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MINI REVIEW

Do heat stress and deficits in DNA repair pathways have a negative impact on male fertility?

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In Europe up to one in four couples experience difficulty conceiving and in half of these cases the problem has been attributed to sub or infertility in the male partner. The development of assisted reproductive technologies (ART) such as *in vitro* fertilization and intra-cytoplasmic spermatozoa injection has allowed some such couples to achieve a pregnancy. Concerns have been raised over the increasing use of ART not least because of the discovery of elevated levels of DNA damage in sperm from subfertile men. The impact of damaged DNA originating in the male germ line is poorly understood, but is thought to contribute to early pregnancy loss (recurrent miscarriage), placental problems and have a long-term impact on the health of the offspring. DNA repair is essential for meiotic recombination and correction of DNA damage in germ cells and proteins involved in all the major repair pathways are expressed in the testis. In this review, we will consider evidence that the production of sperm containing damaged DNA can be the result of suboptimal DNA repair and/or a mild environmental insult, such as heat stress, and how studies in mice may give us insight into the origins and consequences of DNA damage in human sperm.

Keywords: DNA repair; male fertility; scrotal heat stress; spermatogenesis; sperm DNA damage

Introduction

Spermatogenesis is a complex, multi-step process involving the proliferation and differentiation of spermatogonia into mature sperm, which cannot, as yet, be modelled *in vitro* (Cooke and Saunders, 2002). Germ cell maturation is usually considered to fall into three phases: mitotic (replicative), meiotic and post-meiotic (spermiogenesis). During this process which takes ~35 days in mice and ~72 days in man, the DNA of the germ cells is vulnerable to the introduction of a range of errors and germ cells containing damaged DNA are routinely eliminated by apoptosis.

The potential for insults to the integrity of sperm DNA to have long-term consequences is most graphically illustrated by the data showing transgenerational effects of radiation exposure. Exposure of mice to X-rays has shown that the maximum increases of DNA damage are seen in differentiating spermatogonia (Haines *et al.*, 2002). One of the first studies demonstrating transgenerational effects of ionizing radiation showed that injection of males with plutonium salts gave rise to offspring with increased levels of dominant lethal mutations (Luning and Eiche, 1976). Further, more recent studies have demonstrated elevated frequencies of chromosomal aberrations in the offspring of irradiated male mice and rats. For example, radiation-induced damage to the genome of male mice was manifested in the

F1 and F2 generations where regeneration of liver tissue was impaired (Slovinska *et al.*, 2004). Irradiation of parents has also been shown to result in the generation of offspring with reproductive problems. For example, spermatozoa from non-irradiated F1 offspring of gamma-irradiated male mice, exhibit a significantly reduced fertilization rate (Burrue *et al.*, 1997). Gamma ray treated sperm has also been shown to result in fetal malformations (Muller *et al.*, 1999).

Treatment of male cancer patients with alkylating drugs such as cyclophosphamide is associated with increased incidence of oligo- and azoospermia and male infertility (Charak *et al.*, 1990; Kenney *et al.*, 2001). Treatment of rats with cyclophosphamide resulted in an elevated level of abnormalities in their offspring (Hales *et al.*, 1992). Cyclophosphamide has also been shown to cause DNA damage in sperm (detected using the sperm chromatin structure assay) and to alter the composition of sperm head basic proteins (Codrington *et al.*, 2007). Pre-conception paternal cyclophosphamide exposure has also been shown to induce aberrant epigenetic programming in early embryos sired by these males (Barton *et al.*, 2005). In addition, it has been shown to alter the expression of important DNA repair genes in preimplantation rat embryos (Harrouk *et al.*, 2000).

