Azoospermia

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6.1 Definition

Azoospermia is defined as the complete absence of sperm in the ejaculate even after centrifugation. Yet even a patient with rare nemasperms after centrifugation in the seminal fluid test is erroneously considered *azoospermic*. This latter condition is instead more correctly defined as *cryptozoospermia*. Instead, severe *oligospermia* is considered a sperm count less than 5 millions/ml. *Azoospermia* must also be differentiated from *aspermia*, which is, instead, the complete absence of seminal fluid emission during orgasm. In this situation the causes are very different and depend on defects of the bladder neck and urethral and ejaculatory ducts system (retrograde ejaculation, urethral stricture, neurological alterations) [1].

6.2 Epidemiology and Classification

Approximately 15 % of couples are unable to procreate after a year of unprotected intercourses and are therefore defined *infertile*. An isolated male factor is present in about 20–30 % of these cases, while in another 20–30 %, there is an association between the male and female factors [2]. Therefore, approximately in half of the cases of couple infertility is the male factor present. The prevalence of azoospermia is approximately 1 % among the general male population and ranges between 10 and 15 % among infertile men [3].

The different types of azoospermia are commonly classified in two large groups: obstructive azoospermia (OA) caused by an obstruction of the passage of sperm

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G. Cavallini, G. Beretta (eds.), *Clinical Management of Male Infertility*,
DOI 10.1007/978-3-319-08503-6_6

along the seminal pathways and secretory or non-obstructive azoospermia (NOA) due to a sperm production deficit. The latter can then be subdivided into pre-testicular and testicular according to whether the problem derives from an alteration of the hypophisary hormonal system which stimulates spermatogenesis (very rare condition) or from damage intrinsic to the testis (more frequent condition). The prevalence of OA and NOA regarding the total of the different types of azoospermia is very variable according to authors and geographical areas. In countries or cultures where vasectomy is very diffused as a contraception system, a prevalence of slightly higher OA (40 %) [4] can be seen compared to countries, like Italy, where this is rarely done (25–30 %) (Franco G. 2008, unpublished data). It is clear, therefore, that on the whole, the NOAs constitute the majority of the different types of azoospermia (60–75 %). As will be seen in this chapter, even OA and NOA prognosis is rather different and more favourable to OA.

6.3 Aetiology

6.3.1 Obstructive Azoospermia

Obstructive azoospermia is usually classified according to the location of the obstruction (Table 6.1).

6.3.1.1 Testicular Obstruction

The isolated form, with total absence of spermatozoa in the epididymis and normal spermatogenesis, is an extremely rare condition, more often due to a congenital malformation (complete detachment of testicle from epididymis, vasa efferentia and rete testis atresia). The form associated with patchy epididymal obstruction with the presence of some epididymal tubules containing spermatozoa is more frequent and usually due to an acquired post-phlogistic base.

6.3.1.2 Epididymal Obstruction

Obstruction usually happens in the epididymis. It affects about 30–67 % of azoospermic men with normal serum FSH values. The congenital form of it most often appears as bilateral agenesis of the vas deferens (CBAVD), which is associated with a cystic fibrosis gene mutation in 82 % of cases. This form is often correlated to the absence of the distal part of the epididymis (body and tail) and to the agenesis or atresia of the seminal vesicles. Other congenital forms include Young's syndrome which

Table 6.1 Obstructive azoospermia: obstruction location

Testicular obstruction (often congenital, extremely rare)
Epididymal obstruction (post-flogistic, postvasectomy, congenital, Young's syndrome)
Vas deferens obstruction (congenital: partial or complete aplasia; iatrogenic: vasectomy, hernioplasty)
Ejaculatory duct obstruction (cysts, post-flogistic stenosis)

typically is associated with chronic pulmonary infections, normal spermatogenesis and dilation of the head of the epididymis which is full of spermatozoa and amorphous matter, with the absence of spermatozoa in the epididymal corpus region. Among the acquired forms, the most frequent are the obstructions from sexually transmitted infections (gonococcus, chlamydia) and the postvasectomy obstructions from blow-out of the epididymal tubule due to secondary hyperpressure.

