Chapter 18
Sperm DNA Damage and Oocyte Repair Capability

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18.1 Introduction

Approximately one in every six couples suffers from infertility (17%), and male factor contributes to 40–50% of these cases [1, 2]. The causes of almost half of these male factor-associated infertilities are unexplained/idiopathic, and within this group, 5–10% men had high amount of sperm DNA fragmentation despite having normal semen parameters. In fact, DNA fragmentation is observed in 5–10% of infertile normozoospermic men [3–5]. Today, *routine* semen analysis is the "gold standard" test used in the evaluation of male infertility; however, it is unable to identify the causes of some of the cases [6]. Therefore, in order to distinguish infertile men from the fertile population and to predict the success of in vitro fertilization (IVF) cycle outcomes, a new diagnostic test is required. The use of DNA fragmentation tests as a part of the routine analysis in fertility investigation remains controversial [7–9], despite the fact that many research groups are greatly in favour of these test [10–13].

Some studies have shown that sperm DNA fragmentation is correlated with poor reproductive outcomes including miscarriages, chromosomal aberrations, congenital malformations, genetic disorders, neurological defects and cancer in offspring [14, 15]. Understanding the mechanisms after fertilization in the zygote is therefore important.

This chapter reviews closely the process of DNA damage in spermatozoa, origin of DNA damage, the effect of sperm DNA damage on reproductive outcomes and the selection methods for spermatozoa as well as the DNA repair mechanisms in the oocyte.

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18.2 Sperm DNA Damage

Sperm DNA damage results from any modifications of the molecular structure of DNA, including a chemically changed base such as 8-OHdG, a base missing from the backbone of DNA or single-stranded DNA breaks (SSBs) and/or double-stranded DNA breaks (DSBs). DNA fragmentation of sperm has been assessed in epididymal, testicular and ejaculated sperm [16]. In male germ cells, DNA repair is highly effective during mitosis and meiosis until the last 3 weeks of spermatogenesis. Therefore, spermatozoa are very susceptible to DNA damage towards the end of spermatogenesis in the haploid and compacted nucleus of spermatid due to insufficient DNA repair system, and these lesions would be transmitted unrepaired into the egg [16, 17]. Errors in maternal repair of sperm DNA damage may, thus, lead to chromosomal abnormalities in zygotes [18].

In general, there are two pathways in which sperm DNA fragmentation is originated: intrinsic and extrinsic factors.

18.3 Origin of DNA Fragmentation in Sperm Nucleus

18.3.1 Intrinsic Factors

18.3.1.1 Aberrations in Recombination During Spermatogenesis

DSBs are formed by specific nucleases during meiotic crossing-over process. These breaks should be ligated prior to meiosis II. Generally, before the DNA is fully fixed, the recombination checkpoint of the prophase does not permit the cells to proceed to meiosis I [19]. However, the defects that occur or persist at the checkpoint may result in permanent DNA fragmentation in ejaculated spermatozoa [20].

18.3.1.2 Abnormal Spermatid Maturation (or Abnormal Protamination Defects) During Spermatogenesis

Both SSBs and DSBs occur during the maturation process of spermatozoa into spermatids. These breaks are necessary for the packaging of sperm DNA with protamines [21] and are usually repaired and restored prior to the epididymal transit [20].

18.3.1.3 Apoptosis During Spermatogenesis

Male germ cells are regulated by Sertoli cells; nearly half of them undergo apoptosis at meiosis I during spermatogenesis. These selected cells are labelled with the Fas-type apoptotic markers and should be phagocytosed and removed by the Sertoli cell [22].

18.3.1.4 Oxidative Stress

Reactive oxygen species (ROS) are extremely unstable particles that comprises of oxygen metabolites [23]. Sources of ROS can be exogenous or endogenous. Exogenous sources of ROS originate from outside of the cells/environment, including radiation (x-rays, UV light), cigarette smoking, herbicides, alcohol abuse, chronic stress, drugs (acetaminophen) and air pollution. On the other hand, the endogenous sources are those arise from within the cell, including mitochondrial respiration and enzymatic systems such as xanthine oxidase and NADPH oxidase [18].

