Original Article

Microdissection testicular sperm extraction: Overall results and impact of preoperative testosterone level on sperm retrieval rate in patients with nonobstructive azoospermia

Shahbaz Mehmood, Shima Aldaweesh, Noor Nabi Junejo, Waleed Mohamed Altaweel, Said Abdulghani Kattan, Naif Alhathal[1]

King Faisal Specialist Hospital and Research Center, [1]Department of Urology, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia

Abstract

Objective: The main objective is to review the overall result and impact of preoperative testosterone level on sperm retrieval rate (SRR) by microdissection testicular sperm extraction (micro-TESE) in patients with nonobstructive azoospermia (NOA).

Materials and Methods: We retrospectively reviewed the files of patients who underwent micro-TESE for NOA from August 2013 to December 2014. All patients were evaluated with history, physical examination, and hormonal assessment. Patients who had previous micro-TESE, obstructive azoospermia, or who took hormone therapy were excluded from the study. Patients were classified into two groups. Group A included patients who had low testosterone (<10 nmol/L), and Group B included patients with normal testosterone (>10 nmol/L). The primary endpoint was to review the overall results of the procedure and the impact of preoperative testosterone level on sperm retrieval.

Results: A total of 264 patients with NOA underwent micro-TESE. Group A included 133 patients with low testosterone (<10 nmol/l) with a median age of 36 ± 6.59 years, and Group B included 131 patients with normal testosterone (>10 nmol/L) with a median age of 33 ± 7.88 years ($P = 0.1350$). There was no significant difference in follicle-stimulating hormone ($P = 0.2467$), luteinizing hormone ($P = 0.1078$), prolactin ($P = 0.5619$), and testicular volume ($P = 0.4052$), whereas a significant difference was found in testosterone level ($P = 0.0001$) in both groups. Overall, sperm were successfully retrieved in 48.8% of men. SRR in Group B was significantly higher (57.25%) than that in Group A (40.60%) ($P = 0.0068$). SRR in patients with Sertoli-cell-only pathology was 30.35%, hypospermatogenesis was 89.74%, and maturation arrest was 32.43%.

Conclusion: Micro-TESE is a successful and safe procedure in NOA patients with a poor prognosis. Preoperative testosterone level has a significant impact in the SRR by micro-TESE.

Keywords: Microdissection testicular sperm extraction, nonobstructive azoospermia, Sertoli-cell-only syndrome, sperm retrieval rate

Address for correspondence: Dr. Naif Alhathal, Department of Urology, King Faisal Specialist Hospital and Research Center, PO Box 3354, Riyadh 11211, Kingdom of Saudi Arabia.
E-mail: alnaif@yahoo.com
Dr. Shahbaz Mehmood, King Faisal Specialist Hospital and Research Center, Riyadh, Kingdom of Saudi Arabia.
E-mail: shahbazmalik49@gmail.com
Received: 12.03.2018, **Accepted:** 20.11.2018

Access this article online	
Quick Response Code:	Website: www.urologyannals.com
	DOI: 10.4103/UA.UA_36_18

How to cite this article: Mehmood S, Aldaweesh S, Junejo NN, Altaweel WM, Kattan SA, Alhathal N. Microdissection testicular sperm extraction: Overall results and impact of preoperative testosterone level on sperm retrieval rate in patients with nonobstructive azoospermia. Urol Ann 2019;11:287-93.

