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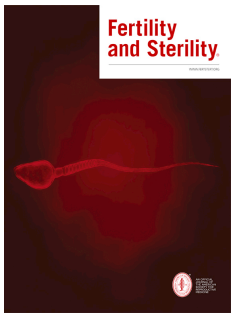

Optimal Restoration of Spermatogenesis following Testosterone Therapy using hCG and FSH

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Running Title: hCG and FSH Therapy after Testosterone Use

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CRediT Author Statement

Blair T. Stocks: Conceptualization, Formal analysis, Writing. **Amelia G. Oppenheimer:** Investigation, Formal analysis, Writing. **Kevin J. Campbell:** Conceptualization. **John P. Lindsey**: Conceptualization. **Taylor P. Kohn**: Formal analysis. **Juliet M. Alexander:** Investigation. **Jason B. Huang:** Investigation. **Larry I. Lipshultz:** Conceptualization. All authors contributed to Writing-Review & Editing.

Structured Abstract

Objective: To study improvements in spermatogenesis in men with a history of testosterone therapy using a novel fertility treatment regimen.

Design: A single-center retrospective cohort analysis.

Subjects: Seventy-seven men with previous testosterone use seeking fertility treatment from January 2020 to March 2024.

Exposure: A treatment regimen of 3000IU human Chorionic Gonadotropin (hCG) and 75IU Follicle Stimulating Hormone (FSH) three times a week was utilized.

Main Outcome Measures: The primary outcome measured was change in sperm concentration during hCG/FSH therapy. The secondary outcome measured was whether concurrent testosterone therapy during hCG/FSH therapy affected recovery of spermatogenesis.

Results: Within the entire cohort (n=77), 74% of men demonstrated improvements in their sperm concentrations. There was not a significant difference in recovery of sperm concentration in men who stayed on testosterone therapy during hCG/FSH reboot (No testosterone therapy [n=50], 74% improved vs. Concurrent testosterone therapy [n=27], 74% improved).

Conclusion: We report optimal recovery of spermatogenesis with hCG/FSH therapy in infertile men with a history of testosterone use. Concurrent testosterone therapy does not impede hCG/FSH-mediated spermatogenic recovery.

Keywords: Human Chorionic Gonadotropin (hCG), Follicle Stimulating Hormone (FSH), Testosterone Therapy, Male Infertility, Spermatogenesis

Introduction

Three percent of US males under 40 utilize testosterone therapy, which unbeknownst to many, can result in testicular atrophy, decreased sperm count, and reduced fertility(1–3). Current guidelines from the American Urological Association and the American Society for Reproductive Medicine recommend against testosterone monotherapy for individuals trying to conceive(4). Despite this recommendation, 25% of men are not aware of the negative impact of testosterone on fertility(5), and more worrisome, 25% of urologists incorrectly believe that testosterone can improve male fertility(6). Due to the limited number of high-quality studies, there currently exists no standardized treatment regimen for restoring sperm parameters in infertile men with prior testosterone use.

Clinicians have designed and utilized various regimens of off-label spermatogenic restoration therapies. The simplest therapy is discontinuing testosterone with the hope of a return to normospermia. Liu published an analysis of over 30 studies from 1990-2005 emphasizing the utility of testosterone as a form of reversible hormonal male contraceptive given for 16-78 weeks(7). Men included in this study were eugonadal, normal on physical exam, and had two semen analyses with sperm concentrations of at least 20 million [M] per mL before initiating testosterone. Two-thirds of men returned to normospermia within 6 months, and all men at 2 years following testosterone cessation. However, this study carries important caveats. Many infertile males have used testosterone for longer than the few months to one year timeframe described in this study. A significant number may also harbor undiagnosed forms of male factor infertility before testosterone use.

Another treatment utilized is hCG injection monotherapy. hCG works as an LH analog stimulating Leydig cells to produce testosterone in the testicles, a requirement for spermatogenesis(8). Previous studies have demonstrated that hCG taken every other day preserved spermatogenesis in men on testosterone therapy(9). Treatment regimens range from 1000-3000IU one to three times a week. A second regimen utilized is the combination of hCG and a Selective Estrogen Receptor Modulator (SERM) or an Aromatase Inhibitor (AI). SERMs (clomiphene or tamoxifen) and AIs (anastrozole) block the negative feedback of endogenous estradiol, which increases production of LH and FSH, the latter of which stimulates Sertoli cells which are critical for sperm maturation(10–12). Wenker illustrated the utility of testosterone cessation and combination therapy with hCG (dosed 3000IU every other day) and a SERM or AI for the treatment of azoospermic or severely oligospermic

men with a history of testosterone use. Forty-seven of the 49 patients (95.9%) demonstrated improved semen parameters(13).

