Reproductive Biology and Endocrinology

REVIEW

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Molecular regulation of DNA damage and repair in female infertility: a systematic review

Xiuhua Xu[1,2†], Ziwei Wang[1†], Luyi Lv[1], Ci Liu[1], Lili Wang[1], Ya-nan Sun[1], Zhiming Zhao[1], Baojun Shi[1], Qian Li[2*] and Gui-min Hao[1*]

Abstract

DNA damage is a key factor affecting gametogenesis and embryo development. The integrity and stability of DNA are fundamental to a woman's successful conception, embryonic development, pregnancy and the production of healthy offspring. Aging, reactive oxygen species, radiation therapy, and chemotherapy often induce oocyte DNA damage, diminished ovarian reserve, and infertility in women. With the increase of infertility population, there is an increasing need to study the relationship between infertility related diseases and DNA damage and repair. Researchers have tried various methods to reduce DNA damage in oocytes and enhance their DNA repair capabilities in an attempt to protect oocytes. In this review, we summarize recent advances in the DNA damage response mechanisms in infertility diseases such as PCOS, endometriosis, diminished ovarian reserve and hydrosalpinx, which has important implications for fertility preservation.

Keywords DNA damage, DNA repair, DNA damage response, Infertility, ROS, Oocyte

Introduction

DNA damage is a form of cellular stress, defined as any type of alteration in the DNA that disrupts its primary functions (replication and transcription) [1]. Cells possess DNA damage response (DDR) mechanisms to counter DNA damage. The role of the DDR is to detect damaged DNA and signal repair. The DNA damage response is composed of a variety of proteins, which can be divided into sensors, mediators, transducers and effectors. Cell dysfunction and death, as well as carcinogenesis and the aging process, are all linked to DNA damage. Human genome faces about one million lesions per day, such as adducts, modifications, or fragmentation of the sugar phosphate backbone of DNA [2].Without repaired, mutations such as base substitutions and chromosomal translocations may occur, and interfering normal gene expression and producing abnormal protein molecules. To cope with such damage, cells have a variety

†Xiuhua Xu and Ziwei Wang are the first authors who contributed equally to this work.

*Correspondence:
Qian Li
liqian963@hebmu.edu.cn
Gui-min Hao
haoguimin@hebmu.edu.cn
[1]Hebei Key Laboratory of Infertility and Genetics, Hebei Clinical Research Center for Birth Defects, Hebei Medical Key discipline of Reproductive Medicine, Hebei Collaborative Innovation Center of Integrated Traditional and Western Medicine on Reproductive Disease, Department of Reproductive Medicine, The Second Hospital of Hebei Medical University, Shijiazhuang 050000, China
[2]Cardiovascular platform, Institute of Health and Disease, Hebei Medical University, Shijiazhuang 050000, China

of enzymes and mechanisms for DNA-repair. Defects in one or more of the key components of these pathways can lead to unstable genome spread. This instability can lead to the death of germ cells (a way of eliminating abnormal cells) or cellular changes (a key step in cancer development). Exogenous and endogenous are the two forms of DNA damage.

Exogenous factors of DNA damage

Damage induced by environmental forces is referred to as exogenous damage. Exogenous factors are mainly divided into two categories, namely physical impacts and chemical impacts. The most common ambient physical agents that damage DNA include ionizing and non-ionizing radiation, both natural and manufactured. The majority of ionizing radiation (IR)-induced DNA damage is caused by interactions with hydroxyl radicals produced by radiolysis of water. And the most common DNA damage caused by IR includes 'simple' oxidative damage, for instance, modified bases, DNA single-strand breaks (SSB), or abasic sites, as well as more "complex" clustered lesions, or double-strand breaks (DSB) [3] Ultraviolet (UV) light from the sun is the main source of non-IR-induced DNA damage. Nearby pyrimidines dimerize to create helix-distorting photoproducts, which are the most critical UV targets. In addition, a range of genotoxic compounds present in the environment such as air, soil, water and food. They generate ROS that produce base modifications or SSBs. Occupational exposure to DNA damaging chemicals may occur in particular industrial, laboratory, and clinical settings, in addition to unintentional environmental exposure.

Endogenous factors of DNA damage

The excess of reactive oxygen species (ROS) is one of the most important endogenous factors causing DNA damage. Hydroxyl radicals, oxygen atoms, hydrogen peroxide, and superoxide radicals all belong to ROS. Cellular defense systems, such as ROS-scavenging enzymes, antioxidant enzymes, and vitamins can usually neutralize these reactive chemicals. Free radicals are extremely active and unstable. Oxidative stress ensues when the production of free radicals exceeds the amount of the body's natural antioxidant defenses. And they become stable by absorbing electrons from nucleic acids, lipids, proteins, carbohydrates, or any other adjacent molecule, culminating in a chain reaction that damages DNA and cells. Next, ROS bind to macromolecules such as lipids, proteins, DNA and damage them. Abasic sites and DNA strand breaks are two of the more common lesions caused by ROS action on the glycophosphate skeleton. In the repair process, such lesions are also the result of enzymatic processing of oxidized bases [4].

In the reproductive system, DNA damage and repair are important. Infertile men have abnormal sperm. ROS causes strand breaks in sperm DNA, resulting in base loss or base modifications such as 8-oxy-G. Sperm DNA damage has been positively correlated with lower fertilization rates in IVF, impaired implantation rates, increased incidence of miscarriage and disease in the offspring, including childhood cancer [5]. However, less attention has been paid to oxidative stress and infertility in women, but there is a negative correlation between the ability to fertilize oocytes and elevated DNA damage in cumulus cells [6, 7]. A study performed chromosomal analysis on patients who had two or more spontaneous miscarriages or who had not been pregnant for more than 2 years, in which all known causes of miscarriage were excluded to classify true idiopathic infertility. DNA damage (MN frequency) increased in infertile or aborted couples compared to fertile couples with no history of miscarriage and children younger than 2 years old [8]. In addition, increased MN frequency has been shown to be associated with recurrent miscarriage (at least three consecutive miscarriages) [9]. DNA integrity and stability are important factors for cell survival. This is especially true for female germ cells. DNA integrity is fundamental to successful conception, pregnancy, embryo development and healthy offspring. About 15% of infertility problems in men and 10% in women can be attributed to genetic abnormalities [10]. In this review, we summarize recent advances in the DNA damage response mechanisms in infertility diseases such as PCOS, endometriosis, diminished ovarian reserve and hydrosalpinx, which has important implications for fertility preservation. This article describes the current research status of DNA damage and repair mechanisms in female infertility, emphasizes the important role of oocyte DNA damage in aging and fertility decline. This area of research will potentially lead to new ideas for the prevention of female infertility, early detection of female infertility and treatment of female infertility at the genetic level, further contributing to the knowledge and understanding of female reproductive health.

