Review Report

Sperm deoxyribonucleic acid damage and repair

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Abstract

A greater proportion of inseminated sperm population with fragmented deoxyribonucleic acid (DNA), in either natural service or assisted reproductive technology, has been associated with adverse reproductive outcomes, including abnormal embryonic development and failure of implantation, increasing pregnancy loss and reducing pregnancy rates. However, attempts to establish a causal relationship between sperm DNA damage and pregnancy success have often resulted in conflicting findings. Practical issues include accurate measurement of sperm DNA damage and the necessity to reduce impacts of iatrogenic damage. Sperm DNA repair mechanisms in oocytes and embryos are likely important in addressing sperm DNA damage, promoting normal embryo development, and enhancing reproductive outcomes. However, poor reproductive outcomes are likely caused by unrepaired sperm DNA damage exceeding a critical threshold and adversely affecting embryo development. This review reports sperm DNA damage and repair mechanisms.

Keywords: Sperm, oocyte, embryo, DNA damage, DNA repair

Introduction

Breeding soundness evaluation of bulls is an economically essential element of reproductive management. The prevalence of bulls considered unfit in breeding programs has been investigated and 1 of every 5 bulls in a general population had inadequate semen quality, physical soundness, or both.[1,2] In humans, ∼ 17.5% of the adult population, roughly 1 in 6 worldwide, experience infertility.[3] Males are solely responsible for 20-30% of infertility and contribute to 50% of overall cases.[4]

In clinical settings, sperm fertilization potential is commonly predicted by microscopic assessments of sperm motility and morphology. These regular tests are essential to provide the underlying information on sperm quality under field conditions. However, conventional sperm analysis results are highly variable due to their inherent subjectivity.[5] Further, it is not always possible to achieve an accurate decision regarding male fertility by routine semen evaluations. In bulls, percentage of progressively motile sperm (threshold of ≥ 30% sperm with forward motion) is determined by sperm motility, and percentage of normal sperm (threshold of ≥ 70% normal sperm) by morphological evaluation.[5] Though bulls with ≥ 70% morphologically normal sperm sired more calves and bulls with < 50% normal sperm sired fewer calves, a subpopulation of bulls with ≥ 70% normal sperm failed to sire a calf.[6]

Efforts to standardize assessment of these sperm parameters promoted development of more objective computer-assisted sperm analysis (CASA) and introduction of new technologies enabled investigation of sperm DNA integrity.[7]

Sperm heads contain a condensed haploid nucleus; the deoxyribonucleic acid (DNA) is extremely tightly packed, so that its volume is reduced, facilitating sperm transport.[8] Sperm DNA integrity has emerged as one of the most interesting topics in reproductive medicine. The integrity of a sperm genome is a fundamental factor in development of healthy offspring and can be an effective diagnostic tool for male reproductive potential.[9] Intact DNA is defined as complete absence of nicks and breaks, either single-stranded (SSB) or double-stranded (DSB), or any chemical causing alterations in its structure.[10]

Sperm DNA fragmentation (SDF) is the accumulation of SSBs or DSBs, measured by the DNA fragmentation index (DFI).[11] DFI is calculated by the number of single-stranded DNA in a sample, divided by the total number of sperm with intact DNA, and expressed as a percentage.[11] In many studies, elevated sperm DNA fragmentation was associated with adverse reproductive outcomes.[12] Clinical data demonstrating greater sperm DNA damage is positively associated with sperm morphological defects (Figure 1).[13] The negative

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impact of high levels of sperm DNA damage in natural breeding[13] (Figure 2) and in assisted reproductive techniques (ART) have also been demonstrated.[14,15] Therefore, assessment of sperm DNA integrity could be an independent marker of fertility.[16] Further, there are strong associations between damage to the paternal genome and embryo development, including effects on newborns and subsequent generations.[17]

The increasing use of ovum pick-up (OPU)/in vitro fertilization in cattle[18] and OPU/intracytoplasmic sperm insemination in horses[19] has raised concerns about unknown parental genome modifications affecting embryo development and future diseases in adulthood stemming from these gamete/embryo origins.[20] Subsequently, assessment of DNA damage in the male germ line and influence on reproductive outcome has received attention. An understanding of zygote/blastocyst capacity to repair damage would assist reproduction practitioners, and may help to produce healthy, viable embryos. This review summarizes sources of DNA damage and repair mechanisms in gametes and embryos, plus in silico analysis of DNA repair genes.

DNA damage in sperm

Damaged DNA has been detected in testicular, epididymal, and ejaculated sperm. Elements and assembly of sperm chromatin differ from somatic cells. During spermiogenesis, protamines replace most histones, encompassing chromatin

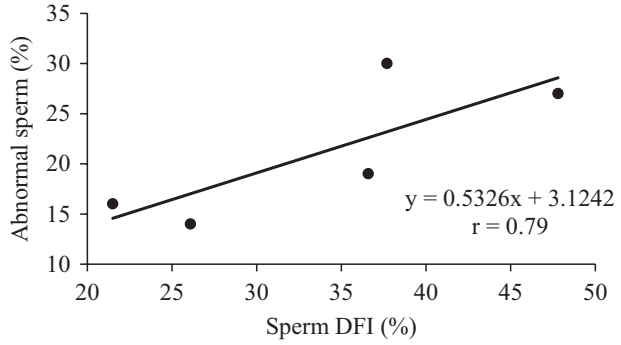

Figure 1. Association between percentage of sperm with abnormal morphology and sperm deoxyribonucleic acid fragmentation index (DFI) in bulls (adapted Borges et al.[12])

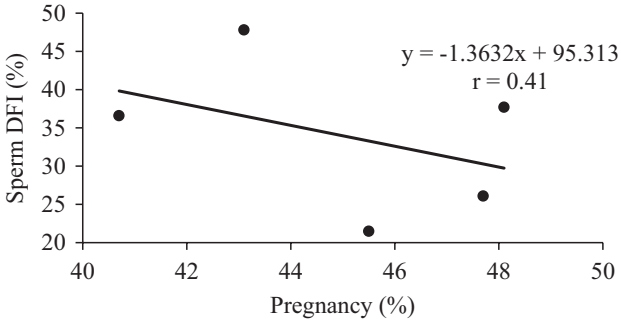

Figure 2. Association between sperm deoxyribonucleic acid fragmentation index (DFI) and pregnancy rate in bulls (adapted Borges et al.[12])

into toroid (a supercoiled structure). As sperm traverse the epididymis, protamines are cross-linked by disulphide bonds, reducing the chromatin to one-sixth the volume in somatic cell nuclei. This dense compaction gives protection against exogenous assaults to sperm DNA.[21-23] Infertile males have relatively high basal sperm DNA damage[16,17] and their sperm are more susceptible to postejaculation damage.[14,15]