18.3.2 Extrinsic Factors

18.3.2.1 Age

Some studies have indicated that men with advanced age have an elevated sperm DNA fragmentation, while DNA fragmentation is considerably lower in younger men (<35 years) [24, 25]. mtDNA is more vulnerable to ROS (generated by electron transport chain) than nuclear DNA. Mutations accumulate in mtDNA and result in mitochondrial dysfunction, which in turn causes an increase in ROS production and oxidative damage and decrease in ATP/ADP ratio. Elevated ROS production, decreased ATP production and apoptosis are three features of dysfunctional mitochondria disrupted by ageing. Some studies have indicated higher DNA fragmentation index (DFI) in older men. Furthermore, high oxidative stress leads to increased apoptosis and spermatozoal DNA damage. Although apoptosis is essential for spermatogenesis under normal conditions, the balance between proliferation of spermatogonia and apoptosis of different germ cell types appears to be disturbed with ageing. This is supported by recent histological and ultrastructural study showing increased apoptosis along with a reduced proliferation in germ cells of the ageing testes. Although the significant decrease in the number of germ cells was found at the late spermatid level, primary spermatocytes did show a numerical decrease in the elderly men compared with the young controls. As the effect of age on sperm DNA single- and double-strand breaks is well documented, the presence of DNA damage repair-associated proteins such as poly (ADP-ribose) polymerase 1 (PARP-1) was also investigated in testicular tissue samples from older men. Statistically significant differences in the expression of DNA repair proteins as well as apoptosis markers, such as active caspase-3 and cleaved PARP-1, were found most markedly in ageing spermatocytes [24].

18.3.2.2 Abstinence Time

Recent studies have claimed that short abstinence period between ejaculations may lead to lower levels of sperm DNA fragmentation (24 h and 3 h) [26]. Sperm DNA fragmentation appears to become considerably elevated during the transit in the

seminiferous tubules towards the epididymis, possibly associated with oxidative stress, and as a result, DNA fragmentation levels rise within the sperm population because of the non-functional DNA repair pathways [27].

18.3.2.3 Scrotal Temperature

The scrotal temperature is 2–8 °C lower compared to the rest of the body, and this is essential for proper spermatogenesis in mammals. In a mouse model study, a high level of DNA fragmentation was observed in spermatocytes retrieved from testes exposed to 40–42 °C [28].

18.3.2.4 Response to Clinical Process, Medications, Environmental Pollutants and Smoking

Some environmental factors including radiation, smoking and alcohol consumption contribute to male infertility [29–31]. Untreated cancer patients [32] as well as those who have been exposed to chemotherapy and radiotherapy [33], environmental pollutants [34, 35] and certain cytotoxic medications [36] may be prone to sperm DNA fragmentation.

Tobacco smoke has known to have mutagenic effects and has been associated with a decrease in semen quality, fertilizing capacity and elevation in the quantity of abnormal cells [29, 37]. Smoking generates reactive intermediates, including reactive nitrogen species (RNS) and reactive oxygen species (ROS), which can induce various genetic and epigenetic alterations. Through the interaction of these intermediates, exposure to tobacco smoke can directly or indirectly cause the formation of DNA and protein adducts, mutations, promoter methylation, sister chromatid exchange (SCE), chromosomal abnormalities and micronucleus formation [29]. Additionally, several studies indicate that the sperm DNA fragmentation index is significantly elevated in fertile smokers [37, 38].

Various studies have demonstrated that alcohol consumption may change both spermatogenesis and the secretion of testosterone. Alcohol consumption produces notable morphological changes in spermatozoa including breakage of the sperm head, distention of the midsection and curled tails. Overall these effects may be based on alterations of the endocrine system controlling the hypothalamic–pituitary–testicular (HPT) axis function and/or testis and/or male accessory glands [30].

Ionizing radiation produces DNA lesions leading to DNA damage, and mutations result in genomic instability that is very harmful for fertility and/or the offspring in adult spermatogenic cells. Although the unique organization of spermatogenic cells within seminiferous tubules makes them less radiosensitive compared to somatic cells, DNA repair rate and frequency of unrepaired lesions are slower in spermatogenic cells compared to somatic cells. Therefore, the use of haploid cells with genomic instability in assisted reproduction could increase the hereditary risk [31, 39].

18.3.2.5 Varicocele

Varicocele affects approximately 15–20% of males and is one of the commonest causes of poor sperm quality (sperm concentration and motility). Significantly higher DNA fragmentation has been observed in patients with varicocele [40, 41] although the underlying mechanism still remains unclear.

18.3.2.6 Microbial Infections and Leucocytospermia

Elevated sperm DNA fragmentation has been found in some patients with genitourinary tract infection such as *Mycoplasma* and *Chlamydia trachomatis* in comparison to fertile controls [42, 43]. The presence of such genital tract infection is associated with a higher concentration of leucocytes and immature germ cell in semen [44] which could lead to the generation of ROS, leading to higher DNA damage. In addition, a higher level of DNA damaged cells were reported [45] in semen samples of leucocytospermic patients.

18.3.2.7 Sperm Preparation Techniques and Cryopreservation

Semen collection techniques and sperm preparation methods affect sperm DNA quality [46, 47]. To preserve spermatozoa with higher motility rates and lower sperm DNA fragmentation, density gradient and swim-up techniques have been suggested to be used for in vitro fertilization (IVF) [48, 49]. *Cryopreservation* of sperm is a useable method to preserve male fertility for utilization in artificial reproduction techniques (ART) in the future prior to chemotherapy, radiotherapy, surgical treatments or vasectomy. However, some studies have demonstrated that this method might have a negative effect on sperm DNA stability [48, 50].

