

Sperm Retrieval Procedures and Intracytoplasmatic Spermatozoa Injection with Epididymal and Testicular Sperms

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Key Words

Intracytoplasmatic sperm injection · Sperm retrieval ·
Microsurgical epididymal sperm aspiration · Testicular
sperm extraction

Abstract

Introduction: Male infertility caused by azoospermia due to non-reconstructable obstruction or non-obstructive azoospermia can be treated by microsurgical epididymal aspiration (MESA) or testicular sperm extraction (TESE) followed by an intracytoplasmatic spermatozoa injection (ICSI). **Material and Methods:** From 9/93 to 6/01, we carried out 1,025 ICSI procedures with aspirated epididymal or testicular sperms in 684 cases. 163 ICSI cycles were performed with epididymal sperms and 862 ICSI cycles with testicular sperms or spermatids. The TESE was carried out by open biopsy, frequently in a multilocular technique. The aspirated spermatozoas were used after cryopreservation (frozen) or immediately after aspiration (fresh). **Results:** 538 patients had obstructive azoospermia or ejaculation failure. In 487 cases the underlying cause of azoospermia was an impaired spermatogenesis, following maldescensus testis, chemotherapy, radiotherapy, or caused by Sertoli-cell-only syndrome, a ge-

netic disorder or an unknown etiology. The transfer rates, pregnancy rates and birth rates per ICSI cycle showed no statistically significant differences between testicular and epididymal sperms in the cases of seminal obstruction (28% average birth rates in both cases). However, highly significant was the difference in birth rates with regard to the underlying cause of infertility. In contrast, in treating non-obstructive azoospermia we observed a birth rate of 19% per cycle. In all patient groups the birth rate with fresh spermatozoas did not differ from those with cryopreserved spermatozoa. 40% of patients after multilocular TESE showed clinical signs of testicular lesion. **Conclusion:** The underlying cause of azoospermia is the most important factor for the outcome of ICSI using epididymal and testicular sperms. In cases of non-obstructive azoospermia, the pregnancy rate is low compared with the results in cases of obstructive azoospermia. There is no difference between fresh and cryopreserved sperms. TESE with ICSI is the most efficient treatment of azoospermia caused by hypergonadotropic hypogonadism. The morbidity of the TESE procedure is highly relevant and must be considered if this technique is indicated.

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0042-1138/03/0702-0119\$19.50/0

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Table 1. Indications for MESA/TESE procedures in 684 patients

Cause of azoospermia	Patients
CBAVD	54
Other obstructions	195
Ejaculation failure	42
Chemotherapy/radiotherapy	28
Cryptorchidism	101
Sertoli cell only syndrome	51
Other testicular causes: spermatocytic arrest, etc.	199
Genetic disorder (AZF/Klinefelter)	14
Total	684

Since its introduction in 1992, intracytoplasmic spermatozoa injection (ICSI) has become a very popular assisted fertilization technique. Especially in the treatment of male-caused infertility, the ICSI technique is very efficient. The ICSI procedure has revolutionized the treatment of male infertility because ICSI is effective not only in cases of severe oligozoospermia but also in cases of azoospermia. In the case of non-reconstructable obstructions of seminal ducts, the sperms can be retrieved from the epididymis or the testicle and then used for ICSI. The techniques of microsurgical epididymal sperm aspiration (MESA) and testicular sperm extraction (TESE) are well-defined uro-andrological procedures. Our experiences and results are discussed in terms of the data in the literature.

Materials and Methods

In the case of posttesticular obstruction of the seminal ducts, the authors have used microsurgical epididymal sperm aspiration (MESA). MESA is the microsurgical incision in the preocclusive region of the epididymal tube, very carefully prepared by microsurgical exploration. The aspiration is performed employing a special microsurgical capillary system. The epididymal tube is then microsurgically closed with stitches of 10–0. This procedure causes minimal trauma to the epididymal tubular system.

When there is a fully or a partially developed spermatogenesis but no outflow of seminal fluid to the epididymis necessary for MESA, the only possibility of obtaining spermatozoa for ICSI is testicular spermatozoa extraction (TESE). TESE is mandatory in all cases of non-obstructive azoospermia with a residual – often focally developed – spermatogenesis.

