

# Prognostic factors for sperm retrieval in non-obstructive azoospermia

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Testicular sperm retrieval techniques associated with intracytoplasmic sperm injection have changed the field of male infertility treatment and given many azoospermic men the chance to become biological fathers. Despite the current use of testicular sperm extraction, reliable clinical and laboratory prognostic factors of sperm recovery are still absent. The objective of this article was to review the prognostic factors and clinical use of sperm retrieval for men with non-obstructive azoospermia.

The PubMed database was searched for the Medical Subject Headings (MeSH) terms azoospermia, sperm retrieval, and prognosis. Papers on obstructive azoospermia were excluded. The authors selected articles that reported successful sperm retrieval techniques involving clinical, laboratory, or parenchyma processing methods. The selected papers were reviewed, and the prognostic factors were discussed.

No reliable positive prognostic factors guarantee sperm recovery for patients with non-obstructive azoospermia. The only negative prognostic factor is the presence of AZFa and AZFb microdeletions.

**KEYWORDS:** Azoospermia; Testicular Sperm Retrieval; Male Infertility; Reproductive Techniques; Prognosis.

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## ■ INTRODUCTION

In the last 20 years, the most important development in male factor infertility has been the introduction of intracytoplasmic sperm injection (ICSI). ICSI brought hope for men with severe male factor infertility who wanted to become biological fathers.

Since Devroye et al.'s (1) report on the use of testicular spermatozoa in an azoospermic male to fertilize human oocytes, physicians have sought a safe and simple method of testicular sperm extraction that is associated with a high recovery rate. Ideally, the best method would only cause minor testicular damage, present a high recovery rate, have a low cost and be reproducible. The first proposed technique was named testicular sperm extraction (TESE) and was simply a testicular biopsy. Further discussions about this technique involved the number of testicular fragments taken, the site of the extraction and in the last decade, the use of microsurgery to improve the results (2).

Despite the current use of testicular sperm extraction, reliable clinical and laboratory prognostic factors of sperm recovery are missing. Prognostic factors have included testis size, follicle stimulating hormone (FSH), inhibin beta, the

etiology of infertility, and genetic alterations; however, the histological testicular pattern remains the best predictor of sperm retrieval, although with the inconvenience of a second invasive procedure (3).

The objective of this article was to review the prognostic factors and clinical use of sperm retrieval for men with non-obstructive azoospermia.

## ■ METHODS

The PubMed database was searched for the Medical Subject Headings (MeSH) terms azoospermia, sperm retrieval, and prognosis. Papers on obstructive azoospermia were excluded. The authors selected articles that reported successful clinical, laboratory, or parenchyma processing methods. The selected papers were reviewed, and the prognostic factors were discussed.

Spermatogenesis is a complex process that involves a mitotic and meiotic cellular division and depends on more than 40 enzymes. While the process occurs in the testicle, part of the process is completely isolated from the immunological system that is hidden behind the blood-testicular barrier (4). Non-obstructive azoospermia is the most serious alteration of the spermatogenesis, and its evaluation and treatment remain a challenge. Although there are well-established treatment protocols, the chances of successful clinical or surgical treatment for non-obstructive azoospermia are small. For most patients, the remaining option is based on sperm retrieval and ICSI.

Sperm retrieval is conducted with testicular aspiration or biopsy for testicular sperm extraction (TESE), and laboratory

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searches are conducted for viable sperm that can be used for ICSI. TESE is an invasive procedure and should be done with the intention of treatment. Sperm retrieval procedures are associated with uncertainties, high costs and the possibility of morbidity for women who suffer from ovarian hyper stimulation during the oocytes retrieval procedure. The prognostic factors of sperm retrieval are important to understand.

Historical prognostic factors for sperm recovery in non-obstructive azoospermia are related to the clinical, laboratory, and surgical technique; the testicular tissue processing method in the gamete laboratory; and the histological pattern of the testis.

