Ovarian Aging: Possible Molecular Mechanisms with Special Emphasis on DNA Repair Gene BRCA1

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Abstract
In last few decades, ovarian aging has been considered as one of the most crucial factors contributing to infertility. Ovarian aging occurs as a consequence of a progressive decline in number and quality of oocytes sequestered in primordial follicle with advancing age and finally leads to menopause with exhaustion of ovarian follicles. Ovarian aging is a multifactorial process in which physiological, genetic and environmental factors play a major role. However, the cellular and molecular mechanism involved in ovarian aging is not fully understood, but continually being explored. The emerging results from clinical and experimental studies suggest that the accumulation of double strand breaks due to DNA damage and decline in DNA repair genes in particular BRCA1 contribute to ovarian aging. The oocytes from women with BRCA1 mutation exhibit early menopause and deficiency in double strand break repair. The recent findings from our group and others demonstrated age-related changes in the efficiency of BRCA1 related DNA repair in primordial follicles. In this review we summarize possible endocrine, genetic and other factors which contribute to ovarian aging along with recent advances which suggest a link between ovarian follicle reserve, double strand breaks and BRCA1 gene expression.

Keywords: Ovarian Aging; Primordial Follicles; BRCA1; Double Strand Breaks; DNA-DSB Repair

Introduction
It has long been known that the female fertility decreases with increasing age and the normal process of reproductive aging varies considerably among women [1-3]. Female reproductive aging appears to be largely based on age related changes in ovarian function. Although quite a bit of information is available on the hormonal changes during ovarian aging, the molecular mechanisms behind the observed gradual decline of the follicle pool and the reduced oocyte quality are only being understood in the past few years. The fertility of mammalian females is dependent upon the synchronized development of follicles and oocytes, contained within them. The duration of the female fertile life span is influenced by the number of primordial follicles containing diploctene oocytes surrounded by one layer of cuboidal granulosa cells arrested in the first meiotic prophase stored in the ovary [4]. In particular, the progression of meiosis, wherein a germ cell-specific cell division decreases the number of chromosomes from diploid to haploid, must be arrested until just before ovulation. It has been known for several years that the somatic cells of follicles arrest the maturation of oocyte until both the follicle and the oocyte are fully developed. Eventually it will undergo ovulation and fertilization [5]. Follicular somatic cells are known to impose this arrest, which is required for co-ordinated oocyte–follicle development. The primordial follicle reserve decreases with increasing age, resulting in cessation of reproduction and menopause. A better understanding of the oocyte biology is essential in this new generation of personalized medicine, as it can contribute to biomarker development, innovative therapeutic interventions, and new model to provide personalized treatments that are most likely to be efficient but with minimal toxicity.

Follicular Depletion and Ovarian Aging
The reproductive aging is associated with a progressive decrease in both the quantity and the quality of the oocytes enclosed in the follicles present in the ovarian cortex [6]. The only estimates of actual primordial follicle numbers across the human female reproductive cycle are based on analysis of fetal ovaries at birth or limited clinical samples and changes in the quality of the granulosal cells surrounding the oocyte [7,8]. The female of all human, primates and domestic species have a finite stock of germ cells that is established in fetal life. Once primordial follicles are recruited from the pool of primordial follicles, they can progress to primary, secondary, antral, and eventually pre-ovulatory follicle stages, the classification of which has been well established during folliculogenesis [9]. For instance, the fetal ovaries of mice, cattle, and humans are born with an average of 250, 210, and 6800 million germ cells, respectively [10]. Despite a large initial follicle pool, only about 1% of the follicles reach the pre-ovulatory stage during the female reproductive life, with the majority undergoing atresia either directly from arrested or activated form throughout postnatal life [9, 11].