Basis

Sperm DNA damage can arise from exogenous or endogenous sources (Table 1) and is due to: 1. faulty chromatin condensation during spermiogenesis, related to an unfitting protamination and inadequate chromatin packaging;[24,25] 2. the frequency of abortive apoptotic processes, (apoptosis cannot be completed due to nuclear and mitochondrial presence in various compartments in mature sperm);[26] and 3. oxidative stress attributable to an imbalance between reactive oxygen species production and antioxidant capacity.[27-30]

Protamination and DNA damage

During the final phases of sperm transformation ~ 80% of histones are replaced by protamines to enhance sperm nucleus compactness.[96] Although beneficial for sperm, protamination can have damaging effects on sperm quality, as errors in the replacement process can result in assembly of sperm with DNA damage.[97,98] While histones are substituted by protamines, temporal breaks occur in the DNA due to topoisomerase II activity that relaxes the DNA structure.[99] If

Table 1. Causes of sperm DNA fragmentation

Cause	References
Intrinsic causes:	
Deficiencies in recombination during spermatogenesis	31-33
Abnormal spermatid maturation	34-36
Protamine 1 and 2 ratios	37-41
Abortive apoptosis	42-46
Oxidative stress	47-64
Extrinsic causes:	
Interval after ejaculation	65-67
Collection method, extender, postejaculation treatment	68-70
Sperm preparation for ART	71,72
Sex-sorting	73-75
Posttesticular oxidative stress	76,77
Varicocele	78,79
Age	80-82
Bacterial infection	83,84
Abstinence	85,86
Testicular thermoregulation	87-89
Reactions to clinical procedures, medications or vaccines	90-92
Environmental chemicals	93-95

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these temporal breaks are not repaired properly before the end of spermiogenesis, they will subsequently appear in mature sperm as fragmented DNA.[100] Alternatively, both the quantity of histones that are replaced by protamines and the proportion of protamines (PRM)1/PRM2 added are typically consistent for each species; therefore, if the proportion is changed, the DNA is likely to be poorly packaged, more susceptible to exogenous agents, and more likely to cause infertility.[101] The ratio between PRM1 and PRM2 is ~ 1 in human, and changes in this ratio may cause male infertility.[102,103] As PRM2 contains fewer cysteine residues than PRM1, it produces fewer disulfide bridges, leaving the DNA slightly more exposed to adverse effects of external agents.[104,105] In addition, abnormal protamine ratios are related to male infertility through aberrant genomic imprinting in infertile humans and bulls.[106,107]

Abortive apoptosis and DNA damage

Apoptosis is genetically controlled, programmed cell death and essential for spermatogenesis. Both pro and antiapoptotic molecules involved in various stages of sperm differentiation are summarized (Table 2).[42] During normal spermatogenesis in mammals, apoptosis results in the loss of up to 75% of prospective sperm,[43] as it limits the germ cell population and optimizes the germ-Sertoli cell ratio.[44] It is a physiological mechanism to eliminate surplus cells during proliferation, removal of derived hormone-dependent cells and elimination of potential harmful cells.

During spermatogenesis, Sertoli cells select germ cells to pass from mitosis to meiosis; thereafter, ~ 60% of germ cells are marked to be eliminated via apoptosis. However, varying percentages of these marked cells escape apoptotic processes called 'abortive apoptosis', in which their DNA is partially fragmented, but they maintain capacity to differentiate into mature and even functional sperm[45] and will appear in the ejaculate as normal sperm with elevated levels of fragmented DNA.[46]

Oxidative stress and sperm DNA damage

An optimal concentration of reactive oxygen species (ROS) in semen is essential for key sperm physiological events, including sperm capacitation, acrosome reaction and sperm-oocyte fusion.[47,48] Generation of ROS in sperm occurs either in the plasma membrane (by nicotinamide adenine dinucleotide phosphate [NADPH] oxidase) or in the inner mitochondrial membrane (in the presence of a nicotinamide adenine dinucleotide-dependent oxidoreductase),[41,47,49] with the latter being the main source of ROS in sperm. Sperm midpiece is rich in mitochondria as they need energy to support motility. Sperm are also affected by ROS in seminal plasma, of either endogenous (leukocytes[50] and immature sperm[50,51]) or exogenous (radiation[52,53] and toxins[53]) origin. Normally, ROS in seminal plasma is counteracted by antioxidants. However, this balance can be unsettled, and oxidative stress causes lipid peroxidation, which reduces membrane fluidity and damages DNA.[14,54,55] Excessive ROS in seminal plasma directly and/or indirectly cause sperm DNA damage, abnormal semen parameters, impaired sperm function, and infertility.[14,56]

Table 2. Pro and antiapoptotic proteins involved in various stages of sperm differentiation

Stages of sperm differentiation	Proapoptotic protein	Antiapoptotic protein
Primordial germ cells	Bax, Bad, Bim, Bak	Bcl-x
Spermatogonia	Bax, p53	c-kit
Spermatocyte	Bax, Fas/FasL	Bcl-x_L, Bcl-x_S
Round spermatid	Fas/FasL	Bcl-x_L, Bcl-x_S
Elongated spermatid	Fas/FasL	Bcl-x
Sperm	Fas/FasL	Bcl-x

The sperm DNA may be damaged due to oxidative stress or from reactions between hydroxyl radicals and involve all components of DNA, including pyrimidine and purine bases and deoxyribose backbone. Reactive oxygen species can further generate DNA damage by production of base-free sites, frame shifts, DNA-protein cross-linkages, deletions, and chromosomal rearrangements.[57] In addition, ROS may stimulate protein kinase and poly(ADP-ribosyl)ation pathways; therefore, oxidative stress can affect signal transduction pathways and modify expression of genes.[58] These collectively may impair sperm functions. However, DNA damage, caused by free radicals, may be repaired by specific and nonspecific repair mechanisms, although there is the potential for ill-repair of DNA that may contribute to infertility.

