase live birth rate, several studies have evaluated different ovarian stimulation protocols associated or not with adjuvant therapies in cases of POR. Pretreatment using coenzyme Q10 (CoQ10), testosterone, dehydroepiandrosterone (DHEA), and myoinositol, as well as, the use of gonadotropins associated with luteinizing hormone (LH), growth hormone (GH), clomiphene citrate, and letrozole, are some of the strategies suggested for the management of patients with POR [41–43]. However, to date, there are no adjuvant therapy that shown convincing results for management of POR patients [41]. A recent meta-analysis has shown that dehydroepiandrosterone (DHEA) and CoQ10 are promising adjuvant therapies for raising the clinical pregnancy rate (OR 2.46, 95% CI 1.16 to 5.23 and OR 2.22, 95% CI 1.08–4.58, respectively), while GH raised the number of oocytes retrieved (weighted mean difference 1.72, 95% CI 0.98 to 2.46) [41]. However, the authors concluded that high-level randomized controlled trial (RCT) studies using uniform standards for POR still need to be performed [41].

It is not uncommon for patients to undergo several IVF cycles with multiple rounds of ovarian hyperstimulation. Thus, since the establishment of IVF as a treatment technique for infertile couples, the medium- and long-term impact of repeated ovarian hyperstimulation on ovarian aging and estrogen-dependent diseases has been investigated [44, 45]. The use of gonadotropins at supraphysiological doses promotes maximum recruitment and maturation of small follicles, but the effects on primordial follicles (unresponsive to physiological levels of FSH) are poorly understood [45, 46].

Most studies have not observed an association between the use of gonadotropins for fertility treatment with accelerated ovarian aging or anticipation of menopausal symptoms [47]. However, women who had low number of aspirated oocytes in IVF cycles are at risk of anticipation of menopause symptoms and early menopause [44, 48–50]. Women who have multiple IVF attempts also have repeated transvaginal ultrasound-guided needle ovarian aspirations, with potential complications (bleeding, infection, and fibrosis) in the short and long term, which can contribute to accelerated ovarian aging.

Currently, female fertility preservation is possible through the vitrification of oocytes, embryos, or ovarian tissue [51, 52]. Among these three methods, vitrification of ovarian tissue, initially proposed for oncologic patients prior to chemotherapy or radiotherapy, and subsequent transplantation can restore not only the female reproductive potential, but also the ovarian hormone function [51]. Ovarian tissue cryopreservation and transplantation (OTCT) was performed for the first time in 1999 in a 29-year-old patient, with surgical menopause at age 28, who did not obtain improvement of menopause symptoms induced using conventional hormone therapy [53].

Recently, the American Society for Reproductive Medicine (ASRM) recognized that OTCT may be indicated for fertility preservation in situations where ovarian hyperstimulation for oocyte retrieval is contraindicated [54]. A systematic review of 309 cases of OTCT revealed that the intention of the vast majority of patients was the restoration of reproductive function, but on nine patients, the procedure was used with non-reproductive purpose, to restore ovarian endocrine function [55]. This same systematic review highlighted that OTCT was able to restore endocrine function in up to 85% of cases [55].

Despite the great progress in ovarian tissue cryopreservation studies, OTCT still has limitations. It is necessary to reduce the percentage of loss of primordial follicles during the freezing–thawing process, especially during revascularization after auto transplantation, increasing graft survival [56]. In addition to the success variables related to technical conditions, the woman's age at the time of freezing and the amount of cryopreserved ovarian tissue are other determining factors for maintaining the endocrine and reproductive function of the thawed and transplanted ovarian tissue [57]. Oktay et al. found that with about a third of an ovary cryopreserved at a mean age of 29.3 (9–44) years, the mean longevity of transplanted

ovarian tissue was 26.9 (4–144) months [55]. Surgical removal of ovarian tissue can also interfere with the age of onset of menopause. Studies are not clear about the real impact of a unilateral oophorectomy on the age of menopause, which may accelerate the onset of menopause from 1 to 7 years before control groups, not submitted to oophorectomy [56]. Thus, in light of early studies in reproductive medicine, OTCT is a promising intervention to delay the onset of menopause. Studies are also needed to identify the best protocols for freezing, thawing, and transplanting ovarian tissue, the ideal age for removing the ovarian cortex, and the number and interval of transplants [57–59].

