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Evaluation of neoadjuvant gonadotropin administration with downregulation by testosterone prior to second time microsurgical testicular sperm extraction: A prospective case–control study

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Abstract

Purpose: The aim of this prospective study was to determine whether there is a beneficial role of combining gonadotropin administration with testosterone downregulation in non-obstructive azoospermia patients prior to a second time microsurgical testicular sperm extraction after a negative one.

Methods: A total of 40 non-obstructive azoospermia men were recruited from a specialized IVF center from 2014 to 2016. Participants were divided equally into two groups: Group A was subjected to testosterone downregulation alone for 1 month and then combined with gonadotropin administration for 3 months prior to second time testicular sperm extraction; Group B (controls) underwent second time microsurgical testicular sperm extraction without prior hormonal therapy.

Results: Mean baseline follicle-stimulating hormone levels of the controls and the cases were 26.9 ± 11.8 and 25.4 ± 8.7, respectively. One month after testosterone downregulation, follicle-stimulating hormone level of the cases was normalized and became 2.4 ± 1.2. There was no statistically significant difference between baseline follicle-stimulating hormone levels of the controls and cases ($p = 0.946$). Remarkably, two cases were positive after downregulation (10%) and no controls were positive at second testicular sperm extraction (0%). There was no statistically significant difference between sperm retrieval after the second microsurgical testicular sperm extraction in the controls and the cases ($p = 0.072$).

Conclusion: Patients who underwent first time testicular sperm extraction with unfavorable outcome due to different techniques may benefit from testosterone downregulation combined with neoadjuvant gonadotropin administration as it had shown positive sperms retrieval in 2 out of the 20 cases, especially those with hypergonadotropic azoospermia.

Keywords

Non-obstructive azoospermia, testosterone downregulation, gonadotropin administration

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Introduction

The incidence of azoospermic males in general population is 1% and in infertile men it ranges from 10% to 20%. Non-obstructive azoospermia (NOA) represents the testicular causes detected by testicular biopsy and has a prevalence range of 40%–60%.[1] Testicular sperm extraction (TESE) gave NOA patients a hope to have a baby.[2] Schlegel (1999) introduced microsurgical TESE (micro-TESE) and improved the results of TESE. Micro-TESE helps in identification of dilated, whitish, and opaque tubules in which

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sperm may be found in opposition to tubules where no sperm production occurs.[3] Although success rate of sperm retrieval by micro-TESE has been improved, methods to stimulate spermatogenesis in NOA men remain unexplored. Plant and Marshal[4] have demonstrated that the actions of follicle-stimulating hormone (FSH) and leutinizing hormone (LH) on germ cells are indirect and mediated by paracrine signals from sertoli and Leydig cells, in addition, close cell–cell interactions are required to maintain normal spermatogenesis. Madgar et al.[5] and Ramasamy et al.[6] have demonstrated that testosterone production in response to human chorionic gonadotropin (HCG) administration has been shown to predict the success of sperm retrieval by micro-TESE, indicating that stimulating Leydig cell function even under hypergonadotropic conditions causes favorable effects on spermatogenesis. Foresta et al.[7] have reported an improvement in sertoli cell function in severe testicular damage after reduction of high FSH plasma concentrations by administration of a gonadotropin-releasing hormone agonist (GnRHa). Selman et al.[8] and Efesoy et al.[9] have done previous successful attempts to improve spermatogenesis in NOA men by treatment with either recombinant human FSH (rh-FSH) or in combination with HCG.

We aimed, in this prospective study, to determine whether there is a beneficial role of combining gonadotropin administration with testosterone downregulation in NOA patients prior to a second time micro-TESE after a negative one.

Methods

Study design and setting

This was a prospective case–control study, conducted from November 2014 to June 2016, to evaluate the sperm retrieval rate after testosterone downregulation for 1 month and then to combine it with gonadotropin administration for 3 months in men with NOA after a negative TESE. A written informed consent was obtained from each participant prior to joining this study followed by approval of the research ethical committee (REC) that conforms to Helsinki declaration 1964. A total of 40 NOA men were recruited from a specialized IVF center. Participants were divided equally into two groups: Group A was subjected to testosterone downregulation for 1 month and then combined it with gonadotropin administration for 3 months prior to a second time TESE; Group B (controls) underwent a second time micro-TESE without prior hormonal therapy.