More recently, two authors have explored hCG and FSH therapy in restoring sperm parameters in infertile men with prior testosterone use. Campbell compared the efficacy of hCG/clomiphene vs. hCG/FSH therapy in azoospermic men with prior testosterone use and found hCG/FSH therapy resulted in faster return of sperm in the ejaculate, averaging 5.5 months vs. 14.8 months in the hCG/clomiphene group(14). In a small series of 10 patients, Hakky explored hCG/FSH stimulation in men undergoing concurrent testosterone therapy and found improvements in sperm concentrations increasing from 2.99M total motile sperm to 98.4M after treatment(15). Complementing these data, Tatem described a practice pattern in which if no improvements in semen parameters are observed after 3 months of hCG/clomiphene therapy, providers may switch patients to hCG/FSH therapy thereafter(16).

We hypothesized that direct gonadotropic stimulation with purified hCG and FSH (in lieu of indirect SERM/AI-mediated FSH release) would result in improved recovery of spermatogenesis in azoospermic, severely oligospermic (<5M/mL sperm), and oligospermic (>5M/mL but <15M/mL sperm) men with a history of testosterone use. Secondarily, we investigated whether concurrent use of testosterone therapy during combination hCG/FSH therapy would dampen spermatogenic recovery as compared to discontinuation of testosterone therapy entirely. As withdrawal of testosterone often leads to the recurrence of hypogonadal symptoms, most patients receiving testosterone are reluctant to stop even for fertility purposes(17). Furthermore, a recently published clinical trial found no difference in spermatogenic recovery in patients with congenital hypogonadotropic hypogonadism continuing testosterone vs. those who did not during hCG/FSH therapy(18). We hypothesized that our patients would respond similarly. We present the largest cohort to date undergoing dual hCG/FSH gonadotropic therapy for the treatment of infertility secondary to prior testosterone use.

Materials and Methods

We conducted a retrospective cohort analysis of men prescribed 3000 International Units (IU) of hCG and 75IU of FSH three times a week (self-administered by subcutaneous injection) who sought infertility treatment at a single andrology clinic at Baylor College of Medicine from January 2020-March 2024. We termed this therapy "hCG/FSH reboot". All patients received these medications from a single local compounding pharmacy with approved access to purified (i.e. non-recombinant) preparations of hCG and FSH. To promote patient compliance and not deviate from the standard three times a week dosing of hCG monotherapy, all patients were instructed to draw up both the hCG and FSH into the same syringe and inject both agents via one injection on Mondays, Wednesdays, and Fridays.

Electronic charts were reviewed to record patient age, race, body mass index (BMI), co-morbidities, female partner age, duration of current conception attempt, and testicular size (exam and/or ultrasound) before hCG/FSH reboot. Prior duration of testosterone use, administration route, and indication for testosterone therapy before and during hCG/FSH reboot were noted. Semen analyses (sperm concentrations) and serum hormone values (FSH, LH, and testosterone) collected at 3-month intervals before and during hCG/FSH reboot were recorded. Sperm concentrations were classified as azoospermic (no sperm), severely oligospermic (<5 M/mL), oligospermic (>5M/mL but <15M/mL), or normospermic (>15M/mL). Duration of hCG/FSH reboot was defined as the interval from the semen analysis analyzed at the initiation of hCG/FSH reboot to the highest documented sperm concentration recorded during therapy. This endpoint was chosen to represent the ceiling for treatment response, or i.e. the "best case scenario" for a patient undergoing treatment, as not all patients had a similar number of semen analyses, nor do we know whether hCG/FSH therapy promotes spermatogenesis linearly with time. Patient data were de-identified, securely stored, and analyzed.

The primary aim of this study was to evaluate the efficacy of hCG/FSH reboot in improving sperm concentrations in infertile men with a history of testosterone therapy. The secondary aim of this study was to determine the impact of concurrent testosterone therapy use on spermatogenic recovery during hCG/FSH reboot. We stratified the entire cohort into two groups: those not on concurrent testosterone therapy during hCG/FSH reboot (No testosterone therapy) vs. those who were (Concurrent testosterone therapy). Although testosterone

dosing was not standardized within this latter cohort, all patients were undergoing 100mg-400mg of injectable testosterone therapy per week. Finally, we determined whether specific patient variables predicted a positive response to hCG/FSH reboot (i.e. moving from a lower sperm concentration group to a higher group).

Statistical analyses were performed using GraphPad Prism (Version 10) and R (Version 4.4.1). Normality of data was assessed via the Shapiro-Wilk test (alpha 0.05). For normally distributed data unpaired student's t-test was performed. For non-normally distributed data unpaired Mann-Whitney test was performed. A complete case sensitivity analysis was performed to account for potential bias due to missing data. To assess an individual's change in sperm concentration during hCG/FSH reboot, a paired Wilcoxon Signed Rank test was performed for these non-normally distributed data. Simple logistic regression analysis was performed to determine the impact of patient variables on the improvement in sperm concentration after hCG/FSH reboot. $P<0.05$ deemed significance. The study was reviewed and approved by the Baylor College of Medicine Institutional Review Board (IRB H-23509: Recovery of Spermatogenesis after Testosterone Administration).