18.4 The Role of Sperm DNA Integrity on Reproductive Success

Model organism reports demonstrated the significance of sperm DNA integrity during prenatal development and implantation [51]. Following studies correlated the level of DNA damage and fertility indexes of the offspring including fertilization success, rate and quality of embryo cleavage, implantation, pregnancy, and live birth rates (Table 18.1).

Table 18.1 The association amongst sperm DNA damage, pregnancy and abortion rate

		High DNA damage			Low DNA damage			
Assay	Cut-off (%)	Pregnancy (%)	Abortion (%)	Total	Pregnancy (%)	Abortion (%)	Total	Reference
SCSA	27	50	0	10	29	0	24	[52]
Comet assay	NI	29	83	30	27	0	22	[53]
SCSA	30	28	NI	57	47	NI	107	[54]
TUNEL	15	32	36	44	36	8	258	[55]
TUNEL	30	27	26	201	30	23	797	[56]
SCSA	30	28	63	29	34	42	77	[57]
TUNEL	15	6	100	18	44	0	18	[58]
TUNEL	10	12	60	43	28	8	89	[59]
TUNEL	10	13	100	18	29	30	34	[60]
SCSA	27	28	NI	25	29	NI	61	[61]
Acridine orange	30	55	33	11	51	12	49	[62]
TUNEL	35	39	35	52	62	10	65	[63]
SCSA	27	51	27	43	52	10	180	[64]
TUNEL	36	42	46	26	56	11	135	[65]
Comet assay	50	19	14	192	33	17	147	[25]
Acridine orange	50	49	37	39	47	25	114	[66]

NI not indicated

18.4.1 Association Between DNA Damage and Basic Semen Criterion

Although a few reports have indicated a slight or non-significant association between semen parameters (sperm count, motility, progression and morphology) and sperm DNA damage, many studies show that sperm from men with abnormal sperm parameters have a higher percentage of DNA damage [52, 67–71] .

There are different causes of DNA damage during spermatogenesis. If sperm DNA damage arises from the failure of DNA break repair (DBR), it would also be correlated with other indications of spermatogenic failure including teratozoospermia and oligozoospermia.

Similarly, if the damage of sperm DNA is primarily a consequence of the negative effects of ROS, sperm motility will also be affected as ROS can induce lipid peroxidation in sperm membrane which contains high amount of unsaturated fatty acids [52, 67–71]. Unrepaired DSB can cause mutations as a result of fixed DNA fragmentation [72].

18.4.2 Natural Fertility

Recent studies have indicated an important relationship between IVF and the integrity of sperm DNA. A few reports have demonstrated significant variation in the degree of sperm DNA damage between infertile and fertile males by using different techniques [52, 71, 73–75]. If the level of spermatozoa with DNA fragmentation is higher than 30% detected by SCSA, the probability of natural conception is almost zero [3, 73]. Couples in whom the man has an elevated level of sperm DNA damage have low natural conception potential, with a long time to pregnancy. The sperm DNA integrity tests may be used to predict pregnancy outcomes of couples who do not know their fertility potential [71, 73].

18.4.3 Intrauterine Insemination

The fertilization potential by intrauterine insemination (IUI) is reportedly low if sperm DNA fragmentation is higher than 30% as detected by SCSA [56, 70]. In addition, sperm samples with sperm DNA fragmentation (SDF) index higher than 12% detected by TUNEL method have demonstrated that no pregnancies were achieved in insemination [76]. Sperm DNA stability and the level of fragmentation effected by insufficient maturation, oxidative damage, apoptosis and other causes may be a marker of poor IUI outcome. Thus, sperm DNA damage has a negative correlation with fertilization, and the evaluation of sperm DNA integrity can be used as a prognostic tool in predicting the outcomes of both natural conception and IUI [56].

18.4.4 In Vitro Fertilization

The correlation between high levels of sperm DNA damage and IVF and intracytoplasmic sperm injection (ICSI) outcomes remains questionable. A negative association between embryo development in IVF cycles and sperm DNA damage has been reported [77]. In addition, several reports have also shown an important relationship between sperm DNA integrity and fertilization success in IVF [15] and ICSI [78, 79]. It has been demonstrated that for a success in pregnancy both by ICSI and IVF, the predictive DFI cut-off value detected by SCSA was 27% [75]. On the other hand, an association between IVF rates and the low level of sperm DNA damage has been shown in several studies. Their results demonstrated that sperm DNA damage has a better prognostic value in IVF compared with ICSI [15, 75, 80]. A few studies reported that a successful pregnancy could still be achieved with severe poor sperm parameters and low sperm chromatin integrity by ICSI using testicular spermatozoa [81–84].

