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Original Citation:

Oxidative stress, DNA damage and the Y chromosome / Aitken RJ; Krausz C. - In: REPRODUCTION. - ISSN 1470-1626. - STAMPA. - 122:(2001), pp. 497-506.

Availability:

The webpage <https://hdl.handle.net/2158/778616> of the repository was last updated on 2019-07-23T17:17:32Z

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Oxidative stress, DNA damage and the Y chromosome

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Recent advances in understanding of male infertility have implicated two major causative factors, oxidative stress and Y chromosome deletions. A major cause of oxidative stress appears to be the high rate of reactive oxygen species generation associated with the retention of excess residual cytoplasm in the sperm midpiece. Other possible causes include the redox cycling of xenobiotics, and antioxidant depletion or apoptosis. Oxidative stress induces peroxidative damage in the sperm plasma membrane and DNA damage in both the mitochondrial and nuclear genomes. Nuclear DNA damage in the germ line of the father may be associated with pathology in the offspring, including childhood cancer and infertility. Gene deletions on the non-recombining region of the Y chromosome account for the infertility observed in about 15% of patients with azoospermia and 5–10% of subjects with severe oligozoospermia. The Y chromosome is particularly susceptible to gene deletions because of the inability of the haploid genome to deploy recombination repair in retrieving lost genetic information. Aberrant recombination, defective chromatin packaging, abortive apoptosis and oxidative stress may all be involved in the aetiology of DNA damage in the germ line. The factors responsible for Y chromosome deletions in spermatozoa remain unresolved but may be one facet of a central reproductive problem: controlling the amount of oxidative stress experienced by germ cells during their differentiation and maturation in the male reproductive tract.

The testis is the engine of evolution (Short, 1997; Hales *et al.*, 1999). The genetic variation that underpins the evolutionary process appears to be created predominantly in the male germ line as a consequence of a mutagenic machinery that is driven by a variety of replication-dependent and -independent factors (Agulnik *et al.*, 1997). Reproduction in human males in particular is characterized by the production of large numbers of spermatozoa by a spermatogenic process that has scant regard for the phenotypic normality of the gametes or their genomic integrity. Human sperm chromatin is often poorly compacted (Sakkas *et al.*, 1999a) and frequently contains DNA strand breaks (Irvine *et al.*, 2000). In addition, the likelihood of damage to the mitochondrial genome during the differentiation and functional lifespan of a spermatozoon is so great that these structures are ubiquitinated and destroyed in the oocyte after fertilization, to overcome the risk of them contributing to the embryonic mitochondrial pool (Kao *et al.*, 1998; Reynier *et al.*, 1998; Cummins, 2000). Although uniparental inheritance of mitochondria is common to nearly all eukaryotes (Birky, 1995), populations of human spermatozoa exhibiting evidence of mitochon-

drial dysfunction also show high rates of nuclear DNA fragmentation (Donnelly *et al.*, 2000).

In contrast to the mass production and genetic instability that characterize the male gamete (Box 1), the female germ line is a model of constancy. Female gametes do not replicate in the adult and spend most of their life in a state of relative dormancy as primordial follicles. This quiescent state is only broken for a brief period when the oocytes enter the growing follicle pool just before ovulation. As a result of this lack of cell division and intense metabolic activity, the ratio (α_m) of male to female mutations in primates is thought to be about 3–6 for humans and higher primates. For example, Shimmin *et al.* (1993) analysed the intron sequences associated with X- and Y-linked zinc finger genes (*ZFX/ZFY*) and recorded a relatively high α_m value of 6. This value may have been influenced by the fact that the pattern of *ZFX/ZFY* expression is known to vary among species. As a consequence, it is possible that the functional significance of this zinc finger protein has changed with time and distortions in mutation rate have been introduced as a consequence of differential selection pressure. Agulnik *et al.* (1997) addressed this criticism by selecting the *SMCY/SMCX* gene pair. The *SMC* gene encodes a minor transplantation antigen that is expressed in all tissues studied and is present in mice, men and marsupials. Significantly, this gene escapes X inactivation, indicating that the X and Y

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Box 1. Evolution of the Y chromosome

The human Y chromosome derives largely from a single autosomal region that was added to the sex chromosomes 80–130 million years ago (Waters *et al.*, 2001). The original X and Y chromosomes were homologous (Delbridge and Graves, 1999). However, most of the genes from the original sex chromosome have decayed on the Y chromosome such that the latter now contains just a small number of genes (largely performing housekeeping functions) with homologues on the X chromosome. The tendency for genes to degenerate on the Y chromosome is not unique to humans; indeed, there has been a general evolutionary tendency for non-paired sex chromosomes to degenerate and even (in some species of *Drosophila*) to disappear entirely (Lahn and Page, 1997). Many of the remaining functional genes on the human Y chromosome appear to have evolved from autosomal homologues, or in one case (*CRY*) retroposition of the corresponding mRNA (Saxena *et al.*, 1996; Chai *et al.*, 1997; Lahn and Page 1997, 1999). Once located on the Y chromosome, there has been a strong tendency for these genes to undergo amplification, presumably because the presence of multiple gene copies creates a buffer against attrition (Burgoyne, 1998). These Y chromosome-specific genes encode molecules that are essential for sex determination (*SRY*) or male fertility (*RBM*, *DAZ*) (Delbridge and Graves, 1999; Marshall Graves, 2000).

copies of *SMC* must be functionally interchangeable, to maintain equal gene dosage in males and females. This functional consistency makes the *SMCY/SMCX* gene pair an excellent candidate for calculating α_m . Analysis of these genes in mice, humans and horses revealed that the *SMCY* copy of the gene is evolving 1.8 times more quickly than *SMCX*. An α_m value of approximately 3 for this gene supports the concept of male-driven evolution. This value is sufficiently small for the mutations to be induced by mechanisms other than replication error, indicating a role for additional factors in the induction of DNA mutation in the male germ line, including methylation and free radical generation (Agulnik *et al.*, 1997).

These observations raise questions about the origins and nature of DNA damage in the male germ line and the impact such damage has on fertility, embryonic viability and the subsequent health and fertility of offspring. These proposed associations form the basis of this review.

Nature of DNA damage in the germ line

Two types of DNA damage appear to characterize the male germ line: replication errors and DNA fragmentation.

Replication errors

Since spermatogonial stem cells replicate throughout life, the spermatozoa of older men will have a history involving many more cell divisions than the gametes of

younger men. As a consequence, the gametes of older men can be expected to exhibit a proportionately higher incidence of mutations as a result of replication errors. It is for this reason that the occurrence of dominant genetic disease (for example, Apert's syndrome, achondroplasia) in a child with genetically normal parents invariably involves a mutation in the germ line of the father and is strongly correlated with paternal age (Crow, 1997).

DNA fragmentation

This kind of damage is characterized by single and double DNA strand breaks and is particularly frequent in the ejaculates of subfertile men (Irvine *et al.*, 2000). Of course, DNA fragmentation does not constitute a mutation in its own right but it is a promutagenic change that has the potential to generate mutations in offspring as a consequence of inadequate or defective repair. Such damage comes from three potential sources: oxidative stress, abortive Fas-mediated apoptosis or deficiencies in natural processes such as recombination and chromatin packaging that involve the induction of DNA strand breaks.

