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BRCA-related ATM-mediated DNA double-strand break repair and ovarian aging

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BACKGROUND: Oocyte aging has significant clinical consequences, and yet no treatment exists to address the age-related decline in oocyte quality. The lack of progress in the treatment of oocyte aging is due to the fact that the underlying molecular mechanisms are not sufficiently understood. BRCA1 and 2 are involved in homologous DNA recombination and play essential roles in ataxia telangiectasia mutated (ATM)-mediated DNA double-strand break (DSB) repair. A growing body of laboratory, translational and clinical evidence has emerged within the past decade indicating a role for BRCA function and ATM-mediated DNA DSB repair in ovarian aging.

OBJECTIVE AND RATIONALE: Although there are several competing or complementary theories, given the growing evidence tying BRCA function and ATM-mediated DNA DSB repair mechanisms in general to ovarian aging, we performed this review encompassing basic, translational and clinical work to assess the current state of knowledge on the topic. A clear understanding of the mechanisms underlying oocyte aging may result in targeted treatments to preserve ovarian reserve and improve oocyte quality.

SEARCH METHODS: We searched for published articles in the PubMed database containing key words, *BRCA*, *BRCA1*, *BRCA2*, Mutations, Fertility, Ovarian Reserve, Infertility, Mechanisms of Ovarian Aging, Oocyte or Oocyte DNA Repair, in the English-language literature until May 2019. We did not include abstracts or conference proceedings, with the exception of our own.

OUTCOMES: Laboratory studies provided robust and reproducible evidence that BRCA1 function and ATM-mediated DNA DSB repair, in general, weakens with age in oocytes of multiple species including human. In both women with *BRCA* mutations and *BRCA*-mutant mice,

primordial follicle numbers are reduced and there is accelerated accumulation of DNA DSBs in oocytes. In general, women with *BRCA1* mutations have lower ovarian reserves and experience earlier menopause. Laboratory evidence also supports critical role for BRCA1 and other ATM-mediated DNA DSB repair pathway members in meiotic function. When laboratory, translational and clinical evidence is considered together, BRCA-related ATM-mediated DNA DSB repair function emerges as a likely regulator of ovarian aging. Moreover, DNA damage and repair appear to be key features in chemotherapy-induced ovarian aging.

WIDER IMPLICATIONS: The existing data suggest that the BRCA-related ATM-mediated DNA repair pathway is a strong candidate to be a regulator of oocyte aging, and the age-related decline of this pathway likely impairs oocyte health. This knowledge may create an opportunity to develop targeted treatments to reverse or prevent physiological or chemotherapy-induced oocyte aging. On the immediate practical side, women with *BRCA* or similar mutations may need to be specially counselled for fertility preservation.

Key words: *BRCA* / *BRCA1/2* / ovarian aging / mutations / oocyte / DNA repair / ovarian reserve / chemotherapy / ovarian response / anti-Mullerian hormone

Introduction

Women are born with a finite number of quiescent primordial follicles that first form in foetal ovaries around the end of the first trimester of pregnancy (Pelosi *et al*., 2015). These follicles are considered as the source of oocytes for entire female reproductive life, and menopause occurs when nearly all follicles are depleted (Faddy *et al*., 1992). During this protracted period, the majority of the primordial oocytes remain arrested in the first meiotic prophase and may be subjected to various endogenous and exogenous insults that may cause DNA damage (Roos and Kaina, 2013). DNA damage can occur in the form of both single- and double-strand breaks (DSBs) (Lips and Kaina, 2001). DNA DSBs represent the most deleterious and complex type of DNA damage, which may result in chromosomal instability and failed rearrangements (Vilenchik and Knudson, 2003). If DNA DSBs are not promptly and accurately repaired, they may cause lethal consequences for cells, including severe mutagenesis, carcinogenesis and apoptotic cell death (Roos and Kaina, 2006; Cohen *et al*., 2015). There are two main mechanisms of DNA DSB repair: nonhomologous end joining (NHEJ) and homologous recombination (HR) repair (Petersen and Cote, 2004). HR is based on using genetic information from a corresponding undamaged region on homologous chromosomes to replace the deleted information on the damaged strand, whereas two broken ends of the chromosome are 'glued' back together in NHEJ (Hustedt and Durocher, 2016). Because NHEJ cannot always replace the lost genetic information as it typically does not have access to a homologous strand for replication, with few exceptions, it is considered the error-prone repair mechanism for DNA DSBs (Cannan and Pederson, 2016). Although NHEJ is the main mode of DSB repair in mitotic cells and those that are in the G0–G1 phase of the cell cycle, HR plays the predominant role in cells that are in S-G2/M phase of the cell cycle and is likely to be the main pathway of choice responsible for DNA damage repair in oocytes (Kujjio *et al*., 2010). As HR uses an intact sister chromatid for regenerating the lost information in DNA and is tightly regulated by the Ataxia telangiectasia mutated (ATM)-mediated signalling pathways, it is a high fidelity, 'error-free' repair mechanism. Because it requires the availability of sister chromatids for repair and because primordial follicles are arrested at the G2/M phase of the cell cycle, and because error-free repair of genetic information is critical in germ cells, HR is hypothesised to be the physiologically dominant DNA repair mechanism in oocytes. However, recent data suggest that the NHEJ and HR pathways may not be entirely exclusive as BRCA may also be involved in the NHEJ type repair (Wu *et al*., 2010; Saha and Davis, 2016). Although it is not likely to be a main mechanism of repair in oocytes, further investigation is warranted on the role of NHEJ in ovarian aging.

BRCA1 and 2 are involved in homologous DNA recombination and play essential roles in ATM-mediated regulation of the DNA DSB repair (Venkitaraman, 2012). Numerous mutations in *BRCA* genes are associated with increased susceptibility to breast, ovarian and other cancer types (Lowery *et al*., 2018; Rao *et al*., 2018). While developing an ovarian stimulation protocol with aromatase inhibitors to reduce oestrogen exposure in women with breast cancer undergoing *in vitro* fertilisation for fertility preservation, we observed that women with *BRCA* mutations produced fewer oocytes and proposed that altered DNA DSB repair may be responsible for accelerated ovarian aging in these women (Oktay *et al*., 2010). Subsequent to these observations, we and others have completed numerous basic, translational and clinical studies investigating that hypothesis. In this manuscript, we will systematically review the evidence originating from those studies and summarise the current understanding of the role of DNA repair and *BRCA* mutations in human reproduction.

Methods

We searched for published articles in the PubMed database containing key words, *BRCA*, *BRCA1*, *BRCA2*, Mutations, Fertility, Ovarian Reserve, Infertility, Mechanisms of Ovarian Aging, Oocyte and Oocyte DNA Repair, in the English-language literature until May 2019. We did not include abstracts or conference proceedings because the data are usually difficult to assess. Out of 2972 articles identified initially, by cross-referencing 96 were found to be relevant and were evaluated carefully. Of these articles, 69 were laboratory studies, 26 were clinical studies and 1 was translational, including both laboratory and clinical data (Titus *et al*., 2013) (Fig. 1).

