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.

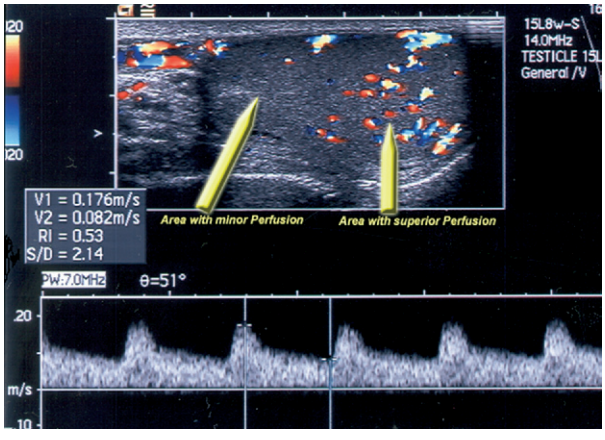

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FIGURE 2

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