Commentary

Testing of sperm DNA damage and clinical recommendations

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Agarwal *et al.* in their most recent paper (1) propose a clinical guideline on the use of sperm DNA damage testing in infertility treatment. This guideline is extremely relevant for fertility specialists and further insight into the topic is in high demand as the debate regarding the role of DNA damage testing is still ongoing (2,3).

We believe that some of the controversies in the field are due to misunderstandings which might be prevented by a more careful communication. In our opinion, the term "fragmentation" is misleading as it implies that the sperm DNA has already been broken into "fragments"—i.e., DNA with double-stranded breaks. Double-stranded DNA breaks represent an irreversible change which is highly unlikely to be repaired by the oocyte.

The initial discovery that sperm DNA damage affected the outcome of natural intercourse negatively (4) led to the assumption that there would be a similar impact on the outcome of intrauterine insemination (IUI), *in vitro* fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment. However, several clinical studies subsequently demonstrated that this is not the case and that the outcome of IVF and especially ICSI treatment are less affected than is the case for IUI treatments and natural intercourse. The term "fragmentation" and our perception of the type of sperm DNA damage have led to a number of misunderstandings. As an example, the results of sperm DNA testing have been regarded as "false positive results" in the area of IVF and ICSI treatments.

A study by Bungum *et al.* (5) demonstrated that the impact on treatment outcome depended on the type of fertility treatment. IUI was affected to the same extent as natural intercourse, IVF to a lesser extent, and the smallest impact was seen for ICSI treatments. The work by Bungum *et al.* (5) and other publications during the past decade have resulted in the "two-step-hypothesis" proposed by Aitken and De Iuliis (6): sperm DNA testing concerns the integrity of the DNA. If DNA integrity is poor, the sperm DNA is fragile and may become damaged after the sperm becomes motile. This is due to increases in the level of reactive oxygen species (ROS) following the oxidative production of energy in the mid-piece. Clearly, the extent of DNA damage depends on the length of the "journey" which the sperm make to the oocyte as well as the demanding process of fertilization. Reducing the length of the journey to the oocyte (IVF) as well as bypassing the process of fertilization (ICSI) will minimize the extent of sperm DNA damage. It is, therefore, not surprising that treatment success rates vary for the different types of fertility treatment.

Agarwal *et al.* (1) provide a comprehensive review of the literature with evidence based recommendations including the role of sperm DNA damage on recurrent miscarriage. A recent review and meta-analysis by Zhao *et al.* (7) highlighted the importance of sperm DNA damage in relation to miscarriage following IVF and ICSI treatment. In addition, a new review and meta-analysis by Simon *et al.* (8) also showed that sperm DNA damage negatively affects the outcome of ICSI treatment.

The recommendations by Agarwal *et al.* (1) also include the use of testicular sperm in men with high DNA fragmentation index (DFI) and repeated IVF failure. We agree but would also suggest that factors known to increase the level of sperm DNA damage are identified and recommend that these are treated or corrected prior to fertility treatment. Factors which should be considered

include varicocele, smoking, obesity, pollution and treatment with antioxidants. More recently, metabolic syndrome (or insulin resistance) has been added to the list of factors that cause sperm DNA damage (9).

We believe that it is very important to improve the integrity of sperm DNA and consequently select the most appropriate treatment to minimize the amount of sperm DNA damage at the time of fertilization. This strategy is likely to increase the success rates for IUI, IVF and ICSI treatments, and is also likely to reduce the risk of complications for the offspring.

Paternal smoking has been associated with increased levels of sperm DNA damage. However, it has also recently been shown to result in *de novo* mutations in the offspring (10). Mutations in the offspring may result in complications such as childhood cancer (11) or mental illnesses such as autism or schizophrenia (12). The study by Kong *et al.* (12) demonstrated that 94% of all mutations in the newborn were of paternal origin. More recently it has been shown that children born after fertility treatment have an increased risk of various mental illnesses (13). Clearly, reduction of sperm DNA damage prior to assisted reproductive technology (ART) should be recommended to reduce the disease-risk for the offspring.

Interestingly, interventions to reduce the level of sperm DNA damage may also have a positive effect on male health in general, as it has been demonstrated that there is a clear link to DNA damage in somatic cells (14). In contrast to mature sperm, somatic cells are able to repair DNA damage but a likely outcome is mutations or cell necrosis leading to various diseases, including cancer. In this regard, it is interesting that a large follow-up study of more than 40,000 men with reduced fertility showed an increased risk of various diseases, including cardio-vascular disease and cancer (15).

Agarwal *et al.* (1) in their guideline also provide a review of current methods for detection of sperm DNA damage. Some methods are described as "rapid, simple or inexpensive" with the note that they may suffer from "lack of reproducibility or intra-observer variability". Considering the future possible health implications for both the offspring and the male with sperm DNA damage, we suggest that the time has now come to move to the most precise detection method which seems to be flow cytometry performed by skilled technicians. Although more expensive, a high level of precision for any test of sperm is essential and a poor level of precision is a well-known problem with the classic assessment of sperm count and percentage of motile sperm (16). Flow cytometric assessment of sperm DNA damage has been demonstrated to provide highly reliable and precise results (17,18). Obviously, it is essential that the same level of quality control (QC) is used for detection of DNA damage as for assessment of conventional semen parameters (19,20).

