Isolation of Ejaculated Spermatozoa Six Hours After Unsuccessful Testicular Biopsy in Nonobstructive Azoospermia

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Azoospermia, the absence of sperm in ejaculated semen, is the most severe form of male factor infertility and is present in approximately 5% of all investigated infertile couples. The condition is currently classified as obstructive and nonobstructive subgroups. In nonobstructive azoospermia, testicular sperm extraction (TESE) is usually necessary for sperm recovery. This is a case report of pregnancy and subsequent birth of healthy babies following intracytoplasmic sperm injection (ICSI) of ejaculated motile spermatozoa presented 6 h after unsuccessful testicular biopsies in four subsequent in vitro fertilization (IVF) cycles with infertility due to nonobstructive azoospermia.

KEY WORDS: Ejaculated spermatozoa; ICSI; nonobstructive azoospermia; testicular biopsy.

INTRODUCTION

Intracytoplasmic sperm injection (ICSI) is a successful treatment for severe male subfertility (1) enabling fertilization with the use of ejaculated semen even with very poor conventional sperm parameters (2) or with the use of different surgical sperm retrieval techniques (3). For the benefit of patients, sperm recovery should be minimally invasive. In nonobstructive azoospermia, testicular sperm extraction (TESE) is usually necessary for sperm recovery. Biopsies with focal spermatogenesis may detain embryologist in a prolonged search for viable spermatozoa lasting many hours.

In men with no previous sperm-positive testicular histology, bilateral or multiple biopsies may be

required. Occasionally, spermatozoa can be found in the ejaculate of men previously diagnosed as azoospermic and it is defined as virtual azoospermia or transient state of azoospermia. Repetitive semen sampling in such cases prior to ART cycle can enable to pool frozen spermatozoa. The introduction of semen banking for patients with transient azoospermia may increase the chance of pregnancy using their own sperm (4). Patients with nonobstructive azoospermia may have lower fertilization, embryo quality, and pregnancy rates than those with obstructive azoospermia following TESE and ICSI (5).

We present a case report of pregnancy and subsequent birth of healthy babies following ICSI with ejaculated spermatozoa presented 6 h after testicular biopsies. The unpleasant procedure was performed with the same couple resulting in the birth of a healthy female (1997) and a male (2000) baby. They weighed 2950 and 3080 g, respectively.

CASE REPORT

A 27-year-old male with azoospermia presented to our Center for evaluation of infertility and

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therapeutic options. The couple had an anamnesis of infertility for 3 years. The objective urological examination highlighted mild male tract infection treated with appropriate medication according to the antibiogram. Testis and epididymis were normal. The patient confirmed normal erection, sexual activity, and ejaculation. The serum levels of FSH and LH were within the higher limits of the normal value. Y chromosome evaluation was not available at the time of the intervention. According to the protocol of our center, it was decided to perform an open testicular biopsy which guaranteed a higher chance of sperm recuperating for possible fecundation. Testicular biopsies and sperm extractions were synchronized with oocyte retrieval and ICSI procedures were performed on the same day.

The fertility examination of the patient's 27-year-old wife revealed polycystic ovarian syndrome (PCOS) confirmed by phenotypic, ultrasound, and endocrine criteria. The partner was stimulated following oral contraceptive premedication with clomiphencitrate (CC) (Clostilbegyt; Richter, Budapest, Hungary) and human menopausal gonadotrophin (hMG) (Humegon; Organon, Rotterdam, Holland). Stimulation was monitored by means of a series of transvaginal ultrasound examinations and by measuring the plasma concentration of β -oestradiol. When a leading follicle reached 18 mm or more in diameter, 7500 IU of hCG (Choriogonin; Richter, Budapest, Hungary) was administered. Ultrasound guided transvaginal oocyte retrieval was performed under general anaesthesia 35 h after hCG injection. From eight aspirated follicles, six oocytes were recovered.