The authors apply TESE by carrying out an open testicular biopsy. In cases of non-reconstructable obstructive azoospermia, the testicular tissue is extracted on an unilocular basis, and in all cases of non-obstructive azoospermia on a multilocular basis (3–10 incisions per testicle).

In 1997, we introduced genetic screening of patients with a primary testicular lesion with regard to the azoospermic factor (AZF), a deletion of the Y-chromosome. All patients with a congenital bilateral aplasia of the vas deferens (CBAVD) underwent screening to determine mutations of the CFTR gene. At the beginning of the study period, the aspirated spermatozoa or spermatids were used immediately after aspiration. Since 1994, sperms have been used whenever possible after cryopreservation.

Patients

From September 1993 to June 2001, we treated 684 azoospermic patients by carrying out a MESA/TESE operation. The indications for MESA were chiefly non-reconstructable obstruction of the seminal ducts, either acquired or congenital.

Indications for TESE were chiefly non-obstructive azoospermia caused by maldescensus testis, chemotherapy or radiation therapy, complete fibrosis of the epididymis, or genetic disorder (table 1).

A small group of patients had severe ejaculation failure caused by spinal cord injury, retroperitoneal lymphadenectomy or severe psychogenic problems not treatable by conventional methods. All of them showed normal spermatogenesis. In most of these cases we carried out the TESE procedure.

Preoperative genetic screening of patients with a primary testicular lesion showed in 11 cases a numeric disorder (9 patients with Klinefelter syndrome and 2 patients with XO) and in 3 patients a deletion of the Y-chromosome (AZF). 38 of the 54 patients with congenital bilateral aplasia of the vas deferens (CBAVD) showed a mutation of the CFTR gene.

Results

From September 1993 to June 2001, 737 sperm retrieval procedures were carried out in 684 patients. In 93 procedures sperm retrieval was combined with a concomitant reconstruction of the seminal ducts retrieving the sperms optionally. In 156 of 392 operative procedures (40%) carried out because of testicular lesions, no sperms were found. Testicular histology was available in all of these patients.

In 31 ICSI procedures, epididymal sperms were used immediately on extraction (synchronous procedure) having regard to the stimulated female cycle. In 132 other ICSI procedures, epididymal sperms were used at a later date using cryopreserved sperms.

Testicular spermatozoa were used on 96 occasions simultaneously and on 735 occasions on a cryopreserved basis (table 2).

In 31 ICSI procedures, testicular spermatids (fresh or frozen) were used in default of spermatozoa.

A total of 1,025 ICSI procedures were carried out with the epididymal or testicular sperms. 949 ICSI procedures (93%) resulted in at least one oocyte fertilization and embryo transfer. Pregnancy occurred in 296 cases – an

Table 2. Results of ICSI with retrieved sperms in relation to sperm origin (testicular/epididymal) and type of application (fresh/frozen)

Sperm origin	ICSI cycles	Embryo transfer rate % per cycle	Birth rate % per cycle
<i>Epididymal spermatozoa</i>			
Simultaneous procedure	31	97 (n = 30)	16 (n = 5)
Cryopreservation	132	96 (n = 127)	33 (n = 43)
<i>Testicular spermatozoa</i>			
Simultaneous procedure	96	94 (n = 90)	25 (n = 24)
Cryopreservation	735	92 (n = 676)	24 (n = 175)
<i>Testicular spermatis</i>			
Fresh or frozen	31	84 (n = 26)	0

overall pregnancy rate of 29% per oocyte retrieval. However, in 47 of these cases an abortion was observed, so the abortion rate per cycle was 16%. The overall birth rate was thus 24% per cycle.

245 deliveries were noted (this figure includes ongoing pregnancies). 40 of these were after twin pregnancies and 4 were after triplet pregnancies.

In 84% of the 31 ICSI cycles with spermatis, embryo transfer could be obtained, even if clinical pregnancy could not be observed in any case. The transfer rates, pregnancy rates and birth rates per ICSI cycle showed no significant differences between testicular and epididymal sperms in the cases of seminal obstruction (27–28% birth rates per cycle).

However, the difference in fertility rates was highly significant in relation to the underlying causes of infertility (table 3). Thus, in cases of testicular lesions we have observed a birth rate of only 19% per cycle.

In all patient groups the birth rate with fresh spermatozoa did not differ from those with cryopreserved spermatozoa.

