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CASE REPORT

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Are hormone measurements and ultrasounds really predictors of sperm retrieval in testicular sperm extraction? A case report and literature review

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Summary

Azoospermia can be diagnosed in about 10%–15% of the infertile male population. To overcome the problem of failure to produce spermatozoa in the ejaculate in patients with nonobstructive azoospermia (NOA), testicular sperm extraction (TESE) may be performed to find the focal area of spermatogenesis. A 47-year-old man with NOA presented for treatment of secondary couple infertility. The patient underwent a first TESE 7 years earlier with cryopreservation, and an intracytoplasmic sperm injection–embryo transfer ended in a term pregnancy. He reported a history of repeated testicular traumas. At the present time, a complete medical workup was carried out, including clinical history, general and genital physical examination, scrotal and transrectal ultrasounds. Hormone measurements showed follicle-stimulating hormone level of 42.7 IU/L, luteinising hormone of 11.4 IU/L, total testosterone of 2.6 ng/ml and right and left testicular volume, respectively, of 4 and 3.9 ml. He underwent a second TESE, with successful sperm retrieval and cryopreservation. The histological pattern was hypospermatogenesis. In cases of extreme testicular impairment, although in the presence of very high follicle-stimulating hormone value and small testicular volume, estimating poor sperm recovery potential, the integration of clinical and anamnestic data, could help the surgeon to practise the more appropriate method of treatment.

KEYWORDS

azoospermia, male infertility, sperm retrieval, testicular sperm extraction

1 | INTRODUCTION

Azoospermia is characterised by the complete absence of spermatozoa in the ejaculate (World Health Organization, 2010) after assessment of centrifuged semen on at least two occasions. It can be diagnosed in about 1% of men and in 10%–15% of the infertile male population (Jarow, Espeland, & Lipshultz, 1989; Willott, 1982). Sperm recovery after testicular sperm extraction (TESE) for infertility treatment by intracytoplasmic sperm injection (ICSI) is the main opportunity to father their own biological child in cases of nonobstructive azoospermia (NOA) (Hessel et al., 2015). An unsuccessful sperm retrieval may happen in up to 57% of TESE attempts (Devroey et al., 1995; Friedler et al., 1997; Rosenlund et al., 1998; Schlegel et al., 1997).

Focal testicular spermatogenesis may explain the failure rates of these procedures (Silber, 2000). The seminiferous tubules of patients with NOA display differing degrees of deficient spermatogenesis, including hypospermatogenesis, Sertoli cell-only syndrome

(SCOS) and maturation arrest (MA) (McLachlan, Rajpert-De Meyts, Hoei-Hansen, de Kretser, & Skakkebaek, 2007; Salehi et al., 2017). Physical examination, detailed medical history, hormonal analysis and genetic studies can help the clinician to determine the type of azoospermia and predict the chance of sperm retrieval (Schlegel, 2004). Particularly, testis histology has been demonstrated to be the most reliable predictive factor of successful sperm retrieval (SSR) in NOA patients, since the late nineties (Tournaye et al., 1997). It has been proposed that the previously obtained testicular histology could help in predicting SSR in a further surgical attempt, in all cases with previous testicular surgery and unsuccessful sperm retrieval (Abdel Raheem et al., 2013; Sokmensuer et al., 2015).

2 | CASE REPORT

A 47-year-old Caucasian man referred to the Assisted Reproductive Technologies (ARTs) Centre of the University of Florence (Italy), for treatment of secondary couple infertility. The female had 38 years old and presented no ongoing pathology. In the past, 7 years earlier, the patient underwent a TESE procedure with positive sperm recovery and cryopreservation of 10 Cryo Bio System straws. The sperm concentration was 0.1×10^6/ml, and the total sperm motility was 5%. Subsequently, the couple performed a first cycle of ICSI embryo transfer with a pregnancy ended in abortion. A second ICSI cycle was successful and concluded in a term pregnancy of a healthy baby. At the time of first TESE, the man performed semen analysis that detected an absolute azoospermia, also after semen centrifugation. Serum hormone measurements were collected, showing a follicle-stimulating hormone (FSH) value of 24.5 IU/L, with a range 1.5–8.0 IU/L being taken as normal. Serum luteinising hormone (LH) was 8.23 IU/L, total testosterone (TT) was 3.5 ng/ml, considering in order the normal range 1.0–9.0 IU/L and 3.0–10.6 ng/dl respectively. 17 beta-estradiol was <20 pcg/ml, considering a regular reference value less than 51.1 pcg/ml. Prolactin was 23.36 ng/ml (normal range 3–18 ng/ml) (Table 1). Scrotal and tranrectal ultrasounds performed, revealed the absence of abnormalities in the seminal tract, a right testicular volume of 6 ml and a left testicular volume of 5 ml. Genetic studies were negative for alterations, including the chromosome analysis of the karyotype with the examination of microdeletions for chromosome Y and mutation of cystic fibrosis transmembrane conductance regulator (CFTR) genes. The histopathological analysis of a single testicular biopsy taken simultaneously with TESE showed a pattern of hypospermatogenesis.

