# INFERTILITY TREATMENT OF MEN WITH NON-OBSTRUCTIVE AZOOSPERMIA

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Male infertility can be treated by several methods with varying degree of success. We present evidence that „open" testicular biopsy is favorable for men suffering from non-obstructive azoospermia (NOA). Moreover, any NOA patient may be subjected to this treatment even though his past histopathological examinations suggest that it is likely no sperm will be found in the testicular tissue. Thus, we recommend the testicular sperm extraction (TESE) procedure for NOA patients.

## INTRODUCTION

In obstructive azoospermia (OA) sperm can be retrieved by microsurgical sperm aspiration (MESA), percutaneous sperm aspiration (PESA), testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) in more than 90% of cycles. The method of intracytoplasmic sperm injection (ICSI) is able to ensure fertilization of an ovum by a single sperm. Fertilization and pregnancy rates are comparable to a conventional *in vitro* fertilization (IVF).

Non-obstructive azoospermia (NOA) is caused by severe impairment of spermatogenesis and is considered the most critical case of male infertility. Here the epididymis is devoid of spermatozoa and only few foci with spermatogenesis may be found in the testis. Even if a Sertoli cell syndrome is the only one diagnosed, sperm can be retrieved in about 30% TESE cycles.

In this communication we are discussing the possibility of predicting results of TESE based on previous medical tests and before a patient undergoes the procedure.

## MATERIALS AND METHODS

33 azoospermic male patients, who were included in our study, underwent a battery of hormonal (FSH, LH, testosterone, DHEAS, and PRL), urological (testical palpation, ultrasonography of testes, epididymis and prostate) and genetic tests (chromosomal evaluation). All were also tested for the presence of the 19 most common mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene.

Open testicular biopsy was performed under general anesthesia. A small, one centimeter incision was made in the scrotal skin and carried through the peritoneal tunica vaginalis. Another half centimeter incision was made in the tunica albuginea and a small piece of extruding testicular tissue was excised. The Cook needle (K-TESE-20-3.0) was used for testicular tissue aspiration.

Tissue obtained in the biopsy was placed in a Petri dish under a microscope and then shredded using two needles or minced using two blades. The homogenate was centrifuged for 10 minutes at 300 g and the resulting pellet was resuspended in a drop of IVF media. The suspension was incubated for about 2 hours. A special ICSI pipette with 7 μm inner diameter was used for transferring sperm into a polyvinylpyrrolidone (PVP) droplet.

Microinjection procedure was performed under an inverted microscope (NIKON – diaphot 300, Japan) with heated stage set at 37 °C. The microscope was also equipped with a Hoffman condenser and a micromanipulation set (Narishige, Japan). A chosen sperm was immobilized by touching its midpiece with the tip of injecting micropipette and then aspirated into it. The micropipette was inserted through the zona pellucida and oolema well into the cytoplasm of an ovum. Aspiration of the cytoplasm followed, the sperm was slowly injected and the micropipette withdrawn. Rate of cleavage and morphological appearance of embryos were monitored. On day two after the fertilization were the embryos transferred using a CCD catheter. Clinical pregnancy was verified by the presence of a gestational sac.

The following groups of conditions were compared: i) TESE and TESA; ii) mincing and tubuli preparation; iii) time between testicular biopsy and ICSI procedure; and iv) the pregnancy rate with testicular and donor sperm. Students t-test or chi-square (P) were employed in statistical analysis of data in all the groups.

## RESULTS

All male patients included in this study were classified as NOA, with a single case of obstructive epididymis. Three cases of cystic fibrosis (CF) carriers were diagnosed, all of whom had the delta F508 mutation in the CFTR

gene. One patient suffered unilateral congenital absence vas deferens (CAVD), two other were not obstructive. There were two cases of Klinefelter syndrome and three cases of azoospermia after successful oncologic treatment. In all, 15% of genetic anomalies were found in patients with NOA. Sperm was located in 17 cycles (57%).

Our data suggest that urologic examination carried no prognostic value. Even in patients suffering from testicular hypotrophia was sperm found in 56% of biopsies. Results of pathologic hormonal tests are summarized in Tab 1. High occurence of elevated FSH and PRL levels was observed in NOA. Despite abnormal hormonal levels being common for impaired spermatogenesis, sperm was found in all of the groups (Tab. 1).

**Table 1.** Comparison of sperm retrieval rate and abnormal hormonal levels.

| Hormone | % | sperm found | sperm not found |
|---|---|---|---|
| High FSH | 56.6 % | 41.2 % | 58.8 % N |
| High LH | 16.6 % | 20.0 % | 80.0 % N |
| High PRL | 46.6 % | 64.3 % | 35.7 % N |
| Low testosterone | 23.3 % | 42.9 % | 57.1 % N |

N = not significant

Histologic examination was not directly correlated to sperm retrieval (Tab. 2). Sperm was found in testicular tissue in 38% of NOA cases regardless of histologic examination discovering mature or immature sperm. On the other hand, histologic examination confirmed the presence of mature sperm in 25% of patients but no sperm was located in the tissue preparations. We conclude that pre-existing histologic examination cannot predict sperm retrieval by TESE procedure in NOA patients.

