


Submitted on January 10, 2005; accepted on January 24, 2005

Ruben Alvero and James Segars

1University of Colorado Health Sciences Center, Aurora, Colorado, USA and 2Unit on Reproductive Endocrinology, Reproductive Biology and Medicine Branch, National Institute of Child Health and Human Development, NIH, DHHS, Bethesda, MD 20892, USA

E-mail: ruben.alvero@uchsc.edu
doi:10.1093/humrep/de813

Letter to the Editor

Sir,

I have read with great interest the article by Greco et al., entitled ‘Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular sperm’, published in the January issue of Human Reproduction.

The study shows that DNA fragmentation levels (assessed by the TUNEL test) in testicular sperm from normozoospermic and oligozoospermic males were significantly lower than those found in ejaculated sperm from these same males (4.8 ± 3.6 versus 23.6 ± 5.1%, P < 0.001). More importantly, pregnancy rates in ICSI cycles using ejaculated sperm from males with DNA fragmentation levels in semen >15% were significantly lower than those obtained using testicular sperm (5.6% versus 44.4%, P < 0.05). With the exception of one sample, all testicular sperm samples had TUNEL test values ≤6% (range 1–6%). In sharp contrast, all ejaculated sperm samples had TUNEL test values >15% (range 15–37%). This suggests that DNA fragmentation is, for the most part, a post-testicular event.

Previous studies have postulated that one of the mechanisms involved in sperm DNA fragmentation is ROS-induced DNA damage during co-migration of mature sperm with ROS-producing immature sperm through the seminiferous tubules and epididymis (Ollero et al., 2001; Alvarez, 2003; Alvarez et al., 2004). In the study reported by Ollero et al. (2001), a strong correlation was found between reactive oxygen species (ROS) production by immature sperm and DNA fragmentation in mature sperm from the same semen samples. This is consistent with the observation that centrifugation of semen samples containing high levels of ROS-producing immature sperm results in significant DNA damage of mature sperm (Twigg et al., 1998). This is also consistent with the fact that in vitro exposure of mature sperm to high levels of ROS resulted in significant DNA damage (Aitken et al., 1998; Lopes et al., 1998). Steele et al. (1999) have reported that DNA fragmentation levels (as determined by the COMET assay) are significantly higher in epididymal compared to testicular sperm from patients with obstructive azoospermia.

It is well known that testicular sperm are more vulnerable to DNA damage due to the fact that the protamines of sperm chromatin are not fully cross-linked by disulphide bonds. Completion of disulphide cross-linking takes place in the epididymis (Bedford et al., 1973). Therefore, exposure of mature testicular sperm to ROS, produced either by immature sperm or by the epithelial cells lining the epididymis, could result in damage of these sperm before disulphide cross-linking takes place. This is consistent with the recent report by Dalzell et al. (2004) who found that testicular sperm are more sensitive to DNA fragmentation following aerobic incubation.

All this evidence strongly suggests that DNA fragmentation could occur during sperm transport from the seminiferous tubules to the epididymis.

Although the number of patients included in the study is relatively small and the results should be independently confirmed in other studies, the data reported by Greco et al. would suggest that DNA fragmentation testing could be of great value in couples with failed pregnancy in two or more IVF cycles. Those couples with DNA fragmentation levels in semen >15% could benefit from ICSI using testicular sperm, and therefore should be offered the option of performing testicular sperm extraction or testicular sperm aspiration. This would apply to normozoospermic males, as well as to males with oligozoospermia. However, since DNA fragmentation levels in semen have been shown to vary with the spermatogenic cycles (Alvarez et al., 2004), DNA fragmentation testing should first be performed in several semen samples obtained from at least two consecutive spermatogenic cycles before recommending testicular biopsy. If all samples have DNA fragmentation values >15%, testicular biopsy would be indicated. If one or more of the samples evaluated have values ≤6%, then they should be cryopreserved for use in future IVF cycles.

The authors’ suggestion of developing new selection techniques to enrich the sample in ‘healthy sperm’ with low or absent DNA damage is attractive but inconsistent with their hypothesis of the ‘tip of the iceberg effect’ in samples with DNA fragmentation levels >15%.

