Evaluation of a serum 17-hydroxyprogesterone as a predictor of semen parameter improvement in men undergoing medical treatment for infertility

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Cite as: Lima TFN, Rakitina E, Blachman-Braun R, et al. Evaluation of a serum 17-hydroxyprogesterone as a predictor of semen parameter improvement in men undergoing medical treatment for infertility. *Can Urol Assoc J* 2021;15(7):E340-5 http://dx.doi.org/10.5489/cuaj.6846

Published online December 15, 2020

Abstract

Introduction: The goal of medical therapy for infertile men with testosterone deficiency (TD) is to improve intratesticular testosterone (ITT). There is a gap in knowledge to identify those who will respond with semen parameter(s) improvement. We hypothesized that serum 17-hydroxyprogesterone (17-OHP) — a marker of ITT — can be used to predict improvement of semen parameter(s).

Methods: Between July 2018 and January 2020, we conducted a prospective study of 31 men with primary infertility, TD, and secondary hypogonadism receiving clomiphene citrate (CC) and/or human chorionic gonadotropin (hCG) for three months. We assessed baseline and followup hormones, including testosterone, 17-OHP, semen parameter(s), and demographics. Semen quality upgrading was based on assisted reproduction eligibility: in-vitro fertilization (<5 million), intrauterine insemination (IUI) (5–9 million), and natural pregnancy (>9 million). Variables were compared using the Mann-Whitney U or Wilcoxon rank test.

Results: Twenty-one men received CC and 10 received CC/hCG. Median followup was 3.7 (3.3–5.1) months. Sixteen men upgraded semen quality. Six of 10 men with baseline total motile sperm count (TMSC) of 0 had motile sperm after treatment, and 11/20 men with TMSC <5 upgraded semen quality into TMSC >5 range. Low 17-OHP was the only factor that predicted semen quality upgrading. Men with 17-OHP ≤55 ng/dL upgraded semen quality and improved hormones, whereas men with 17-OHP >55 ng/dL did not upgrade semen quality.

Conclusions: Medical therapy for infertile men with TD resulted in the improvement of sperm concentration, TMSC, testosterone, and 17-OHP. Semen quality upgrading appears to be more significant in patients with low 17-OHP, suggesting that ITT can be used as a biomarker to predict semen parameter(s) improvement.

Introduction

According to the U.S. Department of Health and Human Services, for every 100 couples in the U.S., 12–13 have trouble conceiving a child.[1] Although many times the cause of infertility is a combination of both male and female factors, male infertility still accounts for over one-third of such cases. Medical therapy for male factor infertility remains largely empiric[2] and relies on off-label use of oral medications known to increase intratesticular testosterone (ITT) levels, for example selective estrogen receptor modulators such as clomiphene citrate (CC) and gonadotropins such as human chorionic gonadotropin (hCG).[3]

Spermatogenesis is controlled by Sertoli cells and responds to multiple paracrine factors and steroidogenic factors secreted from Leydig cells.[4] Roth et al[5] showed with their mouse models that ITT level is positively correlated with the extent of spermatogenesis and the latter ceases when ITT falls below 75% of baseline. Coviello et al[6] also showed that males receiving a contraceptive regimen of testosterone enanthate and levonorgestrel presented with markedly suppressed gonadotropins and dramatic suppression of ITT (reduction of 98%) associated with severe sperm parameter[s] decline (since almost all patients became azoospermic). Additionally, fertile males receiving exogenous testosterone combined with hCG 500 IU every other day had no impact on semen parameter(s) in a long-term followup (up to one year), showing that direct stimulation of Leydig cells to produce ITT was able to maintain spermatogenesis.[7] These results support Zirkin et al's[8] findings that ITT (in higher levels than serum testosterone) is necessary for spermatogenesis. In this study, reductions of up to 80% of ITT were related to a quantitative decrease in sperm concentration, whereas further decrease of ITT resulted in inability to maintain complete spermatogenesis.

