

DNA damage in human spermatozoa; important contributor to mutagenesis in the offspring

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Abstract: Nuclear DNA damage in spermatozoa could potentially have a major impact on the fertilizing capacity of these cells, their ability to establish a normal pattern of embryonic development as well as the health and wellbeing of subsequent offspring. Many laboratory techniques have been developed to assess this damage focusing on strand breaks, chromatin stability following exposure to extreme pH conditions and the formation of DNA adducts. Of particular importance may be the dominant role played by oxidative stress in the etiology of defective sperm function and DNA damage. The oxidative base lesions created via this mechanism have the potential to generate genetic and epigenetic mutations in the offspring that could have a profound impact on the latter's long-term health trajectory. Moreover if oxidative stress is the cause of DNA damage in spermatozoa then there is a potential role for antioxidant therapy in the resolution of this problem, which deserves investigation.

Keywords: spermatozoa; DNA damage; oxidative stress; mutation

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DNA damage in human spermatozoa is a conundrum. We do not know where it is induced, why it is induced, how it is induced, how to measure it or what it means for fertility or for the health and wellbeing of the offspring. The paper by Agarwal *et al.* (1) represents an extremely comprehensive capture of our current understanding with respect to measurement methodologies, clinical association and diagnostic implications. However, it is inevitably impacted by our lack of understanding concerning the etiology of DNA damage and the manner in which such damage is ultimately processed by the oocyte and early embryo. Moreover, the key relationship between DNA damage in human spermatozoa and the mutational/epimutational load subsequently carried by the offspring is still a matter for conjecture. We do know that as men age, the spontaneous mutation rate in their children increases in a linear fashion (2) presumably as a consequence of age-dependent damage to the DNA in their spermatozoa. If

we can demonstrate that DNA damage in the male germ line is associated with genetic or epigenetic changes in the progeny then we shall have gone a long way towards making the case for conducting routine assessments of DNA damage in spermatozoa, as recommended in Agarwal's article (1).

Methodology

The review at the heart of this commentary (1) has beautifully summarized and presented the various techniques that are available to measure DNA damage in human spermatozoa, paying attention to the nature of the DNA damage being measured, the chemical underpinnings of the respective techniques and the potential clinical relevance of the measurements made. The authors rightly point out that the list of methodologies they describe either measure pre-existing DNA damage in the spermatozoa

(Halo test, TUNEL) or damage induced or revealed on exposure of the gametes to very high (Comet) or very low (SCSA) ambient pH. They also correctly emphasize the importance of assays that measure the efficiency of DNA packaging and compaction such as the aniline blue or chromomycin A3 staining methodologies. Many authors have pointed out the vulnerability of poorly remodelled sperm chromatin to DNA damage particularly when the damage is oxidatively induced (3). Indeed, in a fascinating experiment of nature, marsupial spermatozoa are much more vulnerable to oxidative DNA damage than their eutherian counterparts precisely because their protamines lack the cysteine residues needed to compact and protect sperm chromatin through the creation of disulphide bridges (4).

Two-step hypothesis

Reflecting on the importance of chromatin compaction is the aetiology of DNA damage in mammalian spermatozoa, we have proposed a two-step hypothesis for how such damage might occur in our own species (5). This hypothesis posits that the first stage in the etiology of DNA damage is a defect at the spermatid stage of germ cell differentiation leading to a defect in the chromatin remodelling process that accompanies spermiogenesis. This generates a vulnerable cell that, in the second step, succumbs to a free radical attack that influences the structure and integrity of the sperm nuclear DNA. Such an oxidative attack could occur at any time during the life of a spermatozoon from its differentiation during spermiogenesis (6) to its maturation and storage in the epididymis (3,7). Moreover, as Agarwal *et al.* (1) point out, the source of the oxidative stress could be anything from a specific clinical condition such as the presence of a varicocele, to age, obesity, smoking and environmental exposure to toxicants (8).

Importance of oxidative stress

Central to the two-step hypothesis is the important role played by oxidative stress in the etiology of DNA damage in human spermatozoa (5), reactive oxygen species (ROS) attack sperm DNA in several different ways. Firstly, an oxidative attack on sperm DNA can lead to the formation of oxidative base adducts such as 8-hydroxy-2'-deoxyguanosine (8OHdG). In responding to such damage spermatozoa can only call upon the first enzyme in the base

excision repair pathway, 8-oxoguanine DNA glycosylase, OGG-1 (9). This glycosylase cleaves the oxidized base out of the DNA duplex to generate a corresponding abasic site that destabilizes the ribose-phosphate backbone leading to a β -elimination or a ring opening reaction of the ribose unit and a consequential strand break. If this limited DNA repair pathway does not complete its task, then 8OHdG residues persist in both the spermatozoa (10) and (because the oocyte is poorly endowed with OGG1) well into S-phase of the first mitotic division following fertilization (9). The significance of such persistence is that 8OHdG residues are highly mutagenic, potentially causing an increase in the mutational load carried by the embryo (11) particularly, but not exclusively, GC-AT transversions (12). Similarly, oxidative stress in the germ line can result in the formation of lipid aldehyde adducts on DNA involving compounds such as 4-hydroxynonenal and 4-hydroxyhexenal, both of which are also powerfully immunogenic (13,14) and could be responsible for increasing the mutational, as well as the epimutational, load carried by the offspring (15).

The associations between oxidative stress in the germ line, DNA damage in spermatozoa and genetic/epigenetic mutational changes in the offspring that potentially impact the latter's health trajectory are clearly critical for the future of sperm DNA damage testing in male patients. The observed increases in mutational load associated with children as a function of their fathers' age (2) is an example of such a mechanism-in-action which resonates with abundant evidence linking paternal age with oxidative DNA damage to spermatozoa (16) and the impact of paternal age on a range of pathologies in the offspring including dominant genetic diseases such as achondroplasia to neurodevelopmental disorders such as autism, bipolar disease or spontaneous schizophrenia (11,17). Similarly, there is a clear link between the high levels of oxidative DNA damage observed in the spermatozoa of male smokers and the significantly increased risk of cancer seen in their offspring (18). Oxidative stress in spermatozoa has also been linked with an increased risk of recurrent miscarriage (19) which could again be due to genetic/epigenetic changes in the zygote, subsequent and consequent to increased DNA damage in spermatozoa.

Conclusions

This volume captures much of the current thinking around the nature of DNA damage in spermatozoa, how this

damage can be measured and how such measurements should be deployed in a clinical setting. Nevertheless one might add to the list of methods used to detect DNA damage in spermatozoa, the analysis of 8OHdG lesions for three major reasons: (I) measurement of oxidative DNA may reflect the potential impact of the male germ line on the mutational load carried by the embryo; (II) these mutations, whether they are genetic or epigenetic are likely to have a significant impact on the health and wellbeing of the progeny and (III) if oxidative damage to the sperm DNA is responsible for inducing genetic/epigenetic changes in the offspring that impact the latter's health then there are important therapeutic possibilities to explore in the form of appropriate antioxidant therapy (20).

Acknowledgements

None.

Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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