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Intratesticular Doppler flow, seminal plasma nitrites/nitrates, and nonobstructive sperm extraction from patients with obstructive and nonobstructive azoospermia

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Objective: To prospectively evaluate the role of intratesticular vascular flow in modulating sperm function in men with obstructive and nonobstructive azoospermia. The correlation of testicular Doppler values with nitric oxide and testicular sperm extraction was further evaluated.

Design: Prospective study.

Setting: Assisted reproduction unit at a university center.

Patient(s): Twenty-eight men with azoospermia undergoing sperm extraction for intracytoplasmic sperm injection.

Intervention(s): Ultrasound and color Doppler scanning of the testes. Testicular sperm retrieval and nitrite/nitrate assay.

Main Outcome Measure(s): Doppler analysis of testicular transmediastinal artery, plasma and seminal plasma nitrite/nitrate values, and sperm extraction histopathology.

Result(s): The pulsatility index (PI) of the transmediastinal artery was higher in patients with nonobstructive azoospermia (PI = 1.40 ± 0.13) than in those with obstructive azoospermia (PI = 1.09 ± 0.15; $P=.011$). Seminal plasma nitrite/nitrate concentrations were more elevated in cases of obstructive azoospermia than in gonadal failure. Unsuccessful sperm recovery was observed in four patients who showed the worst indices of gonadal failure. In this subgroup, a transmediastinal PI value >1.50 was always observed.

Conclusion(s): Doppler analysis of the transmediastinal artery and nitrite/nitrate seminal plasma concentrations are useful for distinguishing between obstructive and nonobstructive azoospermia and allow the identification of the presence of spermatozoa within the testes. (Fertil Steril® 2001;75:1088–94. ©2001 by American Society for Reproductive Medicine.)

Key Words: Azoospermia, Doppler, ultrasound, nitric oxide, sperm retrieval, assisted reproduction

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Azoospermia affects approximately 1% of the male population and 10% of men who seek fertility evaluation. In 20% of cases, a bilateral obstruction of the male genital tract is responsible for the azoospermia. If the patient has a history of scrotal or inguinal surgery or recurrent genital infection, an obstruction should be suspected. These patients are generally characterized by small volume of ejaculate, normal testis volume, and normal concentration of gonadotropins. In about 2% of the infertile male population, congenital bilateral absence of the vas deferens is found.

A definitive diagnosis of obstructive azoospermia may be made only by showing normal spermatogenesis with a testicular biopsy. However, most patients with azoospermia have the nonobstructive type and present with poor spermatogenesis, small testes, normal ejaculate volume, and elevated serum follicle-stimulating hormone (FSH). Furthermore, on testicular biopsy, gonadal failure is associ-

ated with a Sertoli cell–only pattern, maturation arrest, or hypospermatogenesis (1, 2).

The use of testicular biopsy began more than 60 years ago as the most reliable procedure to distinguish between obstructive and nonobstructive azoospermia (3). Recently, however, its use has been focused on extracting, by testicular sperm aspiration (TESA) or testicular sperm extraction (TESE), testicular spermatozoa for intracytoplasmic sperm injection (ICSI) (4, 5). TESE is an invasive procedure that may have important side effects and complications. Thus, the need to restrict TESE to those patients with azoospermia who have the best chance of yielding testicular spermatozoa and the need for finding new prognostic factors are widely acknowledged.

Among prognostic indicators, the assessment of testicular volume, by palpation or ultrasound, could be important (seminiferous tubules represent 80%–85% of the testicular mass) because a small testicular volume may suggest an impairment of seminiferous tubules and may be associated with raised FSH plasma concentrations (6). High plasma FSH levels, probably due to decreased Sertoli cell inhibin secretion, are considered reliable indicators of germinal epithelial damage. However, other than FSH, testicular paracrine (i.e., inhibin, activin, and nitric oxide) products may be implicated in abnormal sperm function.

Nitric oxide (NO) is a labile and diffusible molecule, which forms stable metabolites (nitrite/nitrate, NO_2^-/NO_3^-) detectable in many biological fluids. NO is synthesized from a guanidine nitrogen atom of L-arginine either by a constitutive calcium-dependent or a proinflammatory cytokine-inducible NO-synthase (NOS) (7). The precise role of NO has not been elucidated, however, it is thought to be involved in a wide array of biological processes: neuronal transmission, immune modulation, vascular wall contractility, hormone production, cell differentiation, apoptosis, and defense against free radicals. NO has also been implicated as a mediator of penile erection, and its localization at the level of the male testes, epididymis, prostate, and seminal vesicle suggests a role in vascular modulation of reproductive organs, spermatogenesis, and sperm maturation (8–10).