18.4.5 Embryo Growing Quality and Blastulation Rate

Results of several clinical reports have proposed an association between sperm DNA damage/poor sperm quality and embryo development/maturation [62, 72, 85]. The impact of DNA damage on the embryo seems to be related to the development of embryo more than the embryo quality [86]. Virro and colleagues have suggested that fertilization rate was not statistically distinctive between the patients groups with low and high DNA fragmentation level. However, high DNA fragmentation (> or =30% DFI) caused a lower blastocyst and pregnancy rates [54]. The blastocyst development is controlled by maternal genes during the first few steps of development, while paternal gene expression starts at four- to eight-cell stage (approximately 48–56 h after fertilization process) [85]. Thus, during this stage, fragmented DNA inherited by father may affect negatively on the embryo development and/or blastocyst formation. Interestingly, a study has demonstrated that the adverse paternal effect on development of embryo may occur at a later stage even if there are no morphological anomalies at the zygote stage [17]. Repeated failures of assisted reproduction without any evident defective zygote formation and cleavage of embryo are frequently correlated with high sperm DNA fragmentation levels.

18.4.6 The Role of DNA Damage on Embryo Progress After IVF

Blastocyst development is negatively affected by the degree of sperm DNA fragmentation in prepared ejaculated spermatozoa used in IVF. An important inverse relationship has been reported between the apoptotic activity of sperm specimens and blastocyst progress after either ICSI or IVF [77]. Second- and third-day embryo scoring was unaffected because the paternal genome is activated after the four-cell stage, until which point embryo development is mainly controlled by maternally inherited mRNA [17, 87].

The early paternal effects were not related with sperm DNA fragmentation; however, the late paternal effects were correlated with sperm DNA integrity; therefore, analysis of sperm DNA integrity may be helpful to predict late paternal effect. The early paternal effect has been suggested to be mediated by deficiency of oocyte-activating factors or centrosome dysfunction and commence at the four-cell stage [17]. It is well documented that the incidence of pronuclear stage defect is higher in couples with female factor infertility [88]. The late paternal effect may comprise sperm DNA damage, sperm aneuploidy or abnormal chromatin packaging of paternal genome, which can affect the proper activation of paternal gene expression [17]. The role of sperm DNA integrity on the embryo quality is reportedly less important during conventional IVF process compared to ICSI [86]. Sperm DNA repair in the oocyte and the natural selection that occurs during IVF may

result in lack of influence of sperm DNA damage on IVF embryo quality. In fact, the sperm DNA integrity is associated with sperm membrane and motility; therefore, the chance of fertilization with DNA-fragmented sperm at conventional IVF is low compared to ICSI [89].

18.4.7 The Role of DNA Damage on Embryo Quality After ICSI

Highly fragmented sperm DNA can escape from the natural selection and fertilize the oocyte. Despite a range of DNA damage are repairable by the oocyte after fertilization, excessive damage may possibly cause poor embryo development. A study has shown that high levels of DNA damage were present in semen samples with teratozoospermia and also those with normal morphology [72, 90, 91]. The distinction between the IVF and ICSI studies has shown that the impact of sperm DNA fragmentation on embryo quality/growing rate is more remarkable with ICSI compared to conventional IVF [86].

18.4.8 The Impact of DNA Damage on Pregnancy and Pregnancy Loss

An inverse relationship has been reported between elevated sperm DNA fragmentation and pregnancy rate using SCSA [3, 75], TUNEL [3, 58, 59] and Comet assay [92], although a few reports have shown no relation between pregnancy and sperm DNA damage [74, 86]. Two systemic reviews have also shown an important correlation between high DNA damage and decreased pregnancy rate [93, 94]. The reported relationship between sperm DNA damage and pregnancy loss may be caused by abnormal embryo development as a result of abnormal paternal genome [59]. In fact, the oocyte can easily repair SSBs; however, the repair capability of high levels of DSBs is limited; therefore, these DSBs may lead to chromosomal rearrangements and mutations that may subsequently block or modify embryo development leading to pregnancy loss [87].

18.5 Management of Infertile Patients with Elevated Sperm DNA Fragmentation

The relationship between sperm DNA integrity and fertility potential is a growing interest amongst researchers [3]. As mentioned before, there are significant differences in the sperm DNA fragmentation levels between infertile and fertile

men [71]. Both in vivo and in vitro, fertility capacity has been found to be lower in men with elevated level of DNA fragmentation [73, 95]. DNA fragmentation also has an impact on sperm parameters [96], embryonic development [17], chromosomal aneuploidy [97], implantation [11, 76, 98–100] and recurrent miscarriages [94, 101–103].

18.5.1 Antioxidant Treatment

The presence of high polyunsaturated fatty acids (PUFA) in the sperm membrane makes them highly susceptible to oxidative stress. Previous reports, using indirect assays, have demonstrated that oral antioxidant treatment could reduce the elevated levels of sperm DNA fragmentation in ejaculated spermatozoa [104, 105].

Harmful outcomes of ROS on sperm DNA have been reported in different studies [58, 106–109]. ROS generation can be controlled in some degree by the seminal plasma antioxidants. The favourable impact of antioxidants including reduction of DNA fragmentation level in ejaculated spermatozoa can be detected following 2 months of oral antioxidant therapy [109]. Additionally, dietary antioxidants are an appropriate therapeutic option to alleviate sperm DNA damage for infertile men [108].