A number of recent reviews have dealt with the evaluation of sperm DNA damage, the impact of DNA damage on offspring and the

evaluation of tools for DNA damage detection (Agarwal and Allamaneni, 2004, 2005; Sharma *et al.*, 2004; Erenpreiss *et al.*, 2006; Evenson and Wixon, 2006; Muratori *et al.*, 2006; Zini and Libman, 2006). In the subsequent sections of this review, we will focus on evidence that deficits in DNA repair pathways and/or a mild environmental stress (heat) can both have a negative impact on the integrity of DNA in sperm and discuss how this can have an impact on both fertility and development of the embryo.

DNA repair and spermatogenesis

DNA repair is required for meiotic recombination and correction of DNA damage in developing germ cells (Baarends *et al.*, 2001). During spermatogenesis germ cells also produce high levels of reactive oxygen species (Fisher and Aitken, 1997) which can induce a variety of DNA lesions. One of the most abundant lesions, 8-oxoG (Lindahl, 1993), is strongly mutagenic and also blocks transcription. Fortunately a complex anti-oxidant defence system exists in the testis (Bauche *et al.*, 1994) which complements the DNA repair systems. In humans, more than 130 genes have been identified that are involved in protecting genome integrity (Wood *et al.*, 2001). These include proteins involved in mismatch repair, which repairs small mismatches or loops; nucleotide excision repair, which is principally associated with repair of UV-induced DNA lesions, but is also active against some bulky and oxidative DNA lesions; base excision repair, which is involved in replacement of aberrant (including oxidized) bases in DNA; single-strand break repair and double-strand break repair. Proteins involved in all of these processes are expressed within the testis [reviewed in Jaroudi and SenGupta (2007)]. For example, histone variant H2AX (2AX) is involved in the response to double-strand breaks recruiting DNA repair factors to DNA damage sites where it is rapidly phosphorylated, resulting in formation of γ -H2AX foci (Rogakou *et al.*, 1999). In addition to anti-oxidant and repair pathways the testis also expresses proteins that are involved in both the intrinsic and extrinsic apoptotic pathways. The cell cycle (mechanism of cell replication and proliferation) is directly linked to apoptosis via a number of cell cycle checkpoints at the G1/S, S and G2/M phases (MacLachlan *et al.*, 1995). These checkpoints are a series of control systems that enable continued proliferation only if the conditions are favourable: DNA damage and chromosomal misalignment can activate these checkpoints (Weinert and Hartwell, 1989). The cell cycle is then paused for DNA repair but if the damage cannot be repaired then apoptosis of the cell is initiated (Waldman *et al.*, 1996). However, the response to damage is cell type-specific and the amount of damage required for initiation of apoptosis varies between cell types.

During meiotic prophase parts of the DNA from the paternal and maternal homologous chromosomes are recombined. The recombination events result in the exchange of genetic information between non-sister chromatids resulting in crossovers that are essential for the correct segregation of the chromosomes and play an important role in creating genetic diversity among individuals within a population by creating new and potentially beneficial combinations of paternal and maternal alleles (Baarends *et al.*, 2001). The recombination process is initiated by the formation of double-strand breaks early in prophase I, (Sun *et al.*, 1989) and this is mediated by the topoisomerase Spo11 (Keeney *et al.*, 1997; Celerin *et al.*, 2000). The importance of forming double-strand breaks to allow the process of recombination is illustrated by the observation that deletion of *Spo11*, results in infertility in both sexes. In males, loss of Spo11 results in failure of germ cells to progress beyond the zygotene stage of meiotic prophase and is associated with increased rates of germ cell apoptosis (Baudat *et al.*, 2000; Romanienko and Camerini-Otero, 2000).