Oxidative stress in the male germ line

The susceptibility of male germ cells to oxidative stress has been appreciated since MacLeod (1943) observed that human spermatozoa incubated under high oxygen tensions *in vitro* lost motility via mechanisms that could be reversed by the presence of catalase in the incubation medium. MacLeod (1943) concluded from these experiments that human spermatozoa must be able to generate hydrogen peroxide from ground state oxygen and that the former must be damaging to sperm viability. Jones *et al.* (1979) demonstrated subsequently that the mechanism by which oxidative stress induced motility loss in mammalian spermatozoa involved the induction of peroxidative damage to the sperm plasma membrane. Human spermatozoa are particularly vulnerable to such stress because their plasma membranes are so enriched with unsaturated fatty acids, particularly decosohexaenoic acid with six double bonds per molecule (Jones *et al.*, 1979). These unsaturated fatty acids are essential to give the plasma membrane the fluidity it needs to participate in the membrane fusion events associated with fertilization. However, when reactive oxygen species attack the double bonds associated with unsaturated fatty acids, a lipid peroxidation chain reaction is initiated that if not arrested leads to a loss of membrane fluidity and a consequent loss of sperm function (Fig. 1).

There are two important features of this peroxidative damage that are relevant to the aetiology of male infertility. Firstly, the lipid peroxidation cascade is catalysed by transition metals such as iron and copper (Jones *et al.*, 1979; Aitken *et al.*, 1993). Addition of ferrous iron to suspensions of human spermatozoa results in a dose-dependent acceleration of lipid peroxidation and a concomitant decrease in the motility and fertilizing potential of these cells (Aitken

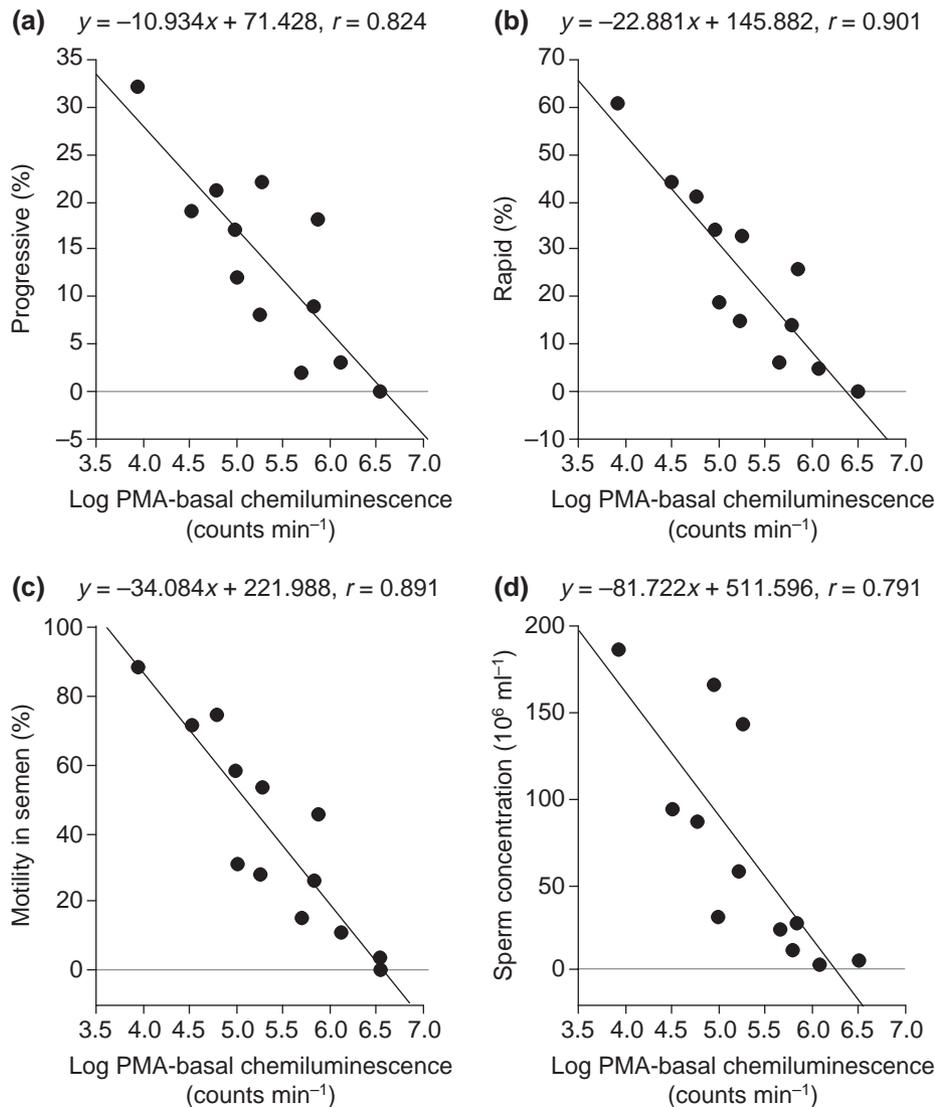


Fig. 1. Relationships between the intensity of the signal generated by phorbol ester (PMA)-positive leucocyte-free samples and semen quality. (a) Percentage of spermatozoa with progressive motility in semen. (b) Percentage of spermatozoa rapid in semen. (c) Percentage of spermatozoa motile in semen. (d) Concentration of spermatozoa in semen (Gomez *et al.*, 1998).

et al., 1989). Moreover, human seminal plasma appears to contain sufficient free iron and copper to catalyse this process (Kwenang *et al.*, 1987).

A second major feature of oxidative stress in the germ line is that its occurrence is profoundly influenced by the presence of antioxidants in the secretions of the male reproductive tract. These antioxidants include highly specialized protective enzymes that are secreted into the extracellular space. Examples include glutathione peroxidase (GPx5) and extracellular superoxide dismutase (SOD), produced in the caput and cauda epididymides, respectively (Perry *et al.*, 1993; Vernet *et al.*, 1996, 1997). Seminal plasma also contains small molecular mass free radical scavengers such as vitamin C, alpha tocopherol, tyrosine,

hypotaurine and uric acid that contribute significantly to the antioxidant protection of spermatozoa (van Overveld *et al.*, 2000). In addition, the albumin present in human seminal plasma is a sacrificial antioxidant that protects spermatozoa from peroxidative damage by absorbing lipid peroxides from the sperm plasma membrane (Twigg *et al.*, 1998a). In view of the importance of oxidative stress in the aetiology of sperm dysfunction, it is not surprising that seminal antioxidant activity has been shown to be depressed in the ejaculates of infertile men and to exhibit an inverse correlation with fertility (Smith *et al.*, 1996; Barbieri *et al.*, 1999; Hendin *et al.*, 1999). In addition, underexpression of mitochondrial phospholipid hydroperoxide glutathione peroxidase in spermatozoa has been linked to the motility

loss exhibited in cases of asthenozoospermia (Imai *et al.*, 2001).