Reactive oxygen species concentration in seminal plasma is maintained in a physiological range by numerous biological antioxidant mechanisms, including enzymatic antioxidants[59] (e.g. catalase, superoxide dismutase, and glutathione peroxidase), plus nonenzymatic compounds[60] such as vitamins C and E, carotenoids, lactoferrin or coenzyme Q10. Prostasomes in seminal plasma decrease superoxide radical release by leucocytes, thus reducing oxidative stress.[61,62] This antioxidant activity occurs through inhibition of NADPH oxidase activity in polymorphonuclear cells (PMNs). This arises by rigidification of the PMN plasma membrane by lipid transfer after fusion with a prostasome.[63,64]

Fragmented DNA in mature sperm may result from a combination of these processes. For example, apoptosis can occur due to ROS-induced sperm damage. Furthermore, synergistic effects among damaging effectors are likely to contribute to DNA damage and cause sperm death.

Single-stranded versus double-stranded breaks

In normal, healthy sperm, the chromatin is exemplified by a linear arrangement of nucleotides along each DNA strand and a lack of SSB and DSB, nucleotide modifications, or base loss.[108] Sperm chromatin has abundant alkali-labile sites, mainly localized in repetitive DNA sequences, prone to DNA torsion during chromatin packing. Chromatin damage is a comprehensive term that accounts for any defects in DNA structure, including: 1. SSB or DSB; 2. base deletion or modification; 3. interstrand or intrastrand DNA cross-linkage; and 4. protamine deficiency and/or mispackaging via defective DNA-protein cross-links (reviewed[109]). It may occur during spermatogenesis, spermiogenesis, epididymal transit or postejaculation. SDF relates to breaks at DNA strands that are termed SSBs or DSBs. Single-strand breaks

give rise to free 5′-3′ ends, affecting only 1 DNA strand, whereas its template remains undamaged. By contrast, DSBs are characterized by blunt 5′-3′ ends affecting both DNA strands.

Tests currently available to evaluate SDF include:

Sperm chromatin structure assay (SCSA)[14-16]
Terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labelling (TUNEL) and in situ nick translation (ISNT)[110-112] assays
Breakage detection-fluorescence in situ hybridization (DBD-FISH) test[113]
Comet assay[114,115]
Single-cell pulsed-field gel electrophoresis technique[116]
Sperm chromatin dispersion test (SCDt)[117,118]

These tests can be grouped in methods that use:

1. enzymatic reactions to label DNA breaks: either using a terminal transferase (e.g. TUNEL) or specific enzymes such as the Klenow fragment (e.g. in situ nick translation assay; ISNT) to label free 3-OH ends of the nucleotide at the DNA break. In the latter, a 5′- 3′ polymerase activity is combined with a 3′-5′ exonuclease activity for elimination of precoding nucleotides and proofreading. Both TUNEL and ISNT assays detect SSBs and DSBs indistinctively.
2. controlled DNA denaturation combined with protein depletion as intermediates to reveal DNA breaks: these tests apply DNA denaturation and/or controlled protein depletion. The SCSA relies on controlled DNA denaturation to target preexisting DNA breaks, whereas the alkaline Comet assay and the SCDt are based on DNA denaturation and controlled protein depletion. Like TUNEL, these assays determine the global SDF without discriminating between sperm with SSB versus DSB. By contrast, the neutral Comet assay only uses a controlled protein depletion to exclusively detect DSB whereas the 2-dimensional Comet assay uses 2 electrophoretic runs, one in a neutral buffer and another in an alkaline buffer to simultaneously map SSBs and DSBs.[119]
3. dyes that bind to relaxed guaning cytocine (GC)-rich motifs: includes tests that spot abnormal chromatin packaging by means of the fluorescent antibiotic chromomycin A3 staining, given its preference to bind relaxed DNA GC-rich motifs, toluidine blue staining, acridine orange test and aniline blue staining.[120] Notably, given that histone-complexed DNA (stained by acridine orange) fluoresces twice as brightly as protamine-complexed DNA,[121] this high DNA stainability sperm fraction, which represents sperm with excess nuclear histones and faulty chromatin condensation, can also be detected by the SCSA. Hence, this test also provides information about chromatin compaction.

The SCSA, Comet, SCDt, and TUNEL provide reliable information about sperm DNA integrity in subfertility.[122] However, optimal thresholds are still to be defined and are vital to understand how each test reports results. The SDF tests can detect sperm with evident DNA fragmentation, but sperm with undetectable damage may remain hidden or ambiguous within that population. The latter might not have yet fully expressed SDF at the time of analysis, representing sperm with a DNA fragmentation predisposition termed 'iceberg effect'.[65-67,109,123] All of these are valuable tools to acquire information on the state of the sperm DNA, but mechanisms of restitution in the oocyte and embryo may provide a confusing view of the real impact of DNA damage on pregnancy, contributing to discrepancies about impacts of SDF on pregnancy rates.

DNA repair mechanisms

An intricate balance of genome stability and instability is necessary, controlled by interactions of many DNA repair mechanisms. In germ cells, there are some levels of defenses that avoid the production and persistence of DNA damage, such as base mismatches, SSBs and DSBs, bulky adducts (disturb DNA transcription and replication, and prompt mutations), etc.

Male and female gametes, and embryos have an array of DNA repair pathways including:

1. nucleotide excision repair (NER): corrects a wide variety of helix-distorting DNA lesions and crosslinks, primarily caused by environmental agents such us ultraviolet light.[123,124]
2. mismatch repair (MMR): eliminates DNA mismatches produced from recombination between imperfectly matched sequences or from errors during DNA replication.[125] Mismatch repair can also repair oxidative damage and provide maintenance of repeated sequences.[123,126,127]
3. base excision repair (BER): corrects small DNA alterations that only affect 1 DNA strand and that do not distort the structure of the DNA helix such as the incorporation of uracil or oxidized bases induced by reactive oxygen species or the presence of SSBs[123,128-130]
4. homologous recombination (HR); a process that occurs during meiosis but is also used to repair DSBs as well as inter-strand DNA crosslinks (mainly due to ionizing radiation).[123,131]
5. nonhomologous end joining (NHEJ): repairs DSBs without using the homologous sequence as a template and thus can cause insertions and deletions.[123,132,133]

The DNA direct repair mechanisms, photolyase, alkyltransferase, and dioxygenase-mediated repair processes, provide cells with simple yet efficient solutions to reverse covalent DNA adducts.[134] These DNA repair mechanisms are active in practically all somatic cell types, as well as germ cells,[135] but mature sperm and oocytes have differential organization of their repair mechanisms.