Therefore, at the present time, the couple presented a history of secondary couple infertility for about 6 years. The couple was sent to us after a complete medical workup, including a woman gynaecological evaluation, confirming the absence of ongoing pathologies and a clinical andrology consultation. Newly, general and genital physical examination, semen analysis and endocrin profile were performed. The semen analysis, on at least two determinations, after sedimentation, confirmed the diagnosis of absolute azoospermia. A clinical history was recorded, including weight and height with body mass index (BMI) count of 24.7. The patient had a normal sexual development and a physiologic descended of both testes in the scrotum. He did not suffer from medical diseases, such as diabetes or cardiovascular disease. There was no prevalence of smoking and drinking, history of mumps orchitis, varicocele, previous chemotherapy or radiotherapy, hormonal therapy or exposure to gonadotoxins. The patient reported a history of repeated testicular traumas during the past years, since childhood. On physical examination, testicular volume measurement was evaluated with the Prader orchidometer scale, showing a right testicular volume of 4 ml and left testicular volume of 3 ml. Scrotal and transrectal ultrasounds (US) were performed to evaluate the current testicular volume bilaterally. It was detected a small right-sided testis, measuring 27 × 15 × 17.7 mm (volume of 4 ml) with inhomogeneous echogenicity and a 10 mm epididymal head with an inhomogeneous hypoechoic echotexture (Figure 1), while left-sided testis measured 32 × 13 × 17.7 mm (volume of 3.9 ml) (Figure 2). How showed in Table 1, FSH value was much higher than previously (42.7 IU/L). LH was 11.4 IU/L, TT was 2.6 ng/dl, 17 beta-estradiol concentration was 27 pcg/ml, and prolactin was 17.4 ng/ml.

TABLE 1 Parameters of the patient at the time of first and second testicular sperm extraction

Parameter	First TESE	Second TESE
FSH (IU/L) [normal range]	24.5 [0.9–15]	42.7 [1.5–8.0]
LH (IU/L) [normal range]	8.23 [2.4–5.9]	11.4 [1.0–9.0]
TT (ng/ml) [normal range]	3.5 [3.0–14]	2.6 [3.0–10.6]
Prolactin (ng/ml) [normal range]	23.36 [3.0–30]	17.4 [3.0–18]
17 beta-estradiol (pcg/ml) [normal range]	<20 [5.0–56]	27 [5–51.1]
TV left (ml)	5	3.9
TV right (ml)	6	4
Bio system straws cryopreserved	10	14
Sperm concentration ($\times 10^6$/ml)	0.1	0.01
Sperm motility (%)	5	1

FSH, follicle-stimulating hormone; LH, luteinising hormone; TT, total testosterone; TV, testicular volume; TESE, testicular sperm extraction.

He was diagnosed with NOA and nevertheless repeated conversations with the patient about the poor sperm recovery potential, we decided to undergo a conventional bilateral TESE procedure. The surgical procedure has been performed under a spinal anaesthesia. An informed consent was obtained from the patient. A 2.5-cm median raphe scrotal incision was made in the scrotum, tunica vaginalis opened, and both the testicles delivered through the incision. The left testicle showed the scarring of the previous TESE and a condition of widespread vaginalitis (Figure 3). One single large transversal incision was made on an avascular