Early Predictors and Hormonal Control of Ovarian Aging
The event that can be easily recognized in ovarian aging are cycle irregularity and menopause which are mainly due to serum hormonal changes. The first subtle clinical sign of advancement in the reproductive aging process is a decrease of the length of the menstrual cycle by 2–7 days [3,6]. It is only when menstrual cycles become irregular one can first observe the signs of the continued reduction in follicle numbers. The onset of the menopausal transition period occurs on average at the age of 46 years, with a range of 34 to 54 years. The final menstrual period (menopause) can only be recognized by an increase in serum follicle-stimulating hormone (FSH) and decreases in estradiol and inhibin. These are the main endocrine changes that occur at the time of transition to menopause [1,12]. FSH levels are higher than luteinizing hormone (LH) levels, and these two hormones will rise to even higher values than those seen in the surge during the menstrual cycle [1,13]. The FSH rises prior to LH rise; FSH is the main predictor for ovarian failure [14]. A large cyclical variation of estradiol and estrone is witnessed during the years of menstrual cessation. Discrepancy in levels is small and inconsequential, with the mean value being considerably lower. It is clear that the changes in serum hormonal levels indicate the status of follicle as well as the beginning of the menopause when there is a rapid fall in the follicle number. Thus the decreasing numbers of follicles, coinciding with decline in oocyte quality, dictate the progressive changes in menstrual cycle regularity and monthly fecundity. With female aging, the decline in primordial follicle numbers parallels the decrease in size of the FSH-sensitive antral follicle cohort [15]. Generally a rough estimate can be made from the time of occurrence of menstruation to menopause as 150 to 200 maturing follicles [Antral]. The only available clinical method to determine the actual number of follicles cohort is responsiveness to stimulation of external FSH. The responsive follicles show a morphologically normal oocyte and it is assumed to be healthy.

Anti-Mullerian hormone (AMH) is a glycoprotein dimer mainly produced by granulosa cells of preantral (primary and secondary) and small antral follicles [16]. When follicles undergo transition from the primordial to the primary stage, production of AMH commences and extended until the follicles have reached the mid-antral stages with diameters of 2–6 mm [16]. Although AMH primarily controls follicle development, measurable quantities of AMH also appear in serum. The small antral follicles count is directly related to the overall volume of the primordial follicle pool. The decrease in AMH serum levels is associated with decrease in antral follicles number with increasing age and AMH is not detectable when women
experience menopause [17]. AMH serum levels represent quantitative ovarian reserve in the patients undergoing in-vitro fertilization (IVF) and may provide an indication of age at menopause [17,18].

The inhibins are heterodimeric protein hormones containing two subunits α,β, and β; inhibin B, and inhibin B, respectively. The biology of the inhibin-B containing cell products, with inhibin B secreted mainly at the time of follicular phase by the developing cohort of antral follicles. Inhibin inhibits the production of FSH and is mainly involved in the control of the production of gametes and embryonic and fetal development [19,20]. The decline in the secretion of inhibin B levels results in reduction of cohort size with increasing age, and it is also associated with elevated FSH levels and with diminished oocyte quality and reproductive capacity [21]. Apparently Inhibin B may be a good indicator of ovarian activity than of ovarian reserve, because it controls follicular development and dominant follicle selection. However, both combination of AMH and inhibin B might be used as an indicator for ovarian aging and also to assess integrity of the follicle recruitment process in predicting the possibility of ovulation.

Ovarian aging refers to a process in hypothalamic–pituitary–ovarian (HPO) axis that controls ovarian cycles resulting in menopause [22-25]. The pulsatile gonadotropin secretion in women is continuously influenced by ovarian follicles, while others affect the recruitment, activation, and various decrease in pulsatile release of GnRH and LH with increasing age and a loss of positive feedback response to estradiol, is one of the reasons for arrest of follicular growth and maturation in spite of the presence of enough numbers of functional ovarian follicles [26,27]. Altered pituitary response to estradiol feedback has been demonstrated in peri-menopausal women. This altered feedback maybe due to a lack of steroid action and also as a result of contribution of ovarian factors. The functional changes in the hypothalamic-pituitary unit as described in the pre- and peri-menopausal women are not a main process, but merely represent alterations in ovarian feedback.