The susceptibility of human spermatozoa to oxidative stress is exacerbated by the capacity of these cells to generate reactive oxygen species (ROS). When all traces of leucocyte contamination have been removed from the ejaculate, the amount of ROS generation correlates inversely with semen quality and sperm function (Aitken *et al.*, 1992; Gomez *et al.*, 1998). This association between ROS generation and poor semen quality is particularly evident when the spermatozoa are stimulated with phorbol esters (PMA). Normal, functional, mature human spermatozoa do not generate ROS in the presence of this reagent. However, in the infertile population, responses to PMA are obtained that correlate extremely well with the quality of the original ejaculate (Gomez *et al.*, 1998; Fig 2). A powerful discriminator of infertility in patients not exhibiting leucocytospermia is a combined measure of ROS generation and antioxidant activity in the seminal plasma (Sharma *et al.*, 1999). Analysis of the key antioxidant enzymes (SOD and catalase) in seminal plasma has not revealed any significant differences between the ejaculates of fertile and infertile men (Zini *et al.*, 2000a). Thus, if there is an association between the overall antioxidant activity in seminal plasma and oxidative damage to the spermatozoa, it is presumably due to deficiencies in the presence of small molecular mass free radical scavengers such as vitamin C.

Origins of oxidative stress

The factors responsible for the excessive generation of ROS by the spermatozoa of infertile men have not yet been elucidated. In some cases, it may be the presence of xenobiotics that are induced to redox cycle by the spermatozoa and generate toxic free radicals. In other cases, there may be defects in the cellular mechanisms that normally regulate free radical generation by these cells. The most significant lead to date in resolving the aetiology of excess free radical generation by spermatozoa has been the observed association with defective cytoplasmic extrusion. Defective sperm function is associated with excessively high cellular concentrations of enzymes such as lactic acid dehydrogenase (Casano *et al.*, 1991), creatine kinase (Huszar *et al.*, 1988), SOD (Aitken *et al.*, 1996) and glucose-6-phosphate dehydrogenase (Aitken *et al.*, 1994; Gomez *et al.*, 1996). The feature that all these enzymes hold in common is that they are cytosolic. In keeping with this observation, the cellular content of these enzymes is correlated with the retention of excess residual cytoplasm by human spermatozoa (Gomez *et al.*, 1996). The loss of sperm motility and fertilizing potential associated with varicoceles and idiopathic male infertility is associated with the retention of excess residual cytoplasm by the spermatozoa (Zini *et al.*, 1998, 1999, 2000b), as is the loss of fertility associated with heavy smoking (Mak *et al.*, 2000). Recent studies of patients undergoing IVF therapy have demonstrated a strong negative correlation between

fertilization rate and the presence of residual cytoplasm in the sperm midpiece (Keating *et al.*, 1997).

In light of these observations, it is possible that the generation of excess free radicals by the spermatozoa of infertile patients reflects an underlying defect in Sertoli cell function, the latter failing to remove sufficient residual cytoplasm before spermatozoa are discharged from the germinal epithelium. The presence of excess residual cytoplasm then enhances the free radical generating system of the spermatozoa via mechanisms that are still poorly understood. One possibility is that the presence of excess glucose-6-phosphate dehydrogenase enhances the cellular generation of NADPH that, in turn, fuels the production of free radicals by a proposed sperm NADPH oxidase (Aitken *et al.*, 1994). Further studies will be needed to determine whether this or other, equally plausible, mechanisms are involved.

DNA damage

Oxidative stress does not simply disrupt the fertilizing capacity of human spermatozoa, it also attacks the integrity of the DNA carried in the sperm nucleus and mitochondria. A variety of techniques has been used to demonstrate the presence of DNA fragmentation in human spermatozoa, including comet, nick translation and sperm chromatin structure assays (Hughes *et al.*, 1996; Evenson *et al.*, 1999; Irvine *et al.*, 2000). DNA fragmentation appears to be inversely correlated with semen quality, particularly sperm count, morphology and motility (Shen *et al.*, 1999; Irvine *et al.*, 2000; Muratori *et al.*, 2000; Shen and Ong, 2000). Moreover, negative correlations have been observed between the stability of DNA in the sperm nucleus and the fertilizing capacity of spermatozoa *in vivo* and *in vitro* (Sun *et al.*, 1997; Aitken *et al.*, 1998; Evenson *et al.*, 1999; Host *et al.*, 2000). The ability of the embryo to survive to term also appears to be negatively correlated with the level of DNA fragmentation in the germ line (Host *et al.*, 2000).

That oxidative stress is correlated with DNA fragmentation has been demonstrated in many independent studies. Firstly, the DNA in the ejaculates of infertile men is commonly associated with oxidative damage as reflected by measurements of 8-hydroxydeoxyguanosine (8-OHdG) (Kodama *et al.*, 1997; Irvine *et al.*, 2000; Shen and Ong, 2000). Secondly, correlations have been observed between oxygen radical generation and DNA damage in ejaculated spermatozoa (Barroso *et al.*, 2000; Irvine *et al.*, 2000). Oxidative stress in the male germ line can also be promoted by the presence of transition metals such as iron, copper and nickel that stimulate free radical generation and DNA damage (Liang *et al.*, 1999; Wellejus *et al.*, 2000). Some protection against metal-catalysed DNA damage may be afforded by protamination of sperm chromatin. The N terminus of human protamine P2 contains a heavy metal trap with particular affinity for Ni(II) and Cu(II) (Liang *et al.*, 1999). Therefore, protamines may serve a protective function by sequestering metals capable of promoting the