6.3.1.3 Vas Deferens Obstruction

The most common cause of congenital obstruction is vas deferens agenesis, almost always associated with the cystic fibrosis gene mutations. In Italy, it has been calculated that about one out of three cases of obstructive azoospermia is caused by vas deferens agenesis. The most frequent form is the complete bilateral one in which the vas cannot be palpated by physical examination, but conditions of partial or unilateral atresia can be observed in which at least one vas deferens or a section of it can be palpated. Unilateral complete or partial aplasia is associated with irregularities of the ejaculatory ducts with to contralateral renal ageneses in 80 and 26 % of cases, respectively.

In the countries where vasectomy is widespread, the most common cause of acquired obstruction is represented by vasectomy as a contraception method. In the United States, almost 500,000 vasectomies are performed per year and approximately 2–6 % of the patients require a conversion (from 10 to 30 thousand reversals/year). In Italy, vasectomy is rarely used; therefore, it is a very less frequent cause of obstruction. Even inguinal hernioplastic surgery can induce vas deferens obstruction via accidental direct damage of it or of its blood supply during surgery. Fibroblastic reaction induced by contact with the polypropylene of the mesh can also determine delayed vas obstruction.

6.3.1.4 Obstruction of the Ejaculatory Ducts

This represents almost 10 % of the obstructive forms of azoospermia and can be congenital (cyst, atresia) or acquired (post-flogistic or dysfunctional).

The utricle, Mullerian and Wolffian cysts are localized in the prostate between the ejaculatory ducts and can be communicating or noncommunicating with the semen pathways and normally cause obstruction by compressive phenomena and lateralization of the ejaculatory ducts themselves. Postinflammatory obstructions, decisively more rare, are secondary to acute, subacute or chronic prostatovesiculitis. When complete, the ejaculatory duct obstructions are associated with a reduced volume of seminal fluid (<1.5 ml) with reduction or absence of fructose, acid pH and dilatation of the seminal vesicles.

6.3.2 Non-obstructive Azoospermia

The non-obstructive forms of azoospermia (secretory) are usually classified according to their cause (Table 6.2). In almost all cases, the cause is at the gonad level (*testicular cause*), while very rarely, only in the case of hypogonadotropic hypogonadism is

Table 6.2 Non-obstructive forms of azoospermia: aetiological classification

1. Idiopathic
2. Genetic
3. Cryptorchidism
4. Testicular torsion
5. Orchitis (viral or bacterial)
6. Varicocele
7. Chemo- or radiotherapy, medicines, toxics
8. Hypogonadotropic hypogonadism

azoospermia attributed to an alteration of the hypophisary gonadotropin secretion (*pre-testicular cause*).

6.3.2.1 Idiopathic

Unfortunately, in almost half of the non-obstructive types of azoospermia, it is difficult to pinpoint the cause connected to the condition, and therefore we speak about an idiopathic or unknown cause. Many of these situations probably have a base of genetic defects which are still unknown, congenital aberrations or a previous unknown exposition to gonadotoxins.

6.3.2.2 Genetic

Karyotype aberrations can be recognized in 10–15 % of azoospermia cases. *Klinefelter's syndrome* is characterized by the presence of a supernumerary X chromosome (47 XXY). One male out of 600 is affected. The patients manifest small and hard testicles, gynecomastia, azoospermia and high levels of gonadotropins. The only possible therapy is testicular sperm extraction (TESE) for intracytoplasmic sperm injection (ICSI). Apparently the best probabilities of success are in younger patients in whom spermatogenesis may not be entirely compromised.

Thanks to recent techniques of molecular biology (PCR), it has been possible to demonstrate that almost 5–10 % of azoospermic patients are a carriers of a *microdeletion of chromosome Y*. This is characterized by alterations of the genes localized in the AZF region (azoospermia factors a, b and c) which have a determining role in spermatogenesis. The complete deletion of the AZFa and AZFb loci is always associated with the absence of spermatozoa in the testicles and therefore with a worse fertility prognosis [5, 6].