18.6 The Use of Different Sperm Sources

18.6.1 The Use of Testicular Sperm

Two recent reports have indicated the lack of pregnancy and birth when the sperm subpopulation manifesting DNA fragmentation is higher than 20% and 15%, respectively, using TUNEL [55, 110]. When comparing testicular sperm samples to the ejaculate samples, a significantly decreased level of sperm DNA fragmentation was demonstrated in patients with fragmented sperm DNA (≥15%) during the treatment with ICSI. Additionally, high implantation, pregnancy and birth rates are reported in ICSI by using testicular spermatozoa [55, 110]. It has been shown that retrieved testicular spermatozoa have a reduced level of DNA damage than ejaculated sperm in men with continuously high DFI after previously ineffective oral antioxidant treatment [107]. Recently, we reported that ICSI using testicular spermatozoa retrieved by TESA appears to be an effective option for patients with elevated DNA fragmentation (>30%) and repeated pregnancy lost [111]. The reproductive outcomes of testicular and ejaculated spermatozoa were analysed using ICSI. The pregnancy rate using testicular and ejaculated spermatozoa was

44% and 6%, respectively. Implantation percentage was found as 22% and 2% using testicular spermatozoa and ejaculated spermatozoa, respectively [110, 112].

18.6.2 Utilization of the Second Ejaculation

An ongoing pregnancy rate of higher than 30% can be achieved by taking a second consecutive sperm ejaculate on the day of oocyte pick-up to increase the total motile sperm number for IVF treatment. Invasive sperm processing techniques and unnecessary micromanipulation can be avoided by this method [113].

Some studies have suggested that spermatozoa are significantly exposed to ROS and reactive nitrogen species (RNS) during epididymal transfer and storage; therefore, low intracellular ROS values may be an indication of efficient epididymal function and the short duration in the epididymis [114]. The reduction of intracellular ROS has been seen in samples received after only 1 day of abstinence; this can be explained by the fact that these spermatozoa spent a shorter period of time in the epididymis and that their intracellular antioxidants have not been fully consumed. Hence, recurrent ejaculations can potentially be an approach to reduce sperm DNA damage and improves IVF treatment success rate [26, 115]. Nevertheless, frequent daily ejaculation of 2 weeks has no major adverse effects on both conventional and functional sperm parameters. Therefore, frequent daily ejaculations can be utilized as an alternative treatment option in male infertility cases with high oxidative stress.

18.7 Sperm Preparation Techniques

18.7.1 Conventional Sperm Preparation Techniques

Different sperm centrifugation and sedimentation techniques are routinely used in the semen sample preparation for the ART for separating sperm from seminal plasma. Density gradient centrifugation and swim-up techniques have been used for separation of fragmented sperm DNA. Swim-up is a better sperm preparation method to eliminate fragmented sperm DNA. The fragmentation level reduced from 12% to 5.5% after swim-up [14, 77, 116]. Sperm DNA quality in neat sperm or prepared samples is important in the success of ARTs. The sperm obtained by density gradient separation provide spermatozoa with higher progressive motility, viability and lower fragmented DNA as compared to those which are prepared by the other conventional sperm separation techniques [117]. Consequently, a combination of swim-up and density gradient separation methods has been suggested to reduce sperm with damaged DNA during sperm preparation in IVF treatment [118].

18.7.2 Alternative Sperm Preparation Techniques Before ICSI

Magnetic-activated cell sorting (MACS) is a technique used in separation of apoptotic sperm [119] and reduction of sperm with fragmented DNA [120]. This method is based upon the property of spermatozoa in expressing the apoptotic signal phosphatidylserine that attached to annexin-V-combined micro-beads. Spermatozoa with apoptotic signal and fragmented DNA could be distinguished by a magnetic field to annexin-V-positive and annexin-V-negative fractions. This technique is recommended to use in IVF laboratories for sperm preparation [119]. The utilization of hyaluronic acid is another way of selection. The method is based on binding of spermatozoa with DNA fragmentation to hyaluronic acid [121]. Electrophoretic separation of spermatozoa for sperm selection is another advanced technique, which is based upon detection of mature spermatozoa because of the negatively charged glycocalyx rich in sialic acid remnants [122].

18.8 Advanced Sperm Selection for ICSI

18.8.1 Morphological Selection

Motile and morphologically normal sperm are selected for ICSI process; however, these sperms may have an elevated level of DNA fragmentation. Therefore, analysis of DNA fragmentation is suggested for motile and morphologically normal spermatozoa before ICSI procedure [67].