Color Doppler ultrasound is a recent advance in sonographic imaging that provides simultaneous display of tissue morphology in gray scale and blood flow in color. Recent reports showed that color Doppler facilitates the detection of small intratesticular vessels and allows the measurements of impedance to flow in this vascular tree (11, 12).

The aim of the present study was to prospectively evaluate the role of intratesticular vascular flow in modulating sperm function in men with obstructive and nonobstructive azoospermia. The correlation of testicular Doppler values with NO and TESE was also evaluated. The outcome of the sperm retrieval/ICSI procedures has been analyzed.

PATIENTS AND METHODS

Study Population

The study protocol was approved by the departmental ethics review committee. Twenty-eight couples attending the infertility clinic with primary infertility due to azoospermia participated in the study after giving informed consent. The diagnosis of azoospermia (no spermatozoa in the ejaculate) was made on the basis of two semen analyses performed according to the WHO recommended procedure. After history, physical examination, sperm analysis (including assessment of sperm volume and pH and evaluation of fructose concentration), endocrine profile (FSH, luteinizing hormone, testosterone, androstenedione), ultrasound testicular volume, and seminal vesicle evaluation, the patients with azoospermia were divided into groups: nonobstructive (group 1; $n = 16$) and obstructive (group 2; $n = 12$).

All patients were tested for deletion in the genes (sY84, sY125, sY143, sY254) on the long arm of the Y chromosome. Furthermore, men with obstructive azoospermia with congenital absence of the vas deferens were screened for mutations of the cystic fibrosis gene (1717-1G, ΔF508, R1162X, N130K, G542X, 2183AA, 711+5G, G85E, R553X, 2789+5G→A, Q552X, 3132delTG, 3849+10KbC→T, ΔI507, W1282X).

Excluded from the study were patients with a history of drug or alcohol abuse, ongoing medical treatments (anabolic steroids, gonadotropins, cancer chemotherapy), a heavy smoking habit (>10 cigarettes/day), diabetes, hypertension (systolic blood pressure >140 mmHg and/or diastolic pressure >90 mmHg), leukocytospermia (>1^6 leukocytes/mL), ultrasound abnormalities of scrotal content (varicocele, focal hypo- or hyperechoic lesions, local hemorrhage, microlithiasis, intratesticular cysts), or unilateral testicular atrophy.

The female partners had regular menstrual cycles (28 ± 4 days) and patent tubes on X-ray hysterosalpingography. Furthermore, hormonal and ultrasound evaluations confirmed ovulatory cycles in all patients.

Couples with women presenting with endometriosis, functional cysts, polycystic ovarian syndrome, unilateral ovarian resection, or ovariectomy were not included in the study. Women had not received hormonal treatment for at least 4 months before the study, and their mean age was similar (33 ± 4.9 vs. 36 ± 2.8 years) in both groups.

The female patients underwent the same controlled ovarian hyperstimulation (COH) protocol. Briefly, COH was achieved by an injection on day 20 of the cycle of gonadotropin-releasing hormone agonist (Decapeptyl 3.75; Ipsen, Milan, Italy) and pure FSH (pFSH) (Metrodin HP; Serono, Rome, Italy) administered intramuscularly (IM) after pituitary desensitization (plasma estradiol concentrations <100 pmol/L; ovaries with no follicles >5 mm in diameter and endometrial thickness <5 mm) in an individual assessed

dosage. When at least three follicles >17 mm in diameter were present, pFSH was withdrawn and 10,000 IU of human chorionic gonadotropin (hCG; Profasi; Serono) was administered IM. Ultrasound oocyte recovery was carried out transvaginally 35 hours after hCG injection.

Embryo transfer (ET) was performed using a Frydman catheter (SCS International; Genoa, Italy), ~72 hours after oocyte retrieval. Between one and three embryos were replaced at the 8- to12-cell stage. The remaining cleaved embryos with <20% fragmentation were allocated to a cryopreservation protocol. Vaginal progesterone (Esolut Crema; Angelini, Rome, Italy) was daily prescribed as luteal support. Patients with a clinical pregnancy (ultrasound evidence of embryonic heart activity) were followed up until after delivery.