Classic DDR and DNA repair mechanisms in somatic cells

DDR involves many cellular responses, including cell cycle arrest, chromatin remodeling, damage repair, and apoptosis, which are one of the more comprehensive cellular responses to stimuli [11]. The DDR mechanism is mainly activated by cells in the G1/S and G2/M phases. Ataxia telangiectasia mutated (ATM) and ATM and Rad3 related (ATR) proteins are important DNA damage checkpoint kinases. In the G1 phase, ATM and ATR kinases are recruited to DNA damage sites and undergo phosphorylation, immediately activating downstream

checkpoint kinase1 (CHK1) and checkpoint kinase2 (CHK2) [12, 13]. The CHK1 and CHK2 kinases continue to activate downstream effecter p53. Under the mediation of p53, p21 binds and inhibits the activity of cyclin-dependent kinase (CDK), thereby causing cell cycle arrest [14, 15]. In the G2 phase, DNA damage sites also recruit and activate ATM/ATR kinases and CHK1/CHK2 kinases. The difference is that the CHK1/CHK2 kinase inhibits the activation of CDK1 by inhibiting cell division cyclin25 (CDC25) phosphatase, leading to cell cycle arrest [15]. During cell cycle arrest, cells repair damaged DNA through complex mechanisms. After DNA repair is completed, the DNA damage checkpoint kinase undergoes dephosphorylation and the cell cycle resumes [15]. When DNA damage cannot be fully repaired, p53 activates the transcription of pro apoptotic genes such as p53 upregulated modulator of apoptosis (Puma) and phorbol-12-myristate-13-acetate induced protein 1 (Pmaip1/Noxa), thereby inducing cell apoptosis [16, 17].

For different types of DNA damage, cells can be repaired by appropriate DNA repair mechanisms. Base mismatches that occur during DNA replication can be corrected by mismatch repair (MMR) mechanisms; Bases that undergo minor chemical changes can be removed by the base excision repair (BER) mechanism; Larger DNA lesions can be removed through the nucleotide excision repair (NER) mechanism; The repair process of DNA single strand breaks involves a series of enzyme cascade reactions; Homologous recombination (HR) and non homologous end joining (NHEJ) are two mechanisms for repairing DSBs; The mechanism for elimination of ICLs involves a complex set of reactions related to Fanconi anemia proteins [18]. It is generally believed that DSBs are the most severe types of DNA damage, which can lead to genome rearrangement and structural changes, such as deletions, translocations, fusion, etc. [11, 18]. The repair mechanisms of DSBs include HR and NHEJ. In the HR repair process, MRN complexes are first recruited to the ends of DSBs and the DNA ends are processed and cleaved to produce single stranded DNA [19]. Afterwards, replication protein A (RPA) wraps single-stranded DNA to protect it from nuclease action and remove its secondary structure. Under the mediation of breast cancer protein 2 (BRCA2), RPA is replaced by DNA repair protein RAD51 (DNA repair protein RAD51). Subsequently, RAD51 mediated the invasion of single strand DNA into the uninjured sisters' chromosome [20, 21]. Finally, under the action of polymerase, nuclease, helicase, and other molecules, DNA is extended and repaired [22, 23]. In contrast to HR, NHEJ directly connects the ends of DSBs through DNA ligases. First, Ku70/Ku80 proteins recognize and bind to the ends of DSBs, followed by recruitment and activation of DNA dependent protein kinase catalytic subunits (DNA-PKcs) [24]. Afterwards, DNA-PKcs recruits the recombinant enzyme Artemis to process the DNA ends, while also recruiting a protein complex composed of X-ray repair cross complementing protein4 (XRCC4) and DNA ligase 4 (DNAligase4, LIG4) to connect the DNA ends [24, 25]. Finally, under the action of polymerase, nuclease, helicase, and other molecules, DNA is extended and repaired [22, 23]. Unlike HR, NHEJ directly connects the ends of DSBs through DNA ligases. Firstly, Ku70/Ku80 proteins recognize and bind to the ends of DSBs, followed by recruitment and activation of DNA dependent protein kinase catalytic subunits (DNA-PKcs) [24]. Afterwards, DNA-PKcs recruited the recombinant enzyme Artemis to process the DNA ends, while also recruiting a protein complex composed of X-ray repair cross complementing protein4 (XRCC4) and DNA ligase 4 (DNAligase4, LIG4) to connect the DNA ends [24, 25]. HR occurs in the S and G2 phases of the cell cycle and is repaired with undamaged sister chromosomes, making it more precise. NHEJ directly connects the two ends of DSBs together, although imprecisely, it can operate throughout the entire cell cycle [23, 26]. It is widely believed that the accumulation of DNA damage and incorrect DNA repair can easily cause gene mutations and chromosomal aberrations, leading to the decline and loss of cellular function, which may promote aging and the occurrence of diseases [11, 27]. Therefore, it is essential that cells maintain the stability and integrity of their genome.

DNA damage and oxidative stress in female reproductive system

Researchers believe that 40% of infertility is caused by male factors with abnormal sperm. Low sperm count, poor motility, and aberrant morphology are all examples of abnormal sperm. 40% of infertility is caused by female factors. The reason for the last 20% is uncertain. The main contributors to the genetic reasons of infertility and recurrent miscarriage are chromosomal abnormalities.

