Predictive factors of successful sperm retrieval in azoospermia

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Abstract

Introduction: Testicular sperm extraction with intracytoplasmic sperm injection is the standard treatment for azoospermia. The objective of this study is to identify predictive factors of successful sperm retrieval.

Materials and methods: Between June 2003 and May 2011, we tried testicular sperm extraction (TESE) in 74 azoospermic patients in the Reproductive Medicine Unit of Son Espases Hospital (Palma de Mallorca). Serum follicle stimulating hormone (FSH) and inhibin B levels, testicular histology, genetic study, presence or not of cryptozoosperma and testicular volume were examined.

Results: Spermatozoa were successfully recovered in 47.2% of the total patients, in 36% of non-obstructive azoospermic patients and in 100% of obstructive azoospermic patients. Low inhibin B and high FSH were correlated to sperm retrieval failure. The cutoff points were determined using ROC curves that were 67 pg/mL for inhibin B and 12.2 mU/mL for FSH. Spermatozoa were not successfully retrieved in any patient with Y microdeletions in AZFa,b regions. Spermatozoa were successfully retrieved in 100% of the patients with CFTR mutations. The highest sperm retrieval rate was for hypospermatogenesis, followed by maturation arrest and Sertoli-cell-only. Spermatozoa were successfully retrieved in all cryptozoospermaic patients. Although using a non-significant test, there seems to be a correlation between higher testicular volume and a higher probability of successful sperm retrieval.

Conclusions: Except for Y microdeletions in AZFa,b regions, there is no predictive factor of testicular sperm retrieval to rule out a patient for TESE. Lower inhibin B is more related to sperm retrieval failure than higher FSH. Sperm retrieval is possible for all cases of CFTR mutations but in any case of microdeletion Y in AZFa,b. The lack of germ cells is correlated with a high probability of sperm retrieval failure. The presence of cryptozoosperma is correlated with a high probability of sperm retrieval success. We do not find a statistically significant relation between testicular volume and successful sperm retrieval.

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PALABRAS CLAVE
Azooceptia; Factores predictores; Recuperación espermatológica testicular; Hormona foliculoestimulante (FSH); Inhibina B

Factores predictivos de recuperación espermatológica en las azooceptias

Resumen
Introducción: El tratamiento estándar de las azooceptias es la recuperación espermatológica del testículo para inyección intracitoplasmática. El objetivo de este estudio es identificar factores predictores de recuperación espermatológica.

Material y métodos: Intentamos recuperar espermatozoides mediante extracción espermatológica del testículo (TESE) en 74 pacientes azooceptéricos. Se estudiaron los niveles séricos de FSH e inhibina B (INHB), la histología testicular, la genética, la criptozoospermia y el tamaño testicular.

Resultados: La recuperación espermatológica fue del 47,2% para el total de pacientes, del 36% para las azooceptias no obstructivas y del 100% para las obstructivas. La INHB baja y la FSH alta se correlacionaron con el fracaso en la recuperación espermatológica. Los puntos de corte obtenidos mediante Curvas ROC fueron de 67 pg/ml para la INHB y de 12,2 mUI/ml para la FSH. En ningún paciente con microdelección Y en AZFa,b se recuperaron espermatozoides. En el 100% de los pacientes con mutaciones CFTR se obtuvieron espermatozoides. La mayor tasa de recuperación espermatológica fue para las hipoespermatogénesis, seguidas de los bloqueos madurativos y de los Sólo Sertoli. En todos los pacientes con criptozoospermia se recuperaron espermatozoides. Se encontró una relación entre el tamaño testicular y la recuperación espermatológica pero no resultó estadísticamente significativa.

Conclusiones: Salvo las microdeleciones en AZFa,b ningún factor predictor descarta a un paciente para TESE. La INHB baja se relaciona mejor con la FSH alta con el fracaso en la recuperación espermatológica. La recuperación es posible en todos los casos de mutaciones CFTR. La ausencia de células germinales se correlaciona con una alta probabilidad de fracaso en la recuperación espermatológica. La presencia de criptozoospermia se correlaciona con una alta probabilidad de éxito en la recuperación espermatológica. No encontramos relación significativa entre el tamaño testicular y la recuperación espermatológica.

