Role of Optimizing Testosterone Before Microdissection Testicular Sperm Extraction in Men with Nonobstructive Azoospermia

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Abbreviations and Acronyms

AZF = azoospermia factor
FSH = follicle-stimulating hormone
hCG = human chorionic gonadotropin
ICSI = intracytoplasmic sperm injection
KS = Klinefelter syndrome
LH = luteinizing hormone
MA = maturation arrest
micro-TESE = microdissection testicular sperm extraction
NOA = nonobstructive azoospermia
SCO = Sertoli-cell-only
SRR = sperm retrieval rate
T = testosterone
TESE = testicular sperm extraction

Submitted for publication November 29, 2011.
Study received institutional review board approval.

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Purpose: Although optimizing endogenous testosterone production before testicular sperm extraction is commonly practiced, whether improved preoperative testosterone levels enhance sperm retrieval remains unclear. We evaluated the influence of preoperative medical therapy in men with nonobstructive azoospermia before microdissection testicular sperm extraction.

Materials and Methods: A total of 1,054 men underwent microdissection testicular sperm extraction from 1999 to 2010. Patients with preoperative testosterone levels less than 300 ng/dl were treated with aromatase inhibitors, clomiphene citrate or human chorionic gonadotropin before microdissection testicular sperm extraction with the goal of optimizing testosterone levels. Patient demographics, preoperative testosterone levels, sperm retrieval rate and pregnancy outcomes were recorded and compared in men with different baseline testosterone levels.

Results: Of the 736 men who had preoperative hormonal data 388 (53%) had baseline testosterone levels greater than 300 ng/dl. The sperm retrieval rate in these men was 56%. In the remaining 348 men with pretreatment testosterone levels less than 300 ng/dl, the sperm retrieval rate was similar (52%, $p = 0.29$). In addition, the sperm retrieval, clinical pregnancy and live birth rates were similar between men who responded to hormonal therapy and those who did not.

Conclusions: Men with nonobstructive azoospermia and hypogonadism often respond to hormonal therapy with an increase in testosterone levels, but neither baseline testosterone level nor response to hormonal therapy appears to affect overall sperm retrieval, clinical pregnancy or live birth rates.

Key Words: testosterone, testis, sperm retrieval, pregnancy

Microdissection testicular sperm extraction has become a procedure of choice for men with nonobstructive azoospermia. Micro-TESE combined with ICSI allows men with nonobstructive azoospermia the opportunity to have children of their own while minimizing testicular damage.[1] Hypogonadism, defined here as serum testosterone less than 300 ng/dl, is frequently observed in men presenting with NOA, and there are many therapeutic options available for low testosterone.[2] Unfortunately in most of these cases no specific, targetable cause of spermatogenic failure is identifiable. As a result, various empirical medical treatments are often used to treat idiopathic male infertility with limited success. Controversy exists regarding the efficacy of preoperative hormonal manipulation.[3] However, it is possible that hormonal therapy increases intratesticular testosterone levels and there is evidence that improvement in serum testosterone levels may improve sperm

0022-5347/12/1882-0532/0
THE JOURNAL OF UROLOGY®

http://dx.doi.org/10.1016/j.juro.2012.04.002
Vol. 188, 532-537, August 2012
Printed in U.S.A.

production. Hussein et al showed return of sperm to the ejaculate with clomiphene citrate in men with nonobstructive azoospermia.[4] We previously reported that in men with nonobstructive azoospermia and Klinefelter syndrome the sperm retrieval rate with micro-TESE was better in those who responded to hormonal therapy.[5]

In this study we analyzed the results of sperm retrieval surgery relative to preoperative testosterone optimization. We determined the effect of preoperative medical therapy on optimizing serum testosterone levels in men undergoing micro-TESE in a large population. We compared SRRs and pregnancy outcomes in men with NOA who underwent micro-TESE who were and were not treated preoperatively for hypogonadism with hormonal therapy.