Lessons from animal studies

Ovarian aging, evaluated from a comparative perspective between rodents and humans, also results from follicle loss starting in the prenatal period and an exponential depletion of primordial follicles associated with loss of fertility in middle age [60]. Furthermore, both species share progressively increasing irregularities in ovulatory cycles and increased pregnancy loss as oocyte depletion becomes imminent [60]. In C57BL/6 mice, the ovarian reserve is reduced in half around 10 months of age compared to 2-month-old mice [60], and at 18 months is reduced approximately 10 times [61]. Our group also observed a 75% reduction in primordial follicle density in C57BL/6 mice from 3 to 12 months of age [62] and severely compromised fertility around 14 months of age. Around this age, mice start to present irregularities in the estrous cycle and may enter a persistent anovulatory state, known as estropause [63]. This reinforces the idea that the ovarian reserve in mice, as in humans, is depleted with age and this process is completed in first half of the lifespan [60, 61].

The loss of ovarian function and the resulting decline in circulating estrogen levels are associated to various metabolic changes during induced menopause in rodents, similar to observed in women during natural menopause, such as increased adiposity, changes in lipid metabolism, hypertension, and insulin resistance [64, 65]. Although mice do not naturally enter in menopause, it can be induced chemically or by ovariectomy. Chemical menopause induction can be achieved by treatment with 4-vinylcyclohexene diepoxide (VCD). Treatment with VCD causes atresia of primordial and primary follicles leading to exhaustion of the ovarian reserve but retention of ovarian tissue [66]. One study showed that as young as 6-month-old rats induced to menopause by ovariectomy have a slight increase in systolic blood pressure (SBP), body weight, insulin resistance, and plasma cholesterol compared to intact females of the same age. This reinforces the notion that metabolic changes are driven by ovarian function independent of age [64]. These metabolic changes observed in ovariectomized rodents are a consequence of the hypoestrogenic state, similar to observed in women [65].

As estradiol reduction is one of the main effects of menopause, several studies have evaluated the benefits of exogenous estradiol replacement. Studies in murine models point to some beneficial effects of estradiol replacement therapy. These effects include improvement of glucose homeostasis and insulin sensitivity [67], improvement of the innate immune response [68], and reduced arterial pressure [69]. Interestingly, the transplantation of young ovaries into older females can increase lifespan [70], suggesting a protective effect of the ovarian tissue itself. Transplantation of young ovarian tissue to post-reproductive mice was able to significantly restore the cardioprotective benefits, similarly to what is seen at reproductive age [71]. These protective effects of ovarian tissue transplantation were also observed even when the young ovaries were follicle depleted with VCD prior to transplantation [72], suggesting that other factors beyond estrogen levels may play a role. Therefore, novel therapies to replace estrogen could be developed, improving quality of life without side effects of hormone replacement therapies.

Faced with the hormonal and physiological changes induced by menopause, some alternatives also aim at delaying the onset of menopause in preclinical models, such as ovarian transplantation of young mice into old mice [70, 72], dietary strategies [61, 73], and use of drugs, such as rapamycin [74, 75] and metformin [76, 77] have been developed. Dietary strategies have been proved widely effective in preserving the decline of follicle reserve in mice with aging. Caloric restriction (CR), ranging from 10 to 30% reduction of calorie intake [78], is an intervention that has been proven efficient in promoting overall longevity [79] as well as extended fertility in females, due to its impact on the control

of oxidative stress, insulin resistance, and reduction of the inflammatory state [80, 81]. CR can extend fertility by preserving the ovarian reserve [78, 82], reducing mTOR and FOXO3 activation [77, 83, 84], the main regulators of primordial follicle activation [83]. Mice under 30% CR can still reproduce successfully around 15–16 months of age, when ad libitum-fed mice could not [61]. In addition, CR also regulated DNA repair mechanisms, preventing the accumulation of DNA damage [85, 86], which could impact oocyte quality. However, some studies have associated the positive effects of CR to the restriction of protein [87]. Protein restriction also results in lower activity of the mTOR pathway in the ovary, decreasing the activation of primordial follicles and preserving the ovarian reserve [88]. The reduction in protein supply also results in lower adiposity and greater insulin sensitivity in humans and rodents, similar to that observed with 30% CR [87, 89]. On the other hand, hypercaloric diets (high-fat and/or high-carbohydrate diets) cause harmful metabolic adaptions, decreasing the ovarian reserve. Mice exposed to a high-fat diet have decreased ovarian reserve and increased insulin resistance and inflammation in the ovarian tissues [90, 91]. Interestingly, this unhealthy diet decreased ovarian reserve even in the absence of body weight gain [90].