During spermiogenesis post-meiotic germ cells are extensively remodelled during which histones are replaced, first by transition proteins and subsequently by protamines to form the characteristic condensed protamine–DNA toroid structure (McPherson and Longo, 1993). Although normal round spermatids contain no DNA strand breaks during elongation transient DNA breaks are introduced. This coincides with histone hyperacetylation, and is thought to be essential for removal of DNA supercoils before binding of transition proteins (Laberge and Boissonneault, 2004). Studies in knockout mice have suggested that transition protein 1 may play a role completing the repair of DNA breaks (Kierszenbaum, 2001). Although most breaks occurring during spermatid remodelling are repaired by ligation (Kovtun and McMurray, 2001; Boissonneault, 2002), sperm appear to contain higher levels of DNA strand breaks than most somatic cells (Haines *et al.*, 1998; van der Schans *et al.*, 2000) and there is no information on the impact of DNA damage arising before the spermatid phase on subsequent DNA condensation. The spermatids that are formed after meiotic division II are transcriptionally and translationally silenced during the condensation of the chromatin. Thus these post-meiotic spermatids have a minimal capacity for DNA repair (Sega, 1979; Sotomayor and Sega, 2000) and therefore their DNA could be damaged in an accumulative manner, as the sperm cannot respond by inducing either apoptosis or DNA repair, as they are transcriptionally silent.

Following remodelling of the round spermatids to spermatozoa they are released from the testis into the epididymis. Sperm can also incur DNA damage in the epididymis. For example, leukocytes found in male genital tract infections have the potential to produce reactive oxygen species (ROS) (Wolff, 1995), and in significant numbers can overwhelm the anti-oxidant defence system in place in the epididymis and could cause DNA damage as a result of oxidative stress (Aitken *et al.*, 1995). Infections in the female genital tract may also have adverse effects on sperm chromatin condensation. For example, Chlamydia has been shown to cause fragmentation in human sperm DNA (Satta *et al.*, 2006). In addition colonization of the upper female reproductive tract with *Ureaplasma urealyticum* has been associated with adverse pregnancy outcomes (Andrews *et al.*, 1995). It has been demonstrated that this infection causes premature de-condensation of sperm chromatin and DNA damage to human sperm (Reichart *et al.*, 2000).

Deficits in DNA repair pathways can influence germ cell survival and production of sperm

A number of mice deleted in genes encoding proteins involved in DNA repair have been generated and provide invaluable information about the impact of DNA repair on spermatogenesis (and other processes).

ERCC1 is essential for nucleotide excision repair and is also involved in homologous recombination, double-strand break repair and the repair of interstrand crosslinks (Schiestl and Prakash, 1990; Davies *et al.*, 1995; Hsia *et al.*, 2003). Highest expression occurs in post-meiotic round spermatids (Hsia *et al.*, 2003). In *Ercc1* null mice, consistent with the stochastic nature of DNA damage, germ cell loss was highly variable and, even in the absence of ERCC1, very few germ cells in Day 22 males had the appearance of pachytene spermatocytes (Hsia *et al.*, 2003). *Ercc1* nulls die before weaning with liver failure, but are rescued by a liver-specific *Ercc1* transgene. Strikingly, the low level of transgene-derived *Ercc1* expression (<10% of normal) in the testes of transgene-positive *Ercc1* null mice (*TG-Ercc1*−/−) was sufficient to markedly increase the numbers of germ cells and allowed some to complete meiosis and mature into sperm. Staining of meiotic spreads for synaptonemal complex

protein 3 (SCP-3) and γ -H2AX showed that, contrary to the situation in controls, in *TG-Ercc1*^{-/-} testes many double-strand breaks persisted into the pachytene stage (Paul *et al.*, 2007). Animals were infertile with low sperm counts. Increased levels of apoptosis and higher levels of DNA-damaged sperm were detected in both knockout and heterozygote mice (Hsia *et al.*, 2003; Paul *et al.*, 2007). Furthermore, there was a 3-fold increase in the level of the oxidized base 7,8-dihydro-8-oxoguanine (8-oxoG) in the testis (Hsia *et al.*, 2003) suggesting a role for ERCC1 in the repair of 8-oxoG. *Xpa* knockout mice have a nucleotide excision repair pathway defect and become very sensitive to ultraviolet B (Boonstra *et al.*, 2001), *Xpa* null males appear to have normal spermatogenesis (de Vries *et al.*, 1995; de Vries and van Steeg, 1996) but are subfertile compared to wild-type controls (Tsai *et al.*, 2005), with smaller average surviving litters. Germ cell loss (Sertoli cell only phenotype) is also observed in another such nucleotide excision repair mutant, the *mHR23B*^{-/-} mice (Ng *et al.*, 2002); these mice are subfertile with reduced litter size resulting from a high rate of intrauterine or neonatal death (Ng *et al.*, 2002; Tsai *et al.*, 2005). Mutations in RPA (also involved in the nucleotide excision repair pathway) result in defects in DNA double-strand break repair and homozygous deletion in mice results in embryonic lethality before implantation and impaired cell proliferation (Wang *et al.*, 2005).