Several studies have been performed to investigate the relationship between ITT and male infertility.[9] Until now, ITT has been measured through testicular aspirations and

surgical biopsies, which inherently harbor the risk of damage to the testicles and cause potential complications, such as infection, bleeding, and infertility.[10] Amory et al[11] showed a positive correlation between serum levels of serum 17-hydroxyprogesterone (17-OHP) and ITT concentrations in response to hCG administration as confirmed by testicular aspiration analysis. In a recent study, we evaluated serum 17-OHP as a biomarker for ITT in hypogonadal men who were treated with medications that alter ITT.[12] We also demonstrated that it can be used to monitor response in men with hypogonadotropic hypogonadism.[13]

Our current study focuses on the response of infertile men with testosterone deficiency to targeted therapy with CC and CC combined with hCG. The current gap in knowledge is determining predictors of semen parameter(s) improvement following medical therapy using CC[14] and hCG. We hypothesized that men with lower baseline 17-OHP will experience a more significant improvement in semen parameter(s) (semen quality upgrading) after medical therapy based on the correlation between restoration of adequate levels of 17-OHP (ITT) and quantitative spermatogenesis.[8,15] Our objective was to evaluate serum 17-OHP's reliability as a serum biomarker of ITT and its response to hormonal therapy, as well as to identify ideal candidates for medical therapy in infertile men as a step toward personalized medicine.

Methods

We prospectively followed men who complained of primary infertility, had abnormal semen parameter(s) according to the World Health Organization (WHO) 5th edition criteria[16] on at least two properly collected semen analyses, and had testosterone deficiency (total testosterone <300 ng/dL drawn on separate occasions before 10 am) between July 2018 and January 2020 under an institutional review board-approved protocol. We compared pre- and post-treatment values of testosterone, 17-OHP, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and semen parameter(s) in men who received agents that increase ITT levels (CC and CC + hCG). Men with idiopathic infertility or secondary hypogonadism received CC (25 mg orally every other day) if ITT was <300 ng/dL (on two separate occasions) and LH was 2–8 IU/mL. Patients with a history of testosterone abuse and interested in spermatogenesis recovery were prescribed oral CC 50 mg + hCG 2000 IU subcutaneously every other day if baseline semen analysis and hormone levels were not in normal range.

We included men with a history of testosterone abuse and azoospermia interested in spermatogenesis recovery, men with azoospermia or total motile sperm count (TMSC) <9 associated with idiopathic infertility, and men with secondary hypogonadism with TMSC <9. We excluded men with idiopathic hypogonadotropic hypogonadism, men who had varicocele repair during the followup period, patients who were on testosterone replacement therapy (TRT), hCG, or CC therapies during the baseline assessment, those with azoospermia due to Klinefelter syndrome or Y-chromosome microdeletion, those who were lost to followup, or those who changed therapy before followup evaluation to guarantee homogeneity of patients. Also, these patients were excluded because these groups of men require specific regimens of medication to improve ITT production.

All men underwent full clinical evaluation, including evaluation of semen parameter(s) (performed at the same laboratory by the same technician according to the WHO 5th edition criteria[16]), serum 17-OHP, and testosterone, which were drawn at the same lab from 6:00 am to 10:00 am. All semen samples were collected with an abstinence period of 2–7 days. Patients using medications that altered ITT, which included exogenous testosterone, CC, or hCG, stopped treatment for eight weeks prior to baseline evaluation. The patients were evaluated at baseline and three months after beginning therapy.

