

Clomiphene Administration for Cases of Nonobstructive Azoospermia: A Multicenter Study

ALAYMAN HUSSEIN,* YASAR OZGOK,† LAWRENCE ROSS,‡ AND CRAIG NIEDERBERGER‡

From the *Minia Infertility Research and Treatment Unit, El-Minia University, Egypt; the †Gulhane Military Medical Faculty, Ankara, Turkey; and the ‡University of Illinois at Chicago, Illinois.

ABSTRACT: Clomiphene citrate is a well-established agent that has been empirically used in cases of idiopathic oligospermia. Clomiphene increases endogenous gonadotropin-releasing hormone secretion from the hypothalamus and gonadotropin hormone secretion directly from the pituitary and, thus, increases intratesticular testosterone concentration. Using intracytoplasmic sperm injection (ICSI), very few sperm may be required for fertilization. The objective of this study was to determine if the application of clomiphene citrate in males with nonobstructive azoospermia might produce sufficient sperm for ICSI, either by resulting in sperm identified in the ejaculate or by potentially improving outcomes of surgical testicular sperm extraction. Forty-two patients with nonobstructive azoospermia (age range, 25–39 years) from 3 international centers were evaluated with routine history, physical examination, and hormonal assessment. Initial testicular biopsy demonstrated maturation arrest in 42.9% and hypospermatogenesis in 57.1% of patients. Clomiphene citrate was administered, with the dose titrated to achieve serum testosterone levels between 600 ng/dL and 800 ng/dL, and semen analyses were

performed at periodic intervals. In patients remaining azoospermic on semen analysis, surgical testicular biopsy and sperm extraction were performed. After clomiphene citrate therapy, 64.3% of the patients demonstrated sperm in their semen analyses ranging from 1 to 16 million sperm/mL, with a mean sperm density of 3.8 million/mL. Sufficient sperm for ICSI was retrieved by testicular sperm extraction in all patients, even though 35.7% remained azoospermic. Additionally, clomiphene citrate administration resulted in a statistically significant increase in testis biopsy patterns associated with greater likelihood of sperm obtained by surgical extraction ($P < .05$). We conclude that clomiphene citrate administration may result in sperm in the ejaculate of patients with nonobstructive azoospermia or the simplification of testis sperm retrieval. Surgeons may consider a course of clomiphene citrate administration prior to surgical sperm retrieval in patients with nonobstructive azoospermia.

Key words: Hypospermatogenesis, maturation arrest, ICSI, TESE. **J Androl 2005;26:787–791**

With advances in assisted reproduction techniques, only 1 sperm per ovum is necessary for fertilization with intracytoplasmic sperm injection (ICSI). Sperm may be surgically retrieved from the testis (Bourne et al, 1995). Men with nonobstructive azoospermia due to hypospermatogenesis and maturation arrest at the level of spermatids may, thus, father children by surgical sperm retrieval and ICSI. Focal areas of spermatogenesis may be missed in unilocal testis biopsy (Plas et al, 1999). Specimens from several testicular sites may yield sperm (Weiss et al, 1998). Testicular fine needle–aspiration mapping may predict these focal areas of spermatogenesis and aid in sperm retrieval (Turek et al, 1999, 2000). Microsurgical testicular sperm extraction (micro-TESE) allows for sperm retrieval in cases of nonobstructive azoospermia by observing and resecting the dilated seminiferous tubules, resulting in a higher probability of finding sperm (Schlegel, 1999).

Clomiphene citrate is a well-established agent that has been described to empirically treat idiopathic oligospermia (Sigman and Jarow, 2003). Clomiphene citrate increases pituitary secretion by blocking the feedback inhibition of estradiol, thus increasing serum follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels, with the latter gonadotropin stimulating the testicular synthesis of testosterone. Numerous studies report improvement in semen quality and increased pregnancy rates among the partners of men in whom clomiphene citrate was administered (Check et al, 1989). The objective of this investigation was to determine if the application of clomiphene citrate in males with nonobstructive azoospermia may result in sufficient sperm for ICSI, either by resulting in sperm identified in the ejaculate or potentially increasing the probability of successful surgical testicular sperm extraction.

Materials and Methods

The study was conducted between June 2001 and June 2002 at 3 institutions. Subjects were recruited from the Minia Infertility Research and Treatment Unit, El-Minia University, Egypt and the IVF and Infertility Center, Gulhane Military Medical Faculty,

Correspondence to: Alayman fathy Hussein, Urology Department, Minia University Hospital, El-Minia 61111 Egypt (e-mail: alaymanh@hotmail.com).