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Introduction

Azooceptia is the absence of sperm in the semen after centrifugation (World Health Organization [WHO] 2010). It can be a consequence of testicular failure at the start or during the spermatogenesis due to intrinsic causes or exogenous abnormalities in the case of non-obstructive azooceptias (NOA), or it may be due to obstruction of the seminal duct in the case of obstructive azooceptias (OA). Men with NOA can become fertile by means of intracytoplasmic sperm injection (ICSI), even in cases with high FSH. The only condition is that sperm can be retrieved by means of testicular sperm extraction (TESE) or other techniques. The sperm retrieval probabilities range from 20 to 50% depending on the technique used. The procedure can cause testicular damage or stoppage of the spermatogenesis. In order to avoid unnecessary procedures, various authors have attempted to determine which patients are best candidates for TESE using predictors of success/failure in sperm retrieval:

1. High levels of serum FSH have been associated with a high probability of lack of sperm. Recent studies establish a correlation between low levels of inhibin B (INHB) with impaired spermatogenesis. Furthermore, it has been found that the INHB is a better marker of spermatogenesis than the FSH.
2. Histopathology is the best predictive factor for successful TESE, although with some limitations: it is not available before the TESE, it is invasive, and it is not exempt from complications.
3. The karyotype investigates the presence of chromosomopathies. Mutations of the cystic fibrosis gene (CFTR) are very common in obstructive azooceptias by deferens agenesia. Y microdeletions in AZFa,b,c regions are associated with abnormal spermatogenesis.
4. Cryptozoospermia is the presence of a sperm after centrifugation and reassessment of an azooceptific semen. Some studies have found a relationship with sperm retrieval.
5. Testicular size is another parameter that could be correlated with spermatogenesis. Some studies report a high likelihood of sperm retrieval for larger testes.

The aim of our study is to analyze together all the sperm retrieval predictors: the FSH, the INHB, the INHB/FSH ratio, the histopathology, the genetic alterations (Y microdeletions, karyotype abnormalities, and mutations of the CFTR gene), the cryptozoospermia, and the testicular size to predict the success/failure of sperm retrieval for ICSI.

Materials and methods

Patients

Between June 2003 and May 2011 in the Reproduction Unit of the Son Espases Hospital, Palma de Mallorca, we studied 74 azooceptic patients (61 NOA and 13 OA) who underwent
TESE. The basic study consisted of: anamnesis, physical examination (presence of deferens, subjective categorization of testicular size in normal, small, and atrophic ones), 2 spermiograms, general blood and urine analysis, serologies of sexually transmitted diseases (HIV, HBV, HCV, syphilis), hormonal study (LH, FSH, LRP, testosterone, INHB, estradiol, SHBG), and genetic study (karyotype and Y microdeletions in all and CFTR gene mutations in suspected OA).

**Hormone analysis**

Serum INHB was measured using the commercially available ELISA kit DSL-10-84100ACTIVES (Diagnostic Systems Laboratories). The FSH was determined by direct chemiluminescence assay (AdviaCentauroms, Bayer). The INHB values within the range 80–300 pg/ml were considered normal. For the FSH, the normal values were those located between 3 and 17 mIU/ml.

**Genetic analysis**

The karyotype study was performed on peripheral blood lymphocytes. For Y microdeletions, we carried out amplification by PCR in the regions AZFa,b,c. The CFTR gene study was conducted using the PCR-OLA technique, using the CFV3 (Abbott) commercial kit.

The samples were obtained by masturbation after 3–4 days of abstinence. The azoospermias were confirmed in at least 2 spermiograms, always carrying out a post-centrifugation analysis. We assessed the concentration, motility, and sperm morphology with the current WHO criteria (1999 criteria to 2010 and 2010 criteria after that date). It was considered that there was cryptozoospermia when the presence of sperm in the semen after centrifugation was taken into account.

**Testicular sperm retrieval and histopathological study**

TESE was performed in the 74 azoospermic patients. Under local anesthesia of the spermatic cord and the scrotal skin, a skin incision was made and 5 fragments were obtained open for sperm retrieval and a fragment for the histological study in Bouin’s fluid. When sperm were not obtained, the procedure was repeated on the contralateral testis in the same surgery.

**Statistical analysis**

The data were analyzed using SPSS for Windows (Version 11.5, Inc., Evanston, IL). We used contingency tables for the INHB, FSH, the presence or absence of germ cells, the cryptozoospermia, testicular size, and genetic alterations regarding the success/failure of the TESE. Each of these factors was correlated with sperm retrieval using the Chi-square test, with 95% confidence interval (CI) and a p < 0.05. We also calculated the positive predictive value (PPV) and negative predictive value (NPV) for the same factors.

In the case of the determinations of INHB, FSH, and INHB/FSH quotient, ROC curves were obtained as well as the optimal cut-off point for each of the variables.

**Results**

Of the 74 patients who underwent TESE in our study, 61 were NOA, and 13 OA. Sperms were retrieved in 35 patients, 22 cases of NOA, and 13 of OA. The sperm retrieval rate was 47.29% for the total of patients, 36% for the NOA cases, and 100% for the OA cases.

The relationship between the INHB and the sperm retrieval was significant (p < 0.001, 95% CI), with 80% sensitivity and 79.4% specificity. Out of 38 patients with INHB > 67 pg/ml, in 31 sperm were not retrieved (NPV: 81.5%) and out of 36 patients with INHB < 67 pg/ml, in 8 of them sperm were retrieved (PPV: 77.7%) with 15.5 OR (Tables 1 and 2).