Overview of ATM-Mediated DNA DSB Repair via HR

While a detailed review of the ATM-mediated DNA DSB repair does not belong to this manuscript, we provide a brief explanation of the current understanding of this pathway to enhance our interpretation of the translational and clinical data (Fig. 2). As the focus of this review is human reproduction, we also highlighted the aspects of the pathway relevant to oocytes and meiotic cells in this brief description. DSBs

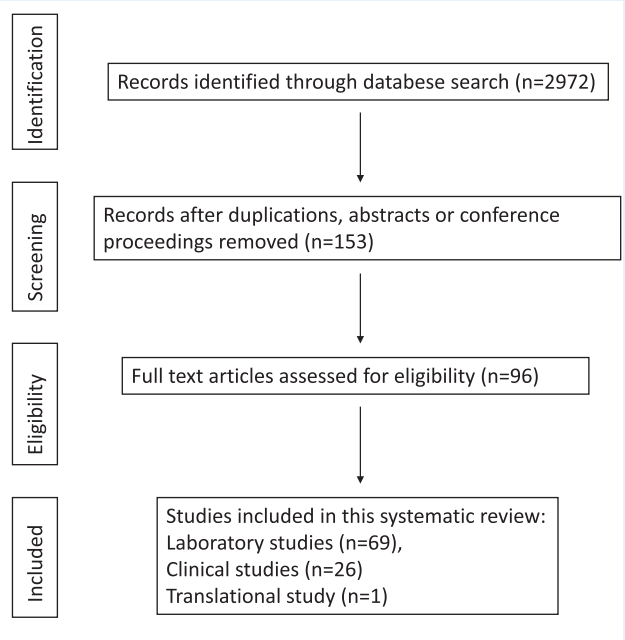

Figure 1 Flow chart of study selection.

represent a type of DNA damage in which two complementary strands of the double helix of DNA are damaged due to physical, chemical and biological stressors (Ashwood-Smith and Edwards, 1996). These severe brakes are first sensed by the MRN complex (MRE11, Rad50 and NBS1 complex) which not only activates ATM, the subsequent orchestrator of the HR repair pathway, but also serves as a platform for DNA repair (Lamarche *et al*., 2010; Dinkelmann *et al*., 2009; Titus *et al*., 2013). At the same time, Histone H2AX protein is phosphorylated (and is now called γ-H2AX) and attracted to DNA DSB sites to facilitate repair. This binding is on a one-to-one basis, and as a result, DNA DSBs can be quantitated by visualisation of γ-H2AX foci by immunohistochemical methods and this serves as a reliable method of DNA DSB assessment (Lowndes and Toh, 2005). γ-H2AX also has an activating effect on ATM which in turn activates other repair proteins, such as the BRCA1 and BRCA2 as well as check point regulators in the pathway. A partner in sensing DNA DSBs is 53BP1 which is also attracted to DNA DSB sites and activates ATM (Jackson and Bartek, 2009). BRCA1 has a more complex role in the pathway, compared to BRCA2, as it plays roles in damage sensing and HR as well as check point activation, while BRCA2 is mainly involved in the HR repair. This physiological distinction is important because, as will be seen later in the discussion, *BRCA1* dysfunction appears to have more prominent and earlier impact on reproductive aging than *BRCA2* dysfunction.

The activation of the ATM-mediated DNA DSB Repair Pathway leads to three potential outcomes. First and most desirable is the successful repair of the DSBs, as explained in Fig. 2. However, when the DNA damage is beyond repair, two other mechanisms have evolved to prevent cells with severe mutagenic information propagating themselves. One of these outcomes is c-ABL and/or TAP 63-alpha mediated activation of apoptotic pathways which result in the elimination of the damaged cells (Kerr *et al*., 2012; Hutt *et al*., 2013), although the role of ABL1 and ABL2 in activating apoptosis in response to cisplatinum treatment in oocytes has been challenged (Kim *et al*., 2018). However, commonly used gonadotoxic drugs in cancer treatment are cyclophosphamide and doxorubicin, and it is possible that different drugs may elicit different repair and damage responses. Regardless, this outcome may represent the main mechanism of age- and chemotherapy-induced accelerated primordial follicle loss, as will be discussed later. Another potential outcome is the prevention of these cells from progressing in the cell cycle by activation of cell cycle checkpoint proteins such as the CHK1. The latter results in what is called the cell senescence. This may represent the few primordial follicles that remain in the postmenopausal ovary and which never activate to result in follicle growth or ovulation.

Preclinical Assessment

Declining DNA DSB repair and accumulation of DNA DSBs in aging oocytes

Recent evidence, including our own results, suggests that the DNA DSBs accumulate with age, possibly due to reduced DNA repair capacity with age in the oocytes of humans and mice (Titus *et al*., 2013; Govindaraj *et al*., 2015; Oktay *et al*., 2015; Govindaraj *et al*., 2017). Following our initial observations of low ovarian response to stimulation in women with *BRCA* mutations (Oktay *et al*., 2010), we hypothesised that declining DNA DSB repair deficiency may be a factor responsible for age-induced accelerated primordial follicle loss and aging. To investigate this hypothesis, we took a combined laboratory and clinical approach (Titus *et al*., 2013). First, we showed that an increased percentage of primordial follicle oocytes were stained for γ-H2AX in ovaries of older females. Second, by single-cell real-time quantitative PCR, we showed that the expression of key ATM-mediated DNA DSB repair pathway members, such as *BRCA1*, *ATM*, *MRE11* and *RAD51*, declined with age in human germinal vesicle (GV) oocytes, particularly after age 37. The latter finding correlates with the accelerated loss of human primordial follicles after age 37 (Faddy *et al*., 1992) as well as the sharp decline of fecundity and oocyte quality after that age (Oktay *et al*., 2015). Interestingly, even though we did not detect an overall significant decline in the expression of *BRCA2* in GV oocytes with age in this group of women aged 23–41, (r-squared: 0.0253, slope −0.0171, $P = 0.75$), further analysis of the data revealed that *BRCA2* expression showed a trend for decline after age 36 (r-squared 0.4201, slope −0.0161, $P = 0.08$), i.e. in the terminal years of reproductive life and later than that of *BRCA1*. This difference may explain the seemingly lesser impact of *BRCA2* dysfunction on ovarian aging. While the above findings do not prove that the decline in the function of ATM-mediated BRCA-related HR repair is a vital mechanism of oocyte aging, our subsequent gene manipulation studies supported this notion. When key members of the ATM-mediated DNA repair pathway were knocked down, oocytes have become more susceptible to genotoxic stress, as will be discussed later (Titus *et al*. 2013).

Our finding of age-induced accumulation of DNA DSBs and reduced expression of DNA DSB repair genes has further been confirmed in several *in vivo* and *in vitro* studies in various species (Govindaraj *et al*., 2017; He *et al*., 2018). Govindaraj *et al*. recently showed considerable differences in the expression patterns of over 1000 genes involved