Received for publication November 29, 2004; accepted for publication May 31, 2005.

DOI: 10.2164/jandrol.04180

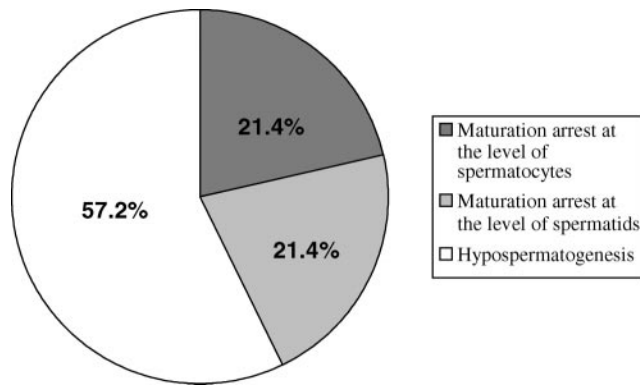


Figure 1. Distribution of histopathologic patterns on initial biopsy.

Ankara, Turkey. Data analysis was performed by the Division of Andrology, Department of Urology, University of Illinois at Chicago. Patients with nonobstructive azoospermia diagnosed by testis biopsy and who were referred from other centers were included if they remained azoospermic at an average of 6–10 semen analyses for at least 12 months. We re-evaluated those patients with routine history and physical examination. At minimum, 2 semen analyses were performed in each patient, with centrifugation of the specimen and microscopic examination of the pellet to confirm azoospermia. Morning plasma testosterone and FSH assays (enzyme-linked immunosorbent assay [Sorim] method at El-Minia University Central Laboratory, El-Minia, Egypt, and Gulhane Military Medical Laboratory, Ankara, Turkey) were performed in each patient. After microscopic examination of previously performed testicular biopsy for each patient was undertaken by a single investigator in each center, 42 patients (age range, 25–39 years) with nonobstructive azoospermia due to hypospermatogenesis or maturation arrest at the levels of spermatocytes and spermatids were selected. Histopathologic diagnosis was based on the predominant histology. Patients with Sertoli cell-only syndrome and testicular malignancy noted in the biopsy were excluded from the study, as were patients with varicocele and those receiving chemotherapy or radiotherapy. Patients with contraindications to clomiphene citrate such as severe depression, hepatic impairment, and heart failure were also excluded.

Clomiphene citrate was administered to azoospermic patients, with hypospermatogenesis and maturation arrest noted on testicular biopsy with an initial dose of 50 mg every other day. After a minimum of 2 weeks, plasma testosterone was assayed. The dose of clomiphene citrate was titrated in increments of 25 mg every other day (eg, daily alternating 50 mg/25 mg, then 50 mg daily, then daily alternating 50 mg/75 mg, then 75 mg daily) until morning serum testosterone was determined to be between 600 ng/dL and 800 ng/dL. In cases where the serum testosterone was noted to exceed 800 ng/dL, the dose of clomiphene citrate was decreased to 50 mg every third day. The lower limit of serum testosterone (600 ng/dL) was chosen as twice the lower limit of the Federal Drug Administration's (FDA) normal range (300–1000 ng/dL), and the upper limit (800 ng/dL) was chosen as a margin of safety of 200 ng/dL under the upper limit of the FDA's normal range.

Duration of treatment ranged from 3 to 9 months (mean du-

Table 1. Initial clinical characteristics in the patient population

	Minimum	Maximum	Mean \pm SD
Age (y)	22.0	39.0	29.6 \pm 4.54
Right testis size (mL)	10.2	28.6	18.6 \pm 4.52
Left testis size (mL)	10.4	27.3	18.4 \pm 4.07
FSH (mIU/mL)*	0.80	18.0	7.21 \pm 7.85

* FSH indicates follicle-stimulating hormone.

ration of treatment, 5.15 \pm 2.38 months). In cases where the serum testosterone was noted to exceed 800 ng/dL, the dose of clomiphene citrate was decreased to 50 mg every third day. Semen analyses were performed at periodic intervals. If at 6 months of treatment sperm was not noted in the ejaculate, testicular sperm extraction using an incisional testis biopsy technique and microdissection of the seminiferous tubules was performed in an attempt to obtain testicular sperm for ICSI. Histopathologic examination of the specimen was performed in a sample of 15 patients who remained azoospermic after clomiphene citrate administration and compared with the biopsy performed before treatment.