DNA repair mechanisms in sperm

Spermatogenesis is a complex process that produces sperm, which are unique in structure and function. Spermatogenesis can be divided into 3 sequential steps:

1. Mitotic proliferation: produces large numbers of sperm over the reproductive lifetime; DNA mismatches produced during this step are repaired via the MMR pathway.[136]
2. Meiotic recombination: Meiotic recombination and chromosome segregation produce genetically diverse haploid gametes.[137,138] Regarding recombination, HR and NHEJ seem to divide their tasks based on the phase of the

cell cycle and the landscape of the DNA break.[139] Homologous recombination mainly works during S phase and on replication-derived 1-ended DSBs to accurately resolve the damage, whereas the more error-prone NHEJ process functions primarily during G1 assembly of 2-ended DSBs.[139-141] Perhaps programmed DNA DSBs during meiosis is mainly repaired by HR with high reliability. Repair of these breaks is tightly controlled to favor HR (the only repair pathway that can form crossovers).

3. Cytodifferentiation of spermatids: a complex remodeling of the haploid genome that involves replacing the majority of histones with protamines.[100] DNA compaction, an outcome of this process, is achieved by the transient formation of SSBs and DSBs in sperm DNA.[100,142] Spermatids resolve exogenous and programmed DSBs using the alternative NHEJ pathway[143,144] due to their haploid character and the absence of the main components of the classical NHEJ pathway. It is critical that these transition strand breaks are fixed during this step because persistence of DNA breaks in mature sperm can increase sperm DNA fragmentation.[145,146]

In general, mature sperm were deemed incapable of DNA damage restoration due to maximum compaction of their DNA and reduced transcriptional capacity.[147,148] However, human sperm retain a truncated but functional BER pathway containing only the 8-oxoguanine DNA glycosylase-1 protein.[147,148] The presence of this enzyme is adequate for sperm to detect and remove oxidized base adducts, specially 8-hydroxy-2′-deoxyguanosine (8-OHdG) residues, a prevalent product of oxidative stress.[149,150] Due to the rest of the pathway being shortened, the abasic (apurinic/apyrimidinic) site produced after excision of 8-OHdG must be then repaired by the oocyte after fertilization and prior to the first round of cell division during early embryo development.[149,150] At the end of spermiogenesis, disulfide cross-links are created between protamines, while spermatids pass through the epididymis. This process may be considered an inherent screening mechanism directed at eliminating genetically defective sperm, as the greater the DNA damage, the lower disulfide cross-linking is established, resulting in lower-quality sperm with a reduced capacity to fertilize an ovum or produce a good embryo.[151,152]

DNA repair mechanisms in the oocyte

Oogenesis can be divided into 3 phases:

1. Multiplication phase: primordial germ cells initiate differentiation into female germ cells (oogonia) in the early postimplantation embryo
2. Growth phase: oogonia divide through mitosis and enter meiosis I until they stop developing at the diplotene stage, in prophase I
3. Maturation phase: oocytes complete the first division of meiosis I during ovulation

The integrity of the oocyte genome is affected mainly by 2 processes:

1. meiotic recombination during the fetal period
2. the long postnatal period of meiotic arrest (dictyate stage) before meiotic division

Physiological DSBs are fabricated in association with meiotic recombination during the fetal period; however, this damage is generally repaired at the end of the meiotic prophase I by the oocyte through HR.[153,154] Failure to repair DNA damage caused by recombination directs meiotic checkpoints and activates apoptosis.[155,156] Regarding the prolonged postnatal interval of meiotic arrest prior to meiotic division, oocyte DNA is subjected to a wide range of potential damage that can reduce fertility.[157,158]

Several studies provided strong evidence that oocytes, from the primordial follicle stage in metaphase II (MII), have the capacity to repair damaged DNA and maintain genome integrity.[159,160] During oogenesis, genes related to DNA repair are expressed at high levels and their mRNAs and proteins are accumulated inside oocyte cytoplasm.[161] Transcripts from all DNA repair pathways including direct lesion reversal, BER, MMR, NER, HR, and NHEJ are represented in mouse, monkey, and human MII oocytes.[157,158] These transcripts and proteins have roles during fertilization to address changes in chromatin remodeling and maintain chromatin integrity, and are also used in the zygote until the embryo genome becomes active and can transcribe its own DNA repair genes.[162,163] Metaphase II stage oocytes have potential to DNA repair via NHEJ in mice.[164] However, another study suggested that there may be species differences in the ability of GV (germinal vesical stage) and MII oocytes to undertake DNA repair.[165] In this study, the overall expression patterns of genes involved in the repair of DNA DSBs differed between primates and mice. Therefore, it was proposed that rodent oocytes have better DNA repair potential than primates.[165] DNA repair efficiency in the oocyte also decreases with maternal age due to a reduction in mRNA levels for DNA repair genes.[166,167]

Sperm with DNA damage is mostly functional; hence, the damage is transmitted to the oocyte after fertilization.[161,164,168] This damage must be rapidly repaired to ensure that genetic information is transmitted reliably and to avoid paternal transmission of chromosomal structural abnormalities and/or mutations.[168] As mature sperm have limited DNA repair capabilities, oocytes are initially responsible for repair and remodeling of both paternal and maternal genomes. As demonstrated in mouse models, women with deficiencies in DNA repair mechanisms produce embryos with more frequent chromosome/chromatid aberrations and microsatellite instabilities.[157,169,170] After fertilization, DNA damage repair transcripts and proteins produced by the oocyte are used by the zygote until the embryonic genome is activated and can begin transcribing its own DNA repair genes.[157,162] This maternal-zygotic transition is species specific; in humans, it typically occurs at the 4-8 cell stage.[157,171] In due course, exhaustion of stored maternal mRNAs is compensated by zygotic transcription, leading to activation of cell cycle checkpoints and expression of DNA repair proteins.[157,168] However, impaired oocytes, especially those from older women, may not be able to adequately compensate for paternally derived DNA damage. Further, as oocytes age, their DNA repair efficiency diminishes due to lower expression of key DNA repair genes, such as *BRCA1*, *MRE11*, *RAD51*, and *ATM*.[172] This results in more DSB, chromosomal abnormalities, and increased sensitivity to oxidative stress.[157] These findings led to the hypothesis that lower-quality human oocytes may be unable to repair paternally derived DNA damage adequately, accounting for poor reproductive outcomes. Highly expressed DNA repair genes in MII oocytes are provided (Table 3).

