

Effect of hormonal manipulation using clomiphene citrate prior to microdissection testicular sperm retrieval

Khalid Alrabeeh^{1,2,3}, Abdulmalik Addar^{1,2,3}, Ali Alothman^{1,2,3}, Saad Abu Melha^{1,2,3}, Abdullah Alkhalayal^{1,2,3}

¹Department of Surgery, Division of Urology, Ministry of the National Guard - Health Affairs, ²King Abdullah International Medical Research Center, ³Department of Surgery, College of Medicine, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

Abstract

Introduction: Microdissection Testicular Sperm Extraction (micro-TESE) is a surgical method used for retrieving sperm from men with non-obstructive azoospermia. Clomiphene citrate (CC) is a selective estrogen receptor modulator (SERM) that stimulates luteinizing hormone (LH) and follicle-stimulating hormone (FSH) production. It is believed that treating patients with CC prior to micro-TESE increases the chance of sperm retrieval.

Methods: This retrospective study was conducted in a tertiary care center in Riyadh, Saudi Arabia and included all patients who underwent Micro-TESE from August 2015 to November 2018. Data related to the pre-surgery hormonal levels, testicular volume, and associated genetic abnormalities were collected. chi-square and t-test were used to compare variables. A p-value of less than 0.05 was considered significant.

Results: A total of 122 patients were included in this study, with 30.0% (n=37) treated with CC. The overall sperm retrieval rate was 44.3%. Micro-TESE results were not statistically affected by age, testosterone levels, Klinefelter syndrome, or taking CC. However, higher testicular volumes and lower LH and FSH levels had more positive micro-TESE results.

Conclusion: In conclusion, Micro-TESE results were not affected by CC, age or testicular volume.

Keywords: Azoospermia, infertility, male infertility

Address for correspondence: Dr. Abdulmalik Addar, 2682 Prince Muteb bin Abdulah Street, Ar Rimayah, Riyadh 14611, Saudi Arabia.

E-mail: AbdulmalikAddar@gmail.com

Received: 08.08.2020, **Accepted:** 30.12.2020, **Published:** 14.07.2021.

INTRODUCTION

In men with nonobstructive azoospermia (NOA), microdissection testicular sperm extraction (micro-TESE) is a surgical method used for obtaining spermatozoa in males seeking fertility. The success rate for this procedure ranges from 25% to 60%.^[1,2] The minimally invasive procedure is safe and aims to preserve testicular function.^[2] It involves exposing and uncovering the larger testis. A microscope is used to visualize and examine the testicular

parenchyma and identify and examine the larger tubules to find spermatozoa.^[3]

Specific drugs are used as medical treatment to improve sperm motility, increase spermatogenesis, and adjust testosterone levels. This is accomplished by stimulating Leydig cells to produce more testosterone and increasing follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels to stimulate the Sertoli cells to increase sperm production. Clomiphene citrate (CC) is a

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Alrabeeh K, Addar A, Alothman A, Melha SA, Alkhalayal A. Effect of hormonal manipulation using clomiphene citrate prior to microdissection testicular sperm retrieval. *Urol Ann* 2021;13:254-7.

Access this article online	
Quick Response Code:	Website: www.urologyannals.com
	DOI: 10.4103/UA.UA_129_20

selective estrogen receptor modulator that stimulates LH and FSH secretion and stops the central estrogen feedback system, increasing sperm production. CC has been used for decades to treat male infertility with positive results and may increase the chance of pregnancy. However, this application is still off label as it is only approved to treat female infertility.^[4,5] Hussein *et al.* reported that hormonal treatment, such as CC, to treat male infertility patients, prior to micro-TESE, improves the chance of sperm retrieval compared to patients not receiving CC.^[6] In addition, Shiraishi *et al.* compared the effect of human chorionic gonadotropin (hCG) treatment in men with a negative micro-TESE.^[7] With the second micro-TESE attempt, 21% of the patients that received hCG had a positive sperm collection, with all the untreated patients having no sperm.

In this retrospective study, we investigated all patients who underwent micro-TESE at King Abdulaziz Medical City (KAMC) in Riyadh from August 2015 to November 2018. Retrieved data included presurgical hormonal levels, testicular volume, associated genetic abnormalities, and intra-surgical reports, and a comparison of the effect of using or not using CC on the micro-TESE results was done.

