New frontiers in fertility preservation: a hypothesis on fertility optimization in men with hypergonadotrophic hypogonadism

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Abstract: Strategies exist that can mitigate the risk of causing iatrogenic infertility when men require testosterone replacement therapy (TRT). This article reviews the current medical therapies that preserve spermatogenesis when TRT is indicated. Furthermore, we highlight the re-emerging concept of hypothalamic-pituitary-gonadal (HPG) axis reset in hypergonadotrophic, hypogonadal infertile men who are planning sperm retrieval procedures. Finally, we present our hypothesis for a novel protocol to optimize hypergonadotrophic hypogonadal men before sperm extraction surgeries hormonally.

Keywords: Male fertility; testosterone; hormonal therapy; non-obstructive azoospermia; testosterone therapy; Klinefelter syndrome

Introduction

Hypogonadism is defined by the Endocrine Society as a testosterone level <300 ng/dL and is accompanied by associated symptoms, such as low energy and impaired libido (1). Testosterone therapy (TRT) is an effective treatment option that ameliorates symptoms of hypogonadism. Exogenous testosterone, however, can impair spermatogenesis by disrupting the hypothalamic-pituitary-gonadal (HPG) axis. This adverse effect makes testosterone therapy challenging in men who wish to have children. The Endocrine Society recommends against TRT in men planning fertility in the near term (1).

Fortunately, strategies exist that can mitigate the risk of developing infertility associated with TRT. Some therapeutic strategies may even enhance the fertility potential of men with certain diagnoses. This article reviews the current medical therapies that preserve spermatogenesis when TRT is indicated, highlights the re-emerging concept of HPG axis reset in hypergonadotrophic, hypogonadal

infertile men planning sperm retrieval procedures, and presents our hypothesis for a novel protocol in the optimization of hypergonadotrophic hypogonadal infertile men.

Methods

A literature review was performed in MEDLINE using the terms infertility, hypogonadism, testosterone, luteinizing hormone, and follicle stimulating hormone. Both basic and clinical studies were included.

Physiology of the HPG axis

Male fertility and proper functioning of the HPG axis are closely intertwined. Spermatogenesis depends on the pulsatile release of gonadotropins, follicle stimulating hormone (FSH), and luteinizing hormone (LH), from the pituitary, and their action on their cells in the testis. FSH signals to FSH receptors (FSHR) on Sertoli cells

to initiate spermatogenesis, whereas LH signals to LH receptors (LHR) on Leydig cells to produce intratesticular testosterone (2). The qualitative and quantitative integrity of these receptors plays an equally significant role in the regulation of spermatogenesis. Unsurprisingly, inactivating mutations of the FSHR and LHR results in impaired fertility (3,4). However, gonadotrophic overstimulation can also have a detrimental effect on spermatogenesis by downregulating the expression of LHR and FSHR (5). This effect may explain the clinical observations of Ross *et al.*, who observed a reversible decline in sperm motility following high dose administration of selective estrogen receptor modulators (SERMs), which function by increasing the gonadotropin levels (6).

High intratesticular testosterone (ITT) levels maintain the spermatogenic process and prevent germ cell death (7). Low ITT levels result in an impaired blood-testis barrier permitting immune cells to enter the seminiferous tubules and attack autoantigenic germ cells [reviewed by Walker (8)]. Low intratesticular testosterone levels also block the conversion of round spermatids to elongating spermatogonia and prevent spermiation, leading to phagocytosis of spermatids by Sertoli cells (8,9).

It is well established that exogenous testosterone therapy causes azoospermia in the majority of men (10). Exogenous testosterone's contraceptive effect occurs through its suppression of the HPG axis, preventing LH and FSH release and their respective gonadal functions (11). Studies examining the reproductive outcomes and rates of azoospermia development of various testosterone treatment modalities show less detrimental effects with testosterone patches [24% rate of azoospermia (12)] compared to intramuscular injections of testosterone enanthate, which result in up to an 98% rate of combined azoospermia and oligozoospermia (≤3 million/cc) after 1 year of therapy (13).

In a male contraceptive study, Coviello *et al.* followed seven healthy men with serum hormone assessments and percutaneous testicular aspirates to assess intratesticular hormone levels (14). They showed a 98% suppression of LH, 97% suppression of FSH, and 93% suppression of intratesticular androgenic bioactivity levels following weekly administrations of intramuscular testosterone enanthate (TE) 100 mg with levonorgestrel over a 6-month treatment period compared to baseline. Levonorgestrel was administered to suppress the HPG axis further. One subject failed to suppress spermatogenesis with a nadir sperm count of 3.4 mill/mL despite a reduction in his ITT level from 1,607 to 29 nmol/L (98% reduction). This study demonstrates the heterogeneity in TRT's contraceptive effect and the variability in intratesticular testosterone levels necessary for spermatogenesis to occur. Conversely, recovery of the HPG axis function following testosterone therapy cessation is possible but can take between 110 days and 2 years (15,16).

Strategies to preserve fertility when testosterone therapy cannot be stopped

Hypogonadal men desiring fertility can become symptomatic beyond the capacity of current non-TRT medical therapies [see review by McBride and Coward (17) for non-TRT medical therapy]. Fertility can be maintained and potentially improved by the combination of exogenous testosterone therapy and adjunctive therapies to preserve spermatogenesis. Fertility data is available for the use of concomitant use of human chorionic gonadotropin (HCG) and aromatase inhibitor (AI) therapy with TRT. Below is evidence supporting the use of HCG, SERMs, and AI therapy.

