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Successful pregnancy in double factor infertility: Sperm in vitro culture by modified testicular fine-needle aspiration

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Abstract

Introduction: Infertility may depend up to 27% of couples on both partners. In patients with obstructive azoospermia, testicular fine-needle aspiration represents a good option to retrieve spermatozoa, in order to perform an assisted reproductive treatment. In vitro maturation of testicular spermatozoa could be the better choice of treatment in view of the increased motility, improving fertilization and pregnancy rates.

Case description: A 34-year-old azoospermic man and his 33-year-old partner referred for treatment of simultaneous male and female infertility factor. The woman presented a diminished ovarian reserve, with serum follicle stimulating hormone value of 27.151IU/L. The man underwent trans-rectal and testicular ultrasounds that detected the congenital absence of proximal vas deferens on the right side and the absence of seminal vesicle and distal vas deferens on the left side. We proposed a chance to have their own biological child. The man underwent modified testicular fine-needle aspiration using a 18-gauge butterfly needle. Sperm retrieval was successful with 0.001×10^6 spermatozoa/mL and absence of motility. Testicular sperm suspension was cultured for 24 h to identify sperm viability, achieving 10% of sperm motility. Two metaphase II oocytes were retrieved and processed with intracytoplasmic sperm injection. Clinical pregnancy with live birth was obtained.

Conclusion: Performing modified testicular fine-needle aspiration increases successful sperm retrieval. Testicular sperm in vitro culture for 24 h proved to be a real and practical technique to increase sperm motility, in order to select mature and viable spermatozoa and improve successful intracytoplasmic sperm injection outcomes.

Keywords

Azoospermia, in vitro culture, intracytoplasmic sperm injection, motility, testicular fine-needle aspiration

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Introduction

Infertility may depend up to 27% of couples on both partners.[1] Among male factors, azoospermia (OA) interests almost 10%–20% of all cases.[2] In patients with OA, testicular fine-needle aspiration (TEFNA) represents a good option to retrieve spermatozoa, in order to perform an assisted reproductive treatment (ART).[3] However, testicular spermatozoa recovered by sperm retrieval (SR) techniques are immature physiologically and often are immotile or with finely shaking movements.[4] Indeed, the

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Table 1. Male baseline parameters prior to TEFNA.

Parameter	Value
FSH (IU/L)	5.72
(Normal range)	(1.5–8.0)
LH (IU/L)	1.61
(Normal range)	(1.0–9.0)
TT (ng/mL)	4.03
(Normal range)	(3.0–10.6)
Prolactin (ng/mL)	23.50
(Normal range)	(3.0–30)
17-beta estradiol (pcg/mL)	<20
(Normal range)	(5.0–56)
Left TV (mL)	17
Right TV (mL)	16.5

FSH: follicle stimulating hormone; LH: luteinizing hormone; TT: total testosterone; TV: testicular volume; TEFNA: testicular fine-needle aspiration.

achievement of progressive motility is just the final step of sperm maturation in the seminal tract, after spermatozoa pass through epididymis where structural changes occur in the lipid and protein membrane composition.[5] Immediately after SR, finding of sufficient motile sperm cells for fresh intracytoplasmic sperm injection (ICSI) could be very difficult. Consequently, selecting just motile spermatozoa might be extremely important, since it allows to choose those viable for injection. In this regard, in vitro maturation of testicular spermatozoa could be the better choice of treatment in view of the increased motility, improving fertilization, and pregnancy rates.[6]

Otherwise, female infertility factor may depend on many causes, including diminished ovarian reserve, chromosome aberration, or premature ovarian failure.

Case description

A 34-year-old Caucasian man and a 33-year-old female presented for treatment of primary couple infertility for at least 2 years. Baseline characteristics of the patient were collected (Table 1). He had normal sexual development and no history of cryptorchidism. In anamnesis, he reported a post-traumatic hematoma of the epididymis, varicocelectomy, and trans-rectal ultrasound guided aspiration of prostatic cyst. The semen analysis diagnosed an absolute OA. The men underwent a preliminary physical examination, revealing testicular volume of about 18 mL bilaterally (by orchidometer scale) and impalpable right vas deferens. Scrotal and trans-rectal ultrasounds were performed showing (a) right-sided testis of $44 \times 28 \times 26$ mm and a 10-mm epididymal head with an inhomogeneous hyperechoic echotexture, (b) left-sided testis of $45 \times 28 \times 26$ mm, and (c) congenital absence of proximal vas deferens on right side and the absence of seminal vesicle and distal vas deferens on left side. Genetic studies showed a normal karyotype, the absence of microdeletions for chromosome Y, and the presence of ΔF508 mutation of cystic fibrosis transmembrane conductance regulator (CFTR) genes. Thus, he was diagnosed for OA. The woman had very low ovarian reserve (follicle stimulating hormone (FSH) level of 27.15 IU/L) due a familiar risk factor and pelvic endometriosis.

