ANDROLOGY

Is Diagnostic Testicular Fine Needle Aspiration Necessary in Azoospermic Men Before Sperm Aspiration/Extraction for Intracytoplasmic Sperm Injection Cycles?¹

Z. ZUKERMAN,^{2,3,7} R. ORVIETO,^{2,3} O. M. AVRECH,^{2,3} D. B. WEISS,⁴ S. GOTTSCHALK-SABAG,⁵ E. BAR-ON,⁶ O. RUFAS,^{2,3} I. BAR-HAVA,^{2,3} Z. BEN-RAFAEL,^{2,3} and B. FISCH^{2,3}

Submitted: December 14, 1998

Accepted: October 11, 1999

Purpose: To determine whether diagnostic testicular fine needle aspiration (TEFNA) sampling needs to be performed in azoospermic men prior to obtaining testicular sperm cells for IVF-ICSI procedures.

Methods: Ten azoospermic patients underwent TEFNA in 1993–1996. During 1997, all patients underwent testicular sperm aspiration (TESA) and/or testicular sperm extraction (TESE) to retrieve spermatozoa for IVF-ICSI cycles. The results of the two procedures performed in two separate hospitals were compared.

Results: Diagnostic TEFNA revealed spermatozoa in five patients; identical results in four were found during IVF-ICSI cycles. In three patients, only Sertoli cells were found on TEFNA, in two of them TESA/TESE showed identical results, and in one, two spermatozoa were detected by CytoSEM. In the remaining two patients, spermatids or spermatoocytes were found on both procedures.

Conclusions: There was a very good correlation between the diagnostic and therapeutic procedures. We suggest that

in azoospermic patients, diagnostic TEFNA is valuable in order to avoid unnecessary controlled ovarian hyperstimulation in the female partner for IVF. In patients in whom spermatozoa are detected, cryopreservation may be performed for later IVF-ICSI cycles.

KEY WORDS: TEFNA; TESA; TESE; IVF-ICSI cycle.

INTRODUCTION

The paramount importance of testicular spermatogenesis determination in the evaluation of azoospermic males makes adequate testicular sampling imperative. Sampling methods are also used to retrieve testicular sperm for fertilization and possible pregnancy (1). The most common ones are percutaneous and open testicular biopsy and testicular fine needle aspiration (TEFNA).

TEFNA is a simple and reliable method for obtaining testicular cytologic elements (2–4). The procedure is well tolerated with no complications or adverse effects. Lewin *et al.* (5) reported the first delivery following intracytoplasmic sperm injection (ICSI) of mature live testicular sperm cells obtained by TEFNA from a patient with nonobstructive azoospermia. Recently Lewin *et al.* (6) summarized experience with TEFNA in 85 patients who underwent 111 TEFNA cycles, and mature testicular spermatozoa were recovered in 65 (58.5%).

The aim of the present study was to present our experience with testicular sperm aspiration (TESA) or testicular sperm extraction (TESE) in patients with

¹ Presented at the Annual Meeting of the Israeli Fertility Association, April 27–28, 1998, Tel Aviv, Israel.

² Department of Obstetrics & Gynecology, Rabin Medical Center, Petah Tiqva, Israel.

³ Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

⁴ Me'uhedet Sick Fund, Sha'are Zedek Medical Center, Jerusalem, Israel.

⁵ Department of Cytology, Sha'are Zedek Medical Center, Jerusalem, Israel.

⁶ Male Infertility Unit, Sha'are Zedek Medical Center, Jerusalem, Israel.

⁷ To whom correspondence should be addressed at Andrology Unit, Department of Obstetrics and Gynecology, Rabin Medical Center, Beilinson Campus, Petah Tiqva 49100, Israel.

nonobstructive azoospermia who previously underwent TEFNA. We compared the cytological results of bilateral TEFNA with those of TESA/TESE done later to retrieve spermatozoa for ICSI and reviewed the necessity of diagnostic TEFNA in the evaluation of azoospermic candidates for IVF-ICSI cycles.

PATIENTS AND METHODS

This study was carried out in 1997 at Rabin Medical Center in Israel. Ten patients undergoing TESA/TESE to recover spermatozoa for ICSI, who had previously undergone TEFNA at the Sha'are Zedek Medical Center, were included. All participants had azoospermia due to primary testicular failure.