6.3.2.3 Cryptorchidism

Cryptorchidism is the undescent of one or both testicles into the scrotum, often linked to a development deficit of the testicles and their ligament connections. In 8 % of cases the testicle remains in the abdomen, in 70 % in the inguinal canal and in 20 % in the pre-scrotum. The greater the degree of retention, the more serious the testicular dysfunction; there is an absence of germinative cells in 20–40 % of testicles in the inguinal or pre-scrotum region and in 90 % of testicles in the intra-abdominal location. In the case of bilateral cryptorchidism, the probability of infertility is 50–90 %, while in the case of monolateral forms, the probability is 20–70 %. Azoospermia caused by cryptorchidism is almost always due to

alterations of spermatogenesis connected to the testicular dysgenesis syndrome or due to damage caused by the elevated temperature the retained testicle is exposed to, in particular when not operated on immediately (before 1–2 years of age). Other possible causes of azoospermia are epididymal aberrations often associated with the cryptorchid testicle (didymo-epididymal detachment of testicle from epididymis) which could theoretically cause an obstructive-based azoospermia or mixed secretory-obstructive azoospermia.

6.3.2.4 Testicular Torsion

Testicular torsion can result in azoospermia in the case of bilateral torsion, in monorchid patients or when contralateral testicle is already compromised by other conditions.

6.3.2.5 Orchitis

The parotitis virus can be responsible for orchitis in almost 30 % of affected patients, above all in the post-puberty age, with bilateral interest in 10–30 % of cases. As a result of the infection, an atrophy or permanent testicular hypotrophy can occur with consequent azoospermia. The introduction of the anti-parotitis vaccination has made this event rare. Other bacterial and viral infections or infections from other microorganisms can cause non-obstructive azoospermia from direct damage of spermatogenesis. These germs, however, more frequently cause epididymitis which determine a condition of obstructive azoospermia.

6.3.2.6 Varicocele

The relationship between varicocele and azoospermia is still a matter for discussion. According to most authors, the two conditions could be coexistent only, but for others there is a direct correlation between them. On the basis of this, some suggest the treatment of varicocele which can lead to the reappearance of spermatozoa in the ejaculate, particularly in the presence of a histological pattern of a late maturation arrest or hypospermatogenesis [7].

6.3.2.7 Exposure to Drugs, Toxic Substances and Radiation

Chemotherapy can exercise a negative effect on spermatogenesis. The most affected cells are the spermatogonia and spermatocytes up to the preleptotene stage. The type of drug, its dosage and patient age at time of treatment assume a relevant importance. It seems that alkylating agents and procarbazine are the most toxic for the testicles. During chemotherapy many patients become azoospermic with elevated levels of serum FSH, but the majority of them recover a normal spermatogenesis months or years afterwards. Instead, in other patients, azoospermia is permanent. Also radiation exposure plays a negative role on spermatogenesis; in fact, spermatogonia and spermatocytes are very sensitive to this. Lastly, many toxic substances can cause a serious reduction in spermatogenesis, leading to azoospermia.

6.3.2.8 Hypogonadotropic Hypogonadism

This is a very rare cause of azoospermia (less than 1 % of cases), even if it is the only condition treatable with medical therapy. In hypogonadotropic hypogonadism

the alterations of the hypothalamus or the pituitary gland compromise the correct release of gonadotropins, determining seminal alterations that extend to cases of azoospermia. The Kallmann syndrome is characterized by hypogonadotropic hypogonadism associated with anosmia. An insufficient secretion of GnRh from the hypothalamus with consequent gonadotropin reduction and secondary testicular insufficiency is recognized as the cause of this syndrome. A delayed puberty is a pathognomonic sign. In this condition testicles are usually very small, under 2 cm in longitudinal diameter. Other pathologic conditions affecting the hypophysis diseases such as ischaemia, tumours or infections can cause hypogonadotropic hypogonadism. The Prader-Willi syndrome is characterized by hypogonadism, obesity, muscular hypotonia, mental retardation, reduced development of hands and feet and short stature. These patients have an FSH and LH deficit caused by an insufficiency of GnRh. Therapy for these types of hypogonadism is pharmacological with administration of gonadotropins sometimes associated with GnRh.

