21

ICSI with Epididymal and Testicular Sperm Retrieval

SHERMAN J. SILBER

Introduction

Silber et al. and Tournaye et al. initially developed the use of intracytoplasmic sperm injection (ICSI) to treat obstructive azoospermia due to congenital absence of the vas deferens (CAV), failed vasopepididymostomy (V-E), and otherwise irreparable obstruction, using microsurgically retrieved *epididymal* sperm (1,2). We coined this procedure "MESA" (i.e., *m*icrosurgical *e*pididymal *s*perm *a*spiration). Devroey et al., Silber et al., and Schoysman et al. then demonstrated the systematic use of ICSI with *testicular* sperm in cases where there is either no epididymis or no motile sperm in the epididymis (3–5). Several months later, Devroey et al. and Silber et al. demonstrated that intracytoplasmic sperm injection using frozen–thawed epididymal spermatozoa retrieved from a previous attempt at fresh MESA was as successful as using freshly retrieved sperm (4,6). The present state-of-the-art appears to be that there are very few cases of obstructive azoospermia that cannot be successfully treated with sperm retrieval methods and ICSI, as long as the wife has adequate eggs (7). This may involve the use of epididymal sperm, or, if epididymal sperm cannot be retrieved, the use of testicular sperm.

Sperm Retrieval Methods

There have been many trivial debates over how best to collect epididymal or testicular sperm from azoospermic patients for ICSI. The reader can decide what works best in a particular setting. Our preference is as described in the following.

For cases of obstructive azoospermia, there is usually some epididymis present. If so, then we prefer to perform MESA via a very small "window"

incision in the scrotum under local anesthesia using 0.5% marcaine. By injecting both the spermatic cord as well as the anterior scrotal skin, we can easily expose the epididymis, and with an operating microscope, complete the procedure in about 15 min. The advantage of epididymal sperm retrieval performed in this fashion is that huge numbers of the most motile sperm can readily be obtained from the most proximal ducts, and then frozen for an unlimited number of future ICSI cycles. There is often only one specific area of the proximal epididymis where the most motile sperm can be retrieved, and this can be found more easily through microsurgery than through a blind needle stick. The disadvantage, particularly for the gynecologist, is that it requires skills the infertility physician may not possess.

For cases of obstructive azoospermia where there is no epididymis (most unusual), a simple needle stick into the testis will usually retrieve enough sperm for ICSI, but not enough for reliable freezing for future cycles. Because our open biopsies are so simple, quick, and painless, we still prefer it to a needle stick in these cases. For nonobstructive azoospermia, an open biopsy under local anesthesia is clearly the preferred approach.

Nonobstructive Azoospermia

Men with the most severe spermatogenic defects causing complete azoospermia often have a minute number of sperm, or mature spermatids, very sparsely present in an extensive testicular biopsy (which could then be used for ICSI) (8). This approach was based on quantitative studies of spermatogenesis dating back to the late 1970s (9–11). Testicular histology of azoospermic, oligospermic, and normospermic men has shown that the number of sperm in the ejaculate is directly correlated to the number of mature spermatids found quantitatively in the testis. Although there is a wide variation in each tubule, the average mature spermatid count in a large number of tubules was very clearly always predictive of the sperm count in the ejaculate. It is intriguing that many patients with complete azoospermia in the ejaculate were found to have extremely low numbers of mature spermatids per seminiferous tubule. These studies of quantitative spermatogenesis in the late 1970s and early 1980s gave the impetus for our efforts to extract sperm, however few, from men with azoospermia caused by Sertoli cell only or maturation arrest, and to use these few sperm for intracytoplasmic sperm injection (ICSI).

Applying the technique of testicular sperm extraction (TESE), which was developed originally for obstructive azoospermia, it was found that even in azoospermic men with apparently absent spermatogenesis (diagnosed as "Sertoli cell-only syndrome"), there is very frequently a tiny focus of sperm production still to be found somewhere in the testicles (6,12,13). This went undiscussed in those early papers, but it is now apparent that an extremely diminished quantity of sperm production in the testes will result in absolute azoospermia in the ejaculate, even though there is some sperm being pro-

duced. A certain tiny threshold of sperm production is necessary before any sperm can actually appear in the ejaculate. It was quite possible, therefore, that very small, tiny numbers of spermatozoa might exist in the testes sufficient for an ICSI procedure, seen in patients who are azoospermic apparently from "absence" of spermatogenesis. This observation led us to perform successful TESE for patients with azoospermia due to Sertoli cell-only syndrome, or those with cryptorchid testicular atrophy, who had high FSH levels, very small testes, apparently absent spermatogenesis, and no obstruction (6).