METHODS

This was a retrospective cohort study investigating all men with nonobstructive azoospermia who underwent micro-TESE at KAMC, Riyadh, from August 2015 to November 2018. Patients were prescribed CC 50 mg daily for 3–6 months prior to the micro-TESE. If sperm was not noted in the ejaculate 6 months after the treatment as per pre-established department protocol for men with low TT, testicular sperm extraction by micro-TESE was performed. Data were retrieved from medical records of all men who had undergone micro-TESE. The data included testicular volume, presurgical hormone levels (LH, FSH, and testosterone), genetic testing, hormonal treatment, and procedure results (presence of sperm). All cases of nonobstructive azoospermia, who remained azoospermic after 12 months and underwent a micro-TESE for the first time in the study period, were included. Data were entered in an Excel Sheet, and SPSS IBM SPSS version 24; USA software was used for statistical analysis. t-test was used to compare the means of continuous variables, and a Chi-square test was used to compare categorical variables. $P < 0.05$ was considered statistically significant.

RESULTS

The sample size was 122 patients. Table 1 displays the baseline characteristics including age, LH, FSH, and

testosterone levels. The mean age was 37 ± 8.84 years, the mean LH was 8.32 ± 6.08 IU/L, the mean FSH was 15.83 ± 11.64 IU/L, and the mean testosterone level was 15.84 ± 9.50 nmol/L. A third (30.32%, $n = 37$) of the sample was prescribed CC. Karyotyping was done, indicating that 9% ($n = 11$) of the patients had Klinefelter syndrome with two prescribed CC and 2.5% ($n = 3$) had Y chromosome micro deletion and none was prescribed CC. All patients with abnormal cytogenetics did not have successful sperm retrieval. The overall sperm retrieval rate was 44.3%. Excluding the patients with abnormal cytogenetics increased the sperm retrieval rate to 50%. The micro-TESE results were not statistically affected by age, testosterone levels, and taking CC [Tables 2 and 3]. However, higher testicular volumes and lower LH and FSH levels were more prevalent in the positive micro-TESE group, which was statistically significant [Table 2]. Taking

Table 1: Demographics and baseline characteristics (n=122)

Variable	Mean±SD
Age	36.69±8.84
LH (IU/L)	8.32±6.08
Follicular stimulating hormone (IU/L)	15.83±11.64
Testosterone	15.84±9.50
Right testicle volume (mL)	10.17±5.31
Left testicle volume (mL)	9.59±4.76

SD: Standard deviation

Table 2: The association between positive sperm retrieval and other demographic variables

Variable	Mean±SD		P
	Sperm found	No sperm found	
Age	37.98±9.81	35.68±7.20	0.139
Right testis volume (mL)	12.40±5.84	7.95±4.11	0.000
Left testis volume (mL)	11.50±4.77	7.68±3.96	0.001
LH (IU/L)	6.45±3.25	9.72±7.25	0.005
FSH (IU/L)	13.14±8.96	17.78±12.98	0.035
Testosterone (nmol/L)	16.1±11.09	15.64±8.29	0.793

SD: Standard deviation, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone

Table 3: The association between taking clomiphene citrate and sperm retrieval

Variables	Sperm found, n (%)	Sperm not found, n (%)	P
Taking CC	15 (40.5)	22 (59.5)	0.695
Not taking CC	39 (45.9)	46 (54.1)	
Total	54 (44.3)	68 (55.7)	

CC: Clomiphene citrate

Table 4: The association between taking clomiphene citrate and hormonal levels

Variables	Mean±SD		P
	Taking CC	Not taking CC	
LH (IU/L)	8.42±5.28	8.28±6.44	0.912
FSH (IU/L)	16.37±8.72	15.59±12.77	0.741
Testosterone (mean±SD) nmol/L	14.21±7.60	16.58±10.20	0.224

SD: Standard deviation, LH: Luteinizing hormone, FSH: Follicle-stimulating hormone, CC: Clomiphene citrate

CC was associated with higher levels of LH and FSH levels, which was not statistically significant [Table 4].

DISCUSSION

In the current study, we investigated the outcome of presurgical treatment with CC for men with nonobstructive azoospermia to enhance sperm retrieval via micro-TESE. Taking presurgical CC did not improve sperm retrieval. Patients taking CC had higher LH and FSH and lower testosterone levels, which was not statistically significant. Patients who had a successful sperm retrieval had larger testes bilaterally and lower presurgical LH and FSH, which was statistically significant ($P < 0.05$). CC is one of the most frequently used medications to treat idiopathic male infertility and has been studied comprehensively.^[8] It was previously reported that presurgical CC, used by urologists, enhanced pregnancy rates in addition to sperm detection in the ejaculate.^[9,10] Ghanem *et al.* in a review of evidence-based male infertility treatments reported that the use of anti-estrogens such as CC, due its low cost and mild side effect profile, was justified, but no strong evidence exists in support of its use as a single treatment. Most studies related to the topic had major methodological limitations such as a limited sample size and short duration.^[11]