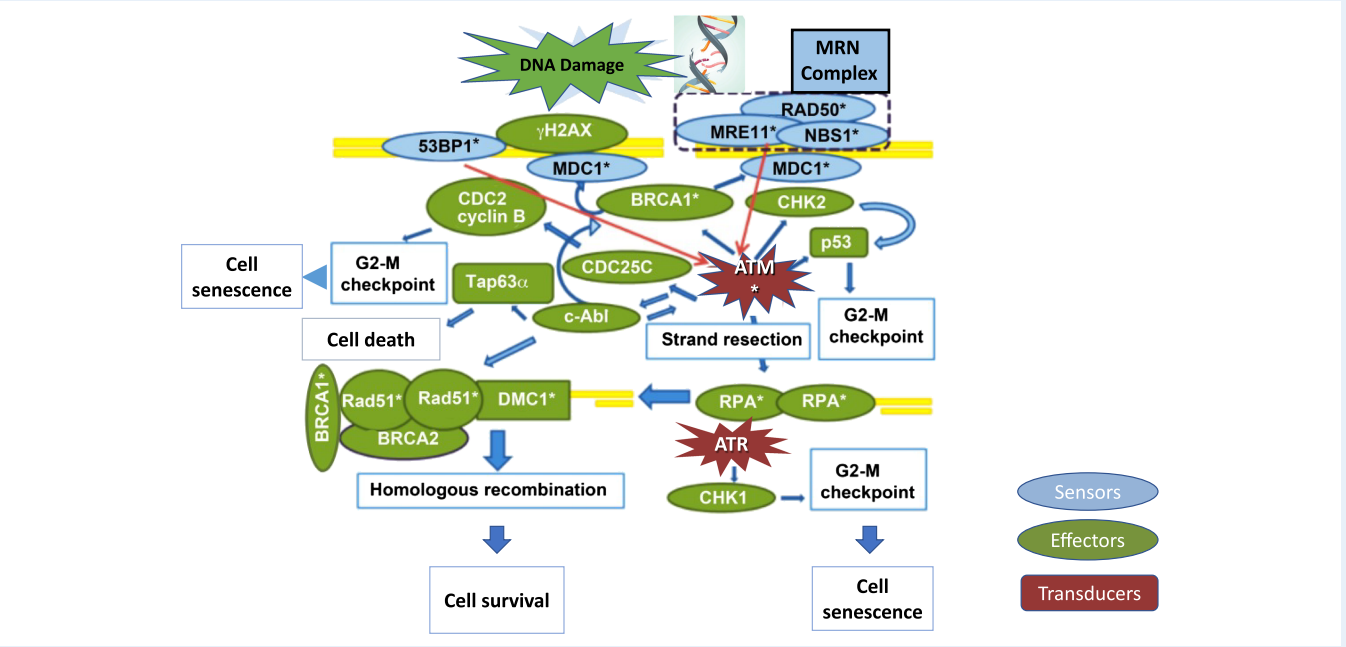

Figure 2 ATM-mediated DNA DSB repair pathway. DNA damage is sensed by the MRN complex (sensor of DSBs, consisting of MRE11, RAD50 and NBS1) and 53BP1 (sensor of changes in chromatin structure) which consequently activate ATM (red arrows). MDC1 binds to γH2AX via BRCA1 and is involved in the retention of the MRN complex to chromatin and accumulation of ATM, as well as mediation of the interaction between ATM and γH2AX. ATM phosphorylates γH2AX and activates downstream pathways leading to cell cycle arrest via CHK2 and inhibition of CDC2 (at the G2/M checkpoint, as applicable to oocyte), DNA repair (via activation of DNA strand resection which leads to homologous recombination) and/or apoptosis (via c-abl and TAp63α). DNA strand resection is necessary to invade in the homologous DNA strand. The resulting single-strand (ss) DNA is coated with RPA which in turn activates ATR and leads to cell cycle arrest (via CHK1). In germ cells, RPA is eventually replaced by Rad51 and DMC1 (the latter being germ-cell-specific) through a BRCA2-mediated process, which results in the initiation of homologous recombination. DSB sensor proteins are shown in blue, effectors are shown in green and transducers are shown in red. Molecules also involved in meiotic recombination are denoted with asterisk.

in the network of oocyte meiosis, chromatin stability, chromosome segregation, spindle formation, DNA repair, gene transcription and apoptosis between immature and aged rat primordial follicles (Govindaraj *et al*., 2017). They also observed a significant age-related decline in the expression of mRNA for *BRCA1* in primordial follicles of rats (Govindaraj *et al*., 2015). The same group extended their studies to water buffalo and studied the expression of *BRCA1* related DNA repair genes in primordial follicles of young and adult buffaloes and showed a significant decrease in mRNA levels of *BRCA1* in adult primordial follicles as compared to the young, which presumably resulted in inefficient DNA DSB repair (Govindaraj *et al*., 2016). While decreased expression does not directly prove that protein function is decreased, it is a highly probable explanation for age-related increase in DSBs in primordial follicles. Should the DNA DSB repair capacity have remained steady throughout life, one would expect steady levels of DSBs in primordial follicles throughout life due to constant repair. Furthermore, it may be questioned why the presence of DNA DSBs in primordial follicles does not immediately trigger apoptosis. Since we are looking at a static picture in tissue sections, we might be capturing the population immediately before apoptosis or, alternatively, there is likely a threshold of DSBs that has to be crossed before oocytes trigger apoptotic mechanisms and give up on repair. In fact, recent studies have suggested that, to enable repair to succeed, cells can tolerate a large number of DNA DSBs before triggering cell death mechanisms (Qiao *et al*., 2018).

In humans, microarray analysis of young and old metaphase II oocytes donated by patients undergoing ovarian stimulation has shown significant changes in the genes related to spindle checkpoint regulation, DNA stability and DNA repair. Moreover, it has been shown that responses to DNA damage and chromosome segregation are significantly affected by age (Grondahl *et al*. 2010). Others have shown that DNA damage in oocytes may result in a delay in meiosis resumption, the activation of spindle assembly checkpoint or germ cell apoptosis (Suh *et al*., 2006; Kerr *et al*., 2012; Marangos *et al*., 2015).

The importance of intact ATM-mediated DNA DSB repair via HR is apparent from many clinical syndromes. In syndromes resulting from mutations of the members of the ATM-mediated DNA DSB repair pathway, such as the Fanconi anemia (FA), ATM and Bloom (Uhrhammer *et al*., 1998; Thompson and Schild, 2002; Taniguchi T *et al*., 2006), females experience premature ovarian insufficiency and early menopause. Ovaries of mice with the Fanconi-gene mutation were found to be hypoplastic and the numbers of primordial follicles were reduced (Luo *et al*., 2004). Interestingly, *FANCD1*, one of the genes responsible for FA, is identical to *BRCA2* and it was demonstrated that other proteins that are involved in the same syndrome, specifically FANCA and FANCD2, interact with BRCA1 (Garcia-Higuera *et al*., 2001). However, there has not been a mechanistic investigation in these groups of patients to determine whether they are born with

lower endowment of primordial follicles or have accelerated follicle loss, or both.

However, genome-wide associations studies (GWAS) investigating the genetic determinants of age at natural menopause have provided further proof for the role of DNA repair in ovarian aging. Multiple GWAS have already identified several DNA repair genes as potential susceptibility genes of early natural menopause (Choi *et al*., 2007; Steuerwald *et al*., 2007; Sharov *et al*., 2008; Chowdhury *et al*., 2009). A large GWAS in ~70 000 women found genes related to DNA DSB repair, particularly *BRCA1*, to be critical in determining age at natural menopause (Day *et al*., 2015). This study also supported a common link between reproductive aging and breast cancer susceptibility.

A novel key component of the HR repair pathway, namely oocyte-expressed protein (Ooep), is required for efficient ATM kinase activation and Rad51 recombinase focal accumulation at DNA DSB locations. Ooep also plays a role in ATM activation and cell cycle checkpoint regulation (Xu *et al*., 2015; Zhu *et al*., 2015). It was recently found that Ooep-null GV oocytes are defective in DNA DSB repair, which results in increased susceptibility to apoptosis and delayed meiotic maturation upon exposure to DNA damaging insults (He *et al*., 2018). Notably, mRNA expression of Ooep in mouse oocytes was found to be reduced with advanced maternal age (He *et al*., 2018), consistent with the finding of an age-related decline in the expression of ATM-mediated DNA repair pathway members in women (Titus *et al*., 2013).

Some groups have also studied age-induced changes in DNA damage and repair in granulosa cells, although they are mitotic renewable somatic cells. A recent study found of γ-H2AX expression that there is age-induced accumulation of DNA DSBs in cumulus cells collected during oocyte retrievals (Sun *et al*., 2018). Aging cumulus cells showed a more frequent occurrence of early apoptosis and shortened telomere length than young cumulus cells (Sun *et al*., 2018). Likewise, Zhang *et al*. showed increased DSBs and decreased DNA repair efficiency in rhesus monkey granulosa cells due to ovarian aging (Zhang *et al*., 2015). However, in that study, the expression of *BRCA1* did not change, suggesting that the function of the *BRCA1* in granulosa cells is intact in middle-aged monkeys (Zhang *et al*., 2015). While the clinical significance of these findings remains to be understood, we have not found an increase in DNA damage in human primordial follicle pregranulosa cells, although we did not study DNA damage in granulosa or cumulus cells (Titus *et al*., 2013). It is, however, physiologically conceivable that the age-induced increased DNA damage in granulosa cells will contribute to declining oocyte health (Almeida *et al*., 2018).

