SHORT COMMUNICATION

LONDON, ENGLAND

In Vitro Maturation of Human Testicular Sperm in Patients with Azoospermia

Submitted: February 6, 1997

Accepted: March 19, 1997

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) has revolutionized the management of male-factor infertility and made it possible for an increasing number of men to become genetic parents. Surgical sperm retrieval, through microepididymal sperm aspiration (MESA), for in vitro fertilization or gamete intra-fallopian transfer, predated the clinical application of ICSI (1) but has since become popular because ICSI makes more efficient use of available sperm than other insemination techniques. The development of percutaneous epididymal sperm aspiration (PESA) and testicular sperm aspiration (TESA) has greatly simplified surgical sperm retrieval (2–5). Several pregnancies have been established through ICSI with epididymal sperm obtained from men with irreparable obstructive azoospermia. Epididymal sperm retrieval in such cases is invariably successful but testicular sperm can also be used for ICSI when there is failure to recover sperm through PESA and/or MESA or if only nonviable sperm are recovered. Surprisingly, sperm can be recovered from the testes in some patients who are clinically diagnosed as having nonobstructive azoospermia. Although fertilization is possible after intracytoplasmic injection of testicular sperm, and births have indeed been reported (6,7), the sperm are physiologically immature and many are immotile or exhibit only feeble shaking movements. Furthermore, testicular sperm tend to remain embedded or trapped within the surgical specimen, and all these become important considerations

in TESA because of the small volume of testicular tissue available for processing. We recently developed a method for maturing human testicular sperm in vitro (8,9) and wish to report our continuing experience with this technique.

MATERIALS AND METHODS

Twenty-nine patients with azoospermia (9 nonobstructive and 20 obstructive) underwent diagnostic PESA and TESA between July and December 1995. Testicular tissue was obtained by the TESA technique (fine-needle aspiration biopsy) as described previously (4,5). Briefly, a size 19G butterfly needle (Venisystems; Abbott Ireland Ltd., Sligo, Republic of Ireland), with vacuum created in the system using a 20-ml syringe (B. Braun Melsungen AG, Melsungen, Germany), was passed through the scrotal skin and used in sampling different parts of the testis. A pair of small artery forceps was applied on the tubing of the butterfly needle nearest the vacuum syringe. This maintained the negative pressure in the system while replacing the 20-ml syringe with a tuberculin syringe (B. Braun Melsungen AG) containing 1 ml of culture medium (IVF Medium; Medi-Cult, Copenhagen, Denmark). After withdrawing the needle from the testis, the aspirated testicular tissue was flushed into sterile Falcon tubes (Becton Dickinson Ltd., Plymouth, UK) with small volumes of culture medium, minced with a pair of clean sterile cutting scissors, and forced through a 25G needle with a tuberculin syringe. The homogenized tissue was washed twice with 5 ml of culture medium, centrifuging each time at 500g for 10 min. The tissue pellet was placed in fresh droplets of culture medium and overlaid with equilibrated mineral oil (Medi-Cult). This was then incubated for 48–72 hr at 37°C in a 5% CO₂-in-humidified air mixture (8,9). The culture droplets were examined daily for evidence of sperm motility using an inverted phase-contrast microscope (Nikon Diaphot 300; Nikon UK Ltd., Kingston,

UK) equipped with Hoffmann modulation contrast (Modulation Optics Inc., Greenvale, USA).

RESULTS

Motile sperm were found in the PESA sample of only one patient with nonobstructive azoospermia (Table 1). Sperm was not found in fresh TESA samples taken from two patients, while immotile sperm were seen in the samples from the other seven patients. After 48–72 hr of culture, sperm were seen to be swimming at the periphery of the culture droplets in five of the seven patients. No motile sperm was found in the PESA samples of two patients with obstructive azoospermia but motile sperm were found, after 48 hr, in the culture droplet containing testicular tissue aspirated from one of them. The remaining 18 patients had motile sperm in both the PESA and the TESA samples but testicular sperm motility became more pronounced and progressive only after 48–72 hr of culture (Table 1).

DISCUSSION

It has been clearly demonstrated that successful pregnancies and normal healthy babies can be generated through ICSI with testicular sperm and even spermatids (6,7,10,11). We, however, wish to stress the physiological immaturity of testicular sperms and the as yet unanswered question of the potential for long-term deleterious effects on the progeny. Following maintenance of testicular tissue and sperm in culture

for a relatively short period of time, we were able to show that this leads to a dramatic improvement in sperm motility. It has been suggested that the acquisition of progressive motility is an indication of sperm maturation (12).

There are a number of reasons why ICSI of in vitro matured testicular sperm may be preferable to injection of freshly recovered testicular sperm. In some countries such as the United Kingdom, practice guidelines stipulate that only documented motile sperm can be injected into oocytes. Liu and colleagues (13) have demonstrated from an analysis of 2732 ICSI cycles that failure of fertilization is caused mainly by the use of nonviable sperm. Observation of sperm motility is the least invasive and most practical method of establishing the viability of sperm that is subsequently selected for injection. Another report (14) has shown that abnormal fertilization, particularly of the one pronuclear variety, is significantly more common when testicular sperm are injected compared to the injection of ejaculated sperm. We wonder whether this difference will disappear if in vitro matured sperm is used for ICSI rather than freshly recovered testicular sperm. Another advantage of in vitro culture is that sperm swim away from the testicular tissue and cellular debris and are usually found at the edge of the culture droplet. This makes it easier to avoid aspirating cells or cellular debris, which may contain DNA, into the injection needle and subsequently introducing them into the ooplasm.