In 1 of the 3 patients with positive testing for azoospermic factor we found testicular sperms, but an ICSI was not performed. Of the 9 patients with Klinefelter syndrome 5 had testicular sperms or spermatis but no pregnancy occurred.

The morbidity of TESE was examined in a prospective study still ongoing with 54 of the 684 patients. Serum hormone levels (testosterone, FSH, LH), serum titer of sperm antibodies, testicular volume, urological ultrasound, clinical examination and other clinical data were documented. The preliminary results demonstrated that 40% of the patients with multilocular TESE showed a significant

Table 3. Birth rates after ICSI with retrieved sperms in relation to the underlying causes of azoospermia

Cause of azoospermia	ICSI cycles	Delivery rate per cycle, %
1 Obstructive azoospermia	455	27
2 Ejaculation failure	83	33
3 Non-obstructive azoospermia	487	19
Total	1,025	24 (n = 245 deliveries)

The differences between groups 1 and 2 are not statistically significant (χ^2 test). 3 differs from 1 and 2 statistically significantly (χ^2 test).

decrease of serum testosterone (>20%) at least in the first 6 months after the TESE operation [Schwarzer et al., unpubl. data].

Discussion

Intracytoplasmic spermatozoa injection (ICSI) has revolutionized the techniques of assisted reproductive technology in the last 9 years. Using the ICSI technique with ejaculated sperms of men with impaired semen quality, a delivery rate of up to 39% per cycle can be achieved [1, 2].

Even in cases of an azoospermia, ICSI can lead to fertility for the couple. Azoospermia can be caused by a non-reconstructable obstruction of the seminal ducts or can be a non-obstructive azoospermia resulting from a testicular lesion. In these cases, sperm retrieval techniques are required before achieving fertility with ICSI. The most common retrieval techniques are MESA and TESE, nevertheless percutaneous aspirating techniques are favored from some groups [3–5].

Obstructive Azoospermia

Based on the experiences of the majority of the major IVF centers worldwide, it is generally accepted that in case of obstructive azoospermia the type of retrieval technique is of secondary importance. In this case it is easy to retrieve sperms at the first attempt (aspiration or biopsy) with any of the techniques mentioned.

The ICSI results with MESA show that MESA seems to be by far the most efficient treatment for male infertility caused by non-operable obstruction of the seminal ducts.

We achieved delivery rates of 27–33% per ICSI cycle. Despite this, we obtained an apparently contradictory result relating to the patient group in which epididymal sperms were used in a synchronous procedure as fresh sperms for ICSI. The birth rate per cycle of 16% was clearly lower than in the patient group with cryopreserved sperms (33%). This phenomenon is explicable in terms of the statistically nonsignificant size of the patient group with fresh sperms (χ^2). A further reason could lie in the learning curve of the ICSI practitioners because epididymal native sperms were used in the first cases of our ICSI series.

Regarding the ICSI results with epididymal sperms, no superiority of epididymal sperms could be demonstrated to be statistically significant. Regarding other factors such as capacity of freezing, higher sperm density, better motility, etc., the probable advantage of epididymal versus testicular sperms must remain a matter of speculation. Also, the possible biological advantage of epididymal sperms, having a epididymal maturation and potentially more motility, is not reflected in the clinical outcome.

A comparison between epididymal and testicular sperms is possible only in the obstructive group, because patients with non-obstructive azoospermia have no epididymal sperms.

Comparing the origin of the sperms, we found no statistically significant differences between epididymal and testicular sperms (25 vs. 24% birth rate).

Our finding is supported by the fact that there is no relevant study in the literature with sufficient patient numbers demonstrating a clear superiority of epididymal sperms as against testicular sperms in obstructive azoospermia.

Similarly, the use of fresh or frozen sperms showed no statistically significant difference in birth rates. This is in agreement with other authors [6, 7–9]. The majority of data in the literature are congruent with our findings.

The epididymal cryopreserved sperms tended to have the best birth rate per cycle (33%), but the difference with regard to the other sperm groups is not statistically significant. It is unclear why the freezing should improve the fertilization capacity of the sperms. Discussion of this point goes beyond the scope of this paper.

The only clear difference in birth rates showing statistical significance related to a comparison of the group with normal spermatogenesis (obstructive azoospermia and ejaculation failure) and the group with impaired spermatogenesis.

