Original Research Article

UJ Urologia Journal

Relevance of testicular histopathology on prediction of sperm retrieval rates in case of non-obstructive and obstructive azoospermia

Urologia Journal
1–8

DOI: 10.1177/0391560318758940
journals.sagepub.com/home/urj
SAGE

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Abstract

Introduction: The aim of our research was to establish the relevance of testicular histopathology on sperm retrieval after testicular sperm extraction in patients with non-obstructive azoospermia and in patients with obstructive azoospermia, who already underwent a previous failure testicular fine needle aspiration.

Methods: We evaluated a total of 82 azoospermic men, underwent testicular sperm extraction, referring to the Assisted Reproductive Technology Centre of the University of Florence, Italy between January 2008 and March 2017. A general and genital physical examination, scrotal and trans-rectal ultrasound, semen analysis, hormone measurements, including follicle-stimulating hormone, luteinizing hormone and total testosterone, were collected.

Results: Successful sperm retrieval was obtained in 36 men of total (43.9%). Successful sperm retrieval was 29.5% in non-obstructive azoospermia patients, while men with obstructive azoospermia, who, underwent a previous failure testicular fine needle aspiration, had sperm retrieval in 86% of cases. Mean luteinizing hormone was 6.55 IU/L, total testosterone 4.70 ng/mL, right testicular volume 13.7 mL and left testicular volume 13.6 mL. Mean Follicle-stimulating hormone was 13.45 IU/L in patients with negative sperm retrieval and 8.18 IU/L in men with successful sperm retrieval. According to histology, 20.7% had normal spermatogenesis, 35.3% hypospermatogenesis, 35.3% maturation arrest and 8.5% Sertoli cell-only syndrome. Successful sperm retrieval was 88.2% in patients with normal spermatogenesis, 24.1% in the maturation arrest group and 48.27% in patients with hypospermatogenesis, while negative sperm retrieval was reported in Sertoli cell-only syndrome patients. Seven cases with maturation arrest showed a successful sperm retrieval.

Conclusion: Testicular histopathology after testicular sperm extraction offers important information on prediction of sperm retrieval and can guide the surgeon in choosing the more suitable therapeutic practice.

Keywords

Azoospermia, male infertility, testicular sperm extraction, testicular histology, sperm retrieval

Date received: 12 December 2017; accepted: 2 January 2018

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Introduction

Azoospermia is defined by the complete absence of spermatozoa in the ejaculate (World Health Organisation, 2009) after assessment of centrifuged semen on at least two analyses. It can be diagnosed in about 1% of men and in 10%–15% of the infertile male population.[1] Known genetic factors are responsible for approximately one-third of cases of azoospermia. Nonetheless, at least 40% of cases are currently categorised as idiopathic and may be linked to unknown genetic abnormalities.[2] Using assisted reproducing techniques, in particular intracytoplasmic sperm injection (ICSI), in combination with fresh or thawed samples from testicular sperm extraction (TESE), azoospermic patients have the opportunity to use the sperm retrieved from their own testis and so be able to father their own biological child.

Azoospermia is classified as obstructive azoospermia (OA) and non-obstructive azoospermia (NOA), each having different aetiologies and treatments. OA occurs as result of a blockage in the epididymis, vas deferens or ejaculatory ducts in which spermatogenesis is normal,[3] while NOA is an intrinsic, often idiopathic, testicular deficiency of sperm production and it represents 60% of all cases of azoospermia.[4]

Usually, the management of patients with OA relies on testicular fine needle aspiration (TEFNA), while patients with NOA undergo a surgical TESE followed by ICSI.[5,6] Alternatively, the retrieved sperm can be cryopreserved for use in future sperm injection attempts.[7,8]

Spermatozoa that were recovered from the testis of azoospermic patients were found to be as effective as ejaculated spermatozoa in ICSI cycles.[9–11] ICSI involves treatment for both partners, that is, the man undergoes surgery for testicular sperm recovery and the woman undergoes ovarian stimulation and possibly oocyte retrieval. An unsuccessful sperm recovery procedure, therefore, has important emotional and financial implications. While sperm retrieval rates (SRRs) for men with OA are excellent (96%–100%), the reported overall SRR for patients with NOA ranges from 30% to 60%.[6] Therefore, it is important to evaluate all clinical parameters that may predict the possibility of sperm retrieval (SR), because they would be indispensable for assisting surgeons and clinicians in the correct counselling of the azoospermic patient.[12] In fact, physical examination, detailed medical history, hormonal analysis and genetic studies will help to determine the type of azoospermia.[13]

Actually, testis histology has been found to be the most reliable predictive factor of successful sperm retrieval (SSR) in NOA patients since the late 1990s.[14] It has been proposed that the previously obtained testicular histology could help in predicting SSR in further surgical attempts, in all cases with previous testicular surgery and unsuccessful SR.[15,16] The aim of our study is to assess the importance of testicular histopathology on SRRs after a conventional bilateral multiple TESE in azoospermic patients with NOA and in men with OA form, in which a previous TEFNA failed.