Accelerated ovarian aging in BRCA-mutant mice

Other evidence on the role of BRCA function and DNA DSB repair efficiency in ovarian aging comes from transgenic mice deficient for *BRCA1* or *BRCA2* (Titus *et al*., 2013). In one study performed by us (Titus *et al*., 2013), the *BRCA1*-mutant mouse carried a deletion of 330 bp in intron 10 plus 407 bp in exon 11 (the largest exon) of the *BRCA1* gene, which was previously shown to result in inefficient DNA repair (Huber *et al*., 2001). Because homozygous mutation of *BRCA1* is lethal, we were only able to study the mice heterozygous for this deletion. For *BRCA2*, we studied mice that carried a deletion in exon 27, which impairs HR repair by preventing the BRCA2-RAD51 interaction (Donoho *et al*., 2003). Since this mutation does not result in lethality, both the heterozygote and homozygote mice were available for analysis. We found that *BRCA1*-mutant heterozygous mice produced fewer oocytes in response to ovarian stimulation compared with wild-type mice (14 ± 7.8 vs. 33.3 ± 0.9; $P < 0.05$) and had smaller litter sizes after mating (5.6 ± 1.5 vs. 7.6 ± 1.4 pups; $P < 0.05$). While we did not study the mechanism of reduced litter size in detail and ascribed it to lower ovarian reserve, this reduction could also be due to impaired embryo development, as our recent work with *BRCA1* mutant male mice (Stobezki *et al*., 2019) showed reduced blastocyst formation and implantation rates when *BRCA1* mutant males were crossed with wild-type females. The total primordial follicle numbers per ovary were lower in both the newborn (5-day) (2292.5 ± 163.8 vs. 3108 ± 96.1 $P < 0.01$,) and 4-month-old (408.3 ± 63.4 vs. 702.9 ± 79.5; $P < 0.05$) *BRCA1* mutant heterozygous mice compared with the wild-type mice. These data could suggest that *BRCA1* mutations may result in a lower endowment of primordial follicles, and since we did not study older mice, we do not know if follicle loss is accelerated in this model. However, while the extent of DNA damage as assessed by γ*H2AX* expression in primordial follicles was similar at birth, by 4 months of life a higher percentage of primordial follicles became γ*H2AX*-positive in *BRCA1* mutant mice compared with wild-type mice (75.7 ± 2.7 vs. 58.8 ± 3.6; $P < 0.01$). In contrast to *BRCA1* mutant mice, there were no differences between the *BRCA2* mutant homo- or heterozygote mice and wild-type mice regarding the same variables. Again, the similarities of findings between the studies in women and these transgenic models are striking. As will be seen in the later sections, ovarian aging appears to be predominantly enhanced in women with *BRCA1* mutations. As previously explained, this difference between BRCA1 and BRCA2 could be explained by the more complex role of the former in the ATM-mediated DNA DSB repair pathway and the much later decline in the function of BRCA2 with age.

BRCA gene manipulation alters sensitivity to genotoxicity

An independent approach to investigate the importance of DNA DSB repair in human oocytes is to use gene manipulation strategies. To achieve this, we knocked down the key ATM-mediated DNA repair pathway members, such as *BRCA1*, *ATM*, *MRE11* and *Rad51*, by small interfering RNAs (siRNA) in mouse GV oocytes and exposed them to genotoxic stress in the form of H_2O_2. These were compared to similarly exposed mock-injected oocytes (Titus *et al*., 2013). We found that in the knockdown group, the survival rates were lower, and the percentage of apoptotic oocytes (as assessed by anti-caspase-3 staining) and the γH2AX foci were higher compared to mock-injected oocytes. Interestingly, the overexpression of *BRCA1* by microinjection of cDNA in oocytes from old mice resulted in resistance to genotoxic stress and brought the *in vitro* survival to levels similar to that in young mouse oocytes. These data collectively show that intact ATM-mediated HR repair is acutely critical for oocyte survival and that, potentially, genetic manipulation of old oocytes can restore their ability to repair DNA DSBs to healthy levels.

Role of DNA DSB repair in chemotherapy-induced ovarian aging

We previously showed, in human ovarian tissue organ culture and xenografting models, that exposure to gonadotoxic chemotherapy

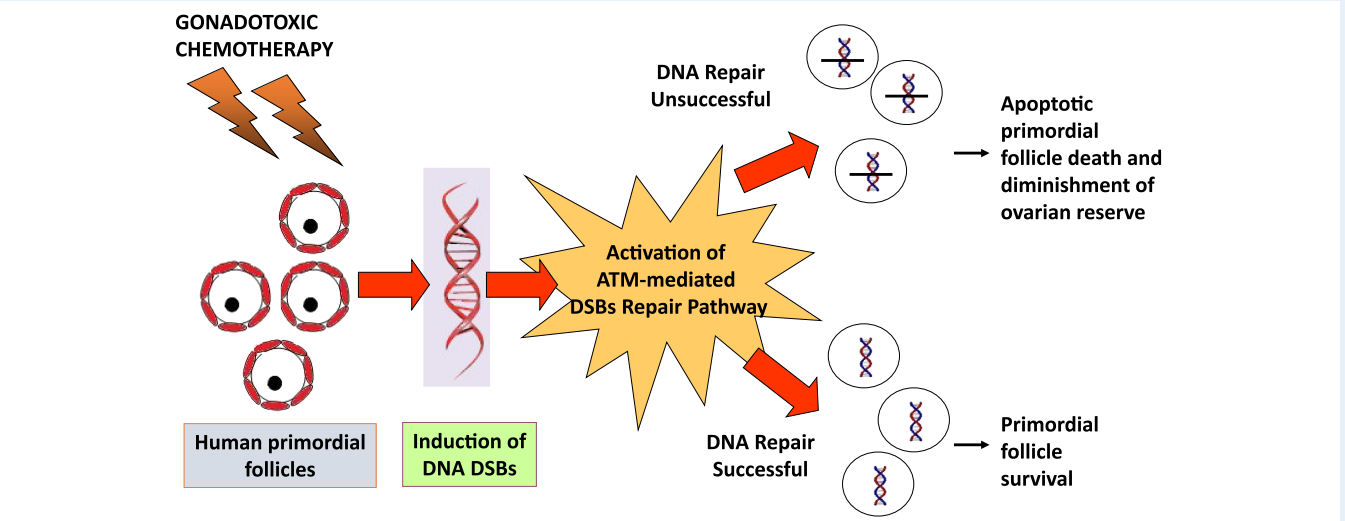

Figure 3 A proposed mechanism of chemotherapy-induced ovarian reserve loss through DNA damage. Primordial follicles have varying abilities to repair DNA double-strand breaks (DSBs) induced by gonadotoxic chemotherapy. When a primordial follicle suffers sufficient DNA damage which cannot be repaired by the ATM-mediated DNA DSB repair pathway, apoptotic pathways will be activated, resulting in follicle death. When on the other hand, DNA DSB repair is successful, the follicle will survive. This mechanism explains why not all follicles suffer the same fate after chemotherapy exposure, in most cases. Recent studies provided strong support for this hypothesis.

agents such as doxorubicin results in the induction of DNA DSBs in primordial follicle oocytes and triggers massive apoptotic death (Soleimani *et al*., 2011). We also observed similar results with cyclophosphamide, a commonly used alkylating agent (Li *et al*., 2014). We additionally found that exposure to gonadotoxic chemotherapy results in the activation of ATM-mediated DNA DSB repair pathways, potentially rescuing some oocytes from chemotherapy-induced death (Soleimani *et al*., 2011). In the study by Soleimani *et al*., we showed that doxorubicin induces DSBs in mouse follicles which results in the activation of ATM-mediated DNA DSB repair mechanisms. However, many follicles with activated ATM pathways remain apoptosis-free (by anti-caspase-3 staining), suggesting that there is reversal of chemo-induced damage. Based on these preliminary findings, we hypothesised that chemotherapy-induced follicle death is determined by the balance between the severity of damage and the ability of the oocytes to repair that damage (Fig. 3). According to this hypothesis, primordial follicle oocytes that cannot repair severe DNA damage are directed to apoptotic death via the ATM-mediated pathway while those that can be repaired via the same pathway survive. In fact, this theory could explain why all primordial follicles are not lost upon exposure to chemotherapy. We further hypothesised that primordial follicles with a better ability to repair DNA DSBs may have a survival advantage (Bedoschi *et al*., 2019; Soleimani *et al*., 2011; Govindaraj *et al*., 2014) and oocytes might first attempt DNA repair then initiate apoptosis if repair fails. Furthermore, since our data suggests that the ability to repair DNA DSBs might decline with age, this may also explain the increased liability to follicle loss in older individuals (Titus *et al*., 2013). Although a recent study is supportive of our hypotheses (Nguyen *et al*., 2019), future laboratory and translational studies will be needed for further confirmation.