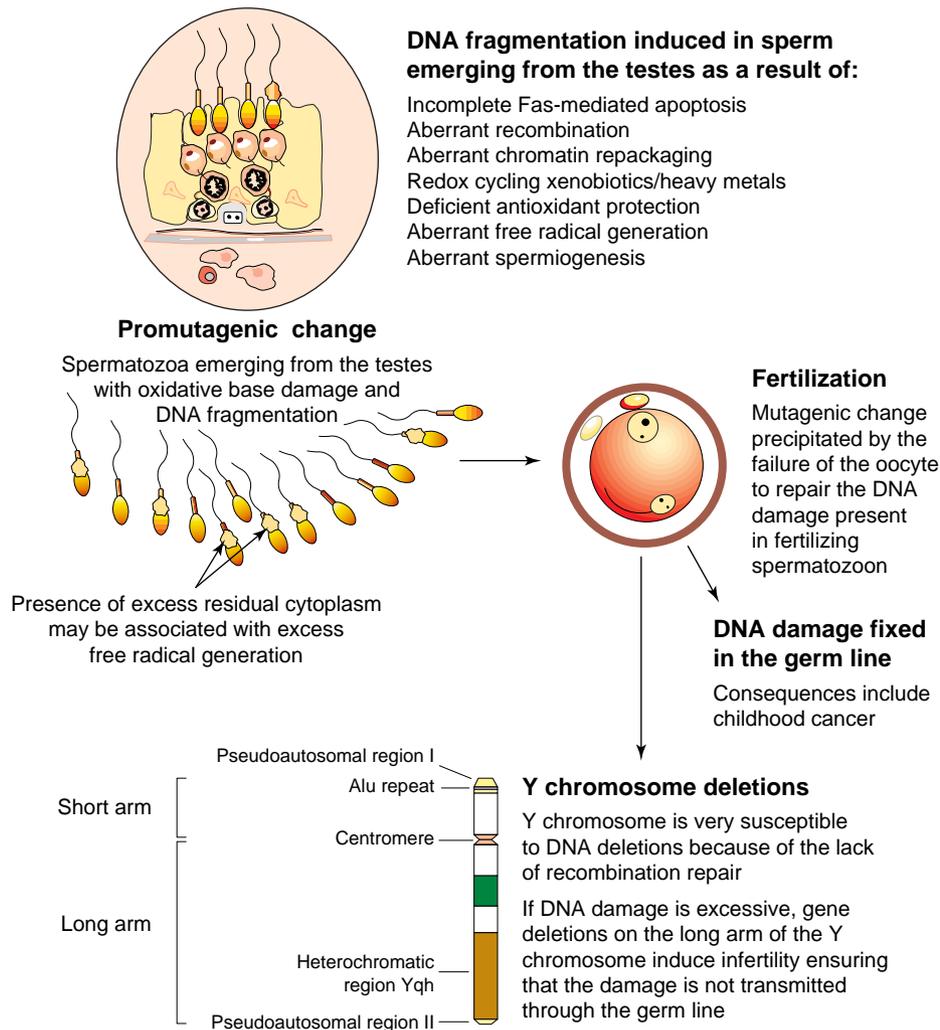


Fig. 2. Proposed associations between DNA fragmentation in the male germ line and the aetiology of human disease. A variety of factors can conspire to induce oxidative stress and DNA damage in spermatozoa emerging from the male reproductive tract. It is proposed that this damage is promutagenic and can give rise to mutations after fertilization as the oocyte attempts to repair the DNA before initiation of the first cleavage division. Any mutations occurring at this point will be fixed in the germ line and may be responsible for the induction of pathology, such as infertility or childhood cancer, in the offspring.

fragmentation of sperm DNA. This function may account in part for the extensive DNA damage observed in poorly packaged spermatozoa in which the protamine–histone transition has been incomplete (Bianchi *et al.*, 1993).

An alternative aetiology for the DNA nicks seen in the spermatozoa of infertile patients involves an abortive apoptotic pathway mediated by Fas. The induction of apoptosis via the Fas pathway is clearly an important mechanism by which Sertoli cells regulate the number of germ cells, particularly in times of stress (Boekelheide *et al.*, 2000). Accordingly, men exhibiting deficiencies in the semen profile, particularly oligozoospermia, possess a large number of spermatozoa bearing Fas, prompting the

suggestion that these dysfunctional cells are the product of an incomplete apoptotic cascade (Sakkas *et al.*, 1999b). Whether defective apoptosis accounts for a significant proportion of the DNA damage seen in the spermatozoa of infertile men is still an open question. A recent analysis of DNA damage in the germ line did not find ultrastructural evidence for apoptosis in association with DNA damage (Barroso *et al.*, 2000), whereas another study found no correlation between DNA damage and Fas expression (Muratori *et al.*, 2000). Of course, Fas binding and ROS generation are not mutually exclusive phenomena; ROS can induce Fas-mediated signal transduction in some types of cell (Huang *et al.*, 2000), whereas Fas-induced apoptosis

appears to be mediated by ROS in other types of cell (Sayers *et al.*, 2000).

Double-stranded DNA breaks also occur naturally in the male germ line both in preparation for recombination and during the process of chromatin packaging (Sakkas *et al.*, 1999a). These physiological strand breaks are normally resolved by the spermatid stage of spermatogenesis. Therefore, it is possible that aberrant recombination–chromatin packaging accounts for unresolved double-strand breaks in mature human spermatozoa; however, evidence to support this contention is currently lacking.

Consequences of DNA damage

The studies cited above indicate that a variety of mechanisms, particularly oxidative stress, conspires to induce DNA strand breaks in the male germ line, particularly in cases of male subfertility associated with poor semen quality. Such samples frequently show depressed fertilization rates *in vitro* in association with the DNA damage (Sun *et al.*, 1997), presumably as a consequence of collateral peroxidative damage to the sperm plasma membrane. Such membrane damage is physiologically important since it constitutes a protective mechanism designed to ensure that spermatozoa with severely damaged genomes cannot participate in the normal process of fertilization. However, this biological safeguard is of limited effectiveness because the genome of the human spermatozoon appears to be more susceptible than the plasma membrane to oxidative damage. As a consequence, it is possible to arrive at levels of oxidative stress at which cells exhibiting significant oxidative damage to their DNA retain the capacity for fertilization (Aitken *et al.*, 1998).

Clinical significance – childhood cancer

The clinical significance of this situation is demonstrated by recent analyses of men who are heavy smokers. The ejaculates of such men are under oxidative stress as indicated by the fact that their semen is characterized by increased levels of oxidative DNA base damage, high chromatin fragmentation and low concentrations of antioxidant vitamins (Fraga *et al.*, 1996). Although fertility is suppressed in such subjects, they are not infertile. As a consequence, DNA-damaged spermatozoa from heavy smokers are able to engage in the process of fertilization, with consequences for the ultimate health and well-being of the embryo. Thus, the offspring of heavy smokers are four to five times more likely to develop childhood cancer than the children of non-smoking fathers (Ji *et al.*, 1997). Furthermore, the possible mutagenic–promutagenic effects of smoking on DNA integrity in the male germ line has been reinforced by another study indicating that 15% of all childhood cancers are directly attributable to paternal smoking (Sorahan *et al.*, 1997). Evidence for this linkage between DNA damage in the male germ line and abnormalities in the developing embryo or child is not

confined to smokers. It can also be found in the wealth of data indicating that powerful associations exist between childhood disease and paternal occupation (Sawyer and Aitken, 2000).

The use of ICSI as a therapeutic technique can only exacerbate this problem. The most highly damaged sperm DNA is generally found in men with the poorest semen quality, whose spermatozoa are incapable of fertilization (Irvine *et al.*, 2000). This could be viewed as a protective mechanism ensuring that the most severely abnormal spermatozoa do not contribute their damaged DNA to the embryo. However, this safeguard is circumvented when ICSI is used to achieve human conceptions. Even if extremely high oxidative DNA damage is induced experimentally in populations of human spermatozoa by co-incubation with activated leucocytes, exposure to hydrogen peroxide or the excessive stimulation of the free-radical-generating system of the spermatozoon itself with NADPH, successful fertilization can still be achieved with ICSI (Twigg *et al.*, 1998b,c). Since DNA damage in the male germ line is associated with an increased incidence of childhood cancer, it is possible that the children of ICSI conceptions will be vulnerable to this disease. Much will depend on the DNA integrity of the spermatozoon selected for injection.

Childhood cancer may not be the only consequence of conceptions involving DNA-damaged spermatozoa. It is also possible that double-stranded DNA breakage induced by oxidative stress, defective apoptosis or aberrant recombination results in infertility in the male offspring as a consequence of irreparable deletions on the long arm of the Y chromosome.