Inclusion criteria

All patients recruited for this study were infertile for more than 1 year, NOA patients with a previous negative TESE, and had high plasma FSH levels.

Exclusion criteria

All NOA patients with low or normal plasma FSH levels, obstructive azoospermia (vasectomy, congenital bilateral absence of vas deferens, low semen volume, semen pH < 7), or suffered from cryptozoospermia were excluded from the study.

All the participants were subjected to the following: All the participants were evaluated for reproductive hormonal profile (FSH, LH, prolactin, total testosterone) where early morning blood samples were taken and radioactive immunoassay (RIA) was done at baseline and 1 month after starting the hormonal treatment. Also, the liver enzymes were measured at baseline and after the end of treatment. Twenty patients who represented Group A were subjected to testosterone downregulation for a duration of 4 months, where testosterone enanthate 250 mg weekly intramuscular (IM) injection was given for 1 month followed by plasma FSH level estimation; if the FSH level lowered to normal or low level, the treatment protocol would continue as HCG 5000 IU weekly IM injection and purified urinary FSH 150 IU IM 3 times/week together with testosterone enanthate 250 mg monthly IM injection for 3 months. At the end of the treatment protocol, semen samples were collected from each participant and ejaculates were assessed for presence of spermatozoa according to World Health Organization (WHO) 2010 guidelines. Sperm cryopreservation was done for positive cases. Cases with negative ejaculate after centrifugation were prepared for a second time micro-TESE 2 months after the end of treatment. In contrast, patients who represented Group B (controls) were prepared for a second time micro-TESE 6 months after the first trial without prior hormonal therapy.

Also, histopathological assessment of testicular tissue was done. Postoperative follow-up of patients to detect any complications and follow-up of wound healing were done.

Micro-TESE procedure

An expert andrologist with outstanding experience in microsurgery was responsible to perform micro-TESE as previously reported.[3,10] The skin was incised through a small incision, and dartos muscle and tunica vaginalis were opened to expose the tunica albugenia. The subtunical vessels were identified under surgical microscope (Leica, Ernst Leitz GmbH, Germany) with optical magnification from 4× to 32×. A stay suture 5/0 prolene was placed into the tunica albugenia, and then a linear transverse 1-cm incision was made carefully to avoid subtunical vessel injury. Testicular tissues were observed under optical magnification 24×. If no morphologically dilated tubules were observed, the incision was extended and blunt dissection performed between the septa of the testicular parenchyma to expose multiple areas. Copious irrigation of the field with

Table 1. Sociodemographic characteristics of the participants.

			Group B, controls	Group A, cases	P-value
Male's age (years)		Mean	36.2	35.9	0.786
		SD	4.3	5.4	
Duration of infertility (years)		Mean	8	7.2	0.634
		SD	4.6	4.3	
Wife's age (years)		Mean	29.2	29.5	0.870
		SD	5.2	4.6	
Special habits	Smokers	Count	7	11	0.204
		%	35	55	
	Non-smokers	Count	13	9	
		%	65	45	
Testicular size	Small	Count	9	9	0.314
		%	45	45	
	Moderate	Count	6	7	
		%	30	35	
	Large	Count	5	4	
		%	25	20	
FSH level	Before testosterone therapy	Mean	26.9	25.4	0.946
		SD	11.8	8.7	
	One month after testosterone therapy	Mean	–	2.4	
		SD	–	1.2	

FSH: follicle-stimulating hormone; SD: standard deviation.

Ringer's lactate solution was carried out to prevent blood from obscuring the field and 6 to 10 small samples were taken from the most dilated tubules. If there was no morphological difference in the appearance of testicular tissue, the small samples were taken regardless of the tubular diameter, all of them were examined fresh for the presence of spermatozoa and one was placed in Bouin's fixative for histopathology. Bipolar diathermy was applied carefully to ensure proper hemostasis. After microscopic examination, cases were classified as successful and unsuccessful TESE according to the presence or absence of spermatozoa. Testicular tissues were taken in Eppendorf tubes containing 1 mL HEPES-buffered Ham's F10 (Gibco-BRL, Grand Island, NY, USA) and transferred within 2 h into a Petri dish (Becton Dickinson, Lakes, NJ, USA; Nunc Cell Culture Petri Dish, Thermo Fisher SCIENTIFIC, Denmark).