MATERIALS AND METHODS

Patient Selection

All 1,054 consecutive patients with NOA who underwent micro-TESE between March 1999 and June 2010 were retrospectively analyzed. Of the 1,054 men who underwent micro-TESE, 736 had perioperative hormonal data available and were included in our study. The study protocol was approved by the institutional review board of Weill Cornell Medical College. Azoospermia in all patients was confirmed by analysis of at least 2 different centrifuged ejaculate specimens according to WHO guidelines.[6] On the day of surgery another ejaculate sample was obtained and azoospermia was confirmed via an extended sperm preparation.[7]

Karyotype analysis and Y chromosome microdeletion analysis was performed on all patients. Micro-TESE was not performed in men with known AZFa, AZFb, AZFb+c and complete Yq deletions. Men with AZFc deletion (31, 4.5%) underwent micro-TESE. Testicular volume was measured by physical examination using an orchidometer and the average volume of both testes was used for analysis. Additionally, physical examination was used to detect the presence of varicocele. Testicular histology was determined based on the results of previous biopsy or intraoperative random sampling of testicular parenchyma during testis exploration via microdissection. Histological category was determined by the most advanced histology present on biopsy. Categories included Sertoli-cell-only syndrome, in which no germ cells are present, maturation arrest, in which germ cells are present and hypospermatogenesis, in which spermatozoa (usually spermatids) are present. Hormonal evaluation included total serum testosterone (measured by radioimmunoassay) and FSH obtained within 2 months before the micro-TESE attempt. Clinical pregnancy per patient in female partners was defined by the identification of at least 1 gestational sac with a fetal heartbeat on transvaginal ultrasound examination 6 to 7 weeks after embryo transfer. Confirmation of live birth was obtained by telephone interviews of couples who achieved clinical pregnancy.

Medical Therapy Before Micro-TESE

We have previously described our algorithm for medical therapy before micro-TESE.[5] The decision to treat was based on initial serum total testosterone with a cutoff of 300 ng/dl (chemiluminescent enzyme immunoassay). Men with low serum testosterone were treated with aromatase inhibitors (50 to 100 mg testolactone orally twice daily or 1 mg anastrozole daily), hCG or clomiphene citrate for at least 2 to 3 months before surgery (table 1). Patients treated for the first time at our institution were preferentially treated with an aromatase inhibitor if the T/E_2 ratio (μg/dl testosterone, pg/μl estradiol) was less than 10 or with clomiphene citrate if T/E_2 was greater than 10 with T less than 300 ng/dl. Patients treated elsewhere first were maintained on their treatments if testosterone levels on treatment were adequate, or were transitioned into our algorithm if testosterone levels were still inadequate. If there was not an adequate response to the oral medical therapy then hCG injections were added to the regimen (at a dose of 1,500 to 2,000 IU 2 to 3 times a week subcutaneously). If patients were on testosterone therapy at the time of initial therapy, treatment was stopped and micro-TESE was not performed until the patient had been off exogenous testosterone for at least 6 months.

Micro-TESE

The micro-TESE procedure has been previously described.[1] Sperm retrieval surgery was typically attempted the day before oocyte retrieval in the female partner. A midline incision was made in the scrotum, and the testis with spermatic cord was preferentially delivered from the hemiscrotum with the larger testis. The tunica vaginalis was opened and the tunica albuginea was visualized. Under an operative microscope the tunica albuginea was widely opened in an equatorial plane, around approximately 270 degrees of the circumference of the testis, with preservation of subtunical vessels. After the tunica albuginea was opened, direct examination of the testicular parenchyma was performed at a magnification of 12 to 18× under an operating microscope. The examination involved microdissection deep into the testicular parenchyma to include as much of the testicular tissue as was necessary until spermatozoa were identified, or to provide a sampling of tissue for analysis after the procedure if no spermatozoa were identified intraoperatively. Small samples (1 to 15 mg) were excised by teasing out larger, more opaque tubules from surrounding Leydig cell nodules or hyperplasia in the testicular parenchyma. Samples were then examined for spermatozoa by an embryologist.

Table 1. *Medical therapies for men with baseline serum T less than 300 ng/dl*

	No.
Anastrozole	180
Anastrozole + hCG	29
Clomiphene citrate	66
Testolactone	14
Testolactone + hCG	12
hCG	9
Other combinations/unknown	38

Table 2. *Baseline patient characteristics*

	Pretreatment T Less Than 300 ng/dl	Pretreatment T Greater Than 300 ng/dl	p Value
Mean ± SD pt age	35 ± 2	34.5 ± 3	0.52
Mean ± SD ng/dl pretreatment T	207 ± 28	–	–
Mean ± SD ng/dl preop T	403 ± 24	459 ± 32	<0.0001
Mean ± SD IU/l FSH	25.7 ± 2.4	22.7 ± 2.1	0.02
No. Klinefelter syndrome (%)	65 (19)	23 (6)	<0.0001
No. varicocele (%)	72 (21)	79 (20)	0.98
No. SCO (%)	194 (56)	214 (55)	0.93
No. MA (%)	53 (15)	80 (21)	0.07
No. hypospermatogenesis (%)	50 (14)	65 (17)	0.43
No. sperm retrieval (%)	182 (52)	219 (56)	0.29
No. pregnancies (%)	93 (51)	105 (48)	0.60
No. live births (%)	72 (40)	94 (43)	0.56