TEFNA was performed by percutaneous puncture of the testis with a 23-gauge needle and aspiration with a 20 ml syringe attached to a syringe holder (5). Each testis was punctured at the upper, middle, and lower poles, and the aspirated material from each puncture was smeared on three slides. One was air-dried and stained with May-Grunwald-Giemsa stain; the second was fixed immediately in 95% alcohol and stained with Papanicolaou stain. The first two smears were examined with light microscopy at a magnification of 250 $\times$. The cells were defined as spermatogonia, spermatocytes, spermatids, spermatozoa, or Sertoli cell groups, and the patients were classified according to the most mature seminiferous tubule spermatogenic cell type element present in the testes, regardless of the number of aspirates in which they appeared. The third smear was fixed with 2.5% glutaraldehyde, centrifuged at 600g for 10 min onto a 10 mm diameter cover slide, and prepared for scanning electron microscope (SEM) observation at 15,000 $\times$ (CytoSEM) (7).

For the ICSI procedure in 1997, no spermatozoa were recovered from the semen sample obtained for reanalysis (using centrifugation at 1800g for 10 min) on the morning prior to oocyte retrieval, so we proceeded to testicular sperm retrieval by needle biopsy, as reported by Craft and Tsirigotis (8), or if needle biopsy failed, by testicular sperm extraction (TESE) (9). TESA/TESE was performed under general anesthesia and the wet preparation obtained was examined at 400 $\times$ as established by Jow *et al.* (10). Sperm analysis was performed according to the criteria of the World Health Organization (WHO) (11), and spermatozoa were recovered for ICSI as previously described (12).

Ovulation was induced with a long gonadotropin-releasing hormone agonist (GnRH-a) (Decapeptyl,

Ferring, Malmo, Sweden) suppression protocol, starting on Day 2 of the cycle. Fifteen days later, when adequate suppression was obtained, the patients underwent ovarian stimulation with human menopausal gonadotropin (hMG) (Pergonal, Teva, Petah Tiqva, Israel). The gonadotropin dosage was adjusted individually according to serum estradiol (E_2) levels and vaginal ultrasound measurements of follicular diameter obtained every 1 or 2 days. Human chorionic gonadotropin (hCG) (Chorigon, Teva, Petah Tiqva, Israel), 10,000 IU, was administered when the leading follicles reached a minimum diameter of 18 mm, with a peak serum E_2 level of >2000 pmol/L. Approximately 34 hr later, the oocytes were aspirated by the transvaginal ultrasonographic route. For the ICSI procedure, the oocytes were treated with 0.5% hyaluronidase (Sigma Chemical, Co., St. Louis, MO) for 30 s to remove the cumulus-oophorus cells. The remaining cumulus complex and the corona radiata were then removed by mechanical dissection. The oocytes were washed four times in IVF medium (Medi-Cult, Denmark) and then cultured in microdrops of IVF medium covered by lightweight paraffin oil (Sigma) for 1 hr before injection. ICSI was performed as previously described (13). Fertilization was confirmed by the appearance of two pronuclei (2PN), a second polar body, or blastomeres.

RESULTS

Light microscopy of the sperm smears from the pretreatment TEFNA yielded three groups according to the most mature spermatogenic cell element found in the tubule: (1) testes with spermatozoa ($N = 5$); (2) testes with spermatocytes and spermatids (maturation arrest) ($N = 2$); and (3) testes with Sertoli cell-only (germ cell aplasia) ($N = 3$). Comparison of these findings with the results of TESA/TESE showed that in four of the patients in group 1, spermatozoa were found for IVF-ICSI cycles, resulting in two pregnancies. No spermatozoa were found in the remaining patient.

One of the three patients with Sertoli cells only, showed on further sperm analysis by CytoSEM very few cells. This patient received gonadotropin treatment (FSH, Metrodin, Teva, Petah Tiqva, Israel) for six months (14), and subsequent TESA and TESE revealed a few spermatozoa for ICSI in three consecutive cycles. The other two patients in this group showed identical results on TESA/TESE to the diagnostic procedure. This was also true for group 2 patients in whom spermatids or spermatocytes were found to be the most mature spermatogenic stage.

CONCLUSIONS

ICSI constitutes a major advance in the treatment of male factor infertility (15). Testicular biopsies in patients with nonobstructive azoospermia who were previously considered sterile have demonstrated that foci of tubules with normal spermatogenesis may exist alongside tubules with Sertoli cells only and maturation arrest. These patients are now able to conceive with IVF-ICSI cycles. Even the presence of elevated FSH levels does not preclude some form of spermatogenesis in these cases (16,17).

In view of the importance of testicular spermatogenesis evaluation in azoospermic males, we compared the results of pretreatment diagnostic TEFNA with those of TESA/TESE, performed later for sperm retrieval. A very good correlation was observed between the two procedures. Our experience indicates that in azoospermic patients, a preliminary diagnostic evaluation of the spermatogenic pattern of the seminiferous tubules in both testes is valuable. Furthermore, the use of CytoSEM in patients with the histological diagnosis of Sertoli cells only on TEFNA, may suggest the administration of gonadotropin in such cases prior to subsequent testicular sperm retrieval for IVF-ICSI cycles, which calls for further prospective-controlled studies.