Material and methods

We retrospectively analysed 82 azoospermic patients, referring to the Assisted Reproductive Technology (ART) Centre of the University of Florence (Italy) between January 2008 and March 2017. The couples were sent to us after a complete medical work up including a woman gynaecological evaluation, a clinical andrology consultation and a genetic visit. General and genital physical examination, semen analysis, serum hormone measurements and genetic studies were performed. The presence of azoospermia was confirmed using at least two semen analyses with proper timing for all patients with centrifugation. The karyotype with the examination of microdeletions for chromosome Y and the research for mutation of cystic fibrosis transmembrane conductance regulator (CFTR) genes were done. Serum follicle-stimulating hormone (FSH) concentrations were measured for each patient, with a value in the range of 1.5–8.0 IU/L being taken as normal. Also serum luteinizing hormone (LH) and total testosterone (TT) were evaluated, considering in order the normal range of 1.8–12 IU/L and 2.7–18 ng/mL. A clinical history was recorded, including age, weight and height with body max index (BMI) count, prevalence of smoking and drinking, history of cryptorchidism, mumps orchitis, varicocele, previous chemotherapy or radiotherapy, surgical procedures, hormonal therapy or exposure to gonadotoxins. All patients underwent scrotal and transrectal ultrasounds to evaluate testicular volume (TV) and to exclude the presence of epididymis head or tail dilatation, unilateral or bilateral absence of vas deferens, ejaculatory duct anomalies, prostate median cysts or seminal vesicles enlargement. We have considered OA forms, for those patients who come to our attention with an ultrasound presentation of hypoplasia or agenesis in the seminal tracts, showed by an ultrasounds findings of congenital bilateral absence of the vas deferens (CBAVD). These patients although in presence of FSH values and TV approximately in the normal range, reported an unsuccessful SR through TEFNA, according to an idiopathic secretive component. Among these patients, seven reported a CFTR mutation and one a history of anejaculation due to a medullary lesion. They had a mean FSH value of 4.52 IU/L (range 1.01–8.8 IU/L), TV right of 16.8 mL (range 9–23 mL) and TV left of 16.42 mL (range 9–24 mL).

TV between 3 and 11 mL and higher FSH levels (>8.0 IU/L) were suggestive of NOA. Most of NOA patients had a history of cryptorchidism, almost all had varicocele, in some cases corrected with surgery. For each patient, we evaluate the presence of positive SR at the time of TESE and the histologic response of testicular biopsy obtained a few days later from the histopathology

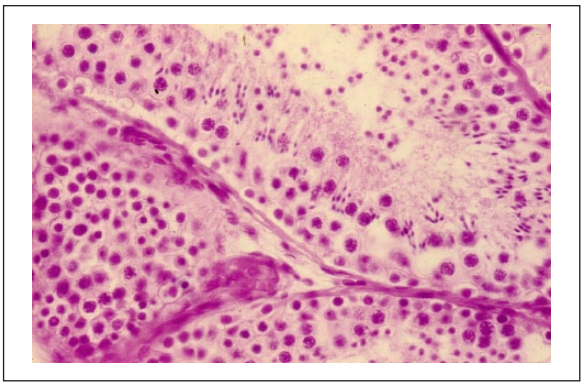

Figure 1. Normal spermatogenesis (NS).

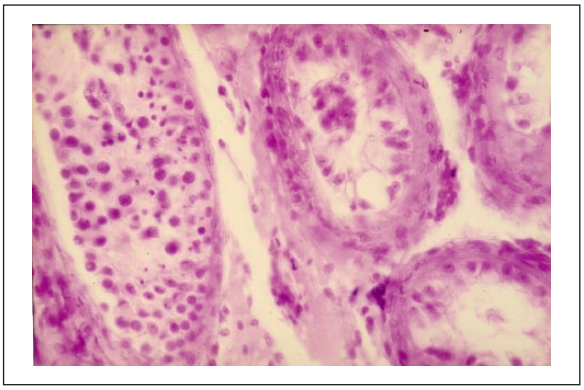

Figure 2. Hypospermatogenesis (HYPO).

laboratory. Each patient signed a written fully informed consent statement before inclusion in the study. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1975 Helsinki Declaration. Institutional review board (IRB) approval was not obtained because the study concerned a retrospective analysis that did not require any exam in addition to the normal clinical practice, without any impact on patients during the study.