Testicular biopsies were minced using sterile glass slides and shredded with two Jeweler Forceps under an Olympus stereo microscope (SZ-PT, Tokyo, Japan) to separate individual tubules and then examined immediately under an inverted microscope (Olympus IMT2) with Hoffman optics modulation (X400) for the presence of testicular spermatozoa in the entire Petri dish. If no spermatozoa were seen, the squeezed tissues were removed from the Petri dish and the remaining medium containing the different tissue cells were collected in a 15-mL Falcon tube (Becton Dickinson) centrifuged at 1800 *g* for 10 min, and then the supernatant was removed. Testicular pellet for each biopsy was then resuspended in 2 mL of erythrocyte lysing buffer (ELB; 155 mm NH_4Cl (0.829 g/100 mL), 10 mm $KHCO_3$ (0.1 g/100 mL), and 2 mm ethylenediaminetetraacetic acid (EDTA; 58.45 mg/100 mL)) for 10 min at room temperature, after which 10 mL of HEPES-buffered Ham's F10 medium was added to the specimen and centrifuged for 10 min at 1800 *g*.[11] The pellet was resuspended in 50 µL of HEPES-buffered Ham's F10 medium, and a second search for spermatozoa was made in 2 µL microdrops covered with mineral oil (Sigma–Aldrich, St. Louis, MO, USA) in Falcon Petri dishes (50 mm × 9 mm; Becton Dickinson).

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann Whitney's U-test for independent samples. For comparing categorical data, chi-square (χ^2) test was performed. Fisher's exact test was used instead when the expected frequency is less than 5.

P-values less than 0.05 were considered statistically significant. All statistical calculations were done using SPSS computer programs (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

Our study showed that the mean ages of controls and cases were 36.2 ± 4.3 years and 35.9 ± 5.4 years, respectively ($p = 0.786$) (see Table 1). Mean baseline FSH levels of the controls and the cases were 26.9 ± 11.8 and 25.4 ± 8.7,

Table 2. Testicular histopathology in Group A before and after testosterone downregulation and gonadotropin administration, and Group B without hormonal therapy prior to TESE.

		C1 arrest	Mixed sertoli	SCO	P-value
First TESE					
Group B, controls	Count	3	7	10	0.320
	%	15	35	50	
Group A, cases	Count	5	3	12	
	%	25	15	60	
Second TESE					
Group B, controls	Count	3	7	10	
	%	15	35	50	
Group A, cases	Count	6	2	12	
	%	30	10	60	

TESE: testicular sperm extraction; SCO: sertoli-cell-only syndrome; C1 arrest: primary spermatocyte arrest.

Table 3. Sperm retrieval rate in Group A after testosterone downregulation and gonadotropin administration versus Group B without hormonal therapy prior to TESE.

Second TESE result		Positive	Negative	P-value
Group B, controls	Count	0	20	0.072
Group B, controls	%	00	100	
Group A, cases	Count	2	18	
Group A, cases	%	10	90	

TESE: testicular sperm extraction.

respectively ($p=0.946$) (Table 1). One month after testosterone downregulation, FSH level of the cases was normalized and became 2.4 ± 1.2 (Table 1). Mean baseline LH levels of the controls and the cases were 12.8 ± 5.7 and 11.4 ± 5, respectively (Table 1). Mean baseline testosterone levels of the controls and the cases were 4.1 ± 2.5 and 3.1 ± 1.7, respectively (Table 1). Different testicular histopathology patterns before and after testosterone downregulation and gonadotropin administration in Groups A and B without hormonal therapy prior to TESE are described in Table 2. Remarkably, two cases were positive after downregulation (10%) and no controls were positive at second TESE (0%) (Table 3). There was no statistically significant difference between sperm retrieval after the second micro-TESE in the controls and the cases ($p=0.072$) (Table 3).

Discussion

This study aims to apply a worthwhile hormonal approach to induce spermatogenesis in azoospermic cases under hypergonadotropic conditions by testosterone downregulation and provides insights into the sperm retrieval rate after this new hormonal treatment protocol during the second time micro-TESE.