Interestingly, the nematode *C. elegans* and the fly *D. melanogaster* can also serve as model for reproductive aging. *C. elegans* also have decreased reproductive potential as they age in both self-fertile and mated reproduction [92]. As reviewed elsewhere, CR, metformin, and rapamycin treatment can also extend reproductive lifespan in *C. elegans* [92], resembling the effects observed in mice. Similarly to the observed in humans and mice, an hyperactive germline in *C. elegans* is linked to reduced lifespan [93], suggesting a central role for reproductive fitness to regulate overall lifespan. The female fly *D. melanogaster* also has decreased reproductive potential with age [94]. In the fly, lifetime egg production was maximized in a diet containing a low protein to carbohydrate content [95]. The overall dietary restriction and rapamycin treatment both reduce daily egg laying, however increasing overall lifespan [96], further confirm a link between reproduction and lifespan in this species.

Repurposing of FDA-approved drugs to target aging

Based on ovarian physiology, animal studies, lessons from menopause and reproductive medicine studies, and the ideal strategy for delaying ovarian aging should have the following characteristics: (1) be initiated in the presence of good ovarian reserve; (2) maintained for a long period; (3) have an action on the dynamics of primordial follicles, controlling the rate of activation and atresia; and (4) safe use in preconception, pregnancy, and lactation.

Recently, Kulkarni et al. suggested the off-label use of FDA-approved drugs to target aging [97]. Based on animal and human studies that evaluated the effect of drugs on hallmarks of aging, healthspan, and lifespan, the authors scored (from 0 to 12) nine drugs according to potential anti-aging effects. Sodium-glucose linked transporter 2 (SGLT-2) inhibitors was the drug with the highest antiaging potential (score 12), followed by metformin (score 11), acarbose (score 9), rapamycin (score 9), methylene blue (score 9), angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ACEi/ARB) (score 8), dasatinib (+quercetin) (score 6), aspirin (score 6), and *N*-acetyl cyeteine (NAC) (score 5) [97].

These nine potential anti-aging drugs belong to different classes. Three of these nine drugs are oral hypoglycemic agents, the SGLT-2 inhibitors, metformin, and acarbose [97]. The impact of these gerotherapeutic agents on ovarian physiology is poorly understood. The benefit of using these hypoglycemic agents for female fertility is essentially due to adequate glycemic control and weight loss, with improvement in the ovulatory function [98]. However, the medium- and long-term effects on ovarian aging are not well known.

The effects of metformin on female reproductive outcomes are extensively studied, especially in patients with polycystic ovary syndrome. In addition to improving spontaneous pregnancy rates, increase implantation rates in IVF cycles, and decrease miscarriage rates among PCOS patients, metformin regulates ovarian mechanisms that may contribute to a delay in ovarian aging [98]. Studies in animal models have revealed that metformin reduces follicular atresia, oxidative stress, and autophagy in granulosa cells via the PI3K/AKT/mTOR pathway [99, 100]. Qin et al. suggested that metformin may delay the ovarian

aging process, probably by inducing SIRT1 expression and reducing oxidative damage [76]. Recently, Landry et al. observed that metformin was able to prevent age-associated ovarian fibrosis by modulating the proportion of fibroblasts, myofibroblasts, and immune cells [101]. At a dosage of 100 mg/kg, metformin was able to prevent the decline in the ovarian reserve with age in older mice [76]. In addition, metformin has chemoprotective effects in the ovaries of young mice exposed to cyclophosphamide [102]. Therefore, metformin due improvement of insulin sensitivity has similar effects to a 30% CR diet, and can be an alternative to dietary interventions.

Rapamycin is a substance originally produced by the bacterium *Streptomyces hygroscopicus*. In the past, rapamycin was used as an antifungal drug, later as an immunosuppressive agent [103]. Rapamycin acts in tissues by inhibiting to mammalian target of rapamycin (mTOR), a member of the phosphatidylinositol 3-kinase family of protein kinases. In this sense, mTOR is a serine/threonine protein kinase that functions as a master regulator of cell growth and metabolism in response to nutritional and hormonal stimuli [104], and is suggested as a CR mimetic. The mTOR signaling is involved in follicular dynamics, and its activation in granulosa cells promotes follicular development [105], and its inhibition can prolong ovarian lifespan [103]. Animal studies have shown that treatment of female mice with rapamycin prevented activation of primordial follicles when compared to untreated controls [82, 106, 107]. Our previous study suggested that treatment with rapamycin (4 mg/kg) prevents the decline in the ovarian reserve of mice similarly to 30% CR [82]. Additionally, rapamycin administered to young animals (5 mg/kg) exposed to chemotherapy also decreased the activation of the mTOR pathway, preserving the ovarian reserve [108].