Results

From January 2020 to March 2024, we identified 226 patients who were prescribed 3000IU of hCG and 75IU of FSH three times a week. Of these 226 patients, 124 patients had follow-up semen analyses after initiating hCG/FSH therapy. Of these 124 patients, 85 had a documented history of testosterone therapy. Eight of these patients had initial sperm concentrations >15 M/mL, were deemed normospermic, and were excluded for a total cohort of 77 patients. Within this cohort of 77 patients, 50 were not on concurrent testosterone therapy during hCG/FSH reboot (No testosterone therapy) and 27 were (Concurrent testosterone therapy).

Patient variables including age, BMI, female partner age, duration of testosterone therapy before hCG/FSH reboot, duration on hCG/FSH reboot, and initial testes size are presented in **Table 1**. There were no statistical differences between the No testosterone therapy and Concurrent testosterone therapy groups (by unpaired student's t-test or Mann-Whitney test as appropriate after Shapiro-Wilk normality testing). Duration of current conception attempt (recorded for 74/77 patients) ranged from 0 to 60 months before initiating hCG/FSH reboot with a median time of trying for 5 months. Of note, 26 patients initiated hCG/FSH reboot before attempting to father a child.

The primary prior indication for testosterone therapy was for the treatment of hypogonadal symptoms (low libido [n=44], decreased energy or fatigue [n=47], reduction in muscle mass [n=35], increased abdominal fat [n= 17], and erectile dysfunction [n=17]). Additional reasons included pituitary pathology (n=6), delayed puberty (n=2), Kallmann Syndrome (n=3), Klinefelter Syndrome (n=2), history of unprescribed anabolic steroid use (n=5), or initiated by an outside practitioner without symptoms documented (n=10). Routes of administration included injection (n=48), pellets (n=5), gel (n=4), or unrecorded (n=20). The primary indication to continue testosterone therapy during hCG/FSH reboot was to treat hypogonadal symptoms (n=20/27). This decision was made through a shared decision-making approach between the patient and provider as documented in the chart. Within these 20 patients, 5 were competitive athletes, 2 had prior pituitary pathologies, 1 had Kallmann Syndrome, and 1 had Klinefelter Syndrome.

Addressing our primary aim, 74% of all patients demonstrated an improvement in their sperm concentration after hCG/FSH reboot. Improvement in sperm concentration was defined as moving from a lower

classification of sperm concentration (azoospermic, severely oligospermic, oligospermic, normospermic) to a higher classification during the duration of hCG/FSH reboot. Median sperm concentrations increased from 0.01 M/mL to 8 M/mL after hCG/FSH reboot (**Figure 1A**, $p<0.0001$ by paired Wilcoxon Signed Rank test). Further stratification of patient sperm concentration changes during hCG/FSH reboot were then characterized (**Figure 1B-D**). Across all patients starting with azoospermia, 65% demonstrated return of sperm and 14% reached normospermia (**Figure 1B**). Of those starting with severe oligospermia, 58% reached normospermia (**Figure 1C**), and of those starting with oligospermia, 88% reached normospermia (**Figure 1D**).

To determine whether specific patient demographics or clinical parameters affected the efficacy of hCG/FSH reboot, we performed a simple logistic regression analysis (**Table 2**). For the purposes of this model, an improvement of sperm concentration was defined as moving from a lower classification of sperm concentration to a higher classification after hCG/FSH reboot (e.g. azoospermic to oligospermic, an improvement, would be assigned 1, whereas azoospermic to azoospermic, a failure, would be assigned 0). Patient age, BMI, time on hCG/FSH reboot, prior duration of testosterone therapy, and starting testosterone and LH did not correlate with sperm concentration improvement. However, patients demonstrated improvements in sperm concentration secondary to hCG/FSH reboot if they started with a larger testicular size, had a higher initial sperm concentration, or recorded a lower serum FSH at reboot therapy initiation ($p<0.05$ deemed significance).

To address our secondary aim, we performed similar analyses within each group: those who were not on concurrent testosterone therapy during hCG/FSH reboot (No testosterone therapy, n=50) vs. those who were (Concurrent testosterone therapy, n=27). In the No testosterone therapy group, 74% of patients demonstrated an improvement in their sperm concentration. Similarly, 74% of patients in the Concurrent testosterone therapy group demonstrated an improvement in their sperm concentration. Overall, Concurrent testosterone therapy during hCG/FSH reboot did not dampen spermatogenic recovery. The No testosterone therapy group demonstrated an overall sperm concentration improvement of 0.005/mL to 6.6M/mL (**Figure 2A**, $p<0.0001$ by paired Wilcoxon Signed Rank test) and the Concurrent testosterone therapy group demonstrated an overall sperm concentration improvement of 0.9M/mL to 12.4M/mL (**Figure 2E**) ($p<0.0001$ by paired Wilcoxon Signed Rank test). There was no significant difference between the starting and ending sperm concentrations of the two groups

(**Table 1**, by unpaired Mann-Whitney test). Of those patients starting with azoospermia in the No testosterone therapy group, 68% demonstrated return of sperm and 12% reached normospermia (**Figure 2B**). Of those patients in the No testosterone therapy group starting with severe oligospermia, 53% reached normospermia (**Figure 2C**) and of those starting with oligospermia 100% reached normospermia (**Figure 2D**). Similar trends were observed in the Concurrent testosterone therapy group. Of those patients starting with azoospermia in the Concurrent testosterone therapy group, 58% demonstrated return of sperm, and 17% reached normospermia (**Figure 2F**). Of those patients in the Concurrent testosterone therapy group starting with severe oligospermia, 67% reached normospermia (**Figure 2G**), and of those starting with oligospermia, 67% reached normospermia (**Figure 2H**).