TESE procedure

All patients underwent a multiple bilateral TESE procedure, under an analgo-sedation. A 1.5-cm median raphe scrotal incision was made in the scrotum, tunica vaginalis was opened and both the testicles were delivered through the incision. Three different transversal incisions were made in tunica albuginea, at equatorially, medium and the cranial part of each testis. Seminiferous tubules will spontaneously extrude with testicular pressure and two large samples of roughly $8 \times 5 \times 3$ mm were sharply excised from each side. A representative sample of each picked specimen was fixed in 4% formaldehyde solution and sent for analysis to the histopathology laboratory. The fragments were washed in human flushing solution with heparin to

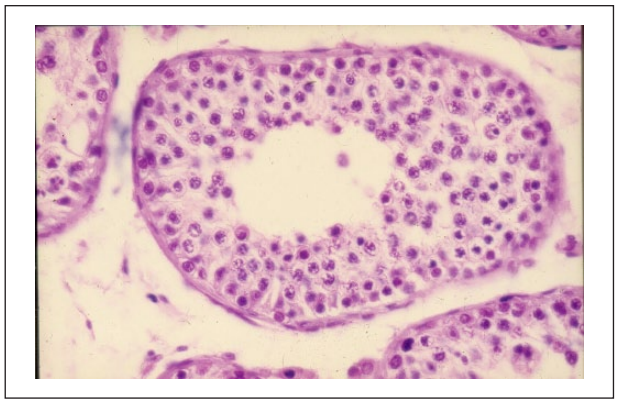

Figure 3. Maturation arrest (MA).

remove the blood and were immediately transferred to the embryologist for the microscopic examination. The incisions of tunica albuginea were repaired using a suture with Vicryl 5-0 polypropylene. Haemostasis was then performed by gently pressing the testicular tissue using gauze wet with Ringer's solution and a very careful bipolar micro-coagulation. Tunica vaginalis was sutured 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. Each testicular tissue fragment was placed in sterile Petri dishes with few millilitre of washing buffered medium and stretched by sterile slides under a stereomicroscopy. Afterwards, the samples were observed under inverted microscopy at 200× to evaluate the presence and number of spermatozoa. When the embryological observation showed more than 2–3 sperms/field (corresponding to an estimated concentration of 0.01×10^6 spermatozoa/mL), the SR was considered to be successful for use. The next step was ICSI if the woman underwent an ovum pick-up procedure simultaneously, otherwise cryopreservation for future use in ICSI. Patients were closely observed and were discharged from the hospital after few hours.

Histological assessment

The histological evaluation of all testis samples was performed by the same pathologist, expert in the uro-andrology field. The tissue and cells sections were stained with haematoxylin and eosin (H&E) colouration and magnified at 20×. Based on the principal histopathological pattern, testicular histology was classified as follows: normal spermatogenesis (NS) as shown in Figure 1; hypospermatogenesis (HYPO), in which there was a reduction in the number of normal spermatogenetic cells, as pictured in Figure 2; maturation arrest (MA), characterised with the absence of the later stages of spermatogenesis (Figure 3); Sertoli cell-only syndrome (SCOS), when there were exclusively Sertoli cells (Figure 4). The evaluation included also the research of germinal cell neoplasia in situ (GCNIS) of the testis.

Statistical analysis

Person's chi-squared and Student's t-test for independent samples was used. Statistical significance in this study was set as $p < 0.05$. Binary and multinomial logistic regression models were evaluated for histopathology and SR as the response variables, respectively, as shown in Table 3 and Table 4. Analyses were performed with IBM SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

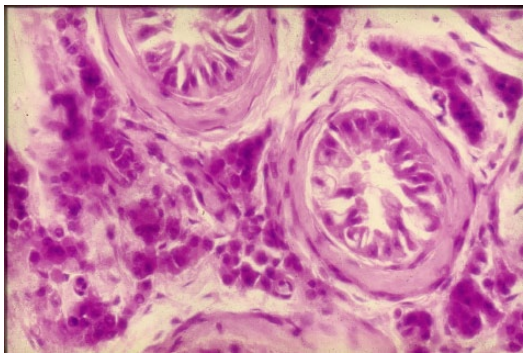

Figure 4. Sertoli cell-only syndrome (SCOS).