Other studies have also supported that oocytes have the capacity for repairing DNA damage induced by chemotherapy and aging through ATM-mediated HR pathway and that such repair mechanisms can be altered by targeting Bax and Rad51 (Kujio *et al*., 2010). Those studies found an inverse relationship between *Bax* and *Rad51* expression. While the percentage of mature oocytes staining positive for Rad51 decreased with age, the Bax levels increased with age, and in a *Bax*-knockout model, oocytes exhibited improved DNA repair. These again suggest that there is a close relationship between chemotherapy-induced aging and ATM-mediated DNA DSB repair mechanisms.

Role of DNA DSB repair and BRCA gene function in oocyte quality

Age-dependent maternal aneuploidy is one of the key manifestations of ovarian aging (Anaya *et al*., 2013; Vaskivuo *et al*., 2001). Although the exact mechanism of the age-related increase in oocyte aneuploidy is not known, oocytes are exposed to numerous factors during the extended years that they remain in the prophase of first meiotic division which may impair oocyte quality (Jones, 2008). As DNA repair efficiency declines with age, DSBs accumulate and an increasing fraction of oocytes are eliminated to prevent genetic transition (Titus *et al*., 2013; Oktay *et al*., 2015; Rinaldi *et al*., 2017). It has been shown that failure to repair DSBs or misrepair may result in deletions, translocations and chromosome loss in oocytes (Di Giacomo *et al*., 2005; Cloutier *et al*., 2015). In mice, it has been shown that the declining function of BRCA1 may result in impaired meiotic spindle assembly and reduced chiasmata (Xiong *et al*., 2008). In addition, BRCA1 and the members of the ATM-mediated DNA DSB repair family are involved in the modulation of cohesins which regulate sister chromatid cohesion and migrate to DSB repair sites (Watrin and Peters, 2006). It has been demonstrated that in mouse oocyte chromosomes, the cohesin complex declines gradually as the mice age (Lister *et al*., 2010). In fact, evidence has been presented to show that weakened centromere cohesion is a leading cause

of age-related aneuploidy in oocytes (Chiang *et al*., 2010). Interestingly, a subunit of the Cohesin complex, SMC1, is regulated by the ATM-mediated HR pathway (Chiang *et al*. 2010), suggesting the possibility that the age-related decline in ATM-mediated DNA repair function may also negatively affect the function of cohesins. Furthermore, it has been demonstrated that old mouse oocytes may have shorter telomeres than those of young oocytes (Yamada-Fukunaga *et al*., 2013). BRCA1 and BRCA2 are also involved in telomere maintenance during aging process (McPherson *et al*., 2006; Cabuy *et al*., 2009; Rosen 2013) and *BRCA1* mutations result in shortened telomeres (Rosen, 2013).

Another mechanism by which declining ATM-mediated HR may affect oocyte quality is through reduced chiasmata maintenance and frequency. At prophase I, when primordial follicle oocytes are arrested, HR occurs between sister chromatids. The same DNA repair mechanisms that naturally occur during HR are used by oocytes to mend DSBs (Shinohara *et al*., 2000). While this process increases genetic diversity, it is possible that it also plays a role in stabilising the metaphase plate by creating physical bonds between the chromatids. In fact, the crossovers associated with the HR events can have stabilising or destabilising effect on the genome. In meiosis, crossovers are highly regulated, such that at least one crossover occurs between each pair of homologous chromosomes, to ensure proper chromosome segregation, but excess numbers of crossovers are suppressed (Champion and Hawley, 2002). In mice, it has long been observed that the frequency of chiasmata decreases with age (Henderson and Edwards, 1968) and, in some strains of mice, age-related increases in aneuploidy rates in the offspring are associated with decreases in the frequency of recombination between chromosome homologs (Jones, 2008).

To sum up, the existing data suggest a regulatory role for ATM-mediated DSB repair/HR mechanisms in oocyte meiotic function and chromatid cohesion. However, further laboratory and translational research will be required to determine whether a decline in the function of the ATM-mediated DNA DSB repair pathway may play a role in age-induced aneuploidy.

Clinical Assessment

Ultimate confirmation of the role of BRCA function and DNA repair in ovarian aging and reproduction should come for clinical studies. Within the past decade, there have been numerous studies that have assessed various aspects of ovarian function in women with *BRCA* mutations. These include those that have studied: (i) the oocyte yield after ovarian stimulation, (ii) age at natural menopausal, (iii) serum anti-Mullerian hormone (AMH) levels, (iv) primordial follicle density and DNA damage in human ovarian tissue and (v) fertility outcomes. It is however important to understand the limitations of studying healthy women with *BRCA* mutations as they may only represent the tip of the iceberg (Fig. 4). Those with most severe BRCA dysfunction would either develop breast, ovarian or other cancers or undergo risk reducing salpingo-oophorectomy and lose their reproductive capacity at earlier ages. This would result in the elimination of individuals with the most severely impaired DNA repair mechanisms from the potential pool of subjects for research. In fact, initial observations on the impact of *BRCA* mutations on response to ovarian stimulation (Oktay *et al*., 2010) and serum AMH levels (Titus *et al*., 2013) were made on a small number of affected women undergoing fertility preservation procedures. These severely affected women would otherwise have received

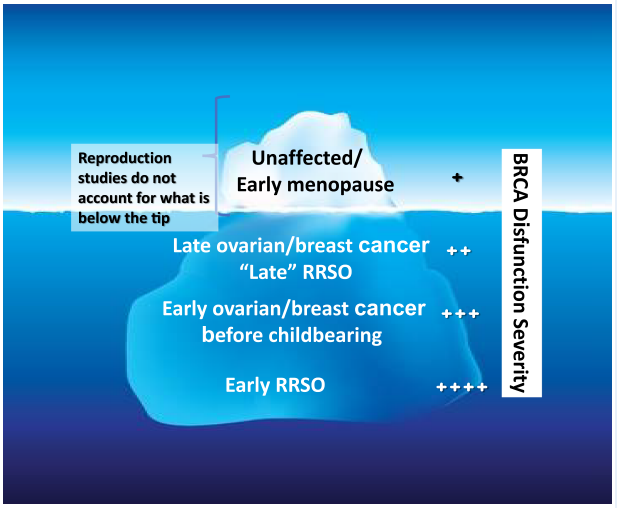

Figure 4 Explanation of the limitations in detecting *BRCA* mutation-related decline in ovarian reserve and fertility. RRSO: risk-reducing salpingo-oophorectomy.

chemotherapy and their ovarian function could not have been properly assessed. Hence, studies that are studying unaffected women with *BRCA* mutations likely represent those with relatively lower impairment in DNA repair capacity and hence the magnitude of difference in ovarian function outcomes may also be smaller than in affected women. Given this caveat, we reviewed the available clinical data.

BRCA mutations and ovarian response to stimulation

We identified five studies comparing oocyte yield after ovarian stimulation between *BRCA* carriers and controls (Oktay *et al*., 2010; Shapira *et al*., 2015; Derks-Smeets *et al*., 2017; Lambertini *et al*., 2018; Turan *et al*., 2018). Oktay *et al*. studied women with breast cancer who underwent fertility preservation before treatment and found that *BRCA1* mutation carriers produced lower number of oocytes compared with controls (7.4 [95% CI, 3.1 to 17.7] in *BRCA1* mutation carriers vs. 12.4 [95% CI, 10.8 to 14.2] in BRCA mutation negative and untested low risk controls; $P = 0.025$) (Oktay *et al*., 2010). However, the number of women with *BRCA2* mutations was small and therefore the conclusions regarding the latter were not robust. In another study where fertility preservation cycle outcomes were compared between women with breast cancer and other malignancy types, it was found that *BRCA* mutation carriers produced lower numbers of oocytes after ovarian stimulation (Turan *et al*., 2018) even though the study was limited by the retrospective design. Lambertini *et al*. reported a trend for a lower number of oocytes (6.5 vs. 9; $P = 0.145$) and cryopreserved oocytes (3.5 vs. 6; $P = 0.121$) in *BRCA*-mutated breast cancer patients compared to controls in a retrospectively designed study. A low response rate occurred more frequently in the *BRCA* mutation group (40 vs. 11%; $P = 0.147$) and they required higher dose of gonadotropins (2775 vs. 2025 IU; $P = 0.085$) and longer stimulation (11.5 vs. 9 days; $P = 0.110$) (Lambertini *et al*., 2018). The authors indicated that although these differences were clinically significant, they

Table I Studies investigating the association between *BRCA* mutation and AMH levels.