Y chromosome and male infertility

The first association between spermatogenic failure and an underlying genetic cause was demonstrated by Tiepolo and Zuffardi (1976) in a report of six azoospermic patients carrying microscopically detectable deletions of the distal portion of Yq. In four cases, the deletion was *de novo* since the fathers of the patients were tested and found to carry an intact Y chromosome. On the basis of this finding, the existence of a spermatogenesis factor, the ‘azoospermia factor’ (AZF) encoded by a gene on distal Yq, was proposed. However, it was not until the mid-1980s, when Y chromosome-specific probes were developed, that the regions associated with spermatogenic failure were defined. Vollrath *et al.* (1992) developed more than 200 sequence-tagged sites (STS: short tracts of DNA that act as a landmark to define position on a physical map) along the length of the Y chromosome and ordered the STS markers using a large panel of individuals with Y chromosome deletions. These markers have permitted simple deletion analysis in infertile males by the polymerase chain reaction (PCR). Subsequently, many STS-based screening programmes have been undertaken in patients affected by azoospermia and severe oligozoospermia to define the AZF locus and isolate

candidate genes for AZF. Vogt *et al.* (1996) observed that Y chromosome microdeletions follow a certain deletion pattern, with three recurrently deleted nonoverlapping subregions in proximal, middle and distal Yq11, designated AZFa, AZFb and AZFc, respectively. In each AZF region, it has been suggested that the deletion is associated with a distinct histopathological profile. Thus, deletions removing the entire AZFa region result in type I Sertoli cell only syndrome (SCOS) (no germ cells present), deletions of the whole AZFb region result in spermatogenic arrest (SGA) usually at spermatocyte stage, and deletions in AZFc are associated with type II SCOS (some spermatogonia present with limited spermatogenesis) or hypospermatogenesis (oligozoospermia).

In each region, a number of candidate genes have been proposed (McElreavey and Krausz, 1999). No single gene-specific deletion has been found in more than 200 oligospermic–azoospermic men screened for six AZF genes by PCR (Krausz *et al.*, 1999a, 2001). This finding indicates that gene-specific deletions are probably rare events and only large Y deletions, removing several genes, are associated with the infertile phenotype. A large study on 576 infertile men describes a *de novo* point mutation of the gene *USP9Y* associated with non-obstructive azoospermia (Sun *et al.*, 1999). This is the first formal demonstration for the aetiopathogenetic role of an AZF gene in spermatogenic failure.

Little is known about the biological functions of the proteins encoded by Y chromosome genes, although most seem to encode for proteins involved in RNA metabolism (*DAZ*, *RBM*, *eIF-1AY*, *DBY*). During the later stages of spermiogenesis, when RNA synthesis is markedly reduced, post-transcriptional regulation plays a primary role. It is possible that factors encoded by Y-linked genes have an important function in this process.

Clinical significance of Y microdeletions

The incidence of Y chromosome microdeletions among infertile men varies among studies from 1 to 55% (Krausz and McElreavey, 1999). The major factor influencing this parameter is the composition of the study population. Since patients affected by idiopathic azoospermia are at higher risk for this genetic defect (approximately 15% of azoospermic men have Yq microdeletions) than are patients affected by oligozoospermia (5–10% oligozoospermic men have Yq microdeletions), a higher proportion of azoospermic men in a given study population will be associated with a higher overall deletion frequency (Krausz *et al.*, 1999b). Yq microdeletions are specific for spermatogenic failure since no microdeletions have been found in normospermic men (Krausz *et al.*, 2001).

Y chromosome deletions are not completely incompatible with fertility because a few cases involving natural transmission of AZFc deletions from father to son have been described (Krausz and McElreavey, 2001; Saut *et al.*, 2000). Since couple fertility is not a synonym of normozoospermia

and sperm analysis of the fathers was not available, it is possible that these 'fertile fathers' may themselves have been oligozoospermic. However, it is also possible that environmental effects or different genetic backgrounds may account for a variable phenotype between a father and his son. For the same reason, the phenotype of an ICSI male offspring from a father with Yq deletions cannot be entirely predicted. Yq microdeletion has been found in two male children conceived through ICSI even though the deletion was not detected in the lymphocytes of their infertile father (Kent-First *et al.*, 1996). Plausible explanations for this situation involve mosaicism in the father's germ line or high rates of DNA fragmentation in the spermatozoa followed by the creation of a post-fertilization Yq deletion. However, in a study of 99 ICSI-conceived sons, no *de novo* Yq deletion was found in the babies (Cram *et al.*, 2000).

In contrast, patients carrying Yq deletions are associated with an increased incidence of 45,XO cells in their lymphocytes and of sperm cells nullisomic for gonosomes, especially for the Y chromosome (Siffroi *et al.*, 2000). Therefore, Yq microdeletions may be associated with Y chromosomal instability leading to the formation of 45,XO cell lines. These findings indicate a possible increased risk for Turner's syndrome in the offspring of men with Yq microdeletion.

A systematic Yq screening of ICSI male babies with long-term follow-up are warranted to understand more about the safety of this technique and the clinical consequence of the transmission of Y deletions. Since certain Y deletions (AZFc) are associated with a progressive change from oligozoospermia to azoospermia (Girardi *et al.*, 1997; Simoni *et al.*, 1997), preventive therapy (cryopreservation of spermatozoa for successive assisted reproductive techniques) could be proposed for affected sons.

Origin and mechanism of Y chromosome microdeletions

Apart from the few inherited cases cited above, most deletions occur as *de novo* events. The cellular origin of Y chromosome deletions is not clear. Presumably, the deletions are preceded by double-strand breaks that, if they occurred premeiotically, could lead to the presence of Y chromosome deletions in the ejaculated spermatozoa. Alternatively, the spermatozoa may harbour a high frequency of double-strand breaks that lead to the creation of deletions after fertilization.

The relatively high frequency of Y deletions indicates that the Y chromosome is particularly susceptible to the spontaneous loss of genetic material. The nature of this susceptibility remains speculative. One possibility is aberrant recombination events between areas of homologous or similar sequence repeats (for example, Alu repeats or gene families) between the X and Y chromosomes or within the Y chromosome itself by unbalanced sister chromatid exchange (McElreavey and Krausz, 1999). The instability of the Y chromosome may also be related to the high

frequency of repetitive elements clustered along the length of the Y chromosome. Deletion interval 6 for example is rich in both inverted and direct repeats, many of which are several hundred kilobases in length (Yen, 1998). A subclass of AZFa deletions appears to have resulted from intrachromosomal crossovers between repeated human endogenous retroviral (HERV) sequences (Blanco *et al.*, 2000; Kamp *et al.*, 2000).

Finally, the particular vulnerability of the Y chromosome to DNA deletions may be the result of its inability to participate in recombination repair. The DNA fragmentation that appears to be commonplace in spermatozoa (Aitken, 1999) has the potential to generate deletions as the chromatin unravels at fertilization. Any double-stranded DNA breaks would normally be repaired by homologous recombination in the few hours that elapse between fertilization and the initiation of the first cleavage division. However, this repair mechanism cannot apply to the non-recombining region of the Y chromosome, where the key spermatogenesis genes are housed and where recombination repair is impossible.

Notwithstanding the evident vulnerability of the Y chromosome, the question remains as to why some men have AZF deletions and others do not. It has been proposed that a particular Y chromosome sequence organization can facilitate deletion formation. However, analysis of Y chromosome haplotypes in men with Y deletions compared with controls with no deletion failed to identify any specific predisposing or protecting haplotype for or against Y deletion formation (Quintana-Murci *et al.*, 2001). In some pathologies characterized by single base-pair substitutions, a paternal age effect has been described. However, paternal age effects do not seem to contribute to the loss of Y sequences in most patients since the father's age at the time of conception of an infertile son with or without Yq deletions is not different from that of control men with normal Y chromosomes.