The more severe phenotypes seen in the *mHR23B*^{-/-} and the *RPA* knockouts compared to that in *Xpa*^{-/-} mice, however, are likely to reflect the additional roles of these proteins beyond nucleotide excision repair. Deficiencies in proteins involved in base excision repair do not appear to have a major impact on male fertility though this may be because many base excision repair mutations are embryonic lethal. For example, *Ogg1* null mice are viable and fertile but have elevated levels of 8-oxoG and an increased mutation rate (Klungland *et al.*, 1999; Minowa *et al.*, 2000) though double polyADP ribose polymerase (PARP1–PARP2) mutant embryos die prior to completion of gastrulation (Menissier de Murcia *et al.*, 2003).

In mice, the mismatch repair pathway involves five proteins (MSH2–MSH6) which function as heterodimers to initiate repair activity; MSH2–MSH6 and MSH2–MSH3 are involved in repairing replicative mismatches whereas MSH4–MSH5 is a meiosis specific complex essential for processing recombination intermediates [reviewed in Kunkel and Erie (2005)]. Mice with deletion of the mismatch repair genes *Msh4* or *Msh5* exhibit reduced testis weights and infertility because the germ cells fail to complete meiosis (Edelmann *et al.*, 1999; Kneitz *et al.*, 2000). In mouse testes MSH2 is highly expressed in spermatogonia and leptotene/zygotene spermatocytes (Richardson *et al.*, 2000) and *Msh2* knockout mice manifest an enhanced predisposition to cancer (de Wind *et al.*, 1995). Although no gross abnormalities in spermatogenesis have been reported we have recently demonstrated a reduction in germ cell complement and the occurrence of Sertoli cell only (SCO) tubules in these mice which we have attributed to an essential role for MSH2 during the first wave of spermatogenesis (Paul *et al.*, 2007). Mutations in the mismatch repair protein MLH1 result in hereditary forms of colorectal cancer (Papadopoulos *et al.*, 1994). In the human testis, expression of MLH1 has been detected predominantly in pre-meiotic germ cells (Velasco *et al.*, 2004) and on spreads of germ cells from mice has been localized to foci at sites of crossing over (Baker *et al.*, 1996). Deletion of *Mlh1* results in both male and female infertility (Baker *et al.*, 1996; Edelmann *et al.*, 1996) and although *Mlh1*^{-/-} spermatocytes are able to complete the pachytene stage of meiosis, at diplotene the central element of the synaptonemal complex breaks down, the chromosomes are no longer held together at their chiasmata, an apoptotic response is triggered and as a result no mature sperm are formed (Eaker *et al.*, 2002). Taken together it would appear that for

the most part, infertility in DNA repair mutants is caused by a failure of meiotic progression though only if the pathway is involved in recombination, for example, in *Msh4* and *Mlh1* mutants which is in contrast to others such as *Ercc1* where no specific stage is affected but the *Ercc1*^{-/-} mutants are still infertile.