INTRODUCTION

Azoospermia is found in approximately 1% of the population and in up to 15% of infertile men. Nonobstructive azoospermia (NOA), which is diagnosed in about 60% of azoospermic men, is detected clinically in men having small-volume testicles, raised follicle-stimulating hormone (FSH), and azoospermia.[1] NOA patients usually seek testicular sperm extraction (TESE) or micro-TESE procedure followed by intracytoplasmic sperm injection (ICSI).[2] Multiple sperm retrieval techniques have been reported in the literature, such as fine-needle aspiration, percutaneous testis biopsy, and open testicular biopsy and micro-TESE. Micro-TESE has become a procedure of choice due to its minimal invasiveness, high safety, and high sperm retrieval rate (SRR).[3] Several studies have suggested that the micro-TESE technique should be the standard technique for treating men with NOA.[4] Several studies have reported that SRR with micro-TESE in NOA patients is between 40% and 60%.[5-7]

Despite micro-TESE's high success rate, preoperative patient counseling regarding the probability of SRR has remained a challenge. It is very important to predict the success of sperm retrieval using a noninvasive method before a definitive procedure. An unsuccessful micro-TESE and ICSI procedure could lead to emotional and financial crises. Hypogonadism, defined as serum testosterone level <10 nmol/L, is frequently observed in 45%–47% of men with NOA who present for treatment in a fertility clinic.[8,9] Testosterone's exact role in spermatogenesis is still controversial. Jarow *et al*. found that a sufficient level of intratesticular testosterone (ITT) is crucial in spermatogenesis.[10]

Further controversy exists regarding the efficacy of preoperative optimization of hormones in patients with NOA having hypogonadism.[11] However, hormonal optimization increases ITT level, and evidence shows that an increase in ITT level may improve sperm production. Hussain *et al*.[12] reported that preoperative optimization of FSH and testosterone with clomiphene citrate or human chorionic gonadotropin increased the likelihood of sperm in ejaculate and increased SRR by micro-TESE in patients with NOA. On the other hand, men with NOA associated with hypogonadism often respond to hormonal therapy, leading to an increase in testosterone level. Neither baseline testosterone nor increase due to response to hormone therapy affects SRR, clinical pregnancy, or live birth rates.[13]

This study had two goals:

1. To investigate the overall result in terms of safety and SRR with micro-TESE
2. To determine the impact of preoperative low or normal testosterone level on SRR by micro-TESE in patients with NOA.

None of these patients were given hormone therapy. Based on the results of this retrospective study, we have started conducting a randomized controlled trial in our prospective study on the outcome of micro-TESE in terms of SRR after preoperative optimization of testosterone and FSH in hypogonadal men with NOA.

MATERIALS AND METHODS

We conducted a retrospective chart review study of all NOA patients who underwent micro-TESE by a single surgeon (NH) at our tertiary care unit between August 2013 and December 2014. The study research protocol was approved by the Office of Research Affairs in King Faisal Specialist Hospital and Research Centre. All patients were confirmed to have azoospermia using at least two centrifuged ejaculate semen analyses according to the World Health Organization 2004 criteria.[14]

NOA was confirmed by history, physical examination, and radiological and hormonal analysis. Patients who had previous micro-TESE or took hormone therapy, men with chromosomal abnormalities, men having a history of disorder such as cryptorchidism, and those having chronic diseases were excluded from the study. Men with obstructive azoospermia were also excluded. Patients were divided into two groups on the basis of serum testosterone. Group A included patients who had low testosterone (<10 nmol/L), and Group B included patients with normal testosterone (>10 nmol/L). A relevant patient history was recorded on preformed pro forma, including patient's age; duration of infertility; and history of undescended testes, mumps orchitis, radiotherapy, chemotherapy, previous urinary tract infection, surgical procedure, or exposure to gonadotoxin. Physical examination included secondary sexual characteristic, testicular size and consistency, the presence of Vas, and varicocele.

Hormonal analysis of FSH, luteinizing hormone (LH), testosterone, prolactin, TSH, and E2 levels was also completed. The reference range of FSH was 1.5–15 mIU/ml, LH was 1.7–8.6 mIU/ml, prolactin was 4.1–18.4 mIU/ml, and testosterone was 10–27 nmol/L. All hormone levels were noted without any hormonal medical treatment within 2 months before micro-TESE. Karyotyping analysis along with Y chromosome microdeletion performed in highly selected cases was also documented. Ultrasound scrotum findings were also noted by confirming testicular volume

and the presence of varicocele. We defined the success of sperm retrieval as getting at least one sperm which was suitable for ICSI. All patients who had a varicocele underwent varicocelectomy minimum of 6 months before proceeding to micro-TESE.

