Controversies in the management of nonobstructive azoospermia

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The fertility potential of patients with nonobstructive azoospermia (NOA) depends on sperm extraction from the tissue sample and then in vitro fertilization with intracytoplasmic sperm injection (IVF/ICSI). Unfortunately, there is no consensus regarding predictors that can identify nonobstructive azoospermic men with a potentially high yield at the time of sperm extraction. This article analyzes two competing approaches to these patients: noninvasive and invasive. The noninvasive approach, based on clinical, laboratory, and ultrasonographic investigations, excludes from IVF/ICSI a significant number of patients owing to errors in predicting the presence of sufficient intra-testicular spermatozoa. The invasive approach, with available percutaneous or surgical testicular biopsy techniques followed by morphologic examination and or sperm recovery, permits many patients with NOA to receive a favorable prognosis and therapeutic trial. However, the available testicular biopsy techniques are so variable that their performance parameters cannot be adequately compared. As a result, any progress in optimizing these techniques must involve delineation of specific selection criteria for each NOA patient. (Fertil Steril 2009;91:963–70. ©2009 by American Society for Reproductive Medicine.)

Key Words: Nonobstructive azoospermia, controversies, needle aspiration techniques, sperm retrieval, male infertility

The diagnosis of nonobstructive azoospermia (NOA) includes idiopathic as well as identifiable etiologies (1). Among the identified causes, there are different congenital and acquired diseases or abnormalities (1). Regardless of the underlying etiology, management of patients with NOA usually relies upon restoring fertility by the recovery of spermatozoa with a testicular biopsy/sperm extraction procedure and a successful in vitro fertilization with intracytoplasmic sperm injection (IVF/ICSI) (2). However, the recovery of sperm cells is successful in only about 50% of cases (1, 3, 4), and the subsequent pregnancy rate after IVF/ICSI is even lower (5). The importance of a successful pregnancy is an incentive to systematically attempt sperm retrieval in almost all men with NOA (1, 3, 5, 6). Because of the significant probability of eventual failure, which includes the 30%–60% of men without sperm plus the 50%–91% of the IVF/ICSI couples who do not obtain a pregnancy (7–9), a different, possibly more efficient, approach is needed. Simply put, this would be to select men with a reasonable probability of successful sperm retrieval before the procedure (10, 11). Unfortunately, no consensus on this patient selection process or the timing of the procedure exists (10, 11).

If an algorithmic approach is to be crafted, both the physician and the patient must consider the safety, invasiveness, cost, and potential results of the proposed evaluations before undertaking them. This process can vary from a clinical examination and a few laboratory tests to bilateral testicular surgery with attendant complication risks. In the present review, we examine the controversial aspects of this topic and focus on two principal, competing types of clinical approach: noninvasive and invasive.

NONINVASIVE APPROACH

Physicians and patients naturally prefer noninvasive medical evaluations, which are inherently safer, even if less accurate, over invasive procedures, which carry relatively greater risk, even if more accurate.

The performance characteristics of the principal tests that can predict sperm retrieval success with testicular biopsy are discussed below and summarized in Table 1.

Clinical and Hormonal Data

Serum FSH concentration reflects testicular volume and germ cell content of the testis. Men with an FSH level of ≥7.6 mIU/mL or a testicular long axis of ≤4.6 cm may be
contemplated to have NOA and counseled accordingly (12). These men are best treated with therapeutic testicular biopsy and sperm extraction, with processing and cryopreservation for usage in IVF/ICSI if they accept advanced reproductive treatment (12). Several reports have reviewed the sperm retrieval success rate as a function of testicular volume and serum FSH values with an upper limit as high as 20 IU/mL (13–15). Various upper limits of serum FSH up to 20 IU/mL were evaluated. For FSH levels, sensitivity varied from 9% to 71% and specificity from 40% to 90%. For the different cut-off values of testicular volume ranging as low as 5 mL, sensitivity varied from 7.6% to 50% and specificity from 6.7% to 71%. Within the range of normal FSH levels, the sensitivity of FSH in predicting successful testicular sperm extraction (TESE) is low. With elevated FSH levels, sensitivity increases and specificity decreases (10). Furthermore, many patients with maturation arrest have a normal FSH level and testicular volume (10, 16). Other reports showed that spermatozoa or mature spermatids were retrieved from, or described in testicular biopsies of, patients with an elevated FSH level and small testicle (e.g., 26 mIU/mL and 5 mL, respectively) (10, 17, 18). More recently, 42 men with Klinefelter syndrome and mean FSH levels of 33.2 IU/L showed a sperm retrieval rate of 72% per TESE attempt (19). There was no lower limit of testicular volume that excluded the presence of spermatozoa; therefore, this clinical marker cannot preclude a trial of sperm retrieval (10).

