

## ORIGINAL ARTICLE

# Microdissection TESE is superior to conventional TESE in patients with nonobstructive azoospermia caused by Y chromosome microdeletions

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## Keywords

Microdissection TESE—nonobstructive azoospermia—Y chromosome microdeletion

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## Summary

Nonobstructive azoospermia is caused in up to 10% by microdeletions of the Y chromosome in the azoospermia factor (AZF) region, which is divided into three nonoverlapping areas (AZFa, AZFb and AZFc). In 25 male patients with AZF microdeletions, the results of two different techniques for surgical sperm retrieval (SR), conventional multilocular TESE and microdissection TESE, were studied retrospectively over a period of 19 years. Conventional multilocular TESE was carried out in 11 patients and microdissection TESE in 14 patients. Successful SR was possible only in patients with isolated AZFc microdeletions, so only the 20 patients with AZFc microdeletions alone were taken into account for the comparison of the both operative techniques. The sperm detection rate for conventional multilocular TESE was 25%, the sperm detection for microdissection TESE was significantly higher with 67%. In all patients, a histological examination of the testicular tissue was carried out, which showed a mixed picture, but Sertoli-cell-only syndrome in most cases. FSH was no prognostic marker for successful SR. In two of six couples performing an intracytoplasmic sperm injection until now, a pregnancy occurred.

## Introduction

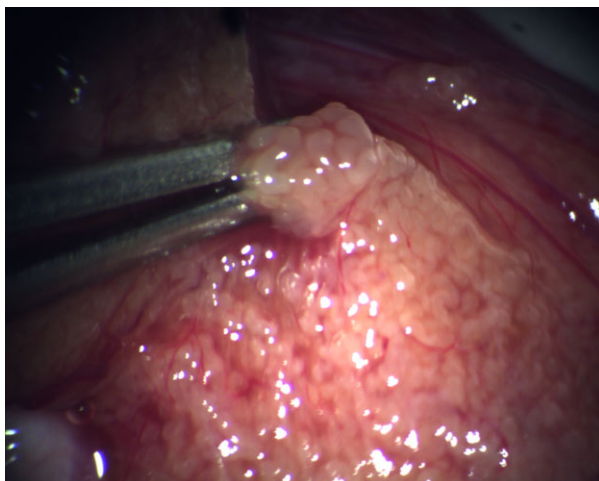
Male infertile patients can present with nonobstructive azoospermia (NOA), which is caused by a spermatogenic failure. Up to 10% of these cases may be caused by microdeletions of the Y chromosome in the azoospermia factor (AZF) region, which is located on the long arm of the Y chromosome. The AZF region is divided into three nonoverlapping areas (AZFa, AZFb and AZFc). A mutation in one of these areas leads to impaired spermatogenesis (Simoni *et al.*, 2008; Navarro-Costa *et al.*, 2010). If AZFc is impaired, successful surgical sperm retrieval (SR) is sometimes possible, so that an intracytoplasmic sperm injection (ICSI) can be performed. This study retrospectively studied the results of two different techniques for SR in patients with AZF microdeletions, conventional multilocular testicular sperm extraction (TESE) (CMT) versus microdissection TESE (MT).

## Materials and methods (surgical technique)

From 1996 to 2005, CMT of one or both testicles (if necessary) was applied with the removal of up to 15 biopsies for each testis in all cases of NOA (Fig. 1). From 2006 to 2015, MT was used with removal of up to 70 microbiopsies of each testicle (Fig. 2). Our surgical technique, similar to that of Schlegel (1999), was carried out in a standardised procedure and has been described in detail by us (Schwarzer *et al.*, 2013). Both multilocular and microsurgical TESE were carried out in an operating room of the IVF centre located next to the IVF laboratory, so that an immediate interaction of the embryologist and the microsurgeon during the surgery was guaranteed. Both techniques of SR were carried out with fractionated tissue removal and an intra-operative feedback about the current state of the tissue preparation and sperm detection by the embryologist. Thus, the surgeon was always



**Fig. 1** Conventional multifocal testicular biopsy (TESE).



**Fig. 2** Microdissection TESE: microsurgical extraction of testicular tissue with dilated seminiferous tubules using a microsurgical forceps.

aware of the state of sperm detection so that an overtreatment by taking too much and undertreatment by removal of too little testicular parenchyma was prevented. In all patients, an additional testicular biopsy from each testicle for histological examination was carried out.

