Tissue perfusion-controlled guided biopsies are essential for the outcome of testicular sperm extraction

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Objective: To determine if there are areas of major and minor perfusion in a single testicle, and if the quality and quantity of sperm are correlated with the level of perfusion, we collected testicular tissue from areas with different levels of perfusion.
Design: Controlled clinical study.
Setting: Consecutive patients with azoospermia.
Patient(s): Patients with azoospermia undergoing testicular sperm extraction (TESE) biopsy for the retrieval of sperm to be used in an assisted reproduction program.
Intervention(s): Perfusion mapping was performed with the use of color Doppler ultrasound. Areas with different levels of perfusion were marked with needles. After incision with radiofrequency cutting, the exposed tissue was examined with a laser Doppler flowmeter, and biopsies were taken for TESE and histology. Sperm were analyzed using World Health Organization criteria, and prepared for intracytoplasmic sperm injection (ICSI).
Main Outcome Measure(s): Correlation of sperm quality and quantity in testicular-tissue biopsies, with tissue-perfusion units (TPU) measured by laser Doppler flowmeter.
Result(s): From 40 biopsies taken from 20 testicles of 12 patients, tissue was analyzed for sperm quality and quantity. Sperm quality was highest in areas of high tissue perfusion. In areas of 70 TPU, 72.3% progressive sperm were detected, whereas in areas of 10 TPU, only 13.3% progressive sperm and elevated numbers of precursor cells could be observed. The number of motile sperm isolated from tissue samples correlated well with the intensity of tissue perfusion.
Conclusion(s): We have shown for the first time that in patients suffering from azoospermia, sperm quality and quantity depend on tissue perfusion within the testicle. (Fertil Steril® 2007;87:1071–6. ©2007 by American Society for Reproductive Medicine.)

Key Words: Laser Doppler flowmetry, perfusion-controlled testicular biopsy testicular sperm extraction (TESE), color Doppler ultrasound, intracytoplasmic sperm injection (ICSI), assisted reproductive technology (ART)

Until recently, various surgical interventions on testicular biopsies were carried out randomly, and focal areas of spermatogenesis were determined (1). Although the outcome of surgical intervention is unpredictable, testicular sperm extraction (TESE) has proved to be a quite reliable and successful sperm-recovery technique for use in intracytoplasmic sperm injection (ICSI) when helping male factor-dependent infertile couples (2–4). However, the difficulty of predicting which patient with azoospermia may have sperm involves the evaluation of hormonal levels and/or testicular histology as variable parameters for surgical sperm retrieval (5). Furthermore, biopsies with focal spermatogenesis may require a prolonged search for viable sperm, sometimes lasting several hours. In some cases, bilateral or multiple biopsies taken randomly are required for sperm retrieval. To improve the success rates of TESE, it would be useful to have a method of determining testicular areas with a high probability of containing normal sperm.

Recently we described a novel approach to improving the chances of sperm retrieval from testicular biopsies, based on testicular tissue perfusion (6,7). Areas of good perfusion in a testicle are identified by external color Doppler ultrasound, marked by precisely placed needles and verified by local laser Doppler flowmetry for measuring perfusion within the opened testicle. The aim of this study was to examine whether there is a correlation between the level of perfusion in testicular areas and the quantity and quality of sperm

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recovered from these areas, after TESE in patients with azoospermia.

MATERIALS AND METHODS

Patients

In a total of 12 patients, perfusion-guided TESE surgery was performed (20 testicles: 10 right side, and 10 left side). Four of the 12 patients had only one testicle because of testicle ablation for treatment of testicular tumors. In 2 patients, a previous biopsy of both testicles revealed a Sertoli-cell-only (SCO) syndrome (Table 1). All patients underwent preoperative staging, including sperm count, hormone analysis, and testicular ultrasound, as well as normal staging for testicular tumors. This study was approved by the Ethics Committee of the Medical University of Innsbruck, Innsbruck, Austria.

Patients with testicular tumors had no sperm in their ejaculate. In the other patients, no sperm had been retrieved from previous TESE biopsies. Chromosomal analysis and search for the azoosperm factor deletion in all 12 patients revealed no cytogenetic abnormalities. The patients' mean age was 36.7 years (age range, 25–47 years). Mean hospitalization was for 1 day. Routinely, testicles from which biopsy samples were obtained were scanned by ultrasound before patients were discharged from the hospital.