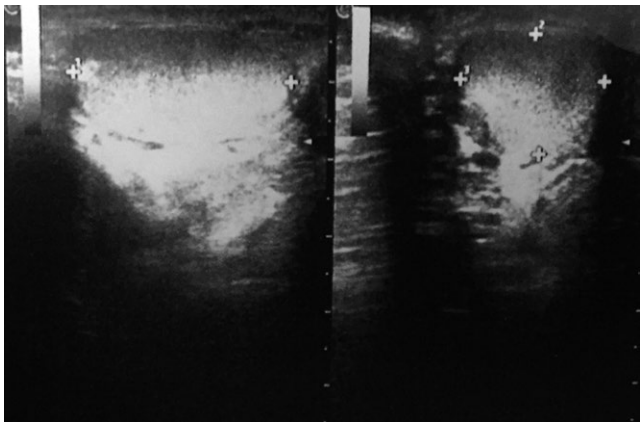

FIGURE 1 Scrotal ultrasound image of right testis revealing an inhomogeneous echogenicity

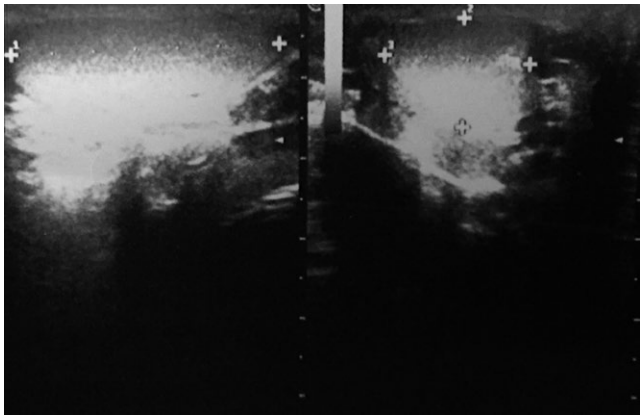

FIGURE 2 Scrotal ultrasound image of left testis

area of the tunica albuginea, at the equatorial portion of each testis, performing a hinged opening and widely exposing the testicular parenchyma. Surgical loupes with a magnification power of 6× were used during the surgical procedure. Seminiferous tubules were sharply excised from areas with enlarged tubules of each testis, which were more likely to contain germ cells. A randomly taken sample of testicular parenchyma was fixed in 4% Formaldehyde solution and sent for histological findings. The fragments were washed in human flushing solution with heparin to remove the blood and were immediately transferred to the embryologist for the microscopic examination. The incisions of tunica albuginea were repaired using a continuous suture with Vicryl 5-0 polypropylene (Figure 4). Tunica vaginalis was close with a running Vicryl 4-0 absorbable monofilament after instillation into the vaginalis cavity of 80 mg gentamicin/100 ml to prevent pain and tunica vaginalis adhesions. Patients were closely observed and were discharged from the hospital after few hours. Each testicular tissue fragment was placed in sterile Petri dishes with 2.5 ml of flushing buffered medium and stretched by sterile slides under a stereomicroscopy and repeatedly sucked through a 20-G safety venous catheter to squeeze tubules. Subsequently, the suspension was directly observed under inverted microscopy at 200× to

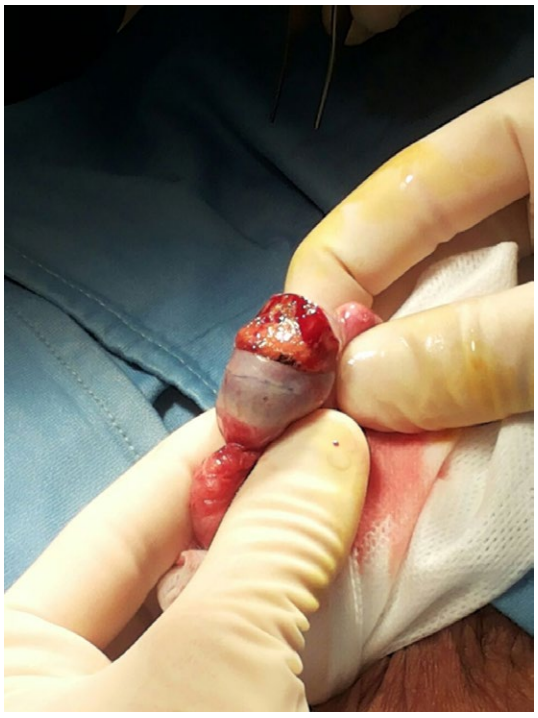

FIGURE 3 Image of surgical procedure showing the scarring of previous testicular sperm extraction

