that it can be induced by six main mechanisms: (i) apoptosis in sperm can affect both mitochondrial and nuclear DNA and test in ART. In that article, I pointed out that DNA damage in women with upper cervical occlusion is intriguing, but it does appear to be a genuine phenomenon since the same observation has been made by others. Our contribution was to measure endometrial thickness using ultrasound, not assess the ultrasound-determined extent of adhesions, in a carefully assessed group of Asherman’s syndrome patients for the first time.

We agree that the ultrasound methodology for assessment of the extent of intrauterine adhesions may not have been ideal by modern standards, but our transvaginal ultrasound data have been collected over nearly two decades, albeit using the same 2D technique. Like Drs Knopman and Copperman, we now use 3D ultrasound for this assessment. Nevertheless, sonographic imaging was not our sole criterion for the classification of patients into different groupings, including the group in which we were mainly interested, the women who solely had uterine outlet occlusion at the level of the internal os (without any intra-cavitary adhesions). These gradings were determined by careful diagnostic hysteroscopy in all cases (as noted in our publication). Hence, we can refute the statement by Drs Knopman and Copperman that ‘one must wonder if the authors under-diagnosed the extent of disease as a result of their limited screening modality (2D TVUS), thereby affecting the validity of their results’.

Ian S. Fraser¹

Faculty of Medicine, College of Health Sciences, Queen Elizabeth II Research Institute for Mothers and Infants, University of Sydney, DO2, NSW 2006, Australia

¹Correspondence address. Tel: +61-2-9351-2478; Fax: +61-2-9351-4560; E-mail: helena@obsgyn.usyd.edu.au

doi:10.1093/humrep/den074
Advance Access publication on March 12, 2008

Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive of the outcome of ART

Sir,

We read with great interest the article recently published by Bungum et al., in Human Reproduction (Bungum et al., 2008) entitled, ‘Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive of the outcome of ART’.

Two years ago I, Alvarez, published a letter to the editor in this journal commenting on the predictive value of the SCSA test in ART. In that article, I pointed out that DNA damage in sperm can affect both mitochondrial and nuclear DNA and that it can be induced by six main mechanisms: (i) apoptosis during the process of spermatogenesis; (ii) DNA strand breaks produced during the remodelling of sperm chromatin during the process of spermiogenesis; (iii) post-testicular DNA fragmentation induced mainly by oxygen radicals, including the hydroxyl radical and nitric oxide, during sperm transport through the seminiferous tubules and epididymis; (iv) DNA fragmentation induced by endogenous endonucleases; (v) DNA damage induced by radio and chemotherapy and (vi) damage induced by environmental toxicants. Of these six mechanisms, the one that appears to play a major role in causing sperm DNA fragmentation is post-testicular damage during sperm transport through the epididymis. This is supported by previous reports that demonstrate that DNA fragmentation is higher in epididymal and ejaculated (Ollero et al., 2001; Greco et al., 2005) compared with testicular spermatozoa. More recent reports have confirmed this hypothesis (Suganuma et al., 2005).

In that letter, I also pointed out that, to a first approximation, two types of DNA fragmentation tests can be considered: (i) tests that measure ‘real’ DNA damage, such as TUNEL, ISNT or COMET under neutral pH conditions (n-COMET); and (ii) tests that measure ‘potential’ DNA damage and susceptibility to DNA denaturation, such as the SCSA, DBD-FISH, SCD, Chromomycin A3 or COMET under denaturing conditions. Tests that measure real DNA damage should have a higher predictive value than tests that measure potential DNA damage.

