



PREGNANCIES RESULTING FROM INTRACYTOPLASMIC SPERM INJECTION OF SPERMATOZOA FROM FROZEN-THAWED TESTICULAR BIOPSY SPECIMENS

To the Editor: The introduction of intracytoplasmic sperm injection (ICSI) in assisted reproduction has revolutionised the treatment of male infertility,^{1,2} to the extent that fertilisation and pregnancy are even possible when the man suffers from obstructive and non-obstructive azoospermia,^{3,5} where epididymal and testicular spermatozoa have to be used.

Recently there have been reports of successful fertilisation and pregnancies after the use of frozen-thawed spermatozoa obtained from testicular tissue in ICSI.^{6,7} The problem, however, is the relatively low number of motile sperm obtained after thawing. A number of authors have subsequently investigated the possibility of cryopreserving spermatozoa still contained within the testicular tissue.⁸⁻¹¹ These authors reported obtaining adequate yields of testicular spermatozoa after thawing using similar cryopreservation protocols. The cryopreservation of supernumerary testicular spermatozoa adds a new dimension to the treatment of these patients, as it reduces the need for repeated scrotal surgery.

We report on our success using spermatozoa from frozen-thawed testicular tissue samples for the ICSI procedure in 5 cases of obstructive azoospermia.

The female partners were superovulated using standardised regimens, as previously described.¹² An open testicular biopsy was performed 24 hours before or on the same day of the sonographic oocyte aspiration. The testicular tissue (2 - 5 biopsy specimens) was thoroughly dissected and the resultant supernatant examined for the presence of spermatozoa. An aliquot of the testicular homogenate was prepared for the ICSI procedure and the rest of the testicular homogenate was prepared for cryopreservation. The homogenate (including testicular tissue) was diluted 1:1 with cryoprotectant and thoroughly mixed. The mixture was drawn into cryopreservation straws and frozen with a Planar (Kryo 10 Series) cryopreserver, using a stepwise controlled freezing programme.

Cryopreservation of a homogenate of the tissue rather than whole tissue ensures better access for cryoprotectant. The inclusion of all the testicular tissue in the cryopreserved sample is, however, beneficial as it serves as a natural milieu for the maturation of immature testicular spermatozoa.¹⁰

In the initial ICSI cycles, using 'fresh' spermatozoa, fertilisation (60.2%) was achieved but no pregnancies were obtained after the transfer of embryos. In subsequent cycles the female partners were again superovulated and the oocytes obtained by transvaginal sonographic aspiration. On this occasion the cryopreserved testis homogenate was prepared and used in the ICSI procedure. In all cases motile spermatozoa were obtained after a mini-Percoll gradient centrifugation

procedure. Thirty-five metaphase II oocytes from the 5 couples were successfully injected with motile but immobilised spermatozoa. Thirty oocytes fertilised and cleaved normally (85.7%). Three to four embryos were transferred into each patient, and 2 clinical pregnancies were obtained (40%). Both are ongoing — one is a triplet and the other a singleton pregnancy. Although the numbers are small, our results indicate that the fertilisation and pregnancy rates using spermatozoa from cryopreserved testicular tissue compare favourably with the results obtained when using fresh testicular spermatozoa.

The cryopreservation of testicular tissue as described has the advantages of lower cost, lower patient stress and lower surgical risk (decreased surgical procedures), and facilitates clinical management of the couple. There is also speculation that the freeze-thaw procedure may help to select the most viable sperm.

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1. Palermo G, Joris H, Devroey P, Van Steirteghem AC. Pregnancies after intracytoplasmic sperm injection of single spermatozoa into an oocyte. *Lancet* 1992; **430**: 17-18.
2. Van Steirteghem AC, Nagy Z, Joris H, et al. High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum Reprod* 1993; **8**: 1061-1066.
3. Schoysman R, Vanderzwalmen P, Nijs M, et al. Pregnancy obtained with human testicular spermatozoa in an *in vitro* fertilization program. *J Androl* 1994; **15**: 105-135.
4. Silber SJ, Van Steirteghem AC, Liu J, Nagy Z, Tournaye H, Devroey P. High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum Reprod* 1995; **10**: 148-152.
5. Nagy Z, Liu J, Janssenwillen C, Silber S, Devroey P, Van Steirteghem A. Using ejaculated, fresh, and frozen-thawed epididymal and testicular spermatozoa gives rise to comparable results after intracytoplasmic sperm injection. *Fertil Steril* 1995; **63**: 808-815.
6. Gil-Salmon M, Romero J, Minguez Y, et al. Pregnancies after intracytoplasmic sperm injection with cryopreserved testicular spermatozoa. *Hum Reprod* 1996; **11**: 1309-1313.
7. Podsiady BT, Woolcott R, Stanger JD, Stevenson K. Pregnancy resulting from intracytoplasmic injection of cryopreserved spermatozoa recovered from testicular biopsy. *Hum Reprod* 1996; **11**: 1306-1308.
8. Hovatta O, Foudila T, Sieberg R, Johansson K, von Smitten K, Reima I. Pregnancy resulting from intracytoplasmic injection of spermatozoa from frozen-thawed testicular biopsy specimen. *Hum Reprod* 1996; **11**: 2472-2473.
9. Fisher R, Baukloh V, Naether O, Schulze W, Salzburn A, Benson DM. Pregnancy after intracytoplasmic sperm injection of spermatozoa extracted from frozen-thawed testicular biopsy. *Hum Reprod* 1996; **11**: 2197-2199.
10. Khalifeh FA, Sarraf M, Dabit ST. Full-term delivery following intracytoplasmic sperm injection with spermatozoa from frozen-thawed testicular tissue. *Hum Reprod* 1997; **12**: 87-88.
11. Oates RD, Mulhall J, Burgess C, Cunningham D, Carson R. Fertilization and pregnancy using intentionally cryopreserved testicular tissue as the sperm source for intracytoplasmic sperm injection in 10 men with non-obstructive azoospermia. *Hum Reprod* 1997; **12**: 734-739.
12. Kruger TE, Menkveld R, Stander FSH, et al. Sperm morphologic features as a prognostic. *Fertil Steril* 1986; **46**: 1118-1123.