

## ORIGINAL PAPER

# Predictive factors for successful testicular biopsy

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**Summary** *Introduction: Infertility, the inability to conceive, constitutes a major problem in modern societies. It affects 10 to 15 percent of couples in the United States. Evaluation of infertile men is usually complex and often demands a testicular biopsy.*

*Materials and methods: We reviewed all azoospermic men submitted to testicular biopsy, in our center, during infertility investigation between January 2015 and December 2021.*

*Results: A total of 117 patients with a mean age of 36.5 was considered. Biopsy was positive, as defined by the presence of viable spermatozooids by microscopy, in 48.7% of patients (n = 57). Patients were divided in two separate groups based on positive (PB) or negative biopsy (NB) and compared. PB-group had normal serum total testosterone levels and higher than NB-group (3.7 ng/mL vs. 2.85 ng/mL,  $p = 0.021$ ), and normal serum FSH levels and lower than NB-group (6.0 mIU/mL vs. 16.0 mIU/mL,  $p < 0.001$ ). The groups were similar concerning serum LH levels (3.9 mIU/mL vs. 6.3 mIU/mL,  $p = 0.343$ ).*

*Conclusions: Predicting outcomes of testicular biopsy is a difficult task. Our study found that men with normal testicular volume, normal levels of testosterone and FSH and those with type 1 diabetes mellitus had a higher probability of positive testicular biopsy.*

**KEY WORDS:** Male infertility; Azoospermia; Testicular biopsy; Predictive factors.

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## INTRODUCTION

Infertility, the inability to conceive after one year of regular unprotected intercourse, constitutes a major problem in modern societies. It affects 10 to 15 percent of couples in the United States, with male component being the isolated cause in about 20% of cases and representing part of the aetiology in another 30% (1-3). Multiple factors contribute for male infertility, from anatomic obstruction and genetic alterations to hormonal disturbances, among many others. Evaluation of infertile men is usually complex and requires a careful clinical history, physical exam, semen analysis, hormonal profile and imaging and genetic testing (4).

Azoospermia, the complete absence of spermatozoa in the ejaculate, is present in 10-20% of all infertile men and can be classified as obstructive or non-obstructive (5). The evaluation of these men often demands a testicular biopsy, with associated morbidity. A non-invasive diagnostic technique predicting the presence of spermatozoa in the testis would be useful to avoid surgical intervention

in cases of absence of spermatozoa. This would decrease surgical risks, patient discomfort and likely the costs of infertility workup and treatment.

In men with non-obstructive azoospermia, spermatozoa may be found in testicular tissue, but their complete absence in testicular biopsy makes the couple unable to conceive (6, 7). Although several testicular biopsy techniques are described, the most currently used are *testicular sperm aspiration* (TESA), *open testicular sperm extraction* (TESE) and *microscopic testicular sperm extraction* with similar outcomes being reported (4, 6-8).

Previous studies correlated FSH levels with the rate of success in testicular biopsy but other factors remain to be established of important predictive value, as spermatozoa can still be found in patients with high FSH levels and patients with low volume testicles (7-9).

Besides, in cases of non-obstructive azoospermia, pregnancies are achieved in 30-50% of couples when spermatozoa are found after sperm harvesting (10).

Infertile couples usually go through a hard psychological pathway during pregnancy process and predictive factors for positive testicular biopsy may help identifying patients with higher probabilities of successful pregnancy and help to manage expectations (4, 7, 8).

Therefore, our aim was to identify possible predictive factors for a successful testicular biopsy in men with azoospermia after a diagnostic sperm analysis during infertility evaluation.

## MATERIAL AND METHODS

### *Patient selection, surgical technique and variables definition*

All men submitted to testicular biopsy during infertility investigation between January 2015 and December 2021 in *Centro Hospitalar e Universitário de Coimbra, Portugal*, were evaluated. A complete medical history and physical examination was performed. Testicular volume was evaluated and classified as normal or decreased by physical exam and further confirmed by testicular ultrasonography. Serum FSH, LH, total testosterone, free testosterone, and prolactin morning levels for endocrinologic evaluation were assessed and patients had at least two semen analysis confirming azoospermia, according to *World Health Organization Guidelines* (11). All patients were testicular biopsy naïve. Patients with microdeletions and cariotype alterations were excluded.

Patients were submitted to testicular biopsy under local anaesthesia after spermatic cord blockage as an outpatient procedure. A step-by-step approach is done in our institution, as forward described. *Percutaneous epididymal sperm aspiration* (PESA) is performed as a first procedure in one testicle and then in the contralateral in the case of no spermatozoa retrieval). If no spermatozoa are obtained after PESA, open TESE is done. A small scrotal incision is performed without exteriorization of the testicle and testicular parenchyma is excised from at least two different sites. If no spermatozoa are identified the same approach is performed in the contralateral testicle.

The extracted tissue is then preserved in sperm preparation substrate and samples are analysed by an experienced biologist. Sperm retrieved by PESA or TESE was classified as of good quality (sperm concentration > 1/HPF; with mobility *in situ* > 10%; Progressive motility), medium quality (sperm concentration < 1/HPF; with mobility *in situ* < 10%) or bad quality (rare sperm or spermatid; without mobility). Biopsy was considered positive when sperm of good or medium quality sperm was obtained.