Table 3. Highly expressed DNA repair genes in metaphase II stage (MII) oocytes

DNA repair genes in MII oocytes	References
ABH2	161,162
APEX1	161,162,173
BLM	162
CCNH	161,162
CDK7	161,162
CHAF1A	161,162
DCLRE1A	161,162
DUT	161,162
FANCL	162
FEN1	161,162
GTF2H2	161,162
GTF2H5	161,162
H2AFX	161,162
KIAA1794	162
LIG1	161,162
MBD4	161,162
MGMT	161,162
MSH2	161,162
MSH6	161,162
PCNA	161,162
POLB	161,162
RAD17	161,162
RAD50	161,162
RAD51	161,162
RAD51C	161,162
RAD52	161,162
RBBP8	162
REVL1	162
RPA2	161,162
TDP1	161,162
UBE2A	161,162
UNG	161,162
XPA	161,162
XRCC4	161,162
XRCC6	162

Prediction and analysis of differentially expressed DNA repair genes in MII oocytes

The miRNet (http://www.mirnet.ca/, accessed on 4 August 2024) platform was used to perform interaction analysis[174] and to select the top 10 DNA repair genes in MII oocytes with high degree (number of connections a node has to other nodes) and betweenness (number of connections occurring upon a node) centrality. Betweenness centrality measures the extent to which a miRNA/gene lies on paths between other miRNAs/genes. MicroRNAs/genes with high betweenness may have substantial influence within a regulatory network by virtue of their control over passing information between others.[175] Genes with a high degree centrality are important for diagnosis of disease, whereas proteins with a high betweenness are important for drug discovery.[175]

Thirty-four DNA repair genes in MII oocytes interacted with 45 transcription factors and 2050 miRNA (Figure 3). The top 10 DNA repair genes in MII oocytes with high degree and betweenness centrality are illustrated (Table 4) and their tissue and single-cell expressions, associated genes, and functions are listed (Table 5).

DNA repair mechanisms in the zygote

Fertilization induces a cascade of critical events including fusion of paternal and maternal DNA and development of the zygote. During this stage, DNA has 3 main processes:

1. parental chromosomes initially exist separately as distinct maternal and paternal pronuclei,
2. remodeling of chromatin assembly with active demethylation of paternal DNA versus passive demethylation of maternal DNA[176]
3. reparation of SSBs and DSBs in paternal DNA[177,178]

The DNA repair in the zygote is considered a maternal trait because until embryonic genome activation occurs (4-cell stage in humans),[179] zygote development is supported by maternal transcripts and proteins. As mature sperm have reduced DNA repair capacity, a few DNA lesions will inevitably remain in sperm DNA and will need to be reduced/eliminated when gametes are enlisted in the zygote. Following fertilization, the effects of sperm DNA damage depend on the balance between the amount and/or type of DNA damage present and the capacity of the fertilized oocyte to fix the damaged sperm DNA. It has been suggested that an oocyte has the capacity to repair 8% sperm DNA damage[180]; higher levels of sperm DNA damage are associated with a failure to reach the blastocyst stage[180] and embryonic loss between embryonic genome activation and blastocyst stages,[181] known as a 'late effect' from paternal DNA damage.[182,183] An early paternal effect can compromise reproductive outcomes without increased sperm DNA fragmentation. Further, sperm DNA fragmentation evaluation is useful to detect late paternal effect, which is not associated with morphological abnormalities at the zygote and early cleavage stages.[183]

Sperm DNA fragmentation repair in embryos during/after implantation

Unrepaired DNA damage beyond a critical threshold in embryos with normal karyotype generated in vivo and in vitro may cause failure in embryo development after embryo implantation. This type of damage is expressed during/after implantation and has been characterized as late paternal effect.[183] There are also indicators of high levels of DNA damage in a sperm sample with failure to obtain blastocysts[162] and it is believed that some loss of preimplantation embryos occurs between postembryonic genome activation and the blastocyst stage.[184-186] Short G1 and G2 phases in rapidly dividing blastomeres support the assumption that HR is the dominant DSBR mechanism in the blastocyst. The first step in HR involves *RAD52*, which competes with KU to direct DSBR toward HR rather than NHEJ.[164,165] Many animals lacking DNA repair enzymes are not viable, with preimplantation death being a common result; however, much of the embryonic loss occurs around implantation, indicating the necessity for DNA repair ability when embryonic cells proliferate rapidly and differentiate.[166] Many DNA repair genes involved in DNA damage response pathways seem to be expressed in postimplantation mammalian embryos, especially mid-gestational stage onwards. At this stage, spatial