In our study, the treated group was given HCG treatment after 1 month of starting testosterone therapy to induce testosterone downregulation. Furthermore, we did not find a significant relation between the testicular size and the patients' response to the hormonal therapy. Similarly, Tunc et al.[12] stated that sperm extraction was successful in 31 of 52 patients (59.6%) with NOA which was unrelated to their testicular volumes. Interestingly, our study had shown improvement in two cases out of the 20 patients who received testosterone for downregulation for 1 month followed by adjunctive administration of gonadotropins for 3 months. Our findings can be explained by either the potential effective role of the combined regimen we prescribed to the cases or it may be explained by the fact that these two patients underwent the previous TESE at different centers with different techniques using surgical loop instead of surgical microscope with the lack of high magnification power and lack of experience of the operators with subsequent improper search for sperms in their first TESE. In the same context, Matthiesson et al.[13] have demonstrated that increased intratesticular testosterone level following HCG treatment promotes the stages of spermiogenesis. Shiraishi and Ascoli[14,15] and Tai et al.[16] have demonstrated that HCG stimulation not only promotes proliferation of Leydig cells but also inhibits apoptosis of Leydig cells by in vitro studies. Aggarwal et al.[17] and Wistuba et al.[18] have demonstrated a possible method to restore Leydig cell function by generation of an HCG pulse through exogenous high-dose HCG administration. Although there is no established method to obtain sperms in cases of failed first micro-TESE, yet, hormonal therapy has been advocated in some cases with NOA prior to a second micro-TESE and demonstrated that testicular Leydig cells could respond positively to exogenous gonadotropins even under hypergonadotropic conditions.[19] Furthermore, rescue of spermatogenesis arrest has been reported in azoospermic men after long-term gonadotropin treatment as reported by Selman et al.[8] These authors found that after 6 months of gonadotropin therapy, testicular sperms were found in 11 of 49 patients (22.4%).[8] In this study, it should be noted that the two cases that became positive after downregulation were having mixed

pathology. Consistently, Tournaye et al.[20] reported that testicular histology is the only effective predictor of successful sperm recovery. In addition, Shiraishi et al.[19] found that the success of sperm retrieval at the second micro-TESE was more likely if histology at the first micro-TESE showed hypospermatogenesis categorized by McLachlan's criteria.[21] Recently, two studies had demonstrated contradictory values of adding hormonal therapy to azoospermic patients, where the first multi-institutional prospective one conducted by Shiraishi et al.[22] had revealed limited impact of salvage HCG after first negative micro-TESE especially in patients with sertoli cells only. However, Oka et al.[23] had shown antifibrotic effects of salvage HCG in NOA patients. Admittedly, our sample was small which can be considered a main imitation of this study. Furthermore, some patients in Group A, who received testosterone downregulation and gonadotropin administration, underwent the first TESE at different centers with different techniques using surgical loop instead of surgical microscope can be considered as another limitation.

Conclusion

Patients who underwent first time TESE with unfavorable outcome due to different techniques may benefit from testosterone downregulation combined with neoadjuvant gonadotropin administration as it had shown positive sperm retrieval in 2 out of the 20 cases, especially those with hypergonadotropic azoospermia.

Author contributions

M.K.A. and H.E.H.A. contributed to study conception and design. A.R.A. and A.F.M. contributed to recruitment of cases and statistical analysis. S.F.G.D. drafted the manuscript intellectually and critically revised the data.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

All procedures performed were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was approved by the local ethical committee.

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Informed consent

Informed consent was obtained from all individual participants included in the study.

