

3. Silber SJ, Van Steirteghem AC, and Devroey P: Sertoli cell only revisited. *Hum Reprod* 10: 1031–1032, 1995.
4. Devroey PI, Liu J, Nagy Z, Tournaye H, Silber S, and Van Steirteghem A: Normal fertilization of human oocytes after testicular sperm extraction and intracytoplasmic sperm injection. *Fertil Steril* 62: 639–641, 1994.
5. Silber SJ, Van Steirteghem AC, Liu J, Nagy Z, Tournaye H, and Devroey P: High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum Reprod* 10: 148–152, 1995.
6. Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M, Van Steirteghem A, and Silber S: Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod* 10: 1457–1460, 1995.
7. Stone IC: *Forensic Science Gazette*. Dallas, Southwestern Institute of Forensic Sciences, 1977.
8. Tulloch WS: Varicocele in subfertility: results of treatment. *Br Med J* 2: 356–358, 1955.
9. Del Castillo EB, Trabucco A, and De La Balze FA: Syndrome produced by absence of the germinal epithelium without impairment of the Sertoli or Leydig cells. *J Clin Endocrinol* 7: 493–502, 1947.
10. Levin HS: Testicular biopsy in the study of male infertility; its current usefulness, histologic techniques, and prospects for the future. *Hum Pathol* 10: 569–584, 1979.
11. Jarow JP, Espeland MA, and Lipshultz LI: Evaluation of the azoospermic patient. *J Urol* 142: 62–65, 1989.
12. Bar-On E, Weiss DB, Gottschalk-Sabag S, and Zukerman Z: The relationship between plasma levels of gonadotropins, androgens, and prolactin in azoospermic men with their testicular spermatogenic pattern. *Fertil Steril* 64: 1043–1045, 1995.
13. Mendoza C, and Tesarik J: The occurrence and identification of round spermatids in the ejaculate of men with non-obstructive azoospermia. *Fertil Steril* 66: 826–829, 1996.
14. Fishel S, Green S, Bishop M, Thornton S, Hunter A, Fleming S, and Al-Hassan S: Pregnancy after intracytoplasmic injection of spermatid. *Lancet* 345: 1641–1642, 1995.
15. Tesarik J, Rolet F, Bramei C, Sedbon E, Thorel J, Tibi C, and Thebault A: Spermatid injection into human oocytes. II. Clinical application in the treatment of infertility due to non-obstructive azoospermia. *Hum Reprod* 11: 780–783, 1996.
16. Araki Y, Motoyama M, Yoshida A, Kim S-Y, Sung H, and Araki S: Intracytoplasmic injection with late spermatids: a successful procedure in achieving childbirth for couples in which the male partner suffers from azoospermia due to deficient spermatogenesis. *Fertil Steril* 67: 559–561, 1997.

COMMENT

Spermatogenic failure is incurable at present, with the rare exception of maturation arrest occurring in conjunction with a varicocele.⁸ The seminiferous tubules contain germ cells in hypospermatogenesis and maturation arrest (both complete and incomplete arrest). By contrast, germinal cell aplasia, or Sertoli cell-only syndrome, was originally described as the complete absence of the germinal epithelium. It was characterized as a constellation of findings that included total azoospermia, somewhat reduced testicular size bilaterally, and testicular histology revealing seminiferous tubules with a reduced diameter, containing no germinal epithelium and lined by Sertoli cells.⁹ It is clear, however, that germinal cell aplasia is not necessarily global within the testes. Levin¹⁰ initially described the histologic findings of germinal cell aplasia with focal spermatogenesis, a condition that is distinguishable from classic germinal cell aplasia by foci of spermatogenesis within the testis. Sampling error in either a surgical biopsy or the subsequent pathologic examination may miss such foci of spermatogenesis if they are rare.

Hence, the identification of germ cells in an ejaculate does not guarantee that they will be found on surgical biopsy. So, too, identifying spermatogenesis on one biopsy specimen from a patient with germinal cell aplasia does not guarantee that foci will be present in future biopsies (as might be planned for ICSI with testicular sperm extraction). The probability of such sampling errors will decrease if multiple and larger biopsies are performed. However, surgical biopsy through the tunica albuginea risks vascular compromise of the testis because the testicular blood supply is centripetal; this risk increases when multiple biopsies are performed.

Although the identification of rare spermatozoa in an ejaculate does not confirm a specific pathologic diagnosis, it establishes the presence of spermatogenesis. As Jarow *et al.*¹¹ found, an FSH level less than twice normal in the absence of bilateral testicular atrophy is 100% sensitive for identifying patients with obstructive azoospermia. The corollary to this finding, therefore, is that an FSH level of greater than or equal to two times normal (ie, 20 IU or greater) in the presence of bilateral testicular atrophy is 100% specific for nonobstructive azoospermia (as all patients with obstructive etiologies will have been eliminated by the aforementioned criteria). Similarly, it has been shown that FSH elevations that are threefold greater than normal preclude the existence of full, global testicular spermatogenesis.¹²

We would therefore propose that under appropriate circumstances, spermatozoa found on NF-

PICS may be used to direct the clinical sequence of events. In patients with azoospermia on routine semen analysis, a significantly elevated FSH level, and bilateral testicular atrophy (or an atrophic solitary testis), nonobstructive azoospermia is present. If such a patient has mature, formed spermatozoa on NF-PICS, then that patient might be spared from undergoing a purely diagnostic testis biopsy on the grounds that some degree of complete spermatogenesis is present.