Hussein *et al.* researched the effect of CC on 42 men with nonobstructive azoospermia and reported that all patients had sufficient sperm for intracytoplasmic sperm injection (ICSI) in their ejaculate (64.3%) or by testicular sperm extraction (35.7%).^[12] However, due to the lack of research and despite being used for decades to treat male infertility, its use is still considered off label.^[4] Hussein *et al.* also investigated the effect of CC and reported that 10.9% of patients who received CC had visible sperm on semen analysis posttreatment and sperm was retrieved for 57.7% who went for micro-TESE sperm compared to 33.7% in the control group ($P < 0.001$).^[6]

Our findings differ from that of Hussein *et al.*,^[12] which is attributed to the population investigated as they excluded men with Sertoli cell only syndrome and a varicocele. Also, the maximum age for patients in their study was 39 years with a mean age of 29.6 years. In the current study, the mean age was 36.9 years with a maximum of 65 years. Age is proposed to play a role in infertility and decreases pregnancy rates.^[13] In addition, males with small testes were not included as the mean volume for the right and left testes was 18.6 mL and 18.4 mL, respectively, and the minimum 10.2 and 10.4 mL. In the current study, the mean for the right and left testes was 10.17 mL and 9.59 mL and the minimum testes volume included was as small as 5 mL.

In the current study, presurgical LH and FSH levels were found to be higher in the group who had negative sperm retrieval. This coincides with Eken *et al.* who also reported higher LH and FSH levels in negative micro-TESE sperm retrieval which was not statistically significant.^[14] In contrast, Ramasamy *et al.* concluded that the FSH level does not affect micro-TESE results and had successful sperm retrieval in patients with an FSH >90 IU/mL.^[15]

It has been reported that micro-TESE is an effective method to retrieve sperm in patients with Klinefelter syndrome, even in cases of nonmosaic Klinefelter syndrome.^[16-18] In our findings, all men with Klinefelter syndrome had negative micro-TESE results, which may be due to the small sample size ($n = 11$). A major concern with sperm retrieved from men with Klinefelter syndrome is the ability of the spermatozoa to fertilize and develop an embryo and eventually a healthy baby.^[19] Ramasamy *et al.* reported that ICSI in couples with infertility due to Klinefelter syndrome, had a fertilization rate of 54.2% with the pregnancy rate per embryo transfusion 27.2%; however, only one of 12 couples in their study delivered a healthy baby girl. Infertile couples due to Klinefelter syndrome deserve a chance to achieve fertility and potentially father healthy children. Ramasamy *et al.* studied the effect of the pretreatment of men with Klinefelter syndrome for at least 3 months with anastrozole, hCG, CC, or testolactone. Their findings revealed a sperm retrieval rate of 55.5%, 75%, 100%, and 65%, respectively.^[16]

Men with hypogonadism (defined as a serum testosterone level less than 300 ng/dl) usually present with NOA, and there are many medical treatment options available. It should be noted that these patients have no targetable or specific cause of infertility, and controversy exists regarding the treatment options and their efficacy as presurgical hormonal stimulation.^[20] Reifsnnyder *et al.* reported in a retrospective study, over 11 years, with 1054 patients who underwent micro-TESE, that men with NOA and hypogonadism had an increased serum testosterone level after the hormonal treatment, which did not affect the overall sperm retrieval rate, pregnancy rate, and successful live births.^[21] Other hormonal treatment for male infertility, other than CC, exists. Some of these are aromatase inhibitors such as anastrozole and testolactone, which aim to correct the abnormal testosterone-to-estrogen ratio in infertile men.^[22] Pentoxifylline acts by inhibiting phosphodiesterase and results in increasing cyclic adenosine monophosphate levels and is thought to increase testicular blood flow and decreasing oxidative stress on sperm.^[23] All these treatments are still considered off-label and the only

FDA-approved class of drug to treat male infertility is gonadotropins which include FSH, LH, and GnRH.^[5]

The current study has some limitations due to its retrospective nature and limited sample size. In addition, we did not determine the pregnancy rate for the patients with successful sperm retrieval as for most patients, the sperm is still frozen and ICSI is planned in future or the ICSI was done elsewhere. Future studies with a larger sample size are required to explore this topic in-depth. In addition, the reason for the low sperm retrieval rate in our patients may be attributed to the high incidence of abnormal cytogenetics (11.5% in total). Excluding the abnormal cytogenetic group increased the sperm retrieval rate to 50% in both patient group, with or without CC.