Serum inhibin B levels also have been proposed as a marker of spermatogenesis (20), and some reports have suggested its use in predicting successful sperm retrieval by testicular biopsy (21, 22). A large series that compared serum inhibin B levels with sperm retrieval results after TESE found that the best discriminating concentration, 13.7 pg/mL, had a sensitivity of 44.6% and a specificity of 63.4% (23).

Therefore, they are inadequate to determine whether or not a patient should undergo a TESE solely on the basis of a serum inhibin B concentration (23).

In a different series of 100 patients with idiopathic NOA who underwent microdissection TESE (mTESE; see below), nine parameters (age, testicular volume, LH, FSH, total T, free T, E2, PRL, and inhibin B) correlated well with sperm retrieval outcome using a multivariate logistic regression model (10). Of the nine parameters, FSH, total T, and inhibin B were most predictive of successful sperm retrieval, with a sensitivity and specificity of 71% and 71.4%, respectively (10). A different analysis was performed based on eight similar variables in predicting successful TESE in 303 NOA patients (11). The LH level and testicular volume were the only parameters significantly lower in the sperm negative than in the sperm positive group. In this study, mathematical modeling using an artificial neural network was used for predicting the outcome of TESE (11). The results were compared with those obtained by the analysis of the same data using a standard logistic regression model. The neural network analysis correctly predicted the outcome in 80.8% of the patients, whereas the logistic regression model did so only in 65.7% of the same cases (11). Thus, clinical and hormone data elaborated by artificial intelligence–based models can yield an accurate prediction of sperm retrieval after TESE in NOA patients. At present, this method as well as the use of other biomarkers, such as activins and antimullerian hormone, have limited utility owing to their novelty (11, 24, 25).

### Nonhormone and Ultrasonographic Data

It is controversial whether the detection of spermatids in the ejaculate can predict the probability of sperm retrieval (26, 27). It has been suggested that NOA patients with Y chromosomal AZF region microdeletions have a poor prognosis of sperm retrieval with TESE (1, 28). Moreover, among the different AZF microdeletions of the Y chromosome, the isolated AZFc deletion seems to be associated with a good probability of sperm detection after testicular biopsy or TESE (29). Furthermore, the importance of a karyotypic abnormality is reduced based on the recent finding of a high sperm retrieval rate with TESE in patients with Klinefelter syndrome (19).

Recently, Doppler ultrasound imaging has been proposed to identify regions of the testis in which spermatozoa are most likely to be retrieved by TESE (30, 31). In a study of 24 men with NOA having 107 regions biopsied, sensitivity and specificity of this Doppler ultrasound technique in

<table>
<thead>
<tr>
<th>Parameter or exam</th>
<th>Reference</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Overall predictive value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testicular volume</td>
<td>15</td>
<td>7.6–50</td>
<td>6.7–71</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>15</td>
<td>9–71</td>
<td>40–90</td>
<td></td>
</tr>
<tr>
<td>Inhibin B</td>
<td>23</td>
<td>44.6</td>
<td>63.4</td>
<td></td>
</tr>
<tr>
<td>FSH, total T, inhibin B</td>
<td>10</td>
<td>71</td>
<td>71.4</td>
<td></td>
</tr>
<tr>
<td>Testicular volume + hormones</td>
<td>11</td>
<td>47.3</td>
<td>89</td>
<td>80.8</td>
</tr>
<tr>
<td>Doppler ultrasound imaging</td>
<td>31</td>
<td>47.3</td>
<td>89</td>
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</table>

Sertoli cell–only syndrome have been described (39). A histo-
logic pattern of Sertoli cell–only can confound the perfor-
mance of TESE (39). In addition, a cytologic finding of se-
miferous tubules without any cells score as 1 and seminif-
erous tubules with ≥5 spermatozoa score as 10. Multivariate anal-
ysis of these independent factors was able to predict success-
ful testicular sperm extraction in patients with NOA 71% of
the time (32). Overall, testicular histology proved to be the
current best predictor of successful or unsuccessful TESE (32–35).