Mendoza and Tesarik¹³ reported that round spermatids may be detected in the ejaculates of up to 69% of men with nonobstructive azoospermia. Although the use of round spermatids for ICSI has been reported, these techniques are not widely practiced,¹⁴⁻¹⁶ whereas use of spermatozoa for ICSI is. Unlike Mendoza and Tesarik, we also identified spermatozoa in the semen of men with documented spermatogenic failure. Because this study is retrospective, we cannot directly address the possibility that these rare spermatozoa might have been isolated from the ejaculate, and themselves used for in vitro fertilization procedures. Another limitation of our study is that our sample size is too small to compare the relative prevalence of ejaculated spermatozoa among the different categories of spermatogenic failure.

CONCLUSIONS

We found that rare formed spermatozoa may be present in the ejaculates of men with testicular failure, regardless of the histologic diagnosis. In patients with azoospermia, the finding of rare formed spermatozoa in the semen will confirm that there are foci of spermatogenesis in the testes. Ideally, testicular biopsy should be performed under circumstances that maximize the potential benefits to the patient while minimizing the risks, which include further testicular atrophy. If the clinical evaluation suggests testicular spermatogenic failure and formed spermatozoa are present in the semen, then testis biopsy may be performed for histologic diagnosis as well as testicular sperm extraction at the time of in vitro fertilization.

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REFERENCES

1. Kim ED, Gilbaugh JH III, Patel VR, Turek PJ, and Lipshultz LI: Testis biopsies frequently demonstrate sperm in men with azoospermia and significantly elevated follicle-stimulating hormone levels. *J Urol* 157: 144-146, 1997.
2. Gil-Salom M, Remohi J, Minguez Y, Rubio C, and Pellicer A: Pregnancy in an azoospermic patient with markedly elevated serum follicle-stimulating hormone levels. *Fertil Steril* 64: 1218-1220, 1995.

TABLE I. Clinical diagnosis and seminal findings in azoospermic men

Clinical Diagnosis/Histologic Findings	No. of Patients	Nuclear-Fast Red/Picroindigocarmine Staining of Semen	
		Positive for Mature Germ Cells*	Spermatozoa Identified
Spermatogenic arrest	20	15 (75%)	9 (45%)
Germinal cell aplasia	7	4	2
Complete maturation arrest	6	5	2
Incomplete maturation arrest	3	3	3
Hypospermatogenesis	4	3	2
Congenital bilateral absence of the vas deferens	10	0	0

* Test considered positive if spermatozoa, sperm heads, or round spermatids were identified.

tified on microscopic examination of at least three wet preparations of semen from a single specimen. Special staining of these azoospermic semen specimens was performed with Nuclear-Fast Red and picroindigocarmine solution (NF-PICS). This technique assists in the microscopic identification of rare sperm by staining sperm heads red and sperm tails green. Azoospermic specimens were also examined with Wright's staining to evaluate for the presence of white blood cells, which might be confused with round spermatids.

The clinical charts of azoospermic patients were reviewed to identify those with nonobstructive azoospermia. Additionally, patients with congenital bilateral absence of the vas deferens were identified, and their NF-PICS staining results were used as negative controls. Pathology reports were reviewed for all patients who had undergone surgical testis biopsy. Specimens revealing germinal cell aplasia, when available, were reviewed again by one pathologist (HSL) to identify any foci of spermatogenesis.

Slide preparations consisted of two long smears, two cytospin slides, and a cytospin slide without sample that served as a blank control. The cytospin slides, containing 2 drops of sterile saline each, were prepared simultaneously and centrifuged at 310g in a cytospin unit (SCA-0031, Shandon-Southern Instruments Inc., Sewickley, Pa). The blank slide was used to detect potential sperm contamination of the cytospin sample chambers. The working solutions were checked weekly to ensure staining quality, and monthly for contaminants. The pathology reports as well as the original NF-PICS slides were reviewed.

For NF-PICS staining, the slides were fixed with absolute ethanol for 15 minutes, and air dried. Nuclear-Fast Red solution was placed over the slide for 15 minutes, after which the slides were carefully rinsed with distilled water. The slides were then covered with picroindigocarmine stain for 15 seconds and immediately rinsed with absolute ethanol. They were then air dried and covered with coverslips.⁷ The Nuclear Fast Red solution consisted of 73.1 mM aluminum sulfate with 1.49 mM Nuclear-Fast Red. The picroindigocarmine solution contained 7.15 mM indigo carmine in a saturated picric acid solution. An NF-PICS test was considered positive if some form of germ cell or fully formed spermatozoa were identified.

A statistical analysis comparing the results of NF-PICS staining between different histologic diagnosis categories was performed using Fisher's exact test. In assessing whether the NF-PICS results might have been confounded by the presence of leukocytes, Fisher's exact test was used to compare the findings of leukocytes by Wright's test with "round spermatids" by NF-PICS.