Perfusion-Controlled Testicular Biopsy

Patients underwent TESE for assisted reproduction (ICSI), as previously described (6,7). Preoperative testicular perfusion mapping using contrast-enhanced, high-resolution color Doppler ultrasound was performed (Fig. 1) and repeated intraoperatively using a −12 MHz probe of Acuson Sequoia 512 (Acuson, Mountainview, CA), fitted with a high-frequency linear ultrasound probe (15LW40).

FIGURE 1

Contrast-enhanced color Doppler ultrasound of left testicle of patient 5. Arrows indicate areas of major and minor perfusion.

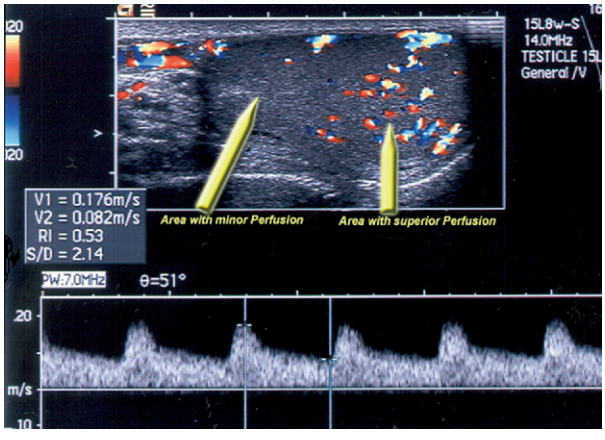

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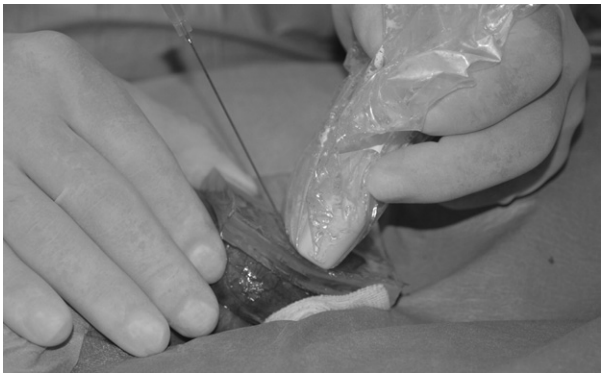
FIGURE 2

Needle placement before radiofrequency cutting and opening of the testicle.



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The reason for using an invasive method in combination with our novel technology is that noninvasive needle biopsy does not permit precise localization of high spermatogenic areas, and therefore leads to a random search for sperm retrieval. Furthermore, tissue perfusion cannot yet be measured via noninvasive needle biopsy. This consideration is of greater importance in patients with nonobstructive azoospermia.

A 22-gauge needle was placed in the area of best perfusion. Afterwards, a small incision was made with radiofrequency cutting (Fig. 2). The exposed tissue was additionally screened with a 3-mm laser Doppler probe, and perfusion rates were determined using a BLF21 laser Doppler flowmeter (Transonic Systems, Inc., Ithaca, NY). The laser Doppler flowmeter converts the "Doppler shift" of a laser light beam, which is frequency-shifted and reflected by a moving column of blood. Tissue perfusion units (TPU) were defined arbitrarily, and were based on mean cell velocity and average concentration of moving blood (mL/min/100 g).

Tissue biopsies were obtained for TESE from the left and/or right testicle, respectively (Fig. 3). Additionally, a random biopsy was taken from the same testicle, and TPU were measured as previously described (6,8). Four biopsies (two perfusion-controlled, and two taken randomly) correspond to the average of four biopsies routinely taken blindly from patients with nonobstructive azoospermia (9).

Measurement of tissue perfusion levels was performed before TESE. Radiofrequency cutting was used, because this method provides the best intact surface in deeper testicular regions.

Testicular biopsy-score counts (Johnsen score) (10) were determined from random biopsies. The size of testicular

TABLE 1

Patients with TESE with different underlying causes of infertility, and their hormonal status.