Table 1. Baseline characteristics of men with NOA and OA who underwent TESE (total n = 82).

Parameter	Mean	Standard deviation
Age (years)	37.7 (27.0–52.0)	±5.7
FSH (IU/L)	11.14 (1.01–47.80)	±8.86
LH (IU/L)	6.55 (1.2–36.0)	±5.37
TT (ng/mL)	4.7 (1.44–9.5)	±1.67
TV right (mL)	13.7 (3.4–25.0)	±5.0
TV left (mL)	13.6 (1.0–30.0)	±5.3
Weight (kg)	80.8 (47.0–136.0)	±13.6
Height (cm)	177 (150–193)	±7
BMI	22.8 (15.7–38.4)	±3.5

NOA: non-obstructive azoospermia; OA: obstructive azoospermia; TESE: testicular sperm extraction; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TT: total testosterone; TV: testicular volume; BMI: body mass index.

Results

We collected data of 82 azoospermic patients, of which 61 patients (74.4 %) had a clinical presentation of NOA, while 21 (25.6%) presented OA. All the men with OA performed a previous TEFNA with a negative sperm recovery. The patients had a mean age of 37.7 years (range 27–52 years). The mean weight was 80.8 kg and the mean height was 177 cm, with a mean BMI calculated of 22.8 (range 15.7–38.4). Prevalence of smoking and drinking was only in 16 patients (19.5%), while the majority (80.4%) did not ever smoke and/or drink in their life. Eighteen patients had a history of cryptorchidism, 33 men had varicocele, of which eight underwent varicocelectomy. Other possible type of previous surgery was inguinal hernia repair and hydrocele. Two patients had chromosome abnormalities in karyotype, in particular a Robertsonian translocation (13–14 and 20–21). Ten patients had a CFTR gene abnormality, such as the more common poly T tract 7T/7T anomalies. Four patients had Y-chromosome azoospermia factor (AZF) region's microdeletions. All OA patients (21/82) had an ultrasound presentation of CBAVD.

Positive SR was obtained in 36/82 men (43.9%). In patients with a clinical presentation of OA form (21/82), who underwent a previous failure TEFNA, the SR percentage with TESE was 86%, while in patients with NOA (61/82) was 29.5%. In 82 men (Table 1), mean FSH serum level was 11.14 ± 8.86 IU/L (range 1.01–47.8 IU/L), mean LH serum level was 6.55 ± 5.37 IU/L (range 1.2–36.0 IU/L), TT levels were in mean 4.70 ± 1.67 ng/mL (range 1.44–9.5 ng/mL) and the mean right testicular volume was 13.7 ± 5.0 mL (range 3.4–25 mL), while the mean left testicular volume 13.6 ± 5.3 mL (range 1.0–30.0 mL). FSH mean level was 13.45 ± 8.45 IU/L in patients with unsuccessful sperm recovery (46/82), whereas patients with SSR (36/82) had a mean FSH concentrations of 8.18 ± 7.11 IU/L (Table 2). As shown

Table 2. Comparison of clinical parameters, hormonal plasma concentrations parameters and testicular volume with sperm retrieval in azoospermic patients after TESE.

Parameter	Positive sperm retrieval	Negative sperm retrieval	*p* value
Patients, n (%)	36 (43.9%)	46 (56.0%)	
Age mean, years (SD)	37.8 (±5.7)	37.5 (±5.7)	0.79
BMI mean (SD)	22.3 (±3.1)	23.2 (±3.8)	0.28
FSH mean, IU/L (SD)	8.18 (±9.45)	13.4 (±7.11)	*<0.005*
LH mean, IU/L (SD)	5.65 (±5.2)	7.25 (±5.44)	0.18
TT mean, mL (SD)	4.57 (±1.57)	4.86 (±1.79)	0.43
TV right mean, mL (SD)	15.7 (±4.92)	12.1 (±4.62)	*<0.005*
TV left mean, mL (SD)	15.3 (±5.82)	11.9 (±4.65)	*<0.005*

TESE: testicular sperm extraction; BMI: body mass index; FSH: follicle-stimulating hormone; LH: luteinizing hormone; TT: total testosterone; TV: testicular volume; SD: standard deviation. Significant at *$p<0.05$.

by the logistic regression model in Table 3, the high levels of FSH and reduced testicular size were significantly associated with lower potential of SSR.