Study (year)	Design	Population	Findings	Limitations
Titus *et al*., (2013)	Prospective	Affected women *BRCA1*+ ($n=13$, mean age: 34.8 ± 4.8 years) *BRCA2*+ ($n=9$) *BRCA1* + 2 ($n=2$) *BRCA*− ($n=60$, mean age: 36.3 ± 3.5 years)	Lower serum AMH with *BRCA1* ($P=0.01$) but not *BRCA2* ($P=0.127$) mutations compared to BRCA− controls	Small sample size.
Wang *et al*. (2014)	Cross-sectional	Unaffected women *BRCA1*+ ($n=62$, mean age: 35.5 ± 5.2 years) *BRCA2* + ($n=27$, mean age: 35.6 ± 6.2 years) *BRCA*—($n=54$, mean age: 39.3 ± 3.7 years)	After adjusting for age and BMI, AMH was lower in *BRCA1* ($P=0.026$) but not *BRCA2* carriers ($P=0.470$) vs. *BRCA*− controls	No adjustment for potential confounders such as oral contraceptive use and smoking.
Phillips *et al*. (2016)	Cross-sectional	Unaffected women *BRCA1*+ ($n=172$, mean age: 34.2 ± 5.7 years) *BRCA1*− ($n=216$, mean age: 35.8 ± 5.8 years) *BRCA2*+ ($n=147$ mean age: 34.4 ± 5.6 years) *BRCA2*− ($n=158$ mean age: 35.8 ± 5.6 years)	After adjusting for smoking, age and body mass index, AMH was lower in *BRCA1* ($P=0.02$) but not in *BRCA2* carriers ($P=0.94$) compared to *BRCA*− controls.	The results may not be generalisable to those with severe mutations since unaffected women were included.
Johnson *et al*. (2017)	Prospective cohort	Unaffected women *BRCA1* + ($n=55$ mean age: 31.4 ± 5.5 years) *BRCA2* + ($n=50$ mean age: 30.9 ± 6.2 years) *BRCA*− ($n=26$ mean age: 34.3 ± 6.7 years) Low risk controls ($n=64$ mean age: 30.9 ± 5.6 years)	Significantly lower AMH among *BRCA2* mutation carriers compared with low-risk control women	Relatively younger study groups. BMI was available for only 41% of the subjects. Affected women and women with RRSO were excluded.
Lambertini *et al*. (2018)	Secondary analysis of database from two previous studies	Affected women *BRCA* + ($n=29$) *BRCA*− ($n=72$) Median age 31 years [interquartile range 28–33 years]	Reduced reproductive potential in *BRCA*+ cohort. Median AMH 1.8 µg/l and 2.6 µg/l in the *BRCA*+ and *BRCA*− cohorts, respectively ($P=0.109$). Trend for fewer oocytes being retrieved ($P=0.145$) and cryopreserved ($P=0.121$) in *BRCA*+ cohort 0.121).	Retrospective design, small sample size.
Michaelson-Cohen *et al*. (2014)	Retrospective	Unaffected women *BRCA* 1/2 + ($n=41$ mean age: 33.2 ± 3.9 years) Healthy controls? ($n=324$)	No difference in AMH	No adjustment for potential confounders Women with polycystic ovary syndrome were not excluded, *BRCA1* and 2 mutation carriers were analysed collectively. AMH was not log-adjusted.
Giordano *et al*. (2016)	Prospective	Unaffected women *BRCA1*+ ($n=68$, 66% aged under 35 years) *BRCA*− ($n=56$, 41% aged under 35 years)	With adjustment for BMI, duration of birth control, smoking, gravidity, parity and age >35, *BRCA1* was strongly associated with a low AMH ($P=0.037$)	Small sample size.

(Continued)

Table 1 Continued.

Study (year)	Design	Population	Findings	Limitations
Van Tilborg *et al*. (2016a)	Cross-sectional	Unaffected women *BRCA 1/2*+ ($n = 124$, median age: 29 [20–45] years) Non carriers ($n = 131$, median age: 31 [18–44] yrs)	Linear regression analysis adjusted for age, current smoking and current hormonal contraceptive use and did not detect lower AMH levels in BRCA mutation carriers	Relatively young mean age. *BRCA1* and 2 mutation carriers analysed collectively.
Ben-Aharon *et al*. (2018)	Prospective	Unaffected women *BRCA*+ ($n = 33$, median age: 35 years) *BRCA*− ($n = 15$, median age: 34 years)	Lower serum AMH level in *BRCA* mutation carriers compared to negative controls	Small sample size. No adjustment for potential confounders.
Gunnala *et al*. (2019)	Retrospective cohort	Affected and unaffected women *BRCA*+ ($n = 57$, mean age: 32.4 ± 3.6 years) *BRCA1*+ ($n = 31$, mean age: 32.0 ± 3.5 years) *BRCA2*+ ($n = 18$, mean age: 33.4 ± 3.5 years) *BRCA*− ($n = 738$, mean age: 35.5 ± 4.3 years)	With adjustment for age and BMI, no difference was found in AMH levels.	Small sample size No adjustment for potential confounders Lack of log transformation of AMH Assume the controls are in fact *BRCA*-negative even though they were not tested. Selection bias (including patients that underwent fertility preservation and those with severe mutations that underwent RRSO were not taken into account)

Abbreviations: AMH, anti-Mullerian hormone; BMI, body mass index; RRSO, risk reducing salpingo-oophorectomy

could only show a statistical trend due to inadequate study power. In the study by Shapira *et al*., however, *BRCA* mutation carriers showed normal ovarian response to ovarian stimulation (Shapira *et al*., 2015). However, that study had several limitations, including the retrospective design, relatively young age of the cohort, inclusion of both affected and unaffected women, and inclusion of those who underwent *in vitro* fertilisation for preimplantation genetic diagnosis. In addition, varying ovarian stimulation protocols were included in that study. In another study, ovarian stimulation outcomes were compared between the unaffected *BRCA* mutation carriers aged 31.4 ± 3.7 years and controls who had mean age of 32.1 ± 4.1 years (Derks-Smeets *et al*., 2017). Although ovarian response rate was similar between the groups, the number of mature oocytes was found to be reduced in *BRCA1* but not *BRCA2* mutation carriers. In summary, although there are some limitations, the preponderance of evidence suggests that response to ovarian stimulation is reduced in women with BRCA mutations. However, randomised controlled studies comparing uniform groups are required to achieve more conclusive results.

BRCA mutations and menopausal age

We found five studies which assessed the influence of *BRCA* mutation status on age at natural menopause (Rzepka Gorska *et al*., 2006; Collins *et al*., 2013; Finch *et al*., 2013; Lin *et al*., 2013; van Tilborg *et al*., 2016a), and three of those found that age at natural menopause was lower in women with *BRCA* carriers compared with controls (Rzepka Gorska *et al*., 2006; Finch *et al*., 2013; Lin *et al*., 2013). Lin *et al*. compared unaffected *BRCA1/2* mutated women in the Study of Women's Health Across the Nation (SWAN) group and found that the median age at natural menopause in *BRCA1/2* carriers was significantly earlier than in the control group (50 vs. 53 years, *P* value <0.001) (Lin *et al*., 2013). In another study by Finch *et al*., while the mean age at natural menopause was 48.8 and 49.2 years for unaffected *BRCA1 BRCA2* mutation carriers, it was 50.3 years for controls (Finch *et al*., 2013). Different from these two studies, Rzepka Gorska *et al*. compared menopause age between carriers of the *BRCA1* mutation and patients without mutation of *BRCA*. All women had a breast cancer diagnosis and it was found that the mean age at menopause was 45.3 years in carriers of the *BRCA1* mutation while it was 48.2 in patients without the mutation (Rzepka Gorska *et al*., 2006). Two studies found no difference for age at natural menopause between the groups (Collins *et al*., 2013; van Tilborg *et al*., 2016a). In the study by Van Tilborg, the authors indicated that they could not support or refute that women with *BRCA* mutations have earlier age at natural menopause due to various types of selection bias including more women being censored at age >40 years if using oral contraceptive pills and a higher incidence of surgical menopause among carriers due to risk reducing bilateral salpingo-oophorectomy (28.5 vs. 7.5% in controls). Interestingly, when the authors separately analysed the earliest born cohort, which was less likely to be subjected to the aforementioned censoring biases (such as early prophylactic risk reducing bilateral salpingo-oophorectomy) due to lack of availability of *BRCA* mutation testing at that time, they did detect earlier age at natural menopause among women later found to be *BRCA* mutation carriers (van Tilborg *et al*., 2016a). In the case of the study by Collins *et al*., the outcome was the percentage of women reaching natural menopause during the study period. Although 445 women with *BRCA1*