There are various histologic criteria that have been used to
analyze spermatogenesis within the testis (32–38). In several
studies, the presence of mature spermatozoa or late sperma-
tids had the highest predictive value (77%–100%) (32, 35,
36–38). Furthermore, two different histologic patterns of
Sertoli cell–only syndrome have been described (39). A histo-
logic pattern of Sertoli cell–only can confound the perfor-
mance of TESE (39). In addition, a cytologic finding of se-
miferous tubulas in surgical or percutaneous biopsy of the same testicle (41, 42).

This approach also permits sperm retrieval and cryopreser-
vation. Retrospective studies performed in patients with
NOA showed that cryopreservation does not adversely affect
ICSI outcomes (44–49).

### Invasive Approach

#### Testicular Biopsy—General Data

Ezeh et al. (32) evaluated age, body mass index, various hor-
monal levels, testicular volume, the presence of spermatids in
a testicular biopsy, and the Johnsen score of testicular biopsy
histology for predicting successful sperm retrieval with
TESE. The Johnsen score ranges from 1 to 10, where seminif-
erous tubules without any cells score as 1 and seminiferous
tubules with ≥5 spermatozoa score as 10. Multivariate anal-
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Moreover, some authors have reported that FNAB cytology was more
sensitive than surgical or percutaneous biopsy histology for
sperm detection (41–43). FNAB for the selection of testicles
containing sperm can be easily performed in an ambulatory
setting with one or two percutaneous punctures (43). In con-
trast, FNA for testicular area selection (mapping) requires 20–
30 needle excursions in more than four testicular areas (42).

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### Testicular Biopsy—Techniques

The techniques for sperm retrieval in patients with NOA have
evolved considerably in the last decade. In NOA patients, the
function and histology within the testicle are heterogeneous
(50). Therefore, the likelihood of sperm retrieval depends
on regional sperm production. This likelihood can be in-
creased if a greater volume of testicular tissue is sampled.
Testicular biopsy techniques can be grouped into broad cate-
gories: percutaneous and open (surgical).

#### Percutaneous techniques

Percutaneous biopsy techniques can be performed in the office setting and avoid the need
for most traditional surgical equipment. However, there is
a large variation among the techniques in terms of materials,
technical aspects, and general conditions of use that make a comparison of their performances difficult. Table 2 clas-
sifies the principal percutaneous testicular biopsy techniques
used for sperm recovery in NOA patients and includes the
needle and biopsy specimen size.

**Table 2**

<table>
<thead>
<tr>
<th>Percutaneous biopsy technique</th>
<th>Reference</th>
<th>Needle size, gauge</th>
<th>Specimen size, no. of ST, range or mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fine needle aspiration biopsy</td>
<td>51, 52</td>
<td>23–19</td>
<td>10–20</td>
</tr>
<tr>
<td>Coarse or cutting needle biopsy</td>
<td>59–66</td>
<td>18–16</td>
<td>15, 20</td>
</tr>
<tr>
<td>Large needle aspiration biopsy</td>
<td>41, 43, 67–70</td>
<td>22–18</td>
<td>52</td>
</tr>
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</table>

Note: ST = seminiferous tubules.

a case of testicular bleeding after FNAB with 21-gauge butterfly needle that was managed conservatively (57).

Owing to differences in the various FNAB and open surgical techniques, it is difficult to assess how FNAB can supplant open surgery for sperm retrieval. Although FNAB can be successful in sperm retrieval (26), when compared head-to-head studies showed that open surgery was successful more often than FNAB and that after unsuccessful FNAB, open surgery could still yield sperm (53–54).