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References

1. Jarow JP, Espeland MA and Lipshultz LI. Evaluation of the azoospermic patient. *J Urol* 1989; 142(1): 62–65.
2. Jow WW, Steckel J, Schlegel PN, et al. Motile sperm in human testis biopsy specimens. *J Androl* 1993; 14(3): 194–198.
3. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod* 1999; 14(1): 131–135.
4. Plant TM and Marshall GR. The functional significance of FSH in spermatogenesis and the control of its secretion in male primates. *Endocr Rev* 2001; 22(6): 764–786.
5. Madgar I, Dor J, Weissenberg R, et al. Prognostic value of the clinical and laboratory evaluation in patients with non mosaic Klinefelter syndrome who are receiving assisted reproductive therapy. *Fertil Steril* 2002; 77(6): 1167–1169.
6. Ramasamy R, Ricci JA, Palermo GD, et al. Successful fertility treatment for Klinefelter syndrome. *J Urol* 2009; 182(3): 1108–1113.
7. Foresta C, Bettella A, Spolaore D, et al. Suppression of the high endogenous levels of plasma FSH in infertile men are associated with improved Sertoli cell function as reflected by elevated levels of plasma inhibin B. *Hum Reprod* 2004; 19(6): 1431–1437.
8. Selman H, De Santo M, Sterzik K, et al. Rescue of spermatogenesis arrest in azoospermic men after long-term gonadotropin treatment. *Fertil Steril* 2006; 86(2): 466–468.
9. Efesoy O, Cayan S and Akbay E. The efficacy of recombinant human follicle-stimulating hormone in the treatment of various types of male-factor infertility at a single university hospital. *J Androl* 2009; 30(6): 679–684.
10. Amer M, Ateyah A, Hany R, et al. Prospective comparative study between microsurgical and conventional testicular sperm extraction in non-obstructive azoospermia: follow up by serial ultrasound examination. *Hum Reprod* 2000; 15(3): 653–656.
11. Nagy ZP, Verheyen G, Tournaye H, et al. An improved treatment procedure for testicular biopsy specimens offers more efficient sperm recovery: case series. *Fertil Steril* 1997; 68(2): 376–379.
12. Tunc L, Kirac M, Gurocak S, et al. serum inhibin B and FSH levels, testicular histology and volume predict the outcome of testicular sperm extraction in patients with non-obstructive azoospermia. *Int Urol Nephrol* 2006; 38(3–4): 629–635.
13. Matthiesson KL, McLachlan RI, O'Donnell L, 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(10): 3962–3969.
14. Shiraishi K and Ascoli M. Activation of the lutropin/choriogonadotropin receptor (LHR) in MA-10 cells leads the stimulation of classical tyrosine kinase cascades that participate in the activation of Ras and the phosphprylation of the extracellular signal regulated kinases (ERK1/2). *Endocrinology* 2006; 147(7): 3419–3427.
15. Shiraishi K and Ascoli M. Lutropin/choriogonadotropin stimulate the proliferation of primary cultures of rat Leydig

cells through a pathway that involves activation of the ERK1/2 cascades. *Endocrinology* 2007; 148(7): 3214–3225.
16. Tai P, Shiraishi K and Ascoli M. Activation of the lutropin/choriogonadotropin receptor inhibits apoptosis of immature Leydig cells in primary culture. *Endocrinology* 2009; 150(8): 3766–3773.
17. Aggarwal A, Misro MM, Maheshwari A, et al. Adverse effects associated with persistent stimulation of Leydig cells with hCG in vitro. *Mol Reprod Dev* 2009; 76(11): 1076–1083.
18. Wistuba J, Luetjens CM, Stukenborg JB, et al. Male 421, XX Y mice as a model for Klinefelter syndrome: hyperactivation of Leydig cells. *Endocrinology* 2010; 151(6): 2898–2910.
19. Shiraishi K, Ohmi C, Shimabukuro T, et al. Human chorionic gonadotrophin treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia. *Hum Reprod* 2012; 27(2): 331–339.
20. Tournaye H, Verheyen G, Nagy P, et al. Are there any predictive factors for successful testicular sperm recovery in azoospermic patients. *Hum Reprod* 1997; 12(1): 80–86.
21. McLachlan RI, Rajpert-De Meyts E, Hoei-Hansen CE, et al. Histological evaluation of the human testis—approaches to optimizing the clinical value of the assessment: mini review. *Hum Reprod* 2007; 22(1): 2–16.
22. Shiraishi K, Ishikawa T, Watanabe N, et al. Salvage hormonal therapy after failed microdissection testicular sperm extraction: a multi-institutional prospective study. *Int J Urol* 2016; 23(6): 496–500.
23. Oka S, Shiraishi K and Matsuyama H. Effects of human chorionic gonadotropin on testicular interstitial tissues in men with non-obstructive azoospermia. *Andrology* 2017; 5(2): 232–239.