

CASE REPORT

Changes in motility patterns during in-vitro culture of fresh and frozen/thawed testicular and epididymal spermatozoa: implications for planning treatment by intracytoplasmic sperm injection

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The present report describes the motility changes *in vitro* (percentage motile and progressively motile) of freshly collected testicular and epididymal spermatozoa and following freeze/thaw of the same spermatozoa from a man with obstructive azoospermia. Washed spermatozoa were cultured in micro droplets under paraffin oil or in test tubes using HEPES-buffered or bicarbonate-buffered medium containing 10% human serum. In fresh testicular sperm cultures 60–65% of the sperm cells became motile within 2 days of culture; the motility was maintained for a further 4–5 days before a decline was observed. The progressive motility improved markedly on the third day of culture and it peaked around day 5. Only a small number of frozen/thawed testicular spermatozoa became motile during in-vitro culture (15–20%) and the motility was maintained for only 2–3 days before it declined. Furthermore, only 10–12% of the spermatozoa showed progressive motility. Spermatozoa recovered from micro-epididymal sperm aspiration (MESA) showed a gradual decrease in progressive motility and in 5 days all sperm cells were found to be immotile in both freshly collected and frozen/thawed spermatozoa. All culture systems supported sperm motility. It is clear that testicular spermatozoa, particularly from men with obstructive azoospermia, can be collected and maintained *in vitro* for up to 1 week before the oocyte retrieval but when frozen testicular or epididymal spermatozoa are used it is more reliable to thaw these spermatozoa on the day of intracytoplasmic sperm injection.

Key words: epididymal spermatozoa/ICSI/*in vitro*/motility/testicular spermatozoa

Introduction

For azoospermia, extraction of spermatozoa from either the testis or the epididymis and its use in intracytoplasmic sperm injection (ICSI) to achieve fertilization and pregnancy has become one of the main treatment options (Schoysman *et al.*, 1994; Silber *et al.*, 1995; Tucker *et al.*, 1995). However, the fertilization rates obtained with testicular or epididymal spermatozoa seem to be lower than with ejaculated spermatozoa

(Nagy *et al.*, 1995). In some instances with testicular spermatozoa, due to the poor motility, selection of viable spermatozoa for ICSI may be difficult and this may result in poor fertilization rates. Culture of freshly collected testicular spermatozoa *in vitro* for 3 days has been shown to improve the progressive motility (Zhu *et al.*, 1996), although a drop in motility of frozen/thawed testicular spermatozoa following a few days in culture can occur (Edirisinghe *et al.*, 1996). At present the information available on in-vitro culture of testicular spermatozoa is limited. This case report examines the in-vitro changes in sperm motility patterns of testicular and epididymal spermatozoa collected freshly and following freeze/thaw of the same batch of spermatozoa from a man who had exploratory surgery for reversal of vasectomy and sperm collection for ICSI. The results have implications for the planning of sperm collection and preparation prior to oocyte collection for in-vitro fertilization (IVF)-related procedures.

Case report

A 52 year old man and his 32 year old wife were referred for treatment. The man had had a vasectomy 21 years previously after having three children in his first marriage. The initial plan was to have the vasectomy reversed and to collect spermatozoa for possible future IVF with ICSI. During the surgery it was confirmed that the vasovasostomy was not possible but that bilateral scrotal explorations indicated good sperm production from both testes with distended epididymal loops and vasa deferentia. Micro-epididymal sperm aspiration (MESA) and testicular biopsies were carried out. The testicular biopsies were homogenized using a pair of sterile scissors and a scalpel blade and resuspended in HEPES-buffered T6 culture medium (H-medium) containing 10% human serum. The majority of epididymal and testicular spermatozoa recovered were cryopreserved for the couple's future ICSI attempts. For cryopreservation, both sperm preparations were mixed in a 1:1 ratio with the sperm cryopreservation medium containing 15% glycerol, loaded into sterile plastic straws and cooled gradually by placing straws 10 min each at 4°C and –20°C, in upper liquid N₂ vapour (30 cm above liquid N₂) and lower liquid N₂ vapour (15 cm above liquid N₂) respectively, before plunging into liquid N₂ for storage. Small aliquots of epididymal and testicular spermatozoa were washed twice in bicarbonate-buffered human tubal fluid medium (B-medium) containing 10% human serum and the final sperm pellets were divided into two fractions. Each fraction was resuspended in either H-medium or B-medium and 100 µl sperm droplets from each

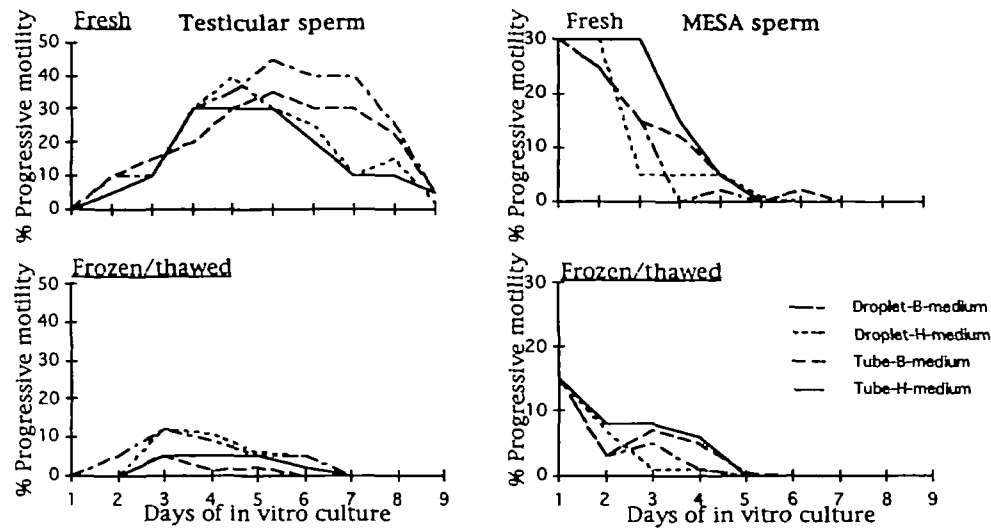


Figure 1. Changes in the progressive motility fraction of fresh and frozen/thawed testicular spermatozoa and spermatozoa collected from micro-epididymal sperm aspiration (MESA) during in-vitro culture under various conditions.