All patients with azoospermia underwent semen analysis and ultrasound and color Doppler scanning of the testes on the day of hCG administration. Nitrite/nitrate concentrations were assayed in seminal plasma. Furthermore, on the same day, peripheral blood was obtained from all patients between 8:00 and 11:00 A.M., and different hormonal and biochemical parameters were analyzed. On the day of ovum pickup, testicular sperm retrieval, by testicular aspiration and/or extraction, was performed.

Semen Analysis and Seminal Plasma NO_2^-/NO_3^- Assay

After 2–3 days of sexual abstinence, semen samples were produced by masturbation, collected into sterile specimen cups, and allowed to liquefy at room temperature. Semen volume, pH, and sperm concentration were determined following the WHO guidelines for semen analysis (13).

For assessment of NO_2^-/NO_3^- (stable oxidation products of NO metabolism) concentrations in the seminal plasma, the whole semen sample was centrifuged at 1,500 × *g* for 5 minutes and the seminal plasma was removed and stored at −70°C until bioassay. Since very little or no NO_2^- is normally found in the plasma, we did not attempt to differentiate between the respective amounts of NO_2^- and NO_3^-; therefore, results are reported as NO_2^-/NO_3^-. The concentrations of NO_2^-/NO_3^- were assayed with the Greiss reaction with procedures previously described (14, 15).

Hormonal and Biochemical Assay

Peripheral blood samples, obtained from an antecubital vein, were immediately centrifuged, and the plasma was separated and stored at −20°C and subsequently assayed as previously reported (15). Plasma concentrations of luteinizing hormone (LH), FSH, testosterone, androstenedione were determined by radioimmunoassay (Radim; Pomezia, Italy). The NO_2^-/NO_3^- plasma concentrations were determined with the same method used for seminal plasma.

Ultrasound and Color Doppler Examinations

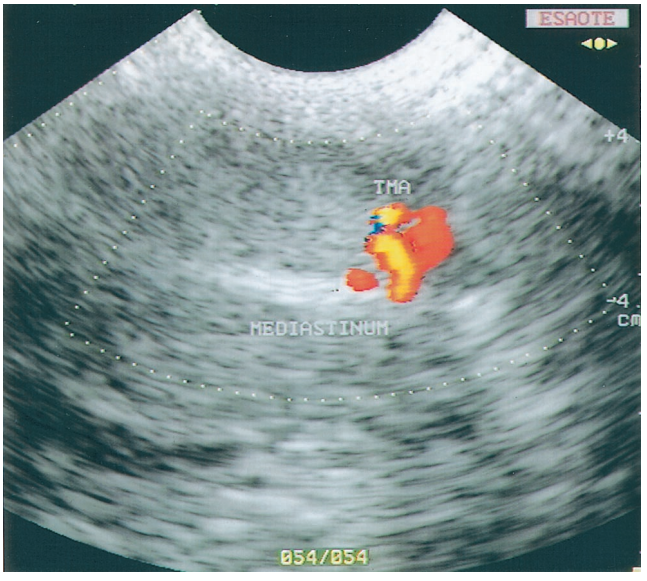
Scrotal ultrasound assessment of testicular volume was performed in a warm room with the patients in the supine position and with the penis resting on the lower abdomen, using a 6.5-MHz digit probe (AU4 Idea, Esaote; Milan, Italy). All three dimensions of each testicle were measured, and the volume was electronically calculated by machine. No significant differences between the volumes of the left and right testicle were observed, and, therefore, the average value of testicular volume was used.

Doppler flow measurements of transmediastinal arteries (TMAs) were performed in each testis with a trans-scrotal approach using a 6.5-MHz (AU4 Idea) color Doppler system. A 50-Hz filter was used to eliminate low-frequency signals originating from vessel wall movements. The maximum ultrasound energy was <80 mW/cm^2. The intensity is within the safety limits suggested by the American Institute for Ultrasound in Medicine (16). Color flow images of TMAs were sampled, in a longitudinal plane, at the level of the testicular mediastinum (Fig. 1) (17). As previously reported, the TMAs present blood flow directed away from the mediastinum.