DNA damage in oocyte

The sensitivity of the oocyte to DNA damage is less well documented than that of the spermatozoon, possibly due to the difficulty of acquiring oocytes for research. However, oocytes are known to be more susceptible to external stimulation at certain times. Oocytes are more susceptible to DNA damage during division.

Oogenesis is the process of producing mature oocytes after mitosis and meiosis. During prenatal development, oogenesis begins in the ovary and then stops. Rapid cell division creates 7 million oogonia during the second and seventh month of pregnancy, which are either destined to develop and form germ cell cysts, or disappear through natural atresia. Breakdown of the germ cell cysts is accompanied by the transition of precursor oocytes,

enveloped by a single layer of flattened follicular epithelial cells, into primordial follicles [28]. Immediately after birth, the first phase of meiosis begins. The primordial follicle is particularly susceptible to DNA damage due to its extreme longevity and the unique design of the primordial nucleus [29].

As mentioned above, DNA damage and apoptosis may be related to fertility and posed a certain threat to fertility. Currently, it is more common in DNA damage caused by anticancer therapy. In detail, the major molecular mechanism of the depletion of ovarian reserve due to exposure to genotoxic stressors is apoptosis mediated by transactivation of p63 (TAp63) [30]. The effect of TAp63 on the apoptotic pathway is mainly achieved through several processes. Ataxia telangiectasia (mutated) (ATM) kinase and checkpoint kinase 2 (CHK2) trigger the phosphorylation process required for TAP63 activation. This process promotes the induced transcription of BH3-only pro-apoptotic factors, PUMA, and NOXA. The upregulation of these pro-apoptotic factors promotes their interaction with the pro-apoptotic BCL2 family members BAX and BAK. As a result, mitochondrial apoptotic proteins are released, and the crucial proteolytic enzyme caspase-9 for apoptosis and death is activated [31]. Under the premise of evolution, removing damaged oocytes avoids the risk of passing the mutation on to offspring. This will undoubtedly affect reproductive function and lead to infertility. It is expected to be able to cope with damage or repair through this period of its developmental arrest.

Primordial follicles are released from arrest after birth and continue to develop into primary oocytes. TAP63 levels remain high in primary oocytes, which are also dependent on TAp63-mediated DNA damage responses, but not found in advanced follicles after ovulation [32]. Taken together, these findings suggest that TAp63 has a conserved function in the removal of defective oocytes from the germline in both primary and primordial follicles.

There may be a way of genetic protection of the female reproductive system without TAp63. Through experiments with rats, researchers found that when the ovaries were exposed to bisphenol A (BPA), a group of DNA repair genes involved in the classic double-strand break (DSB) DNA repair pathway were considerably up-regulated in just 24 hours. However, it was also found that there is a certain threshold of exposure source, beyond which the repair effect fails and apoptosis begins. This can lead to impaired ovarian function [33].

In contrast to the growing follicle, oocyte at the germinal vesicle stage is transcriptionally dormant. Studies in mice have shown that oocytes in preovulatory follicles have the ability to recognize DNA damage. This can be seen from the development of γH2AX foci after exogenous DSB induction. The phosphorylation of H2AX requires both MRN complex and ATM activation. ATM kinase, the master regulator of DNA damage response pathway, cannot be effectively activated in mature GV oocytes, so oocytes carrying DSB DNA have the opportunity to enter the first meiotic M-phase (MI), unless the degrees of damage are quite high [34]. This explains why oocytes are particularly vulnerable to the accumulation of DNA damage, because of their prolonged pause in meiotic prophase.

In addition, during the G2/M stage of meiosis, the spindle assembly checkpoint (SAC) protects the integrity of the female germline, by examining the state of kinetochore–microtubule attachment and inhibiting the activity of the anaphase-promoting complex (APC) before chromosomes are ready for full division to prevent the occurrence of aneuploidy [35]. The SAC acts as a gatekeeper. Ovotoxins, UV-B, and ionizing radiation can activate the SAC, which in turn leads to the failure of oocytes from MI to MII. In mice, researchers discovered that roughly 53 proteins are involved in DNA repair, replication, and recombination in oocytes. Including double-strand break (DSB) DNA repair, base excision repair (BER), single-strand break (SSB) repair and any other proteins associated with similar pathways [36]. There is a basal BER activity in MII oocytes, and this pathway repairs oxidation-induced DNA damage. MII oocytes may have proteins necessary to carry out this repair. Thus, this pathway reduces the burden of oocytes carrying oxidative DNA damage. Taken together, this is the susceptibility of oocytes to DNA damage, and the mechanisms of their defense.

Oxidative stress in oocyte

In the ovary, oocytes are exposed to a variety of reactive oxygen species. This is necessary for normal reproductive activity. Oocyte maturation and ovulation can be compared with an inflammatory response, resulting in the production of large amounts of ROS [37]. Oocytes have a certain resistance to it, so as we can see the high levels of antioxidants in follicular fluids may be related to that. The biomarkers of the oxidative stress (OS) have already been found nowadays. The expression of antioxidants such as Cu-Zn superoxide dismutase (SOD), Mn-SOD and glutathione peroxidase(GSH-Px) in the follicular microenvironment, which may be related to folliculogenesis, maturation and luteal function, and they are well expressed in MII period [38]. Reduced glutathione (GSH) is found in abundance in all mammalian cells and acts as a potent antioxidant. Decreased GSH levels have been linked to increased oxidative stress. Low fertilization was associated with downregulation of GSH-Px. Nitric oxide (NO) is also an unstable free radical that can participate in ROS reactions. It also directly acts on DNA

to deaminate it. Low levels of NO in the follicular fluid are associated with the eventual fertilization success of the oocytes. And it is negatively correlated with embryo quality and cleavage rate [39]. H_2O_2 is a very weak oxidant. DNA damage caused by H_2O_2 cannot be mediated by H_2O_2 alone. H_2O_2 can penetrate cell membranes quickly, and once inside, it binds to iron and copper ions to produce more harmful substances, such as hydroxyl radicals. Hydroxyl radicals effectively interact with DNA, resulting in single- and double- strand breaks. Certain substances and ionizing radiation induce DNA strand breaks by producing hydroxyl radicals.