Testicular Tissue Processing and ICSI

In cases in which spermatozoa were not found intraoperatively, testicular tissue was processed with collagenase and DNase I, and examined using a microdroplet approach under modulation optics as previously described.[8] ICSI was performed by microinjection technique on mature oocytes as previously described.[9]

Statistical Analysis

Microsoft Excel 2008, GraphPad Prism® 5 and Stata® version 11.0 software were used to perform all statistical calculations, with $p < 0.05$ considered statistically significant. Student's t test (unpaired) and chi-square analysis were used to compare factors between men with successful and failed sperm discovery. Fisher's exact test rather than a chi-square analysis was used when the sample size was less than 10 in 1 of the cells of the 2×2 contingency table.

RESULTS

Of the 736 men 348 (47%) had initial testosterone levels less than 300 ng/dl and 388 (53%) had initial testosterone levels greater than 300 ng/dl (table 2). Serum FSH was higher and the proportion of patients with Klinefelter syndrome was higher in men with a low serum testosterone ($p < 0.0001$). However, there were no differences between the patients with low or higher initial testosterone with respect to the proportion of men with varicocele or testicular histology. Among the 348 men with initial testosterone levels less than 300 ng/dl, testicular spermatozoa were retrieved in 52%. Among men with initial testosterone levels greater than 300 ng/dl, testicular spermatozoa were retrieved in 56%. Mean female age was 31 ± 5 years, mean number of oocytes injected was 6 ± 5 and mean number of fertilized eggs was 3 ± 4. There were no differences in clinical pregnancy per patient or live birth rates between the patients with low initial testosterone levels and those with normal levels.

Of the 348 men with serum testosterone less than 300 ng/dl, 307 (88%) were treated with hormonal therapy and 41 (12%) were not treated. There were no differences in sperm retrieval, pregnancy or live birth rates between men treated for low testosterone and those with low testosterone but not treated (table 3). Of the 307 men who were treated for low testosterone, 252 (82%) responded and had serum testosterone levels of at least 250 ng/dl before micro-TESE, while 55 (18%) did not. Men with a history of Klinefelter syndrome or varicocele responded more often to hormonal therapy, with a preoperative testosterone greater than 250 ng/dl, compared to those men without this history ($p < 0.05$). In the complete cohort of men treated with hormonal therapy there were no differences in SRR, clinical pregnancy or live birth rates between the patients who responded to hormonal therapy and those who did not (table 4).

DISCUSSION

NOA is characterized by impairment of the endocrine (testosterone producing) and/or exocrine (sperm producing) functions of the testis. The phenotype of primary exocrine testicular failure is impaired spermatogenesis leading to male infertility. Spermatogenic failure may result from hypothalamic, pituitary, testicular or post-testicular disorders. Specific and effective medical treatment is available for men with gonadotropin deficiencies, but these patients represent a small proportion of men with NOA. The more common variant of severe male infertility is primary testicular failure, which is characterized by low serum testosterone as well as increased serum FSH (and often LH) levels. In most of these men no specific cause of NOA is identifiable, leaving no specific medical therapy. Therefore, various empirical medical treatments such as hormonal agents and antioxidants are often used to treat idiopathic male infertility with varying degrees of success.[10] These therapies act at a variety of points on the hypothalamic-pituitary axis. Clomiphene is a selective estrogen receptor modulator that prevents negative feed-

Table 3. *Preoperative testosterone optimization for men with baseline serum T less than 300 ng/dl*

	Untreated	Treated	p Value
Mean ± SD pt age	37 ± 3	34 ± 4	0.11
Mean ± SD ng/dl pretreatment T	249 ± 19	199 ± 23	0.15
Mean ± SD ng/dl preop T	–	423 ± 21	–
Mean ± SD IU/l FSH	28 ± 2	25 ± 2	0.02
No. Klinefelter syndrome (%)	7 (17)	58 (19)	1.00
No. varicocele (%)	7 (17)	65 (21)	0.68
No. SCO (%)	21 (51)	169 (55)	0.77
No. MA (%)	4 (10)	54 (18)	0.27
No. hypospermatogenesis (%)	7 (17)	43 (16)	0.63
No. sperm retrieval (%)	25 (61)	157 (51)	0.31
No. pregnancies (%)	14 (56)	79 (50)	0.75
No. live births (%)	12 (48)	60 (38)	0.54

