Sins of the fathers: sperm DNA damage in the context of assisted reproduction

João Ramalho-Santos*1,2
1Biology of Reproduction and Stem Cell Group, CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
2Department of Life Sciences, University of Coimbra, Coimbra, Portugal

A permanent challenge in Assisted Reproduction is the uncovering of methodologies to allow for better gamete and embryo evaluation and selection, which may in turn lead to higher success rates in general, and to more predictable evaluations of specific cases. In this regard, sperm analysis holds several interesting paradoxes. While the number of male gametes ultimately required for fertilization almost always allows material to be available for analysis, it does not necessarily follow that the same cells analysed may then be of clinical use. From another perspective, while classical sperm evaluation via the standard spermogram (WHO, 2010) provides a technically easy and affordable measure of ejaculate quality, the notion that the data obtained are relatively poor at predicting the success of any intervention is as unanimous a statement as is likely to be found in the field. Besides sperm quantity, motility and morphology (and viability) the need for better assessment tools and parameters has been clear for many years (Said and Land, 2011; Sousa et al., 2011).

In addition to standard spermogram measurements, the sperm parameter that arguably has received the most attention is DNA integrity, given its potential importance on the quality of the genetic information transmitted to a developing embryo, and thus on the likelihood that impaired sperm DNA may negatively impact the success of Assisted Reproduction. Additionally, sperm DNA status may also serve as an important indicator to evaluate both defects in spermatogenesis, or the possible effects of different environmental exposures on male reproductive function (Perry, 2008; Sousa et al., 2009; Tavares et al., 2013). Although there seems to be a general agreement as to the importance of measuring sperm DNA integrity, the literature is controversial (if not downright confusing), with some studies suggesting a modest predictive power that does not support general implementation of the parameter (Collins et al., 2008), while others show more promising outcomes. Namely, sperm DNA integrity was shown to have predictive power (higher for IUI and IVF, lower for ICSI) in terms of pregnancy outcomes (Evenson and Wixon, 2006; Simon et al., 2010, 2014) and to be useful in terms of predicting pregnancy loss (Zini et al., 2008; Robinson et al., 2012), but not in the prediction of embryo quality (Zini et al., 2011).

No doubt the lack of agreement in the literature is partially due to the many variables involved, and of the main issues that need tackling have been identified (Zini and Sigman, 2009; Barratt and De Jonge, 2010; Barratt et al., 2010; Said and Land, 2011). How is DNA integrity specifically measured, and how can measurements be robustly standardized? What does each test monitor, which is more appropriate, what controls need to be performed? How many groups of samples with varying DNA damage does each test define, and how should they be established? What are the cut-off values that identify potential problems and that should function as ‘red flags’ for clinicians in terms of therapeutic options? In which samples should sperm DNA integrity be measured (native versus prepared sperm)? Were published tests carried out with any degree of blinding, and what strategies were employed to deal with potential outliers? What exactly can sperm DNA integrity help predict (fertilization rates, embryo quality, embryo development, pregnancy, pregnancy loss)? In terms of the specific Assisted Reproduction technologies, are there differences in the predictive power of sperm DNA integrity for IUI, IVF or ICSI? When should measurements be performed, and in what samples, in order to be cost-effective in a clinical setting? What other parameters/factors should be taken into account in models of increasing complexity to allow for relevant predictions? What statistical analyses should be performed and what is their true predictive power? This final point is often overlooked; much more than a ‘mere’ P-value below 0.05 (still a ‘holy grail’ in most papers), what needs to be determined is to what extent sperm DNA evaluation can be truly relevant.