In some cases, such as certain diseases or ovarian lesions, elevated levels of oxidants in the oocyte or disrupted of the balance between oxidative stress and antioxidants resulting in higher ROS than normal levels, which can affect the oocyte quality, altering its cytoskeleton and microtubules, lead to chromosomal abnormalities that ultimately affect fertilization. Guanine is the most easily to oxidation of the four bases. 8-oxoG is the most often oxidized form of guanine, which is the causal molecule for spontaneous and inheritable germ lineage cell mutations. And it is endogenously produced by ROS [40]. In addition, increased ROS levels in the tubal and peritoneal environment may affect gametes in the fallopian tube, and their ability to interact and syngamy.

Infertility and DNA damage

Polycystic ovary syndrome and DNA damage

Polycystic ovary syndrome (PCOS) is one of the most common endocrine diseases in women of reproductive age. It is a heterogeneous disease with different clinical and endocrine manifestations. It is a disorder with a wide range of phenotypes such as obesity, hyperandrogenism, irregular menstrual cycles, anovulation, ovarian cysts, and low-grade chronic inflammation-related stress indicators.

Testosterone(T)

Plasma lipid peroxides are increased in PCOS patients, and testosterone has an inductive influence on lipid peroxidation. Hyperandrogenism combined with reduced catalase activity causes free O_2 and peroxynitrite accumulation in PCOS women. These endogenous free radicals can cause lipid peroxidation. In turn, faster lipid metabolism leads to increased oxidative damage. The results showed that H_2O_2, hydroxyl radical production and lipid peroxidation were enhanced and GSH levels decreased after treatment of androgen-responsive prostate cancer cell lines with physiological levels of androgens [41]. In animal experiments, it was found that after long-term combined use of testosterone and estrogen, the DNA strand of the dorsolateral prostate in rats was broken and lipid peroxidation was increased [42]. In humans, free testosterone is positively correlated with DNA strand breaks, as is H_2O_2-induced DNA damage. Therefore, it is believed that women with PCOS may have more DNA strand breaks as a result of increased androgen production.

Estrogen(E2)

Estrogen levels are elevated in PCOS patients. Although estrogen is considered a kind of antioxidant, some of its compounds also metabolize to produce ROS [43]. Estrogen can interconvert between reduced and oxidized states, potentially generating ROS that can damage DNA [44]. Studies of estrogen-induced development of DNA adjuncts in Syrian hamster kidneys suggested possible mechanisms of estrogen-induced DNA damage. It was found that estrogen could be metabolized to catechol estrogens, which then undergo an oxidative cycle to produce hemiquinones, reactive oxygen species that are extremely destructive to DNA. Other oxidation-generating processes, such as prostaglandin synthesis or the phage/leukocyte infiltration pathway, may be activated in response to the combined effects of testosterone and estrogen, leading to DNA damage and lipid peroxidation [42].

Glutathione (GSH)

In PCOS patients, ROS is induced by increased androgen production, which may lead to depletion of GSH. GSH can protect cells by antagonizing and react with ROS through its thiol group in the reduced state. Glutathione plays an important role in cell metabolism. Activation of transcription factors that affect gene expression has been shown to depend on intracellular GSH levels [45]. In addition, decreased GSH levels may enhance the oxidative stress vulnerability of biological components such as DNA.

Body mass index(BMI)

In addition to effects of hormones on PCOS patients, obesity is also related to the development of PCOS. The average frequency of DNA damage is positively correlated with waist circumference (WC) [46]. Obesity may be linked to certain oxidants [47]. Obesity is known to induce an increase in free radicals, which can contribute to cellular DNA damage. The free radicals may be the link between DNA damage and obesity status. Some researchers have found that high BMI leads to increased frequency of micronuclei, a form of chromosomal aberration in interphase cells that may be associated with loss of centromeres during anaphase and DNA damage. Meanwhile, there may also be a correlation between oxidative stress and inflammation in PCOS patients, which are also factors inducing DNA damage. Among them,

low-grade inflammation with obesity is most likely to contribute to the pathogenesis of PCOS [48].

In addition, PCOS patients are often accompanied by metabolic syndrome (MS), and there is also a strong link between obesity and metabolic syndrome, each of them makes patients suffer from genetic damage. Obese women with MS have significantly higher levels of DNA damage compared to healthy non-obese people [46], and with the progression of MS disease severity, antioxidant capacity decreases and DNA damage increases. Meanwhile, studies have shown that MS patients have lower levels of total antioxidant capacity (TAC) and higher levels of DNA damage and oxidative stress index (OSI). Insulin resistance exists is a characteristic of PCOS and PCOS patients with MS. Insulin resistance causes oxidative stress by activating NADPH oxidase, which response to free radicals or extremely reactive oxygen species. Elevated blood glucose caused by abnormal glucose metabolism may exacerbate oxidative stress by promoting the generation of reactive oxygen species and diminishing the body's natural antioxidant defenses [47].

Animal studies have also found that a high-fat diet can cause weight increase in mice and DNA damage in many organs [49]. There is a correlation between DNA damage and overweight. Weight gain impacts the DNA repair system through many molecular pathways. The brain and ovary are the organs with the lowest DNA repair activity. In addition, increased body weight can lead to DNA damage through decreased telomere length, inflammation, hormonal effects and reactive oxygen species formation [50]. It is now known that nucleotide excision repair (NER) proteins play an important role in cellular regulation of oxidative DNA damage. In peripheral blood lymphocytes, a negative correlation was found between NER levels and BMI in humans. Therefore, the lower activity of this repair pathway in obese animals may be related to the oxidation of DNA bases. Impaired repair processes lead to genomic instability in obese animals. Weight loss reduces the oxidative stress associated with obesity, and after weight loss, an increase in NER activity has been found in multiple organs such as liver, colon, testes, and ovaries.