6.4 Diagnosis

A careful and accurate andrological evaluation can immediately pinpoint the genesis of azoospermia: personal history, for example, can reveal previous cryptorchidism, testicle infections (orchitis, mostly from epidemic parotitis) or previous chemo- or radiotherapy treatments.

Particular attention is to be paid to:

- Family medical history (even reproductive)
- Personal reproductive medical history and alterations of the ejaculate
- Pathological personal medical history
  - Congenital aberrations (e.g. cryptorchidism)
  - Inflammatory diseases
  - Traumas
  - Inguinal-scrotal and pelvic surgery
  - Systemic diseases
  - Endocrinopathies
  - Chronic obstructive bronchopulmonary diseases
  - Drugs and chemo- and radiotherapy
  - Environmental and professional exposure to heat sources, radiations and toxics

Physical examination can reveal small testicles (<10 ml) with reduced consistence or eunuchoid look of the patient in NOA, while the presence of a normal testicular volume, or unpalpable vasa, will orient towards OA. Physical examination can also show the presence of a varicocele.

The seminal fluid exam can reveal the nature of OA/NOA via the evaluation of volume, pH and fructose. Serum levels of FSH and inhibin B supply further indications for the differential diagnosis between OA and NOA. In fact, an elevated

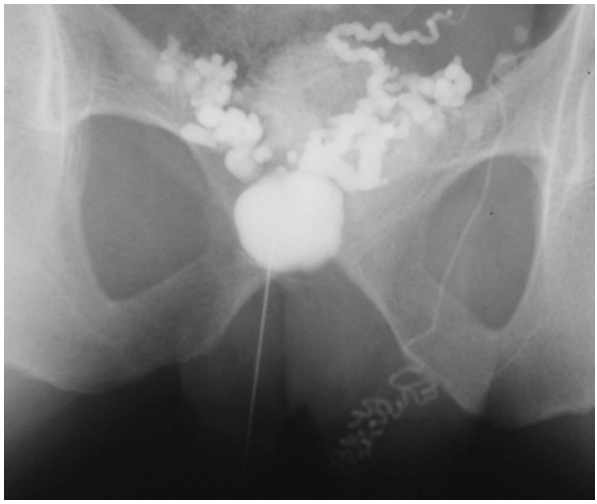

Fig 6.1 Ultrasound-guided transperineal vesiculodeferentography with fine needle puncture of a median prostatic cysts communicating with the seminal tract

FSH value and a low inhibin B value certainly point to a NOA condition. Scrotum ultrasounds must always be done for the study of the testicle (volume, echogenicity) and epididymis (cribriform pattern suspect for obstruction) but also for the screening of testicular tumours, more frequently found in azoospermic subjects and, in general, in the infertile male. Scrotum echo Doppler or colour duplex scanning must be done in the presence of clinically evident varicocele. TRUS (transrectal ultrasound of the prostate) is recommended in cases of oligoposia, when obstruction of the ejaculatory ducts and agenesis of the vas deferens or seminal vesicles are suspected. As concerns genetic screening, karyotype and chromosome Y microdeletions screening must be done when NOA is suspected and in any case before assisted reproductive techniques (ART). On the other hand, cystic fibrosis gene mutations screening is advised for patients with suspected congenital obstruction but also for the partner, in order to verify the risk of development of cystic fibrosis in the newborn. The invasive diagnostic study, instead, is represented by testicular fine needle aspiration, open testicular biopsy and vasography and vesiculography, which can be performed transscrotally or transperineally via ultrasound-guided needle puncture of the distal seminal tract (Fig. 6.1).

6.5 Therapy

6.5.1 Obstructive Azoospermia

In obstructive azoospermia, when possible, recanalization of the seminal tract and restoration of spontaneous fertility are indicated. Obstruction location and characteristics and partner age influence the choice of treatment.