Despite an accurate counselling on the very few possibilities of successfull pregnancy, so to recommend firstly a heterologous ART program, the couple was particularly motivated to have a chance with their gametes, since they asked for own biological child.

The man underwent a bilateral TEFNA with a modification of classic technique. An informed consent was obtained from the patient. The scrotal skin was cleaned with 10% Povidone-iodine solution. Each testis was grasped with the fingers with epididymis and vas deferens directed posteriorly, safe from injury. Testes was systematically screened and aspirated twice at three different sites, upper, middle, and lower part, using a larger disposable 18-gauge butterfly needle with 60 mL Luer Lock syringe attached to it. The needle was inserted into the testis to a depth of about 2 cm. Every testicular puncture was composed of multiple precise rapid in and out movements until the yellowish fluid aspirated ceased to flow or if bloody fluid appeared. The tubing was then occluded with an artery forceps, maintaining a negative pressure. Subsequently, removing very slowly the needle from the testis and scrotal skin, we extracted some intact seminiferous tubules, protruding from the site of puncture. This tissue was carefully cut with sharp scissors and placed in the Petri dishes, using two pairs of fine tweezers. Following each puncture, the contents were washed in a 15-mL centrifuge tube containing 1 mL of flushing medium, using a 20 mL syringe filled with Hams medium. Retrieved tissue was instantaneously brought to the adjacent embryology laboratory to rule out the presence of spermatozoa.

Each sample was processed by centrifugation at 1800 r/min for 7 min. The pellet obtained was observed under inverted microscopy at 200× to evaluate the presence and motility of spermatozoa: 1–2 sperms/field (corresponding to an estimated concentration of 0.001×10^6/mL spermatozoa/mL) with the total absence of motility. Thereafter, the testicular sperm suspension had been cultured with 500 µl of Sequential Fert™ medium (ORIGIO®) that promotes sperm function and supports both gametes during fertilization. Testicular sperm culture was incubated with 6% CO_2, 5% O_2 at 37°C, until the ICSI procedure was performed the next day.

A personalized ovarian antagonist stimulation protocol with high dosage of FSH 450 UI/die (rFSH Gonal-F® 900 IU, Merck) was performed. In presence of 14 mm dominant follicle antagonist Ganirelix 0.25 mg (Fyremadel®) was started. Only two follicles growth and 999 pg/mL estradiol was observed on the 11th day of human chorionic gonadotropin (hCG) dosage (Ovitrelle 250 µg/mL). The