We classified men with baseline TMSC into three groups: TMSC <5, TMSC 5–9, and TMSC >9 according to Samplaski et al.[17] The TMSC groups were created based on the eligibility of assisted reproductive technology (ART) and are useful to guide couples on success rates of ART procedures. Males with TMSC <5 are usually recommended to proceed with in vitro fertilization (IVF) because of the reduced amount of motile sperm. Males with TMSC 5–9 are candidates for intrauterine insemination (IUI), but also IVF. Males with TMSC >9 are eligible for all types of ART and natural conception. TMSC was calculated using the formula: ejaculate volume (mL) x concentration (million/mL) x motile fraction. Semen quality upgrade was defined by the improvement of the TMSC group after treatment or by patients with TMSC of 0 having motile sperm in the ejaculate during followup.

To develop a "standard" level for serum 17-OHP, we performed a cross-sectional analysis of a representative sample of 247 fertile controls (men who were evaluated for vasectomy reversal, erectile dysfunction, Peyronie's disease, orchialgia, and vasectomy evaluation) aged 39.5±11.93 years. The fifth, 25th, 50th, and 75th percentile for serum 17-OHP was 34.2, 55, 72, and 105.5ng/dL, respectively. We used 55 ng/dL as the threshold to determine the lower limit of normal. The lower limit of normal established by commercial laboratories ranges from 27–199 ng/dL (LABCORP*; www.labcorp.com)* and from 33–195 ng/dL (QUEST; *www.questdiagnotics.com*). These ranges were validated internally in each company and based on studies in the pediatric population.[18-20] We preferred to use the cutoff of 55 ng/dL because we wanted to provide the best threshold that demonstrated improvement in semen parameters. Therefore, we evaluated men based on serum 17-OHP ≤55 ng/dL (low 17-OHP) or 17-OHP >55 ng/dL (normal 17-OHP).

Statistical analysis

Statistical analysis was performed with SPSS version 24.0 software. Categorical variables were presented as absolute values and frequencies. For continuous variables, means and standard deviations (± SD) or medians and interquartile ranges (25–75) were calculated according to the data distribution as indicated by the normality test. A comparison of numerical variables between groups was performed using the Mann-Whitney U or Wilcoxon rank test as required. A p-value <0.05 was considered statistically significant. Based on a statistical power calculation using ClinCalc software (*https://clincalc.com/stats/samplesize.aspx*), and a study published on changes in sperm concentration after CC therapy,[14] we needed at least 30 men to achieve acceptable statistical power of 80% and α=0.05.

Results

Between July 2018 and January 2020, a total of 21 men received CC and 10 received the combination of CC and hCG. The clinical and demographic characteristics, as well as history of testosterone treatment, indication for treatment, and followup time of the analyzed men are shown in Table 1. Comparisons between hormonal levels and semen analysis parameter(s) at baseline and during followup are illustrated in Table 2. Overall, there was a significant elevation of all hormone levels, in addition to sperm concentration and TMSC. At baseline, most men had 17-OHP ≤55 ng/dL (19/31, 61.3%) and TMSC <5 (20/31, 64.5%). Males with previous history of steroid anabolic use had similar baseline levels of 17-OHP and mean testicular volume when compared to those without previous history (24 [16–88] ng/dL vs. 49 [28.5–72] ng/dL, respectively, p=0.306 and 14.7 [10.5–16.5] cc vs. 12 [10.8–14] cc, respectively, p=0.208).

A total of 17 of 23 (73.9%) men upgraded semen quality after treatment. Of the 17 men, 14 had TMSC <5, including seven men with TMSC of 0 at baseline that presented with motile sperm during followup. Also, the three patients with baseline TMSC 5–9 improved to TMSC >9 range. Eight of 31 (25.8%) men had baseline TMSC >9 and therefore could not have semen quality upgrade. In addition, males with oligozoospermia had significant improvement in sperm concentration (1.7 [0.68–6.9] to 9.3 [4.9–15] million/cc, p=0.003). Of the patients that improved semen quality, 47% (8/17) had a previous history of anabolic steroid use. Also, among men who upgraded semen quality, 17-OHP improved in 16/17 patients, with a median improvement of 56 (28–102) ng/dL.