When no sperm is found a sample is sent for histological confirmation and diagnosis.

### Statistical analysis

A demographic analysis of the entire cohort was performed. Patients were divided into two groups for comparative analysis: patients who had a positive biopsy (PB-group) and patients who had a negative biopsy (NB-group). Pearson chi-square and Mann-Whitney tests were used to compare quantitative and categorical variables across groups. Unconditional binary logistic regression was used to evaluate the independent association between possible predictors and detection of spermatozoa in the biopsy. Statistical significance in this study was set as  $p < 0.05$ . Statistical analysis was performed using IBM SPSS®, version 27.0 for Windows.

## RESULTS

We had a total of 117 patients with a mean age of  $36.5 \pm 6.0$  years. Study sample features are described in Table 1. Concerning the main conditions that may affect testicular function 22.2% patients ( $n = 26$ ) presented reduced testicular volume and 20.5% ( $n = 24$ ) presented with left varicocele. Twenty patients (17.1%) had previous history of inguinal or scrotal surgery: four had inguinal hernia correction, eight had radical orchiectomy, four had orchiopexy and four the excision of hydrocele or epididymal cysts. Six patients (5%) had been previously treated with systemic chemotherapy. Regarding metabolic factors, 32% of patients ( $n = 37$ ) had overweight or obesity (body mass index > 25 kg/m<sup>2</sup>), 14.5% ( $n = 17$ ) had type 1 diabetes mellitus and 36.8% ( $n = 43$ ) were active smokers. Testicular biopsy was positive in 48.7% of patients ( $n = 57$ ) and these form the PB-group. The remaining 60 patients with negative biopsy constitute NB-group. Groups were similar regarding demographic and clinical features, with no difference in age, history of smoking, presence of varicocele and history of inguinal and scrotal surgery (data not shown). On the other hand, the study groups were statistically different in testicular volume

**Table 1.**  
Patients characteristics.

Variable	Frequency (%)
Decreased testicular volume	26 (22.2)
Varicocele	24 (20.5)
Cryptorchidism	4 (3.4)
Previous systemic chemotherapy	6 (5.1)
Type 1 diabetes mellitus	17 (14.5)
Body mass index > 25 kg/m <sup>2</sup>	37 (31.6)
Active smoker	43 (36.8)
Inguinal or scrotal procedures	20 (17.1)
Radical orchiectomy	8
Orchiopexy	4
Hydrocele or epididymal cyst correction	4
Inguinal hernioplasty	4
Positive testicular biopsy	57 (48.7)

( $p < 0.001$ ) history of cryptorchidism ( $p = 0.047$ ) and history of type 1 diabetes mellitus ( $p = 0.015$ ) with normal testicular volume, no history of cryptorchidism and type 1 diabetes mellitus being predictive factors for successful testicular biopsy. From the 24 patients with imaging diagnosis of varicocele only 8 had a clinical varicocele and these were the patients submitted to repair (varicocele embolization). From those who received varicocele correction, 7 patients (88%) had a positive testicular biopsy ( $p = 0.059$ ) (Table 2).

In terms of endocrine evaluation (Table 3) PB-group had normal serum total testosterone levels and higher than NB-group (3.7 ng/mL vs. 2.85 ng/mL,  $p = 0.021$ ), and normal serum FSH levels and lower than NB-group (6.0 mIU/mL vs. 16.0 mIU/mL,  $p < 0.001$ ). The groups were similar concerning serum LH levels (3.9 mIU/mL vs. 6.3 mIU/mL,  $p = 0.343$ ) and serum prolactin levels (1.5

**Table 2.**  
Potential predictive factors for positive testicular biopsy.

Variable	PB-group <sup>a</sup>	NB-group <sup>a</sup>	p value
Testicular volume - normal	52	39	< 0.001
Cryptorchidism	0	4	0.047
Previous systemic chemotherapy	2	4	0.439
Type 1 diabetes mellitus	13	4	0.015
Body mass index > 25 kg/m <sup>2</sup>	21	16	0.237
Active smoker	19	24	0.455
Varicocele embolization	7	1	0.059

<sup>a</sup> frequency, in absolute number.  
PB: positive biopsy; NB: negative biopsy.

**Table 3.**  
Hormonal differences between positive biopsy (PB) and negative biopsy (NB) groups.

Hormone	PB-group	NB-group	p value
Total testosterone (ng/mL)	3.7	2.85	0.021
FSH (mIU/mL)	6.0	16.0	< 0.001
LH (mIU/mL)	3.9	6.3	0.343
Prolactin (ng/mL)	1.5	1.2	0.274

FSH: follicle-stimulating hormone; LH: luteinizing hormone.

ng/mL vs. 1.2 ng/mL,  $p = 0.274$ ). Normal FSH serum levels and normal total testosterone levels are predictive factors for positive testicular biopsy.