The angle of insonation was altered to obtain the maximum color intensity. When good color signals were obtained, blood flow velocity waveforms were recorded by placing the sample volume across the vessel and entering the pulsed Doppler mode. The pulsatility index (PI) was calculated electronically by the machine. The PI has been shown to reflect blood flow impedance downstream of the point of sampling and may be used when the end diastolic shift is

FIGURE 1

Color flow image of the transmediastinal artery (TMA). The TMA is sampled, in longitudinal plane, at the level of the testicular mediastinum.



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absent or reversed. For each examination, the mean value of three consecutive waveforms was obtained. No significant differences between the PI of left and right TMAs was observed. Therefore, the average value of both arteries was used. Ultrasound and color Doppler analyses were performed by a single examiner (C.B.).

Sperm Retrieval

In patients with obstructive azoospermia, TESA was initially performed under general anesthesia, by directing a 21-gauge butterfly needle, connected to a 20-mL syringe, into the immobilized testis. Under negative pressure, the needle was repeatedly introduced into the testicular parenchyma until testicular fluid was obtained. Then the needle was slowly withdrawn and the aspirate flushed into a sterile Petri dish, using IVF culture medium. The specimen was then microscopically examined and, in the absence of sperm, the procedure was repeated up to three times per testis.

In patients with nonobstructive azoospermia, TESA was performed as an initial procedure. If no sperm was obtained after two specimens per testis, we continued with TESE. Scrotal exploration was performed, under general anesthesia, through a median raphe incision, and sperm was retrieved using an open testicular biopsy technique (5). When no sperm was retrieved from the harvested testicular parenchyma, a controlateral biopsy was done. The collected spermatozoa were used for ICSI and eventually frozen for subsequent use.

Histopathology

Testicular tissue (~5 mg) was prepared for histology. Semi-thin paraffin wax testicular sections fixed in Bouin's solution were dewaxed and rehydrated by transfer through graded alcohol/xylene, stained with hematoxylin and eosin, and examined under a light microscope at ×100–1,000 magnification using standard techniques. Testicular histology was classified into normal spermatogenesis, hypospermatogenesis (reduction in the degree of normal spermatogenic cells), maturation arrest (absence of the later stages of spermatogenesis), Sertoli cell only (absence of germ cells in the seminiferous tubules), and tubular sclerosis (no germ cells or Sertoli cells in the tubules).

STATISTICAL ANALYSIS

A statistical analysis (SPSS software; SPSS, Inc., Chicago, IL) was performed using the Mann-Whitney test and a one-way analysis of variance. The relationship between the parameters analyzed was assessed using the stepwise linear regression method. $P \leq .05$ was considered the limit of statistical significance. Data are presented as mean ± SD unless otherwise indicated.

RESULTS

All 28 couples completed the study. The mean male age was 39.8 ± 5.7 years, and no significant differences were observed between the groups. The semen volume was significantly higher in group 1 (2.8 ± 1.2 mL) than in group 2 (0.7 ± 0.5 mL; $P=.033$). The patients' plasma testosterone (319 ± 92 vs. 382 ± 88 ng/100 mL) and androstenedione (141 ± 59 vs. 169 ± 70 ng/100 mL) did not differ between the groups. Plasma FSH (16.3 ± 11.4 vs. 3.6 ± 1.5 IU/L; $P<.0001$) and LH (6.6 ± 3.9 vs. 2.7 ± 0.8 IU/L; $P=.03$) was significantly higher in cases of nonobstructive azoospermia than in cases of obstructive azoospermia.

The assessment of ultrasound volume showed smaller testes in group 1 (9.9 ± 2.6 mL) than in group 2 (16.9 ± 4.3 mL; $P=.03$) patients. On color Doppler analysis, TMAs were observed in at least one testicle in 100% of the cases, and bilaterally in 96.5% of the cases. Higher resistances were observed in TMAs of patients with nonobstructive azoospermia (PI = 1.40 ± 0.13) than of those with obstructive azoospermia (PI = 1.09 ± 0.15; $P=.011$).

The NO_2^-/NO_3^- plasma concentrations were similar in both groups (Fig. 2). Seminal plasma NO_2^-/NO_3^- concentrations were significantly higher in men with obstructive azoospermia than in patients of other the group (Fig. 2). Normalizing the NO_2^-/NO_3^- seminal plasma concentrations to testes volume, we obtained an NO_2^-/NO_3^- seminal concentration of 1.56 ± 0.6 μM/mL testis and 1.84 ± 0.3 μM/mL testis ($P=.038$), respectively, in nonobstructive and obstructive azoospermia. In addition, in patients with obstructive azoospermia, the NO_2^-/NO_3^- plasma concentrations were significantly lower ($P=.002$) than the concentrations in the seminal plasma. No significant differences were observed in the other group.