Inflammation

Low-grade chronic inflammation is closely associated with PCOS. Increased inflammation can lead to insulin resistance, leading to a "vicious cycle" of metabolic disorders in PCOS patients [51]. Inflammatory markers and genetic markers were higher in PCOS patients. Women with PCOS had elevated CRP, interleukin 18(IL-18), interleukin 6(IL-6), and tumor necrosis factor (TNF-α) and white blood cells (WBC) compared with age- and BMI-matched controls. Its hyperinsulinemia, obesity, hyperandrogenism, and inflammatory states are interrelated. Inflammatory factors can also cause endothelial cell dysfunction, which in turn leads to the risk of cardiovascular disease. In addition, Iron overload exists in PCOS patients, and high levels of ferritin and transferrin can lead to decreased levels of anti-inflammatory factors and antioxidant molecules [52].

These all prove that the inflammatory response is higher in PCOS patients. Some studies suggest that this is inseparable from the disease characteristics of PCOS. If the WBC of PCOS patient is higher than that of the normal population, it may be positively correlated with androgens, insulin resistance and BMI. Meanwhile, with a multiple regression analysis, the researchers found that testosterone was one of the predictors of WBC [53]. Thus, androgens play an important role in the development and activation of WBCs, as well as in low-grade inflammation. In addition to androgens, as we mentioned above, obesity is also one of the triggers of inflammation. As obesity increases, so does its inflammatory state. In obese women, an imbalance between classically activated macrophage (MI) and alternatively activated macrophage (M2) is observed. The higher concentration of MI-type macrophages, which are associated with pro-inflammatory factors, suggests that pro-inflammatory processes dominate, leading to low-grade chronic inflammation throughout the body [54]. In addition, elevated levels of leptin may also be associated with obesity status, and leptin also has pro-inflammatory effects. Hyperleptinemia further promotes the production of pro-inflammatory cytokines [55]. Insulin resistance and hyperglycemia also have similar effects in PCOS patients.

Inflammation can lead to accelerated mutagenesis and gene instability [56]. DNA damage caused by inflammation is mostly related to reactive oxygen and nitrogen species (RONS). RONS secreted by immune cells destroy pathogens, but can also damage neighboring human cells. Most importantly, RONS can cause efficient mutagenesis in DNA. Inflammatory responses may exacerbate their production or effect. For example, NO is a pleiotropic RONS that is an indispensable signaling molecule at concentrations below 400nM. However, during inflammation, immune cells produce high levels of NO, which can induce apoptosis through protein nitrosation, nitration, and alkylation when NO concentrations approach or exceed 1μM [57]. In addition, macrophages and neutrophils can produce superoxide and participate in enzymatic reactions, producing a series of RONS [58]. Apart from that, proinflammatory cytokines can also lead to the production of intracellular RONS. RONS can cause DNA damage in multiple ways as RONS are strong oxidants. As we mentioned above, strong oxidants can cause DNA damage. Nitrosated RONS can cause deamination of DNA bases. Deamination products are especially mutagenic, and the corresponding chemical reactions

can occur on hydrogen bonds, eventually leading to base mismatches. Inflammatory cells, such as neutrophils, can also produce hypohalous acid [59]. These hypohalous acids easily react with DNA to form adducts during inflammation, which is even higher than the accumulation of DNA damage caused by oxidation, deamination, or lipid peroxidation.

In conclusion, the DNA damage in PCOS patients is higher than that in the normal population. This may be associated with increased levels of estrogen, androgen, obesity, decreased glutathione levels, and high levels of inflammation.

Endometriosis and DNA damage

Endometriosis (EMS) is a common benign and estrogen dependent disease in fertile women. The incidence among women in fertility is about 10–15%, which is often accompanied by chronic pelvic pain and infertility. Its main characteristic is the growth of endometrioid tissue outside the uterus.

Estrogen(E2)

Estrogen and its metabolites have been recognized as genotoxic mutagens. Evidence from animal studies suggests a causal relationship between exposure to environmental factors, such as dioxins, and endometriosis. Although the serum estrogen in patients with endometriosis is not high, the local estrogen concentration in ectopic lesions is significantly increased. Estrogen receptors α and β are encoded by the ESR1 and ESR2 genes located on non-homologous chromosomes. The expression of ERα is higher than that of ERβ in normal endometrium, but in endometriosis, the opposite is true [60, 61]. This may be related to the hypermethylation of ESR1 promoter. In endometriosis, the main manifestation is the high expression of local estrogen mainly mediated by ERβ. In addition, the expression of enzymes that mediate estrogen synthesis also plays a crucial role. The expression level of steroidogenic acute regulatory protein(StAR), aromatase, 17β-hydroxysteroid dehydrogenase(17β-HSD) in ectopic lesions of patients with endometriosis is significantly higher than that in normal endometrial tissue [62].

Natural estrogen plays an important role in regulation. Physiological concentrations of estrogen are essential for maintaining cell growth and several other biological activities. In addition, elevated estrogen levels are known to have adverse effects such as embryotoxicity, teratogenicity, and carcinogenicity. Estrogens are genotoxic and reactive estrogen metabolites may act at the genetic and chromosomal levels. Some researchers found that hamsters exposed to E_2 also occurred in vivo-induced DNA single-strand breaks [63]. Although it is known that estrogen and its metabolites can cause a lot of DNA damage, whether estrogen in patients with endometriosis can cause corresponding damage still lacks relevant evidence-based medicine evidence, and further research is needed.