18.8.2 Sperm Selection Under High Magnification

Recently, to increase the reproductive outcomes of ICSI, non-invasive methods have been requested for patients with poor sperm quality [119, 122, 123], especially, selection of morphologically best sperm for injection based upon motility and morphology of organelles analysed at over 6000 magnification [124] to improve pregnancy and abortion rates. Intracytoplasmic morphologically selected sperm injection (IMSI) is a sperm selection method based on selection of motile spermatozoa without head vacuoles simultaneously under high magnification (>6000×). The presence of vacuoles in the nuclear region of the sperm head is one of the most prognostic indicators of poor sperm quality. These vacuoles seem to be related with fragmentation and/or denaturation of sperm DNA and lead to poor embryo development [125, 126].

18.8.3 Human Motile Sperm Head Birefringence

Sperm head birefringence (SHBF) is used as a criterion for the selection of best sperm to use in ICSI. A few studies reported a significant higher DNA fragmentation in spermatozoa with sperm head birefringence total (SHBF-T) than in those with sperm head birefringence partial (SHBF-P) [127].

18.9 DNA Repair Mechanisms

DNA damage/lesions arise as a result of spontaneous errors during DNA replication and spontaneous cellular metabolism. Approximately 105 DNA lesions are generated in a cell each day [128]. A number of mechanisms in our body are able to recognize and repair these DNA lesions. The DNA repair rate of these lesions is based on the cell types, the age of the cell and the extracellular environment of the cell. Following the DNA damage, the cell can enter one of three states, namely, (i) apoptosis, (ii) mutations or (iii) rearrangement and DNA repair (Fig. 18.1).

DNA repair mechanisms have evolved to compensate the DNA damage to maintain genomic integrity and stability. These mechanisms are base nucleotide excision repair (NER), excision repair (BER), mismatch repair (MMR), DSB repair (DSR) and post-replication repair. These mechanisms detect and correct the DNA lesions regardless of the cause (Fig. 18.2).