Ejaculated sperm outcomes were compared by subpopulation characteristics including testicular volume, testosterone, FSH, dose of clomiphene citrate, and duration of therapy using independent sample Student's *t* tests. Biopsy patterns of spermatogenesis prior to and subsequent to clomiphene citrate administration were compared using χ^2 analysis.

Results

As shown in Figure 1, hypospermatogenesis was noted in the initial biopsy in 24 patients (57.2%), maturation arrest was noted at the level of spermatocytes in 9 patients (21.4%), and maturation arrest was noted at the level of spermatids in 9 patients (21.4%). Table 1 delineates clinical characteristics including patient age, testis volume, and FSH. Mean age was 29.6 \pm 4.5 years, mean right testicular volume was 18.6 \pm 4.5 mL, mean left testicular volume was 18.4 \pm 4 mL, and mean FSH was 7.21 \pm 7.85 mIU/mL. Of the 42 patients, in 18 patients (42.9%) initial testosterone level was less than 300 ng/dL.

After clomiphene citrate therapy, 27 (64.3%) of the patients demonstrated sperm in their semen analysis with densities ranging from 1 to 16 million sperm/mL. Mean sperm density in these patients was 3.8 million/mL, mean motility was 20.8%, and mean total motile count was 2.6 million (Table 2). One partner achieved spontaneous preg-

Table 2. Seminal parameters in patients manifesting ejaculated sperm subsequent to clomiphene citrate therapy

Seminal Parameter	Minimum	Maximum	Mean \pm SD
Density (million/mL)	1.0	16.0	3.81 \pm 4.99
Motility (%)	0.0	40.0	20.8 \pm 16.4
Total motile count (million)	0.0	11.2	2.56 \pm 3.92

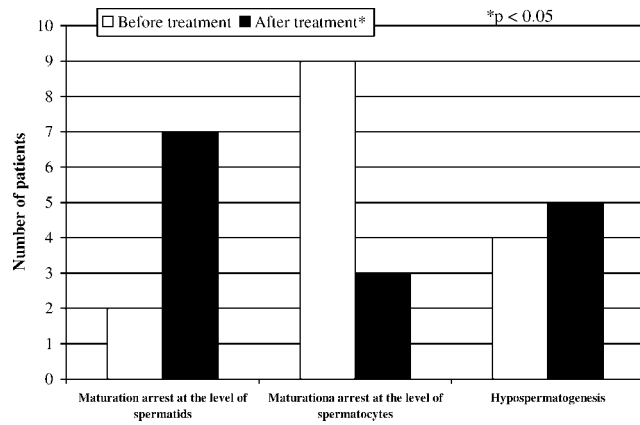


Figure 2. Biopsy histologic patterns prior to and subsequent to clomiphene citrate therapy.

nancy (3.7%), while the remainder underwent IVF or ICSI with ejaculated sperm. Fifteen (35.7%) of the patients remained azoospermic after clomiphene citrate therapy, but sufficient sperm for ICSI was retrieved by testicular sperm extraction in all.

In those patients remaining azoospermic and in which testicular sperm extraction was performed, simultaneous biopsy was also performed for histopathologic analysis and compared with initial biopsy results (Figure 2). A statistically significant difference was noted subsequent to clomiphene citrate therapy in patterns of spermatogenesis toward those with higher probability to yield mature sperm on surgical extraction ($\chi^2, P < .05$; Cramer phi, $r = 0.52$).

In those patients manifesting sperm in the ejaculate after clomiphene citrate therapy, semen outcomes were stratified by patient age, testicular volume, serum FSH, final clomiphene citrate dose after titration, and duration of clomiphene citrate treatment (Table 3). No statistically significant difference between patients manifesting sperm in the semen analysis was noted by comparing patient

Table 4. Seminal outcomes by initial biopsy pattern*

Initial Biopsy Result	Azoospermic	Sperm in Ejaculate
Maturation arrest	11 (61.1%)	7 (38.9%)
Level of spermatids	2 (22.2%)	7 (77.8%)
Level of spermatocytes	9 (100%)	0 (0%)
Hypospermatogenesis	4 (16.7%)	20 (83.3%)
Total	15 (35.7%)	27 (64.3%)

* Chi square, $P < .0005$; Cramer phi, $r = 0.70$.

age, testicular volume, or serum FSH. Interestingly, although no statistically significant difference was noted in outcomes when final clomiphene citrate dose was compared, a longer duration of clomiphene citrate therapy was associated with a greater likelihood of finding sperm in the ejaculate ($P = .010$). This finding argues that as long as the dose of clomiphene is titrated to a serum testosterone between 600 ng/dL and 800 ng/dL, the final dose is not as consequential as the duration of therapy, with longer treatment durations favored.