Finally, we compared changes in serum hormone levels (FSH, LH, and testosterone) from the initiation of hCG/FSH reboot to the conclusion of therapy between the two groups (**Table 1**). There was a significant difference in serum FSH levels after therapy between the two groups (No testosterone therapy 2.1IU/L vs. Concurrent testosterone therapy 1.7 IU/L, p=0.03 by unpaired Mann-Whitney test). Complete case sensitivity analyses for missing hormone data (as not all patients had complementary serum hormones drawn at each semen analysis despite clinical efforts) found a higher rate of sperm concentration improvement in men with missing pre-hCG/FSH reboot FSH and LH. However, no other missing hormone levels had differing sperm concentration improvement rates (**Supplementary Table 1**).

Discussion

Three-quarters of infertile men with prior testosterone therapy demonstrated sperm concentration improvements after hCG/FSH reboot. Concurrent use of testosterone therapy did not dampen hCG/FSH-mediated spermatogenic recovery. Patients with larger testicular volumes, higher starting sperm concentrations, and lower serum FSH levels were more likely to respond to hCG/FSH reboot. Patient age and prior testosterone therapy duration did not predict therapy success contrary to previous studies(19). Overall, we present a novel and efficacious treatment strategy for restoring spermatogenesis in men with prior testosterone use.

A major limitation to hCG/FSH therapy is cost and availability. These medications are either purified from the urine of pregnant (hCG) or post-menopausal (FSH) donors or made recombinantly. Using GoodRx as a benchmark, examples of purified hCG include Pregnyl and Novarel which cost ~$300 per 10,000IU ($0.03/IU). Now discontinued Bravelle (urofollitropin) was a form of purified FSH and cost ~$750 for 750IU ($1/IU). A 250-microgram (~6000IU) Ovidrel vial (recombinant choriogonadotropin alfa) costs ~$250 ($0.04/IU). A 900IU cartridge of Follistim (recombinant follitropin beta) averages $3000 ($3.33/IU). A single month's supply of brand hCG and FSH dosed at 3000IU and 75IU three times a week would cost ~$4000. Currently, 81 503b compounding pharmacies across the US are approved to compound hCG and FSH(20). Only 4 supply both hCG and FSH and are limited to Nevada, Texas, Pennsylvania, and Massachusetts. Reported costs are $50-$80 per 10,000IU of purified hCG and $165 per 1500IU of purified FSH.

Optimal dosing of hCG and FSH for male factor fertility remains to be determined. As hCG administration does not augment serum LH levels, clinicians will often monitor changes in serum testosterone and semen analyses to assess efficacy and adjust dosing accordingly. Interestingly, work by Roth demonstrates that far lower doses of hCG than those used clinically (just 125IU of hCG every other day for 5 days) can adequately restore intratesticular testosterone levels required for spermatogenesis(21). Perhaps current hCG treatment regimens are supratherapeutic. Fortunately, reported side effects of hCG are minimal(22,23). Conversely, dosing oligospermic men with the 75IU of FSH three times a week employed in this study may be subtherapeutic. A meta-analysis by Canarella assessed the efficacy of FSH dosing in men with idiopathic oligospermia and grouped dosing regimens into low (175-262.5IU/week), intermediate (350-525IU/week), and high (700-1050IU/week)(24). Those on the

low-dosing regimen demonstrated improvements only in sperm motility. Those on the high-dosing regimen demonstrated increases in sperm concentration, total sperm count, and motility.

Complementing clinical indications to continue testosterone therapy during hCG/FSH reboot, we hypothesized that any negative pituitary feedback signaled by exogenous testosterone (thereby dampening endogenous LH and FSH release) is overcome by adequate if not superior gonadotropic stimulation with exogenous hCG and FSH. Men with azoospermia and oligospermia have been shown to harbor anti-FSH antibodies(25). Thus, one could speculate that the therapeutic introduction of antigenically foreign hCG/FSH, to which an individual's immune system has yet to develop neutralizing antibodies, could temporarily restore adequate gonadotropic signaling. Looking toward the future, recombinant technology has led to hCG and FSH molecules with enhanced biologic activity and longer serum half-lives secondary to N-glycosylation and sialylation modifications and often escape immunogenicity(26–28). For the infertile male requiring prolonged gonadotropic treatment, recombinant formulations may escape immunologic surveillance and permit longer-term spermatogenic signaling. Future clinical studies may benefit from measuring serum anti-Mullerian hormone and inhibin-B for Sertoli cell response(18) and 17-hydroxyprogesterone (a promising biomarker for intratesticular testosterone levels) for Leydig cell response(29).