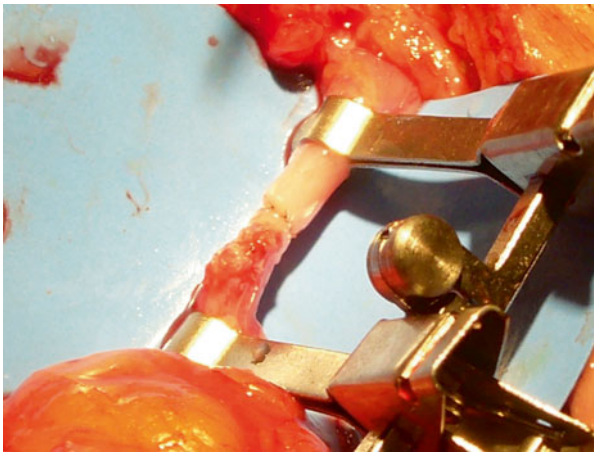

Fig. 6.2 Microsurgical vasovasostomy in two layers according to Silber

- *Microsurgical recanalization of the proximal seminal pathways*
  This treatment is indicated in case of azoospermia, confirmed by at least two recent spermiograms and normal spermatogenesis at least on one side, documented by histology or testicular cytology. Microsurgical reconstruction (epididymovasostomy, vasovasostomy) should be indicated as the first therapeutic option in azoospermia due to epididymal or vasal obstruction. In the majority of patients, it consents the achievement of spontaneous pregnancies by avoiding ART techniques which carry high costs and invasivity to the female partner. In a recent revision of over 4,000 operated cases, Silber reports patency and pregnancy percentages after microsurgical reconstruction at, respectively, 96 and 81 % for vasovasostomy and 84 and 67 % for vasoepididymostomy [8]. The recent introduction of simpler microsurgical anastomosis techniques has further improved results (Figs. 6.2 and 6.3) [9]. When the female partner is older than 37 years, there is, instead, a priority indication for immediate ICSI. This might also be associated with a contextual microsurgical recanalization of the seminal tract.
- *Recanalization of the distal seminal tract*
  Endoscopic resection of the ejaculatory ducts (TURED) or obstructing prostatic cysts is the treatment of choice in distal obstruction. However, its indications have recently been reduced due to the introduction of less invasive techniques and the known possibility of negative postsurgical consequences such as urinary reflux in the seminal tract during micturition [10, 11].

  In the presence of prostatic cysts obstructing the ejaculatory ducts but not communicating with the seminal pathways, a recanalization is possible with a minimally invasive approach of transperineal ultrasound-guided injection and schlerotization of the cysts with alcohol (TRUCA) [10, 11].
- *Sperm retrieval for ICSI*
  When recanalization is not feasible, sperm retrieval and ICSI are indicated [12, 13].

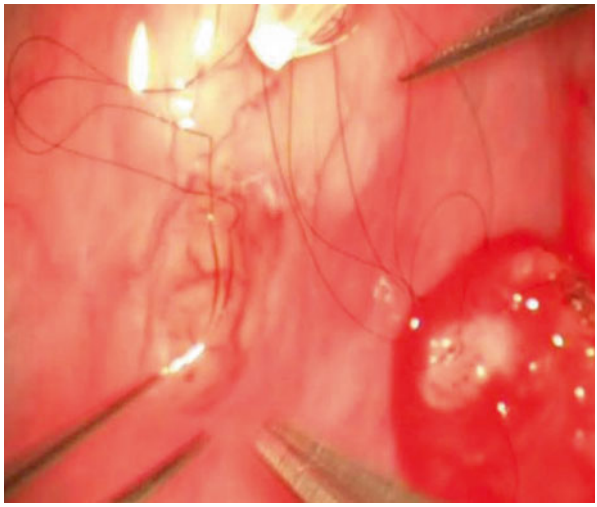

Fig. 6.3 Microsurgical terminolateral vasoepididymostomy (tubulovasostomy): simplified technique with invagination of the epididymal tubule according to Monoski

Table 6.3 Retrieval techniques of male gametes and their acronyms

MESA	Microsurgical epididymal sperm aspiration
PESA	Percutaneous epididymal sperm aspiration
TESA	Testicular sperm aspiration
TESE	Testicular sperm extraction
MicroTESE	Microsurgical testicular sperm extraction

6.5.2 Non-obstructive Azoospermia

Except in the rare cases of hypogonadotropic hypogonadism which can be treated with medical therapy, in patients with NOA the only possible treatment is sperm retrieval for assisted reproductive techniques (ART).