Semen outcomes after clomiphene citrate treatment in all patients are delineated in Table 4 by initial testis biopsy result. The finding of sperm in the ejaculate subsequent to clomiphene citrate was highly correlated to initial testis histologic pattern (chi square, $P < .0005$; Cramer phi, $r = .70$). In cases of hypospermatogenesis, 83.3% of patients had sperm in their ejaculate after clomiphene citrate treatment, while 16.7% remained azoospermic. However, although 77.8% of patients with maturation arrest at the level of spermatids manifested sperm in their ejaculate after treatment, no patient with maturation arrest at the level of spermatocytes did so.

Discussion

Coupled with surgical retrieval of testicular sperm, intracytoplasmic injection represents the only currently avail-

Table 3. Semen outcomes after treatment stratified by patient age, testicular volume, serum FSH, final clomiphene citrate dose after titration, and duration of clomiphene citrate treatment

Clinical Feature	Semen Outcomes After Clomiphene Citrate Treatment	Mean \pm SE	P
Age (y)	Patients remaining azoospermic	32.00 \pm 4.43	.081
	Patients demonstrating sperm	29.09 \pm 4.55	
Right testis size (mL)	Patients remaining azoospermic	17.25 \pm 3.85	.322
	Patients demonstrating sperm	18.59 \pm 3.66	
Left testis size (mL)	Patients remaining azoospermic	17.13 \pm 3.23	.404
	Patients demonstrating sperm	18.15 \pm 3.41	
FSH (mIU/mL)	Patients remaining azoospermic	9.75 \pm 13.27	.364
	Patients demonstrating sperm	6.94 \pm 4.26	
Clomiphene citrate dose (mg/d)	Patients remaining azoospermic	31.25 \pm 14.60	.119
	Patients demonstrating sperm	20.46 \pm 20.62	
Clomiphene citrate duration (mo)	Patients remaining azoospermic	3.81 \pm 0.69	.010
	Patients demonstrating sperm	5.90 \pm 2.22	

able treatment for patients with nonobstructive azoospermia (Devroey et al, 1995; Silber et al, 1995). Surgeons may use one of a number of specific techniques to retrieve testicular spermatozoa including open or percutaneous biopsy, fine-needle aspiration, and microsurgical extraction (Friedler et al, 1997; Sheynkin et al, 1998; Sigman and Jarow, 2003). Many surgeons consider fine-needle biopsy to be the least invasive method of sperm retrieval. However, with the exception of the systematic multiple aspiration approach described by Turek et al (1999), controlled studies indicate that in patients with nonobstructive azoospermia who harbor scant testicular sperm, needle biopsy is much less likely to identify rare foci of spermatogenesis than would an open procedure (Rosenlund et al, 1998).

In this study, we investigated the use of clomiphene citrate to stimulate the presence of ejaculated sperm in azoospermic men and, in those cases of persistent azoospermia after treatment, to improve the probability of obtaining sperm by testicular extraction. The rationale for this use of clomiphene citrate was based on its effect in increasing endogenous gonadotropin-releasing hormone secretion from the hypothalamus and gonadotropin-hormone secretion from the pituitary, thus increasing intratesticular testosterone concentration, a fundamental requirement for spermatogenesis. In a similar manner, many investigators have described the use of clomiphene citrate in cases of idiopathic male infertility (Check et al, 1989).