Our article is timely and complements recent work by Ledesma(30). Ledesma demonstrated that 28% of azoospermic men with a history of testosterone use remained azoospermic despite treatment with hCG/clomiphene. We demonstrate that a comparable 35% of azoospermic men with prior testosterone use remained azoospermic despite hCG/FSH. Potential etiologies in our cohort included delayed puberty, orchidopexy, left testicular cancer and chemotherapy, pituitary adenoma, empty sella syndrome, LH beta chain mutation, Crohn's Disease on high-dose corticosteroids, severe obesity, and Klinefelter syndrome. Whereas their study found that only 6% of azoospermic men returned to normospermia after hCG/clomiphene(30), we found that 14% of azoospermic patients returned to normospermia after hCG/FSH. We hypothesize that hCG/clomiphene therapy requires more than just 6 months treatment, as supported by Campbell, who demonstrated faster resolution of azoospermia in men undergoing hCG/FSH vs. hCG/clomiphene(14). When grouping both azoospermic and severely oligospermic patients, Ledesma found that 17% returned to

normospermia after hCG/clomiphene. In our patient cohort of severely oligospermic men undergoing hCG/FSH, we found that 58% of these patients returned to normospermia. Our findings may support that hCG/FSH is a superior treatment regimen for the recovery of spermatogenesis in infertile men with a history of testosterone use.

The inherent limitation of our study is its retrospective nature without strict inclusion criteria, randomization, or patient monitoring (i.e., some data, such as hormone levels, are not completely captured). Specific limitations and selection biases also include the following: 1) exclusion of patients not undergoing a subsequent semen analysis after initial prescription of hCG/FSH therapy (n=102, potentially missing those patients unable to afford the high cost of hCG/FSH), 2) exclusion of patients without a documented history of testosterone use (n=39, raising the question of whether hCG/FSH therapy would benefit testosterone naïve infertile patients), and 3) exclusion of patients documented to be normospermic (>15M/mL) despite being treated for infertility (n=8, highlighting whether gonadotropic therapy could improve not only sperm quantity but quality). Accordingly, one should not generalize our findings and propose that hCG/FSH therapy would benefit all infertile male patients.

Our study is also limited by its relatively short follow-up. Although the median time on hCG/FSH reboot to achieve the highest documented sperm concentration was 4.7 months, the upper quartile of therapy duration ranged from 8.7 to 29.4 months. Within this upper quartile, however, 73% of patients still demonstrated an improvement in their sperm concentrations vs. 74% of patients in the lower three quartiles. This raises the possibility that some patients may take longer to respond to hCG/FSH reboot therapy. Longer follow-up will be required to better understand this regimen's safety and efficacy profile. Finally, we did not assess fertility outcomes in patients undergoing hCG/FSH reboot therapy as our IRB did not include calling patients for follow-up. However, men with isolated hypogonadotropic hypogonadism treated with human Menopausal Gonadotropin, a combination of urinary purified LH and FSH, can reliably achieve pregnancy despite having severe oligospermia or oligospermia(31). Thus, we posit that any improvement in sperm concentration secondary to hCG/FSH therapy represents a means to restore fertility in men with prior testosterone use.

Key to implementing standardized, guideline-based treatment protocols for the treatment of infertility secondary to testosterone use are prospective trials. To confirm these data, the authors are currently designing a

multi-center, randomized, and controlled trial to assess hCG/FSH therapy in infertile men with prior testosterone use. Studies of importance should also include prospective trials comparing the efficacy of hCG monotherapy or hCG/Clomiphene to hCG/FSH, as well as the efficacy of hCG/FSH therapy in testosterone naïve patients(18).

Ultimately, the strength of this study is its size, representing the largest study to date investigating the utility of gonadotropic hCG/FSH therapy in restoring spermatogenesis in men with a history of testosterone use. Subjects followed a consistent medication regimen throughout the study, allowing appropriate analyses with semen analyses and hormonal testing at 3-month intervals. With hope of increased availability and reduced cost, hCG/FSH gonadotropic therapy may represent the optimal regimen for spermatogenic recovery in infertile men with a history of testosterone use.

Conclusions

We report optimal recovery of spermatogenesis with hCG/FSH reboot therapy in infertile men with a history of testosterone use. Moreover, we demonstrate that concurrent testosterone therapy does not impede hCG/FSH-mediated spermatogenic recovery. Although further prospective trials are needed, we present an efficacious treatment strategy to optimally restore semen parameters in infertile men with a history of testosterone use.