## ■ CLINICAL

Clinical aspects related to sperm recovery include the etiology of infertility and the age and testicular volume of the patient. For non-obstructive azoospermia, these three prognostic factors are typically considered alone or combined with endocrinologic data. For the etiology of azoospermia, there are data on Klinefelter syndrome, cryptorchidism, and varicocele.

*Klinefelter syndrome* is a karyotype alteration that results from a supernumerary X chromosome that can be expressed in two forms: mosaic and non-mosaic. Klinefelter syndrome is usually associated with infertility. The sperm recovery rate in Klinefelter patients is approximately 50% (5-7). Previous literature suggests that age (5) and testicular volume (4) are prognostic factors, but a more recent study showed no correlation between clinical parameters and sperm retrieval (7). However, these studies used limited numbers of patients, ranging from 20 to 51. In 2010, a review of 13 papers involving 373 Klinefelter patients with azoospermia who underwent sperm retrieval showed that clinical parameters were not good prognostic factors (8).

*Cryptorchidism* is one of the most common child malformations, and it affects 3% of full term male infants. Cryptorchidism is related to male infertility, testicular tumors, testicular torsion, and inguinal hernias (9). Studies on the clinical factors related to sperm retrieval in cryptorchid patients have small numbers of individuals, and the conclusions remain controversial. Negri et al. (10) compared 30 azoospermic cryptorchid men with 77 men with various causes of non-obstructive azoospermia and concluded that bilateral orchidopexy was a positive predictive factor for sperm finding after TESE. However, the authors did not compare unilateral and bilateral orchidopexy, which invalidates the final conclusion. In a study of 38 azoospermic men who had previously undergone surgical treatment for cryptorchidism, Raman and Schlegel (11) found correlations between sperm recovery rate and age at orchidopexy and testicular volume. Men who had had surgery before they were ten years old had a better sperm recovery rate than men who had had orchidopexy at ten years of age or higher. More recently, Wiser et al. (12) studied 40 patients who had undergone orchidopexy and were presenting with azoospermia and found no statistically significant differences between men who had undergone surgery before or after ten years of age. The authors also found no significant relationship between sperm recovery rate and testicular volume.

*Varicocele* is an important male infertility factor that has a high incidence. While surgical treatment is possible, the

discussion about surgical treatment and in vitro fertilization (IVF) remains active in the literature. The benefits of treatment in terms of sperm quality are difficult to question, but the issue becomes complicated for patients with azoospermia associated with varicocele who want to naturally conceive after surgical correction (13,14). Lee et al. (14) analyzed the cost effectiveness of live birth after varicocele treatment for patients with azoospermia after testicular microdissection and ICSI. The authors concluded that ICSI was the most cost effective. The goal of the present article was to discuss whether varicocele treatment before TESE improves the chances of retrieving sperm and serves as a positive prognostic factor. Inci et al. retrospectively studied 96 patients with varicocele and non-obstructive azoospermia. Sixty-six patients had undergone a previous surgical varicocele treatment with TESE or ICSI at least five months previously, and 30 patients had not previously undergone treatment. The mean patient ages, testicular volumes, FSH levels, and partner ages were not significantly different between the two groups. The results showed a significantly better recovery rate for the treated group (53% versus 30%). There were no statistically significant differences in embryo quality, implantation rates, miscarriage rates, or live birth rates (15).

## ■ LABORATORY

Laboratory investigations are based on tests that verify the hypothalamic-pituitary-gonadal axis by measuring follicle-stimulating hormone (FSH) levels and the feedback regulator inhibin B. Other tests include the genetic detection of chromosome alterations.