In 1993, J.W. Akin reported the case of an infertile male with a deletion within the androgen receptor gene who presented with nonobstructive azoospermia and, after treatment with clomiphene citrate treatment, was found to have sperm within his ejaculate. However, ICSI was not available at that time and the ultimate goal of pregnancy was not achieved, nor were there enough sperm present to warrant an IVF attempt (Akin, 1993). In the current study of patients with nonobstructive azoospermia, use of clomiphene citrate allowed for both the appearance of sperm in the ejaculate and, in those patients who remained azoospermic after therapy, successful sperm retrieval for ICSI. After duration of therapy ranging from 3 to 9 months, 64.3% of patients manifested sperm in their ejaculate. Sperm concentrations in these patients ranged from 1 to 16 million sperm/mL, with a mean sperm density of 3.8 million/mL and the partner of 1 patient achieving a spontaneous pregnancy. Ejaculates of the remainder of these patients contained sufficient sperm for either IVF or ICSI. In the 35.7% of patients who remained azoospermic after therapy, enough sperm for ICSI was retrieved by testicular sperm extraction in all of them.

A literature review revealed an overall success rate in obtaining sperm in men with nonobstructive azoospermia by conventional TESE to be 16.7% and, when a microsurgical approach was employed, 44.6% (Silber, 2000;

Okada et al, 2002). That clomiphene citrate use in the current study resulted in ejaculated sperm in 64.3% of cases and sperm was successfully retrieved in all cases who remained azoospermic is likely due, at least in part, to the exclusion of cases of Sertoli cell-only syndrome, the normal mean testis size, and relatively lower FSH. Our results are, thus, consistent with Okada et al (2002) who reported the retrieval of spermatozoa by testicular microdissection in 100% of patients with hypospermatogenesis and 75% of patients with maturation arrest. Because of the relatively small number of our patients, we are aware that further prospective randomized studies are needed to assess the role of clomiphene in the treatment of azoospermic patients.

In the current study, treatment with clomiphene citrate resulted in significantly improved outcomes in cases of hypospermatogenesis and maturation arrest at the level of spermatids when compared with cases of maturation arrest at the level of spermatocytes. After treatment, 83.3% of patients with hypospermatogenesis and 77.8% of patients with maturation arrest at the level of spermatids manifested sperm in their ejaculate, compared with no patient with maturation arrest at the level of spermatocytes. In addition to the outcome of ejaculated sperm in the current study, a significant difference was observed after therapy in patients in whom testis biopsy results were available before and after therapy in patterns of spermatogenesis toward those with higher probability to yield mature sperm on surgical extraction. This was demonstrated by the findings of Tournaye et al (1996) who noted a sperm recovery rate of 100% in cases of hypospermatogenesis, 84% in maturation arrest at the level of spermatids, and 76% in maturation arrest at the level of spermatocytes.

In a literature review of the use of clomiphene citrate in the treatment of idiopathic oligospermia, differing doses and protocols are reported with the most common being 50 mg daily, 50 mg every other day, 25 mg every other day, or 25 mg daily for 25 days followed by a drug-free interval of 5 days (Empeaire et al, 1979; Ronnberg, 1980; Abel et al, 1982; Micic and Dotlic, 1985; Pusch et al, 1986; Homonnai et al, 1988; Sokol et al, 1988). Outcomes are variable. Some studies reported improvement in sperm count after clomiphene treatment (Empeaire et al, 1979; Ronnberg, 1980; Micic and Dotlic, 1985; Pusch et al, 1986; Homonnai et al, 1988). Other studies failed to identify any efficacy of clomiphene over placebo (Abel et al, 1982; Micic and Dotlic, 1985; Sokol et al, 1988). In the current study, no significant difference was noted in outcomes relative to the dose of clomiphene citrate if the dose was titrated to a serum level of testosterone between 600 ng/dL and 800 ng/dL.

Advantages of clomiphene citrate therapy prior to consideration of testicular sperm extraction include that in

those patients with sufficient sperm manifest in the ejaculate for ICSI (64.3% in the current study) surgery is obviated, that the likelihood of obtaining sperm is increased if surgical extraction is necessary, and that the therapy is relatively inexpensive compared with surgery. A disadvantage of instituting clomiphene citrate therapy prior to testicular extraction is the delay prior to ICSI (ranging from 3 to 9 months in the current study, with longer therapeutic durations favoring improved outcomes), especially concerning partners of advanced maternal age. As with any protocol, treatment regimens are always the individual choice of physician and informed patient.

Conclusion

The current study offers a rational basis for the use of clomiphene citrate therapy in cases of nonobstructive azoospermia prior to surgical extraction of sperm for ICSI. Based on our findings, in approximately two thirds of patients without Sertoli cell-only syndrome, the physician may expect sufficient sperm in the ejaculate for use in artificial reproductive techniques, obviating the need for scrotal surgery. In the one third of patients for whom testicular sperm extraction is required, a greater likelihood of obtaining sperm suitable for ICSI is achieved.