6.5.2.1 Sperm Retrieval Techniques for ART

Sperm retrieval techniques with acronyms are listed in Table 6.3. There is general consensus that in the case of obstructive azoospermia any technique allows a sufficient sperm retrieval for ICSI [14–19]. In fact, by definition, in OA spermatogenesis is normal and sperm can be easily retrieved from the testicle or epididymis even with percutaneous techniques (TESA, PESA) [20, 21]. The original MESA technique is today rarely used because of its high costs and longer surgical times, but its simplification (Mini-MESA), introduced in 1996 [22–24], combines advantages and simplicity of percutaneous techniques with the precision and accuracy of microsurgical procedures. Using a small scrotal incision, the head of the epididymis is exposed and dislocated in the wound, anchoring it at the edges. The procedure continues with the direct puncture of the more dilated and whitish epididymal tubules with a TB syringe (Fig. 6.4). This technique allows one to obtain high counts of sperm and therefore facilitate cryoconservation of an adequate number of paillettes for subsequent ICSI cycles.

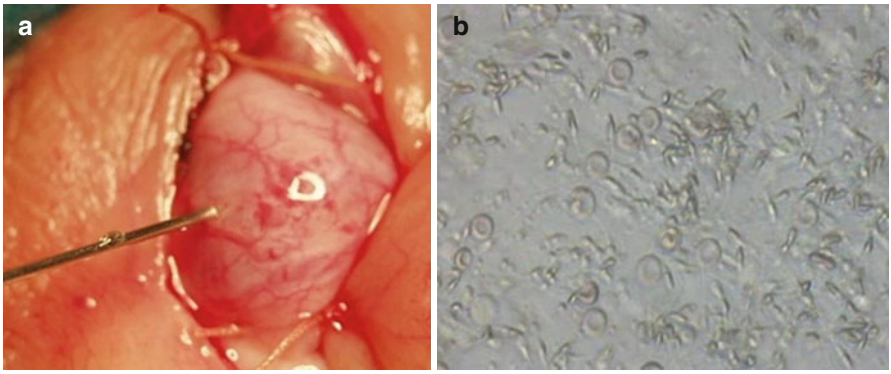

Fig 6.4 Mini-MESA: (**a**) TB needle aspiration from the head of the epididymis, (**b**) sperm retrieval

TESA (or TEFNA, testicular fine needle aspiration) is the simplest percutaneous technique and in OA allows an immediate retrieval of sufficient spermatozoa for one or more ICSI cycles. With a 21G needle butterfly, the testicle is punctured, and with slight movements, testicular fluid is aspirated and sent to the lab for sperm search. PESA is a similar percutaneous technique but performed by inserting the needle into the head of epididymis in order to obtain a more sizeable and clean sperm retrieval compared to TESA. PESA is particularly indicated in congenital absence of the vas deferens. In TESE one or more open surgical biopsies are performed on one or both testicles. TESE is particularly indicated in NOA and when the percutaneous techniques have failed [38].

Many studies have compared ICSI results using freshly retrieved or frozen-thawed sperm, and the majority of these have concluded that there is no difference in terms of fertilization, implantation and pregnancy rates [25].

In NOA the standard treatment is represented by single or multiple TESE [36, 37]. In fact, success rate with percutaneous techniques is extremely low [26, 27]. Overall, in nearly 50–60 % of NOA patients, it is possible to retrieve spermatozoa with TESE [38–40]. Microsurgery has regained interest even in NOA after the introduction of the MicroTESE technique proposed by Schlegel et al. in 1999 [28]. With this technique, many authors have reported a higher rate of sperm retrieval, with less complications compared to multiple TESE [29–31, 36–40]. The technique is performed with an equatorial incision of the tunica albuginea and clam opening of the testicle. Using the magnification of an operating microscope, it is possible to spare the blood supply and to extract single seminiferous tubules with jeweller's forceps. This is done in different areas of the exposed parenchyma, trying to identify the more dilated tubules which more likely harbour sperm (Fig. 6.5).

