Baseline FSH Predicts Semen Parameter Response in Infertile Men on Clomiphene Citrate

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Abstract

Background: Clomiphene citrate (CC) is a selective estrogen receptor modulator that increases gonadotropin production and may improve spermatogenesis. The purpose of the study was to examine the effects of CC on hormone levels and spermatogenesis in men treated for infertility, and to determine whether it is possible to predict positive treatment outcomes, in terms of hormonal or spermatogenic response to CC prior to treatment.

Methods: The cases of 90 men, who were prescribed CC for infertility, were retrospectively reviewed. Serum values for follicle stimulating hormone (FSH), luteinizing hormone (LH), total testosterone (TT), free testosterone (FT), sex hormone binding globulin (SHBG), estradiol (E) as well as complete semen analyses were collected before and after CC. Exclusion criteria included azoospermia and men treated with testosterone, human chorionic gonadotropin, or recombinant FSH during the previous 6 months.

Results: Forty-two men (46.7%) with a mean age of 35±6years met the inclusion criteria for analysis. Serum hormone values significantly ($p<0.01$) increased for FSH (Δ3.4 mIU/mL), LH (Δ2.7 mIU/mL), TT (Δ250.0 ng/dL), FT (Δ5.2 ng/dL), SHBG (Δ5.2nmoI/L) and E (Δ1.9 ng/dL). Patients with a baseline FSH of ≤2 mIU/mL had no change in sperm density (Δ-13.1±32.0 million/mL) or total motile count (Δ-20.6 ± 45.5 million). Men with an initial FSH>2 (n=32) had a mean change in density of +1.68±7.46 million/mL and demonstrateda significant improvement in total motile sperm (Δ+3.4±13.96 million). There were no significant differences between the serum hormone levels in men with baseline FSH levels of ≤2 or >2 mIU/mL.***Conclusions:*** Infertile men exhibit significant increases in serum hormone levels with CC. Those with FSH >2 mIU/mL had improved sperm density and motility relative to men with FSH ≤2mIU/mL. FSH prior to initiating CC therapy may be a useful predictor of improvement in semen parameters.

Keywords: Infertility, Male; Clomiphene Citrate; Idiopathic Infertility; Hypogonadism, Male

This article has been peer reviewed.

Larry I. Lipshultz is a paid consultant to the following companies: Eli Lilly Pharmaceuticals - Consultant/Advisor/Speaker/Clinical Trial Investigator, Endo Pharmaceuticals - Clinical Investigator/Meeting Participant/Speaker, Pfizer - Meeting Participant/Speaker, Auxilium Pharmaceuticals - Clinical Trial Investigator/Speaker

Introduction

The prevalence of male infertility is approximately 12%.[1] A male factor alone is involved in 20% of cases of couple fertility and a combination of male and female factors involved another 30-40% of cases.[1,2] Despite modern diagnostic assessments, much of male infertility remains uncharacterized and classified as idiopathic.[3,4] Two-thirds of surveyed urologists use empiric medical therapy to treat idiopathic male fertility. Clomiphene citrate (CC) represents the most commonly used empiric therapy by both general urologists and those fellowship-trained in male infertility.[5]

Clomiphene citrate, a selective estrogen receptor (SERM), inhibits the negative feedback of estrogen on the hypothalamic-pituitary-gonadal axis. The resultant increases in gonadotropins, luteinizing hormone (LH), and follicle stimulating hormone (FSH), are hypothesized to raise intratesticular testosterone, maximizing the hormonal milieu for sperm production and maturation.[6] It has been used off-label for over 40 years in the treatment of infertile males and has been found to variably impact spermatogenic activity, either showing an increase in spermatogenesis or no beneficial effect.[7-12] Rare studies have shown a possible negative impact; however, the majority of studies demonstrate a significant increase in sperm concentration of infertile men treated with CC.[8,12] In 1992, the World Health Organization (WHO) completed a multi-center, double-blinded study to assess the effect of low-dose CC on idiopathic male fertililty. This study found no significant difference in semen quality between the treatment and control groups among 190 couples with idiopathic male infertility.[13] A prior Cochrane review concluded that CC, and a similar SERM tamoxifen, appear to have a beneficial effect on hypogonadal parameters; however, there was no enough evidence to evaluate the use of anti-estrogens for increasing fertility in men with idiopathic oligo/asthenospermia.[14]