Conclusions

In conclusion, the male germ line appears to be particularly susceptible to mutagenic and promutagenic change. This variability is biologically useful in that it creates the genetic diversity that fuels the evolutionary process, but the genetic instability characteristic of the male germ line is harmful in that it helps create the mutations responsible for genetic disease including cancer and infertility. The mutagenic mechanisms involve replication errors that are heavily correlated with paternal age and responsible for the spontaneous appearance of dominant genetic diseases such as Apert's syndrome and achondroplasia. In addition, the male germ line is highly susceptible to DNA fragmentation via mechanisms that are independent of paternal age. The aetiology of these strand breaks may involve aberrant recombination, defective chromatin packaging, abortive apoptosis and oxidative stress. Oxidative stress appears to be particularly important and may be induced by a variety

of mechanisms including antioxidant depletion, redox cycling xenobiotics and defective cytoplasmic extrusion during spermiogenesis. Such oxidatively induced DNA fragmentation constitutes a promutagenic change that, in its most severe form, does not have an impact on the quality of the germ line because collateral oxidative damage to the sperm plasma membrane prevents fertilization. When there is less oxidative stress, fertilization can occur, but the oocyte must repair the DNA strand breaks before the initiation of the first cleavage division. It is at this juncture that deletions or sequence errors may be introduced (Fig. 2). The Y chromosome is particularly vulnerable to DNA damage, partly because of its genetic structure and partly because it cannot correct double-stranded DNA deletions by homologous recombination. The fact that such damage to the Y chromosome frequently results in infertility might be regarded as another safety mechanism that serves to limit the extent to which mutations are propagated in the germ line. If the DNA damage does not induce infertility through an effect on the Y chromosome but involves an oncogene, the result will be an increased risk of cancer in the offspring. Such associations are illustrated by the increased risk of childhood cancer seen in the children of men who possess high DNA fragmentation in their spermatozoa as a consequence of heavy smoking. Moreover, because the mutation is fixed in the germ line, it has the potential to impact upon the health and well-being of all the future descendants of a given individual.

C. Krausz received a grant from the Italian Telethon (No. 281/6) and a travel grant from the University of Newcastle (Australia).

References

- Key references are identified by asterisks
- Agulnik AI, Bishop CE, Lerner JL, Agulnik SI and SolovyeV VV** (1997) Analysis of mutation rates in the SMCY/SMCX genes shows that mammalian evolution is male driven *Mammalian Genome* **8** 134–138
- ***Aitken RJ** (1999) The Amoroso Lecture: the human spermatozoon – a cell in crisis? *Journal of Reproduction and Fertility* **115** 1–7
- Aitken RJ, Clarkson JS and Fishel S** (1989) Generation of reactive oxygen species, lipid peroxidation and human sperm function *Biology of Reproduction* **41** 183–187
- Aitken RJ, Buckingham D, West K, Wu FC, Zikopoulos K and Richardson DW** (1992) Differential contribution of leucocytes and spermatozoa to the high levels of reactive oxygen species recorded in the ejaculates of oligozoospermic patients *Journal of Reproduction and Fertility* **94** 451–462
- Aitken RJ, Harkiss D and Buckingham DW** (1993) Analysis of lipid peroxidation mechanisms in human spermatozoa *Molecular Reproduction and Development* **35** 302–315
- Aitken RJ, Krausz C and Buckingham DW** (1994) Relationships between biochemical markers for residual sperm cytoplasm, reactive oxygen species generation and the presence of leucocytes and precursor germ cells in human sperm suspensions *Molecular Reproduction and Development* **39** 268–279
- Aitken RJ, Buckingham DW, Carreras A and Irvine DS** (1996) Superoxide dismutase in human sperm suspensions: relationships with cellular composition, oxidative stress and sperm function *Free Radical Biology and Medicine* **21** 495–504
- Aitken RJ, Gordon E, Harkiss D, Twigg JP, Milne P, Jennings Z and Irvine DS** (1998) Relative impact of oxidative stress on the functional