We then evaluated the infertile men according to baseline serum 17-OHP levels and found that men with low 17-OHP had a significant improvement in sperm concentration and TMSC (1 [0–10] to 9.2 [0.7–19] million/cc, p=0.002, and 0.3 [0–9.6] to 10.2 [0.1–23] million, p=0.014, respectively). On the other hand, men with normal 17-OHP did not have a significant change in sperm concentration and TMSC (p>0.05). Besides that, only one of three (33.3%) men with TMSC of 0 and normal 17-OHP improved semen analyses during the followup. Conversely, five of seven (71.4%) men with TMSC of 0 and low 17-OHP had motile sperm during the followup. Also, men with low 17-OHP had a significant increase in 17-OHP and testosterone, as opposed to men with normal 17-OHP, who only experienced significant amelioration in serum testosterone levels (Fig. 1, Table 3).

Discussion

The management of idiopathic infertility in patients with low testosterone is challenging. Since testosterone replace-

Table 1. Clinical and demographic characteristics of the analyzed patients

Characteristics	Patients (n=31)
Age (years)	39.6±6.6
BMI (kg/m²)	29.9 (27.3–33.1)
Mean testicular volume (cc)	13.1±3.1
Testosterone abuse	
No	22 (71%)
Yes	9 (29%)
Indication for treatment*	
Infertility	28 (90.3%)
Hypogonadism	10 (32.2%)
Time baseline to followup bloodwork (months)	3.7 (3.3–4.4)
Time baseline to followup SA (months)	3.7 (3.3–5.1)

*In 7 patients, the indication was both infertility and hypogonadism. Mean ± standard deviation, Median (interquartile range 25–75). BMI: body mass index; SA: semen analysis.

Table 2. Comparison between hormonal levels and semen analysis parameter(s) at baseline and during followup

	Baseline (n=31)	Followup (n=31)	p
Hormones			
17-OHP, ng/dL	41 (24–72)	88 (61–131)	**<0.001**
Testosterone, ng/dL	213 (137–367)	415 (324–669)	**<0.001**
FSH, mIU/mL	4.4 (3.4–15.8)	7 (3.4–19.4)	**0.024**
LH, mIU/mL	3.7 (2.7–5.8)	5.2 (1.9–7.7)	**0.022**
Semen analysis			
Volume	2.3 (1.5–3.3)	2.6 (1.8–3.2)	0.368
Sperm concentration, million/cc	1 (0–10)	9.2 (0.7–19)	**0.002**
Total motility, million	0.17 (0–0.58)	0.45 (0.20–0.56)	0.115
TMSC	0.3 (0–9.6)	10.2 (0.1–23)	**0.014**
TMSC category			
<5	20 (64.5%)	9 (29%)	
5–9	3 (9.7%)	5 (16.1%)	
>9	8 (25.8%)	17 (54.8%)	**0.001**

Median (interquartile range 25–75). Wilcoxon rank test was used to analyze the change in variables over time. Bolded values=statistically significant. 17-OHP: 17-hydroxyprogestrone (ng/dL); FSH: follicle-stimulating hormone (mIU/mL); LH: luteinizing hormone (mIU/mL); SA: semen analysis; TMSC: total motile sperm count (million).

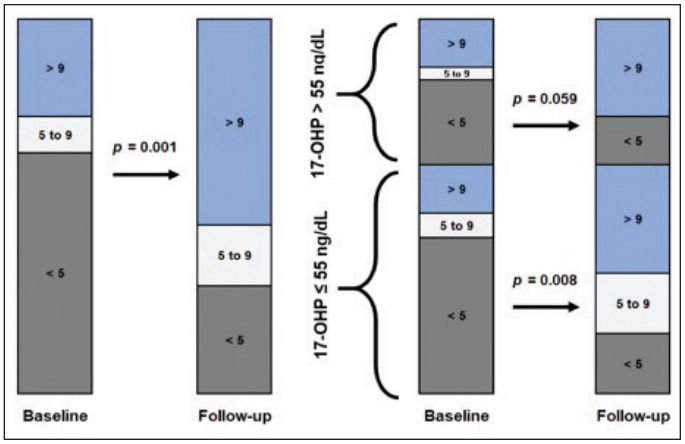

Fig. 1. Representation of changes of total motile sperm count (TMSC) category in all the patients and among men with low 17-hydroxyprogesterone (17-OHP) (≤55 ng/dL) and normal 17-OHP (>55 ng/dL), between baseline and followup.

