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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 spermatozoon pick-up (direct sperm aspiration) (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 in patients where testicular spermatozoa are used to allow in-vitro maturation which could improve both morphology and motility, thereby reducing the possibility of fertilization failure. We now report our experience with in-vitro maturation of testicular spermatozoa in two patients with secretory azoospermia who underwent the operation of testicular sperm aspiration and one patient with obstructive azoopermia who underwent percutaneous epididymal (PESA) and testicular sperm aspiration (TESA).

The first two patients (aged 34 and 41 years) had a history of azoospermia with follicle-stimulating hormone (FSH) concentrations of 9.6 IU/l and 16.5 IU/l respectively (normal range 7–9 IU/l). Both underwent a diagnostic TESA cycle 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 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 microeffusion 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 pellets were suspended in 100 µl of IVF medium. 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 converting 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.

These results indicate that in-vitro maturation of testicular spermatozoa can be achieved and maturation takes ~3 days, as indicated by progressive motility. They also show considerable maturation, which is indicated by both morphology and motility compared to the testicular spermatozoa recovered on the day of the aspiration procedure, avoiding the need to inject immature forms, i.e. spermatids (Fishel *et al.*, 1995) with unknown long-term effects.

We therefore conclude that, in view of the improved morphology, motility and forward progression following in-vitro maturation of human testicular spermatozoa, extraction/aspiration should be carried out 3 days prior to the day of the oocyte retrieval improving patients' safety and fertilization and pregnancy rates.

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