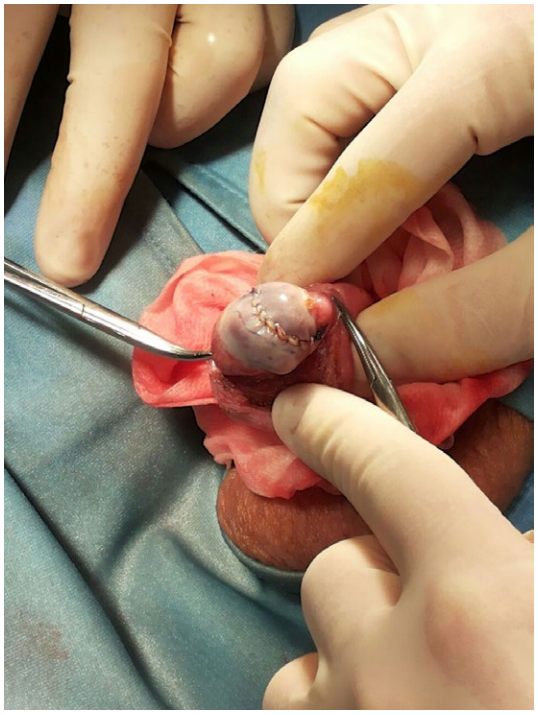

FIGURE 4 Reparation of tunica albuginea using a continuous suture 5/0 Vicryl

a first evaluation of the presence of spermatozoa. Afterwards, all the suspension was transferred to tubes and, after sedimentation of solid tissue, the supernatant containing free sperm cells were

processed by centrifugation at 1800 rpm for 10 min. The pellet was suspended another time in a flushing medium, and a large representative drop was observed on a Petri dish under inverted microscopy at 200×. The embryological processing showed 1–3 spermatozoa/field (corresponding to an estimated concentration of 0.01×10^6 spermatozoa/ml). Consequently, sperm retrieval was considered to be successful for cryopreservation and a subsequent use. Thus, a total of 14 Cryo Bio System straws were cryopreserved. The sperm concentration was 0.01×10^6/ml, and the total sperm motility was 1%. The female partner underwent a pickup procedure: 10 oocytes were retrieved; eight oocytes were injected by ICSI. From three "two pronuclear" (2PN) fertilised oocytes, the transfer of two embryos was performed. At the fourteenth day after embryo transfer serum beta HCG no increased.

The histopathological examination confirmed a pattern of hypospermatogenesis, with spermatogenesis strongly impaired, characterised by seminiferous tubules atrophy and sclerosis (Figure 5). No evidence of germ cell neoplasia in situ (GCNIS) was found.

3 | DISCUSSION

We report a rare clinical case of positive sperm retrieval in a man presenting a severe impairment of spermatogenesis and a very poor sperm recovery potential. Our aim was to assess the effective power of some predictive factors of sperm retrieval (SR) in NOA patients with an extreme bilateral testicular impairment.

In front of a man with a severe degree of NOA, it is fundamental to evaluate all clinical parameters that may predict the chance of SR, in order to have a more appropriate counselling of the azoospermic patient. In fact, the evaluation of genetic factors, hormones, ultrasounds and histopathological status of patients could guide the surgeons in the correct management.

Salehi et al. supported that high levels of FSH and small testicular volume were significantly associated with lower chances of SR, while age, testosterone and prolactin serum levels not. In fact, the levels of FSH were significantly higher in the SCOS and MA groups (respectively, in mean 22.59 and 18.27 IU/L) when compared to the hypospermatogenesis group (9.73 IU/L) (Salehi et al., 2017). Also Aydin, Sofikerim, Yucel, Karadag, and Tokat (2015) demonstrated a significantly higher level of FSH and LH in patients with SCOS.

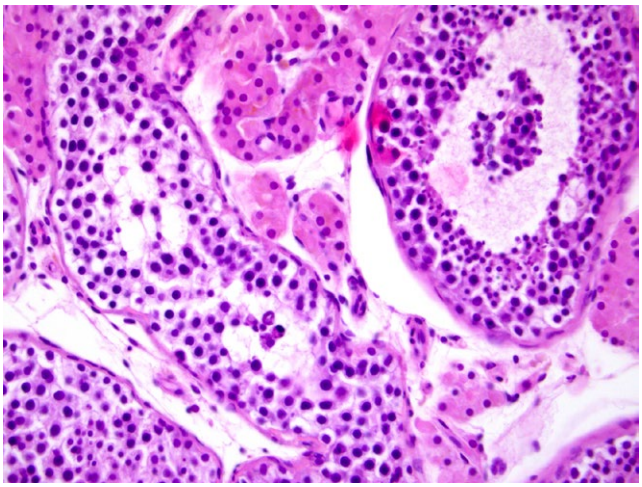

FIGURE 5 Histological evaluation of testicular biopsy revealing a pattern of hypospermatogenesis