Finally, since post-testicular sperm DNA fragmentation appears to be related to ROS-induced damage during sperm transport through the seminiferous tubules and epididymis, patients with high DNA fragmentation levels could also benefit from the use of oxygen radical scavengers and antioxidants.
as the authors suggested. Preliminary data from our laboratory indicate that administration of diclofenac significantly reduces DNA fragmentation in males with high DNA fragmentation values (Alvarez et al., unpublished data). Diclofenac, in addition to being an anti-inflammatory agent, is also a known scavenger of the hydroxyl radical (Aruoma and Halliwell, 1988) and readily crosses the blood–testis barrier.

References


Submitted on December 31, 2004; accepted on January 25, 2005

JUAN G. ALVAREZ

Centro de Infertilidad Masculina ANDROGEN, La Coruña, Spain and Harvard Medical School, Boston, Massachusetts, USA

E-mail: jalvarez@androgen.es

doi:10.1093/humrep/deh814

Reply: ‘Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa’

Sir,

We have read with interest the letter by Alvarez concerning our recent paper about the use of ICSI with testicular sperm in the treatment of infertility due to sperm DNA damage (Greco et al., 2005a). This letter contains stimulating ideas for future research into the mechanism, diagnosis and treatment of this pathological condition.

Sperm nuclear DNA fragmentation is known to reduce implantation and pregnancy rates after ICSI, but the resulting embryo handicap remains latent during the first 3 days after ICSI (Tesarik, 2005). Our observation that testicular sperm show a lower incidence of DNA fragmentation and a higher developmental potential than ejaculated sperm in men with elevated sperm nuclear DNA damage supports the hypothesis that the DNA damage mainly occurs after sperm release from the seminiferous tubules. Alvarez cites several other studies supporting this hypothesis and puts forward the idea that incomplete disulphide cross-linking of the protamines of sperm chromatin, a process which is normally completed during sperm passage through the epididymis, is responsible for this phenomenon. This is a plausible explanation, but it has to be underscored that other possible pathophysiological mechanisms may also come into play. These mechanisms are essentially related to functional disorders of Sertoli cells. In a previous work, in which the relationship between caspase activity and germ cell DNA fragmentation was analysed, we showed that those DNA-fragmented germ cells which remain firmly associated with Sertoli cells also display caspase activity, and their plasma membrane externalizes phosphatidyl serine (Tesarik et al., 2004). The Sertoli-associated germ cells thus appear to undergo a classical apoptotic pathway. The externalized phosphatidyl serine is known to be a surface marker of apoptotic cells to be recognized as targets for phagocytosis. In fact, fragmented DNA was detected in the cytoplasm of Sertoli cells clearly outside their own nuclei, indicating that germ cells with fragmented DNA are recognized and actively removed by Sertoli cells (Tesarik et al., 2004). In contrast, DNA-fragmented germ cells that are detached from Sertoli cells do not display caspase activity and do not externalize phosphatidyl serine (Tesarik et al., 2004). It is thus tempting to speculate that the premature detachment of germ cells makes them particularly susceptible to oxidative DNA damage which may begin during their passage through the seminiferous tubules. Because most of the Sertoli-associated germ cells undergoing the classical apoptotic pathway are probably not released from the testis, the DNA damage detected in ejaculated sperm can be supposed to result mostly from non-apoptotic oxidative DNA damage occurring in prematurely released late spermatids.

The recourse to testicular biopsy to recover sperm for ICSI makes it possible to shorten the Sertoli cell-unprotected time-period during which maturing spermatids and sperm are particularly vulnerable to DNA-damaging agents. However, the need for testicular biopsy makes this therapeutic approach more invasive and expensive than ICSI with ejaculated sperm. A search for more conservative treatment alternatives, aimed at the limitation of the extent of DNA damage in ejaculated sperm, is thus warranted. Alvarez has suggested an approach based on the use of diclofenac, a prostaglandin synthesis inhibitor and hydroxyl radical scavenger. Interestingly, earlier work has demonstrated a significant activation of dog spermatogenesis by subcutaneous application of diclofenac for 42 days (Moskovitz et al., 1987). We look forward to seeing the publication of the new data on the application of this...