Iron overload

Iron is essential for cell growth and metabolism. Low molecular weight iron pools are a major source of toxic iron, which can be reduced by binding to corresponding proteins. This is also one of the ways in which iron regulates the production of reactive oxygen species. Reflux of menstrual blood leads to rapid increase of iron and heme content in EM lesions in a short period of time. Compared with women without endometriosis, patients with endometriosis had significantly higher levels of iron and ferritin in the peritoneal fluid, so as the lipid peroxidation in low-density lipoprotein (LDL) [64]. Hemorrhage and iron overload are common in the tissues of endometriotic lesions, and the expression level of transferrin gene receptor is higher. Besides, extensive ferritin staining was also observed in macrophages [64, 65]. From this perspective, there is a certain imbalance of iron homeostasis in patients with endometriosis. As mentioned above, this can directly induce the occurrence of oxidative stress, as well as the generation of ROS, which may be one of the reasons of iron-induced DNA damage. In addition, iron can directly act as a catalyst to indirectly promote the generation of a series of free radicals through the fenton reaction, which further promoting the generation of oxidative stress [66]. At the same time, iron further activates intracellular tyrosine kinase or casein kinase II, and activates the p50/p65 NF-kappaB dimer, which can bind to DNA in the nucleus and participate in transcription, thereby mediating the production of a series of cytokines and involving in the development of EMS disease. From this perspective, EMS and oxidative stress are mutually reinforcing [67]. Direct contact of iron with DNA causes toxic lesions in a dose- and time-dependent manner. In the process of iron-induced DNA oxidative damage, it may lead to the transversions of G to T, transversions of G: C to A: T, and transitions of G: C to C: G in DNA. It also causes coding errors and reading errors in DNA polymerases [68]. This may also cause errors in base pairing. 8-Oxoguanine is the most common DNA oxidation marker, and Fe-NTA, an iron chelate, generates hydroxyl radicals through the Fenton reaction, which greatly induces the hydroxylation of guanine and pyrimidine and simultaneously produces 8-oxoguanine. In addition, iron can form 8-hydroxyguanine adducts, which can lead to point mutations and DNA strand breaks, as well as induce DNA hypermethylation and shorten telomere length. Furthermore, hemoglobin, heme, and iron derivatives helped to fine-tune the expression of several genes associated with oxidants and antioxidants, resulting in high levels of nuclear factor erythroid 2(Nrf2) and

heme oxygenase-1(HO-1) expression. These two counteract inflammation and oxidative stress, which in turn induces malignant transformation of endometriotic cells with persistent DNA damage [69]. In summary, in the development of endometriosis, severe hemolysis occurs that results in the production of free hemoglobin–haptoglobin(Hb), heme, and iron, which are toxic and act as inflammatory molecules, oxidatively modifying lipids and proteins, and ultimately causing cellular and DNA damage.

Changes in metabolism

Studies have found that the concentration of lipid peroxides in the peritoneal fluid of women with pelvic endometriosis is significantly increased [70]. The product of unsaturated fatty acid peroxidation promoted by free radicals in cell membrane is lipid peroxide. For example, malondialdehyde (MDA), because it is relatively stable, can be used as a cumulative measure for this process. Vitamin E is one of the non-enzymatic antioxidants that prevent lipid peroxidation or its spread. Some studies have found vitamin E levels are reduced after pituitary blockade with GnRH-a [71]. One of the antioxidant activities of glutathione is the elimination of oxidized tocopherols, which is important for recycling and maintaining physiological levels of vitamin E, which is essential for fighting oxidative stress [72]. Vitamin E and glutathione levels were significantly lower in women with moderate and severe endometriosis compared with women with mild disease, speculated that the reduction in antioxidants may be due to their ability to protect against endogenous oxidative stress and therefore consume more in the endometrium [73]. Women with endometriosis had lower glucose levels and up-regulated pyruvate, both indicative of enhanced glycolysis in these women. Consequently, two TCA cycle intermediates, citrate and succinate, were also elevated which may be related to impaired mitochondrial respiration, and the possibility of ROS generation by the mitochondrial electron transport chain is also increased. This may be another important reason for the increase of ROS in the lesions of patients with endometriosis, leading to oxidative stress [74].

Inflammatory

Endometriosis is essentially an inflammatory response. Endometriosis is often accompanied by changes in the quantity and function of inflammatory factors, including TNFα, IL-1, IL-6, IL-8, macrophage migration inhibitory factor(MIF), CC chemokine monocyte chemoattractant protein-1(MCP-1) and serum amyloid A(SAA). Among several inflammatory factors, researchers have found that TNF-α plays a role in endometriosis. The expression of TNF-α is increased in the tissues of patients with endometriosis. It is regulated by urocortin 2 and urocortin 3 secreted by the endometrium, thus further illustrating that TNF-α may be a key cytokine in the inflammatory aspects of endometriosis [75]. At the same time, neutrophils and macrophages were determined to have higher chemotaxis in endometrial proliferative and luteal phase biopsies compared with normal endometrium. However, neutrophil activation is associated with disease severity in endometriosis, and only in patients with stage III and IV disease does the activation signal show a relevant response. It further illustrated that endometriotic tissue has a pro-inflammatory effect[76]. For macrophages, it may be regulated by estrogen. The expression of the estrogen receptor ER-a is positively correlated with the expression of inflammatory factors in macrophages of endometriosis [77]. RANTES, a chemotactic protein, is a chemotactic cytokine for a variety of inflammatory cells and plays an active role in recruiting leukocytes to sites of inflammation. However, increased RANTES was found in both the peritoneal fluid and endometrial tissue of patients with endometriosis. The expression of RANTES is also induced by TNF-α, which in turn promotes the recruitment of macrophages to endometriotic tissues. In addition, increased expression of T cells was found in patients with endometriosis. In animal models of endometriosis, there is a decrease in peripheral regulatory T cells and an increase in peritoneal fluid, which may lead to endometriosis associated infertility [78]. In macrophage MI and MII patterns, MI is characterized by a pro-inflammatory phenotype, while MII is an anti-inflammatory phenotype. We have found that polarization in favor of MII macrophages is observed in endometriosis [79]. Iron-induced ROS mentioned above can induce cellular and DNA damage by activating KB and increase the expression of pro-inflammatory genes NFκB. Various indications show that there is an inflammatory response in patients with endometriosis, including initial infection and subsequent sterile inflammation. Abnormal increasing in inflammation leads to some degree of genetic damage.

Diminished ovarian reserve and DNA damage

DNA damage is especially problematic for cells that do not divide or divide slowly, such as oocytes. DNA damaged cells undergo complex reactions such as cell cycle arrest, DNA repair and apoptosis. DNA damage accumulates over time. During the formation of the ovarian reserve, germ cells are in an active phase of DNA replication, proliferation and meiotic recombination. This stage is prone to DNA damage, which may lead to the loss of primordial follicles. This in turn affects ovarian reserve. From this perspective, diminished ovarian reserve (DOR) and even premature ovarian failure (POF) are one of the outcomes of DNA damage. DNA damage repair is crucial. The accumulation of DNA damage, and the impair

function of DNA damage repair, ultimately lead to a decrease in ovarian reserve.