Among many adaptations with aging, there is an accumulation of senescent cells with age in several species [6, 109]. Senescent cells have decreased capacity for proliferation [110–112], however remain metabolically active, secreting pro-inflammatory factors known as senescence-associated secretory phenotype (SASP) [113]. Our group demonstrated an accumulation of senescent cells with aging also in the ovary of mice [62]. Furthermore, we demonstrated that obese mice accumulate more senescent cells in the ovary than normal littermates [114], suggesting that obesity accelerates the ovarian aging phenotype. Interestingly, senolytic drugs, such as dasatinib and quercetin (D + Q), were able to reduce cellular senescence in the ovary of obese mice, without affecting the ovarian reserve. [114]. In young ovaries exposed to chemotherapy, an increase in cellular senescence was also observed, which was prevented by treatment with D + Q [115]. Furthermore, metformin along with D + Q was able to reduce ovarian senescence even more, maintain regular estrous cycle, and prevent excessive primordial follicle activation [116].

NAC is a stable form of the essential amino acid L-cysteine. NAC is converted to L-cysteine after ingestion and subsequently converted to glutathione [117]. NAC has important antioxidant and anti-inflammatory properties [117]. Animal studies have observed that NAC is able to reduce ovarian damage by upregulating local antioxidant capacity and reducing ovarian secretion of pro-inflammatory cytokines [118, 119]. In humans, the antioxidant action of NAC contributed to reduce cell damage in ovarian tissue cryopreservation protocols [120, 121]. NAC also has an antifibrotic action, already described in tissues such as muscle, aorta, and kidney [122–124]. Therefore, although there are no studies evaluating the long-term effect of NAC on ovarian physiology, these properties may contribute to delaying ovarian aging. There are no animal or human studies evaluating a possible impact of SGLT-2 inhibitors, acarbose, methylene blue, ACEi/ARB, and aspirin on ovarian aging.

We found no clear evidence in the literature that these intervention strategies can have a negative impact on overall fertility in animal models and humans. However, it should be noted that interventions that prevent ovarian aging result in retention of follicles in the primordial stage, which will have a negative effect on fertility during the period of the intervention. For example, in mice under 30% CR, follicles progress slower to secondary and tertiary stages, and, therefore, we observe a preserved pool of primordial follicles [82], which is desirable in the long term. However, as these follicles are not progressing to the secondary and tertiary stages, we observe a reduction in ovulation rate, reflecting in decreased fertility during the course of CR [78]. However, once mice are put back in ad libitum, feeding pregnancy rate increases [78] and is maintained above control group in aged mice due to preserved

ovarian reserve [61]. Therefore, in a proposed dietary/pharmacological intervention protocol to preserve the ovarian reserve, one should consider removing the treatment once pregnancy is desired. However, more studies are necessary to understand how long these interventions should be applied and how long before pregnancy is desired they should be interrupted, in order to maximize preservation of the ovarian reserve without compromising fertility.

New possibilities for ovarian anti-aging interventions

Recently, numerous studies, including those by Johnson et al. and White et al., have described the presence of ovarian germline stem cells (GSCs) in ovaries of non-mammals, mammals, and humans [125–128]. These findings began to question the dogma, established by Zuckerman in 1951, of the inability of new ovarian germ cells to arise [129].

Currently, studies seek to define protocols for ovarian rejuvenation or ovarian regeneration, through interventions that stimulate the emergence of new follicular units from GSCs. Injection of platelet-rich plasma (PRP) and stem cells into the ovaries are the two most studied treatments at present, especially in patients suffering from ovarian insufficiency and low ovarian reserve or undergoing IVF treatments with a poor prognosis [130–132].

In 2018, Sills et al. were the first to describe that intraovarian injection of autologous PRP significantly improved the response of four patients with diminished ovarian reserve as determined by at least one prior IVF cycle canceled for poor follicular recruitment response. Decreased FSH and increased AMH levels were also observed after the intervention. All four patients had at least 1 day five blastocyst available for cryopreservation [130]. Currently, numerous studies are being conducted to validate the use of PRP as an alternative treatment for female infertility associated with low ovarian reserve [133, 134].