Tables

	Total Cohort			No Testosterone Therapy			Concurrent Testosterone Therapy			p-value
Parametric Distributions	N	Range	Mean (StdDev)	N	Range	Mean (StdDev)	N	Range	Mean (StdDev)	
Female Partner Age (years)	68	22-43	32 (4)	45	25-39	32 (4)	23	22-43	32 (5)	0.73
Initial Testis Size (cc)	76	3-26	14 (5)	49	4-22	14 (5)	27	3-26	15 (6)	0.19
	Total Cohort			**No Testosterone Therapy**			**Concurrent Testosterone Therapy**			**p-value**
Non-parametric Distributions	N	Range	Median (IQR)	N	Range	Median (IQR)	N	Range	Median (IQR)	
Age (years)	77	15-63	35 (33-40)	50	15-63	35 (34-40)	27	25-49	35 (30-39)	0.24
BMI (kg/m2)	77	18-57	30 (26-33)	50	18-57	29 (26-33)	27	25-44	31 (27-35)	0.21
Duration of Time on Testosterone before Reboot (years)	69	0.2-20	5 (2-9)	45	0.2-20	6 (2-10)	24	1-20	5 (3-8)	0.8
Sperm Concentration at Initiation of Reboot (M/mL)	77	0-12.4	0.01 (0-3.8)	50	0-12.4	0.005 (0-4.1)	27	0-10.8	0.9 (0-3.6)	0.67
Sperm Concentration at End of Reboot (M/mL)	77	0-110	8 (0.4-19.4)	50	0-110	6.6 (0.4-20.2)	27	0-50	12.4 (0.01-18.8)	0.89
Serum T at Initiation of Reboot (ng/dL)	64	48-3000	417 (256-1038)	43	48-3000	367 (226-898)	21	61-3000	945 (278-1356)	0.11
Serum T at End of Reboot (ng/dL)	62	42-3000	454 (227-657)	42	42-919	440 (227-607)	20	92-3000	513 (192-1008)	0.2
Serum LH at Initiation of Reboot (IU/L)	54	0.01-16.4	0.4 (0.3-3.5)	38	0.03-16.4	0.5 (0.3-4.6)	16	0.01-7.6	0.3 (0.3-1.2)	0.48
Serum LH at End of Reboot (IU/L)	55	0-30.2	0.3 (0.3-2.4)	39	0-21	0.4 (0.3-2.4)	17	0.1-30.2	0.3 (0.3-1.7)	0.31
Serum FSH at Initiation of Reboot (IU/L)	54	0.05-24	1 (0.3-3.3)	38	0.05-24	1.1 (0.3-3.7)	16	0.13-12.4	1 (0.3-1.9)	0.42
Serum FSH at End of Reboot (IU/L)	56	0.2-32.4	2 (1.1-3.4)	39	0.24-24.7	2.1 (1.3-3.6)	17	0.3-32.4	1.7 (0.5-2.1)	**0.03**
Duration of Reboot to Outcome (months)	77	0.7-29.4	4.7 (3.2-8.7)	50	0.7-29.4	5.3 (3.3-9.9)	27	1.5-16.1	4.0 (3.1-8.0)	0.36

Table 1. Patient Variables. Patient cohorts are divided into three columns: Total Cohort (n=77), then grouped into No testosterone therapy (n=50) and Concurrent testosterone therapy (n=27). The rightmost column compares the differences of means and medians between the No testosterone therapy group and the Concurrent testosterone therapy group using unpaired Student's t-test (normally distributed data) or Mann-Whitney test (non-normally distributed data), with $p<0.05$ deeming significance. The only significant difference found between the No testosterone therapy group and the Concurrent testosterone therapy group was serum FSH level at the end of hCG/FSH reboot ($p=0.03$), with the Concurrent testosterone therapy group demonstrating lower serum FSH levels than the No testosterone therapy group.

	Odds Ratio	95% Confidence Interval	p-value
Age	1.035	0.9560-1.131	0.40
BMI	0.942	0.8723-1.014	0.11
Testicular Size	1.130	1.017-1.271	**0.02**
Initial Sperm Concentration	1.210	1.004-1.581	**0.04**
Time on Reboot	1.000	0.9971-1.004	0.87
Duration of TRT Prior	1.022	0.9053-1.168	0.73
Starting Testosterone	1.001	0.9997-1.002	0.24
Starting FSH	0.859	0.7303-0.9652	**0.01**
Starting LH	0.897	0.7438-1.064	0.21

Table 2. Simple Logistic Regression Analysis. Patient demographics (age and BMI) and clinical parameters (testicular size, initial sperm concentration, time on hCG/FSH reboot, duration of prior testosterone use, and starting serum hormones) and their impact on the likelihood of improved sperm concentrations after hCG/FSH reboot were assessed. For the purposes of this model, an improvement of sperm concentration was defined as moving from a lower classification of sperm concentration to a higher classification after hCG/FSH reboot (e.g., azoospermic to oligospermic, an improvement, would be assigned 1, whereas azoospermic to azoospermic, a failure, would be assigned 0). Overall, patients demonstrated sperm concentration improvements on hCG/FSH reboot therapy if they started with a larger testicular size, had a higher initial sperm concentration, and had a lower starting serum FSH value ($p<0.05$ deemed significance).