## Patients

From April 1996 to April 2015, 25 patients with NOA and a Y chromosome microdeletion in the AZF region

underwent TESE for retrieval of testicular spermatozoa. In 20 patients, an AZFc microdeletion was detected alone, in one patient an AZFb microdeletion alone, two of the operated patients had a microdeletion in the AZFb and AZFc region (AZFb + c), two patients had an AZFc microdeletion in combination with other chromosomal disorders (chromosomal mosaic and structural aberration of Y chromosome). The genetic studies were carried out with an amplification of genomic DNA by microdeletions by polymerase chain reaction (PCR) with subsequent fragmentation and analysis by different institutes of human genetic in Germany.

The three patients with AZFb + c microdeletions and AZFb microdeletion alone were operated on explicit patient's request despite the minimal chance of successful SR.

Even in the two patients with combined disorders, TESE was performed at the explicit wish of the patient regarding the unclear situation of scientific data concerning the chances of positive SR in these combined problems. The latter patients were aware that the chances of successful TESE were considered significantly worse than in the situation of an AZFc microdeletion alone.

From April 1996 to February 2005, TESE was performed by a bilateral multilocular testicular biopsy in 11 patients, and from March 2005 to April 2015, micro-TESE was performed in 14 patients. The testicular volume was 4–20 ml (average 15 ml).

None of the patients was pre-treated with any kind of hormonal manipulation, as described for other forms of NOA (Reifsnnyder *et al.*, 2012). The preoperative FSH level ranged between 5 and 53 mU ml<sup>-1</sup> with an average of 16 mU ml<sup>-1</sup>.

All interventions were performed on an outpatient basis under general anaesthesia. The surgical approach was performed through a midline incision in the scrotal raphe. The average operating time was 50 min for the conventional multilocular biopsy and 90 min for the micro-TESE. No perioperative or post-operative complications were recorded.

## Results

In 10 of the total 25 patients, testicular spermatozoa were detected and cryopreserved (Tables 1 and 2). Successful SR was possible only in the patients with AZFc alone (10/20 patients). In all cases with positive SR, a heterogeneous testicular damage with only focal spermatogenesis in very few areas of the testicular parenchyma was found.

Evaluation of the 20 patients with AZFc microdeletion alone without additional chromosomal disorder showed a significantly better SR rate in the period after 2005, when in all patients with NOA consistently, the micro-TESE

**Table 1** Sperm retrieval rates in patients with AZFc alone without additional chromosomal disorders ( $n = 20$ ) in correlation to two different techniques of TESE (chi-square test,  $P = 0.0339$ )

Surgical technique	Patients ( $n$ )	Spermatozoa: yes ( $n$ )	Spermatozoa: no ( $n$ )	Detection rate (%)	Significance
Multilocular TESE	8	2	6	25	$P = 0.0339$
Micro-TESE	12	8	4	67	

**Table 2** Sperm detection (by multilocular and microsurgical TESE) in 25 patients with Y deletion of various characteristics: azoospermia factor (AZF)c vs. AZFb vs. AZFb + c vs. AZFc combined with other chromosomal disorders

Genetic constellation	Patient ( $n$ )	Sperm detection ( $n$ )	Sperm detection (%)
AZFc	20	10	50
AZFb	1	0	0
AZFb + c	2	0	0
AZFc + other chromosomal disorder	2	0	0

was performed (Table 1). The statistical analysis was carried out by chi-square test.

In all patients, histological examination of testicular tissue was performed (Prof. Köhn). In 14 of 25 patients, a Sertoli-cell-only syndrome (SCOS) was found histologically, and in seven of 25 patients, a spermatogenetic arrest (SA) was found, and in four of 25 patients, a mixed atrophy (MA) was found (Table 3).

FSH levels were  $13 \text{ mU ml}^{-1}$  on average in the group with successful SR and at  $16 \text{ mU ml}^{-1}$  in the group without success ( $p > 0,076$ ).

In the five patients in whom a combination of Y microdeletion and other alterations occurred (e.g. AZFb, AZFc + b or AZFc plus other chromosomal disorders) spermatozoa were detected in no case.

Of the 10 patients with evidence of spermatozoa and cryopreservation, an ICSI procedure (1–5 cycles) was carried out in six couples (average of 2.3 cycles) in two cases a pregnancy could be reached, one led to an abortion and one to the birth of a healthy girl.