The testicular biopsy was performed in local anaesthesia prior to a transversal incision of 1 cm of the scrotum. A piece of testicular pulp was extracted for TESE. Hystopathologic test was performed prior to the IVF-cycle. The testicular biopsy was placed on a petri dish with IVFTM-100 (Scandinavian IVF Science Gothenburg, Sweden) at 37°C. Under a dissecting microscope, the seminiferous tubules were teased apart using a 21-gauge needle and the contents were squeezed into the surrounding media. After the incubation period no spermatozoon could be isolated after a prolonged and cautious search. After 6 h following the biopsy our last suggestion was to ask the husband to try to produce semen for testing the sample for viable sperm. The semen sample was put on two-layer Percoll gradient (Irvine Scientific, Santa Ana, CA), centrifuged at 300g for 20 min, and the final pellet was washed and centrifuged for 10 min at 300g. After meticulous search a few motile and morphologically

normal sperm could be isolated, all the six oocytes were microinjected (1). Two oocytes were fertilized and both the two good quality embryos were transferred on day 2 resulting in a singleton pregnancy.

In 1998 the couple decided to restart the treatment for a second child. Following two unsuccessful attempts with gonadotrophin releasing hormone analogues (GnRHa) (Decapeptyl; Ferring, Malmo, Sweden) and follicle stimulating hormone (FSH) (Metrodin-HP; Serono, Aubonne, Switzerland) the third stimulation in 1999 with the same stimulation protocol (CC + HMG) resulted in seven oocytes. The oocytes were microinjected with spermatozoa isolated 6 h after sperm-negative testicular biopsy from semen sample. Three oocytes were fertilized and all the three good quality embryos were transferred on day 2.

After normal vaginal delivery the female baby weighed 2950 g (1997) and the male 3080 g (2000). Both the two neonates were healthy and showed no sign of malformation after the 1-year follow up.

DISCUSSION

ICSI has been used world-wide and successfully to treat infertility due to impaired testicular function or obstruction of the excretory ducts resulting severe oligo-, astheno-, teratozoospermia or even azoospermia in the ejaculate (6). It must be added that the ICSI procedure cannot be carried out in approximately 3% of the scheduled cycles. Either no cumulus-oocyte complexes or metaphase-II oocytes are available, or no spermatozoa are found in patients with nonobstructive azoospermia (7). Although less invasive techniques such as testicular fine needle aspiration and percutaneous needle biopsy are efficacious, particularly in cases of obstructive azoospermia, studies in the current literature support that open testicular biopsy is a more reliable method to obtain testicular specimen (8). Testicular sperm extraction (TESE) is a therapeutic technique that has revolutionized the treatment of severe male infertility presenting as nonobstructive azoospermia. However, the procedure is not without side effects, involving at least a transient effect on spermatogenesis and potentially adverse local consequences like decrease in seminiferous tubular volume within the testicular parenchyma adjacent to the biopsy site (9). It was confirmed that men with nonobstructive azoospermia have a mean of 0–3 mature spermatids per seminiferous tubule in contrast with 17–35 mature spermatids per tubule in men with normal spermatogenesis and obstructive

azoospermia. The use of mapping by multiple biopsy and microsurgical removal of contiguous strips of testicular tissue to determine the distribution of spermatogenesis in different regions of the testis in nonobstructive azoospermia revealed a diffuse, rather than regional, quantitative distribution of spermatogenesis (10). This result can explain the possibility of finding sperm in 25% in the repeated TESE cycle after failing to obtain sperm in the first extraction attempt in nonobstructive azoospermia cases (11).

The unexpected phenomenon that motile spermatozoa could be prepared from the ejaculate 6 h after testicular sperm extraction also can be the effect of the transient change in the micromilieu of the testis. The diffuse distribution of spermatogenesis and the stress of the intervention could also have certain effects that elevated the quantitative spermatogenesis to exceed the threshold of enough spermatozoa to spill over into the ejaculate (10). This could result in this repetitive, transient motile sperm-positive semen sampling. The rather unpleasant event helped the couple to deliver two healthy child following four TESE–ICSI cycles with unsuccessful biopsies in each cycle and with only azoosperm previous semen samples. The motile and morphologically normal spermatozoa could in all the four cases be extracted only from the postbiopsy semen samples. The same therapeutic option was suggested in five other nonobstructive azoospermia cases in the following period but only in one single cycle could we detect motile sperm in the ejaculate after 6 h following the unsuccessful testicular biopsy. Good quality embryos were transferred, but no pregnancy occurred.

This method can be the last and in a few cases helpful effort in such apparently hopeless situation when the couple is not open for fertilization with donor sperm.

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