Patient no.	Disease	Testosterone (ng/mL)	LH (mU/mL)	FSH (mU/mL)
1	Nonobstructive azoosprmia	5.5	6.8	10.4
2	Nonobstructive azoospermia	4.3	3.0	6.5
3	Testicular tumor	4.2	3.0	7.9
4	Testicular tumor	6.4	4.8	8.2
5	Hypogonadism	2.1	14.3	27.5
6	Nonobstructive azoospermia	5.0	2.3	3.9
7	Testicular tumor	7.2	4.4	6.2
8	SCO-Syndrome	3.6	4.2	12.0
9	SCO-Syndrome	3.1	3.4	4.6
10	Nonobstructive azoospermia	3.3	1.4	3.8
11	Testicular tumor	5.3	2.7	5.5
12	Nonobstructive azoospermia	4.9	4.1	6.4

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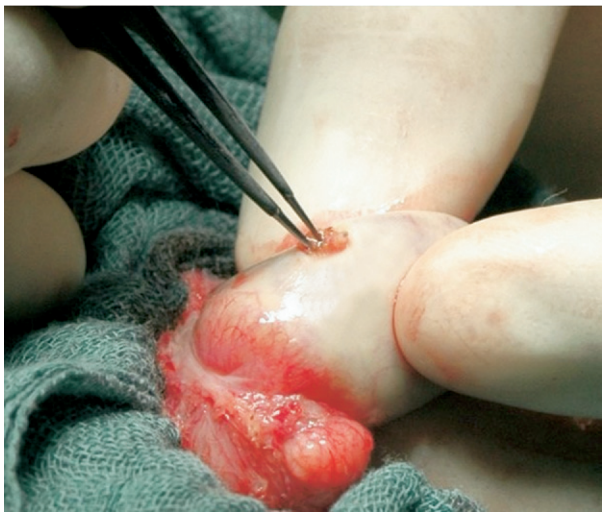
biopsy samples was the same as in conventional TESE (about 2 × 1 mm). Tissue pieces were separately kept in preincubated Sperm-Prep medium (MediCult, Jyllinge, Denmark) for further processing.

Sperm Preparation

Blinded morphology assessment of sperm was done independently in our Department of Gynecology, Medical University Innsbruck, Austria, according to World Health Organization (WHO, 2006) quality standards. Testicular biopsies were manually dissected into very small pieces. After 3 hours of incubation in IVF medium (MediCult) at 37.5°C and 6 % CO_2, 15-μL aliquots were transferred into microdrops of IVF medium under mineral oil, providing a volume of 25 μL per microdrop. For each area from which biopsies were obtained, four microdrops were randomly selected for counts and the morphological evaluation of sperm. The manually dissected tissue pieces were further processed using sperm-freeze medium (MediCult) and cryopreserved in straws with the use of a freeze-control device with a cryoprogram for sperm (Cryologic, Victoria, Australia). Motile sperm with apparently normal morphology retrieved from the biopsies were used for ICSI.

FIGURE 3

Small testicle of patient 5. A small incision was made to extract small parts of testicular tissue.



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Statistical Analysis

The software SPSS for Windows 11.5 (SPSS, Chicago, IL) was used for all analyses. Data are expressed as means and standard deviation (SD), with minimum and maximum values. Comparisons with respect to age, Johnsen score, TPU, and sperm quality and quantity were performed using Student's *t*-test. The Pearson correlation coefficient was calculated. Statistical significance was defined as $P<.05$.

RESULTS

To compare well-perfused and randomly chosen testicular areas with regard to the quality and number of sperm retrieved, small pieces of tissue were surgically removed from the testicle. After individual processing of tissue samples (see Materials and Methods), sperm evaluation was undertaken according to WHO (1999) standard criteria.

We were able to find sperm in all 12 of our patients. In 9 of 12 patients, motile sperm with apparently normal morphology suitable for ICSI could be isolated from the biopsies. In 2 of 3 patients with previously diagnosed SCO

TABLE 2

Descriptive statistics of sperm count evaluation obtained by TESE.

Descriptive statistics	N	Mean count	SD	Minimum count	Maximum count
Normal sperm	40	16.12	17.99	0	64
Abnormal sperm					
Abnormal head	40	12.77	18.72	0	78
Abnormal tail	40	11.23	14.91	0	63
Abnormal midpiece	40	7.40	6.62	0	29
Total sperm count	40	47.50	46.39	0	185

Note: N = number of TESE procedures.

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syndrome, enough sperm suitable for ICSI (5–16 sperm/100-μL microdrop count) were found. In 3 of 12 patients, no normal sperm could be found. One patient had been previously diagnosed with SCO syndrome, one patient had previously undergone chemotherapy because of a testicular tumor, and in one patient, the reason for azoospermia remained unclear.