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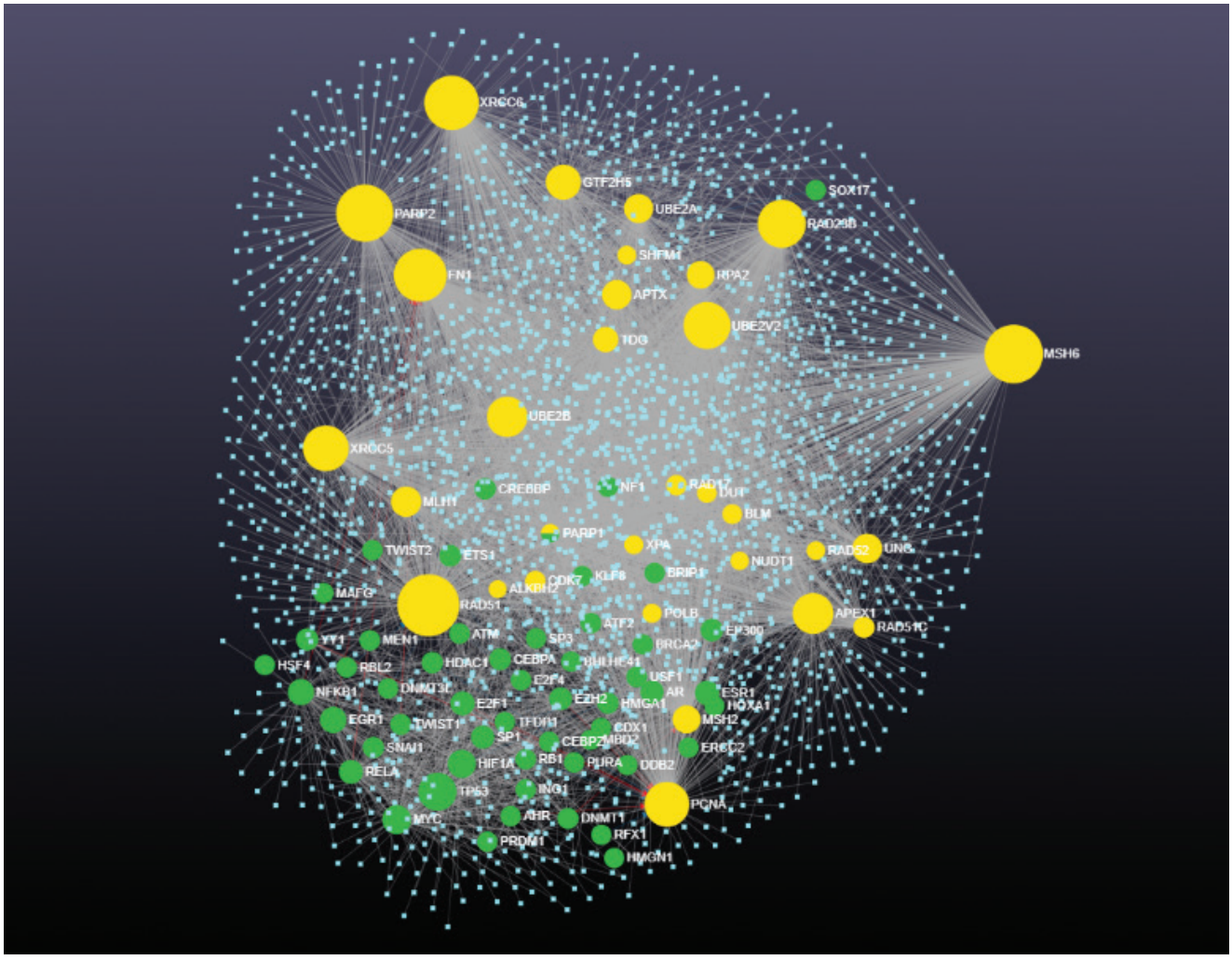

Figure 3. Gene-miRNA-transcription factor interaction network of DNA repair genes in MII oocytes; 34 genes interacted with 2050 miRNAs and 45 transcription factors ($p < 0.05$). Yellow circles denote genes; green circles denote transcription factors; and size indicates significance. Blue squares denote miRNAs

Table 4. Top 10 DNA repair genes in metaphase II stage (MII) oocytes with high degree and betweenness centrality

High degree centrality				High betweenness centrality			
#	ID	Degree	Betweenness	#	ID	Degree	Betweenness
1	*MSH6*	424	352737.6	1	*RAD51*	375	403488.3
2	*XRCC6*	382	290840.3	2	*MSH6*	424	352737.6
3	*RAD51*	375	403488.3	3	*XRCC6*	382	290840.3
4	*PCNA*	296	226486.4	4	*PCNA*	296	226486.4
5	*APEX1*	283	199468.7	5	*RAD50*	275	216615.7
6	*RAD50*	275	216615.7	6	*APEX1*	283	199468.7
7	*MBD4*	229	167614.4	7	*H2AFX*	192	173755.7
8	*GTF2H5*	226	172415.7	8	*GTF2H5*	226	172415.7
9	*MSH2*	221	100570.1	9	*MBD4*	229	167614.4
10	*FEN1*	208	121183	10	*FEN1*	208	121183

Table 5. Top 10 genes (in metaphase II stage (MII) oocytes with a high degree and betweenness centrality) and their characteristics

Gene	Tissue expression	Single-cell normalized expression (nTPM)	Associated Genes	Functions
APEX1 (BER)	Ovary Testis	Oocyte 95.6 Spermatocytes 11.7 Spermatogonia 55.0	*ANP32A* *FEN1* *LIG1* *POLB* *XRCC1*	Vital role in DNA repair and redox regulation
FEN1	Ovary Testis	Oocyte 27.9 Spermatocytes 98.9 Spermatogonia 67.2	*APEX1* *PCNA* *POLD1* *LIG1* *WRN*	DNA replication and repair
MSH6 (MMR)	Ovary Testis	Oocyte 27.9 Spermatocytes 44.7 Spermatogonia 66.0	*PCNA* *MSH2* *MLH1* *MSH3* *PMS2*	DNA mismatch repair
RAD50 (HR)	Ovary Testis	Oocyte 8.8 Spermatocytes 8.5 Spermatogonia 6.6	*BRAD1* *BRCA1* *MRE11* *NBN* *RBBP8*	Vital role in DSB repair, DNA recombination, maintenance of telomere integrity and meiosis
XRCC6 (NHEJ)	Ovary Testis	Oocyte 233.1 Spermatocytes 584.0 Spermatogonia 303.1	*APLF* *XRCC4* *XRCC5* *LIG4* *PRKDC*	Repair of nonhomologous DNA ends, e.g. as required for DSB repair, transposition, and V(D)J recombination.
RAD51 (HR)	Ovary Testis	Oocyte 67.6 Spermatocytes 65.6 Spermatogonia 28.7	*XRCC3* *BLM* *BRCA2* *RAD52* *ABCD3*	Homologous strand exchange, a key step in DNA repair through homologous recombination
MBD4 (BER)	Ovary Testis	Oocyte 116.2 Spermatocytes 79.6 Spermatogonia 93.8	*DNMT* *DNMT3A* *DNMT3B* *MLH1 TDG*	DNA repair
GTF2H5 (NER)	Ovary Testis	Oocyte 241.2 Spermatocytes 336.5 Spermatogonia 162.9	*ERCC2* *ERCC3* *GTF2H1* *GTF2H2* *GTF2H4*	General and transcription-coupled NER of damaged DNA. In NER, it opens DNA around lesion to allow excision of the damaged oligonucleotide and its replacement by a new DNA fragment. Essential role in transcription initiation.
MSH2 (MMR)	Ovary Testis	Oocyte 19.9 Spermatocytes 80.0 Spermatogonia 59.0	*ATM,* *MLH1* *MSH3* *MSH6* *PMS2*	DNA mismatch repair system; 2 formed heterodimers bind to DNA mismatches to initiate repair.