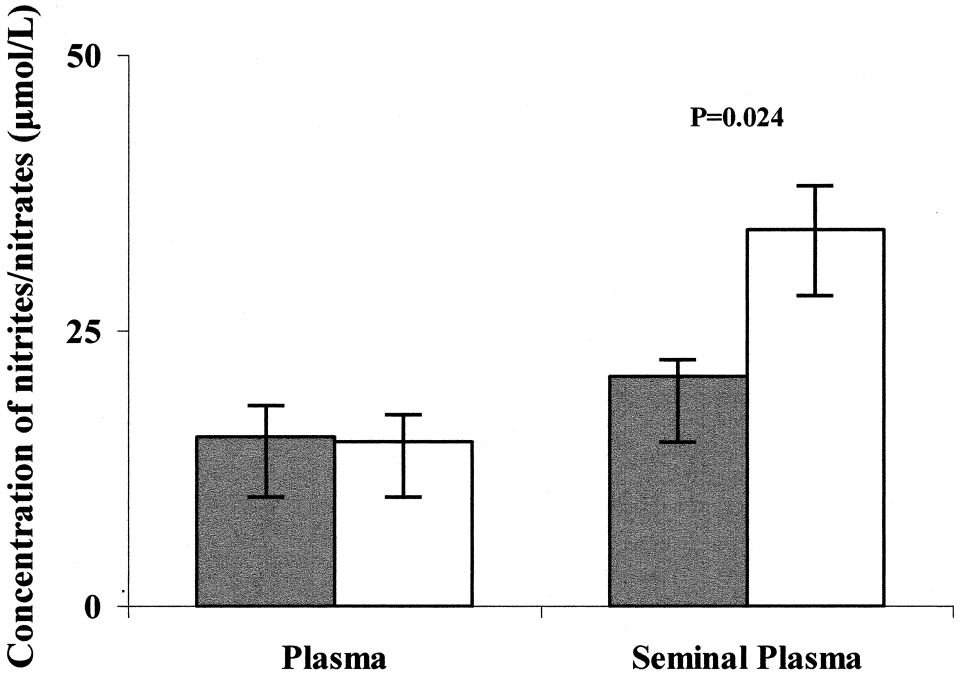
Plasma FSH was inversely correlated with testicular volume ($r = -0.756$; $P=.002$), and positively correlated with TMA PI ($r = 0.647$; $P=.011$). Furthermore, the NO_2^-/NO_3^- seminal plasma concentrations were correlated with testicular volume ($r = 0.559$; $P=.029$).

All patients were negative for the tested deletion in the genes on the long arm of the Y chromosome. Among men with obstructive azoospermia with congenital absence of the vas deferens (n = 4), two were positive for mutation of the cystic fibrosis gene ΔF508. Because the partners were negative for the mutation, the couple, after counseling, was not excluded from the study.

Sperm retrieval was obtained with TESA in all (100%) men with obstructive azoospermia and in 3 (18%) men with nonobstructive azoospermia. TESE was successful in 9 out of the 13 patients with nonobstructive azoospermia, giving a sperm retrieval rate of 69%. The retrieved testicular tissue was always considered suitable for histological analysis. In nonobstructive azoospermia, the relative frequencies of the testicular histological patterns were maturation arrest in 8 (50%), hypospermatogenesis in 5 (31%), Sertoli cell only in 2 (12.5%), and tubular sclerosis in 1 (6.5%). All the specimens from men with obstructive azoospermia revealed a normal spermatogenesis.

FIGURE 2

Nitrites/nitrates concentrations in plasma and seminal plasma of men with obstructive and nonobstructive azoospermia. Shaded bars, nonobstructive azoospermia; open bars, obstructive azoospermia.



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On sperm retrieval, among subjects with gonadal failure, as above reported, we did not find spermatozoa in 4/16 cases (25%). In these patients the histological examination showed tubular sclerosis (n = 1) and early maturation arrest (n = 3). Extrapolating this group we observed significantly higher TMA PI values and plasma FSH concentrations and lower testicular volume and NO_2^-/NO_3^- seminal plasma concentrations than in other men with nonobstructive azoospermia (Table 1).