- competence and genomic integrity of human spermatozoa *Biology of Reproduction* **59** 1037–1046
- Barbieri ER, Hidalgo ME, Venegas A, Smith R and Lissi EA** (1999) Varicocele-associated decrease in antioxidant defenses *Journal of Andrology* **20** 713–717
- Barroso G, Morshedi M and Oehninger S** (2000) Analysis of DNA fragmentation, plasma membrane translocation of phosphatidylserine and oxidative stress in human spermatozoa *Human Reproduction* **15** 1338–1344
- Bianchi P, Manicardi GC, Bizzaro D, Bianchi U and Sakkas D** (1993) Effect of DNA protamination on fluorochrome staining and *in situ* nick-translation of murine and human mature spermatozoa *Biology of Reproduction* **49** 1038–1043
- Birky CW, Jr** (1995) Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution *Proceedings of the National Academy of Sciences USA* **92** 11 331–11 338
- Blanco P, Shlumukova M, Sargent CA, Jobling MA, Affara N and Hurles ME** (2000) Divergent outcomes of intrachromosomal recombination on the human Y chromosome: male infertility and recurrent polymorphism *Journal of Medical Genetics* **37** 752–758
- Boekelheide K, Fleming SL, Johnson KJ, Patel SR and Schoenfeld HA** (2000) Role of Sertoli cells in injury-associated testicular germ cell apoptosis *Proceedings of the Society for Experimental Biology and Medicine* **225** 105–115
- Burgoyne PS** (1998) The mammalian Y chromosome: a new perspective *Bioessays* **20** 363–366
- Casano R, Orlando C, Serio M and Forti G** (1991) LDH and LDH-X activity in sperm from normospermic and oligozoospermic men *International Journal of Andrology* **14** 257–263
- Chai NN, Salido EC and Yen PH** (1997) Multiple functional copies of RBM gene family, a spermatogenesis candidate on the human Y chromosome *Genomics* **45** 355–361
- Cram DS, Ma K, Bhasin S, Arias J, Pandjaitanc M, Chu B, Audrins MS, Saunders D, Quinn F, deKretser D and McLachlan R** (2000) Y chromosome analysis of infertile men and their sons conceived through intracytoplasmic sperm injection: vertical transmission of deletions and rarity of *de novo* deletions *Fertility and Sterility* **74** 909–915
- ***Crow JF** (1997) The high spontaneous mutation rate: is it a health risk? *Proceedings of the National Academy of Sciences USA* **94** 8380–8386
- Cummins JM** (2000) Fertilization and elimination of the paternal mitochondrial genome *Human Reproduction* **15** Supplement 2 92–101
- Delbridge ML and Graves JA** (1999) Mammalian Y chromosome evolution and the male-specific functions of Y chromosome-borne genes *Reviews of Reproduction* **4** 101–109
- Donnelly ET, O'Connell M, McClure N and Lewis SE** (2000) Differences in nuclear DNA fragmentation and mitochondrial integrity of semen and prepared human spermatozoa *Human Reproduction* **15** 1552–1561
- Evenson DP, Jost LK, Marshall D, Zinaman MJ, Clegg E, Purvis K, de Angelis P and Claussen OP** (1999) Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic *Human Reproduction* **14** 1039–1049
- Fraga CG, Motchnik PA, Wyrobek AJ, Rempel DM and Ames BN** (1996) Smoking and low antioxidant levels increase oxidative damage to DNA *Mutation Research* **351** 199–203
- Girardi SK, Mielnik A and Schlegel PN** (1997) Submicroscopic deletions in the Y chromosome of infertile men *Human Reproduction* **12** 1635–1641
- Gomez E, Buckingham DW, Brindle J, Lanzafame F, Irvine DS and Aitken RJ** (1996) Development of an image analysis system to monitor the retention of residual cytoplasm by human spermatozoa: correlation with biochemical markers of the cytoplasmic space, oxidative stress and sperm function *Journal of Andrology* **17** 276–287
- Gomez E, Irvine DS and Aitken RJ** (1998) Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function *International Journal of Andrology* **21** 81–94
- Hales DB, Diemer T and Hales KH** (1999) Role of cytokines in testicular function *Endocrine* **10** 201–217
- Hendin BN, Kolettis PN, Sharma RK, Thomas AJ, Jr and Agarwal A** (1999) Varicocele is associated with elevated spermatozoal reactive oxygen species production and diminished seminal plasma antioxidant capacity *Journal of Urology* **161** 1831–1834
- Host E, Lindenberg S and Smidt-Jensen S** (2000) The role of DNA strand breaks in human spermatozoa used for IVF and ICSI *Acta Obstetrica Gynecologica Scandinavica* **79** 559–563
- Huang C, Li J, Zheng R and Cui K** (2000) Hydrogen peroxide-induced apoptosis in human hepatoma cells is mediated by CD95(APO-1/Fas) receptor/ligand system and may involve activation of wild-type p53 *Molecular Biology Reports* **27** 1–11
- Hughes CM, Lewis SEM, McKelvey-Martin J and Thompson W** (1996) A comparison of baseline and induced DNA damage in human spermatozoa from fertile and infertile men, using a modified comet assay *Molecular Human Reproduction* **2** 613–619
- Huszar G, Vigue L and Corrales M** (1988) Sperm creatine phosphokinase quality in normospermic, variablespermic and oligospermic men *Biology of Reproduction* **38** 1061–1066
- Imai H, Suzuki K, Ishizaka K, Ichinose S, Oshima H, Okayasu I, Emoto K, Umeda M and Nakagawa Y** (2001) Failure of the expression of phospholipid hydroperoxide glutathione peroxidase in the spermatozoa of human infertile males *Biology of Reproduction* **64** 674–683
- Irvine DS, Twigg J, Gordon E, Fulton N, Milne P and Aitken RJ** (2000) DNA integrity in human spermatozoa: relationship with semen quality *Journal of Andrology* **21** 33–44
- ***Ji BT, Shu XO, Linet MS, Zheng W, Wacholder S, Gao YT, Ying DM and Jin F** (1997) Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers *Journal of the National Cancer Institute* **89** 238–244
- Jones R, Mann T and Sherins RJ** (1979) Peroxidative breakdown of phospholipids in human spermatozoa: spermicidal effects of fatty acid peroxides and protective action of seminal plasma *Fertility and Sterility* **31** 531–537
- Kamp C, Hirschmann P, Voss H, Huellen K and Vogt PH** (2000) Two long homologous retroviral sequence blocks in proximal Yq11 cause AZFa microdeletions as a result of intrachromosomal recombination events *Human Molecular Genetics* **9** 2563–2572
- Kao SH, Chao HT and Wei YH** (1998) Multiple deletions of mitochondrial DNA are associated with the decline of motility and fertility of human spermatozoa *Molecular Human Reproduction* **4** 657–666
- Keating J, Grundy CE, Fivey PS, Elliott M and Robinson J** (1997) Investigation of the association between the presence of cytoplasmic residues on the human sperm midpiece and defective sperm function *Journal of Reproduction and Fertility* **110** 71–77
- Kent-First MG, Kol S, Muallem A, Ofir R, Manor D, Blazer S, First N and Itskovitz-Eldor J** (1996) the incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers *Molecular Human Reproduction* **2** 943–945
- Kodama H, Yamaguchi R, Fukuda J, Kasai H and Tanaka T** (1997) Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients *Fertility and Sterility* **68** 519–524
- Krausz C and McElreavey K** (1999) Y chromosome and male infertility *Frontiers in Bioscience* **4** 1–8
- Krausz C and McElreavey K** (2001) Y chromosome microdeletions in “fertile” males *Human Reproduction* **16** 1306
- Krausz C, Bussani-Mastellone C, Granchi S, McElreavey K, Scarselli G and Forti G** (1999a) Screening for microdeletions of Y chromosome genes in patients undergoing intracytoplasmic sperm injection *Human Reproduction* **14** 1717–1721
- ***Krausz C, Quintana-Murci L, Barbaux S et al.** (1999b) A high frequency of Y chromosome deletions in males with nonidiopathic infertility *Journal of Clinical Endocrinology and Metabolism* **84** 3606–3612
- Krausz C, Rajpert-De Meyts E, Frydelund-Larsen L, Quintana-Murci L, McElreavey K and Skakkebaek NE** (2001) Double blind Y chromosome microdeletion analysis in men with known sperm parameters and reproductive hormone profiles: microdeletions are specific for spermatogenic failure *Journal of Clinical Endocrinology and Metabolism* **86** 2638–2642
- Kwenang A, Kroos MJ, Koster JF and Van Eijk HG** (1987) Iron, ferritin and copper in seminal plasma *Human Reproduction* **2** 387–388
- Lahn BT and Page DC** (1997) Functional coherence of the human Y chromosome *Science* **278** 675–680