Sperm from hereditary non-polyposis colon cancer patients, heterozygous for an *Msh2* mutation, have an increased frequency of aneuploidy, indicating an important role for MSH2 in male fertility (Martin *et al.*, 2000). Although it is true that some patients with rare inherited DNA repair deficiency disorders are fertile, we are not aware of studies in man investigating the consequences of repair deficiency in the male germ line on resulting embryos. Although the frequency of individuals in the human population homozygous for any one of these will be low, the cumulative frequency of heterozygotes will be much higher. Although it does not necessarily follow that heterozygotes will have reduced repair capacity, increased sensitivity to sperm DNA damage in *Ercc1* heterozygotes has been detected (Hsia *et al.*, 2003).

Evidence that heat stress can influence male fertility

Normal testicular function is temperature dependant and in most mammals the testes are kept between 2 and 8°C below core body temperature by virtue of being held outside the body cavity in the scrotum (Harrison and Weiner, 1948; Ivell, 2007). In man, raised testicular temperature may occur as a result of occupational exposure, lifestyle or a clinical disorder (Mieusset *et al.*, 1987). For example, occupational exposure can occur in men who work in high temperature environments such as bakers and welders [reviewed in Thonneau *et al.* (1998)] and also occupations that involve long periods in a sedentary position such as professional drivers. Recent studies have also reported that posture and clothing can cause increased scrotal temperature (Mieusset *et al.*, 2007). Clinical disorders including cryptorchidism, where one or both testes fail to descend into the scrotum and remain in the abdominal cavity, can also result in the exposure of the testes to higher than normal temperatures.

Men with scrotal temperatures above the normal range are reported to exhibit increased rates of sub or infertility and their ejaculates contain an increased incidence of abnormal and immature spermatozoa (Mieusset *et al.*, 1987). In mice, heat stress has been reported to result in germ cell loss, poor quality sperm with altered DNA integrity and chromatin packaging as well as early embryo loss (Jannes *et al.*, 1998; Rockett *et al.*, 2001; Zhu and Setchell, 2004; Banks *et al.*, 2005) and spermatocytes and spermatids are believed to be the germ cells most sensitive to heat stress (Setchell, 1998). A number of animal models designed to study the impact of heat stress on the testis have been developed. These include transient exposure of the testes to elevated temperatures (typically greater than 40°C) or placing the testes and epididymides within the body cavity (surgical inducing cryptorchidism) resulting in long-term exposure of the testes to core body temperature (37°C). Both methods have been reported to cause a variety of disturbances in testicular function, including a decrease in testis weight, increased apoptosis, germ cell loss and altered fertilization capability of sperm (McLaren *et al.*, 1994; Setchell *et al.*, 1996, 1998; Lue *et al.*, 1999). For example, localized scrotal heating of mice at 40 and 42°C for 60 min is reported to cause a decrease in testicular weight and an increase in DNA damage in sperm at 40°C at 3, 7, 11 and 14 days after heat stress as determined by the sperm chromatin structure assay (SCSA). Those heated to 42°C have no sperm at these time points for use in the SCSA (Sailer *et al.*, 1997). In another study, heat stress (42°C for 30 min) has been shown to cause an increase in DNA damage in sperm as analysed by the COMET assay with maximum damage observed at 4 h after heat

stress suggesting that this could have been mediated by alterations in epididymal function. Rockett *et al.* (2001) observed not only an increase in apoptosis of spermatocytes but also increased expression of the stress-inducible proteins Hsp70-1 and Hsp70-3 in spermatocytes 4 h after heating for 20 min at 43°C. Further investigations (our unpublished results) on the impact of heat stress on the spermatocytes revealed that heat stress resulted in an increased incidence of DNA stand breaks in pachytene spermatocytes as measured by γ H2AX immunostaining.