During controlled ovarian stimulation, no significant differences in term of pFSH ampoules, days of stimulation, follicles with a diameter >1.7 cm, and endometrial thickness were observed between the groups. After ovum pickup, a total of 196 oocytes were obtained and 174 (88%) were injected. Excluding the four cases in which it was not possible to find spermatozoa, the fertilization rate in patients with gonadal failure (53%) and with obstructive azoospermia (68%) was not different. Similarly, the mean number of transferred embryos (2.3 ± 0.4 vs. 2.8 ± 0.3) did not differ in the groups with gonadal failure and obstructive azoospermia. In the group with nonobstructive azoospermia, 1 cycle had no embryos for transfer. The pregnancy rate/ET was not statistically different between group 1 (9%) and group 2 (33%).

Sperm cryopreservation was performed in 4 (44%) out 9 patients who successfully underwent TESE and in 12 (80%) out 15 patients who underwent TESA. The frozen semen was subsequently used for further ICSI cycles.

TABLE 1

Men with gonadal failure: Comparison between patients presenting or not presenting spermatozoa on sperm retrieval.

Parameter	Sperm absent (n = 4)	Sperm present (n = 12)	Significance
Testicular volume, mL	7.6 ± 0.8	11.8 ± 2.0	*P*=.011
FSH, IU/L	27.5 ± 13.7	10.7 ± 3.1	*P*=.006
Seminal plasma nitrites/nitrates, µmol/L	14.9 ± 7.0	27.0 ± 8.8	*P*=.049
Doppler TMA, PI	1.62 ± 0.09	1.25 ± 0.11	*P*=.016

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DISCUSSION

When azoospermia is present, obstruction should be distinguished from seminiferous tubular damage because men with nonobstructive azoospermia have different genetic problems from men with obstructive azoospermia (1). In

addition, the chance of conceiving with ICSI is higher for obstructed than nonobstructed patients with azoospermia (18, 19). A reduced bilateral testicular volume, associated with normal ejaculate volume and raised concentrations of circulating FSH, is considered to be a reliable indicator of germinal epithelial damage and nonobstructive azoospermia. Conversely, obstructive azoospermia is usually characterized by normal testicular volume, normal circulating FSH values, and reduced ejaculate volume.

Our data showed higher ejaculate volume, lower testicular volumes, and higher FSH circulating levels in patients with nonobstructive azoospermia than in those with obstructive azoospermia, confirming that the above parameters are useful for the differential diagnosis between secretive and obstructive azoospermia. In addition, an inverse relationship between testicular volume and FSH has been found. As the seminiferous tubules represent approximately 80%–85% of the testicular mass, a small testicular volume and elevated FSH may suggest a significant impairment of the seminiferous tubules with subsequent testicular fibrosis, damaged spermatogenesis, and inhibin deficiency.

Plasma FSH values were positively correlated with TMA PI, and TMA resistances resulted in higher rates of patients with nonobstructive than with obstructive azoospermia. Since the testicular mediastinum is the entry site for the main testicular vessels and PI is used as a measure of blood flow impedance distal to the point of sampling, elevated PI values express a low testicular vascularization. We speculated that in patients with nonobstructive azoospermia, the severely altered testicular structure is associated with fibrotic processes and significant decrease of blood flow supply to the testes, whereas in obstructive azoospermia, testicular perfusion is normally represented. These data make the color Doppler analysis a novel and useful clinical parameter (in addition to FSH, testicular volume, and ejaculate volume) for distinguishing between obstructive and secretive azoospermia.

Recently, Foresta et al. (12) utilized a semiquantitative score (number of vessels "randomly" visualized by color and power Doppler) to analyze the testicular blood supply. We believe that the method is not objective and is less reproducible than the measurement of blood flow impedance of the TMA; the PI has been shown to accurately reflect blood flow impedance of the studied structures. Furthermore, in contrast with Middleton et al., who, in 1989, affirmed that ". . . it is unusual for arteries to pass directly through the mediastinum of the testis" (20), and, in a subsequent paper, visualized the TMA in only about one-half of normal testes (17), our data showed that, although studied in patients with azoospermia, the TMA is present in at least one testis in 100% of the cases and bilaterally in 96.5% of the cases. We think that the progressive improvements of color Doppler machine and the skill of the ultrasonographer allow an easy and reproducible visualization of the main testicular vessels.