In all 3 patients, abnormal sperm as well as round and elongated spermatids were found in perfusion-controlled biopsies, but not in random biopsies. These abnormal sperm were not used for ICSI. The perfusion levels in these testicles were below 10 TPU.

No significant difference according to area of biopsy was observed for age, TPU, Johnsen score, and relative or absolute count for normal, abnormal, or total sperm. The distribution of sperm per 100-μL microdrops found in TESE biopsies is shown in Table 2.

A significant difference, however, was found between perfusion-controlled and randomly taken biopsies. In perfusion-controlled biopsies, elevated TPU levels (P=.008) were measured, and a larger number of normal sperm (P=.003) were found, when compared with randomly obtained testicular samples. Furthermore, the best sperm quality and larger numbers of motile sperm were found in areas with high tissue perfusion (0.85, P=.001) (Fig. 4).

FIGURE 4

Correlation between TPU and count of normal sperm per 100 μL. Each circle represents data from one particular TESE.

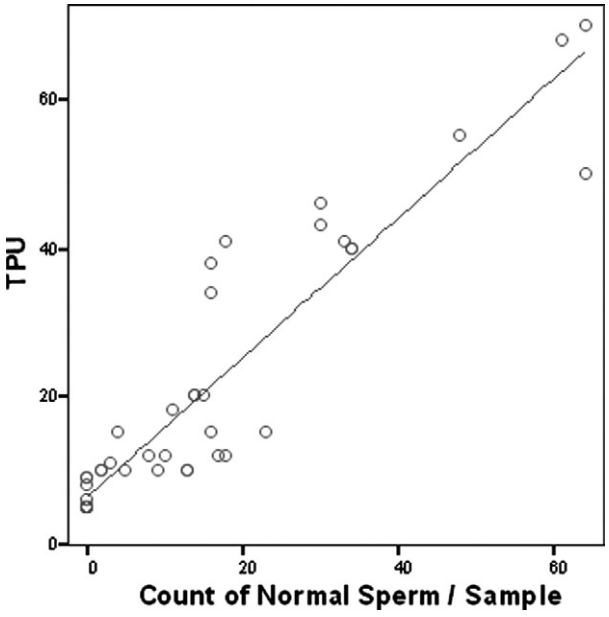

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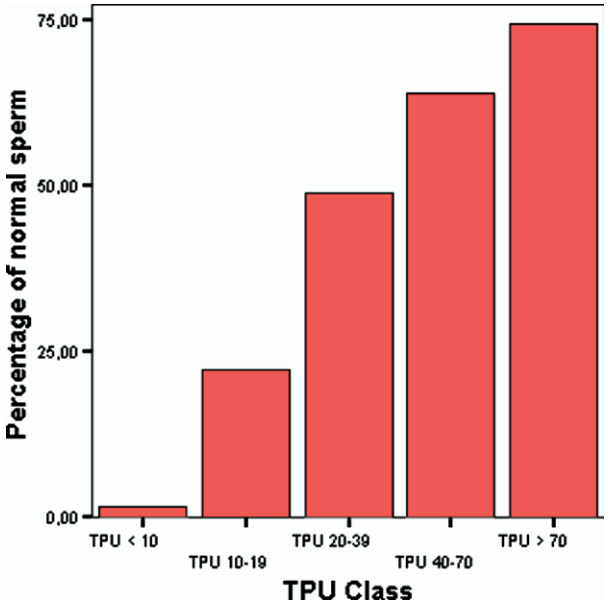
FIGURE 5

Percentage of normal sperm count in relation to TPU.



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In tissue areas with >70 TPU, 74.42% progressive A-quality sperm (Fig. 5) were found. In tissue areas with about 40 TPU, 65.94% ± 4.93% motile sperm were found, along with nonmotile sperm and elongated spermatids as well as sperm with various kinds of abnormal morphology. Tissue from areas with 20–40 TPU contained a lower number of sperm (48.84% ± 6.02%) that were suitable for ICSI. In addition, many abnormal sperm (double head, pinhead, double and short tail, and defective midpiece) and elongated spermatids were detected. Only very few normal sperm (2.75% ± 4.7%) were isolated from samples obtained from areas of about 10 TPU; the rest were abnormal sperm and precursor cells. Using motile sperm with apparently normal morphology for ICSI, the fertilization rate (2 pronuclei formation) ranged between 76%–89%. We did not observe a significant difference in fertilization rate with the use of sperm from areas with different TPU levels, although these data must be considered preliminary.