An intact hypothalamic-pituitary-gonadal axis is necessary for correct sperm production. The FSH level was initially used as a predictor of sperm recovery, but its use remains controversial. Ramasamy et al. evaluated 792 men with non-obstructive azoospermia who underwent testicular microdissection. The men were divided into four groups according to FSH levels: less than 15 IU/mL, between 15 and 30 IU/mL, 31 to 45 IU/mL, and greater than 45 IU/mL. Compared with the group with less than 15 IU/mL FSH, the recovery rate for mature sperm was significantly higher in the groups with greater than 15 IU/mL FSH (16). Chen et al. conducted a prospective study with 208 patients who underwent an FSH test and TESA. To establish an FSH cut-off limit, the men were divided into two groups based on mature sperm recovery. The authors found a cut-off value of 19.4 mIU/mL. Sperm was not found in men with FSH levels at or above the cut-off limit (17). Both of these studies have a large number of patients and had the prime objective of analyzing the relationship between FSH levels and sperm finding; in the second study, the mean FSH levels for successful sperm recovery were in the normal range and did not characterize the histological findings in each group. A predominance of hypospermatogenesis in the successful group may explain the findings.

Inhibins, anti-Mullerian hormone (AMH), and activins are glycoproteins that are transforming growth factors (TGF). These glycoproteins cause the pituitary gland to take part in the feedback mechanism at the hypothalamic-pituitary-gonadal axis. For this reason, they can be used as spermatogenesis markers. Plasma levels of inhibin fraction B and seminal levels of AMH can be used as predictive parameters for sperm recovery in non-obstructive azoospermia



(18). Inhibin B and AHM can be measured in the plasma or seminal plasma, and their clinical use is debated in the literature (19). A prospective study of 139 men with non-obstructive azoospermia was conducted by Mitchell et al. The authors measured FSH, inhibin B in the plasma and AMH in the seminal plasma before TESE, and they compared the mean levels of successful sperm retrieval with the failed group. The recovery sperm rate was 43% (60/139), and there was a statistically significant correlation between serum and seminal plasma inhibin B levels and seminal plasma AMH and inhibin B levels. However, the mean inhibin B seminal plasma levels and mean AMH seminal plasma levels did not show statistically significant differences between the successful and failed sperm retrieval groups. On the contrary, the mean serum levels of FSH and serum inhibin B differed between the two groups; the statistical significance is difficult to explain and justify (19).

Tiepolo and Zuffardi postulated the involvement of deletions in the long arm of the Y chromosome. Using a cytogenetic analysis, they identified a region known as azoospermia factor (AZF). That region was later subdivided into three regions (AZFa, AZFb, and AZFc), and the relationship with infertility was established. Y chromosome microdeletions occur in 6-8% of severely oligozoospermic men and 3-15% of azoospermic men. The importance of Y microdeletions as a prognostic factor for sperm recovery is based on the absence of mature sperm in azoospermic men with AZFa and AZFb microdeletions who underwent sperm retrieval techniques. Fortunately, AZFc is the Y microdeletion most often found in azoospermic men (60%), and sperm can be retrieved for these patients. For this reason, the presence of AZFa or AZFb is a negative predictive factor for sperm retrieval in azoospermic men (20).

## ■ SURGICAL TECHNIQUES

To analyze the surgical technique as a prognostic factor, it is necessary to compare existing techniques. Sperm can be retrieved from testicles using percutaneous (testicular sperm aspiration, TESA; testicular fine needle aspiration, TFNA) or open (testicular sperm extraction, TESE; testicular microdissection, TM) methods (21). Comparisons between these techniques are difficult, as patients are not the same, and the efficacy of the method varies according to the testicular histology. Percutaneous methods are less invasive, but the laboratory has less tissue to search for sperm. Open techniques provide larger samples to work with but are more invasive. Finally, TFNA mapping attempts to combine the qualities of the anterior techniques by mapping the parenchyma and making a target opening, but an expert cytologist is still required to correctly identify mature sperm on the cytology sample. In general, percutaneous techniques are more successful in hypospermatogenesis than in other histological patterns (21). The sperm recovery rates range from 11-47% for TESA, 30-63% for TESE, and 43-63% for TM; the rate for TFNA is approximately 47% (21).