**Molecular Aspects of Oocyte/Ovarian Aging**

**Genetic Factors**

Several factors can affect the oocyte quantity and quality in the aging ovary. In recent years, the role of genetic factors regulating the complex genetic trait of ovarian aging has received wide attention. The age of natural menopause is highly heritable [28]. The results of estimation of heritability of normal menopausal age revealed an average of 60% of known hormonal factors (genes such as FSH, LH, etc.) affect the ovarian follicular function [29,30] while others affect the recruitment, activation, and various stages of folliculogenesis (genes such as BMP15, GDF9, etc.) [1,29-31]. Any changes due to mutation of these genes can give rise to variation in reproductive life span. For example, NOBOX (new-born ovary homeobox gene) is a homeobox gene that is specific for oocyte and plays a critical role in early folliculogenesis and mutation of this gene was observed in women with early menopause (POF) [32,33]. Mutations in GDF9 and BMP15 were also observed in women with POF [34]. Recent genome-wide association studies (GWAS) have identified several genetic variants that are associated with menopause and primary ovarian insufficiency; the identified genes were mainly involved in DNA repair and maintenance as well as in immune function [35].

**Changes in Chromosomes**

After the sequence of events, like oogenesis, DNA replication and ovarian steroids and peptides, and recombination occur and the meiotic cell cycle arrest takes place in oocytes at the diplotene stage in fetal life [36]. Depending on the species, the primordial follicles are in dormant stage for years/months and they are recruited for growth and ovulation during reproductive life [37]. In the prophase-arrested oocytes, a noticeable bivalent homologous chromosome connected with each other by the chiasma is observed. This chiasma is formed by sister chromatid cohesion and maintenance of chiasmata in prolonged-arrested oocytes is major cause for increased chromosome miss segregation including aneuploidy (abnormality in chromosome number) with increasing maternal age [5,9,38]. Recent studies using mouse models explored that the weak centromere and/or sister chromatid cohesion is responsible for the increasing errors in chromosome segregation in oocytes with increasing age [39]. This is mainly due to separated sister chromatids and aneuploidy with single chromatids. The damage in cohesion exhibit reduced expression of centromeric protein called Shugoshin-2 (SGOL2) in chromosomes. This protein is mainly involved in preserving the integrity of the multi-protein cohesion complexes [40]. In the mouse model, 90% aneuploidy in oocytes from old mice are related to weakened centromere cohesion. Degradation of cohesion over time (often due to failure to replace new cohesion complexes), may cause premature partition of sister chromatids leading to age-dependent increase in aneuploidy. The naturally aging mice models revealed not only chromosomal segregation errors but also increased aberrations in spindle organization and defects in histone modifications with increasing maternal age [41]. Similar age related defects were observed in human oocytes and the association of age, aneuploidy and miscarriage has been established in women undergoing IVF [38].

**DNA damage, DNA-DSB repair and BRCA1 in ovarian aging**

DNA damage has potential role to induce infertility. The integrity of the nuclear DNA is constantly being insulted by environmental factors and also by various physiological processes that take place within the cell like increased free radical generation, oxidative damage and aging [42]. In the mammalian cell, it has been estimated that as many as several thousand and DNA lesions can occur every day and the complex network of control mechanisms also called genome surveillance system is involved in nuclear DNA damage. There are several types of DNA damage and number of mechanism that can detect and repair various types of damages, and each one is dedicated to a particular category of DNA lesions. In humans, several DNA repair genes have been identified. In parallel to activation of DNA repair mechanisms, the various types of apoptosis are also triggered when the DNA repair fails [42]. Moreover, the DNA damage is particularly problematic for non-dividing cells, especially oocytes arrested in primordial follicles, because the oocyte genome is constantly challenged by two important processes such as the meiotic recombination during the fetal life and very long post-natal period of meiotic arrest (diplotene stage) leading to completion of the meiotic divisions. Of the various type of DNA damage, double strand breaks (DSBs) are most lethal form because it not only difficult to repair but also re-joining of unrelated ends [43]. During meiotic recombination hundreds of DSBs are formed, if left unrepaired the damage will tend to accumulate over time due to the absence of the mechanisms to repair and will lead to cell death. In fact, even a single DNA-DSB is sufficient to cause apoptosis, or can directly inactivate key genes, lead to chromosomal abnormalities and these effects can be highly detrimental for female fertility [43].