Table 4. *Characteristics of men with NOA treated medically for testosterone optimization before micro-TESE*

	No Response (preop/posttreatment T less than 250 ng/dl)	Response (preop/posttreatment T greater than 250 ng/dl)	p Value
Mean ± SD pt age	35 ± 3	34 ± 2	0.71
Mean ± SD ng/dl pretreatment T	194 ± 23	214 ± 19	0.27
Mean ± SD ng/dl preop T	202 ± 6	472 ± 18	<0.0001
Mean ± SD IU/l FSH	30 ± 7	23 ± 8	0.14
No. Klinefelter syndrome (%)	65 (44)	23 (13)	<0.0001
No. varicocele (%)	72 (11)	79 (23)	0.04
No. SCO (%)	194 (65)	214 (53)	0.12
No. MA (%)	50 (9)	80 (19)	0.08
No. hypospermatogenesis (%)	53 (11)	65 (15)	0.53
No. sperm retrieval (%)	182 (51)	219 (51)	0.97
No. pregnancies (%)	93 (54)	105 (50)	0.86
No. live births (%)	72 (43)	94 (37)	0.52

back by sex hormones and, as a result, increases the expression of gonadotropins. hCG is an analog of the gonadotropin LH that stimulates Leydig cell production of testosterone. Other drugs such as anastrozole and testolactone increase the effective concentration of endogenous testosterone by preventing its conversion to estradiol by aromatase. These various hormonal therapies are used to increase endogenous testosterone production and are associated with improved sperm retrieval rates for men with KS undergoing micro-TESE. However, the role of optimizing testosterone in all men with NOA undergoing micro-TESE has yet to be studied. The rationale for optimizing serum testosterone is that spermatogenesis in the testis is related to intratesticular testosterone levels. Therefore, an increase of endogenous testosterone production via hormonal manipulation could lead to improved sperm production in the testis. While it is unknown whether there is a threshold level at which production of spermatozoa becomes likely, we have used a threshold based on the serum level at the low end of the normal range.

In a case series from 3 international centers comprised of 42 patients with nonobstructive azoospermia, 64% of highly selected patients with NOA were reported to respond to clomiphene citrate with sperm in the ejaculate sufficient for ICSI.[4] The patients with NOA were highly selected, including men with MA (43%) and hypospermatogenesis (57%) only, and no patients with SCO syndrome. The posttreatment sperm concentration ranged from 1 to 16 million sperm per ml (mean 3.8).[4] Since no control group was evaluated, and the patients with NOA were selected as those most likely to have sperm in the ejaculate on occasional semen analyses, it is likely that the reported changes in sperm concentration were not necessarily related to medical therapy.

There are conflicting data regarding the effect of preoperative testosterone levels on sperm retrieval outcome with TESE.[11–13] An artificial neural network model based on age, duration of infertility, preoperative serum testosterone, FSH and LH levels, and testicular volume had fair sensitivity for predicting outcomes with TESE.[12] The remaining 2 studies reported that preoperative testosterone did not aid in predicting TESE outcome.[11,13] Our previous study of patients with KS demonstrated that patients who responded to medical therapy with an increase in serum testosterone to greater than 250 ng/dl had a higher SRR than those men with KS who did not respond.[5]

In our study there was no difference in SRR between men with NOA who presented with serum testosterone greater than 300 ng/dl, and those who presented with serum testosterone less than 300 ng/dl and were treated to optimize testosterone. The success of micro-TESE is based on the presence of focal regions of the most advanced stage of spermatogenesis. Serum testosterone levels could be indicative of the testis function as a whole that may not reflect focal areas of spermatogenesis. The failure of preoperative treatment to improve sperm retrieval in 46,XY cases compared to KS may represent a difference in the etiology of NOA. Additional work is necessary to determine this difference.

It is interesting that men who showed increases in serum testosterone following medical therapy had a SRR similar to that of those who were not treated and those who did not respond to hormonal therapy. It is possible that the patients with a low baseline testosterone had a lower chance of retrieval than those with a higher baseline testosterone. These observations are in contrast to the subset of patients diagnosed with KS, since those men with KS who responded to therapy (preoperative testosterone greater than 250 ng/dl) had a higher sperm retrieval rate.[5] It has been suggested that testicular tissue in men with KS may reflect varying degrees of low grade gonosomal mosaicism, and that patients with KS with an increased incidence of XY cells in their testes may be more likely to harbor germ cells.[14] It is possible that patients with KS possess varying lev-

els of normal XY cells that are able to respond to hormonal therapy, while men with 46,XY with NOA harbor unknown abnormalities and are less likely to respond to treatment. The difference in SRR between responders with KS and responders without KS could also be explained by the much smaller subset of men left untreated before micro-TESE, which made an appropriate statistical comparison not possible between men with low baseline serum testosterone (less than 300 ng/dl) who remained untreated and those with low baseline serum testosterone who underwent treatment.