Since Schlegel's 1999 publication of the TM technique (2), studies have attempted to find its superiority over previous methods, but definitive conclusions are difficult to make. The comparative studies that found statistically significant differences between TESE and TM used different testicles (22), took one to three TESE samples per testicle (22,23) or did not explore two testicles in the previous TESE (24).

In 2000, Amer et al. compared classic biopsy on one side and testicular microdissection on the other, in 116 non-obstructive azoospermic men. The study aimed to verify the sperm retrieval rates and parenchymal changes in the Doppler ultrasound during the first six months after the procedure. The sperm retrieval rate was significantly higher on the side that underwent testicular microdissection, and at the end of the follow-up, there were no permanent devascularization areas in the testes. Biopsies were performed on the same patients. Authors used 100 patients with similar histology for comparison, however, only one testicular fragment was retrieved in classic biopsy side and evaluated (22).

In a retrospective comparative study, Okada et al. analyzed two groups of patients with obstructive and non-obstructive azoospermia who had undergone TESE and testicular microdissection. The authors reported a better sperm retrieval rate with testicular microdissection. This advantage was present for all patterns of testicular histology; however, TESE was performed using three 5-mm incisions (23).

In a retrospective study with 46 patients who had undergone traditional TESE and whose sperm retrievals had failed, Tsujimura et al. found a 44% sperm retrieval rate with testicular microdissection and concluded that the technique was superior. When analyzing the results, the authors highlighted the difference between the sperm retrieval rates in patients with one (47%) or three (33%) testicular fragments; only nine patients had multiple bilateral fragments collected in the first TESE procedure, which suggests that the amount of tissue removed should be considered (24).

In 2002, Tsujimura et al. compared several conventional TESE and testicular microdissection techniques in different patients and found no significant differences in the sperm retrieval rates between the groups (25).

## ■ TESTICULAR TISSUE PROCESSING METHOD

After tissue has been removed, two methods can be used to open the seminiferous tubules to separate the structural tissue from the viable sperm: enzymatic tissue digestion and mechanical tissue disruption.

Mechanical preparation consists of using needles or scalpels to mince and shred the tissue to open the tubules and separate the sperm from the tubular epithelium. The procedure is conducted in the operating room at the same time as the surgery and later in the manipulation laboratory, and the procedure is typically associated with a microdroplets search for viable spermatozoa (26). Enzymatic preparation uses a collagenase tissue exposure to liberate sperm from the tubules so that they can be manipulated in the laboratory (26). A comparison between the two techniques was conducted by a multicenter German study. The data were from eleven centers that used testicular sperm from obstructive and non-obstructive azoospermia, but the authors did not conduct separate analyses of the techniques; thus, the results are inconclusive (26).

Enzymatic tissue digestion can be used in the laboratory after a mechanical preparation is conducted to rescue negative initial searches by digesting the testicular parenchyma and freeing sperm. A study of 501 negative testicular samples from testicular microdissection showed that an enzymatic preparation could rescue sperm in seven percent of men after a mechanical preparation (27).





## HISTOLOGIC PATTERN

The most reliable factor for predicting sperm retrieval in non-obstructive azoospermic patients is the testicular histological pattern. However, its use is limited, as the patient must undergo a prognostic biopsy, which adds a surgical procedure. The worst pattern for sperm recovery is Sertoli cell-only syndrome, which has a rate of sperm retrieval ranging from 4-51%; maturation arrest (8-80%) affects sperm recovery adversely, while hypospermatogenesis produces high rates of sperm recovery (80-100%) (3,28,29).

Non-obstructive azoospermia remains the most challenging diagnosis for andrologists, and there are no positive prognostic factors that guarantee sperm recovery for these patients. The only reliably negative prognostic factor is the presence of AZFa and AZFb microdeletions.

## AUTHOR CONTRIBUTIONS

Glina S and Vieira M conducted the literature search and prepared the manuscript.

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