- Lahn BT and Page DC (1999) Retroposition of autosomal mRNA yielded testis-specific gene family on human Y chromosome *Nature Genetics* **21** 429–433
- Liang R, Senturker S, Shi X, Bal W, Dizdaroglu M and Kasprzak KS (1999) Effects of Ni(II) and Cu(II) on DNA interaction with the N-terminal sequence of human protamine P2: enhancement of binding and mediation of oxidative DNA strand scission and base damage *Carcinogenesis* **20** 893–898
- McElreavey K and Krausz C (1999) Male infertility and the Y chromosome *American Journal of Human Genetics* **64** 928–933
- MacLeod J (1943) The role of oxygen in the metabolism and motility of human spermatozoa *American Journal of Physiology* **138** 512–518
- Mak V, Jarvi K, Buckspan M, Freeman M, Hechter S and Zini A (2000) Smoking is associated with the retention of cytoplasm by human spermatozoa *Urology* **56** 463–466
- Marshall Graves J (2000) Human Y chromosome, sex determination, and spermatogenesis – a feminist view *Biology of Reproduction* **63** 667–676
- Muratori M, Piomboni P, Baldi E, Filimberti E, Pecchioli P, Moretti E, Gambera L, Baccetti B, Biagiotti R, Forti G and Maggi M (2000) Functional and ultrastructural features of DNA-fragmented human sperm *Journal of Andrology* **21** 903–912
- Perry AC, Jones R and Hall L (1993) Isolation and characterization of a rat cDNA clone encoding a secreted superoxide dismutase reveals the epididymis to be a major site of its expression *Biochemical Journal* **293** 21–25
- Quintana-Murci L, Krausz C, Heyer E *et al.* (2001) The relationship between Y chromosome DNA haplotypes and Y chromosome deletions leading to male infertility *Human Genetics* **108** 55–58
- Reynier P, Chretien MF, Savagner F, Larcher G, Rohmer V, Barriere P and Malthiery Y (1998) Long PCR analysis of human gamete mtDNA suggests defective mitochondrial maintenance in spermatozoa and supports the bottleneck theory for oocytes *Biochemical and Biophysical Research Communications* **252** 373–377
- Sakkas D, Mariethoz E, Manicardi G, Bizzaro D, Bianchi PG and Bianchi U (1999a) Origin of DNA damage in ejaculated human spermatozoa *Reviews of Reproduction* **4** 31–37
- Sakkas D, Mariethoz E and St John JC (1999b) Abnormal sperm parameters in humans are indicative of an abortive apoptotic mechanism linked to the Fas-mediated pathway *Experimental Cell Research* **251** 350–355
- Saut N, Terriou P, Navarro A, Levy N and Mitchell MJ (2000) The human Y chromosome genes BPY2, CDY1 and DAZ are not essential for sustained fertility *Molecular Human Reproduction* **6** 789–793
- Sawyer DE and Aitken RJ (2000) Male mediated developmental defects and childhood disease *Reproductive Medicine Review* **8** 107–126
- Saxena R, Brown LG, Hawkins T, Alagappan RK, Skaletsky H, Reeve MP, Reijo R, Rozen S, Dinulos MB, Distche CM and Page DC (1996) The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned *Nature Genetics* **14** 292–299
- Sayers TJ, Brooks AD, Seki N, Smyth MJ, Yagita H, Blazar BR and Malyguine AM (2000) T cell lysis of murine renal cancer: multiple signaling pathways for cell death via Fas *Journal of Leukocyte Biology* **68** 81–86
- Sharma RK, Pasqualotto FF, Nelson DR, Thomas AJ and Agarwal A (1999) The reactive oxygen species-total antioxidant capacity score is a new measure of oxidative stress to predict male infertility *Human Reproduction* **14** 2801–2807
- Shen H and Ong C (2000) Detection of oxidative DNA damage in human sperm and its association with sperm function and male infertility *Free Radicals in Biology and Medicine* **28** 529–536
- Shen HM, Chia SE and Ong CN (1999) Evaluation of oxidative DNA damage in human sperm and its association with male infertility *Journal of Andrology* **20** 718–723
- Shimmin LC, Chang BH and Li WH (1993) Male-driven evolution of DNA sequences *Nature* **362** 745–747
- Short RV (1997) The testis – the witness of the mating system, the site of mutation and the engine of desire *Acta Paediatrica* **86** 3–7
- Siffroi JP, Le Bourhis C, Krausz C *et al.* (2000) Sex chromosome mosaicism in males carrying Y chromosome long arm deletions *Human Reproduction* **15** 2559–2562
- Simoni M, Gromoll J, Dworniczak B *et al.* (1997) Screening for deletions of the Y chromosome involving the DAZ (*deleted in azoospermia*) gene in azoospermia and severe oligozoospermia *Fertility and Sterility* **67** 542–547
- Smith R, Vantman D, Ponce J, Escobar J and Lissi E (1996) Total antioxidant capacity of human seminal plasma *Human Reproduction* **11** 1655–1660
- Sorahan T, Lancashire RJ, Hulten MA, Peck I and Stewart AM (1997) Childhood cancer and parental use of tobacco: deaths from 1953 to 1955 *British Journal of Cancer* **75** 134–138
- Sun C, Skaletsky H, Birren B, Devon K, Tang Z, Silber S, Oates R and Page DC (1999) An azoospermic man with a *de novo* point mutation in the Y-chromosomal gene USP9Y *Nature Genetics* **23** 429–432
- Sun JG, Jurisicova A and Casper RF (1997) Detection of deoxyribonucleic acid fragmentation in human sperm: correlation with fertilization *in vitro*. *Biology of Reproduction* **56** 602–607
- Tiepolo L and Zuffardi O (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm *Human Genetics* **34** 119–124
- Twigg J, Irvine DS, Houston P, Fulton N, Michael L and Aitken RJ (1998a) Iatrogenic DNA damage induced in human spermatozoa during sperm preparation: protective significance of seminal plasma *Molecular Human Reproduction* **4** 439–445
- *Twigg J, Fulton N, Gomez E, Irvine DS and Aitken RJ (1998b) Analysis of the impact of intracellular reactive oxygen species generation on the structural and functional integrity of human spermatozoa: lipid peroxidation, DNA fragmentation and effectiveness of antioxidants *Human Reproduction* **13** 1429–1437
- Twigg JP, Irvine DS and Aitken RJ (1998c) Oxidative damage to DNA in human spermatozoa does not preclude pronucleus formation at ICSI *Human Reproduction* **13** 1864–1871
- van Overveld FW, Haenen GR, Rhemrev J, Vermeiden JP and Bast A (2000) Tyrosine as important contributor to the antioxidant capacity of seminal plasma *Chemical and Biological Interactions* **127** 151–161
- Vernet P, Rigaudiere N, Ghyselinck N, Dufaure JP and Drevet JR (1996) *In vitro* expression of a mouse tissue-specific glutathione peroxidase protein lacking selenocysteine can protect stably transfected mammalian cells against oxidative damage *Biochemistry and Cell Biology* **74** 125–131
- Vernet P, Faure J, Dufaure JP and Drevet JR (1997) Tissue and developmental distribution, dependence upon testicular factors and attachment to spermatozoa of GPX5, a murine epididymis-specific glutathione peroxidase *Molecular Reproduction and Development* **47** 87–98
- Vogt PH, Edelmann A, Kirsch S *et al.* (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11 *Human Molecular Genetics* **5** 933–943
- Vollrath D, Foote S, Hilton A, Brown LG, Beer-Romero P, Bogan JS and Page DC (1992) The human Y chromosome: a 43-interval map based on naturally occurring deletions *Science* **258** 52–59
- Waters PD, Duffy B, Frost CJ, Delbridge ML and Graves JA (2001) The human Y chromosome derives largely from a single autosomal region added to the sex chromosomes 80–130 million years ago *Cytogenetics and Cell Genetics* **92** 74–79
- Wellejus A, Poulsen HE and Loft S (2000) Iron-induced oxidative DNA damage in rat sperm cells *in vivo* and *in vitro*. *Free Radical Research* **32** 75–83
- Yen PH (1998) A long range restriction map of deletion interval 6 of the human Y chromosome: a region frequently deleted in azoospermic males *Genomics* **54** 5–12
- Zini A, O'Bryan MK, Israel L and Schlegel PN (1998) Human sperm NADH and NADPH diaphorase cytochemistry: correlation with sperm motility *Urology* **51** 464–468
- Zini A, Buckspan M, Jama IM and Jarvi K (1999) Effect of varicocelectomy on the abnormal retention of residual cytoplasm by human spermatozoa *Human Reproduction* **14** 1791–1793
- Zini A, Garrels K and Phang D (2000a) Antioxidant activity in the semen of fertile and infertile men *Urology* **55** 922–926
- Zini A, Defreitas G, Freeman M, Hechter S and Jarvi K (2000b) Varicocele is associated with abnormal retention of cytoplasmic droplets by human spermatozoa *Fertility and Sterility* **74** 461–464