The main question that arises from the report by Bungum et al. is why DNA fragmentation levels in the pellet of the gradient, as measured by the SCSA test, are not predictive of pregnancy outcome, if these are the actual sperm used in ART? One explanation could be that the actual DNA damage that interferes with embryo implantation and/or the development of a viable pregnancy is related to a DNA property that the SCSA test does not measure. As pointed out above, the SCSA test measures ‘susceptibility’ to DNA denaturation. But, in fact, even DNA fragmentation values in neat semen are not predictive of pregnancy outcome after IVF or ICSI according to the present report by Bungum et al. In contrast, the results reported by Borini et al. (2006) and by Duran et al. (2002), cited by Bungum et al. in the present article under discussion, provide strong evidence for the predictive value of DNA fragmentation values in ART in sperm from the gradient pellet, as measured by the TUNEL test. This is even more significant in the report by Duran et al., where the predictive value of TUNEL was applied to IUI cycles, where a limited number of oocytes are available compared with IVF. That is, while in IVF the probability that a mature oocyte be fertilized by a spermatozoon with intact DNA or that a spermatozoon with damaged DNA fertilize an oocyte with a high DNA repair capacity is relatively high, given the high number of oocytes usually obtained after oocyte retrieval, this probability is much lower in IUI where usually 1 to 2 oocytes are available. But, why may TUNEL test values in the gradient pellet be predictive of pregnancy outcome and not SCSA’s? One of the main modes of post-testicular sperm DNA damage is most likely that induced by oxidative stress via the hydroxyl radical resulting in the formation of 8-OH-guanine and 8-OH-2’-deoxyguanosine (8-OHdG) in a first stage followed by double-stranded DNA
fragmentation thereafter (Cui et al., 2000). While DNA damage of the first type could be repaired to some extent by the oocyte and/or the embryo, double-stranded DNA damage is virtually irreversible and incompatible with the development of a viable pregnancy. The TUNEL and n-COMET tests measure single- and/or double-stranded DNA fragmentation and, therefore, should provide more meaningful biological information concerning embryo’s implantation potential than the SCSA test. In addition, DNA fragmentation values, as measured by COMET (Agbaje et al., 2008) and TUNEL are associated to DNA damage of the 8-OH-guanine and 8-OHdG type.

In fact, as predicted by the study of Ollero et al. (2001), the levels of 8-OH-guanine and 8-OHdG in sperm DNA should correlate with the levels of oxidative damage in sperm from the gradient pellet, since ROS-producing immature sperm, isolated from the lower density layers, could induce oxidative damage of the mature sperm, isolated from the gradient pellet, during co-migration through the epididymis. On the other hand, Drevet (2006) has recently postulated that the risk of post-testicular oxidative damage in the epididymis increases from the caput to the cauda and that this damage may be related, at least in part, to glutathione peroxidase content in the lumen and epithelial cells of the epididymis and in spermatozoa, and to the oxygen radical recycling equilibrium beyond the caput epididymis related to sperm protamine and flagellar protein disulfide crosslinking, which also increases from the caput to the cauda epididymis. Those sperm with a lower degree of disulfide crosslinking would be more vulnerable to sperm DNA damage.

In conclusion, additional studies are required to evaluate the predictive value of sperm DNA damage levels in the gradient pellet in ART, but using tests that measure real DNA damage, such as TUNEL by flow cytometry. In addition, to give a more complete picture, we should also include measures of strand break precursor damage, e.g. 8-OHdG levels in these sperm. This is especially important in disease conditions, such as diabetes, where oxidative stress is overtly manifested and where this type of oxidative DNA damage may lead to double-stranded DNA fragmentation (Agbaje et al., 2008).

References


Juan G. Alvarez1,2,4 and Sheena Lewis3

1Institute Marques, Barcelona, Spain,

2Harvard Medical School, Boston, MA, USA and

3Queen’s University, Belfast, UK

4Correspondence address.

E-mail: juan.alvarez@institutomarques.com

doi:10.1093/humrep/den053

Advance Access publication on March 5, 2008

Reply: Sperm chromatin structure assay parameters measured after density gradient centrifugation are not predictive of the outcome of ART

Sir,

We thank Drs Alvarez and Lewis (2008) for their interest in our paper focusing on the predictive value of the Sperm Chromatin Structure Assay (SCSA) on the outcome of ART (Bungum et al., 2008). Their interesting and adequate thoughts contribute to the ongoing scientific debate on the precise nature of human sperm DNA damage and may help in understanding the discrepancies among the variety of tests available to measure sperm DNA strand breaks. Their speculation may also add to clarify why SCSA performed on gradient centrifuged semen was not predictive for the ART outcome. We also agree with them on the requirement of ‘additional studies to evaluate the predictive value of sperm damage levels’ within the framework of more standardized interlaboratory trials.

Reference


Mona Bungum1,3, Aleksander Giwercman1 and Marcello Spano2

1Centre of Reproductive Medicine, Malmö University Hospital, Malmö, Sweden and

2Malmo¨ University Hospital, Malmo¨, Sweden and

3Queen’s University, Belfast, UK