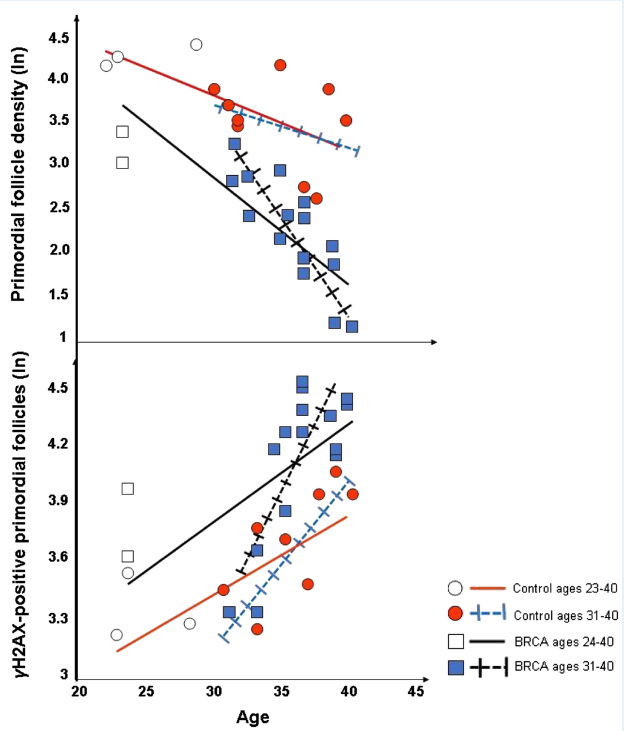

Figure 5 Impact of BRCA mutations on ovarian aging. In women with BRCA mutations, (**A**) primordial follicle density is lower and declines faster with age compared to controls, and (**B**) a higher fraction of primordial follicles accumulate DNA DSBs with age (as indicated by γH2AX expression). Both processes are accelerated after age 30.

and 361 women with *BRCA2* mutations were initially included in the study, only a small fraction of these women reached natural menopause (11 and %13, respectively) due to risk reducing bilateral salpingo-oophorectomy (Collins *et al*., 2013). Moreover, higher number of carriers (40% for *BRCA1* carriers and 29% for *BRCA2* carriers) than controls (7% for both) were censored at cancer diagnosis, highlighting the difficulty in monitoring age at natural menopause with *BRCA* mutation carriers. Interestingly, a later study on the same cohort by the same group of investigators found that AMH levels were lower in women with *BRCA* mutations (Phillips *et al*., 2016). In summary, despite the fact that risk reducing salpingo-oophorectomy limited the methodology in some studies, women with BRCA mutations appear to experience menopause at earlier age.

BRCA mutations and AMH levels

There is a direct relationship between the number of early-stage follicles and serum AMH levels, and it is one of the best predictors known today to evaluate ovarian reserve (van Rooij *et al*., 2002). We identified 10 studies which investigated the association between *BRCA* mutations and AMH levels. Although there were some contradictory studies, the majority found lower AMH levels in women with mutations (Titus *et al*., 2013; Michaelson-Cohen *et al*., 2014; Wang *et al*., 2014; Giordano *et al*., 2016; Philips *et al*., 2016; van Tilborg *et al*., 2016b; Johnson *et al*., 2017; Ben-Aharon *et al*., 2018; Lambertini *et al*., 2018; Gunnala *et al*., 2019). These studies showed heterogeneity and differences in design (Table I). First, of the 10 publications, affected women with *BRCA* mutations were studied in only three (Titus *et al*., 2013; Gunnala *et al*., 2018; Lambertini *et al*., 2018). Second, studies compared various combinations of untested healthy women and women who tested negative for *BRCA* mutations in control groups to affected and/or unaffected women with or without *BRCA* mutation-type specification as the study groups. This heterogeneity makes it difficult to compare these studies. Third, in numerous studies, no adjustment was made for important confounding factors such as smoking, oral contraceptive use and body mass index. Fourth, AMH levels are not normally distributed and can vary by as much as three orders of magnitude. Negative studies, other than the one by Von Tilborg *et al*., did not adjust data for non-normal distribution. And finally, some studies included relatively younger women with mutations (van Tilborg *et al*., 2016b; Johnson *et al*., 2017) and one did not have sufficient power identifying a trend for lower AMH in affected women with *BRCA* mutations (Lambertini *et al*., 2018). Studying younger women will reduce the likelihood of detecting differences in ovarian reserve as the decline in the function of intact *BRCA* allele may not be significant until after age 35 years (Oktay *et al*., 2014; Giordano *et al*., 2016).

We analysed the impact of mutations in *BRCA1* versus *BRCA2* on AMH level compared to those who tested negative and found that AMH levels were lower in young women with germline *BRCA1* mutations but not in women with *BRCA2* mutations (Titus *et al*., 2013). However, our study was not sufficiently powered for subgroup analysis. In 2014, Wang *et al*. found lower AMH levels in unaffected *BRCA1* carriers compared with noncarriers. Similar to Titus *et al*. and Wang *et al*., Giordano *et al*. showed that women who are *BRCA1* mutation positive had significant decline in AMH levels especially after age 35 compared to women without *BRCA* mutations after adjusting for confounders such as BMI, duration of birth control, smoking, gravidity, parity and age $>$ 35 (Giordano *et al*., 2016). The findings of two other studies were consistent with the aforementioned three studies (Philips *et al*., 2016; Ben-Aharon *et al*., 2018). While the studies where subgroup analysis was feasible showed lower AMH levels in women with *BRCA1* mutations, one study found significantly lower AMH levels in *BRCA2* but not *BRCA1* mutation carriers compared with low-risk control (Johnson *et al*., 2017). In summary, although some studies pose weaknesses, the majority of studies support that women with *BRCA* mutations, particularly of the *BRCA1* gene, have lower serum AMH levels.

BRCA mutations and histopathologic examination of ovarian tissues

The ultimate proof of reduced ovarian reserve in women with *BRCA* mutations should come from the direct assessment of the primordial follicle reserve. There have been multiple studies that have looked at the primordial follicle density in women with *BRCA* mutations. Pavone *et al*. examined ovaries of women who underwent risk reducing salpingo-oophorectomy because of *BRCA* (+) status or a strong family history of breast or ovarian cancer (mean age: 37.3 years) and compared them to those which were removed for benign indications (mean age: 36.5 years) (Pavone *et al*., 2014). They found that women with *BRCA* mutations had significantly decreased follicle counts compared to controls. We investigated primordial follicle density and DNA

