CASE REPORT

Full-term delivery following intracytoplasmic sperm injection with spermatozoa extracted from frozen–thawed testicular tissue

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This report describes the full-term delivery of a female baby by Caesarean section following intracytoplasmic sperm injection (ICSI) using spermatozoa extracted from thawed previously frozen testicular tissue of a patient with azoospermia due to congenital bilateral absence of the vas deferens (CBAVD).

Key words: cryopreservation of testicular tissue/intracytoplasmic sperm injection (ICSI)/testicular exploration and sperm extraction (TESE)

Introduction

Azoospermia is found in 2% of infertile men, of which obstructive azoospermia is diagnosed in about half of the cases (Hull et al., 1985). However, azoospermia may make up to 20% of the cases referred to an in-vitro fertilization (IVF) centre (Van Steirteghem et al., 1993a). Obstructive azoospermia may be due to congenital bilateral absence of the vas deferens (CBAVD) or to post-infectious obstruction.

Patients with azoospermia had almost no chance of treatment until Temple-Smith et al. (1985) reported the use of microsurgical epididymal sperm aspiration (MESA) from a man who had received an irreversible vasectomy. However, the chance of pregnancy with MESA remained in the range of 6.9–11% per cycle (Silber et al., 1994).

The introduction of intracytoplasmic sperm injection (ICSI) (Palermo et al., 1992; Van Steirteghem et al., 1993b) made MESA/ICSI a very effective solution for patients with CBAVD (Tournaye et al., 1994). When spermatozoa were not obtainable from the epididymis, testicular exploration and sperm extraction (TESE/ICSI) provided an additional solution. This procedure can be used in cases of extensive scarring secondary to previous surgery on the epididymis, spermatogenic dysfunction and ejaculatory dysfunction (Tournaye et al., 1994). Thawed–frozen testicular spermatozoa have been used for ICSI with high fertilization rates (Romero et al., 1996).

Freezing of testicular tissue at time of testicular biopsy or exploration is advisable when possible in order to provide testicular spermatozoa when ICSI is performed later (Salzbrunn et al., 1996). Pregnancies from frozen–thawed testicular tissue have been reported recently (S.Al-Hassani, personal communication).

Case report

An infertile couple, of whom the wife was 35 years of age and the husband 42 years of age, were seen for the first time in our IVF unit in August, 1994. They had suffered from primary infertility for 9 years due to CBAVD. Previously, they had received different modalities of treatment including epididymal cup application, two trials of intrauterine insemination (IUI) from the epididymal cup aspirate and one trial of MESA with IVF, but no fertilization occurred.

The hormonal profile of the husband was normal; physical examination showed no respiratory problems, and examination of the genitalia showed normal sized testes with excessive scarring of the epididymis. The hormonal profile of the wife was also normal, while physical examination showed multiple small uterine fibroids of a size corresponding to 14 weeks growth. Abdominal myomectomy was performed in August 1994.

The couple underwent the first IVF trial by mid-luteal long suppression protocol with gonadotrophin-releasing hormone agonist GnRHa (Triptorelin 3.75 mg; Beaufour Ipsen International, Paris, France). Ovulation was induced using follicle stimulating hormone (FSH, Metrodin; Serono, Geneva, Switzerland) and human chorionic gonadotrophin (HCG; Serono).

Oocyte retrieval was carried out in November 1994, by vaginal-guided ultrasonography. Ten eggs were collected, eight of them being metaphase II (MII). On the day of egg retrieval, the husband underwent MESA after removal of the epididymal cups, but no spermatozoa were retrieved. It was therefore decided to attempt a TESE procedure and five small pieces of testicular tissue from both testes were obtained.

Sperm collection and preparation

Five pieces of testicular tissue were removed and each placed in a 5 ml plastic test tube (Falcon 2003; Becton Dickinson, Lincoln Park, NJ, USA) with 1 ml of IVF medium (Medicult; International Supplies, Amman, Jordan). These pieces were minced with fine scissors. Spermatozoa having sluggish activity were recovered under the microscope directly from one suspension for ICSI. The sperm suspension was kept in an incubator (37°C, 5% CO2) for 4 h before ICSI. A test yolk cryoprotective medium (Irvine Scientific, Santa Ana, CA, USA) was added to the remaining four unused testicular tissue suspensions (1:1) and the resulting mixtures were aspirated into 2 ml vials; each
vial was placed in a container of water at 37°C and then refrigerated at 2–5°C to allow a slow cooling of the mixture (0.5°C/min). The vials were then placed in liquid nitrogen vapour for 25–30 min before being placed in liquid nitrogen.

Eight eggs were injected by ICSI technique, four embryos were formed and transferred to the patient but no pregnancy resulted. In June 1995, the patient underwent a second IVF trial, involving an ultralong protocol (2 consecutive months of suppression with GnRHa) followed by FSH induction and HCG. A total of 11 oocytes were retrieved, nine of them being MII; these were injected with spermatozoa obtained from two vials of frozen testicular tissue suspension which had been thawed according to the protocol described by Tournaye et al. (1991). After thawing 2–3 immotile spermatozoa/slide were recovered.

Five embryos of Grade I were obtained, of which three were transferred to the mother. The remaining two embryos were frozen. Two weeks after embryo transfer, the serum β-HCG concentration was 62 mIU/ml.

At 7 weeks, one gestational sac with fetal echoes and fetal heart activity was seen using ultrasound. The antenatal period was uneventful. A viable healthy female baby weighing 3000 g was delivered by Caesarean section after 37 weeks gestation.

Two vials of testicular tissue suspension and two embryos remain in cryopreservation for this couple for future pregnancy attempts.

Discussion

Since August 1992, when the Brussels group reported higher fertilization and implantation rates after ICSI (Palermo et al., 1992, 1993), this procedure has become a standard practice worldwide. Indications for its use are increasing, one of the most important being obstructive azoospermia mainly due to CBAVD. In the case discussed here, MESA/ICSI was not possible, because of the presence of excessive epididymal fibrosis secondary to previous application of epididymal cups and repeated aspirations. The urologist obtained five small pieces of tissue from both testes and the biologist was able to extract sufficient spermatozoa for ICSI from the first piece. It was decided to freeze the remainder for possible future use. The protocols used for testicular tissue freezing and thawing were the same as for freezing and thawing spermatozoa.

When spermatozoa were needed for the second trial, testicular tissue thawing was carried out on two frozen vials, from which sufficient spermatozoa were removed to perform ICSI.

It was found that the frozen–thawed spermatozoa were of good quality, perhaps because storage within testicular tissue closely resembled its natural milieu. Therefore, it is advisable to perform testicular biopsies in units where testicular tissue freezing facilities are available.

In cases with CBAVD, genetic analysis for the presence of the cystic fibrosis mutation should be performed, especially on patients with chronic bronchitis, pancreatic insufficiency and sweat chloride abnormalities. This was not done in the case reported here because the patient was asymptomatic and because the facility for gene studies was not available in our laboratories.

There have been recent reports of pregnancies from frozen–thawed testicular tissue but, as yet, no full-term delivery. To the best of our knowledge this may be the first full-term delivery following ICSI using spermatozoa from frozen–thawed testicular tissue.

References


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