The relationship between the FSH and sperm recovery was also significant (p < 0.001, 95% CI), with 71% sensitivity and 75% specificity. Of 39 patients with FSH > 12.2 mIU/ml, in 29 of them sperm were not retrieved (NPV: 74.3%), and out of 35 patients with FSH < 12.2 mIU/ml, in 25 sperm were retrieved (VPP: 71.4%), with 7.1 OR (Tables 1 and 2).

By means of ROC curves, we compare the relationship between the INHB/FSH and the FSH with sperm retrieval, finding that the INHB had 0.85 area under the curve (AUC),
Predictive factors of successful sperm retrieval in azoospermia

Table 2  Sensitivity, specificity, and area under the curve (AUC) of inhibin B and FSH to predict the success of sperm retrieval in different series of non-obstructive azoospermia.

<table>
<thead>
<tr>
<th>Studies</th>
<th>n</th>
<th>Hormone</th>
<th>Sperm retrieval rate (%)</th>
<th>Optimal cut-off point</th>
<th>ROC AUC</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nagata et al.8 (2005)</td>
<td>62</td>
<td>FSH</td>
<td>27.4</td>
<td>18.3 IU/l</td>
<td>0.77</td>
<td>58.8</td>
<td>84.4</td>
</tr>
<tr>
<td>Nagata et al.8 (2005)</td>
<td>62</td>
<td>INHB</td>
<td>27.4</td>
<td>34 pg/ml</td>
<td>0.85</td>
<td>70.6</td>
<td>95.6</td>
</tr>
<tr>
<td>Brugo-Olmedo et al.19 (2001)</td>
<td>72</td>
<td>FSH</td>
<td>41.7</td>
<td>19 IU/l</td>
<td>0.65</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Brugo-Olmedo et al.19 (2001)</td>
<td>72</td>
<td>INHB</td>
<td>41.7</td>
<td>53 pg/ml</td>
<td>0.86</td>
<td>91.11</td>
<td>75.86</td>
</tr>
<tr>
<td>Ballesca et al.7 (2000)</td>
<td>17</td>
<td>FSH</td>
<td>59</td>
<td>17 IU/l</td>
<td>0.90</td>
<td>42.8</td>
<td>70.5</td>
</tr>
<tr>
<td>Ballesca et al.7 (2000)</td>
<td>17</td>
<td>INHB</td>
<td>59</td>
<td>40 pg/ml</td>
<td>0.98</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Son Espases (2011)</td>
<td>61</td>
<td>FSH</td>
<td>36</td>
<td>12.2 IU/l</td>
<td>0.75</td>
<td>71</td>
<td>75</td>
</tr>
<tr>
<td>Son Espases (2011)</td>
<td>61</td>
<td>INHB</td>
<td>36</td>
<td>67 pg/ml</td>
<td>0.85</td>
<td>80</td>
<td>79</td>
</tr>
<tr>
<td>Tunc et al.10 (2006)</td>
<td>52</td>
<td>FSH</td>
<td>58.6</td>
<td>4.09 IU/l</td>
<td>0.523</td>
<td>90</td>
<td>19</td>
</tr>
<tr>
<td>Tunc et al.10 (2006)</td>
<td>52</td>
<td>INHB</td>
<td>58.6</td>
<td>6.25 pg/ml</td>
<td>0.557</td>
<td>90</td>
<td>14</td>
</tr>
</tbody>
</table>

The INHB/FSH 0.824, and the FSH 0.752. The optimal cut-off point was INHB< 67 pg/ml, and for the FSH> 12.2 mIU/ml for predicting the failure of sperm retrieval (Fig. 1).

Table 2 shows that the sensitivity of the test is lower than the specificity, with values ranging from 61.5% to 95.4%. The area under the curve (AUC) for both hormones is around 0.75.

With regard to the genetic study, we found 2 patients with AZFa,b microdeletions and 3 with AZFc microdeletions. In none of the first sperm were retrieved, while in the second sperm were retrieved in one case. Out of 13 patients who corresponded to obstructive causes (OA), in 9 we detected mutations of the CFTR gene, obtaining sperm in all of them. One patient with 47XY karyotype and one with a translocation also underwent TESE, but none of them retrieved sperm (Table 3).

In the histopathology, we found 13 OA patients (17.56%) and 61 NOA patients (82.4%). We found a relationship between the presence of germ cells and sperm retrieval (p<0.001), with 95.4% sensitivity and 61.5% specificity. In the NOAs, the sperm retrieval rate was 70% for the 20 patients with hypospermatogenesis, 43.5% for the 16 patients with maturation block, and 4.1% for the 25 patients with Sertoli only syndrome (Table 4).