Histological result of NS was obtained in 17/82 patients (20.7%), while 29/82 patients (35.3%) had HYPO, 29/82 patients (35.3%) had MA and 7/82 patients (8.5%) had SCOS. There were no cases of GCNIS. Testicular histology was a significant predictive model of SR. SRRs were 88.2% in patients with NS, 24.1% in the MA group and 48.27% in patients with HYPO, while negative SR was reported in all patients with SCOS. As shown in Table 4, FSH levels were significantly in excess of the normal ranges in the MA and SCOS when compared to the NS group (MA:OR (odds ratio), 1.368, p 0.017; SCOS:OR, 1.629, p 0.002; HYPO:OR 1.026, p 0.081). No associations were observed among age, LH, TT and testis size. In 7/82 (8.5%) cases with a result of MA, the embryological analysis showed a positive sperm recovery.

Table 3. Multiple logistic regression model with sperm retrieval as the response variable (using successful sperm retrieval as the reference category).

Factor	β	SE	OR	95% CI for OR lower bound	95% CI for OR upper bound
Age	−0.003	−0.003	0.997	0.908	1.095
FSH	0.052	0.038	1.054*	0.978	1.136
LH	0.015	0.049	1.015	0.923	1.116
TT	0.043	0.165	1.044	0.756	1.443
TV right	−0.028	0.115	0.972*	0.775	1.219
TV left	−0.111	0.107	0.895*	0.725	1.103

FSH: follicle-stimulating hormone; LH: luteinizing hormone; TT: total testosterone; TV: testicular volume; SE: standard error; OR: odds ratio; CI: confidence interval.
Significant at *$p < 0.05$.

Discussion

The present study confirms that testicular histology may be a notable predictive factor for SR. In our analysis, men with a clinical presentation of NOA underwent directly TESE. In fact, open testicular biopsy appears to be more efficient than TEFNA for the recovery of testicular spermatozoa in patients with NOA.[14] TESE is an invasive procedure with a, albeit small, risk of complications, including haematoma and loss of a significant amount of testicular tissue.[1,14] However, patients who had OA form, previously performed TEFNA and after a SR failure, underwent a TESE procedure.

According to the literature, we performed to all patients a bilateral multiple TESE. In a study by Plas et al.,[17] they showed that bilateral testicular biopsies are better than unilateral biopsies in the evaluation of azoospermic men.

A testicular biopsy specimen may not also reflect entire testicular tissue, bilaterally; in a study, 28% intra-individual difference in testicular histology was documented in bilateral testicular biopsy specimens.[17,18]

Table 4. Multinomial logistic regression model with histology as the nominal response variable (using normal spermatogenesis as the reference category).

Histology	Factor	β	SE	OR	95% CI for OR lower bound	95% CI for OR upper bound
MA	Age	−0.72	0.080	0.930	0.795	1.088
	FSH	0.313	0.131	1.368*	1.058	1.768
	LH	0.115	0.175	1.122	0.796	1.582
	TT	0.472	0.284	1.604	0.919	2.80
	TV right	−0.141	0.177	0.869	0.614	1.229
	TV left	−0.12	0.165	0.988	0.716	1.365
SCOS	AGE	−0.102	0.122	0.903	0.712	1.144
	FSH	0.488	0.155	1.629**	1.203	2.206
	LH	−0.025	0.271	0.975	0.574	1.658
	TT	0.439	0.417	1.551	0.685	3.51
	TV right	−0.048	0.279	0.953	0.551	1.649
	TV left	0.85	0.254	1.089	0.662	1.792
HYPO	AGE	0.025	0.073	1.026	0.889	1.184
	FSH	0.224	0.128	1.252	0.973	1.610
	LH	0.127	0.173	1.135	0.808	1.594
	TT	0.160	0.270	1.174	0.691	1.994
	TV right	−0.149	0.163	0.862	0.626	1.186
	TV left	0.093	0.151	1.098	0.817	1.475

FSH: follicle-stimulating hormone; LH: luteinizing hormone; TT: total testosterone; TV: testicular volume; MA: maturation arrest; SCOS: Sertoli cell-only syndrome; HYPO: hypospermatogenesis; SE: standard error; OR: odds ratio; CI: confidence interval.
Significant at *$p < 0.05$; **$p < 0.005$.