ment therapy for low testosterone has the potential to inhibit spermatogenesis,[21] studies have described the use of off-label drugs for the treatment of these men.[13,14,22-25] Based on the hypothesis that spermatogenesis disruption is caused by low levels of intratesticular testosterone,[5,16,26] we analyzed the outcomes of men treated with off-label medications that knowingly increase ITT, comparing baseline and followup semen parameter(s) to serum levels of 17-OHP (ITT serum biomarker) and testosterone. We discovered that men with low 17-OHP are better responders to medical treatment and, therefore, are likely the best candidates for medical therapy.

Men with non-obstructive azoospermia have always been a challenge to medical therapy. Hussein et al suggested that after CC therapy, 64.3% of patients demonstrated sperm in their semen analyses ranging from 1–16 million sperm/mL, with a mean sperm density of 3.8 million/mL.[27] These findings concur with our results, where five of nine (55.5%) men with azoospermia presented with sperm in the ejaculate after treatment, ranging from 0.2–21 million/cc. There is also data suggesting that CC therapy could improve sperm retrieval rates in patients with non-obstructive azoospermia. Hussein et al showed that for the 442 patients who remained azoospermic after treatment, successful sperm retrieval was significantly higher (57%) compared with the control group (33.6%).[28]

CC has an important role in the management of oligozoospermic patients. Our study showed the significant response of these men to medical treatment (CC or CC/hCG). This agrees with a recent retrospective analysis of 77 men receiving CC, where 44 (57.14%) oligozoospermic men had a significant improvement of sperm concentration after approximately three months.[14]

Exogenous testosterone use is known to suppress spermatogenesis due to a negative feedback to the hypothalamic-pituitary-gonadal axis and although most men will recover spermatogenesis after cessation, it can take up to two years.[21] In our study, all men with history of anabolic steroid use had sperm after 105 (98–112) days of followup. These findings proved to be better than previously reported by Patel et al,[21] with spontaneous recovery rate of 64–84% after 110 days. Although data about medical therapy is poor, this incongruence can be explained by the use of restorative therapies for recovery of spermatogenesis, which could accelerate the rate of recovery.[29]

Our study's strengths include the novelty of the study's findings, especially defining 17-OHP as a baseline predictor of medical therapy response, as well as analyzing

Table 3. Comparison between hormonal levels and semen parameter(s) at baseline and followup in accordance with the 17-OHP value

