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Improving the recovery and handling of spermatozoa from testicular homogenates

Dear Sir,

The collection of sperm from testicular biopsy material can now enable the treatment by intracytoplasmic sperm injection (ICSI) of couples in which the male partner has azoospermia. It was therefore timely to see the articles by Verheyen *et al.* (1995) and Zhu *et al.* (1996) addressing ways of obtaining the best sperm preparations from testicular tissue.

Verheyen *et al.* (1995) used a variety of dissection methods to disaggregate the testicular tissue and release the spermatozoa, yielding several thousand spermatozoa on average. The subsequent use of Percoll to clean up the preparation resulted in the loss of up to 95% of spermatozoa. However, we have used an alternative method of sperm preparation for cases with even fewer spermatozoa available and achieved fertilization and a pregnancy. A 48 year old man presented with azoospermia, and the diagnosis of spermatogenetic failure was made based on a previous testicular biopsy. During a treatment cycle, biopsies from both testes were taken on the day before oocyte collection. The material was placed into HEPES-buffered T_6 medium supplemented with 10% serum. Each piece of tissue was dissected and cut using sterile scissors and a surgical blade, and the large pieces of tissue then further homogenized by crushing between the frosted ends of two sterile glass slides. The homogenate was then centrifuged at 1800 *g* for 5 min, washed and resuspended in 0.1 ml medium and placed in small droplets under paraffin oil at 37°C for 1–2 h. In the meantime, a thin glass capillary was pulled on a micropipette puller (Campden Instruments Ltd., Loughborough, UK); one half was placed on the microforge and the end broken to give an inner diameter of 10–12 μm and external diameter of 15 μm, with the other half being heated on the microforge to form a small glass bead and a 45° angle made on this one alone. Both glass tools were placed on a Narishige micromanipulator, with the pipette being set at 45° to the horizontal and the beaded pipette set so as to be parallel to the horizontal. These were then lowered into the droplets of testicular homogenate and spermatozoa picked up and transferred to a clean droplet; after scanning the dishes for 5–6 h on the day of biopsy and again for 1–2 h the following day, only nine spermatozoa were found. The majority appeared abnormal, having a cytoplasmic droplet or stumped tail, and exhibited poor shimmering motility. Although 12 mature oocytes were collected from the wife, only nine could be injected, each with a single spermatozoon immobilized by stroking the tail. After overnight culture, six oocytes showed two pronuclei and three 4-cell embryos were subsequently transferred the following day. Pregnancy was diagnosed with a serum human chorionic gonadotrophin (HCG) test 2 weeks later and an ultrasound scan at 7 weeks confirmed a singleton pregnancy which is currently ongoing at 30 weeks.

Zhu *et al.* (1996) described the use of extended culture for testicular spermatozoa to improve motility. We have also found this approach useful. However, we have noted that this method is unsuitable for frozen–thawed testicular spermatozoa. Of the two cases undertaken, both have shown a 50% reduction in motility after overnight culture and a virtual total loss of motility after 3 days. We are now planning the recovery of spermatozoa from fresh biopsy tissue in the treatment cycle a few days prior to oocyte recovery wherever possible but, in cases with frozen tissue, only thawing the material on the day of oocyte collection to avoid deterioration.

In summary, the ICSI technique has made it possible to achieve fertilization and thus pregnancy with testicular spermatozoa. However, it is important to apply the most appropriate techniques to recover spermatozoa from various testicular homogenates. Using the technique described above we were able to isolate a total of nine spermatozoa, inject them successfully into oocytes, achieve fertilization and an ongoing pregnancy for a couple with infertility due to severe spermatogenic failure.

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Internal jugular vein thrombosis: a late complication of ovarian hyperstimulation syndrome

Dear Sir,

We read with great interest the article by Hignett *et al.* (1996). In this study the authors reported a case of severe ovarian hyperstimulation syndrome (OHSS), presenting in a second in-vitro fertilization (IVF) cycle with a late complication of