Microdissection testicular sperm extraction technique

All patients with NOA underwent micro-TESE under general anesthesia. Median raphe incision was made in the scrotum, tunica vaginalis opened, and single testis delivered through the incision. The other testis was delivered if no sperm was found in the first. Tunica albuginea was widely incised in the equatorial plane as originally described by Shlegel[15] under a ×20 magnification surgical operating microscope to avoid vascular injury.[16] Microdissection was then performed to expose seminiferous tubules, and multiple tiny pieces of opaque dilated tubules were taken. These tubules were sent to an embryologist to analyze sperm. Microdissection was done deep in testicular parenchyma following anatomic plane between blood vessels and tubules to take as much dilated opaque tubules for acquisition of sperm, preserve blood supply, and minimize tissues injury. Suitable sperm were cryopreserved for future ICSI.

A separate testicular sample was also taken, placed in Bouin's solution, and sent for histopathology. Based on the most predominant pattern of histology, samples were classified into normal spermatogenesis, hypospermatogenesis (decrease in the number of normal spermatogenetic cells), maturation arrest (MA) (absence of mature stages of spermatogenesis), and Sertoli-cell-only (SCO) pattern (i.e., the absence of germ cells in seminiferous tubules). The surgeon was promptly informed about the microscopic examination of specimen. The albuginea was closed with nonabsorbable sutures. Tunica vaginalis was closed with continuous 5/0 absorbable sutures. Then, dartos muscle was closed with interrupted absorbable suture. Finally, the skin was closed with 5/0 Vicryl Rapide suture and fluffy type dressing, and scrotal support was applied. The procedures were carried out on the contralateral side if no sperm were found on initial microscopic examination. Before discharge on the same day, patients were examined to rule out any bleeding or scrotal hematoma. Bed rest and application of ice packs over the scrotum were advised for the first 48 h. Patients were informed to remove scrotal dressing after 24 h and were encouraged to take warm showers and to wash the incision area with soap and water after 24 h postoperatively. Patients were prescribed analgesic for 3–5 days for pain. Patients were advised to abstain from heavy lifting, physical exertion, and sexual activities for 10 days. All patients were advised to report any adverse sign and symptoms, such as fever, persistent pain and swelling, bleeding, or excessive fluid leaking from the wound.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences version 20 (Armonk, NY, IBM Corp). The unpaired Student's *t*-test and Chi-square test were applied to compare factors between men with successful and failed sperm retrieval at micro-TESE in two groups. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 264 patients underwent micro-TESE by a single surgeon. Group A included 133 patients with median age of 36 ± 6.59 years, and Group B included 131 patients with median age of 33 ± 7.88 years. The median volume of testes on ultrasonography in Group A was 4.6 ± 4.4 ml, and in Group B, it was 4.8 ± 5.2 ml. The median FSH was 15.5 ± 17 and 13.6 ± 14.0 mIU/L ($P = 0.2467$) in Groups A and B, respectively, whereas the median LH was 9.5 ± 7.5 and 9.0 ± 5.8 mIU/L ($P = 0.1078$), respectively. However, the median serum testosterone level in Group A was 6.8 ± 2.6 nmol/L, and in Group B, it was 13.2 ± 4.2 nmol/L ($P = 0.0001$). The median serum prolactin level in Group A was 8.0 ± 8.7, and in Group B, it was 9.3 ± 5.9 mIU/L ($P = 0.5619$). The overall patient biodata is shown in Table 1.

Serum testosterone level 10 nmol/L done by immunoassay technique in King Faisal Specialist Hospital laboratory was taken as the lower normal. The overall successful SRR in our study was 48.8%. SRR in Group A was 57.25% ($n = 75$) and Group B was 40.60% ($n = 54$). We noticed a significant difference in SRR in Group B ($P = 0.0068$). These findings showed a significant positive SRR in patients with preoperative normal testosterone level, and there is a positive correlation in SRR and testosterone level ($P < 0.0068$), as shown in Table 2.

