ICSI with Epididymal and Testicular Sperm in Azoospermic Men

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Abstract. All cases of obstructive azoospermia can now be treated relatively simply with sperm retrieval and ICSI. Our work on the more difficult problem of non-obstructive azoospermia can be summarized as follows: 1) In order for any sperm to reach the ejaculate, >5 mature spermatids must be present in a section of testis; 2) Prior diagnostic testicle biopsy analyzed quantitatively is useful for predicting which patients will have success or failure with TESE-ICSI; 3) Incomplete testicular failure appears to involve a sparse multi-focal distribution of spermatogenesis throughout the entire testicle, rather than a patchy or local distribution in just a few areas; 4) When foci of sperm were present, round spermatids were also present. In the absence of elongated spermatids or sperm, round spermatids could not be found; 5) Successful TESE-ICSI in patients with non-obstructive azoospermia will depend not on the finding of round spermatids in the absence of mature sperm, but rather on finding tiny foci of spermatogenesis with normal-appearing sperm in 60% of cases; 6) Our experience suggests that there are several genes on the non-recombining portion of the Y-chromosome (NRY) other than DAZ that impinge on spermatogenesis. This is consistent with the view that the Y-chromosome has collected, during evolution of higher primates, previously autosomal spermatogenesis genes with resultant amplification and subsequent degeneration.

Key words: azoospermia, ICSI, MESA, TESE, sperm retrieval, Y-chromosome

Introduction

In 1992, we initiated a genome mapping study for men with non-obstructive azoospermia due to germinal failure. Concomitantly the first major breakthrough was being developed for the treatment of male factor infertility, i.e., intracytoplasmic sperm injection (ICSI).[1-3] This breakthrough was extended from cases of severe oligoasthenospermia to actual azoospermia in August 1993, when it was demonstrated that sperm derived from either the epididymis or the testicle was capable of normal fertilization and pregnancy rates in cases of obstructive azoospermia.[3-8] In 1995, the use of testicular sperm extraction (TESE) and ICSI was extended further to men with azoospermia caused by defective spermatogenesis.[9-12] The five-year history of this progression has already been reviewed.[13]

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 vasoepididymostomy (V-E), and otherwise irreparable obstruction, using microsurgically retrieved epididymal sperm.[3,6] We coined this procedure 'MESA', i.e., microsurgical epididymal sperm aspiration. Then, Devroey et al.

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and Silber et al. demonstrated the systematic use of intracytoplasmic sperm injection (ICSI) with testicular sperm in cases where there is either no epididymis, or no motile sperm in the epididymis.[7,14] Several months later, Devroey et al. and Silber et al. demonstrated that ICSI using frozen thawed epididymal spermatozoa retrieved from a previous attempt at fresh MESA was as successful as using freshly retrieved sperm.[7,15] 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 so long as the female has an adequate number of eggs.[9] 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 one's own particular setting. Our preference is as follows:

For cases of obstructive azoospermia, there is usually some epididymis present. If so, we prefer to perform MESA via a very small 'window' incision in the scrotum under local anesthesia using 0.5% bupivacaine. By injecting both the spermatic cord and also the anterior scrotal skin, we can easily expose the epididymis, and with an operating microscope complete the procedure in about 15 minutes. 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 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 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 will usually retrieve sufficient 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 non-obstructive azoospermia, an open biopsy under local anesthesia is clearly the preferred approach.

Non-obstructive azoospermia

Men with the most severe spermatogenic defects causing complete azoospermia were found to have a minute number of sperm, or mature spermatids, very sparsely present in an extensive testicular biopsy (which could then be used for ICSI). This approach was based on quantitative studies of spermatogenesis dating back to the late 1970s.[16-18] 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. Intriguingly, 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 ICSI.

Applying the technique of testicular sperm extraction (TESE) developed 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.[4,10,15] The original studies on quantification of spermatogenesis by histology demonstrated a puzzling number of completely azoospermic patients who had nonetheless demonstrated an average of one or two spermatids per seminiferous tubule.[16-19] This went undiscussed in those early papers, but now it is 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 produced. A certain tiny threshold of sperm production is necessary before any sperm can actually appear in the ejaculate. Therefore, it was quite possible that very small, tiny numbers of spermatozoa might exist in the testes sufficient for an intracytoplasmic sperm injection (ICSI) procedure, seen in patients who are azoospermic apparently from 'absence' of spermatogenesis. This observation led us to perform successful testicular exploration with sperm extraction (TESE) for patients with azoospermia due to Sertoli cell only syndrome or cryptorchid testicular atrophy, who had high FSH levels, very small testes, apparently absent spermatogenesis, and no obstruction.[15]

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 the threshold necessary for a few sperm to 'spill over' into the ejaculate. Thus, 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, might possibly be in a different category.

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

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