CONCLUSION

Our study showed that CC, used in the standard protocol prior to micro-TESE, does not affect the sperm retrieval rate. This topic requires further exploration in a randomized clinical trial with a large sample size, to provide strong evidence of the benefit of CC in male infertility and as hormonal stimulation prior to micro-TESE. Future research, in this setting, should investigate the effect of CC on pregnancy rates and the number of successful live births.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Ashraf MC, Singh S, Raj D, Ramakrishnan S, Esteves SC. Micro-dissection testicular sperm extraction as an alternative for sperm acquisition in the most difficult cases of Azoospermia: Technique and preliminary results in India. *J Hum Reprod Sci* 2013;6:111-23.
- Dabaja AA, Schlegel PN. Microdissection testicular sperm extraction: An update. *Asian J Androl* 2013;15:35-9.
- Bryson CF, Ramasamy R, Sheehan M, Palermo GD, Rosenwaks Z, Schlegel PN. Severe testicular atrophy does not affect the success of microdissection testicular sperm extraction. *J Urol* 2014;191:175-8.
- Dabaja AA, Schlegel PN. Medical treatment of male infertility. *Transl Androl Urol* 2014;3:9-16.
- Chehab M, Madala A, Trussell JC. On-label and off-label drugs used in the treatment of male infertility. *Fertil Steril* 2015;103:595-604.
- 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.
- 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.
- ElSheikh MG, Hosny MB, Elshenoufy A, Elghamrawi H, Fayad A, Abdelrahman S. Combination of vitamin E and clomiphene citrate in treating patients with idiopathic oligoasthenoazoospermia: A prospective, randomized trial. *Andrology* 2015;3:864-7.
- Miyagawa Y, Tsujimura A, Matsumiya K, Takao T, Tohda A, Koga M, *et al.* Outcome of gonadotropin therapy for male hypogonadotropic hypogonadism at university affiliated male infertility centers: A 30-year retrospective study. *J Urol* 2005;173:2072-5.
- Schill WB, Jünger D, Unterburger P, Braun S. Combined hMG/hCG treatment in subfertile men with idiopathic normogonadotrophic oligozoospermia. *Int J Androl* 1982;5:467-77.
- Ghanem H, Shamloul R. An evidence-based perspective to the medical treatment of male infertility: A short review. *Urol Int* 2009;82:125-9.
- Hussein A, Ozgok Y, Ross L, Niederberger C. Clomiphene administration for cases of nonobstructive azoospermia: A multicenter study. *J Androl* 2005;26:787-91.
- Bernie AM, Ramasamy R, Schlegel PN. Predictive factors of successful microdissection testicular sperm extraction. *Basic Clin Androl* 2013;23:5.
- Eken A, Gulec F. Microdissection testicular sperm extraction (micro-TESE): Predictive value of preoperative hormonal levels and pathology in non-obstructive azoospermia. *Kaohsiung J Med Sci* 2018;34:103-8.
- Ramasamy R, Lin K, Gosden LV, Rosenwaks Z, Palermo GD, Schlegel PN. Reprint of: High serum FSH levels in men with nonobstructive azoospermia does not affect success of microdissection testicular sperm extraction. *Fertil Steril* 2019;112:e67-70.
- Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. *J Urol* 2009;182:1108-13.
- Greco E, Scarselli F, Minasi MG, Casciani V, Zavaglia D, Dente D, *et al.* Birth of 16 healthy children after ICSI in cases of nonmosaic Klinefelter syndrome. *Hum Reprod* 2013;28:1155-60.
- Ozveri H, Kayabasoglu F, Demirel C, Donmez E. Outcomes of micro-dissection TESE in patients with non-mosaic klinefelter's syndrome without hormonal treatment. *Int J Fertil Steril* 2015;8:421-8.
- Ulug U, Bener F, Akman MA, Bahceci M. Partners of men with Klinefelter syndrome can benefit from assisted reproductive technologies. *Fertil Steril* 2003;80:903-6.
- Esteves SC, Miyaoka R, Agarwal A. An update on the clinical assessment of the infertile male. [corrected]. *Clinics (Sao Paulo)* 2011;66:691-700.
- Reifsnnyder JE, Ramasamy R, Hussein J, Schlegel PN. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. *J Urol* 2012;188:532-6.
- Schlegel PN. Aromatase inhibitors for male infertility. *Fertil Steril* 2012;98:1359-62.
- Safarinejad MR. Effect of pentoxifylline on semen parameters, reproductive hormones, and seminal plasma antioxidant capacity in men with idiopathic infertility: A randomized double-blind placebo-controlled study. *Int Urol Nephrol* 2011;43:315-28.