**Large Needle Biopsy (LNB)** LNB typically uses relatively larger needles, from 14 to 20 gauge, and provides tissue cylinders or fragments for conventional histologic evaluation as well as sperm retrieval. Testicular LNB techniques can be classified into two groups: large needle cutting biopsy (LNCB) and large needle aspiration biopsy (LNAB) (58).

The LNCB techniques (also called coarse or cutting needle biopsy) use the largest needles (the 14-gauge Tru-Cut disposable needle or the biopsy-gun with the 14- or 18 gauge needle) (59–66).

The Tru-Cut needle and the biopsy Gun are instruments made of two sliding cylinders. The operator maintains sterile technique, because a very small skin incision is usually performed.

In the past, with the use of large needles, prolonged intense pain and large hematomas were reported in some patients (59, 64, 65). More recently, with the use of the smaller needles, almost no clinically evident complications were reported (43, 67). The size of the biopsy tissue fragment was evaluated by the number of seminiferous tubules per histologic section (43). The mean value was reported to vary from 14 to 38.9 (41, 43, 64–67), being slightly higher when ported (43, 67). The size of the biopsy tissue fragment was evaluated by the number of seminiferous tubules per histologic section (43). The mean value was reported to vary from 14 to 38.9 (41, 43, 64–67), being slightly higher when ported (43, 67).

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Percutaneous LNAB is a technique that has been used for evaluation of palpable thyroid nodules and now to obtain testis tissue (68–70). It does not require a skin incision or sterile environment and uses a commonly available disposable 18-, 20-, or rarely 22-gauge needle (67) attached to a 20–30-mL syringe placed in the usual “pistol.” With local anesthesia, the same procedure as for FNAB is performed except more vigorously in the case of LNAB. In >100 men with NOA, the technique safely obtained testicular fragments larger than those of other percutaneous LNCB techniques and similar to those of open surgical techniques (70). The mean number of seminiferous tubules was 52, with a section length of 0.2 × 0.3 × 0.6 cm and a weight of 385 mg (70). A recent study (71) described how LNAB obtains relatively large biopsy specimens in a safe manner. In this study, the internal and external cross-sectional area and volume of eight dispos-
vasculature. This requires the use of the operating microscope, which also helps in identifying the larger and more opaque tubules which might indicate sites of active spermatogenesis (73, 78, 80). High sperm retrieval rates (35%–77%) (10, 78–87) and relatively high quantities of retrieved sperm (160,000) from relatively small tissue fragments (72 mg) have been reported with this technique in NOA (78). Furthermore, this technique was associated with a reduced risk of testicular tissue damage with long-term ultrasound and endocrinologic evaluation (74, 75, 88). The mTESE technique was more successful in sperm retrieval than the standard surgical biopsy when sampling either a single large specimen (81) or multiple specimens (79, 82). Therefore, the mTESE technique seems to increase the probability of sperm retrieval even if it prolongs