The tumor suppressor gene BRCA1 (Breast Cancer 1) promotes homologous recombination (HR), a mechanism to repair DNA-DSBs that develop during normal replication and in response to DNA damaging agents. BRCA1 and BRCA2 gene belong to the family of ataxia telangiectasia-mutated (ATM)-regulated DNA-DSB repair genes that play a major role in protecting DNA integrity. Recent studies based on rodents and human from our laboratory and others demonstrated that a decrease in BRCA1 and BRCA2 expression levels (HPO) axis that controls ovarian cycles resulting in menopause [22-25]. The decline in the secretion of inhibin B levels results in reduction of cohort size with increasing age and a loss of positive feedback response to estradiol, is one of the reasons for arrest of follicular growth and maturation in spite of the presence of enough numbers of functional ovarian follicles [26,27]. Altered pituitary response to estradiol feedback has been demonstrated in peri-menopausal women. This altered feedback maybe due to a lack of steroid action and also as a result of contribution of ovarian factors. The functional changes in the hypothalamic-pituitary unit as described in the pre- and peri-menopausal women are not a main process, but merely represent alterations in ovarian feedback.

The existing information on the role of BRCA1 in reproductive aging and ovarian function mostly come from BRCA1 mutation carriers [47,48]. Women with BRCA1 gene mutation show increased risk of breast and ovarian cancer and exhibit rapid ovarian aging in addition to several other malignancies, such as pancreatic cancer, and melanoma. A decline in ovarian follicle reserve, tendency to premature menopause and decreased AMH level have all been observed in patients with BRCA1 gene mutation compared to women without BRCA1 mutation [49,50]. Also the carriers of mutation of BRCA1 show low response to external ovarian stimulation [51]. The effect of mutation of BRCA2 on ovarian function may be less noticeable and others described decreased and action of normal BRCA2 gene. The results of experimental animals has shown that a mutation of ATM in mice exhibits diminished growth (smaller in body size), meiotic defects in ovary, decreased ovarian follicle reserve and disrupted somatic cell growth in both sexes [52]. Mutations of both BRCA1 and ATM show more resemblance in phenotype, including aberrations in G2/M phase cell cycle checkpoint and response to DNA damage with DNA repair gene 53. Based on recent findings and clinical observations, we suggest a strong link between ovarian follicle reserve, double strand breaks and BRCA1 gene expression.

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In addition to BRCA1, RAD51, and ATM genes analysed in our study, we observed down-regulation of ERCC2 (excision repair cross-complementing group 2) expression in aged rat primordial follicles compared to immature follicles [45]. This is a nucleotide excision repair (NER) gene and its protein product is a member of BTF2/TFIH transcription factor complex which is important for repair of both damaged bases and single strand breaks (SSBs). Our recent comparative proteomic analysis of primordial follicles from immature and aged rat ovaries also revealed disturbance in DNA repair capacity [53]. Down-regulation of fidgetin-like 1 (FIGNL1) and up-regulation of Bcl-2 related ovarian killer (BOK) were found which are involved in DNA repair and apoptosis, respectively in aged primordial follicles. The increase in ERCC2 and Fig11 and decrease in BOK indicate that aged rat primordial follicles have diminished DNA repair capacity and they are prone to apoptosis.

Conclusions
The ovarian follicle number and quality is directly associated with ovarian aging. Several reproductive hormones are closely linked with the oocyte pool and currently the serum levels of FSH, inhibin B and AMH are used as predictors of ovarian reserve, age at fertility loss and menopause. Nevertheless, it is important to understand the molecular mechanism that impact ovarian reserve which may provide greater insight on the process of reproductive aging and the genetic marker that helps in diagnosing women at risk for early ovarian failure and fertility defects. In recent clinical and experimental studies, the accumulation of double strand breaks and decrease in the expression of DSB repair gene BRCA1 are seen with decrease in ovarian follicle reserve and in the aging ovary. This indicates that decline in DNA-DSB repair capacity is a mechanism for ovarian aging. However the detailed study is warranted for mechanism of BRCA1 function in the maintenance of oocyte health and ovarian reserve.

References