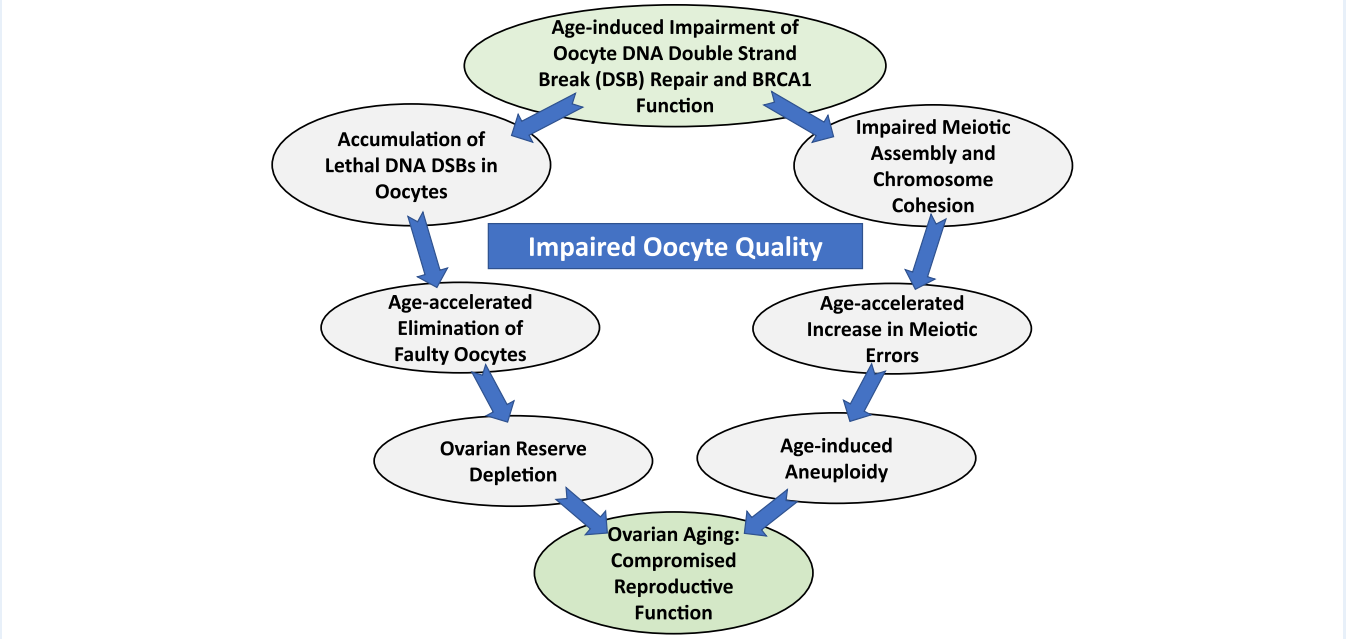

Figure 6 DNA repair theory of oocyte aging. The DNA DSB repair function declines with age, and this decline is accelerated after age 37. As a result, on the one hand an increasing fraction of primordial follicles accumulate severe DNA double-strand DNA breaks, which results in accelerated apoptotic loss of follicles and ovarian reserve diminishment. In parallel, since ATM-mediated DNA DSB repair and BRCA function are important in meiotic function and chromosome cohesion, aneuploidy risk increases with age, again in an accelerated fashion. If proven, this theory may explain age-related decline in oocyte reserve and quality and its acceleration after about age 37.

DSB accumulation in ovarian sections of unaffected *BRCA* mutation carriers (mean age: 36.5 ± 4.7 years) and compared them to the ovarian tissue from age-matched organ donor cadavers (Lin *et al*., 2017). We found that women with *BRCA* mutations had significantly lower primordial follicle density and increased DNA damage in primordial follicles compared to controls. We also found that the differences further diverged after age 30, indicating the acceleration of DNA damage and follicle death in women with *BRCA* mutations (Fig. 5). Lambertini *et al*. investigated the follicle density from women with breast cancer who underwent ovarian cryopreservation and found that women in the *BRCA*-positive cohort tended to have lower number of oocytes per fragment (0.08 vs. 0.14; $P = 0.193$) and per square millimetre (0.33 vs. 0.78; $P = 0.153$) than those in the *BRCA*-negative cohort (Lambertini *et al*., 2018). These differences did not reach statistical significance due to insufficient study power, according to the authors. These findings were further confirmed by Ben-Aharon *et al*., who showed that women with *BRCA* mutations have fewer primordial, primary, secondary and antral follicles in their ovaries compared to noncarriers (Ben-Aharon *et al*., 2018). Moreover, they showed that the levels of protein kinase B and *AMH* mRNAs, markers of cell survival and ovarian reserve respectively, were significantly lower in ovaries of BRCA carriers. In addition, in the same study, *FGF23*, which is known to increase with age, was higher in *BRCA* carriers than in the control group, suggesting that women with *BRCA* mutations may have overall accelerated aging. While none of these studies could practically assess total ovarian follicle counts and are subject to inherent variability of sampling ovarian cortex, these results collectively attest that women with *BRCA* mutations have lower ovarian reserve and possibly an overall accelerated aging status. In summary, there is evidence that primordial follicle numbers are reduced in women with BRCA mutations. While there is some evidence that follicle loss is accelerated in these women with age, we cannot rule out the possibility that women with BRCA mutations have started life with lower primordial follicle endowments.

BRCA mutations and fertility

We identified six studies investigating the association of *BRCA* mutations with fertility (Moslehi *et al*., 2010; Pal *et al*., 2010; Smith *et al*., 2012; Finch *et al*., 2013; Friedman *et al*., 2016; Giordano *et al*., 2016). Although four did not detect any differences between *BRCA* mutation carriers and controls (Pal *et al*., 2010; Moslehi *et al*., 2010; Friedman *et al*., 2016; Finch *et al*., 2013), one study detected lower gravidity and parity in women with *BRCA1* mutations (Giordano *et al*., 2016) and interestingly, one study found increased fertility in women with *BRCA* mutations (Smith *et al*., 2012). However, these studies had numerous limitations. They were questionnaire-based retrospective analyses which may be subject to recall bias. Furthermore, the studies were not uniform in studying affected versus unaffected women and the findings were not adjusted for confounding factors such as oral contraceptive use and smoking as well as age. Only one study found significantly lower gravidity and parity in women with *BRCA1* mutations compared to those without (43 vs. 68%; $P = 0.007$ for pregnancy and 40 vs. 60%; $P = 0.04$ for term birth in *BRCA1* mutation positive versus negative respectively) (Giordano *et al*., 2016). Interestingly, while 66% of women with *BRCA* mutation were under 35 years, it was 41% in *BRCA* negative group in this study, a major limitation in studying

fecundity in a *BRCA* mutation-positive population is again the exclusion of most severely affected cases by previous risk reducing salpingo-oophorectomy, cancer or premature ovarian failure from the study cohort. This leaves a less severely impacted group which may not have clinically significant differences from controls. Moreover, as was discussed before, in women with *BRCA* mutations there is still an intact allele which suffices for the maintenance of DNA repair. The function of the intact allele seems to begin to decline after age 30–35, resulting in clinical consequences probably after age 37–40 (Finch *et al*., 2013; Titus *et al*., 2013; Oktay *et al*., 2014; Oktay *et al*., 2015). This means that clinically significant differences may not be detectable in younger women. In fact, this point was raised in the discussion of the report by Finch *et al*. (2013) and in the accompanying commentary (Santoro, 2013).

Conclusions

In conclusion, available basic science, translational and clinical data indicate that intact BRCA function and related ATM-mediated DNA DSB repair might have an important role in the maintenance of ovarian reserve. Based on these combined data, we generated a hypothesis to explain ovarian aging by the age-related impairment of BRCA-related DNA DSB repair efficiency (Fig. 6). However, it is likely that many other factors and mechanisms are involved in oocyte aging besides diminished DNA repair. These possibly include endocrine, paracrine, genetic and metabolic factors. While clinical studies have not always provided uniform results due to numerous limitations, the preponderance of evidence indicates that women with *BRCA* mutations have reduced ovarian reserve. Evidence is also accumulating that women with pathogenic *BRCA* mutations may also have accelerated somatic aging. Nevertheless, future large prospective studies are needed to better understand the clinical significance of *BRCA* and similar mutations on fertility and aging in general. Moreover, an increasing number of novel mutations are being detected in other members of the ATM-mediated DNA DSB repair pathway in women with breast and ovarian cancers. These discoveries may create an opportunity to clinically study the role of other genes in the ATM-mediated DNA DSB repair pathway in ovarian aging. Furthermore, additional laboratory studies are needed to prove and better delineate the mechanisms by which DNA DSB repair deficiency may cause aneuploidy in oocytes (Fig. 6). If these mechanisms are clearly understood, we may be able to develop targeted treatments to reverse the age-related decline in DNA DSB repair function and slow down ovarian aging.

Authors' roles

Conception of the idea: Kutluk Oktay; manuscript writing: both authors; final approval of manuscript: both authors.

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Conflict of interest

None declared.

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