RESULTS

Of the 3005 semen analyses performed over the study period, 256 (8.5%) were azoospermic. These

256 specimens represented 208 patients, of whom 134 had been clinically evaluated by a urologist. Of these 134 men, 12 who had elevated serum FSH levels did not undergo testicular biopsy because of the combination of presumed spermatogenic failure and lack of patient interest in further workup. Seventy-four men were excluded who had surgically confirmed obstructive azoospermia (postvasectomy, efferent ductal obstruction, or ejaculatory duct obstruction); an additional 10 men had congenital bilateral absence of the vas deferens and were selected as negative controls. Sixteen men did not complete their evaluation. Twenty-two patients had biopsy-proven spermatogenic failure, of whom 2 had a unilaterally obstructed testis with normal spermatogenesis and contralateral maturation arrest (one complete and one partial maturation arrest). Thus, 20 patients had azoospermia caused by spermatogenic failure alone.

Histologic diagnoses for the 20 are listed with the corresponding NF-PICS results in Table I. Of these 20, 15 (75%) had some form of mature germ cell present on NF-PICS, and 9 (45%) had fully formed spermatozoa (with tails); several had both findings. All patients with congenital bilateral absence of the vas deferens had negative NF-PICS findings (Table I). Among the patients with germinal cell aplasia histology, pathologic findings of focal complete spermatogenesis was concordant with NF-PICS identification of spermatozoa in all but 2 cases: 1 patient with a negative NF-PICS result had foci of spermatogenesis identified on testicular biopsy, and 1 patient with positive NF-PICS showed no spermatogenesis on routine diagnostic biopsy slides.

To rule out the possibility that NF-PICS results had been confounded by the presence of leukocytes, results for Wright's stain and NF-PICS were compared among the 50 patients who had nonobstructive azoospermia. Using Fisher's exact test, the data showed no evidence of any dependence between the two findings ($P = 0.572$), indicating that there were no confounding effects.

IDENTIFICATION OF SPERMATOZOA AND ROUND SPERMATIDS IN THE EJACULATES OF MEN WITH SPERMATOGENIC FAILURE

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ABSTRACT

Objectives. As many as 10% of infertile men have azoospermia caused by spermatogenic failure or ductal obstruction. The histologic diagnoses associated with spermatogenic failure—Sertoli cell-only syndrome, maturation arrest, and hypospermatogenesis—do not necessarily represent global changes in the affected testis, as occasional seminiferous tubules may still produce mature germ cells. Intracytoplasmic sperm injection (ICSI) allows individual sperm that have been isolated from testicular tissue to fertilize oocytes. This study assessed whether mature germ cells (either round spermatids or spermatozoa) were present in the ejaculates of patients with spermatogenic failure.

Methods. All semen analyses performed at our tertiary care institution from January 1993 through December 1995 were reviewed to identify azoospermic men with spermatogenic failure. During this period, our laboratory employed Nuclear-Fast Red and picroindigocarmine staining (NF-PICS) of cytospin slides to identify rare spermatozoa and spermatids in otherwise azoospermic ejaculates.

Results. Of 3005 analyses reviewed, 20 azoospermic men whose infertility was solely attributable to spermatogenic failure were identified. The histologic diagnoses were germinal cell aplasia ($n = 7$), complete maturation arrest ($n = 6$), incomplete maturation arrest ($n = 3$), and hypospermatogenesis ($n = 4$). Using the NF-PICS technique, mature germ cells were identified in the ejaculates of 15 men (75%), and 9 men (45%) had fully formed spermatozoa present.

Conclusions. In the clinical management of azoospermic infertile men, the NF-PICS technique may be used to identify men who have some degree of testicular spermatogenesis. This might obviate the need for the purely diagnostic testis biopsy that is performed before therapeutic biopsy for testicular sperm extraction in conjunction with ICSI. *UROLOGY* 51: 816–819, 1998. © 1998, Elsevier Science Inc. All rights reserved.

Recent advances in assisted reproductive technology may allow men with spermatogenic failure to father children. Testicular atrophy and an elevated serum follicle-stimulating hormone (FSH) level do not rule out the possibility of focal spermatogenesis.^{1–3} At least 30% of men with germinal cell aplasia may have foci of complete spermatogenesis in their testes.¹ The combination of testicular sperm extraction with in vitro fertilization by intracytoplasmic sperm injection (ICSI)

has expanded the role of testicular biopsy from a purely diagnostic to a potentially therapeutic procedure.^{2–6}

The purpose of this study was to assess whether mature germ cells are present in the ejaculates of patients with spermatogenic failure. We describe a staining procedure that assists in the identification of rare spermatozoa and spermatids in semen. Further, we correlate the results of this staining technique with the histologic pattern of spermatogenic failure among a group of men with nonobstructive azoospermia.

MATERIAL AND METHODS

The results of all semen analyses performed at our tertiary care institution between January 1993 and December 1995 were reviewed to identify patients with azoospermia. Azoospermia was diagnosed when no spermatozoa were iden-

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