

Andrology Lab Corner*

Diagnosis of Nonobstructive Azoospermia: The Laboratory Perspective

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The definition of male infertility was open for interpretation during the last decade since the introduction of intracytoplasmic sperm injection (ICSI) as an acceptable and routine technique in in vitro fertilization (IVF) and assisted reproduction technology (ART) procedures ([Palermo et al, 1992](#)). It is well documented that once a single living sperm cell has been injected into a normal metaphase II oocyte it has the potential to achieve successful fertilization, and normal embryo development can proceed. The overall pregnancy rate per cycle recently has been estimated to be as high as 39% ([Silber et al, 1995](#); [Craft et al, 1997](#)).

This trend should increase the significant role that the andrology team (physicians as well as embryologists and laboratory technicians) has in ART procedures. The task of isolating a single, viable spermatozoon from a man with azoospermia has become almost a specialty in itself. This task is even more complicated in men with nonobstructive azoospermia (NOA).

The diagnosis of a man with azoospermia should include semen analysis (volume, pH, fructose), careful inspection of the semen sediment obtained by ultracentrifugation of seminal plasma ([Ron-EI et al, 1997](#)), and clinical examination for the presence of vasa deferentia. The next step, differentiation between obstructive azoospermia and NOA, is almost unachievable without testicular biopsy, although other indirect tests such as endocrine evaluation may provide clues ([Matsumiya et al, 1994](#)).

Once NOA is diagnosed, one should address the severity of spermatogenic impairment. Hypospermatogenesis provides good chances for isolating viable, intact spermatozoa with fertilizing capacity. The difficulties occur whenever mature spermatozoa are absent. In such cases, one should be cautious not to quickly conclude the existence of Sertoli cell only syndrome. Minute focal

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spermatogenesis may still take place in the testis without sperm appearing in the selected biopsy specimen or released as part of the ejaculate ([Tournaye et al, 1996a](#)). In such cases it may be possible to isolate sperm cells from additional testicular specimens and to use them in the IVF-ICSI procedure ([Ben-Yosef et al, 1999](#)). The major challenge is to locate the testicular regions with the highest probability of intact spermatogenesis. Precise inspection of the cytology and histology of the specimen is crucial and significant in order to determine the number, location, and size of biopsies to obtain. A skilled pathologist usually performs the evaluation but the process is still prone to errors ([Ezeh et al, 1998](#)).

Few biopsy techniques may use isolated testicular tissue or spermatogenic cells. Basically, they include either tissue extraction (testicular sperm extraction; TESE), needle tissue aspiration (the testicular sperm aspiration; TESA) ([Craft et al, 1997](#)), and the recently developed procedure of microdissection TESE as a method for identifying tubules with foci of spermatogenesis ([Schlegel, 1999](#)). TESE is performed as an open biopsy under local anesthesia. A piece of about 50 mg of the tissue is taken out and minced, and the suspension is carefully assessed for the existence of sperm cells. One piece of the biopsy is also usually taken for histopathology evaluation. TESA is performed by fine needle biopsy ([Fasouliotis et al, 2002](#)). According to a report by Hauser et al ([1998](#)), a multiple TESE is superior in cases of NOA because it may enhance diagnostic accuracy and increase the chance of sperm cell retrieval. On the other hand, a single large biopsy can be sufficient for this purpose ([Silber, 2000](#)). The sperm cells that are isolated from the testis may occasionally have morphology as good as that of ejaculated spermatozoa ([Yavetz et al, 2001](#)).

There is not yet, however, a reliable prebiopsy predictor for determining the chance of locating active spermatogenesis within a person or a testis. We have established a histochemical staining method to find the tubule in which germ cells are present in testis biopsies. This has been performed by the use of anti-RNA binding motif (RBM) protein antibodies. It has been shown that the RBM gene is expressed in germ cells from the spermatogonia stage throughout maturation up to the round spermatid stage ([Maymon et al, 2001](#); [Bar-Shira Maymon et al, 2002](#)). Thus, positive staining in testis with so-called "mixed atrophy" should reveal the presence of focal spermatogenesis rather than pure "Sertoli cell only," allowing direct exploration for active spermatogenesis even to minute regions ([Silber, 2000](#)), and persisting on repeated TESE whenever spermatozoa are not found and when anti-RBM staining is positive.

Recent studies ([Hauser et al, 1998](#); [Yogev et al, 2000a](#); [Bar-Shira Maymon et al, 2002](#)) have shown that a high rate of bivalent formation of homologous chromosomes in spermatocytes increases the prospect of focal spermatogenesis in the testes of men with NOA. Bivalent formation can be analyzed with fluorescence in situ hybridization (FISH) using alpha-satellite centromere probes for the evaluation of autosomal ([Hauser et al, 1998](#); [Yogev et al, 2000a](#); [Bar-Shira Maymon et al, 2002](#)) and sex bivalents. One fluorescent signal indicates normal synapsis, whereas two distant signals indicate failure of synapsis and crossing-over ([Yogev et al, 2000b](#)). Failure of bivalent formation was found to be significantly associated with meiotic arrest. Yet, the opposite is much more meaningful—a higher rate of bivalents in all homologous chromosomes almost ensures the existence of spermatozoa in the testicular specimen ([Yogev et al, 2000a](#)).