When we categorized the patients on the basis of histopathology, we found the following results: 112 (42.42%) patients of 264 had a Sertoli-cell-only pattern in histopathology, and 34 (30.35%) of 112 patients had successful sperm retrieval; 78 (29.54%) patients had a hypospermatogenesis in their histopathology report, of which 70 (89.74%) patients had sperm retrieved during their procedures; and 74 (28.03%) patients were found to have an MA at different stages of spermatogenesis, and 25 (33.78%) patients succeeded in having sperm for ICSI in this group. Previous studies have mentioned that

histopathology is a good predictor for retrieval of sperm, and hypospermatogenesis has a good prognosis in retrieving sperm among three categories, as shown in Table 3.

In Group A, testicular histopathology has been positive sperm retrieval by 40.6% among three categories. In hypospermatogenesis group, retrieval of sperm rate was substantially higher by 18% of 40.6%, whereas it was closer by 11% and 12% in MA and SCO syndrome (SCOS), respectively. In addition to hypospermatogenesis in Group B, the positive sperm rate was higher than MA and SCOS with 35.11% out of 57.25% [Table 4].

When we analyzed our result using Wilcoxon in Group A, we did not find any significant difference between preoperative low testosterone and SRR by micro-TESE ($P = 0.2824$) as shown in Graph 1a. However, in Group B, there was a significant correlation of preoperative normal testosterone and SRR by micro-TESE ($P = 0.0002$) as in Graph 1b.

Regarding postoperative complication, we have not found any patient who reported any significant major postoperative complications, such as bleeding, hematoma formation, infection, or chronic scrotal pain.

Table 1: Biodata of all patients with nonobstructive azoospermia undergoing microdissection testicular sperm extraction

Variable	Number of patients	Median
Age (years)	263	35±7.0
TSH (mIU/L)	262	2.0±3.4
Prolactin (mIU/L)	263	9.0±16.9
FSH (mIU/L)	264	14.8±15.7
LH (mIU/L)	264	9.0±6.6
Testosterone (nmol/L)	264	10.0±6.0
Testes volume (cm^2)	264	8.20±3.8

TSH: Thyroid-stimulating hormone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone

Table 2: Biodata of patients in Groups A and B

Variables	Group A	Group B	*P*
Age (years)	36±6.59	33±7.88	0.1350
FSH (mIU/L)	15.5±17	13.6±14.0	0.2467
LH (mIU/L)	9.5±7.5	9.0±5.8	0.1078
Testosterone (nmol/L)	6.8±2.6	13.2±4.2	0.0001
Prolactin (mIU/L)	8.0±8.7	9.3±5.9	0.5619
Testes volume (cm^2)	8.2±2.9	8.2±3.8	0.4052
Micro-TESE success rate (%)	40.60	57.25	0.0068

FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, Micro-TESE: Microdissection testicular sperm extraction

Table 3: Outcome of microdissection testicular sperm extraction in terms of histopathology and testosterone level

Variable	Successful micro-TESE, *n* (%)	Failed micro-TESE, *n* (%)
Sertoli-cell-only syndrome	34 (30.35)	69.64
Hypo	70 (89.74)	8 (10.25)
MA	25 (32.43)	49 (67.56)
Overall SRR	131 (48.86)	133 (51.14)
Group A SRR	54 (40.6)	79 (59.4)
Group B SRR	75 (57.25)	56 (42.75)

SRR: Sperm retrieval rate, Micro-TESE: Microdissection testicular sperm extraction, MA: Maturation arrest, Hypo: Hypospermatogenesis