Whereas the therapeutic response of men undergoing treatment of CC for secondary hypogonadism is well known, the implications for fertility remain less well understood.[15, 16] We hypothesized that perhaps a subpopulation of men may account for the seminal improvements captured in previous trials and identification of this population, using pretreatment hormone parameters, would allow for more direct and selective use of CC therapy. We therefore sought to determine the effect of CC on hormonal levels and spermatogenesis in infertile men and assess for the presence of predictive factors for hormonal and spermatogenic responses to CC prior to treatment.

Material and Methods

The study population was comprised of patients presenting for male infertility at a large academic center between January 2010 and December 2012. A retrospective chart review was performed following Institutional Review Board approval, which identified 90 men treated with CC. Indications for treatment included: presenting diagnosis of male infertility, documented inability to conceive for ≥ 12 months, and hypogonadism. Hypogonadism was defined as a serum testosterone level <300 ng/dL on 2 consecutive early morning total testosterone measurements. Serum values for follicle stimulating hormone (FSH; reference range 4-10 mIU/mL), luteinizing hormone (LH; reference range 6-19 mIU/mL), total testosterone (TT; reference range 200-1000 ng/dL), free testosterone (FT; reference range 19-26 pg/mL), sex hormone binding globulin (SHBG; reference range 10-55 nmoI/mL), and estradiol (E; reference range 0.5-5 ng/dL) as well as complete semen analyses were collected at baseline and during treatment with CC. Dosing was standardized at 25 mg daily and patients were treated for an average of 111 days. The initial post-treatment hormone estimation was typically performed 6 weeks after commencing CC. Timing of repeat semen analysis was not uniform amongst the cohort. Patient demographics, age of female partner, and whether the infertility was primary or secondary were also recorded. Treatment duration was defined as the time from initiation of CC to the date the final serum hormones were sampled.

Exclusion criteria included azoospermia and men treated with testosterone, human chorionic gonadotropin, or recombinant FSH during the previous 6 months. Those men missing follow-up serum data or semen analyses were similarly excluded. A total of 42 men (46.7%) met the inclusion criteria for analysis. All patients had a discussion with the treating physician regarding risks and benefits of CC therapy prior to initiation of therapy. This included a discussion of its off-label use in the treatment of male infertility and hypogonadism.

Semen analysis and assays for all serum hormones were performed within the same laboratory at the investigating institution. Data were tabulated and organized using

Microsoft Excel™ (Microsoft, Redmond, WA, USA). Statistical significance was established using both paired and unpaired t-tests. A ρ value <0.05 was considered statistically significant, and all values were reported as a mean ± standard deviation unless otherwise noted.

Results

Forty-two men (46.7%) with a mean age of 35±6 years met the inclusion criteria for analysis (Table 1). The majority of patients (71.4%) were Caucasian. The mean age of the patient's female partner was 32±4 years. The majority of men (64.2%) were treated for primary infertility, defined as never having fathered children with the current partner despite 1 year or more of attempting to conceive. Secondary infertility (11.9%) was characterized as previously fathering one or more children with the current partner but presenting with the inability to conceive additional children. Mean treatment duration of treatment was 111±123 days.

Baseline serum hormone levels and semen parameters for the entire cohort (N=42) area captured in Table 2 and Table 3, respectively. All patient were hypogonadal, defined as TT< 300 ng/dL, prior to the initiation of CC. Serum hormone values significantly (ρ<0.01) increased for FSH (Δ3.4 mIU/mL), LH (Δ2.7 mIU/mL), TT (Δ250.0 ng/dL), FT (Δ5.2 ng/dL), SHBG (Δ5.2 nmoI/L), and E (Δ1.9 ng/dL) (Table 2). Men with lower LH at baseline (LH<6; reference range 6-19 mIU/mL) had a significantly lower initial TT compared to those with a normal pretreatment LH (p<0.007). Those men with a lower LH (LH<6), however, showed a greater increase in TT on CC, which trended towards statistical significance (285 vs. 122; ρ<0.10). In contrast to serum hormone values among the entire cohort, semen parameters did not improve significantly while on CC (ρ>0.05) (Table 3).