tube were placed under sterile paraffin oil (BDH, Poole, Dorset, UK) in a Petri dish. The dishes were incubated in a 5% CO₂ incubator at 37°C. The remaining sperm suspensions were left in the test tubes which were either gassed with 5% CO₂ (B-medium) or capped tight (H-medium) at 37°C. The motility was assessed daily in all the sperm fractions according to World Health Organization criteria (WHO, 1992) until no motility or a significant drop in motility was observed.

During the couple's IVF-ICSI treatment, follicular growth was achieved using Lucrin (luprolide acetate, Abbott, Australia) on a flare regimen with pure follicle stimulating hormone (FSH, Metrodin; Serono) and ovulation was triggered with human chorionic gonadotrophin (HCG, Pregnyl; Serono, Australia). Eight oocytes were collected and all were found to be at metaphase II.

Frozen testicular and epididymal spermatozoa were thawed on the day of oocyte collection by placing straws (one straw of each type) for 10 min at 37°C. After two washes in B-medium containing 10% patient's serum, sperm pellets were divided and resuspended in either of the two culture media in test tubes. Epididymal and testicular sperm droplets (100 µl volume) were prepared under paraffin oil and the spermatozoa in H-medium were used for ICSI. The motility in all sperm preparations was monitored daily. Following ICSI, four out of four oocytes were fertilized with epididymal spermatozoa and three out of four with testicular spermatozoa. Two average quality embryos generated from testicular spermatozoa were transferred in to the uterus and a blood test after 2 weeks showed a β-HCG level of 221 mmol/l, indicating pregnancy.

During in-vitro culture of fresh testicular spermatozoa, 60–65% of the sperm cells became motile in 2 days of culture and this motile fraction was maintained for a further 4–5 days before a decline in motility was observed. A small proportion of frozen/thawed testicular spermatozoa showed an improvement in motility during in-vitro culture (15–20%) and the motility was maintained only for 2–3 days before the motility decreased.

The achievement of progressive motility [grade a + b,

WHO (1992) criteria] by testicular spermatozoa during in-vitro culture was studied and, as shown in Figure 1, a large proportion of the fresh testicular spermatozoa showed progressive motility on the third day of in-vitro culture and this proportion peaked around day 5 before a gradual decline was observed over the next 4–5 days. The proportion of frozen/thawed spermatozoa which achieved progressive motility was very small (10–12%) and this was estimated to be ~20 spermatozoa per droplet.

During in-vitro culture of epididymal spermatozoa, the progressive motility declined gradually (Figure 1) and after 5 days all sperm cells were found to be immotile in both freshly collected and frozen/thawed epididymal spermatozoa. All culture systems supported sperm motility *in vitro*. However, the culture droplets using B-medium were found to allow the best motility and survival of testicular spermatozoa *in vitro*.

Discussion

These results indicate that a large proportion of the fresh testicular spermatozoa become motile during in-vitro culture and that the motility is maintained for more than a week under suitable culture conditions. Cultures of testicular sperm homogenates in droplets seem to have improved sperm motility probably due to cells other than sperm cells plating out to form monolayers, which could provide co-culture effects benefiting the sperm maturation process. If gaining progressive motility is considered as an indication of sperm maturity, as shown by Zhu *et al.* (1996), a large proportion of testicular spermatozoa become mature after 3 days in culture, reaching a peak around 5 days. Once they achieve progressive motility or maturity, their duration of survival from there is ~5 days, which is similar for both testicular and epididymal spermatozoa.

The freeze/thaw procedure seems to affect the survival of testicular spermatozoa, thus reducing the number of viable spermatozoa available to show an improvement in motility *in vitro*. We reported earlier that in two cases we observed no improvement in motility but a reduction in motility of frozen/

thawed testicular spermatozoa during culture (Edirisinghe *et al.*, 1996).

For the couple in this study we achieved satisfactory fertilization rates with testicular and epididymal spermatozoa and a pregnancy. This result supports the success rates that have been achieved by other researchers using testicular and epididymal spermatozoa in the treatment of obstructive azoospermia (Schoysman *et al.*, 1994; Silber *et al.*, 1995; Tucker *et al.*, 1995).

The findings from this case report need to be confirmed in a larger patient group in order to assess the differences in *in vitro* motility patterns of testicular spermatozoa recovered from a range of azoospermic men; this study is now underway. Nevertheless, it is useful to know, at least for patients with obstructive azoospermia, that spermatozoa can be collected at a time convenient to all parties concerned and maintained *in vitro* for up to 1 week before the oocyte retrieval. Furthermore, when frozen testicular and epididymal spermatozoa are used, it is more reliable to thaw these spermatozoa on the day of ICSI.

In addition to the motility studies, it is important to assess the fertilizing potential of testicular spermatozoa matured *in vitro*. Thus a randomized study to perform ICSI at different days following the initial testicular sperm culture is required in order to address this issue.

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