DISCUSSION

NOA is one of the challenges andrologists deal with in regard to male infertility. In men with NOA, scattered regions of spermatogenesis within the testes are not unknown. They have highly dysfunctional testes, and although the overall picture is of testicular failure, rare foci of sperm production may exist in up to 60% of these individuals.[4,13] Their only hope to father a biological child is to have these focal regions of spermatogenesis located by various types of sperm retrieval procedures and undergoing sperm harvesting for assisted reproduction. Among all sperm retrieval techniques, micro-TESE has a higher SRR with fewer postoperative complications and negative effects on testicular function than conventional TESE.[3,17]

In our setup, we found micro-TESE to be a promising method of sperm retrieval for ICSI in our infertile men with NOA. An overall SRR of 48.86% was obtained by micro-TESE. Our center is a referral center from all over the Kingdom. Patients had mostly undergone some sort of treatment or procedure for sperm retrieval before being referred to our center. Eighty-nine (33.76%) patients had already undergone sperm retrieval surgeries other than micro-TESE. In various studies, success rates of sperm retrieval in NOA patients with micro-TESE range from 25% to 60%,[18-22] and it has fewer complications than other open techniques, in which taking multiple biopsies of a large amount of testicular tissue may compromise future retrieval attempts and may result in temporary or permanent side effects of hypogonadism.[23]

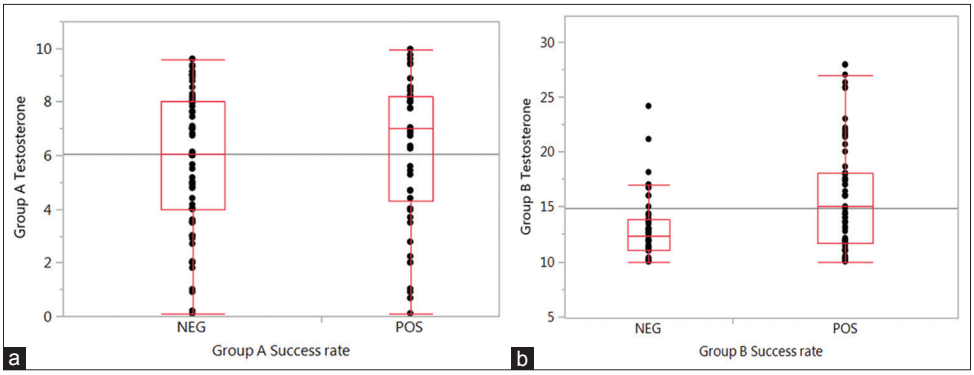
In contrast, micro-TESE in which with the aid of optical magnification of the area of sperm production within the testis is identified based on the size and appearance of seminiferous tubules.[24] It has been advocated that micro-TESE is superior to other methods of sperm retrieval such as TESE and TESA, yielding greater success in obtaining sperm while minimizing tissue removal that ultimately facilitates sperm processing and alleviates testicular damage.[7,24-26]

We have not found any case with postmicro-TESE major complications of bleeding, hematoma formation, and serious infection in our study, which have been seen in various studies. In micro-TESE, the excision of testicular tissue is limited and focused on white opaque

Table 4: Success rate in testicular histopathology in Groups A and B

Histological pattern, *n* (%)	Group A		Group B		*P*
	Positive	Negative	Positive	Negative	
Hypo	24 (18.05)	3 (2.26)	46 (35.11)	5 (3.82)	
MA	14 (10.53)	28 (21.05)	11 (8.40)	21 (16.03)	
SCO	16 (12.03)	48 (36.09)	18 (13.74)	30 (13.74)	
Total	54 (40.60)	79 (59.40)	75 (57.25)	56 (42.75)	<0.0001*