In assessing pretreatment FSH values amongst the cohort, distribution analysis showed that men with FSH ≤2 mIU/mL versus those with baseline FSH >2 mIU/mL had statistically different responses in semen parameters while on CC (Table 4). When compared to serum FSH, pretreatment values of LH, TT, FT, SHBG and E were not statistically associated with changes in semen parameters (ρ>0.05).

There were no significant differences in serum hormone levels (initial, final, or change in) of men with baseline FSH ≤2 mIU/mL versus those with baseline FSH >2 mIU/mL. In the subset of men with baseline initial FSH ≤2 mIU/mL (n=5), there was no statistically significant change in sperm density (Δ-13.12±32.08 million/mL) or total motile sperm count (Δ-20.55±45.53 million). In the subset of men with baseline initial FSH >2 mIU/mL (n=32), there was a significant increase in sperm density (+1.68±7.46 million/mL, p=0.03 and a significant improvement in total motile sperm count (Δ +3.37±13.96 million, p=0.03) compared to their FSH ≤2 mIU/mL counterparts (Table 4). No patient discontinued therapy due to unwanted side effects. No patient had a total testosterone estradiol ratio <10 while on CC.

Table 1. Patient Demographics

Age (years)	**35±6**
Race	
Caucasian	71.4% (N=30)
African American	4.8% (N=2)
Other	23.8% (N=10)
Age of female partner (years)	32±4
Infertility Diagnosis	
Primary infertility	64.2% (N=27)
Secondary infertility	11.9% (N=5)
Other	23.8% (N=10)

Table 2. Pre- and Post-Treatment Hormone Values (All Patients)* (A) Pre Treatment Hormone Values (FSH≤2 group) (B)

A

Serum Hormone	Pre-Treatment	Post-Treatment	Change After Treatment (Δ)	*P* value
Total Testosterone **(200-1000 ng/dL)**	268.9±128	519±194	250±215	<0.01
Free testosterone **(19-26 pg/mL)**	6.3±2.4	11.5±4.5	5.2±4.9	<0.01
LH **(6-19 mIU/mL)**	3.8±4.1	6.2±3.8	2.7±2.7	<0.01
FSH **(4-10 mIU/mL)**	5.1±3.0	8.1±5.1	3.4±3.5	<0.01
Estradiol **(0.5-5 ng/dL)**	2.2±2.0	4.1±2.6	1.9±2.2	<0.01
Sex Hormone Binding Globulin **(10-55 nmol/mL)**	23.3±11.1	28.5±13.9	5.2±7.9	<0.01
*Values recorded as mean ± standard deviation.				

B

Serum Hormone	Pre-Treatment	*P* value compared to FSH>2
Total Testosterone **(200-1000 ng/dL)**	217.67±40.86	0.30
Free testosterone **(19-26 pg/mL)**	5.48±1.27	0.35
LH **(6-19 mIU/mL)**	2.66±1.04	0.48
FSH **(4-10 mIU/mL)**	2	<0.01
Estradiol **(0.5-5 ng/dL)**	1.50±0.55	0.34
Sex Hormone Binding Globulin **(10-55 nmol/mL)**	21.17±8.33	0.615

Table 3. Pre and Post-Treatment Semen Parameters (All Patients)*

Semen Parameter	Pre-Treatment	Post-Treatment	Change After Treatment
Volume (mL)	2.63±1.23	2.59±1.37	0.19±1.16
Density (M/mL)	17.28±22.27	17.11±13.83	-0.97±15.20
Motility (%)	31.09±14.24	31.87±14.86	1.11±11.95
Forward Progression (0-4)	1.94±0.38	1.95±0.44	0.04±0.36
Total Motile Count (M)	17.84±27.54	16.14±16.65	-0.90±23.51
*Values recorded as mean ± standard deviation.			