## Discussion

Infertility diagnostic work-up and treatment is associated with elevated costs for health systems and has a major impact in individual well-being and couples relationship (2). The development and dissemination of new fertilisation techniques allowed men who were previously defined as infertile to father children, in great part due to testicular biopsy (12, 13). About half of all infertile patients has no identified cause for infertility and determining which men will benefit from a testicular biopsy is still controversial (5). Some of the predictive factors for a positive testicular biopsy shown in our analysis are already described in literature but are not strongly established.

Our cohort is similar to other populations in literature regarding age (minimum 24 years old and maximum 54 years old) and number of positive biopsies (14, 15). An interesting point we found is age: the oldest patient in our population is 54 years old but that was not necessarily associated with lower number of positive biopsies (data not shown) indicating the ability of men to preserve fertility even into older age (16).

A total of 8 patients had clinically evident varicocele and were submitted to endovascular embolization. All these patients presented with azoospermia in the spermogram after correction and, despite this, 7 out of 8 had positive biopsy (after correction). Some authors advocate the correction of varicocele as beneficial in all patients with infertility, in particular men with semen parameters alterations, even when fertility is still not a concern but as a way to achieve normal testosterone production (16-19). A comparison to patients with untreated varicocele was not done, so a conclusion regarding formal recommendation for varicocele correction in all azoospermic men cannot be made. Notwithstanding, the results of successful biopsy after varicocele correction highlights the potential role of the procedure, mainly because in most cases it is a low-risk technique.

Hormones play a major role in spermatogenesis and so the idea that hormonal levels could predict the success of biopsy has been postulated. In our analysis, patients with a positive biopsy comparing to the ones with negative biopsy had lower (in normal range) FSH levels (6.0 mIU/mL vs. 16.0 mIU/mL), in line with other studies (9). Some studies define a cut-off of 9.9 mIU/mL as a predictor for a positive biopsy with > 90% sensitivity, but other levels have been proposed. Although it is known that even patients with very high FSH levels can have a positive testicular biopsy, a recent meta-analysis evaluating men with non-obstructive azoospermia reaffirmed FSH levels as a poor predictor for evaluating the success of sperm retrieval (20, 21). This lack of consensus may be due to the fact that FSH levels reflect the amount of testicular germ cells and not mature cells, which does not exclude the presence of mature sperm cells foci (20, 22, 23). In concordance to other studies, our results suggest that normal levels of FSH increase the probability of a positive biopsy but men with increased levels should not be excluded, as sperm may still be found.

Another testicular function hormone, LH, has been investigated as a possible marker: in our cohort, as in most studies, there was no correlation between serum LH levels and the success of biopsy (20, 24). Testosterone, the main hormone produced by the testicular tissue, is hypothesized as a valuable marker of testicular health and fertile capacity (9, 24). Our data found that men with negative biopsies had significant lower mean baseline levels of serum testosterone than patients with a positive biopsy (3.7 ng/mL vs. 2.85 ng/mL). Most literature did not find a relationship between serum total testosterone level and positive biopsy as our data shows (9, 16).

Patients with normal testicular volume in our population presented with a higher likelihood of positive biopsy as it is shown by data from other authors (1, 8).

The analysis of metabolic factors shows that patients with type 1 diabetes mellitus had higher probability of positive biopsy, while smoking and overweight or obesity did not influence these outcomes. High blood glucose levels are toxic to cellular viability, particularly in testis (25). Patients with diabetes in most cases have altered sperm parameters and are more likely to have retrograde ejaculation and atonia of seminal vesicles among other complications (26, 27). Data on sperm alterations in diabetic patients is variable: some studies showed spermatozoa with lower motility, altered morphology or decreased sperm production, while others stated that no pathological alterations were found. The prevalence of infertility may also be increased in cases of pre-diabetes (26, 28, 29). Men with diabetes mellitus have higher probability of azoospermia and need for fertilization techniques as the disease progresses, and most of them present with normal serum levels of FSH, LH and testosterone (29). Our data suggests that type 1 diabetic men benefit from performing testicular biopsy in the presence of azoospermia. A limitation of our analysis was not considering the current drugs used for diabetes treatment as they could affect fertility. Overweight and obesity alter fertility, since excessive fatty tissue interferes with hormonal balance and induces sperm parameters alterations: lower sperm motility and total sperm counts and higher risk of azoospermia. However, few studies evaluate the impact of these factors in sperm retrieval from testicular biopsy (30, 31). In general, the higher the body mass index the greater the risk of infertility worsening (30-34). Our data found no difference in biopsy success concerning body mass index.

Besides the potential bias and limitations previously mentioned, sample size is a limitation of our study. Strong points are the homogeneity of our sample and the steady team of urologist and biologist performing biopsies and sperm analysis, respectively, ensuring a similar technique.

## CONCLUSIONS

Investigating infertile men and predicting biopsy outcomes is a difficult task. Our study found that men with normal testicular volume, normal levels of testosterone and FSH and those with type 1 diabetes mellitus had a higher probability of positive testicular biopsy. More research with larger samples is still needed to ensure more robust data and conclusions.

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