A link between heat stress and DNA repair

Rockett *et al.* (2001) also used DNA microarrays to investigate changes in global gene expression following heat stress at 43°C and reported that expression of a number of DNA repair genes such *Ogg1* (involved in base excision repair), *Xpg* (involved in nucleotide excision repair) and *Rad54* (involved in double-strand break repair) were all down-regulated (Rockett *et al.*, 2001). Other studies have shown decreased expression of polyADP Ribose polymerase (PARP) in the rat testis in response to heat stress (Tramontano *et al.*, 2000); PARP proteins are involved in detection of strand breaks and signalling in both the base excision repair and nucleotide excision repair pathways (Schreiber *et al.*, 2002; Flohr *et al.*, 2003). In addition, decreased expression of oxidative stress-induced anti-oxidants has also been reported (Rockett *et al.*, 2001), this may leave the germ cells more susceptible to oxidative damage during hyperthermia. Heat stress induced by cryptorchidism appears to result in decreased expression of DNA polymerase β and DNA ligase III both of which are involved in the final stages of DNA repair, for example, in both base and nucleotide excision repair (Tramontano *et al.*, 2000).

Impact of sperm DNA integrity on pregnancy outcome

Many DNA repair and damage response genes are expressed in early mammalian embryos [reviewed in Jaroudi and SenGupta (2007)], but low levels of some indicate that the embryo's ability to repair DNA damage may be strictly limited (Zheng *et al.*, 2005). Notably, elevated levels of nucleotide excision repair, mismatch repair and homologous recombination gene transcripts were detected in preimplantation rat embryos developing from DNA-damaged sperm (Harrouk *et al.*, 2000).

Evidence from animal studies

Reduced litter sizes have been reported to result when males are subjected to a variety of heat stress regimes. For example, experiments where the entire body was exposed to 36°C for 24 h, a reduction in the number embryos sired by heated males was recorded. IVF using sperm from males heated 7d (an epididymal or late spermatid effect of heating) earlier showed reduced numbers of embryos developing from the four-cell stage onwards and those from mice heated 21d (a spermatocyte effect) earlier resulted in a reduction from the two-cell stage onwards (Zhu and Setchell, 2004). A further study looking at the effects of increased whole body temperature by exposing male mice twice to 36°C for 12 h on each occasion demonstrated reduced sperm number, pregnancy rate and litter size with maximum effects seen 10 or 14d after heat stress (Yaeram *et al.*, 2006). In the studies by Rockett *et al.* (2001) where control females were mated with males subjected to a transient, acute (20 min), scrotal heat stress 23–28 days previously exhibited reduced litter sizes consistent with an effect on spermatocytes. There are other reports of hyperthermia affecting fertility in mice causing both reduced pregnancy rate and

embryo weight as well as reduced fertilization rate *in vitro*, using sperm from heated males (Jannes *et al.*, 1998). There is evidence that fertilization with sperm exposed to DNA damaging agents can alter the expression of repair genes in the preimplantation embryo as early as the one-cell stage (Harrouk *et al.*, 2000). Previous studies have also shown that paternal DNA damage can be translated into chromosome aberrations at the first-cleavage metaphase stage in the zygote (Matsuda *et al.*, 1989). In mice mutation frequency is reported to increase during spermiogenesis in post-replicative cell types in older mice providing a link to the concerns expressed about increased paternal age (see below) (Walter *et al.*, 1998). Bovine studies have shown that DNA damage induced by X- or gamma-rays does not prevent fertilization or effect early embryonic development but did cause apoptosis in the four- to eight-cell stage thus preventing further development of the embryos (Fatehi *et al.*, 2006). These studies also showed that the same doses of irradiation did not impair sperm function (as measured by motility assays). This demonstrates that changes in spermatozoa following irradiation may not be detectable using the classical sperm parameters (viability, motility and acrosome integrity) but may only be revealed using assays specific for DNA damage.