References

- Abel BJ, Carswell G, Elton R. Randomised trial of clomiphene citrate treatment and vitamin C for male infertility. *Br J Urol.* 1982;54:780–784.
- Akin JW. The use of clomiphene citrate in the treatment of azoospermia secondary to incomplete androgen resistance. *Fertil Steril.* 1993;59:223–227.
- Bourne H, Watkins W, Speirs A. Pregnancies after intracytoplasmic sperm injection collected by fine needle biopsy of the testis. *Fertil Steril.* 1995;64:433–437.
- Check JH, Chase JS, Nowroozi K. Empirical therapy of the male with clomiphene in couples with unexplained infertility. *Int J Fertil.* 1989;34:120–122.
- Devroey P, Liu J, Nagy Z, Goossens A, Tournaye H, Camus M. Pregnancies after testicular sperm extraction and intracytoplasmic sperm injection in non-obstructive azoospermia. *Hum Reprod.* 1995;10:1457–1463.
- Emperaire JC, Riviere J, Ruffie A, Audebert AJ. Clomiphene test and clomiphene therapy in idiopathic male infertility. *Arch Androl.* 1979;2:223–231.
- Friedler S, Raziel A, Straussburger 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;12:1488–1494.
- Homonnai ZT, Yavetz H, Yogev L, Rotem R, Paz GF. Clomiphene citrate treatment in oligozoospermia: comparison between two regimens of low-dose treatment. *Fertil Steril.* 1988;50:801–805.
- Micic S, Dotlic R. Evaluation of sperm parameters in clinical trial with clomiphene citrate of oligospermic men. *J Urol.* 1985;133:221–222.
- Okada H, Dobashi M, Yamazaki T, Hara I, Fujisawa M, Arakawa S, Kamidono S. Conventional versus microdissection testicular sperm extraction for non-obstructive azoospermia. *J Urol.* 2002;168:1063–1067.
- Plas E, Riedl CR, Engelhardt PF, Muhlbauer H, Pfluger H. Unilateral or bilateral testicular biopsy in the era of intracytoplasmic sperm injection. *J Urol.* 1999;162:2010–2013.
- Pusch HH, Haas J, Purstner P. Results of low-dose treatment of oligozoospermia with clomiphene citrate. *Andrologia.* 1986;18:561–566.
- Ronnberg L. The effect of clomiphene treatment on different sperm parameters in men with idiopathic oligozoospermia. *Andrologia.* 1980;12:261–265.
- Rosenlund B, Kvist U, Ploen L, Rozell BL, Sjoblom P, Hillensjo T. A comparison between open and percutaneous needle biopsies in men with azoospermia. *Hum Reprod.* 1998;13:1266–1270.
- Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod.* 1999;14:131–135.
- Sheynkin YR, Ye Z, Menendez S, Liotta D, Veeck LL, Schlegel P. Controlled comparison of percutaneous and microsurgical sperm retrieval in men with obstructive azoospermia. *Hum Reprod.* 1998;13:3086–3091.
- Sigman M, Jarow JP. Male infertility. In: Walsh PC, Retik AB, Vaughan ED, Wein AJ, eds. *Campbell's Urology.* 8th ed. Philadelphia: Saunders Co; 2003;43:1475–1531.
- Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. *Hum Reprod.* 2000;15:2278–2282.
- Silber SJ, Van Steirteghem AC, Devroey P. Sertoli cell only revisited. *Hum Reprod.* 1995;10:1031–1036.
- Sokol RZ, Petersen G, Steiner BS. A controlled comparison of the efficacy of clomiphene citrate in male infertility. *Fertil Steril.* 1988;49:865–870.
- 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;11:2567–2572.
- Turek PJ, Givens CR, Schriock ED. Testis sperm extraction and intracytoplasmic sperm injection guided by prior fine-needle aspiration mapping in patients with non-obstructive azoospermia. *Fertil Steril.* 1999;71:552–557.
- Turek PJ, Ljung BM, Cha I, Conaghan J. Diagnostic findings from testis fine needle aspiration mapping in obstructed and nonobstructed azoospermic men. *J Urol.* 2000;163:1709–1716.
- Weiss DB, Gottschalk-Sabag S, Bar-On E, Zukerman Z. Are testes in oligo/azoospermia homogenous or heterogenous? *Harefuah.* 1998;134:97–101.