There are a variety of factors that can lead to DNA damage and thus affect ovarian function. 8-oxoguanine DNA glycosylase(OGG1) is an important component of the DNA damage repair process. OGG1 plays an important role in OS. Elevated serum OGG1 levels in DOR patients may suggest elevated levels of OS and severe DNA damage in DOR patients. Oxidative stress should be associated with diminished ovarian reserve (DOR) [80]. Excess free radicals can increase DNA damage and cause cellular decline with age [81]. ROS-induced DNA damage may lead to replication errors, base modifications, genomic instability, mutations and cell apoptosis. Among them, 8'OHdG is its marker. This eventually leads to premature ovarian insufficiency (POI), which may also be associated with mitochondrial dysfunction (MD) [82]. A variety of DNA damage can cause impairment of ovarian function and even lead to POI.

Double-strand break(DSBs)

DSBs can significantly alter the genetic integrity, which are difficult to repair and extremely toxic. Therefore, DSBs are the most harmful of all types of DNA damages. DSBs may be caused by genotoxic stress or replication fork defects [83].

Obviously, impaired DSB repair also affects ovarian reserve function. Repair efficiency is the key factor for oocyte loss. In a study of FVB mice, researchers found that the percentage of γH2AX-positive primordial follicles was significantly higher in 11- to 12-month-old compared with 3- to 4-week-old FVB mice, as was the percentage of γH2AX-positive GV-phase oocytes. In contrast, the expression of DNA DSB repair genes was reduced in old mice. The expression of BRCA1, MRE11, Rad51 and ATM was significantly decreased in the aged mice by qRT-PCR. All of these genes are involved in DNA DSB repair. This demonstrates that the reduction of DSB repair in old mouse oocytes results in the accumulation of DSB with age. In addition, the same pattern as in mice was found by studying the expression of key DNA DSBs repair genes in 24 individual human oocytes. Further studies in BRCA1 mutant mice revealed that BRCA1 mutant mice produced fewer oocytes after ovarian stimulation and had fewer litters after mating compared to wild-type mice, and the proportion of γH2AX cells was increased in 4-month-old BRCA1 mutant mice. This indicated inadequate DSB repair in BRCA1 mutant mice and significantly increased accumulation of DNA damage. In humans, people with the BRCA1 mutation have lower AMH levels than people without the BRCA1 mutation. The damage of DSB repair mechanism is associated with the accelerated loss of ovarian reserve, which is related to the accumulation of DSB in oocytes [84]. In addition to BRCA1, Rad51 and MRE11 are also critical in the process of ATM-mediated DNA DSB repair [85, 86]. Therefore, knockdown any of these essential genes reduces the efficiency of DSB repair, which leads to a severe accumulation of DNA damage that triggers the cell death mechanism that explains the diminished function of ovarian reserve. Maintenance complex component (MCM) is also a family of proteins involved in important physiological processes such as DNA replication, meiosis and homologous recombination repair. Especially, both MCM8 and MCM9 promote the aggregation of RAD51 at the site of DNA damage. MCM8 is an important protein involved in the DNA homologous recombination repair mechanism. It is important for the homologous recombination process during the early meiotic prophase of oocytes and DSBs repair during the later developmental stages [87]. MCM8 mutation leads to impaired repair of DSBs, which can lead to excessive regulation of oocyte death. A study found that MCM8 gene polymorphisms were closely associated with early menopause in women, suggesting that MCM8 may play an important role in the maintenance of ovarian function in humans [88]. Female mice with MCM8 gene knockout are infertile and prone to ovarian tumors, as are female mice with MCM9 gene knockout [87]. Several studies have reported that homozygous mutations of MCM8/9 gene have been detected in families of primary amenorrhea. The patients were accompanied by hypergonadotropin, small ovaries and infantile uterus, delayed development of secondary sexual characteristics, short stature and other symptoms, while heterozygous mutation carriers had no phenotype [89]. MutS homologue 5(MSH5) is a member of the MutS protein family. It is mainly involved in the homologous recombination repair mechanism of DSBs mainly through interaction with Holliday Junction. MSH5 knockout female rats develop progressive oocyte loss, ovarian atrophy and infertility after birth, which is very similar to the clinical presentation of human premature ovarian failure (POF) [90]. In addition, meiosis specific with OB-fold (MEIOB) protein stabilizes the binding of the recombinant enzyme to the DSB site. Both female and male mice with MEIOB gene knockout were infertile and sterile. Deletion of this gene has been found in patients with POI [91, 92]. These genes play an indispensable role in the process of DSB repair, and deletion of the corresponding genes will result in ineffective DSB repair, leading to its accumulation in the cells and eventually initiating the program of apoptosis, leading to the loss of oocytes. This process results in diminished ovarian reserve function and even the development of POI.

Base mismatches

DNA damage involves base mismatches. Abnormal repair can also lead to apoptosis. Diminished DNA repair

responses were observed in mice with knockout of the homologous recombination (HR) pathway gene Brca2. There was significant DSB accumulation and chromosome mismatch in their germ cells. The number of follicles was reduced by approximately half [93].

The mismatch repair (MMR) pathway can repair base mismatch. The relationship between the MMR pathway and follicle development needs to be further explored. In addition, the MMR pathway can repair some DNA damage such as base mismatches and may be beneficial in maintaining integrity of germ cell genome. Whole-exome sequencing of family members of a POI patient identified MSH4-pure mutations. This suggests that defects in the MMR pathway lead to germ cell development arrest and genomic instability, which may be associated with POI [94]. The MSH5 protein mentioned above is a member of the MutSγ protein family and is involved in a variety of DNA damage repair processes, especially in correcting base mismatches during DNA replication. Msh5 knockout female mice showed impaired chromosome pairing, meiotic prophase I arrest, and attenuated oocyte numbers. These mice exhibited sterility. MSH5 not only plays an important role in homologous chromosome pairing in oocyte meiosis, but may also accelerate follicle failure by affecting the process of homologous recombination repair during mitosis in granulosa cells [95]. This evidence suggests that base mismatches may affect ovarian function. However, further experimental confirmation is still needed.