Table 4. Pre and Post-Treatment Semen Parameters Among Men with FSH>2 mIU/mL (A), FSH≤ 2 mIU/mL (B) and Comparison of Change after treatment (C).
All values recorded as mean ± standard deviation.

A

Semen Parameters	Pre-Treatment	Post-Treatment
Volume (mL)	2.81±1.22	2.80±1.39
Density (M/mL)	13.39±11.02	15.18±12.34
Motility (%)	30.24±13.79	31.52±12.82
Forward Progression (0-4)	1.93±0.35	1.98±0.37
Total Motile Count (M)	14.53±18.35	16.06±17.28

B

Semen Parameters	Pre-Treatment	Post-Treatment
Volume (mL)	217.67±40.86	0.30
Density (M/mL)	5.48±1.27	0.35
LH **(6-19 mIU/mL)**	2.66±1.04	0.48
FSH **(4-10 mIU/mL)**	2	<0.01
Estradiol **(0.5-5 ng/dL)**	1.50±0.55	0.34
Sex Hormone Binding Globulin **(10-55 nmol/mL)**	21.17±8.33	0.615

C

Semen Parameters	FSH ≤ 2	FSH > 2	*P* value
Change in Volume (mL)	-0.04±0.58	0.24±1.26	0.629
Change in Density (M/mL)	-13.12±32.08	1.68±7.46	0.046*
Change in Motility (%)	-2.40±8.14	1.87±12.65	0.48
Change in Forward Progression (0-4)	-0.20±0.45	0.09±0.33	0.105
Change in Total Motile Count (M)	-20.55±45.53	3.37±13.96	0.037*

Discussion

Idiopathic infertility remains a common diagnosis among men presenting for reproductive evaluation.[14] No standardized treatment algorithm exists for this population of men and use of empiric therapies is prevalent. As characterized by a recent survey of the membership of the American Urological Association, the majority of respondents favor administration of anti-estrogens in this group for 3 to 6 months.[5] Most empiric hormonal therapies are prescribed based on the theory that increasing the amount of serum and intra-testicular testosterone will improve testicular function and ultimately spermatogenesis. The most consistent finding in male patients on CC is an increase in serum FSH, LH, and TT.[17] Indeed, several studies have evaluated the safety and efficacy of CC in the treatment of secondary hypogonadism, with the resultant increases in testosterone and improvements in symptoms.[15,16]

The reliability of improvement in semen parameters has proved more elusive. The World Health Organization completed a multi-center double-blinded study to assess the effect on male fertility with CC. The cumulative life-table pregnancy rates were not statistically significant between the treatment and control groups, and there were no significant changes in semen quality among those with idiopathic male fertility.[13] Subsequent Cochrane collaborative reviews have concluded that there is not enough evidence to support the empirical use of CC for idiopathic male infertility.[14,18] A 2012 review on the use of CC in male idiopathic infertility demonstrated that the majority of studies show an improvement in seminal parameters; however, fewer have been able to show a statistically significant benefit in pregnancy rates.[19] Unfortunately, many of these reviewed studies are uncontrolled and show great variation in study design, outcomes, dosing regimens and treatment duration. The authors concluded that, based on the examined studies, there is insufficient evidence to indicate that CC is effective for the treatment of idiopathic male fertility.[19,20]

In contrast, a more recent meta-analysis, which reviewed 11 randomized controlled trials evaluating estrogen antagonists, reported that the available evidence suggests that estrogen antagonists are useful as empiric medial therapy for idiopathic male infertility. This stemmed from findings of low, non-serious adverse event profiles, increased spontaneous pregnancy rates, and improvements in sperm concentration and motility.[21] Given these discrepant findings and a lack of better treatments, CC retains widespread use as an option for empiric therapy. There may be a subgroup of men in whom its use is advantageous. The purpose of this study was to determine if this subgroup of responders could be delineated based on a comparison of pre-treatment with post-treatment hormone profiles.