(*Continued*)

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Table 5. (*Continued*)

Gene	Tissue expression	Single-cell normalized expression (nTPM)	Associated Genes	Functions
PCNA (BER, NER and MMR)	Ovary Testis	Oocyte 365.4 Spermatocytes 218.6 Spermatogonia 119.5	*FEN1* *LIG1* *POLH* *RFC2* *RFC3*	Increase processivity of leading strand synthesis during DNA replication; involved in RAD6-dependent DNA repair pathway

patterns of expression in the embryo become apparent for a few DNA repair genes.[187]

The capacity of the mammalian embryo to repair damaged DNA and its selective sensitivity to specific lesions is not well understood. Many gaps exist in our current knowledge concerning the roles and expression timings of several DNA repair genes in the early stages of embryonic development. The observed developmental stage-specific variations in DNA repair gene expression transcripts and proteins highlight complexity of the regulation of these pathways during development. Highly expressed genes in embryos are given (Table 6).

Prediction and analysis of differentially expressed DNA repair genes in embryo

The miRNet (http://www.mirnet.ca/, accessed on 4 August 2024) platform was used to perform interaction analysis[174] and to select the top 10 DNA repair genes with high degree and betweenness centrality in blastocysts. Thirty-four DNA repair genes in blastocysts interacted with 249 transcription factors and 2334 miRNAs (Figure 4). The top 10 DNA repair genes in embryo with high degree and betweenness centrality and their tissue and single-cell expressions, associated genes, and functions are in Tables 7 and 8, respectively.

The DNA repair genes expressed in MII oocytes and embryos and their involvement in the repair pathways are listed (Table 9). The gene *PCNA* is expressed in both MII oocytes and embryos, involved in 3 pathways (BER, MMR, and NMR). Similarly, *RAD51* is involved in the HR pathway and is expressed in both MII oocytes and embryos. The *XRCC6* gene is expressed in MII oocytes and embryos and is involved in the NEHJ pathway. Spatiotemporal expression of these common genes could be diagnostic tools to determine the extent of DNA repair by oocytes and embryos.

Conclusion

Excellent quality oocytes and embryos have better DNA repair capabilities, enabling them to compensate for sperm DNA damage and to produce excellent quality embryos that are more likely to develop normally. However, the exact mechanism(s) remain under investigation. Additionally, it is unknown what type and/or degree of sperm DNA damage is too much for an oocyte and/or embryo to overcome.

Table 6. Highly expressed DNA repair genes in embryos

DNA repair genes in embryo	References
ALKBH2	162
APEX1	161,162,173
APTX	161,162
BLM	162
CDK7	161,162
CHAf1A	161,162
DUT	161,162
FN1	162
GTF2H5	161,162
H2AFX	161,162
MLH1	161,162
MMS19L	161,162
MSH2	161,162
MSH6	161,162
NUDT1	161,162
PARP1	161,162
PARP2	161,162
PCNA	161,162
POLB	161,162
RAD17	161,162
RAD23B	161,162
RAD51	161,162
RAD51C	161,162
RAD52	161,162
RPA2	161,162
SHFM1	161,162
TDG	161,162
UBE2A	161,162
UBE2B	161,162
UBE2V2	161,162
UNG	162
XPA	161,162
XRCC5	161,162
XRCC6	161,162
	162

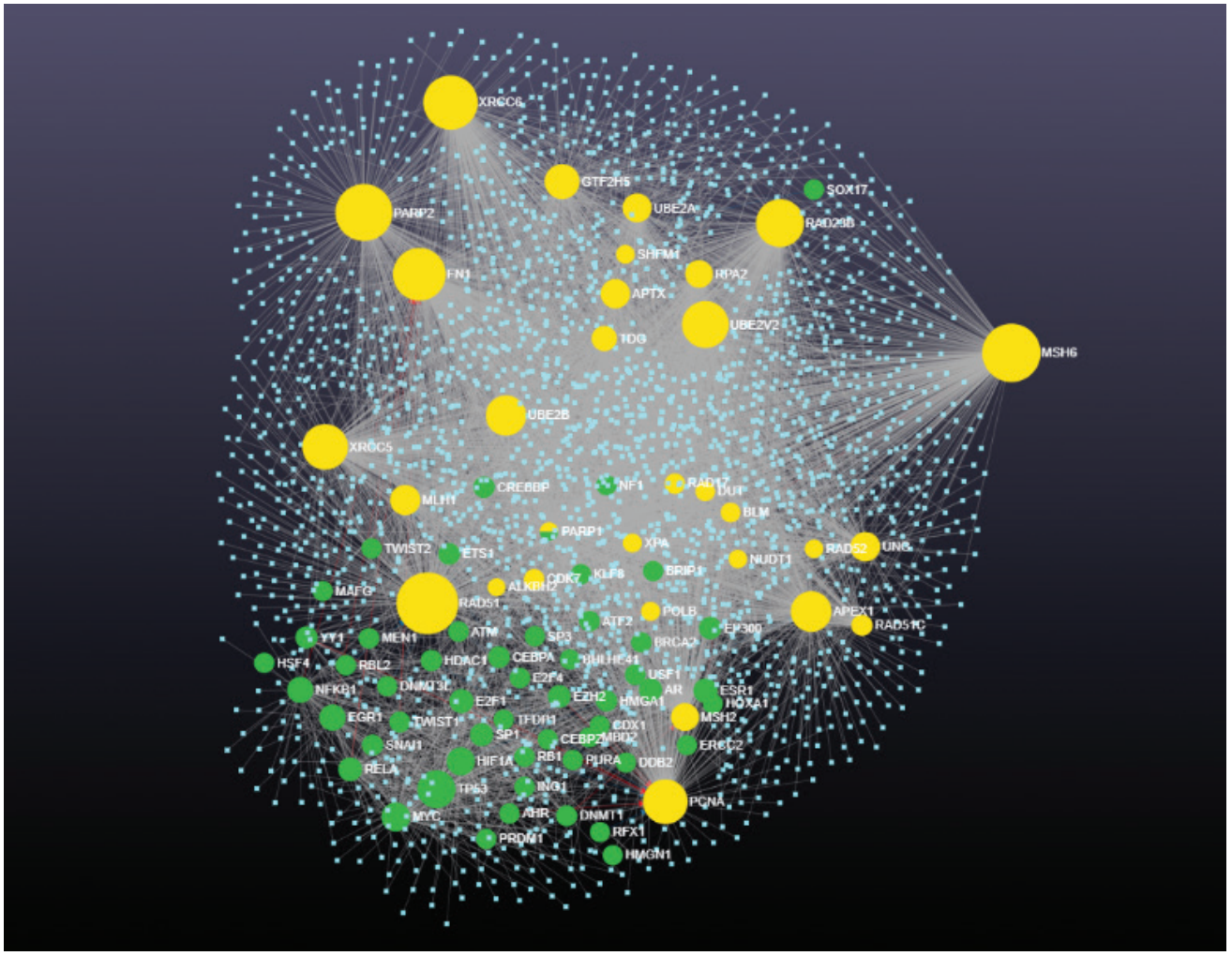

Figure 4. Gene–miRNA-transcription factor interaction network of DNA repair genes in embryos. Thirty-four 34 genes interacted with 2334 miRNAs and 249 transcription factors ($p < 0.05$). Yellow circles denote genes, green circles denote transcription factors and size indicates significance. Blue squares denote miRNAs