Hypo: Hypospermatogenesis, MA: Maturation arrest, SCO: Sertoli-cell-only pattern



Graph 1: (a) One-way analysis (Wilcoxon) of Group A testosterone by Group A success rate. *P* = 0.2824. Negative failed to retrieve sperm, positive successful sperm retrieval, (b) One-way analysis (Wilcoxon) of Group B testosterone by Group B success rate. *P* = 0.0002. Negative failed to retrieve sperm, positive successful sperm retrieval

seminiferous tubules. Moreover, incisions can be made in avascular regions of the tunica albuginea; subtunical vessels can be better appreciated and avoided with the use of a microscope, thus minimizing the incidence of postoperative testicular damage.[27] A comparative study based on testicular ultrasonography within 6 months of TESE showed that micro-TESE was less invasive than conventional TESE.[24] In a large study of 435 NOA patients in whom micro-TESE or conventional TESE was performed, fewer acute and chronic changes in the microdissection group than in the conventional group were noted on postoperative ultrasound.[19] Donoso *et al*.[19] found that 80% of patients had structural changes or intratesticular hematoma on postoperative ultrasound on men who underwent TESE compared to Amer *et al*., who found 30% structural changes and 3.3% fibrosis in micro-TESE patients.[24]

ITT is essential in almost every aspect of spermatogenesis.

A higher percentage of ITT than serum is required for normal spermatogenesis.[28] The presence of testosterone in the intratesticular environment is necessary for normal spermatogenesis in men. The recognition of the high ITT concentrations relative to serum testosterone concentrations has led to the speculation that ITT concentrations must be relatively high to support quantitatively and qualitatively normal spermatogenesis in men.[29] We found a significant positive correlation among patients with normal preoperative serum testosterone and SRR in our study. In Group B, 57.25% of patients who had normal testosterone >10 nmol/L underwent successful retrieval of sperm compared to patients in Group A who had low pre-micro-TESE testosterone <10 nmol/L, in which only 40.60% were able to have their sperm successfully retrieved (*P* = 0.0068).

It can be assumed that optimizing serum testosterone level in hypogonadal men with NOA can increase the success of sperm retrieval with micro-TESE, as suggested in various studies. Shiraishi *et al*.[30] found men with NOA who failed first micro-TESE sperm retrieval and treatment with human chorionic gonadotropin-based hormonal therapy resulted in the successful retrieval of spermatozoa during the second micro-TESE in 20% of cases. Histological data have shown that men with hypospermatogenesis or late MA are likely to respond to hormonal treatment. Even auther further reported that histologic examination revealed that the men who respond to hormonal therapy improved from MA to hypospermatogenesis. In contrast, a group of patients did not respond, did not show any changes in the seminiferous tubules, but, instead, showed changes in the interstitial tissue, including the thickness of basement membrane and interstitial fibrosis. Matthiesson *et al*.[31] found that spermatogenesis, a process requiring high ITT levels, was stimulated by the hormonal therapy in men with hypogonadal NOA.

To date, there are still no absolute preoperative predictive factors for successful SR in NOA. FSH, testosterone

levels, and testes volume reflect global testicular function and not the presence of a site of normal sperm production within a dysfunctional testis.[32,33] Testicular histopathology results, in contrast, confer better prognostic value compared with the aforesaid marker. When examining the histology of sperm retrieval specimens, the pattern seen can often be suggestive of sperm production.

In our study, SRR in patients with SCOS was 30.35%, MA rate was 32.43%, and the rate of hypospermatogenesis was 89.74%. We have more cases of SCOS (n = 112) than hypospermatogenesis (n = 74) and MA (n = 78), which is why the overall success rate of sperm retrieval with micro-TESE is relatively low. SRRs by micro-TESE are significantly higher in hypospermatogenesis (93%) than with MA (64%) and SCOS (20%).[34] Nonetheless, successful retrievals are reported even in the more adverse histopathology pattern of SCOS, as shown in the aforementioned study. This observation indicates that sperm production is distributed in a heterogeneous pattern within the testis, and histologic assessment of a single testicular fragment is limited in its ability to determine the presence of rare foci of sperm production in NOA.[4,35]