Many reports have discussed the role of testicular biopsy before a combined IVF-ICSI cycle for azoospermic patients. Tournay *et al.* (12) suggested that testicular biopsy is predictive only in the presence of normal spermatogenesis or germ-cell hypoplasia, whereas complete or incomplete maturation arrest or germ-cell aplasia preclude a definitive conclusion. They suggested that it is even possible that no spermatozoa will be found in the wet preparation of ICSI when the preliminary histology has shown incomplete maturation arrest or incomplete germ-cell aplasia. Furthermore, in cases of maturation arrest or germ-cell aplasia without focal spermatogenesis, there may still be a chance of recovering testicular spermatozoa by sampling more testicular tissue during the ICSI-TESE cycle. In a subsequent study, the same authors (18) compared two techniques for recovering testicular spermatozoa in 102 patients with obstructive azoospermia: open excisional biopsy and FNA. Although a higher average number of spermatozoa were recovered after open biopsy, there were no significant differences in either fertilization or cleavage rates. They concluded that ICSI performed with testicular spermatozoa recovered by FNA yields results comparable to those obtained with spermatozoa recovered by open biopsy. By contrast, Rosenlund *et al.* (19) noted that percutaneous testicular biopsy is a quick and reli-

able method for demonstrating spermatozoa for ICSI, but material obtained from needle biopsy for a detailed histopathological diagnosis is inferior to that from open testicular biopsies. Moreover, Friedler *et al.* (20) reported a lower sperm recovery rate after FNA (11%) than after open biopsy (43%) in 37 patients with nonobstructive azoospermia.

TEFNA is a simple, reliable, and relatively well-tolerated procedure for obtaining testicular cytologic elements, with no complications or adverse effects (2,3,21). Our findings agree with the study of Craft *et al.* (22), who also found TEFNA a simple alternative to open testicular biopsy for investigating male subfertility and for retrieving spermatozoa for assisted conception during ICSI. We recently reviewed our experience with TEFNA and found that since active spermatogenesis is focal or limited to a small number of seminiferous tubules, TEFNA performed on the upper, middle, and lower poles of each testis is needed to precisely evaluate testicular spermatogenesis (4,5). In addition, since follow up studies of patients who underwent TESA for ICSI-cycle have not shown long-term postoperative complications (23) compared to multiple testicular biopsies, which is associated with sonographic signs of testicular damage (24), TEFNA is the preferred procedure for obtaining testicular samples from multiple sites.

We recommend that preliminary TEFNA be offered to all azoospermic patients to avoid unnecessary controlled ovarian hyperstimulation in the female partner for IVF-ICSI cycles. The results of this diagnostic procedure should be discussed with the couple to enable a more precise prediction of their future success in ICSI cycles. If TEFNA reveals normal spermatogenesis or maturation arrest, cryopreservation may be performed, and the couple may safely proceed to IVF-ICSI (25). Frozen testicular sperm may allow for subsequent cycles of ICSI with or without additional TEFNA. In patients with a histological finding of Sertoli cells only on TEFNA, with or without further sperm analysis by CytoSEM, we offer the male partner three months of gonadotropin therapy and then proceed to IVF-ICSI, using TESA/TESE as an optional donor-sperm backup. This policy still needs to be substantiated in a larger prospective studies.