Caroppo et al. affirmed that patients with SSR had significantly lower serum FSH level (in mean 16.1 IU/L) and higher testicular volume (8.7 ml) compared with sperm retrieval failure, in which FSH value had an average of 22.4 IU/L and a testis volume had a mean of 7.2 ml (Caroppo et al., 2017).

On the other hand, several studies evaluating large cohorts of patients showed that serum FSH concentration and testicular volume are not predictive of SSR when calculated as the unique independent predictive factor (Bryson et al., 2014; Ramasamy et al., 2009, 2013). Indeed, a linear correlation between FSH serum level and spermatogenesis not always can be expected in all patients, because FSH secretion and release are controlled by too many endocrine and paracrine factors that ultimately contribute to its serum levels. In addition, the polymorphisms in FSHB genotype and FSHR genotype can contribute to higher than expected FSH levels in some patients (Tuttelmann et al., 2012). This data could explain the poor relationship between FSH level and sperm retrieval of our patient.

However, only few cases are reported in the literature (Ballescá et al., 2000; Brugo-Olmedo et al., 2001; Guler et al., 2016; Nagata et al., 2005) in which there was a positive sperm recovery in the presence of very high FSH values (Table 2).

Moreover, how explained by Ramasamy et al., in some cases, testicular volume had a poor predictive role, due to the presence of focal area of intact spermatogenesis that could be found even in patients with a severe testicular atrophy (Berookhim, Palermo, Zaninovic, Rosenwaks, & Schlegel, 2014; Ramasamy et al., 2009).

Our patient reported FSH values well above the normal range, which is due to a severe insufficiency of spermatogenesis, according to the markedly reduced testicular volume. Therefore, we considered the chance of sperm recovery as drastically reduced to the minimum, so to recommend at first a heterologous fertilisation, but the patient was particularly motivated to father an own biological child. As widely demonstrated in previous studies (Caroppo et al., 2017; Seo & Ko, 2001; Weedin, Bennett, Fenig, Lamb, & Lipshultz, 2011; Yang et al., 2008), the histopathological patterns could be considered as

TABLE 2 Maximum follicle-stimulating hormone level in which there was successful sperm retrieval

Studies	Serum FSH concentration (IU/L)
Caroppo et al. (2017)	58
Guler et al. (2016)	21.4
Nagata et al. (2005)	45.1
Brugo-Olmedo et al. (2001)	46.3
Ballescá et al. (2000)	18.3

FSH, follicle-stimulating hormone.

a predictive factor of SR. Accordingly, the possibility of SR is higher in patients with hypospermatogenesis compared with patients with MA and SCOS. Salehi et al. have shown an overall mean rate of SR of 48.8% in patients with NOA, with rates of unsuccessful sperm retrieval significantly higher in the MA and SCOS groups rather than in the hypospermatogenesis group. In accordance with the findings of a recent study conducted by Saccà et al. (2016), when histology result differed from hypospermatogenesis, the likelihood of SSR decreased.

However, the application of testicular histology as a predictive factor has limitations in clinical practice, because these criteria can only be applied to those patients with previous testicular surgery, considering the invasiveness of a diagnostic testicular biopsy.

We performed a conventional TESE and not a micro-TESE, according to the results of some systematic reviews, that suggested similar sperm retrieval rates between the two techniques in patients with hypospermatogenesis and better rates of SR only in men with SCOS or MA (Deruyver, Vanderschueren, & Van der Aa, 2014; Donoso, Tournaye, & Devroey, 2007).

4 | CONCLUSIONS

There are predictive factors of SR, before performing TESE, that allow an estimate of sperm recovery possibilities, including histological findings, FSH value and testicular volume. However, when exists an extreme testicular impairment and consequently a damage of spermatogenesis, the integration of clinical and anamnestic data, as in our case a previous TESE with a term pregnancy and the histological pattern of hypospermatogenesis, can help the surgeon to practise the more appropriate method of treatment.

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