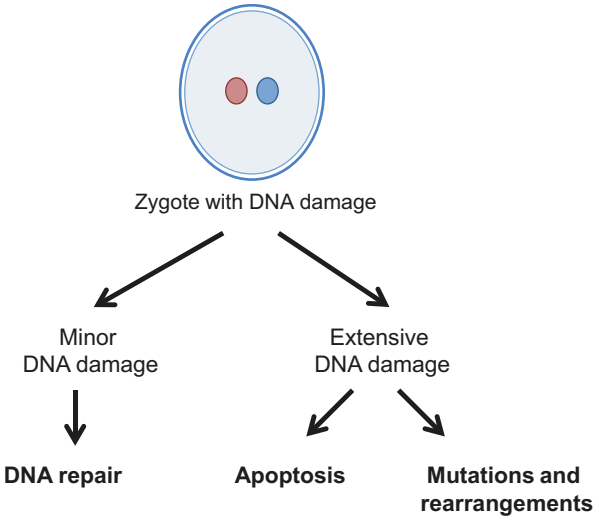

Fig. 18.1 Fate of DNA damage after zygote formation

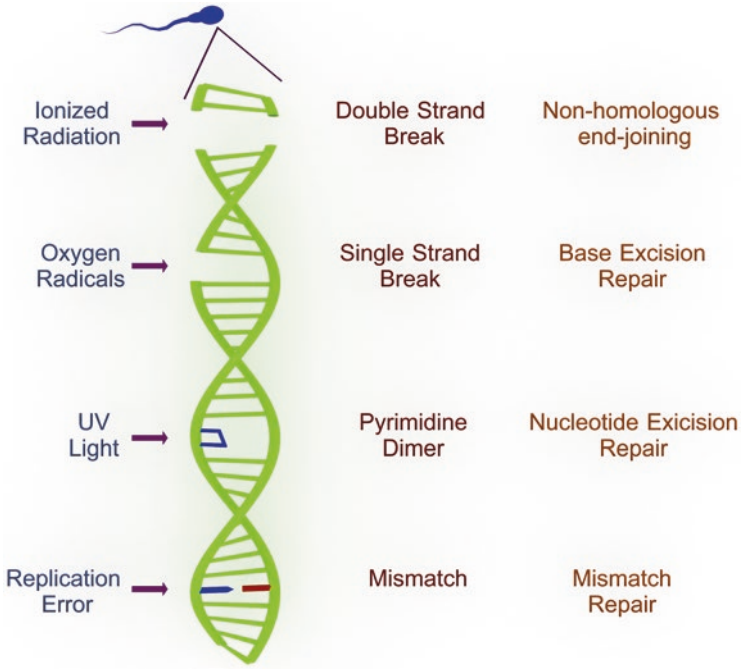

Fig. 18.2 DNA damage and DNA repair mechanisms

18.9.1 Nucleotide Excision Repair

NER mechanism repairs substantial lesions including oxidative damage and DNA intrastrand cross-links, pyrimidine dimers caused by the UV mismatched bases or bulky adducts [129, 130]. These lesions cause distortion of helical structure of DNA [131]. The DNA damage is scanned and detected by roughly 30 different proteins in the NER mechanism. Global genome NER (GG-NER) and transcription-coupled NER (TC-NER) are the two pathways of NER. Each pathway is liable for recognition of different types of damage [18]. GG-NER pathway repairs DNA damage throughout the whole genome [132], while TC-NER pathway is responsible for repairing lesions on the transcribed strand of DNA [130]. DNA damage is scanned and detected by XPC/RAD23B proteins in GG-NER pathway [130, 132–134]. XPC and RAD23B proteins are expressed highly in oocyte [18]. TC-NER activated by DNA distortions block the elongating RNA polymerase II complex [134, 135]. Following damage recognition, both of the pathways utilize the same repair machinery. Firstly, DNA helix unwinds to permit xeroderma pigmentosum (XP) complementation group A (XPA) binding by replication protein A (RPA) to DNA strand for secondary DNA damaged recognition. Subsequently, endonucleases XPG and XPF/

ERCC1 cleave the DNA, leading to removal of lesions [129]. Lastly, DNA polymerase fills the remaining gap and the remaining nick is sealed by DNA ligase. The expression of both XPA and RPA proteins is found at high levels in oocyte [18]. Defects in the NER mechanism may result in autosomal recessive diseases such as Cockayne syndrome (CS), xeroderma pigmentosum and trichothiodystrophy (TTD) [136, 137].

18.9.2 Base Excision Repair

BER is a highly coordinated mechanism in charge of the removal of non-helix-distorting base damages caused by different reactions/mechanisms such as oxidation or adduction [18, 138]. DNA glycosylases recognize specific base substitution in DNA helix and catalyse hydrolytic elimination of altered base [130]. *Uracil* DNA glycosylase (UNG) expression was reported to be high in the oocyte in germinal vesicle (GV) stage [139]. 8-Oxoguanine (80HdG) glycosylase 1 (OGG1) [140, 141] cuts the 80HdG residue and generates abasic sites. AP endonuclease 1 (APE1) incises phosphate backbone of DNA to insert unmodified nucleotide [141, 142]. The expression of OGG1 was found to be moderate; however, ethenoadenosine, 3-methyl adenine and N-methylpurine-DNA glycosylase (MPG) that distinguishes hypoxanthine are highly expressed in oocyte [139]. A recent study has demonstrated that post-translational modification to BER enzymes is initiated by conception such as OGG1 and X-ray repair cross-complementing protein 1 (XRCC1), causing nuclear localization and accelerated excision of 80HdG. The expression level of OGG1 in the oocyte is low compared to the male germ line where it is the only constituent of the BER pathway. Therefore, male germ line cooperates with female germ line to repair oxidative DNA damage, and oocytes are defenceless to high 80HdG levels being transmitted into the zygote by the fertilizing spermatozoon [143].

18.9.3 Mismatch Repair Mechanism

During DNA replication, mismatches occur as a consequence of tautomerization of the DNA strand bases due to inefficient proofreading by DNA polymerase [144]. Mismatches are base–base mismatches, for instance, G/T or A/C, and insertion–deletion loops [145]. MMR enhances fidelity of DNA replication about 100 times and suppresses the genomic instability of a cell. The mechanism is highly conserved evolutionarily to prevent genomic instability [142] in all living organisms. In order to repair the mismatch, MMR proteins first identify the mispaired nucleotides. The differentiation of parental and newly synthesized strands is performed through methylation, where the parental strand is methylated and the newly synthesized strand remains unmethylated in prokaryotes. However, in eukaryotes, MMR is

associated with DNA replication machinery that facilitates discrimination via binding of *proliferating cell nuclear antigen* (PCNA) in the leading strand and free 5′ ends of Okazaki fragments at the lagging strand [130, 144]. MutS protein is responsible for the recognition and binding to mismatched base of the newly synthesized DNA strand. MutL is a latent clamp-structured molecule that binds at unmethylated sites along the newly synthesized strand to induce exonuclease activity of MutH in prokaryotes [146].

There are several homologs of the proteins MutS and MutL in eukaryotes. MutS homologs such as MSH1–MSH6 and MutL include MLH1–MLH3, PMS1 and PMS2 which form heterodimers [146, 147]. Maduro and colleagues showed genomic instability and defects of MLH1 or MSH2 in nonobstructive azoospermia [148]. In meiotic recombination process, MSH4 and MSH5 proteins are essential. There are two types of MutS homolog heterodimers. The first type is MutSa (MSH2⁄MSH6) which plays a role in DNA base–base mispairs. The second is MutSb (MSH2/MSH3) which is involved in insertion–deletion loop mispair repair [147]. The connection of MutL with MutS–DNA complex activates the MutH, which nicks the daughter strand and recruits DNA helicase II to disconnect the DNA double strands [149]. Germ line mutations of these proteins are related to hereditary nonpolyposis colorectal cancer (HNPC). Mutations or aberrant methylation of these genes is also related with sporadic cases [150, 151]. Exonucleases are recruited to digest the SSD tail followed by the formation of a gap. The gap is filled by DNA polymerase and sealed by an unidentified DNA ligase. MLH1 and MLH3 are essential to facilitate recombination and chiasmata separation during pachytene and diplotene.

