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In-vitro culture of spermatozoa induces motility and increases implantation and pregnancy rates after testicular sperm extraction and intracytoplasmic sperm injection

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The aim of this study was to determine the effect of 24-h in-vitro culture of testicular spermatozoa in recombinant follicle stimulating hormone (recFSH) supplemented medium versus simple medium on sperm motility, and to analyse the outcome of intracytoplasmic sperm injection (ICSI) of such spermatozoa. A total of 143 positive testicular sperm extraction procedures in men with non-obstructive azoospermia was evaluated prospectively. Extracted testicular tissue samples were randomized to be cultured *in vitro* for 24 h in simple medium or recFSH supplemented media. ICSI was performed with spermatozoa cultured in recFSH (n = 73) or in simple medium (n = 70). Sperm motility following in-vitro culture, embryo quality after ICSI, and implantation and pregnancy rates were assessed. Of the 898 MII oocytes available in the recFSH group, 646 (71.9%) were injected with spermatozoa showing either twitching or progressive motility. However, only 29.1% of the oocytes in the simple medium group (245/841) were injected with motile spermatozoa (P< 0.05). Fertilization rate (68.8 versus 42.1%), implantation rate per embryo (20.1 versus 13.2%), and clinical pregnancy rate (47.9 versus 30%) were significantly increased in the recFSH group compared with the simple medium group respectively (P < 0.05). In conclusion, in-vitro culture with recFSH appears to increase the motility of testicular spermatozoa, thus increasing the success of ICSI.

Key words: azoospermia/in-vitro culture/motility/testicular sperm extraction

Introduction

The use of testicular spermatozoa for intracytoplasmic sperm injection (ICSI) has become an established treatment for the indication of obstructive or non-obstructive azoospermia (Silber *et al.*, 1995). Testicular spermatozoa are often immotile or only show twitching movements upon extraction (Zhu *et al.*, 1996; Liu *et al.*, 1996). They are presumed to be viable although lower fertilization rates (17–46%) obtained with testicular spermatozoa imply the possible utilization of some non-viable spermatozoa for ICSI (Schoysman *et al.*, 1993; Nagy *et al.*, 1995a; Silber *et al.*, 1995). Injection of motile and thus viable testicular spermatozoa may be theoretically more beneficial regarding fertilization rates. To this end, testicular spermatozoa have been incubated in defined simple culture media which induced progressive motility (Craft *et al.*, 1995; Zhu *et al.*, 1997). Higher fertilization rates obtained with the injection of motile spermatozoa will produce more embryos and afford better embryo selection for transfer. It is currently unknown whether embryo quality will also be favourably affected from such a practice.

Testicular sperm extraction (TESE) procedures may be performed 24–48 h prior to human chorionic gonadotrophin (HCG), thus 3–4 days before oocyte retrieval, and the extracted spermatozoa kept in culture until ICSI. The success of this technique shows the feasibility and safety of culturing testicular spermatozoa. The practice of early sperm retrieval is associated with better patient scheduling in the busy IVF clinic and is appreciated by the operating room personnel and the embryologists (Urman *et al.*, 1998). Testicular spermatozoa may also be obtained at a remote time before a possible oocyte retrieval and frozen for later use. The feasibility of this technique, however, is not well defined, especially in men with very limited numbers of spermatozoa (Nagy *et al.*, 1995b).

Incubation of testicular spermatozoa is normally performed in commercially available defined culture media. Motility of the extracted spermatozoa appears to be increased after in-vitro culture, although it is not known whether implantation and pregnancy rates are affected from such a practice (Nijs *et al.*, 1997). The improvement in motility, however, facilitates the selection of viable spermatozoa for ICSI. To further improve the favourable results obtained with in-vitro culture of testicular spermatozoa, supplementation of culture media with motility-inducing agents such as pentoxifylline has been investigated, but as yet without corroboration of results (Tasdemir *et al.*, 1998).

In this study we examined the effect of supplementing the culture medium with recombinant follicle stimulating hormone (recFSH) on motility of testicular spermatozoa from patients with non-obstructive azoospermia, and compared the results of ICSI with spermatozoa cultured in recFSH medium and in simple medium. FSH is believed to participate in the complex process of spermatogenesis and spermiogenesis (Tesarik *et al.*, 1998). Deficiency of FSH in primates has been associated with oligozoospermia and teratozoospermia. However, whether FSH has any role at all in the process of tail formation and the acquisition of motility is currently unknown.

Materials and methods

All couples with infertility due to non-obstructive azoospermia were eligible for the study. The study, conducted in 1997, included data