REFERENCES

1. Witt MA: Sperm retrieval from testicular tissue. *Assist Reprod Rev* 1995;5:266-271

2. Raywansh A, Indudhara R, Goswami AK, *et al.*: Fine needle aspiration cytology in azoospermic males. *Diagn Cytopathol* 1991;7:3–6
3. Gottschalk-Sabag S, Glick T, Bar-On E, Weiss D: Testicular fine needle aspiration as a diagnostic method. *Fertil Steril* 1993;59:1129–1131
4. Gottschalk-Sabag S, Weiss D, Folb-Zacharow N, Zukerman Z: Is one testicular specimen sufficient for quantitative evaluation of spermatogenesis? *Fertil Steril* 1995;64:399–402
5. Lewin A, Weiss DB, Friedler S, Ben-Shachar I, Porat-Katz A, Meirow D, Schenker JG, Safran A: Delivery following intracytoplasmic injection of mature sperm cells recovered by testicular fine needle aspiration in a case of hypergonadotropic azoospermia due to maturation arrest. *Hum Reprod* 1996;11:769–771
6. Lewin A, Reubinoff B, Porat-Katz A, Weiss D, Eisenberg V, Arbel R, Bar-El H, and Safran A: Testicular fine needle aspiration: The alternative method for sperm retrieval in non-obstructive azoospermia. *Hum Reprod* 1999;14:1785–1790
7. Lederman H, Weiss DB, Heger-Maslansky B, Fisher-Fischbein J, Bartoov B: High power scanning electron microscope, sperm cell observation (CytoSEM)—A novel technique for detection of micro sperm density in azoospermic patients using TFNA. Abstract presented at the 11th Congress of the Israel Society of Obstetrics and Gynecology. Jerusalem, 1995; November 22–23
8. Craft I, Tsirigotis M: Simplified recovery, preparation and cryopreservation of testicular spermatozoa. *Hum Reprod* 1995;10:1623–1626
9. Cha KY, Oum KB, Kim HJ: Approaches for obtaining sperm in patients with male factor infertility. *Fertil Steril* 1997;67:985–995
10. Jow WW, Zielenski J, Schlegel P, Magid MS, Goldstein M: Motile sperm in human testis biopsy specimens. *J Androl* 1993;14:194–198
11. World Health Organization. Laboratory Manual for the Examination of Human Semen and Semen-Cervical Interaction. 3rd Edition. Cambridge, Cambridge University Press, 1993
12. Tournaye H, Liu J, Nagy PZ, Camus M, Goossens A, Silber S, Van Steirteghem AC, Devroey P: Correlation between testicular histology and outcome after intracytoplasmic sperm injection using testicular spermatozoa. *Hum Reprod* 1996;11:127–132
13. Bar-Hava I, Ashkenazi J, Shelef M, Schwartz A, Brenganz M, Feldberg D, Orvieto R, Ben-Rafael Z: Morphology and clinical outcomes of embryos after in vitro fertilization are superior to those after intracytoplasmic sperm injection. *Fertil Steril* 1997;68:653–657
14. Ashkenazi J, Farhi J, Orvieto R, Feldberg D, Shelef M, Bar-Hava I, Ben-Rafael Z: Long-term Metrodin for male factor therapy: Effect on the outcome on in-vitro fertilization-intracytoplasmic sperm injection (IVF-ICSI). Presented at the 2nd Annual Meeting of the American Society for Reproductive Medicine. Boston, Massachusetts, 1996, November 2–6; S41
15. Palermo G, Joris H, Devroey P, Van Steirteghem A: Pregnancies after intracytoplasmic sperm injection of single spermatozoon into an oocyte. *Lancet* 1992;ii:17–18
16. Hauser R, Temple-Smith PD, Southwick GJ, de Krester D: Fertility in cases of hypergonadotropic azoospermia. *Fertil Steril* 1995;63:631–666
17. Kim ED, Gilbaugh JH, Patel VR, Turek PJ, Lipshultz LI: Testis biopsies frequently demonstrate sperm in men with azoospermia and significantly elevated follicle stimulating hormone levels. *J Urology* 1997;157:144–146
18. Tournaye H, Clasen K, Aytoz, Naggy Z, van Steirteghem A, Devroey P: Fine needle aspiration versus open biopsy for testicular sperm recovery: A controlled study in azoospermic patients with normal spermatogenesis. *Hum Reprod* 1998;13:901–904
19. Rosenlund B, Kvist U, Ploen L, Lundh-Rozell B, Sjöblom P, Hillensjö T: A comparison between open and percutaneous needle biopsy/ies in men with azoospermia. *Hum Reprod* 1998;13:1266–1271
20. Friedler S, Raziel A, Strassburger D, Soffer Y, Komarovsky D, Ron-El R: Testicular sperm retrieval by percutaneous fine needle sperm aspiration compared with testicular sperm extraction by open biopsy in men with non-obstructive azoospermia. *Hum Reprod* 1997;12:1488–1493
21. Kessaris DN, Wasserman P, Mellinger BC: Histopathological and cytopathological correlation of percutaneous testis biopsy and open testis biopsy in infertile men. *J Urol* 1995;153:1151–1155
22. Craft I, Tsirigotis M, Courtauld E, Farrer-Brown G: Testicular needle aspiration as an alternative to biopsy in the assessment of spermatogenesis. *Hum Reprod* 1997;12:1483–1487
23. Tsirigotis M, Craft I: Sperm retrieval methods and ICSI for obstructive azoospermia. *Hum Reprod* 1995;10:758–760.
24. Schlegel P, Su LM: Physiological consequences of testicular sperm extraction. *Hum Reprod* 1997;12:1688–1692
25. Ben-Yosef D, Yogev L, Hauser R, Yavetz H, Azem F, Yovel I, Lessing JB, Amit A: Testicular sperm retrieval and cryopreservation prior to initiating ovarian stimulation as the first line approach in patients with non-obstructive azoospermia. *Hum Reprod* 1999;14:1794–1801