18.9.4 DNA Double-Strand Repair

DSBs are caused by several factors including failed DNA replication through replication across a nick and DNA repair, ROS, recombination, meiosis, inadvertent action by nuclear enzymes on DNA including type II topoisomerases, chemotherapeutic agents and ionizing radiation [152]. Unrepaired DSBs can cause chromosomal instability through DNA fusions and chromosomal rearrangements, as well as cell death. Homologous recombination (HR) and non-homologous end-joining (NHEJ) repair are the two major pathways to repair DSBs [130].

18.9.4.1 Homologous Recombination

HR repair mechanism is an error-free repair mechanism that functions primarily during S and G2 phases of cell cycle [18, 130]. In this process, DSBs are protected from exonuclease activity, by the binding of RAD51 to the strands. Ataxia–telangiectasia mutated (ATM) and MRE11–RAD50–NBS1 (MRN) complex are the initiators of DSBs [129, 153], and 3′-ssDNA is generated by resecting the broken DNA ends through interactions with carboxy-terminal-binding protein (CtIP) [154]. The

tail of the ssDNA is coated by replication protein A (RPA) to remove secondary disruptive structures; RPA are replaced with RAD51 homologous sequence on the sister chromatid [155]. RAD51C interacts with BRCA2 to form complexes for homologous pairing [156]. Few studies have suggested a relation between alterations of HR mechanism and infertility. A study conducted by Xu and Baltimore (1996) indicted that men with ataxia–telangiectasia (AT) have azoospermia and gonadal atrophy, due to the failure of primary spermatocytes at the leptotene–zygotene transition [157].

18.9.4.2 Non-homologous End-Joining

The Ku70/Ku80 heterodimers recognize and bind to DSBs in DNA and then recruit dependent protein kinase (DNA–PKcs) [129, 158]. The recruitment of DNA–PKcs induces the removal of non-ligatable termini by an inward translocation followed by replication of DNA polymerases and ligation to create compatible ends. Defects in this repair system, whether in non-homologous end-joining or homologous recombination, predispose a person to cancer and immunodeficiency syndromes [130, 159]. DNA repair proteins associated with germ cells are summarized in Table 18.2.

18.10 Conclusion

Sperm DNA damage has been shown to adversely associate with reduced male reproductive potential including natural fertilization, intrauterine insemination outcomes, IVF pregnancy rates, development of embryo and health of the offspring. However, the ASRM Practice Committee does not recommend routine use of sperm DNA tests [9]. In mice, sperm DNA damage has been found to be associated with chromosomal abnormalities, developmental loss, reduced longevity and birth defects [91]. Identification of a new tool that could help in predicting male fertilizing potential is one of the main areas of male infertility research nowadays. Several assessment techniques have been developed for evaluating sperm DNA damage and integrity [13, 112, 119]. Further studies are required to understand the molecular basis of sperm DNA damage repair and could provide better and tailor-made therapeutic options for couples.

It is well known that ART, especially ICSI, bypasses the natural selection mechanisms and leads to fertilization with spermatozoa with DNA damage, which is not compatible with fertilization under natural circumstances. However, in vivo improvement of spermatozoa before application of assisted reproductive techniques remains of ultimate importance. The repairing capacity of the human oocyte may be insufficient to overcome paternally transmitted damage. Deficiencies in DNA repair mechanisms in oocytes likely contribute to miscarriages, chromosomal aberrations, congenital malformations, genetic disorders, neurological defects and the

Table 18.2 DNA repair proteins associated with germ cells

Gene	Expression level	Phenotype	Reference
MLH1	Medium	Microsatellite instability Failure of crossing over and premature desynapsis of homologous chromosomes Male infertility	[147]
MSH2	High	Loss of germ cells MMR deficiency in somatic cells	[160]
MSH3	High	MMR deficiency in somatic cells Fertile	[161]
MSH4	Not expressed	Failure of spermatogonial maturation beyond zygonema Infertility	[39]
MSH5	Medium	Incomplete and non-homologous chromosomal pairing Infertility	[162]
MSH6	High	MMR deficiency in somatic cells Fertile	[39]
MLH3	Not expressed	Infertile	[161, 163]
PMS2	Medium	Genomic instability Disruption of normal chromosomal synapsis Infertility	[161]
UNG	High (in oocyte)	Removal of adducts	[18]
OGG1	Moderate (in oocyte)	Decrease the level of 80HdG	[143]

development cancer in offspring. However, further research is required to elucidate the precise underlying pathophysiologic mechanisms and thus in the development of potential treatments for DNA repair. The true clinical value of sperm DNA fragmentation and its impact on embryo quality and embryo development are critical areas that need further research.

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