In this latter group we were unable to find sperms in 40% of the operative procedures. This means that in 60%

of the cases with non-obstructive azoospermia we found sperms. In this patient group, the birth rate per cycle was significantly lower (19%) than in the obstructive group where sperm retrieval was not the problem.

Non-Obstructive Azoospermia

Scientific interest should really be focussed to the patient group with non-obstructive azoospermia [10]. It should be remembered that in the patient group with non-obstructive azoospermia are included patients with a previous negative testicular biopsy (no sperms), with a known Klinefelter syndrome, with a Sertoli cell only syndrome and with other negative preconditions relevant to the discovery of testicular sperms.

Thus, the low birth rates reported in the impaired spermatogenesis group should be seen in the light of the fact that many of the patients had very poor fertility prognoses because of poor underlying testicular status.

Comparing our results in this group with the literature, we obtained a sperm detection rate (60%) at the upper end of the range of published results [4, 10–13].

The consistent practice of performing multiple biopsies in a ‘mapping technique’ takes the application of TESE to its limits. We are conscious that this strategy contains the risk of an iatrogenic testicular lesion (see below).

So the TESE procedure is a matter of balancing maximal retrieval of sperms against the risk of an iatrogenic testicular lesion. The patient must be fully informed about this dilemma.

This raises the question as to whether other retrieval techniques could also obtain high sperm detection rates without the risk of testicular damage.

Specificities of Sperm Retrieval

The percutaneous epididymal and testicular sperm aspiration technique using a butterfly needle has been favored by some authors [3–5]. This needle aspiration technique has not been used by us, so we have no empirical data of our own and we must therefore rely on the literature.

Epididymal aspiration using the percutaneous method is very much a blind technique capable of destroying the structure of the vulnerable epididymal tubule. In comparison, the morbidity of MESA is low because it is a minimally invasive microsurgical procedure aiming at minimal trauma of the epididymis. In MESA, the tubule is selectively closed by microsurgical stitches thus preventing the formation of epididymal granulomas or obstructive damage to the epididymal tubule. So the advantages of the percutaneous epididymal aspiration techniques are not obvious.

In the percutaneous testicular aspiration technique the detection rates of testicular sperms in the cases of residual or focal spermatogenesis which have been published are often lower than sperm detection rates (30–60 vs. 60%) using open biopsies [3, 4]. Scepticism is indicated when looking at the morbidity of the percutaneous procedure. If the needle is able to aspirate sperms from the whole volume of the testicle, it must create tissue damage in the area because it is a blind technique without visual control and it is conceivable that this technique leads to the same lesions as the open multilocular biopsy technique.

TESE is not without side effects involving at least a transient or permanent influence on the spermatogenesis. Tash and Schlegel [14] found a relevant decrease in seminiferous tubular volume when examining histologically the testicular biopsies of patients who had had two consecutive TESE procedures.

Our own follow-up results relating to testicular volume, testosterone and FSH after TESE, show significant signs of testicular lesions in about 40% of patients with non-obstructive azoospermia who had had a multilocular TESE in the previous 6 months [unpubl. data]. A longer follow-up study is in progress.

A new technique to prevent secondary testicular damage is the microsurgical TESE procedure, as published by some authors [12, 13].

This microsurgical exploration of the testicle was favored by the authors not only to reduce this damage but

also to maximize the chances of retrieving sperms. The preliminary results do not yet permit a final opinion as to whether the microsurgical TESE leads to higher discovery rates of focal spermatogenetic areas and to lower morbidity than in the non-microsurgical TESE technique.

In the rare cases of a genetically caused non-obstructive azoospermia, we failed to obtain a single delivery. Despite a few cases where successful infertility treatment of Klinefelter patients has been reported [15], we do not recommend the use of testicular sperms in these cases.

Conclusion

Our results show that the most important prognostic factor with regard to the outcome of ICSI after sperm retrieval is the underlying cause of azoospermia. The cases with normal spermatogenesis have good chances of fertility, provided that there is no female sterility factor. In these cases the type of retrieval technique has no great importance.

In contrast, the patients with non-obstructive azoospermia must be informed that the chances of fertility are lower and that the chances of finding no sperms are at 40% or higher.

The patients must be informed about the morbidity of the elected sperm retrieval technique.

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