	17-OHP >55 ng/dL			17-OHP ≤55 ng/dL		
	Baseline (n=12)	Followup (n=12)	p	Baseline (n=19)	Followup (n=19)	p
Followup hormones						
17-OHP, ng/dL	82 (69–106.8)	102.5 (64.5–134)	0.209	27 (19–35)	86 (55–125)	**<0.001**
Testosterone, ng/dL	359.6 (224 3– 406.3)	474.5 (374–617.5)	**0.019**	165 (120–248)	350 (301–671.7)	**<0.001**
FSH, mIU/mL	8.2 (3.4–23.7)	13 (3.4–22.8)	0.241	4.1 (3–8.8)	6 (2.1–16.5)	0.052
LH, mIU/mL	5.1 (3.2–5.8)	5.5 (3.5–7.4)	0.173	3 (1–6.4)	4.1 (1.8–8.6)	0.074
Followup SA						
Volume	2.8 (2–3)	3 (2–3.4)	0.678	2 (1.5–4)	2.6 (1.8–3.1)	0.365
Sperm concentration, million/cc	2.9 (0.2 - 10.1)	8.5 (1.8–15)	0.169	0.7 (0–10)	9.3 (0.7–20)	**0.004**
Total motility, million	0.31 (0.10–0.57)	0.39 (0.06–0.52)	0.102	0.10 (0.00–0.60)	0.50 (0.20–0.56)	0.265
TMSC	1.3 (0.03–16.5)	10.6 (0.7–21.6)	0.139	0.3 (0–9)	7.7 (0.1–25)	**0.049**
TMSC category						
<5	7 (58.3%)	4 (33.3%)		13 (68.4%)	5 (26.3%)	
5–9	1 (8.3%)	0		2 (10.5%)	5 (26.3%)	
>9	4 (33.3%)	8 (66.7%)	0.059	4 (21.1%)	9 (47.4)	**0.008**

Median (interquartile range 25–75). Wilcoxon rank test was used to analyze the change in variables over time. Bolded values=statistically significant. 17-OHP: 17-hydroxyprogesterone; FSH: follicle-stimulating hormone; LH: luteinizing hormone; SA: semen analysis; TMSC: total motile sperm count.

17-OHP changes after hCG/CC and correlating to semen analysis parameter(s). To our knowledge, there are no studies using the concept of semen upgrading for medical therapy in infertile men with idiopathic or secondary infertility. All hormonal level measurements were performed in a single laboratory[30] between 6:00 am and 10:00 am, and all semen analyses were evaluated in our laboratory facilities by a single technician to reduce the heterogeneity of results. Also, all semen samples from azoospermic patients were centrifuged to exclude cryptozoospermia.

However, this is a single-institution study with a modest sample size and we based the correlation of 17-OHP with ITT on Amory et al[11] findings and our previous study,[12] since repeatedly performing testicular aspiration was not feasible. Furthermore, both 17-OHP and testosterone are steroid hormones and measurements can vary within samples[31,32] or with the circadian cycle.[33] Additionally, we did not evaluate pregnancy rates or number of live births as outcomes. We expect that prospective studies in larger populations and with longer followup that include men with oligospermia and non-obstructive azoospermia undergoing medical treatment with CC and/or hCG will help better elucidate the role of 17-OHP in the medical management of male infertility.

Male infertility conveys a large social, psychological, and economic burden.[34] Thus, identifying biomarkers of medical treatment response in infertile men will be critical for counselling couples, setting expectations, and personalizing management. Although the present study provides encouraging results about the potential of 17-OHP as a possible biomarker of medical response, further research needs to be done to confirm our findings and identify the best cutoff value of 17-OHP that provides the highest likelihood of sperm parameter(s) improvement in oligospermic and azoospermic men.

Conclusions

Treatment of infertile men with testosterone deficiency using CC and/or hCG resulted in improvement of sperm concentration and TMSC, concomitantly with serum testosterone and 17-OHP. These improvements appear to be more significant in patients with low 17-OHP. Infertile men with normal 17-OHP seem to benefit less, and prescription of medical therapy should be cautiously evaluated individually. Using serum 17-OHP as a biomarker for ITT and predicting response of semen parameter(s) improvement with medical hormonal therapy could be a step toward personalized medicine.

Competing interests: Dr. Lima participated in a testosterone pellets trial supported by Empower/University of Miami. Dr. Ramasamy has been a consultant for Acerus; has received honoraria and/or grants from Aytu BioSciences, Boston Scientific, Coloplast, and Endo Pharmaceuticals; and participated in a Natesto clinical trial supported by Aytu BioSciences, a testosterone pellets trial supported by Empower/University of Miami, and a shockwave trial supported by the University of Miami. No other authors report competing personal or financial interests related to this work.

This paper has been peer-reviewed.

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