In the present study we found seminal plasma NO_2^-/NO_3^- concentrations to be significantly higher in men with obstructive azoospermia than in those with nonobstructive azoospermia also after normalizing the NO_2^-/NO_3^- seminal plasma concentrations to testes volume. Furthermore, in patients with obstructive azoospermia, the NO_2^-/NO_3^- plasma concentrations were significantly lower than in seminal plasma. This agrees with Schaad et al. (21), who, finding constitutive NOS expression in human spermatozoa, suggested a spermatic release of controlled amounts of NO, which may confirm a spermatozoic release of NO_2^-/NO_3^-, allowing us to speculate that in seminal plasma the NO_2^-/NO_3^- concentrations are related to the number of functionally normal spermatozoa. However, Middendorf et al. (22) showed that NOS is present in Leydig cells and in myofibroblasts of peritubular lamina propria, in Sertoli cells, and in endothelial and smooth muscle cells of testicular blood vessels. In such structures, the above findings suggest a production and a local activity of NO that may be involved, by relaxation of seminiferous tubules and blood vessels, in the regulation and distribution of hormones and nutrients to modulate sperm production and transport. Furthermore, because NO can freely diffuse across membranes, we cannot exclude NO_2^-/NO_3^- production at the level of seminiferous tubules and, through the lamina propria, a subsequent relaxant action at the level of the neighboring vascular smooth muscle cells. This hypothesis could be supported by the relationship we found between NO_2^-/NO_3^- seminal plasma and testicular volume. Further studies will be necessary to elucidate the relationship between NO_2^-/NO_3^- seminal plasma, testicular vascularization, and spermatic production in infertile subjects.

Recovery of testicular spermatozoa from patients with azoospermia for ICSI is a recent advance in the treatment of male-factor infertility. At present there are no diagnostic or prognostic parameters that enable the identification of the presence of spermatozoa within the testes (23, 24). In our series the overall sperm recovery rate, over 28 TESA/TESE procedures, was 86%. An unsuccessful sperm recovery was observed in four patients who presented the worst indices of gonadal failure. In these subjects we found significantly higher circulating FSH values and lower testicular volume and NO_2^-/NO_3^- seminal plasma concentrations than in other patients with nonobstructive azoospermia. Furthermore, TMA PI values >1.50 were observed in this subpopulation. Although the number of patients presenting the above finding is quite low and does not allow us to extrapolate the data for a clinical use, we suggest that the presence of TMA PI values ≤ 1.50 may indicate the possible presence in the testis of residual spermatogenic areas even in the presence of a low testicular volume and an elevated FSH value.

Since the introduction of microsurgical epididymal sperm extraction for patients with obstructive azoospermia, new technologies for sperm retrieval and ICSI have been devel-

oped. Recent studies have shown high fertilization and pregnancy rates, through the use of TESA/TESE, even in patients with germinal failure. Devroey et al. (19) and Kahraman et al. (25), comparing the fertilization rate according to testicular histology, demonstrated a significant increase in the fertilization capacity of spermatozoa retrieved from patients with obstructive versus nonobstructive azoospermia. Although our data showed a higher fertilization rate in obstructive (68%) than in nonobstructive (53%) azoospermia, the difference, in agreement with a previous study of Madgar et al. (2), was not statistically significant. We speculated that even in germinal failure, sperm recovered from well-vascularized testes gain a fertilization capacity similar to those obtained from normal testicular structures of men with obstructive azoospermia.

In conclusion, our study, other than confirming that patients with azoospermia are potentially fertile and that by using TESA/TESE in combination with ICSI it is possible to obtain good fertilization and pregnancy rates independent from the type of azoospermia, demonstrates that, in combination with circulating FSH and testicular ultrasound evaluation, color Doppler analysis of the TMA and NO_2^-/NO_3^- seminal plasma concentrations may be useful to distinguish between obstructive and nonobstructive azoospermia. Furthermore, even though more extensive studies are necessary to elucidate the factors that may influence testicular vascularization and sperm production, color Doppler analysis of the TMA seems to be a diagnostic and prognostic tool that enables the identification of the presence of spermatozoa within the testes.

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