embryo replacement in conventional IVF/embryo transfer, rather than in patients with repeated failures. The statistical critique given by Dr Walters was mistakenly based on the 'small size' of our study. The determination of 'small' or 'large' is subjective and depends on the definition chosen. In clinical studies, an investigation comprising a group of 72 patients (as in our study) would not be considered 'small'.

The issue of which statistical method should be used for analysis is still unresolved. Nevertheless, we do not intend discussing this point, which seems to be less relevant to our paper. Our data clearly show that the transfer of more than five embryos is most useful in patients with chronic failures. Since the publication of our paper, our data have continued to accumulate, and the results confirm the published material.

In conclusion, we firmly stand by our recommendation that in cases of repeated failure, transferring more than the traditional four or five embryos should be performed. After all, it should be remembered that the achievement of pregnancy, even multiple, is the main desire of our patients and our goal as clinicians.

References

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Does high voltage electricity have an effect on the sex distribution of offspring?

For thousands of years the male/female ratio has been almost equal but ever now and then this ratio is altered for one reason or another. The ratio of boys to girls can be decreased by many influence which impairs the viability of the spermatozoa bearing Y chromosomes (Robertson and Sheard, 1973). The possibility that the anti-malarial substance, mepacrine, might have such an effect has been suggested, by implicating factors which give some advantage to X-bearing spermatozoa in the movement of spermatozoa is regulated by calcium and cyclic AMP responsible for sperm movement, with this effect on spermatozoa by interfering with the calcium and cyclic AMP responsible for sperm movement, with this effect occurring to a greater degree in Y-carrying spermatozoa than in X-carrying spermatozoa.

By publishing this isolated observation, I hope that larger surveys may be carried out elsewhere to provide verification and exclude the possibility of this being a chance observation.

References
In-vitro maturation of human testicular spermatozoa

Dear Sir,

Assisted fertilization with intracytoplasmic sperm injection (ICSI) has been successfully used in treating male factor infertility (Palermo et al., 1992; Van Steirteghem et al., 1993; Tsirigotis et al., 1994). One important indication has been for the management of azoospermia due to congenital or acquired causes where ICSI is now increasingly practised using surgically-retrieved spermatozoa (Silber et al., 1994; Craft et al., 1995). However, in patients with mainly secretory azoospermia in whom microsurgical (Silber et al., 1994) or percutaneous (Craft et al., 1995) epididymal sperm aspiration failed to yield sperm, spermatozoa extracted from testicular tissue have been used with ICSI to generate embryos (Craft et al., 1993) and pregnancies (Schoysman et al., 1993; Silber et al., 1995). Nonetheless, the number of spermatozoa retrieved from testicular extraction/aspiration (Silber et al., 1994; Craft and Tsirigotis, 1995) are usually so few and the motility so weak that the only choice for completion of the ICSI cycle is individual spermatozoa manual handling (Craft and Tsirigotis, 1995). In addition, testicular spermatozoa, in view of their immaturity and very weak motility have not been routinely frozen-thawed for subsequent ICSI cycles, although successful cryopreservation has now been reported (Craft and Tsirigotis, 1995).

Since the ICSI technique is comparatively new and the long-term effects on the babies born as a result of this treatment will not be known for some time, it would seem reasonable to assess whether testicular spermatozoa could be retrieved for an in-vitro fertilization (IVF)/ICSI cycle. The third patient, who had two failed vasectomy reversals and had previously had a therapeutic cycle without conception in which spermatozoa were retrieved percutaneously. In all cases, a small piece of testicular tissue was aspirated using a 21 gauge butterfly needle (Venisystems, Abbott Ireland Ltd, Sligo, Republic of Ireland) directly into the testis through the scrotal skin under i.v. sedation with a 10 mm attached syringe to create a strong negative pressure. The needle is moved up and down at various sites within the testis to sample a wide area and an artery forcep is secured across the attached microtubing set before the needle is withdrawn. The aspirate located within the needle or proximal tubing of the microaspiration set is then washed through with a small volume of culture medium into a Falcon tube (Becton Dickinson Ltd., Plymouth, UK) which is then kept at 37°C in a transport incubator (Henning Knudsen, Copenhagen, Denmark). It has also been our experience by processing testicular fluid and the tissue (microtubules) obtained by testicular aspiration, that only few spermatozoa could be retrieved to complete the ICSI cycle. In those cases, direct sperm aspiration (Craft and Tsirigotis, 1995) had to be practised for enough spermatozoa to be recovered. This is the first report of in-vitro fertilization of human testicular spermatozoa although in-vitro maturation of epididymal spermatozoa has been described before (Moore et al., 1992).

We therefore modified the process of testicular tissue handling and the length of incubation period in an attempt to develop a method whereby free, clean spermatozoa could be retrieved. In fact, the testicular tissue was chopped into small pieces with sterilized scissors and forced through a 25 gauge needle. The homogenized tissue was washed twice with IVF culture medium (Medicult a/s, Copenhagen, Denmark) and the tissue suspensions were then cultured in a sterilized Petri dish (Falcon, New Jersey, USA), covered with liquid paraffin oil (Medicult). At that time, under microscopic assessment, it was noted that the testicular spermatozoa were combined with Sertoli cells or embedded in the homogenized tissue. To our surprise, some spermatozoa from all patients showed progressive motility (2–3/4) on day 3 after incubation, which was sufficient for ICSI. These motile spermatozoa were free from Sertoli cells and showed normal morphology under the conventional microscope. It is of significance that, in all patients, spermatozoa became free and motile and showed morphological maturation despite the daily microscopic assessment after 3 days of incubation. The results of performing ICSI on unfertilized aged (24 h old) human oocytes with testicular spermatozoa matured in vitro have been very promising with up to 70% fertilization rates (unpublished data). However, further work is needed before the technique can be applied to fresh oocytes.

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