While we reported on a large cohort of men with NOA treated preoperatively to boost testosterone levels as well as an untreated group, there are several limitations to this large observational experience. This was a retrospective series and, thus, our treated patient populations may have had some inherent selection bias. In addition, the series was not consecutive as the medical records of 20% of the patients who underwent micro-TESE were missing perioperative hormonal data or did not specify the type of treatment received. We were missing data that could shed light on treatment effects in our study population, such as information on preoperative treatment duration, treatment regimens used by patients elsewhere, and complications/adverse effects of treatments. The sample size in several of our subgroups was small, making it difficult to evaluate the effect of some conditions. Finally, those patients with germ cells may be more likely to respond, and these men made up a small subset of our treatment group.

The results from this study serve as a counseling tool for patients and doctors treating infertility who rely on medical therapy to improve the chances of sperm retrieval during TESE. A randomized trial will be necessary to adequately evaluate whether optimizing testosterone before micro-TESE would improve SRR outcomes. However, given the small differences in retrieval rates seen in this retrospective study, it is likely that a prospective study would require a large number of patients to adequately power such an investigation. With a chance of a small effect on outcome, such an undertaking may not be cost-effective. Although other subsets of patients may respond to medical therapy, at present it is only clear that the treatment of men with KS, in whom testosterone levels increased on hormonal therapy before micro-TESE, will result in a better chance of sperm retrieval.

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EDITORIAL COMMENT

Historically, infertile men with NOA have been the most difficult to treat as opportunities for those men to father a child rely on the success of TESE and ICSI. Micro-TESE is currently considered the best retrieval method in NOA, with a mean SRR of 50%.[1] As such, treatment options to improve sperm production are greatly anticipated since nearly half the men with NOA will be halted in their attempt to conceive due to the absence of testicular sperm on retrieval.

This report represents the largest experience to date on the use of micro-TESE to retrieve sperm from treated and untreated men with NOA. The study was intended to analyze the effect of preoperative medical therapy (aromatase inhibitors, clomi-

phene citrate or hCG) to enhance endogenous testosterone levels in hypogonadic men with NOA undergoing micro-TESE. Despite the fact that more than 80% of treated men responded to therapy with increasing endogenous testosterone levels, the authors failed to demonstrate any beneficial treatment effect on SRR.

Intriguingly, contrary results have been achieved by the authors using a similar therapy in men with nonmosaic KS with NOA (reference 5 in article). In the aforementioned study SRR was increased by 1.4-fold in men who responded to medical therapy. Men with KS have hypergonadotropic hypogonadism and very small testis (mean 3 cc). Over expression of aromatase CYP19 in the testis is the likely reason for hypogonadism in KS, thus explaining the high rate of response in increasing endogenous testosterone by using aromatase inhibitors.[2] On the other hand, the overall population of men with NOA is neither hyperestrogenic nor with such dramatic reduced testis compared to men with KS. In fact, men with NOA with hypospermatogenesis and MA have slightly reduced and normal size testes, respectively.[3] As such, it is possible that men with KS respond better to therapy because lower intratesticular testosterone levels and androgenic bioactivity are needed to boost sperm production in such small testes. Intratesticular androgenic bioactivity and intratesticular testosterone, which are approximately 40 times higher than serum testosterone, have key roles in the control of spermatogenesis but are rarely measured.[4] Different subgroups of men with NOA and larger testes may require higher intratesticular testosterone and intratesticular androgenic activity to achieve comparable results. Unfortunately there are no data on intratesticular testosterone to correlate with serum testosterone levels in men with NOA. In the present series the authors titrated their medical therapy for a serum total testosterone of 300 ng/dl used to define hypogonadism.

Therefore, a definitive conclusion cannot yet be drawn on the role of medical therapy in optimizing testosterone production before TESE in men with NOA . Randomized trials including different subsets of men with NOA in whom intratesticular androgenic activity is measured would be ideal to solve this dilemma. However, such studies require a large number of subjects and, thus, are impractical at single institutions. In this sense, long-term collaborative multi-institution efforts are needed. For the time being it is likely that empirical treatments to optimize endogenous testosterone production in men with NOA will continue to have critics as well as supporters among urologists.

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