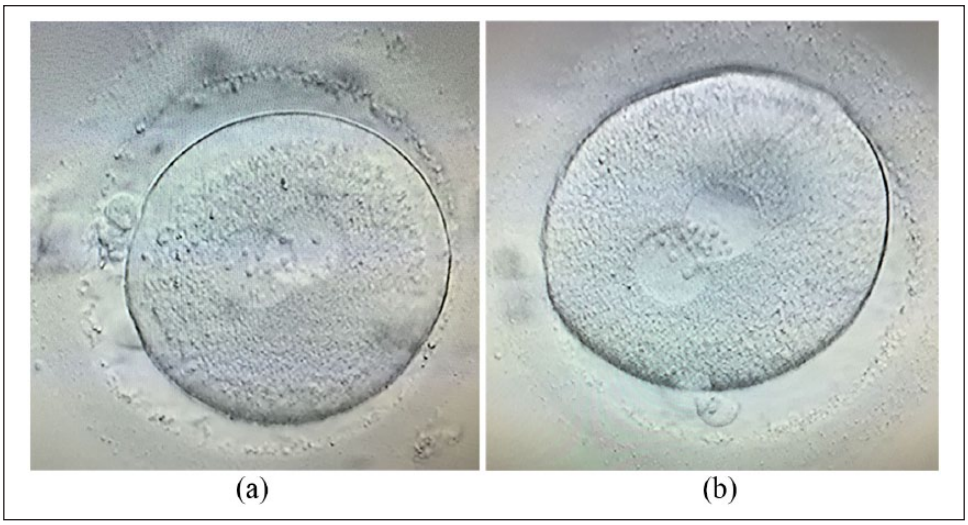

Figure 1. (a) Zygote 17 h post-ICSI, having small size PNs with scattered NPBs and two visible polar bodies (400 × magnification). The zona pellucida appears regular; some debris is present in the perivitelline space. The cytoplasm is light-coloured with a clear cortical zone and (b) the cytoplasm is inhomogeneous, showing cytoplasmic halo.

female underwent oocyte pick-up procedure, resulting in retrieving of two metaphase II oocytes for ICSI procedure. On the day of egg retrieval, total sperm motility increased up to 10%. Thereafter, the aliquots of sperm cells were suspended in SpermCatch (NIDACON®), in order to reduce rates of chromosomal aneuploidies and DNA fragmentation. 17 h post insemination, the fertilization was evaluated: "two pronuclear" (2PN) fertilized oocytes were obtained and two embryos were transferred on day 2 after ICSI (Figure 1). The serum dosing of beta-hCG at the 12th and 14th day after embryo-transfer (ET) showed a value of 783.7 and 2181.9 UI/L, respectively. The clinical pregnancy was verified by the observation of a double gestational sac using vaginal ultrasound at 6 weeks of gestation. The pregnancy ended in live birth of two infants.

Discussion

TEFNA is currently considered an established method of SR for ART. Cost-effectiveness, simplicity, and its minimal invasiveness are the main advantages, if compared with other procedures.[7]

The majority of previous studies described TEFNA technique employing a 21- or 23-gauge needle, but only very few previous researchers depicted alternative methods of testis needle aspiration.[8,9] In our report, it performed a modified TEFNA technique, in order to obtain more testicular tissue available for ICSI. Indeed, we used a larger butterfly needle measuring 18-gauge, with the scope to extract some intact seminiferous tubules and to provide more samples to the embryologist. This technique proved to be simple and safe, as the testicular size of our patient was normal. None of the intraoperative and postoperative complications occurred, like significant hematoma or prolonged orchialgia.

Considering reports that showed improving motility of testicular spermatozoa, we decided to schedule long sperm culturing for 24 h before oocyte pick-up.[6]

The reasons why ICSI of in vitro matured testicular sperm may be preferable to injection of freshly recovered testicular sperm is that it allows to select just viable spermatozoa. Nowadays, the precise mechanism of improving the motion of testicular sperms after culturing remains unknown. In vitro culture could facilitate the interaction between testicular somatic and germ cells, increasing sperm maturation process, and progressive sperm motility, just like the activation of essential factors.[10] However, there are no guidelines to suggest the more appropriate incubation time or temperature. Previous studies showed that 24–48 h was an optimal time for the development of motility.[4] On the other hand, long-term culturing could increase the effect of reactive oxygen species (ROS) which may lead to sperm DNA damages and increase the structural/chromosomal anomalies. In our experience, after 24 h in vitro culture, sperm cells seem to have a successful increasing motility rate (from 0% to 10%), so much that we selected those more viable and proceed to ICSI procedure.

Furthermore, since the couple had also a female infertility factor, enough to minimize the successful of ICSI, a clinical pregnancy was achieved. Indeed, the diminished ovarian reserve proved to be not associated with increased miscarriage among younger women.[11] Equally, elevated FSH values is associated with a higher risk of in vitro fertilisation (IVF) pregnancy loss only among older patients.[12] In this case, a personalized stimulation protocol gave the

chance to have almost two follicles, reducing the risk of cancelled cycle.

Conclusion

Performing a modified TEFNA allowed to obtain more testicular tissue and to increase the chancing of retrieving spermatozoa. Testicular sperm in vitro culture for 24 h proved to be a real and practical method to increase sperm motility, in order to select mature and viable spermatozoa and improve successful ICSI outcomes. Optimizing and personalizing stimulation protocol in the woman and sperm retrieving technique in the man was the strength that gave chance of pregnancy, particularly in presence of ethical or religious reasons.

Declaration of conflicting interests

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