Abdel Raheem *et al*. observed in their study that histopathology in NOA patients is the only strong predictor of SRR with micro-TESE. They further suggest doing preoperative diagnostic testicular biopsy to predict the likelihood of sperm on micro-TESE. The finding of mature spermatozoa upon examination of the histopathologic specimen provides the greatest positive predictor for success of sperm retrieval.[36] Another study by Su *et al*. showed that histopathologic specimen alone has been shown to be the strongest single predictor of successful sperm retrieval with conventional techniques.[37] Thus, testicular biopsies, when positive, provide an accurate predictor of successful sperm retrieval. It is of limited value in most of the cases when it is negative.[38]

Our study has several limitations; one of them was its retrospective nature. Potential bias and reporting errors are the main risks of any retrospective study. We ensured that many of these ambiguities were mostly avoided during data collection. Although hormonal assays were done in the same laboratory, timing of testosterone immunoassay and laboratory errors cannot be ruled out. Prognostic value of testosterone had not evaluated for SRR after micro-TESE. We just compared SRR in two groups of patients according to the presence of a low or a normal preoperative total testosterone level. The result of this study might serve as a counseling tool for patients and doctors for hormone therapy before micro-TESE. A prospective randomized controlled trial has already been started in our institution to adequately evaluate whether optimization of FSH and testosterone before micro-TESE would improve SRRs in patients with NOA.

CONCLUSION

Micro-TESE is a successful and safe technique in patients with NOA with a poor prognosis. Preoperative testosterone level has a significant impact on SRR with micro-TESE. On the findings of this study, we are conducting a randomized controlled trial of our prospective study on the outcome of micro-TESE in terms of SRR after preoperative optimization of testosterone and FSH in hypogonadal NOA patients.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Jarow JP, Espeland MA, Lipshultz LI. Evaluation of the azoospermic patient. J Urol 1989;142:62-5.
2. Silber SJ, van Steirteghem A, Nagy Z, Liu J, Tournaye H, Devroey P. Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. Fertil Steril 1996;66:110-7.
3. Janosek-Albright KJ, Schlegel PN, Dabaja AA. Testis sperm extraction. Asian J Urol 2015;2:79-84.
4. Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod 2000;15:2278-84.
5. Schlegel PN. Testicular sperm extraction: Microdissection improves sperm yield with minimal tissue excision. Hum Reprod 1999;14:131-5.
6. Schlegel PN. Nonobstructive azoospermia: A revolutionary surgical approach and results. Semin Reprod Med 2009;27:165-70.
7. Tsujimura A. Microdissection testicular sperm extraction: Prediction, outcome, and complications. Int J Urol 2007;14:883-9.
8. Sussman EM, Chudnovsky A, Niederberger CS. Hormonal evaluation of the infertile male: Has it evolved? Urol Clin North Am 2008;35:147-55, vii.
9. Bobjer J, Naumovska M, Giwercman YL, Giwercman A. High prevalence of androgen deficiency and abnormal lipid profile in infertile men with non-obstructive azoospermia. Int J Androl 2012;35:688-94.
10. Jarow JP, Zirkin BR. The androgen microenvironment of the human testis and hormonal control of spermatogenesis. Ann N Y Acad Sci 2005;1061:208-20.
11. Kim HH, Schlegel PN. Endocrine manipulation in male infertility. Urol Clin North Am 2008;35:303-18, x.
12. Hussein A, Ozgok Y, Ross L, Rao P, Niederberger C. Optimization of spermatogenesis-regulating hormones in patients with non-obstructive azoospermia and its impact on sperm retrieval: A multicentre study. BJU Int 2013;111:E110-4.