It is crucial to offer to couples, at the first consultation, the chances of SR, because an unsuccessful TESE can have important emotional and financial implications. This may be reduced by having preoperative knowledge of the likelihood of success.[19,20] However, there are no absolute defined prognostic parameters or tests that can accurately predict the spermatogenesis presence in azoospermic patients.[21,22]

FSH concentrations and TV are good clinical parameters for the prediction of SR but less accurate than testicular histopathology.[23] This is supported by the fact that many patients with MA are found to have normal plasma FSH levels and TV.[24] Variations in FSH concentrations can occur for reasons unrelated to spermatogenesis, first of all that the FSH secretion and release is controlled by too many endocrine and paracrine factors.[25] Although the smaller TV is associated with the worse possibility of SR, there is no minimum limit of TV that predicts the presence of spermatozoa.[22] Previous studies also reported that higher serum FSH concentrations were inversely related with the probability of SR in patients with NOA.[26] Indeed, FSH concentrations were inversely related with the total number of testicular germ cells, which has poor predictive value for SR in NOA.[27,28] Therefore, the presence of associated male pathologies cannot be used as predictive factor of success.[29] Nevertheless, in our study, FSH and testis size can be correlated significantly with SR.

Several previous studies have showed that a diagnostic testicular biopsy is the greatest parameter for SR.[5,30,31] In fact, the presence of elongated spermatids or spermatozoa in testicular biopsy is correlated to a high SRR for ICSI.[13,16,32,33]

Based on the fact that testicular histopathology is the most accurate predictor of a positive TESE, some studies have proposed performing a diagnostic biopsy before TESE.[13]. However, a diagnostic biopsy itself is an invasive procedure that may have complications similar to TESE procedure, including infection, bleeding, haematoma and tubular sclerosis.[34] Moreover, because testicular tubules of patients with NOA are usually heterogeneous, the absence of sperm in a single biopsy does not assurance a similar pattern in the entire testis.

Nevertheless, in our centre, we send testis tissue fragments to the pathology evaluation at the time of TESE and we have the histologic result only few days later from the surgical procedure; thus, we cannot use itself for diagnostic purpose. Its role can be proposed as a predictive factor in all cases in which, although there is a negative SR, the histopathological result evidences a NS. Thus, in this way, testicular histology can guide us to repeat surgical procedure a second time to research spermatogenetic cells.

A histopathology showing NS will also accurately diagnose cases of OA that were ignored clinically.[35] It should be noted that patients with OA may have some damage in spermatogenesis. In fact, our azoospermic patients with OA had also a testicular impairment, as demonstrated sometimes by a reduction of testicular size or increased FSH levels. In particular, in two patients with OA secondary to seminal vesicle agenesis, the histopathology showed MA. This may be a result of the long-lasting obstruction causing damage to the spermatogenic tubules. In fact, in our records, the OA patients had a SR of 86%, because the rest of these cases had a component of basic injury and altered spermatogenesis. Differently, it should be noted also that although patients with NOA have a serious injury of spermatogenesis, it may be possible to have areas in the testes showing foci of active NS.[36] This is also able to explain the discrepancy that has occurred in some cases between histology and SR, according to our experience. In fact, seven patients with histological result of MA had SR anyway. This occurs because spermatogenesis can be very unequally distributed in a testis, which may result in a negative finding using a randomly taken testicular open biopsy. This is due to irregular distribution of spermatogenesis on testicular parenchyma that can explain, that is, the presence of focal area of intact spermatogenesis in patients with a severe testicular atrophy. In these cases, in fact, the distribution of a minute quantity of spermatogenesis must be diffusely multi-focal.

Furthermore, another important advantage of histopathology is the revealing of GCNIS, which occurs in 1%–3% of patients with severe male infertility factor.[37–39] In our analysis, we reported no cases of GCNIS. However, one of the main limitations of our study is the reduced number of patients enrolled.

Patients with a negative SR and unfavourable histopathology should be informed that the likelihood of sperm recovery from a second TESE will be extremely low and should have the opportunity to consider other options, including heterologous artificial insemination.

Conclusion

In conclusion, testicular histopathology provides important information on the SRRs after a conventional bilateral multiple TESE. This is best performed at the time of TESE, aiming to negate the need for a second operation. According to the fact that pathologist analysed testis sample only after the surgical procedure, the histologic result can guide the surgeon in choosing the more suitable therapeutic practice for the azoospermic patient.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship and/or publication of this article.

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