Hormonal dynamic testing is more commonly used in the female fertility evaluation. A CC challenge test has been previously described as a predictor of poor ovarian response or pregnancy in women preparing to undergo in vitro fertilization (IVF). In couples undergoing IVF, women who show signs of poor ovarian reserve not only have lower pregnancy rates, but also produce poor ovarian response.

Therefore, identification of these patients before undertaking ovarian hyperstimulation is of high importance.[22] Whether a similar stimulation test could be implemented in men to predict those who may demonstrate an improvement of spermatogenesis upon initiation of CC is unknown. If identified, these predictors could suffice for a CC challenge and allow for appropriate counseling of patients and more selective use of CC in infertile men.

Within the current cohort, men with a pretreatment FSH >2 mIU/mL had significantly improved sperm density and motility relative to men with FSH ≤2mIU/mL, lending some credence to the hypothesis that FSH may serve as a marker of CC response. FSH is regarded as a marker of spermatogenic potential within the tests, and its association with response to CC-induced improvements in spermatogenesis follows conceptually. Semen parameters did not statistically improve among the entire cohort, however, which is in agreement with other studies.[13,14] and suggests that perhaps it is only a subpopulation of men who are deriving benefit in those studies where statistical improvement is noted.[6-12]

We hypothesized initially that men with a lower FSH would see the largest change in semen parameters whereas those with a normal pretreatment FSH would not show as significant an increase in CC. Instead, those men with pretreatment FSH >2 mIU/mL showed a significant change in sperm density and motility suggesting that perhaps there is a preexisting higher baseline potential for spermatogenesis which is actualized due to enhanced secretion of FSH and the raising of testosterone on

CC. **While this relationship achieved statistical significance, the authors acknowledge that a sperm density change of 1.68±7.46 M/mL may be of limited clinical significance. It is important to note, however, that conversely, men with a baseline of FSH ≤2IU/mL had a decline in semen quality, with sperm density significantly affected, while on CC.**

In line with other analyses of the use of CC in hypogonadal men, the infertile patients in this population demonstrated statistically significant changes in FSH, LH, TT, FT, SHBG and E.[10] In particular, hypogonadal parameters of FT and TT improved with corresponding elevations of FSH and LH. Interestingly, men with a lower pretreatment LH demonstrated a greater increase in TT compared to those men with normal pretreatment LH levels. Baseline pretreatment FSH may then represent an indicator of spermatogenic response to CC whereas pretreatment LH may likewise be predictive of endocrine response on CC. **While the current study incompletely evaluated where a CC challenge test could be implemented in men, a follow-up study has been designed to better assess these outcomes.**

One limitation of this study is that variability in LH and FSH assays between laboratories could limit the generalizability of these results to other practices. In addition, the mean duration of treatment in this series was short which may have precluded additional meaningful change in semen analysis parameters. Furthermore, the small sample size in this series may have limited the power to detect significant relationship from the data. **Ideally, a larger patient population could be accrued to further prospectively evaluate the comparisons between these 2 groups.**

Timing of repeat semen analysis following the initiation of CC was variable which similarly may have influenced outcomes. Pregnancy data was not captured within this cohort which prevented assessment of whether semen parameter improvement could translate to improved pregnancy rates.

Conclusions

Infertile men exhibit significant increases in FSH, LH, FT, SHBG and E while undergoing therapy with CC. Men with a pretreatment FSH >2mIU/mL had improved sperm density and motility relative to men with FSH ≤2. Conversely, men with FSH ≤2mIU/mL experienced a drop in semen quality, most notably in sperm density while on CC. No additional hormonal indices proved predictive of improved spermatogenesis on CC. Measure FSH prior to initiating CC therapy may be a useful predictor of improvement in semen parameters and allow for more select use of this treatment in infertile men. Similarly, baseline LH may be an indicator of endocrine response to CC. Larger-scale, prospective, randomized trials are needed to better characterize the effect of CC on spermatogenesis.

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