Evidence from patients

Human semen parameters such as motility, morphology and sperm count are routinely used in fertility analysis. These, however, do not assess for any DNA damage contained within the nucleus of sperm and therefore additional sperm DNA fragmentation detection techniques are required. There have been a number of assays developed to evaluate the integrity of DNA and/or chromatin in sperm (Table I) (Evenson and Wixon, 2006). However, their ability to adequately assess male fertility potential remains under scrutiny (Evenson *et al.*, 2002; Agarwal and Said, 2003; Perreault *et al.*, 2003). The COMET assay involves single cell electrophoresis and evaluates DNA migration in Comet tails using specific software. TUNEL (TdT-mediated-dUTP nick end labelling) detects strand

Table I. Common assays used to detect DNA damage, the principle of the assay and the type of damage the assay detects

Assay	Principle	Type of damage detected
Sperm chromatin structure assay (SCSA)	Susceptibility of sperm chromatin to acid denaturation. Population assay	Abnormal chromatin packaging which is most likely a result of DNA damage
COMET	Migration of DNA out of sperm head. Single cell assay	Miscondensation of chromatin which is most likely a result of underlying DNA damage including breaks
TUNEL	Labels strand breaks in DNA by labelling the free 3'-OH terminus with modified nucleotides in an enzymatic reaction	DNA fragmentation
Sperm chromatin dispersion (SCD)	Following acid denaturation sperm with fragmented DNA do not produce characteristic of dispersed DNA loops seen in native sperm	DNA fragmentation
Enzyme-linked immunosorbent assay (ELISA)	Uses an antibody specific to single stranded DNA. Population assay	Uses the degree of unwound DNA as a measure of extent of DNA damage

breaks by labelling free 3'-OH groups using an enzymatic reaction with TdT, however, useful thresholds have not been established for either of these assays and thus they cannot be used in clinical practice. The sperm chromatin dispersion (SCD) test is described as an inexpensive tool for analysis of DNA fragmentation and is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo when subjected to acid denaturation and removal of sperm nuclear proteins (Fernandez *et al.*, 2003). The sperm chromatin structure assay (SCSA) measures the susceptibility of sperm DNA to acid denaturation and uses the dye acridine orange which fluoresces green when bound to native, double stranded DNA and red when bound to fragmented, single stranded DNA (Evenson *et al.*, 1999) and can analyse many thousands of sperm at one time. Though sperm DNA damage detected by SCSA results has been demonstrated to be directly related to fertilization, blastocyst development and pregnancy outcome in IVF and ICSI in some studies (Virro *et al.*, 2004; Evenson and Wixon, 2006), others have disputed this and shown no correlation between SCSA results and pregnancy outcome (Payne *et al.*, 2005). Therefore, this clearly warrants more studies to establish whether or not this tool should be routinely used in fertility clinics.

Evidence that damaged DNA originating in sperm can result in blocks in embryo maturation, consequent miscarriage or even fetal malformation has been documented. Patients with either idiopathic or male factor infertility have been shown to have higher levels of DNA damage as measured by the SCSA and higher level of oxidative stress in their sperm in comparison to fertile sperm donors (Saleh *et al.*, 2003). In addition, this group also showed a higher level of sperm with DNA damage and oxidative stress in men who failed to initiate a pregnancy and others have shown that oxidative DNA damage reduces male fecundity (Loft *et al.*, 2003). Other studies have revealed that sperm with damaged DNA are capable of fertilization (Twigg *et al.*, 1998; Ahmadi and Ng, 1999) and can produce viable early-stage preimplantation embryos, however, fail to result in a successful term pregnancy (Ahmadi and Ng, 1999). There is concern that parental lifestyle choices such as smoking or delayed parenthood will also contribute to decreased DNA integrity in sperm and have consequences for their children's health (Crow, 1995, 1997; Hemminki and Kyronen, 1999; Hemminki *et al.*, 1999; Zenzes *et al.*, 1999; Zenzes, 2000). A number of studies have shown that DNA damage in sperm, for example, that found in smoking fathers, can be passed to the offspring following ICSI/IVF treatment (Zenzes, *et al.*, 1999; Zenzes, 2000). In addition, this has been reported to lead to an increase in incidence of childhood cancers (Ji *et al.*, 1997; Sorahan *et al.*, 1997). A variety of genetic diseases have been shown to increase with paternal age (Crow, 1995, 1997) and retrospective studies have demonstrated an association between increasing paternal age and the incidence of breast cancer and cancers of the nervous system, however no correlation was shown with other cancers (Hemminki and Kyronen, 1999; Hemminki *et al.*, 1999). A recent study of patients from an IVF clinic demonstrated significant decreases in blastocyst formation and live birth rate where the fathers were more than 50 years old, however this did not appear to affect pregnancy rate and implantation rate (Frattarelli *et al.*, 2007). Early pregnancy loss has also been demonstrated where fathers exhibit increased DNA fragmentation in their sperm and that this affects the post-implantation development of the embryo (Borini *et al.*, 2006). It has been suggested that the paternal genome is important for the development of extraembryonic tissues. For example, one of the first studies on genomic imprinting showed that embryos with two female pronuclei could implant but then underwent resorption and exhibited poor extraembryonic membrane and trophoblast formation (Surani *et al.*, 1984). In addition to this, studies on mice have shown that mutation frequency increases during spermiogenesis