Extracted tubules are then sent to the laboratory for the search of spermatozoa. The incision is then closed with microsurgical running suture. MicroTESE reduces the possibility of vascular lesions with a lower loss of tissue than multiple TESE. Furthermore, postsurgical pain is reduced due to lesser retraction of the tunica albuginea and consequent less compression of the testicular parenchyma [32].

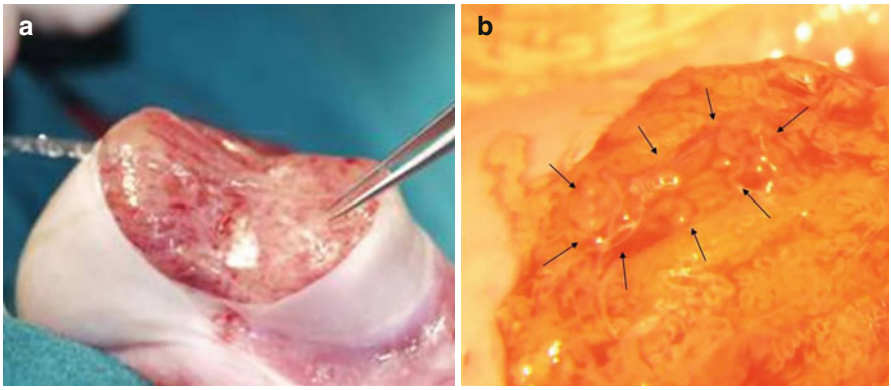

Fig. 6.5 (**a**) MicroTESE: atraumatic extraction of the seminiferous tubule with jeweller's forceps. (**b**) Dilated seminiferous tubules (*arrows* indicate an area of dilated seminiferous tubules)

However, recent reports have shown a reduction in serum levels of testosterone and an increase in LH and FSH after MicroTESE [33].

A New "Stepwise" MicroTESE Approach

Following these considerations, we proposed a "stepwise" approach to MicroTESE in order to reduce invasivity and optimize results, particularly for those patients who did not have previous TESE or histology and whose chance of sperm retrieval is unknown or unclear. Under local anaesthesia (cord block and skin infiltration), a small (10 mm) scrotal window incision is performed and a single testicular biopsy (5 mm) is taken from the mid-portion of the testis and sent to the lab for sperm extraction together with a specimen for histology. If there is presence of sperm, the procedure is terminated and the wound closed. In case of absence of sperm, the scrotal incision is expanded, and the horizontal albuginea incision is also extended equatorially until the testicle is split open and MicroTESE performed. We believe that this approach can optimize the results and reduce the invasivity of sperm retrieval procedures. In fact, although MicroTESE has been shown to be less invasive than multiple TESE [34], a significant hormonal impairment has been described after one or more MicroTESE procedures [33].

Several questions concerning the sperm retrieval techniques in NOA are still being discussed. The possibility of programming the sperm retrieval procedure on the same day of ICSI, in order to use fresh sperm, has been taken into consideration by Verheyen et al. [35]. The authors conclude that there are no significant differences regarding the implant, embryo transfer and pregnancy rate after ICSI with fresh or cryconserved sperm, and therefore they propose, for all patients with NOA, a planned TESE for diagnostic and therapeutic aims, with cryoconservation of the retrieved spermatozoa followed by a differed ICSI. In this way a useless ovarian stimulation of the partner can be avoided in the case of failed sperm retrieval. The superiority of TESE over TESA in NOA has been confirmed by Hauser et al. in a study on the evaluation of the sperm retrieval rate after TESA and multiple TESE in 32 patients with NOA [26, 36–40].

In conclusion, in sperm retrieval techniques, the complexity of the clinical situations and the multiplicity of the therapeutic options presently available suggest the need for a correct evaluation and management of the azoospermic patient. An expert in the field, adequately trained and with competences both in male genital surgery and reproductive medicine, will be able to best carry this out.

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