Thus, severe oligospermia (which is readily treated with ICSI) is just a quantitative variant of azoospermia in that there is some minute presence of spermatogenesis in 60% of azoospermic men, but the amount of spermatogenesis is below that threshold necessary for a few sperm to "spill over" into the ejaculate. For the purpose of comparing Y chromosomal deletions to the degree of spermatogenic defect, azoospermic men with at least a few sperm retrievable from the testes may be in a similar category to very severely oligospermic men. Azoospermic men in whom there was absolutely no sperm retrievable either from the ejaculate or from testicular sperm extraction turn out to be in a different category from azoospermic men who have a minute amount of sperm production (14–16).

In those infertile men who are Y-deleted, larger deletions appear to be associated with a total absence of testicular sperm; however, smaller deletions, limited simply to DAZ, are associated with the presence of small numbers of sperm that are sufficient for ICSI. This implies that there are other modifying genes on the Y that can further affect the severity of the spermatogenic defect in DAZ-deleted infertile men.

References

1. Silber SJ, Nagy ZP, Liu J, Godoy H, Devroey P, Van Steirteghem AC. Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. Hum Reprod 1994;9:705–9.
2. Tournaye H, Devroey P, Liu J, Nagy Z, Lissens W, Van Steirteghem A. Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital absence of the vas deferens. Fertil Steril 1994;61:1045–51
3. Devroey P, Liu J, Nagy P, Tournaye H, Silber SJ, Van Steirteghem AC. Normal fertilization of oocytes after testicular sperm extraction and intracytoplasmic sperm injection (TESE and ICSI). Fertil Steril 1994;62:639–41
4. Silber SJ, Van Steirteghem AC, Liu J, Nagy Z, Tournaye H, Devroey P. High fertilization and pregnancy rate after intracytoplasmic sperm injection with sperm obtained from testicle biopsy. Hum Reprod 1995;10:148–52.
5. Schoysman R, Vanderzwalmen P, Nijs M, et al. Pregnancy after fertilisation with human testicular spermatozoa. Lancet 1993;342:1237.
6. Devroey P, Silber S, Nagy Z, et al. Ongoing pregnancies and birth after intracytoplasmic sperm injection (ICSI) with frozen-thawed epididymal spermatozoa. Hum Reprod 1994;10:903–6.

7. Silber SJ, Nagy Z, Liu J, et al. The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. Hum Reprod 1995;10:2031–43.
8. Silber SJ, Nagy Z, Devroey P, Tournaye H, Van Steirteghem AC. Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure. Hum Reprod 1997;12:2422–28.
9. Steinberger E, Tjioe DY. A method for quantitative analysis of human seminiferous epithelium. Fertil Steril 1968;19:960–70.
10. Zuckerman Z, Rodriguez-Rigau L, Weiss DB, Chowdhury LJ, Smith KD, Steinberger E. Quantitative analysis of the seminiferous epithelium in human testicle biopsies and the relation of spermatogenesis to sperm density. Fertil Steril 1978;30:448–55.
11. Silber SJ, Rodriguez-Rigau L. Quantitative analysis of testicle biopsy: determination of partial obstruction and prediction of sperm count after surgery for obstruction. Fertil Steril 1981;36:480–85.
12. Silber SJ. What forms of male infertility are there left to cure? Hum Reprod 1995;10: 503–4.
13. Silber SJ, Van Steirteghem AC, Devroey P. Sertoli cell only revisited. Hum Reprod 1995;10:1031–32.
14. Lahn BT, Page DC. Functional coherence of the human Y chromosome. Science 1997;278:675–80.
15. Reijo R, Lee T-Y, Salo P, et al. Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. Nat Genet 1995;10:383–93.
16. Tiepelo L, Zuffardi O. Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. Hum Genet 1976;34:119–24.

Serono Symposia USA
Norwell, Massachusetts

Samir Hamamah
François Olivennes
Roger Mieusset
René Frydman
Editors

Male Sterility and Motility Disorders

Etiological Factors and Treatment

A Serono Symposia S.A. Publication

Foreword by R.G. Edwards

With 26 Figures

Springer