13. Reifsnyder JE, Ramasamy R, Husseini J, Schlegel PN. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. J Urol 2012;188:532-6.
14. World Health Organization. Department of reproductive health and research. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: World Health Organization Press; 2010. p. 45-7.
15. Schlegel PN, Li PS. Microdissection TESE: Sperm retrieval in non-obstructive azoospermia. Hum Reprod Update 1998;4:439.
16. Tsujimura A, Matsumiya K, Miyagawa Y, Takao T, Fujita K, Koga M, *et al*. Prediction of successful outcome of microdissection testicular sperm extraction in men with idiopathic nonobstructive azoospermia. J Urol 2004;172:1944-7.
17. Esteves SC, Miyaoka R, Orosz JE, Agarwal A. An update on sperm retrieval techniques for azoospermic males. Clinics (Sao Paulo) 2013;68 Suppl 1:99-110.
18. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. Int Braz J Urol 2011;37:570-83.
19. Donoso P, Tournaye H, Devroey P. Which is the best sperm retrieval technique for non-obstructive azoospermia? A systematic review. Hum Reprod Update 2007;13:539-49.
20. Van Peperstraten A, Proctor ML, Johnson NP, Philipson G. Techniques for surgical retrieval of sperm prior to ICSI for azoospermia. Cochrane Database Syst Rev 2006;3:CD002807.
21. Carpi A, Sabanegh E, Mechanick J. Controversies in the management of nonobstructive azoospermia. Fertil Steril 2009;91:963-70.
22. Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: Which method is to be preferred? Hum Reprod 1999;14 Suppl 1:71-81.
23. Schlegel PN, Su LM. Physiological consequences of testicular sperm extraction. Hum Reprod 1997;12:1688-92.
24. Amer M, Ateyah A, Hany R, Zohdy W. Prospective comparative study between microsurgical and conventional testicular sperm extraction in non-obstructive azoospermia: Follow-up by serial ultrasound examinations. Hum Reprod 2000;15:653-6.
25. El-Haggar S, Mostafa T, Abdel Nasser T, Hany R, Abdel Hadi A. Fine needle aspiration vs. MTESE in non-obstructive azoospermia. Int J Androl 2008;31:595-601.
26. Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. [corrected]. Clinics (Sao Paulo) 2011;66:691-700.
27. Ramasamy R, Yagan N, Schlegel PN. Structural and functional changes to the testis after conventional versus microdissection testicular sperm extraction. Urology 2005;65:1190-4.
28. Coviello AD, Bremner WJ, Matsumoto AM, Herbst KL, Amory JK, Anawalt BD, *et al*. Intratesticular testosterone concentrations comparable with serum levels are not sufficient to maintain normal sperm production in men receiving a hormonal contraceptive regimen. J Androl 2004;25:931-8.
29. McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K, *et al*. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Recent Prog Horm Res 2002;57:149-79.
30. Shiraishi K, Ohmi C, Shimabukuro T, Matsuyama H. Human chorionic gonadotrophin treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia. Hum Reprod 2012;27:331-9.
31. Matthiesson KL, McLachlan RI, O'Donnell L, Frydenberg M, Robertson DM, Stanton PG, *et al*. The relative roles of follicle-stimulating hormone and luteinizing hormone in maintaining spermatogonial maturation and spermiation in normal men. J Clin Endocrinol Metab 2006;91:3962-9.
32. Ramasamy R, Lin K, Gosden LV, Rosenwaks Z, Palermo GD, Schlegel PN, *et al*. High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. Fertil Steril 2009;92:590-3.
33. Esteves SC, Agarwal A. Novel concepts in male infertility. Int Braz J Urol 2011;37:5-15.
34. Practice Committee of the American Society for Reproductive Medicine. Sperm retrieval for obstructive azoospermia. Fertil Steril 2006;86:S115-20.
35. Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN, *et al*. Successful fertility treatment for klinefelter's syndrome. J Urol 2009;182:1108-13.
36. Abdel Raheem A, Garaffa G, Rushwan N, De Luca F, Zacharakis E, Abdel Raheem T, *et al*. Testicular histopathology as a predictor of a positive sperm retrieval in men with non-obstructive azoospermia. BJU Int 2013;111:492-9.
37. Su LM, Palermo GD, Goldstein M, Veeck LL, Rosenwaks Z, Schlegel PN, *et al*. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia: Testicular histology can predict success of sperm retrieval. J Urol 1999;161:112-6.
38. Schoor RA, Elhanbly S, Niederberger CS, Ross LS. The role of testicular biopsy in the modern management of male infertility. J Urol 2002;167:197-200.