Intraovarian injection of mesenchymal stem cells (MSCs) is another promising alternative to stimulate ovarian rejuvenation. MSCs can originate from several sources, such as amniotic fluid, endometrium, germ cells, skin, umbilical cord, bone marrow, and adipose tissue [131]. Stimulation of angiogenesis and cell proliferation, a decrease of apoptosis, modulation of immune cells function, and regulation of

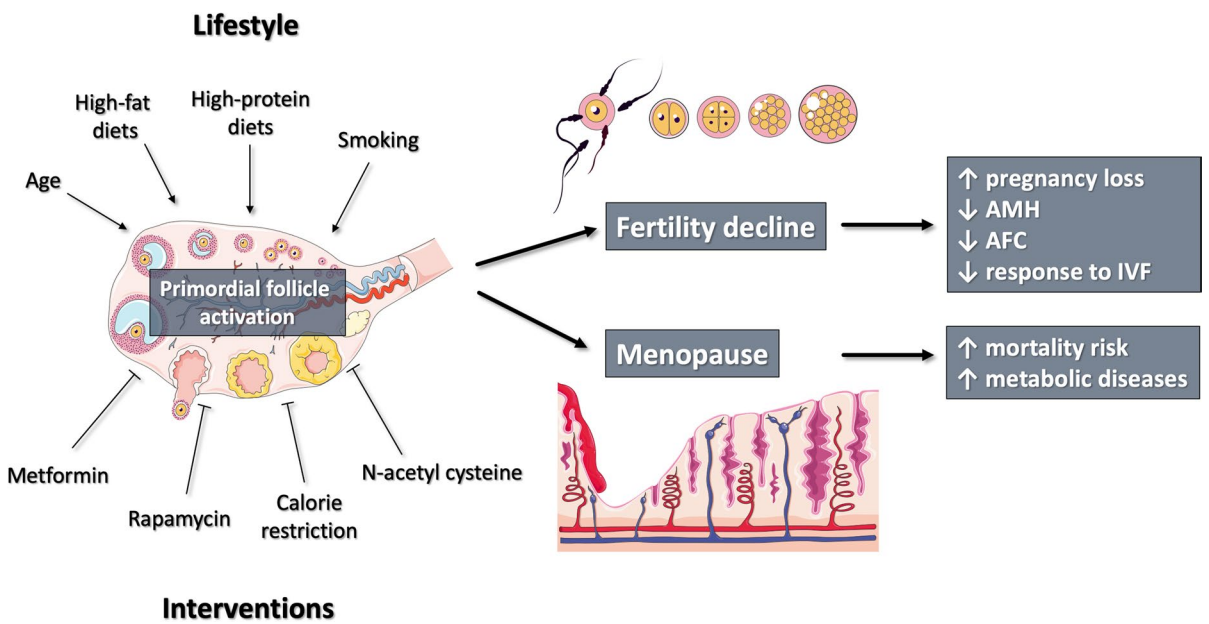

Fig. 1 Ovarian aging results from activation of primordial follicles. This decline in the ovarian reserve leads to reduce fertility and menopause later in the female life. Despite some lifestyle factors are a risk for decreased ovarian reserve, some strategies can prevent this decline

gene expression in the ovary are possible mechanisms involved in the process of ovarian rejuvenation that are observed after MSC transplantation [131]. However, clinical studies are needed to evaluate the impact of treating women with low ovarian reserve undergoing IVF cycles.

Conclusion

Based on the evidence presented in this review, it becomes clear that the ovarian reserve has a central role in the female life. The progressive decline in the ovarian reserve with age is natural; however, it can be accelerated by several factors, like diet and lifestyle. Once the ovarian reserve starts to become severely compromised, females experience a decline in natural fertility, as well as in the response to assisted reproductive technologies. Later in life, once this ovarian reserve is depleted, females experience changes in the body physiology, resulting in increased chronic disease and mortality risk. Therefore, the use of strategies to prevent the decline in the ovarian reserve should be investigated to improve fertility, healthspan, and lifespan. There are several drugs that can be repurposed for this aim; however, we need to better understand the best moment that these therapies must be initiated to promote the desired effects (Fig. 1).

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Declarations

Conflict of interest The authors declare no competing interests.

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