**Table 2.** Comparison of sperm retrieval rate and histologic examination.

| Histology | % | sperm found | sperm not found |
|---|---|---|---|
| normal spermiogenesis | 13.3 % | 75 % | 25 % |
| hypospermatogenesis | 16.7 % | 100 % | 0 |
| immature sperm | 13.3 % | 37.5 % | 62. 5% |
| spermatognesis arrest | 56.7 % | 38.2 % | 61.7 % |

TESE and TESA results are summarized in Tab. 3. TESE procedure was successful in 57% of cycles whereas TESA in only 27%. Open testicular biopsy had a higher yield of sperm than the aspiration method. Although testicular tissue aspiration can reach deep inside the testis the volume of aspirated tissue is small, thus the possibility of finding sperm is lower than in open biopsy.

**Table 3.** Comparison of TESE/TESA.

| Biopsy | sperm found | Number of sperm |
|---|---|---|
| TESE | 57 % | 7.7 |
| TESA | 27 % | 3.0 |

Tab. 4 compares the presence and number of sperm obtained by the two tissue preparation methods. Sperm was found in the same cycles regardless of method used. However, the number of sperm was higher when the tissue was minced instead of shredded. Still, the difference is not statistically insignificant.

**Table 4.** Comparison of two methods for tissue preparation.

| Method | sperm found | Number of sperm |
|---|---|---|
| mincing | 57 % | 3.8 N |
| needle preparation | 57 % | 1.6 N |

N = not significant

Two groups named TESE and donor sperm were formed to indicate the source of sperm. Information on the two groups are summarized in Tab. 5. 27 men in the TESE group underwent 30 cycles of the procedure. ICSI with testicular sperm was performed in 15 cycles. The observed fertilization rate was 29.7%, with three clinical pregnancies. Twins were delivered in one case, the two other pregnancies are in progress. In the donor sperm group were ova fertilized in 21 cycles. The observed fertilization rate was 54%, with 16 cycles of embryo transfer. Embryos were cryopreserved in five cycles. 62.5% was the pregnancy rate in this group. Out of total ten pregnancies three were delivered and six are in progress.

**Table 5.** Comparison of testicular and donor sperm in ICSI cycles.

| | TESE/ICSI | Donor |
|---|---|---|
| number of cycles | 30 | – |
| successful sperm retrieval | 17 | – |
| ICSI cycles | 15 | 21 |
| number of ova | 165 | 291 |
| fertilization rate | 29.7 % | 53.9 % |
| cleavage rate | 91.8 | 93.6 |
| number of transfers | 11 | 16 |
| number of pregnancies | 3 | 10 |
| pregnancy rate/ET | 27.3 | 62.5 |
| deliveries | 1 | 3 |
| miscarriages | 0 | 1 |
| pregnancies in progress | 2 | 6 |

## DISCUSSION

All patients in our study group were diagnosed as non-obstructive azoospermic males with testicular failure. Neither serum FSH nor testicular size were predictive of the probability of finding spermatozoa for ICSI. Testicular parenchymal damage may perpetuate increased serum FSH without necessarily indicating total decay of the germinal epithelium in all patients (3). Therefore, the serum FSH cannot be used as an indicator for absolute absence of spermatogenesis.

Noteworthy is the relationship betweens histopathological reports and the sperm retrieval rate which is only

75% in case of active spermatogenesis and 38% in spermatogenic arrest or partial spermatogenic arrest. Even though there are some spermatozoa produced, an extremely low rate of spermatogenesis in the testes will result in absolute azoospermia in the ejaculate. Consequently, a certain threshold of sperm production is necessary before any spermatozoa appear in the ejaculate (4).

Open testiclular biopsy is more successful than puncture with a special testicular biopsy needle because of the reason stated in the preceding paragraph. In case of NOA only a few foci of spermatogenesis are present and a larger amount of testicular tissue is required for successful location of sperm. Only „open" biopsy is recommended in NOA (1).

Our study shows that spermatozoa were found in 57% of the cycles in NOA and subsequently used for ICSI with fertilization rate of 29.7%. It is lower than fertilization rate in the donor sperm group. The fertilization rates described in literature are 39% in NOA versus 54% in OA (2). Pregnancy rate in our study was lower in the TESE group than in the donor sperm group, but the total number of compared cycles is low.

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## CONCLUSION

Testicular „open" biopsy is a favorable form of treatment in NOA. In our view, no patient should be excluded from TESE procedure based on any prior examinations including: hormonal, urological, genetical, and biochemical. Pregnancy rate in NOA patients can reach the pregnancy rate in ICSI cycles. This is evidenced by delivered twins (1950/42, and 1550/42) and two other pregnancies in progress.

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