<table>
<thead>
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<th>Reference</th>
<th>n</th>
<th>Study design</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Tsujimura et al. (10)</td>
<td>100</td>
<td>Retrospective NOA</td>
<td>41/100 (41%) SRR; total T, FSH, and inhibin-B levels predicted SSR</td>
</tr>
<tr>
<td>Schlegel (78)</td>
<td>49</td>
<td>Prospective NOA</td>
<td>cTESE: 10/22 (45%) SRR; mTESE: 17/27 (63%) SRR ($P &lt; .05$)</td>
</tr>
<tr>
<td>Okada et al. (79)</td>
<td>146</td>
<td>Retrospective comparison</td>
<td>OA: 100% SRR for cTESE and mTESE; NOA: 16.7% SRR for cTESE and 44.6% SRR for mTESE ($P = .0271$); lower complication rate and marked advantage with Sertoli cell–only syndrome with mTESE</td>
</tr>
<tr>
<td>Kamal et al. (80)</td>
<td>116</td>
<td>Prospective NOA</td>
<td>Higher SRR in isolated (dilated and opaque) seminiferous tubules (72.7%) compared with the rest of the specimen (52%) ($P &lt; .05$)</td>
</tr>
<tr>
<td>Raman and Schlegel (81)</td>
<td>38</td>
<td>Retrospective NOA + cryptorchidism</td>
<td>cTESE: 5/8 (63%) SRR; mTESE: 30/39 (77%) SRR</td>
</tr>
<tr>
<td>Tsujimura et al. (82)</td>
<td>93</td>
<td>Prospective NOA</td>
<td>mTESE: 24/56 (42.9%) SRR; multiple cTESE: 13/37 (35.1%) SRR (NS)</td>
</tr>
<tr>
<td>Nudell et al. (89)</td>
<td>26</td>
<td>Prospective OA</td>
<td>mTESE had greater SRR (47%) compared with single cTESE technique (30% SRR) ($P &lt; .05$); patients had identical bilateral histopathology with mTESE on one side and cTESE on the other; mTESE was associated with fewer complications</td>
</tr>
<tr>
<td>Amer et al. (83)</td>
<td>100</td>
<td>Prospective NOA</td>
<td>25/26 (96%) SRR</td>
</tr>
<tr>
<td>Miyazaki et al. (90)</td>
<td>—</td>
<td>Review</td>
<td>70% overall fertilization success rate with mTESE/ICSI</td>
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<tr>
<td>Tsujimura et al. (91)</td>
<td>60</td>
<td>Retrospective NOA</td>
<td>6/60 for AZF deletion (10% detection rate using mTESE)</td>
</tr>
<tr>
<td>Everaert et al. (84)</td>
<td>48</td>
<td>Retrospective NOA</td>
<td>17/48 (35%) SRR with 4/17 (24%) per couple take home baby rate if sperm retrieved; 16% de novo androgen deficiency rate (2.4 ± 1.1 years follow-up)</td>
</tr>
<tr>
<td>Tsujimura et al. (85)</td>
<td>180</td>
<td>Retrospective NOA</td>
<td>134 cTESE: 44% SRR; 46 mTESE after unsuccessful cTESE: 45.7% SRR (NS)</td>
</tr>
<tr>
<td>Talas et al. (86)</td>
<td>68</td>
<td>Retrospective NOA + ICSI</td>
<td>64% SRR first attempt; 82% SRR with repeat attempts (up to four times)</td>
</tr>
<tr>
<td>Camignani et al. (87)</td>
<td>4</td>
<td>Case series</td>
<td>3/4 (75%) SRR in patients with cancerous testicle</td>
</tr>
</tbody>
</table>

Note: cTESE = conventional testicular sperm extraction; ICSI = intracytoplasmic sperm injection; mTESE = microdissection testicular sperm extraction; NOA = nonobstructive azoospermia; NS = not statistically significant; OA = obstructive azoospermia; SRR = sperm retrieval rate.

operative time (82). Nevertheless, hormonal follow up is recommended after mTESE (84).

CONCLUSION
In this review, various methods to evaluate the patient with NOA are critically examined. The noninvasive approach can be used to select NOA patients for possible testicular biopsy and can potentially avert useless interventions. However, these noninvasive tests may exclude a significant number of men from IVF/ICSI.

On the other hand, the invasive approach can identify appropriate patients for IVF/ICSI. However, these invasive approaches can result in a significant number of useless interventions, namely, 30%–40% of testicular biopsies without recovered spermatozoa or 70% of the pregnancies after IVF/ICSI that do not lead to a live birth.

How can these two competing approaches be reconciled and a standard approach be fashioned? Many factors make it difficult to precisely quantify the risks and benefits of each approach. For example, the pathogenesis of NOA and the noninvasive and invasive testing protocols differ greatly among the various clinical studies. Nevertheless, all of the available testicular biopsy techniques allow many NOA patients the opportunity to receive a prognosis and therapeutic trial.

With emergent data using all types of clinical investigative testing in diverse NOA patient populations, we envision a clinical algorithm following this decision tree:

1. Identify patients at risk for NOA, based on clinical history and prior medical evaluation.
2. Perform noninvasive tests to select patients who will benefit from invasive testing, bypassing this step if the clinical scenario alone provides sufficient justification for invasive testing.
3. Decide which invasive test is most appropriate, based on risk-benefit, cost-effectiveness, and local experience with available procedures.
4. Based on the results of the invasive test, provide therapeutic options for the patient.

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