Interstrand cross-linking

DNA interstrand cross-linking (ICL) is a highly toxic form of DNA damage that results from the covalent bonding of two bases on complementary DNA strands. The main features of Fanconi anemia are the occurrence of spontaneous DNA breaks and the ICL that can occur after cross-linking agent induction. Therefore, the classical ICL repair pathway was named the Fanconi anemia (FA) pathway accordingly. It can regulate ICL repair during DNA replication to maintain genome integrity. Both endogenous metabolites and exogenous inducers can lead to the development of ICLs, thereby, impeding DNA transcription and replication [96]. ICLs have been reported to be particularly harmful to rapidly dividing cells, and the genomes of germ cells are particularly vulnerable to widespread DNA damage.

The FA pathway mainly targets the repair of ICLs, including ICL recognition, lesion bypass, DNA excision and DSB repair [97]. Cells with impaired FA pathways are highly sensitive to DNA cross-linking agents and exhibit increased chromosome breaks, decreased cell viability, and cell cycle arrest [98, 99]. Mutations in the biallelic sites of the FANCL gene have now been found to result in the typical FA phenotype. However, FANCL knockout mice exhibited only reproductive defects, and other body systems were not significantly affected. It was also found that FANCL deficiency causes premature ovarian insufficiency in mice [100]. One study confirmed that the level of γH2AX was increased in FANCL knocked out cells, indicating that the deletion of FANCL impaired DNA repair [101]. In addition to the FANCL gene, three FA-related gene mutations have been identified in POI patients, including FANCD1/BRCA2, FANCM, and FANW/XECCR. In summary, the decrease in the number of germ cells may be related to the failure of repair of ICLs during the rapid division phase, leading to reduced proliferation and/or increased mediation.

Replication forks stalling

Replication stress can cause replication fork advancement to slow down or even stall, affecting DNA synthesis. Sustained replication stress can lead to the collapse of replication forks, resulting in double-strand breaks, which can be very damaging and difficult to repair for cells and living organisms. Replication fork stalling due to replication stress is also a major source of genomic rearrangements and mutations in cancer cells. Minichromosome maintenance complex (MCM) 2–7 is an important factor required for DNA replication, and a decrease of MCMs leads to an increase in replication stress, which may be associated with a decrease in the dormant origins. Mice carrying the CHAOS3 allele of MCM4 (Mcm^{c3}) have depleted MCM, had an increased numbers of micronucleated cells and were highly susceptible to cancer. FANCM is involved in DNA replication fork repair and mice lacking FANCM have impaired replication forks and consequently lead to genomic instability in embryos, as confirmed by an increased number of micronucleated cells [102]. The FA pathway we mentioned above also protects the nascent DNA strands of stalled replication forks from lysogenic degradation. There are two ways to restart a stalled replication fork, the 53BP1-dependent cleavage-free pathway and the BRCA1-dependent break-induced replication- (BIR-) like pathway.

Hydrosalpinx and DNA damage

It is well known that hydrosalpinx (HSF) is believed to affect the success rate of IVF. Mechanical scour of the endometrium, endometrial receptivity damage and embryotoxic effects of hydrosalpinx were considered to be the main reasons for the decreased success rate.

Oxidative stress may also play a role in the pathophysiology of HSF. One study excised the affected side of the fallopian tube in patients with HSF, and extracted the fluid. This confirmed the presence of ROS, lipid peroxidation (LPO) and total antioxidant capacity (TAC) in the fluid. It has also been suggested that low concentrations of ROS may be an indicator of normal fallopian

tube secretory function, which may have trophic effects on the embryo. ROS in HSF may originate from immune cells associated with chronic salpingitis. LPO, the product of OS, was detected in all HSFs, indicating that OS occurred in the acute phase of the disease. The embryotoxic effect of HSF was concentration-dependent when the concentration of HSF was greater than 50%. The blastocyst formation rate decreased with the increase of HSF concentration. Although the specific mechanism has not been formalized, DNA damage caused by ROS-induced oxidative stress couldn't be excluded [103].

Conclusions

Increased DNA damage can have an impact on oocytes, spermatozoas, and the developing embryos, leading to infertility, miscarriages, and birth defects. Maintenance of gamete quality is a prerequisite for successful conception, embryo development and pregnancy. Therefore, it is essential to understand whether oocytes respond to DNA damage and which mechanisms of DDR are active to prevent the transfer of genomic damage to the embryo. In this review, we have explored the relationship between infertility related diseases and DNA damage and repair. Researchers have tried a variety of approaches to study the presence and functional mechanism of DDR in mammals, reducing DNA damage and enhancing DNA repair in an attempt to protect female fertility. However, much work remains to be done to elucidate DNA repair pathways in mammalian oocytes and infertility related diseases.

Acknowledgements
Not applicable.

Author contributions
Conceptualization, XH.X. and GM.H.; validation, XH.X., ZW.W., and LY.L.; formal analysis and investigation, C.L. LL.W. and YN.S.; resources, GM.H. and Q.L.; writing—original draft preparation, XH.X. and ZW.W.; writing—review and editing, Q.L. and GM.H.; visualization, XH.X. and ZW.W.; supervision, GM.H. and ZM. Z.; project administration, BJ.S.; funding acquisition, XH.X.,GM.H.,BJ.S. All authors have read and agreed to the published version of the manuscript.

Funding
This study was supported by Hebei Natural Science Foundation (H2022206019). S&T Program of Hebei (21377721D, 21377720D, 22377795D). Medical Science Research Project of Hebei Province (20211494, 20240414). National Key R&D Program of China(2021YFC2700605). China Health Promotion Foundation. Hebei Provincial Government Funded Clinical Medicine Excellent Talent Program (2021).

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
All the authors approved the publication of this manuscript.

Competing interests
The authors declare no competing interests.

Received: 2 January 2024 / Accepted: 31 July 2024
Published online: 14 August 2024

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