The rate of bivalent X-Y has been found to be the most sensitive predictor for detecting spermatozoa as well as the number of mature spermatids per tubule in a histological section ([Yogev et al, 2002](#)). It is recommended that when histologic evaluation of the testis reveals arrest of spermatogenesis that it should be accompanied by a FISH pairing test using at least the X-Y bivalent segregation test. A high rate of bivalent formation may urge one to continue with a meticulous search for mature spermatids/sperm cells in the testes as well as attempting repeated TESE ([Kleiman et al, 1999a](#)).

These methods of evaluation may assist the clinician primarily when there is failure to locate spermatozoa for establishing the prognosis and further management.

Genetic control of spermatogenesis is of particular interest in the era of the human genome project ([Tournaye et al, 1996a](#)). It has been shown that many genes located on both the sex and autosomal chromosomes are essential for the precise control of the process. Accordingly, a man with NOA should be genetically evaluated for major chromosomal abnormalities (numerical and structural) as well as to assess microdeletions in the Y chromosome. One of the major genetic disorders that leads to NOA is Klinefelter syndrome, in which the addition of one X chromosome leads to azoospermia and Sertoli cell only syndrome or testicular atrophy ([Friedler et al, 2001](#)). Recently, it has been shown that if the chromosomes of a man with Klinefelter syndrome exhibits a mosaic pattern, then focal sperm production can be located in the testes ([Tournaye et al, 1996b](#)). In these cases, most sperm are haploid, and normal offspring may be conceived using ICSI.

It has been found that men with NOA have the highest risk for Y chromosome microdeletions ([Krausz et al, 2000](#)). Three close regions were found to have a role in spermatogenesis and are named azoospermia factors (AZFs) a, b, and c ([Vogt et al, 1996](#)). The microdeletions are associated with impairment of spermatogenesis. Deletions in AZFa or an entire AZFb deletion are considered to predict a zero percent chance of finding sperm in the testicular biopsy. Most of the cases are defined by histology as Sertoli cell only syndrome or spermatocyte arrest ([Kleiman et al, 2001a](#)). In cases of AZFc deletion, sperm cells can be found in the testes in approximately 60% of cases. It is clear that a male offspring will bear the same deletion of his father ([Kleiman et al, 1999b](#)).

Recently, the chromodomain Y (CDY) gene, located close to the end of the AZFc region, was found to play a role in determining the presence of matured sperm cells in the testes ([Kleiman et al, 2001b](#)). CDY1-minor expression was detected in all biopsies in which mature spermatids or spermatozoa were found by histological analysis, or in the minced tissue, or both. Recently, it has been shown that the CDY1 gene encodes for histone-acetyltransferase, an enzyme that facilitates the transformation of histones to protamines during spermatid maturation ([Lahn et al, 2002](#)). Accordingly, assessing the expression of the genes encoding for RBM, DAZ, or CDY1 can be of great help in the search for sperm cells in men with NOA, thereby maximizing the efficacy of successful sperm recovery by testicular biopsy ([Tournaye et al, 1997](#)).

Another interesting approach for fertility restoration in men with NOA involves attempts to advance the maturation of spermatogenic cells in vitro, especially in cases of spermatogenic or meiotic arrest. In one report, pieces of testicular tissues were incubated in hormone-supplemented and growth factor-supplemented medium in vitro in order to stimulate maturation of spermatogenic cells ([Tesarik et al, 2000](#)). There is still a great deal of controversy and skepticism regarding the results reported so far, but once the methodological obstacles are resolved this approach may contribute to the effective management of NOA.

In summary, in contrast to a decade ago, a man with NOA is not today considered sterile. The new diagnostic tools that are available in andrology laboratories enable the andrology team to provide better diagnosis and treatment using highly sophisticated diagnostic tools and treatment procedures. The andrology practice and laboratory are not "worn out," but rather, these new diagnostic tools represent a promising start.

Footnotes

Andrology Lab Corner welcomes the submission of unsolicited manuscripts, requested reviews, and articles in a debate format. Manuscripts will be reviewed and edited by the Section Editor. Papers appearing in this section are not considered primary research reports and are thus not subjected to peer review. All submissions should be sent to the *Journal of Andrology* Editorial Offices. Letters to the editor in response to articles as well as suggested topics for future issues are encouraged. [↑](#)

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