Table 7. Top 10 DNA repair genes in blastocysts with high degree and betweenness centrality

High degree centrality				High betweenness centrality			
#	ID	Degree	Betweenness	#	ID	Degree	Betweenness
1	*PARP1*	472	468024.5	1	*PARP1*	472	468024.5
2	*FN1*	461	306674	2	*RAD51*	374	385735
3	*MSH6*	424	355667.7	3	*MSH6*	424	355667.7
4	*RAD23B*	399	264386	4	*PARP2*	325	343441.9
5	*XRCC6*	393	319979.2	5	*XRCC6*	393	319979.2
6	*RAD51*	374	385735	6	*FN1*	461	306674
7	*XRCC5*	371	243838.4	7	*RAD23B*	399	264386
8	*UBE2V2*	364	254464.1	8	*UBE2V2*	364	254464.1
9	*PARP2*	325	343441.9	9	*XRCC5*	371	243838.4
10	*PCNA*	293	237321.9	10	*PCNA*	293	237321.9

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Table 8. Top 10 genes (in blastocysts with a high degree and betweenness centrality) and their characteristics

Gene	Tissue expression	Single-cell normalized expression (nTPM)	Associated genes	Functions
PARP1	Ovary Testis	Oocyte 152.2 Spermatocytes 111.5 Spermatogonia 226.1	*CASP3*, *XRCC5*, *XRCC6*, *TIM1*, *HPF1*	Recovery of cell from DNA damage; regulation of differentiation, proliferation, and transformation
FN1	Ovary Testis	Oocyte 28.7 Spermatocytes 2.6 Spermatogonia 15.2	*ITGAV*, *ITGB3*, *ITGB1*, *ITGA5*, *YES1*	Cell adhesion and migration processes including embryogenesis
MSH6	Ovary Testis	Oocyte 27.9 Spermatocytes 44.7 Spermatogonia 66.0	*PCNA*, *MSH2*, *MLH1*, *MSH3*, *PMS2*	DNA mismatch repair
RAD23B (NER)	Ovary Testis	Oocyte 80.1 Spermatocytes 51.2 Spermatogonia 101.7	*NGLY1*, *PSMD4*, *PSMD2*, *UBC*, *XPC*	Nucleotide excision repair
XRCC6 (NHEJ)	Ovary Testis	Oocyte 233.1 Spermatocytes 584.0 Spermatogonia 303.1	*APLF*, *XRCC4*, *XRCC5*, *LIG4*, *PRKDC*	Repair of nonhomologous DNA ends, e.g. as required for DSB repair, transposition, and V(D)J recombination.
RAD51 (HR)	Ovary Testis	Oocyte 67.6 Spermatocytes 65.6 Spermatogonia 28.7	*XRCC3*, *BLM*, *BRCA2*, *RAD52*, *ABCD3*	Important role in homologous strand exchange, a key step in DNA repair through homologous recombination
XRCC5 (NHEJ)	Ovary Testis	Oocyte 43.4 Spermatocytes 262.4 Spermatogonia 169.7	*APLF*, *NHEJ1*, *PRKDC*, *LIG4*, *XRCC6*	DSB repair and V(D)J recombination
UBE2V2 (error-free postreplication repair)	Ovary Testis	Oocyte 65.4 Spermatocytes 196.3 Spermatogonia 96.0	*RPS27A*, *UBC*, *RAD18*, *UBB*, *UBE2N*	Control of progress through the cell cycle and differentiation, role in error-free DNA repair pathway and contributes to the survival of cells after DNA damage.

(*Continued*)

Table 8. (*Continued*)

Gene	Tissue expression	Single-cell normalized expression (nTPM)	Associated genes	Functions
PARP2 (BER)	Ovary Testis	Oocyte 35.3 Spermatocytes 30.2 Spermatogonia 47.8	*HPF1*, *PARG*, *LIG3*, *POLB*, *XRCC1*	Key role in DNA repair
PCNA	Ovary Testis	Oocyte 365.4 Spermatocytes 218.6 Spermatogonia 119.5	*FEN1*, *LIG1*, *POLH*, *RFC2*, *RFC3*	Increase processivity of leading strand synthesis during DNA replication; involved in RAD6-dependent DNA repair pathway

Table 9. DNA repair genes expressed in MII oocytes and embryos, and their involvement in repair pathways

DNA repair pathways	DNA repair genes in MII oocytes	DNA repair genes in embryo
BER pathway	*APEX1*,[153,173] *MBD4*,[162] and *PCNA*[188]	*PARP1*,[189,190] *PARP2*,[190] and *PCNA*[192]
MMR pathway	*MSH2*,[153,191,182] *MSH6*,[154,161,162,193] and PCNA[178]	*MSH6*[194] and *PCNA*[192]
NER pathway	*GTF2H5* and *PCNA*[188]	*RAD23B*[161,162] and *PCNA*[192]
HR pathway	*RAD50*[161,162,195,196] and *RAD51*[154,162,191]	*RAD51*[161,162,197]
NEHJ pathway	*XRCC6*[162,191,196]	*XRCC5*,[161.162,198,199] and *XRCC6*[153,200]

UBE2V2 - error-free postreplication repair

Further studies are required to elucidate DNA repair capabilities of oocytes and embryos and the extent to which excellent quality oocytes and embryos can overcome high SDF levels. In addition, currently available DNA fragmentation tests cannot provide information regarding 'reparability' of sperm DNA damage. Hence, there is a need for tests that predict the reparability of SDF.

Conflict of interest

None to report.

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