It should be kept in mind that a method with low precision may be useful to describe differences in the average level of sperm DNA damage before and after an intervention for a group of subjects, or between different types of semen samples. However, if the goal is to identify individuals suffering from sperm DNA damage or to monitor the patient's response to intervention, it is mandatory to use a test with the highest level of precision.

We believe that some of the controversies in this field until now were created by the comparison of results from different testing methods without considering the fact that the results and the reliability may vary from one test to another. For a number of testing methods, the relationship between the results of the method has not been shown to relate to the reproductive outcomes of IUI, IVF or ICSI treatments. For this reason, results of such tests should be interpreted with caution.

In conclusion, the review by Agarwal *et al.* (1) provides important knowledge for clinical management of the infertile male, couples with recurrent IVF failure and miscarriage. Moreover, the importance of life-style modification is stressed, and analysis of sperm DNA damage seems to become a future standard testing method in line with standard semen analyses when counselling the infertile couple.

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Footnote

Conflicts of Interest: P. Christensen is the CEO of SPZ Lab A/S which provides sperm DNA testing. P. Humaidan has no conflicts of interest to declare.

References

1. Agarwal A, Majzoub A, Esteves SC, et al. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. Transl Androl Urol 2016;5:935-50.

2. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. Fertil Steril 2015;103:e18-25.
3. Bach PV, Schlegel PN. Sperm DNA damage and its role in IVF and ICSI. Basic Clin Androl 2016;26:15.
4. Evenson DP, Jost LK, Marshall D, et al. Utility of the sperm chromatin structure assay as a diagnostic and prognostic tool in the human fertility clinic. Hum Reprod 1999;14:1039-49.
5. Bungum M, Humaidan P, Axmon A, et al. Sperm DNA integrity assessment in prediction of assisted reproduction technology outcome. Hum Reprod 2007;22:174-9.
6. Aitken RJ, De Iuliis GN. On the possible origins of DNA damage in human spermatozoa. Mol Hum Reprod 2010;16:3-13.
7. Zhao J, Zhang Q, Wang Y, et al. Whether sperm deoxyribonucleic acid fragmentation has an effect on pregnancy and miscarriage after in vitro fertilization/ intracytoplasmic sperm injection: a systematic review and meta-analysis. Fertil Steril 2014;102:998-1005.e8.
8. Simon L, Zini A, Dyachenko A, et al. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. Asian J Androl 2017;19:80-90.
9. Leisegang K, Bouic PJ, Henkel RR. Metabolic syndrome is associated with increased seminal inflammatory cytokines and reproductive dysfunction in a case-controlled male cohort. Am J Reprod Immunol 2016;76:155-63.
10. Laubenthal J, Zlobinskaya O, Poterlowicz K, et al. Cigarette smoke-induced transgenerational alterations in genome stability in cord blood of human F1 offspring. FASEB J 2012;26:3946-56.
11. Ji BT, Shu XO, Linet MS, et al. Paternal cigarette smoking and the risk of childhood cancer among offspring of nonsmoking mothers. J Natl Cancer Inst 1997;89:238-44.
12. Kong A, Frigge ML, Masson G, et al. Rate of de novo mutations and the importance of father's age to disease risk. Nature 2012;488:471-5.
13. Svahn MF, Hargreave M, Nielsen TS, et al. Mental disorders in childhood and young adulthood among children born to women with fertility problems. Hum Reprod 2015;30:2129-37.
14. Baumgartner A, Kurzawa-Zegota M, Laubenthal J, et al. Comet-assay parameters as rapid biomarkers of exposure to dietary/environmental compounds -- an in vitro feasibility study on spermatozoa and lymphocytes. Mutat Res 2012;743:25-35.
15. Jensen TK, Jacobsen R, Christensen K, et al. Good semen quality and life expectancy: a cohort study of 43,277 men. Am J Epidemiol 2009;170:559-65.
16. Schrader SM, Turner TW, Breitenstein MJ, et al. Longitudinal study of semen quality of unexposed workers. I. Study overview. Reprod Toxicol 1988;2:183-90.
17. Sergerie M, Laforest G, Boulanger K, et al. Longitudinal study of sperm DNA fragmentation as measured by terminal uridine nick end-labelling assay. Hum Reprod 2005;20:1921-7.
18. Evenson DP, Jost LK, Baer RK, et al. Individuality of DNA denaturation patterns in human sperm as measured by the sperm chromatin structure assay. Reprod Toxicol 1991;5:115-25.
19. Boe-Hansen GB, Ersbøll AK, Christensen P. Variability and laboratory factors affecting the sperm chromatin structure assay in human semen. J Androl 2005;26:360-8.
20. Björndahl L, Barratt CL, Mortimer D, et al. 'How to count sperm properly': checklist for acceptability of studies based on human semen analysis. Hum Reprod 2016;31:227-32.

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