Figure Legends

Figure 1. Changes in Sperm Concentration in response to hCG/FSH Reboot Therapy (Total Cohort, n=77). In Panel A, changes in sperm concentration of individual patients are paired from the start of hCG/FSH reboot to the completion of therapy. Due to logarithmic limitations, patients deemed azoospermic were assigned a sperm concentration of 0.001 M/mL, and those patients with cryptozoospermia (sperm seen only on pellet analysis) were assigned a concentration of 0.01 M/mL. Although these data demonstrate that some patients did not improve (i.e., azoospermia to azoospermia or cryptozoospermia to cryptozoospermia), one can easily appreciate the overall trend in improvement. In the subsequent panels, patients are grouped as whether they started hCG/FSH reboot azoospermic (no sperm, B), severely oligospermic (<5M/mL, C), or oligospermic (>5M/mL but <15M/mL, D). Pie graphs are color-coded based on the outcome of hCG/FSH reboot therapy: pink – azoospermic, purple – severely oligospermic, yellow – oligospermic, or green – normospermic). Legends calculate the percent of patients in each group demonstrating specific changes in their sperm concentration pre and post hCG/FSH reboot. Significance was determined by Paired Wilcoxon Signed Rank test (****$p<0.0001$).

Figure 2. Changes in sperm concentration in patients not on testosterone therapy (No testosterone therapy, n=50) vs. those on testosterone therapy (Concurrent testosterone therapy, n=27) during hCG/FSH Reboot. Improvements in sperm concentrations of individual patients (Panel A: No testosterone therapy) and (Panel E: Concurrent testosterone therapy) during hCG/FSH reboot are readily apparent. Like Figure 1, in the subsequent panels (No testosterone therapy [B-D] and Concurrent testosterone therapy [F-H]), patients are grouped according to whether they started hCG/FSH reboot therapy azoospermic, severely oligospermic, or oligospermic. Pie graphs are color-coded, as described in Figure 1. Significance was determined by Paired Wilcoxon Signed Rank test (****$p<0.0001$).

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Parametric Distributions	Total Cohort			No Testosterone Therapy			Concurrent Testosterone Therapy			p-value
	N	Range	Mean (StdDev)	N	Range	Mean (StdDev)	N	Range	Mean (StdDev)	
Female Partner Age (years)	68	22-43	32 (4)	45	25-39	32 (4)	23	22-43	32 (5)	0.73
Initial Testis Size (cc)	76	3-26	14 (5)	49	4-22	14 (5)	27	3-26	15 (6)	0.19

Non-parametric Distributions	Total Cohort			No Testosterone Therapy			Concurrent Testosterone Therapy			p-value
	N	Range	Median (IQR)	N	Range	Median (IQR)	N	Range	Median (IQR)	
Age (years)	77	15-63	35 (33-40)	50	15-63	35 (34-40)	27	25-49	35 (30-39)	0.24
BMI (kg/m2)	77	18-57	30 (26-33)	50	18-57	29 (26-33)	27	25-44	31 (27-35)	0.21
Duration of Time on Testosterone before Reboot (years)	69	0.2-20	5 (2-9)	45	0.2-20	6 (2-10)	24	1-20	5 (3-8)	0.8
Sperm Concentration at Initiation of Reboot (M/mL)	77	0-12.4	0.01 (0-3.8)	50	0-12.4	0.005 (0-4.1)	27	0-10.8	0.9 (0-3.6)	0.67
Sperm Concentration at End of Reboot (M/mL)	77	0-110	8 (0.4-19.4)	50	0-110	6.6 (0.4-20.2)	27	0-50	12.4 (0.01-18.8)	0.89
Serum T at Initiation of Reboot (ng/dL)	64	48-3000	417 (256-1038)	43	48-3000	367 (226-898)	21	61-3000	945 (278-1356)	0.11
Serum T at End of Reboot (ng/dL)	62	42-3000	454 (227-657)	42	42-919	440 (227-607)	20	92-3000	513 (192-1008)	0.2
Serum LH at Initiation of Reboot (IU/L)	54	0.01-16.4	0.4 (0.3-3.5)	38	0.03-16.4	0.5 (0.3-4.6)	16	0.01-7.6	0.3 (0.3-1.2)	0.48
Serum LH at End of Reboot (IU/L)	55	0-30.2	0.3 (0.3-2.4)	39	0-21	0.4 (0.3-2.4)	17	0.1-30.2	0.3 (0.3-1.7)	0.31
Serum FSH at Initiation of Reboot (IU/L)	54	0.05-24	1 (0.3-3.3)	38	0.05-24	1.1 (0.3-3.7)	16	0.13-12.4	1 (0.3-1.9)	0.42
Serum FSH at End of Reboot (IU/L)	56	0.2-32.4	2 (1.1-3.4)	39	0.24-24.7	2.1 (1.3-3.6)	17	0.3-32.4	1.7 (0.5-2.1)	**0.03**
Duration of Reboot to Outcome (months)	77	0.7-29.4	4.7 (3.2-8.7)	50	0.7-29.4	5.3 (3.3-9.9)	27	1.5-16.1	4.0 (3.1-8.0)	0.36

Table 1. Patient Variables. Patient cohorts are divided into three columns: Total Cohort (n=77), then grouped into No testosterone therapy (n=50) and Concurrent testosterone therapy (n=27). The rightmost column compares the differences of means and medians between the No testosterone therapy group and the Concurrent testosterone therapy group using unpaired Student's t-test (normally distributed data) or Mann-Whitney test (non-normally distributed data), with $p<0.05$ deeming significance. The only significant difference found between the No testosterone therapy group and the Concurrent testosterone therapy group was serum FSH level at the end of hCG/FSH reboot ($p=0.03$), with the Concurrent testosterone therapy group demonstrating lower serum FSH levels than the No testosterone therapy group.