When considering the tests available, it should be noted that they monitor distinct aspects of sperm DNA integrity (DNA packaging, different types of strand breaks) with varying degrees of robustness, technical complexity and cost. Among the most well established tests are the SCSA (Sperm Chromatin Structure Assay; Evenson et al., 1980), TUNEL (terminal deoxynucleotidyl transferase-mediated dUDP nick-end labelling; Gorczyca et al., 1993), Comet (single-cell gel electrophoresis assay; Hughes et al., 1996) and SCD (sperm chromatin dispersion test, commercially available as the Halosperm® kit; Fernandez et al., 2003). Some of these tests (e.g. TUNEL) can be carried out both by flow cytometry and fluorescence microscopy, with concomitant changes in affordability, degree of potential subjectivity and number of cells that can be analysed; although agreement between measurements is certainly possible and the methodology adaptable (to some extent) to distinct laboratory settings (Varum et al., 2007). Additionally, other tests take advantage of common methodologies normally used to monitor sperm...
morphology, and are therefore more amenable for use on a daily basis in a minimal Andrology lab setting, although quantitative and objective measurements are usually lacking in these cases (Sousa et al., 2009).

Interestingly however, cut-off values that establish when sperm DNA integrity may start to prove problematic, and thus negatively influence Assisted Reproduction outcomes, are remarkably similar in almost all tests/methodologies, around 30% (Tavares et al., 2013, and references therein). Although its exact significance remains to be established, this observation is somewhat reassuring, in that it suggests that there may be some common functional aspects to the different types of sperm DNA damage monitored by the various assays, and that a threshold may actually involve an accumulated multi-causal effect.

Finally, when considering different methodologies to monitor a single variable, another aspect that is worth mentioning includes possible conflicts of interest, common in other fields of biomedical research with similar characteristics, where researchers and clinicians help develop, popularize, evaluate and market different products. However, the extent of potential conflicts of interest could imply that they may not always be understood, or directly disclosed, as such.

In the manuscript ‘Paternal influence of sperm DNA integrity on early embryonic development’, by Simon et al. (2014) published in this issue of Human Reproduction the authors carry out the alkaline Comet assay on sperm samples from over 200 couples and find correlations with embryo development and implantation rates following IVF and ICSI. The Comet assay involves single-cell gel electrophoresis, and damaged sperm is therefore identified by the presence of a visible comet-like tail in a number of cells (in this case 50–100 sperm). In this particular study the authors divided the samples into three groups: ‘low damage’ (<30%), ‘intermediate damage’ (31–70%) and ‘high damage’ (>71%). While it has the advantage of not requiring a flow cytometer (such as SCSA) quantification is not absolute or necessarily unbiased.

To eliminate variability in sperm penetration ability and fertilization timing, only ICSI cases were included in an analysis of pre-fertilization paternal effects of DNA damage. Interestingly the results suggest that female factor (and not sperm DNA) is responsible for the differences found, something that has been described previously (e.g. Tavares et al., 2013). Although specific details varied (notably between the different sperm damage groups established) and clearly more cases were needed for further validation, sperm DNA integrity seemed to influence early embryo development, even when the paternal genome is considered to be inactive, which is an interesting and somewhat unexpected observation. This effect was however more pronounced in terms of later embryo development, implantation rates and pregnancy outcomes, although, as expected, other factors (such as infertility type or female age) were also extremely relevant, underscoring the need for a multifactorial analysis. When available, differences between IVF and ICSI assessed following fertilization were somewhat small in this study.

It is especially noteworthy that in this particular set of experiments native semen was tested, not the prepared samples normally used for Assisted Reproduction (typically involving a combination of differential gradient centrifugation and/or swim-up). While the former approach analyses direct ejaculate quality, the latter actually monitors the sperm cells that are functionally relevant, and differences may arise depending on the strategy employed (Tavares et al., 2013), as the authors themselves note.

In essence, this is another study suggesting that some form of paternal influence at the sperm DNA level is important in early events in human reproduction, and therefore extremely relevant in the context of Assisted Reproduction. However, while reading the many studies in the field furthers the call for some form of sperm DNA evaluation, it is also clear that more definite and consequent institutional efforts need to be made. Specifically, the discussion should focus on promoting a frank and unbiased assessment of the several approaches and methods used. Hopefully these efforts may then lead to consensual guidelines, which can help establish a degree of robustness that still seems to be lacking.

References


Zini A, Boman JM, Belzile E, Ciampi A. Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis. Hum Reprod 2008;23:2663–2668.