the embryos, and the blood (from the cervix) contamination of the embryo transfer catheter in such cases is only a marker of difficult or traumatic embryo transfers.

References
Submitted on November 10, 2004; accepted on January 24, 2005

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doi:10.1093/humrep/deh812

Reply: ‘The presence of blood in the transfer catheter negatively influences outcome at embryo transfer’

Sir,

Visser et al. (1993) commented that embryo transfer was essentially a blind procedure and that indirect observations needed to be made in order to infer causes of failed implantation arising at the time of replacement. Marikinti and colleagues are to be congratulated for providing insight into events that have been previously mysterious, obscured by our inability to visualize post-transfer endocervical and endometrial conditions. By categorizing post-transfer traumatic lesions of the endometrium such as grooving, sub-endometrial haemorrhage and vascular congestion, they have advanced understanding in this critical area of assisted reproduction.

We also concur that the mechanism by which transfer successfully enhances implantation remains to be elucidated. For years, the conventional wisdom has been thatatraumatically delivering the embryos to the cavity enhances the efficiency of implantation (Schoolcraft et al., 2001), yet surrogates for such atraumatic technique have variably reflected pregnancy outcome. Tur-Kaspa et al. (1998) found that difficulty or repeated attempts had no impact on outcome, whereas Spandorfer et al. (2003) discovered the opposite. The flaw in all of these studies, including our own, lies in large part with their retrospective nature and the fact that these correlates may at best each be weakly associated with the true essence of a failed transfer.

Mechanistically, the presence of blood in the cavity or the catheter, possibly arising from injury to the endocervix, remains plausible in predicting poor reproductive outcome. Blood in the catheter may prevent the embryos from escaping or cause them to adhere to the device, thereby drawing them out into the cervix or vagina. The presence of blood in the cavity may reflect endometrial inflammation or other factors that would adversely affect the embryo. In the current correspondence, Marikinti and associates suggest that blood, where it exists, is endocervical. Although not explicitly stated, the bleeding is not implied to be drawn into the uterine cavity. We would welcome clarification on this point.

Supporting this theory, the presence of certain abnormalities, such as submucous fibroids, has shown a strong association with failure in implantation and pregnancy at assisted reproduction (Pritts, 2001). Fibroids, of course, may be associated with endometrial insufficiency and shedding, leading to the presence of blood in the uterine cavity, and this may be one of the mechanisms by which they adversely affect pregnancy outcome. In support of this possibility, we have recently noted the presence of blood after ‘atraumatic’ embryo transfers in several instances where uterine fibroids were present. This is an area in need of further research.

Following the publication of our previous work, we sought to reduce the presence of blood on the catheter and its potential contamination of the endometrial cavity using an ‘afterload’ technique. An initial empty catheter is used to enter the endometrium and the outer sheath is then left in place, traversing the endocervix, and therefore minimizing any effect of cervical trauma or blood. A second, embryo-loaded catheter is then placed directly into the uterine cavity. This requires two catheters but we have noted that pregnancy rates were improved (Neithardt et al., 2005).

Finally, we view ultrasound-guided transfer as the final in a series of optimization steps which also include the saline ultrasound and the trial transfer in an effort to reduce the adventitious nature of the embryo transfer process. Ultrasound helps most importantly to confirm that the catheter is in the cavity. Furthermore, it helps in ‘driving’ the catheter in those significant numbers of cases where the endocervix is able to be visualized. Indeed, it has been demonstrated that the use of ultrasound at transfer is likely to reduce the number of difficult transfers (Mirkin et al., 2003). We therefore view it as essential and worth the marginal additional use of resources.

We would again like to congratulate Marikinti and colleagues for excellent work and look forward to hearing more from them in the future.

References


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

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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 spermatozoic 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.