Because we found a highly significant correlation of TPU with the pathologically determined Johnsen score ($P=.001$), we were able to find sperm in all of our patients, even in those with a Johnsen score far below 8. The mean Johnsen score found in our patients was 7.21 (range, 2.1–9.5).

In routine postbiopsy control of the testicles, a small hematoma was observed in 8 of 40 testicles biopsied. No other complications occurred after the described TESE intervention.

DISCUSSION

Azoospermia is rather common in infertile men, and may occur in 10%–20% of patients with abnormal semen (11). In the past, azoospermic men could fulfill their desire for children only via donor insemination in IVF programs. The technique of ICSI (12) in combination with TESE (13,14) has enabled motile sperm to be selected from azoospermic patients and utilized in assisted reproductive technology (ART). A variety of different techniques of sperm retrieval and biopsy, selected on the basis of patients' infertility syndromes, have enabled appreciable success rates for ICSI, leading to pregnancies and deliveries (15–20). Men who suffer from azoospermia now have the opportunity to fertilize their partners' oocytes with their own sperm, and to have children with their own genetic profile.

An increase in the rate of miscarriages was recently reported for patients using TESE sperm in ART, and this was explained as most likely caused by genetically defective sperm (21). In another report, no increased incidence of miscarriages was found when comparing surgically retrieved testicular sperm with freshly ejaculated sperm (3). Moreover, no difference in pregnancy loss or delivery rates was observed between freshly used and frozen-thawed testicular sperm. On the other hand, parameters such as the maturity, morphology, and motility of testicular sperm were shown to have a major influence on fertilization outcomes and pregnancy rate. Furthermore, maternal age and ovarian function, responsible for oocyte quality and quantity, can significantly determine the outcome of ICSI with the use of testicular sperm (22,23).

Previously, mostly randomly localized testicle biopsies were carried out on azoospermic patients, and were predictably associated with hormonal evaluation and classic histology (24,25). Multiple biopsies were proposed to increase the likelihood of sperm retrieval (26). However, in azoospermic patients, random biopsy for TESE is associated with uncertainty of sperm recovery (27), resulting in cancellations of IVF cycles and discouragement in couples enrolled in ART programs. Fine-needle aspiration (FNA) has been employed for more precise testicle sampling, to gain local information about spermatogenesis and to identify sperm suitable for ART (28,29). It was also suggested that visually identified "fat" tubules containing sperm in patients with nonobstructive azoospermia may be found, during micro-TESE, to be close to the vascular supply (30).

The testicular biopsy score count first described and introduced by Johnsen in 1970 is a method for registration of spermatogenesis in human testes (10). Normally, a minimum Johnsen score of 8 is required to find sperm suitable for ICSI. In our group of patients, a mean Johnsen score of 7.2 (range, 2.1–9.5) in random biopsies was registered. Therefore, we do not consider random biopsy to be an efficient method for TESE.

Constant improvements in focally localizing presumptive testicular areas for sperm retrieval were reported in the past several years. Nevertheless, striking differences in sperm detection and retrieval rates were observed among various clinics, including our hospital, most likely due to different surgical techniques and etiological variations in patients' infertility syndromes. Therefore, we initiated and successfully applied a novel technology by measuring perfusion within the testis to determine whether the level of tissue perfusion correlates with the quality and quantity of sperm retrieved from TESE. We documented for the first time that the level of tissue perfusion matches well with the level of recovered sperm in both quality and quantity. Less perfused areas contain only few sperm of lower quality, whereas well-perfused areas contain a higher number of sperm of higher quality, according to WHO (1999) criteria.

We did not observe a correlation between TPU levels and the patients' underlying diseases, which might be due to the relatively small number of patients analyzed. Whether or not TPU levels correlate with a patient's particular disease needs to be investigated in future studies.

Our novel technique of perfusion-controlled testicular biopsy allows for predictable TESE, and better sperm samples for ICSI and cryopreservation in assisted reproduction. We therefore recommend this new method of sperm retrieval in azoospermic patients. Because the outcome of ICSI is strongly dependent on sperm quality, random TESE should be replaced by perfusion-controlled biopsy of testicle tissue.

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