**Table 3** Histological findings in patients ( $n = 25$ ) with nonobstructive azoospermia and azoospermia factor (AZF) in correlation to the sperm detection (including patients with AZF combined with other chromosomal disorder)

Histology	AZFc ( $n$ )	AZFb ( $n$ )	AZFb + c ( $n$ )	AZFc + other chromosomal disorders ( $n$ )	Spermatozoa: yes ( $n$ )	Spermatozoa: no ( $n$ )
Sertoli-cell-only syndrome	11	1		2	3	11
Spermatogenetic arrest	5	1	1		4	3
Mixed atrophy	4				3	1
Total ( $n$ )	20	1	2	2	10	15

## Discussion

Infertile men may suffer from NOA, which can be caused by a genetic disorder, resulting in spermatogenetic failure. Up to 10% of NOA is caused by Y chromosome microdeletions in the AZF region, which may be present in various variants. A Y chromosome microdeletion can cause a severe oligozoospermia or NOA. In patients with AZFa and b microdeletions, spermatozoa could be found in no single case according to the literature (Simoni *et al.*, 2008; Navarro-Costa *et al.*, 2010), while in the case of AZFc microdeletion, a SR rate of more than 50% can be expected according to the literature (Simoni *et al.*, 2008; Navarro-Costa *et al.*, 2010; Krausz *et al.*, 2014). Some authors believe that the incidence of Y chromosome microdeletions in Germany with 2–3% of men with NOA may be lower than in other countries (Simoni *et al.*, 2008), while the prevalence in infertile men was published for Turkey with 6% and China with up to 13% (Ocak *et al.*, 2014; Elfateh, 2014).

In our single-centre series of 25 patients, we investigated the SR rates in patients with AZF microdeletions using two different techniques of TESE. In the first 11 years exclusively, multilocular biopsies were used, and in the last 8 years, micro-TESE consistently was applied. A comparison of the two groups showed a significantly higher SR rate of the micro-TESE versus the conventional multilocular TESE ( $P = 0.0339$ ).

The present data showed the superiority concerning the SR rate of micro-TESE compared to the conventional multilocular TESE in patients with AZFc, despite the relatively small group of patients. For other forms of NOA, we have already demonstrated the superiority of

micro-TESE compared to conventional multilocular TESE (Schwarzer *et al.*, 2013, Schwarzer *et al.*, 2003).

All patients with Y chromosome microdeletions, in whom the deletion not or not only relates to the AZFc region of the Y chromosome, the SR rates appear extremely low as already described in the literature (Simoni *et al.*, 2008; Navarro-Costa *et al.*, 2010; Krausz *et al.*, 2014). In our three cases with affection of AZFb and also in the two cases with additional chromosomal disorders, this presumption was confirmed. Up to now, only six of 10 couples with positive SR have already performed an ICSI procedure. This can be explained by the fact that the last four micro-TESEs were performed just recently. These couples will begin the treatment in the near future.

However, in three of six cases, the couple did not continue the ICSI treatment after a failure of the 1st cycle. The reasons for the inconsistency of the couples are not known. It can be speculated that fear of genetic disorders in the offspring might be a reason. All patients were counselled in great detail before TESE about the inheritance of the Y chromosome microdeletion, and they were aware of this problem in the case of fathering a son.

Until now, only two couples have continued the ICSI treatment with more than two cycles, so that the pregnancy rates were not statistically comparable with data of other forms of NOA due to the very small number of patients.

Comparison and evaluation of the pregnancy rates with other forms of NOA will only be possible with the existence of much larger patient cohorts, which should, however, be difficult due to the rarity of the disease (Navarro-Costa *et al.*, 2010).

The histological examination of the testicular parenchyma was performed to evaluate the type of the testicular damage and to detect a possible intra-epithelial neoplasia (TIN). It showed a mixed picture of entities (Table 3) with no clear preference of a particular form of testicular failure, but mostly SCOS. No TIN was detected. In the literature, we did not find a relevant number of cases about this point of view (Navarro-Costa *et al.*, 2010).

Pre-operative FSH levels ranged from the standard range to a high pathological range and had no predictive value for the detection of spermatozoa. In the group with successful SR, FSH levels were at an average of 13 mU ml<sup>-1</sup> and in the group without successful SR at an average of 15 mU ml<sup>-1</sup> ( $p > 0,076$ ). So neither histology nor the FSH level was a prognostic marker for a successful SR.

The key message of this study is the fact that micro-TESE is superior to conventional TESE with respect to the SR rate in patients with AZF microdeletions, but many scientific and clinical aspects of NOA caused by Y chromosome microdeletions are yet to be explored.

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