As testicular sperm are physiologically more immature than ejaculated or epididymal sperm, they should be used only in situations where these other sources are not available. This study confirms that epididymal sperm can be retrieved and used for ICSI in a majority of patients with obstructive azoospermia. The likely candidates for testicular sperm retrieval, therefore, are those with nonobstructive azoospermia. It is possible that the quality of testicular sperm depends on whether azoospermia is obstructive or nonobstructive, the pathology in the first group of patients is simply that of blockage to the passage of sperm, while the latter group may have more complex factors operating. The issue left to be decided, then, is whether this culture technique should be applied to all cases where testicular sperm is used for ICSI, in which case testicular sperm retrieval needs to be carried out 24 to 72 hr before oocyte aspiration. From our limited experience, we suggest that in vitro culture of human testicular sperm recovered from patients with nonobstructive may be necessary.

Table 1. Sperm Motility Following Surgical Retrieval and In Vitro Maturation

Sperm retrieval and motility ^a	Obstructive azoospermia (n = 20)	Nonobstructive azoospermia (n = 9)
Progressively motile sperm in PESA sample	18	1
Immotile or weakly twitching sperm in TESA sample	20	7
No sperm found in TESA sample	0	2
Progressive testicular sperm motility following in vitro culture	19	5

^a The definition of sperm motility utilized here is based on the WHO criteria (15). Progressively motility here is equivalent to grades a and b, while immotility and weak twitching are the same as grades c and d.

ACKNOWLEDGMENTS

This study was supported by Life Force Research Ltd., London.

REFERENCES

1. Temple-Smith PD, Southwick GJ, Yates CA, Trounson AO, Kretser DM: Human pregnancy by in-vitro fertilisation (IVF) using sperm aspirated from the epididymis. *J Vitro Fert Embryo Transfer* 1985;2:119-122
2. Shrivastav P, Nadkarni P, Wensvoort S, Craft I: Percutaneous epididymal sperm aspiration for obstructive azoospermia. *Hum Reprod* 1994;9:2058-2061
3. Craft I, Tsirigotis M, Bennett V, Taranissi M, Khalifa Y, Hogewind G, Nicholson N: Percutaneous epididymal sperm aspiration and intracytoplasmic sperm injection in the management of infertility due to obstructive azoospermia. *Fertil Steril* 1995;63:1038-1042
4. Craft I, Tsirigotis M: Simplified recovery, preparation and cryopreservation of testicular spermatozoa. *Hum Reprod* 1995;10:1623-1627
5. Tsirigotis M, Craft I: Sperm retrieval methods and ICSI for obstructive azoospermia. *Hum Reprod* 1995;10:758-760
6. Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M, Van Steirteghem AC: Pregnancies after testicular spermextraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod* 1995;10:101-104
7. Tournaye H, Camus M, Goossens A, Liu J, Nagy P, Silber S, Van Steirteghem AC, Devroey P: Recent concepts in the management of infertility because of non-obstructive azoospermia. *Hum Reprod* 1995;10 (Suppl 1):115-119
8. Craft IL, Tsirigotis M, Zhu JJ: In-vitro culture of testicular sperm [letter]. *Lancet* 1995;346:1438.
9. Zhu JJ, Tsirigotis M, Pelekanos M, Craft IL: In vitro maturation of human testicular spermatozoa [letter]. *Hum Reprod* 1996;11:231-232
10. Fishel S, Green S, Bishop M: Pregnancy after intracytoplasmic injection of spermatid. *Lancet* 1995;345:1641-1642
11. Tesarik J, Mendoza C, Testart J: Viable embryos from injection of round spermatid into oocytes. *N Engl J Med* 1995;333:525
12. Moore HDM, Curry MR, Penfold LM, Pryor JP: The culture of human epididymal epithelium and in vitro maturation of epididymal spermatozoa. *Fertil Steril* 1992;92:776-783
13. Liu J, Nagy Z, Joris H, Tournaye H, Smits J, Staessen C, Devroey P, Van Steirteghem AC: Analysis of 76 total fertilization failure cycles out of 2732 intracytoplasmic sperm injection cycles. *Hum Reprod* 1995;10:2630-2636
14. Hovatta O, Moilanen J, Von Smitten K, Reima I: Testicular needle biopsy, open biopsy, epididymal aspiration and intracytoplasmic sperm injection in obstructive azoospermia. *Hum Reprod* 1995;10:2595-2599
15. World Health Organization: WHO Laboratory Manual for the Examination of Human Sperm and Sperm-Cervical Mucus Interaction, 3rd ed. Cambridge, Cambridge University Press, 1992

Jianjun Zhu¹

Godwin I. Meniru

Ian Craft

London Gynaecology & Fertility Centre

Cozens House

112a Harley Street

London W1N 1AF, England

¹ To whom correspondence should be addressed at Fertility and IVF Center of Miami, Baptist Medical Arts Building, 8950 North Kendall Drive, Suite 103, Miami, Florida 33176-2197.