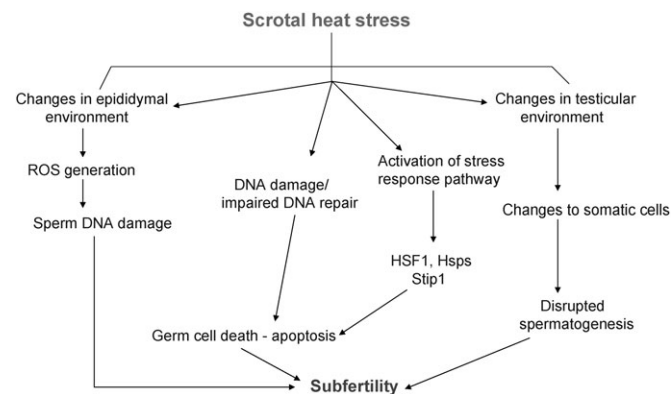


Figure 1: Possible mechanisms involved in male subfertility: scrotal heat stress and other stressors such as cigarette smoke, infection and exposure to xenobiotics may give rise to lesions in sperm DNA which can result in male subfertility if they escape DNA repair and apoptotic death of affected germ cells
ROS, reactive oxygen species; HSF1, heatshock transcription factor 1; Hsps, heat shock proteins; Stip1, stress-induced phosphoprotein 1

in post-replicative cell types in older mice (Walter *et al.*, 1998). A recent study has shown that serum testosterone concentration is reduced in infant boys conceived by ICSI and (Mau Kai *et al.*, 2007) compared to normal plasma testosterone concentrations in boys conceived by IVF. There are also a number of studies documenting the fertility outcomes of cancer survivors as the radiation and chemotherapeutic agents used to treat cancer can cause alterations in DNA and have been shown in animal models to be mutagenic (Epstein 1990; Hales *et al.*, 1992; Codrington *et al.*, 2007). Although many therapies have been shown to cause abnormalities in sperm, data concerning the offspring from cancer survivors does not show an increased risk for genetic disease or alterations in pregnancy outcome (Byrne *et al.*, 1998; Green *et al.*, 2003; Winther *et al.*, 2004). Although this is reassuring to cancer survivors, modern techniques such as ICSI where natural sperm selection is bypassed will require further surveillance of the resulting offspring.

Conclusions

In addition to the mechanisms reviewed above there is also evidence that oxidative stress, such as that in men that smoke, infection and exposure to xenobiotics can all have an impact on the integrity of sperm DNA [reviewed by Aitken and De Iuliis (2007)]. In conclusion, although the testis contains an active DNA repair machinery, backed up by elimination of germ cells with damaged DNA via apoptosis (Fig. 1), lesions on sperm DNA are readily detectable in sperm. Evidence from studies in animal models as well as human data suggest that DNA lesions introduced via the sperm can have life-long impact on offspring. These studies therefore have implications for the growing use of ART to solve problems of male subfertility.

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