Table 3: Spearman’s coefficient of correlation between the presence of germ cells and sperm retrieval.

In 10 patients, at least in one of their semen samples, we found some sperm after centrifugation, defined as cryoprotective. In such cases, in 100% (10 out of 10) sperm were retrieved (p < 0.001).

Regarding testicular size, a subjective categorization was performed according to the physical examination in 3 groups: normal, small, and atrophic. The sperm retrieval rate was 55.5% for normal-sized testes, 39.1% for small ones, and 16.6% for atrophic ones, but the differences found were not statistically significant (p = 0.133) (Table 5).

Discussion

In our study, in the 61 patients with NOA, the sperm retrieval rate was 36%. Tsujimura et al. in 100 patients with NOA retrieved sperm in 41% with the micro-TESE technique.15 Ziaee et al. retrieved sperm in 21.2% with TESE.16 This variability in results is probably due to the different techniques used for sperm retrieval, or the different population size of the studies.

Low INHB (<67 pg/ml) was better correlated with sperm retrieval failure, with 81.5% NPV and 15.5 OR than high FSH (>12.5 mIU/ml), with 74.3% NPV and 7.1 OR. The largest
Table 4  Sperm recovery rate according to the histopathology in non-obstructive azoospermias.

<table>
<thead>
<tr>
<th>Histopathology</th>
<th>Sperm retrieval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Presence of germ cells</td>
<td></td>
</tr>
<tr>
<td>Hypospermatogenesis</td>
<td></td>
</tr>
<tr>
<td>Maturation stoppage</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Absence of germ cells</td>
<td></td>
</tr>
<tr>
<td>Only Sertoli</td>
<td>1</td>
</tr>
</tbody>
</table>

Sperm recovery was probably the only predictor of negative retrieval with sufficient specificity to avoid giving TESE. The problem is that it is a very rare disorder, with a very low sensitivity for the detection of patients that are not candidates for sperm retrieval. Of the 39 patients with NOA in which sperm could not be obtained, AZFa,b microdeletions would have excluded only for 5.1% TESE (2 patients).

According to the histopathological examination, we found 13 patients with OA (17.5%) and 61 with NOA (82.4%). In the NOAs, there was a relationship between the absence of germ cells and the sperm retrieval failure (p < 0.001, 0.95% CI), with 95.4% sensitivity and 61.5% specificity. Only in 4.1% of the cases of Sertoli only (one in 25 patients) sperm were obtained, probably because of the existence of an isolated outbreak of spermatogenesis in the testicular tissue. By contrast, the presence of germ cells correlated with 70% sperm recovery rate for the hypospermatogeneses (20 patients) and 43.7% for maturation blocks (16 patients). Our results are consistent with those of the literature, where the sperm retrieval rates vary around 70% for hypospermatogeneses, around 50% in the maturation arrest, and 20% in Sertoli only. However, although the histological absence of germ cells is a good predictor of failure to obtain sperm, in the routine clinical practice of the Reproduction Units, it cannot be used as a prognostic factor, since testicular biopsy is performed in the same surgery as sperm retrieval, and the result is only known later.

In 50% of the cryptozoospermic patients in whom at least in one of their spermograms some post-centrifugation sperm had been found, even if it was still, sperm were recovered. Other authors found sperm retrieval rates close to 60%. We have no hypothesis to explain this difference, but we believe that in general it can be said that the prognosis for recovery in these cases is good.

With regard to testicular size, there seems to be a tenacious relationship with the likelihood of sperm retrieval, but the statistical test was not significant (p = 0.133), perhaps because of the small number of patients. In any case, we agree with other authors’ claim that there is not a lower limit of testicular volume to exclude the presence of sperm.
Conclusions

So far we do not have a predictor of sperm retrieval sensitive and specific enough to be able to properly select the patients for sperm retrieval. With regard to the factors studied:

1. Low INHB correlates better than high FSH and the INHB/FSH ratio with sperm retrieval failure, but there are still some patients with low INHB and/or high FSH where sperm retrieval is achieved.
2. The complete microdeletions of AZFa or b regions of the Y chromosome are virtually the only negative predictor that can justify excluding a patient for sperm retrieval, but their sensitivity is very low. By contrast, in the mutations of the CFTR gene, as in other cases of OA, the prognosis for recovery is very good.
3. The absence of germ cells in the histopathology correlates with a high probability of failure on sperm retrieval, but in clinical practice it is not useful to predict retrieval procedures.
4. The cryptozoospermia correlates with a high likelihood of sperm retrieval.
5. A small testicular size does not exclude the possibility of sperm retrieval.

Conflict of interest

The authors declare that they have no conflict of interest.

References

of the AZFa AZFb and AZFc regions. Hum Reprod. 2003;18:1660-5.