	Odds Ratio	95% Confidence Interval	p-value
Age	1.035	0.9560-1.131	0.40
BMI	0.942	0.8723-1.014	0.11
Testicular Size	1.130	1.017-1.271	**0.02**
Initial Sperm Concentration	1.210	1.004-1.581	**0.04**
Time on Reboot	1.000	0.9971-1.004	0.87
Duration of TRT Prior	1.022	0.9053-1.168	0.73
Starting Testosterone	1.001	0.9997-1.002	0.24
Starting FSH	0.859	0.7303-0.9652	**0.01**
Starting LH	0.897	0.7438-1.064	0.21

Table 2. Simple Logistic Regression Analysis. Patient demographics (age and BMI) and clinical parameters (testicular size, initial sperm concentration, time on hCG/FSH reboot, duration of prior testosterone use, and starting serum hormones) and their impact on the likelihood of improved sperm concentrations after hCG/FSH reboot were assessed. For the purposes of this model, an improvement of sperm concentration was defined as moving from a lower classification of sperm concentration to a higher classification after hCG/FSH reboot (e.g., azoospermic to oligospermic, an improvement, would be assigned 1, whereas azoospermic to azoospermic, a failure, would be assigned 0). Overall, patients demonstrated sperm concentration improvements on hCG/FSH reboot therapy if they started with a larger testicular size, had a higher initial sperm concentration, and had a lower starting serum FSH value ($p<0.05$ deemed significance).

Total Cohort

	Pre Reboot FSH	Pre Reboot LH	Pre Reboot Testosterone	Post Reboot FSH	Post Reboot LH	Post Reboot Testosterone
With Labs Missing	91.3% (21/23)	91.3% (21/23)	84.6% (11/13)	85.7% (18/21)	85.7% (18/21)	80.0% (12/15)
With Lab Present	66.7% (36/54)	66.7% (36/54)	71.9% (46/64)	69.6% (39/56)	69.6% (39/56)	72.6% (45/62)
p-value (Fisher Exact Test)	**0.0257**	**0.0257**	0.4949	0.2429	0.2429	0.7466

Concurrent Testosterone Therapy

	Pre Reboot FSH	Pre Reboot LH	Pre Reboot Testosterone	Post Reboot FSH	Post Reboot LH	Post Reboot Testosterone
With Labs Missing	90.9% (10/11)	90.9% (10/11)	100% (6/6)	80.0% (8/10)	80.0% (8/10)	71.4% (5/7)
With Lab Present	62.5% (10/16)	62.5% (10/16)	66.7% (14/21)	70.6% (12/17)	70.6% (12/17)	75.0% (15/20)
p-value (Fisher Exact Test)	0.1832	0.1832	0.1548	0.6784	0.6784	1.000

No Testosterone Therapy

	Pre Reboot FSH	Pre Reboot LH	Pre Reboot Testosterone	Post Reboot FSH	Post Reboot LH	Post Reboot Testosterone
With Labs Missing	91.7% (11/12)	91.7% (11/12)	71.4% (5/7)	90.9% (10/11)	90.9% (10/11)	87.5% (7/8)
With Lab Present	68.4% (26/38)	68.4% (26/38)	74.4% (32/43)	69.2% (27/39)	69.2% (27/39)	71.4% (30/42)
p-value (Fisher Exact Test)	0.1466	0.1466	1.000	0.2476	0.2476	0.6622

Supplementary Table 1. Complete case sensitivity analysis accounts for missing hormone data comparing the total cohort, the Concurrent testosterone therapy cohort, and the No testosterone therapy cohort vs. the rates of sperm concentration improvement. For the purposes of this model (and like our logistic regression analysis) an improvement of sperm concentration was defined as moving from a lower classification of sperm concentration to a higher classification after hCG/FSH reboot (e.g., azoospermic to oligospermic, an improvement, would be assigned 1, whereas azoospermic to azoospermic, a failure, would be assigned 0). Fisher exact test was used to compare binary rates of sperm concentration improvement between the groups with and without laboratory data missing ($p<0.05$ deeming significance). Within the total cohort, only men missing pre-hCG/FSH reboot FSH and LH values demonstrated significantly higher rates of sperm concentration improvement. When assessed in the sub-groups of men on Concurrent testosterone therapy vs. No testosterone therapy, those missing initial LH and FSH were not found to be significant.