HCG

HCG is a heterodimeric glycoprotein and an LH analog that binds to the LH receptor [also known as the luteinizing hormone/choriogonadotropin receptor (LHCGR)] to induce steroidogenesis. In studies comparing the intracellular effects of LH and HCG activation of the LHCGR (18,19), HCG activation results in significantly higher cyclic adenosine-monophosphate (cAMP) levels, promoting anti-apoptotic and proliferative cell signaling events. However, there was no significant difference in testosterone production between the two gonadotropins, HCG and LH, in a murine Leydig cell model (18).

Due to its ability to stimulate Leydig cells independent of the HPG axis, HCG has been used as an adjunct therapy in men receiving testosterone supplementation who wish to preserve spermatogenesis. Coviello *et al.* (20) showed increasing doses of HCG administered concomitant with 200 mg TE intramuscular injections in 29 healthy, and eugonadal men resulted in dose-dependent increases in the ITT levels. The men that were given TE and HCG 500 international units (IU) every other day had a 26% increase in their ITT levels over their baseline. Studies examining the ITT levels with high dose HCG are lacking.

In addition to maintaining ITT, low-dose HCG can preserve semen parameters in men on TRT. This was shown

by Hsieh *et al.* (21) in a retrospective review of 26 men on testosterone replacement who were also given HCG 500 IU every other day. At 1 year, none of the patients became azoospermic, and no difference was observed in the semen volume, sperm density, or motility. This stability in semen parameters was not seen in an earlier study by Matsumoto and Bremmer (22), who followed four men administered 200 mg TE and 5,000 IU three times weekly. Men on combination therapy with high-dose HCG showed a decrease in ejaculated sperm concentration from a mean (± SEM) concentration of 79 (±7) million sperm/mL to 25 (±4) million sperm/mL after 6 months of combination therapy.

Selective estrogen receptor modulators and aromatase inhibitors

SERMs and AI exert their action by inhibiting the negative feedback of estrogen on the hypothalamus and the anterior pituitary gland leading to increased LH and FSH production.

Among the SERMs, clomiphene citrate (CC) and tamoxifen citrate are commonly used for male patients with hypogonadal symptoms as monotherapy or in combination with HCG. To our knowledge, no studies are assessing the reproductive outcomes of SERMs given in conjunction with TRT. Therefore, it is unknown if central estrogen inhibition is enough to overcome the HPG suppression of TRT.

AI blocks the conversion of testosterone to estradiol by the enzyme aromatase. Commonly used AI for hypogonadism and male fertility include letrozole and anastrozole. While AI use is well established as monotherapy in hypogonadal men [reviewed by Tan *et al.* (23)], data supporting the use of AI and TRT is less rigorous. In a cohort of ten patients with Klinefelter syndrome, Mehta *et al.* (24) showed a 70% surgical sperm retrieval rate in men given topical testosterone therapy with a goal testosterone of >400 ng/dL combined with 1 mg of anastrozole daily.

Few studies have examined the impact of combined testosterone therapy and AI on the HPG axis. Saki *et al.* (25) studied bone mineral density and HPG axis response in a four-arm sham-controlled study that compared control rats to orchiectomized rats that were given either given no additional therapy *vs.* TE intramuscular injections *vs.* TE and letrozole. The sham-control rats had LH values of 0.5±0.12 mIU/mL, orchiectomized rats given TE had LH-suppression with LH values of 0.21±0.16 mIU/mL, and orchiectomized rats given TE and letrozole had LH

values of 0.45±0.45 mIU/mL. These results suggest the potential for HPG stimulation with AI despite testosterone suppression. However, it is unclear if TRT+AI therapy can achieve enough HPG stimulation to maintain spermatogenesis.

Nasal testosterone

While it is known that testosterone pellets, intramuscular, and transdermal testosterone have detrimental effects on the HPG axis and spermatogenesis, newer modes of testosterone delivery may yield different results. Unique to nasal testosterone gel is its short half-life of only 10–100 minutes and its lack of pituitary gonadotropin suppression. This allows nasal testosterone to normalize androgen levels while maintaining baseline LH and FSH levels (26,27). Masterson *et al.* (28) explored nasal testosterone's effects on semen parameters as part of a phase IV clinical trial. Seventeen reproductive-aged men (mean age 35) received nasal testosterone three times daily. They had reproductive hormone measurements and semen analyses at baseline and intervals of 1 month, 3 months, and 6 months. Fifteen of the 17 enrolled men reached a 1-month follow-up, six reached a 3-month follow-up, and five reached a 6-month follow-up. The authors determined that after 1 month, 93% (14/15) of the men achieved eugonadal testosterone levels (>300 ng/dL) with a median value of 423 ng/dL. For the men who reached the 3-month interval, the median total motile sperm count (TMSC) decreased from 37.5 to 24.8 million. However, at 6 months, the median TMSC increased to 32.5 million. Additionally, they observed no statistically significant change in LH and FSH levels throughout the trial (27). While this preliminary data is promising, more significant and longer-term studies are needed to confirm these findings.

HPG axis reset—a new tool in our armamentarium?

Among men with hypergonadotrophic, hypogonadal non-obstructive azoospermic (NOA) men, excess gonadotropin exposure carries the potential for desensitizing Leydig and Sertoli cells (5). This is believed to occur due to a decreased responsiveness of LH and FSH-responsive adenylyl cyclase following hyperstimulation. Increased cAMP catabolism due to FSH stimulated phosphodiesterase activity results in a refractory period for Sertoli cells (29,30). Thus, a therapeutic dilemma with these men is the limited number

Table 1 Demographics and the impact of HPG axis reset on perioperative hormone levels and microsurgical sperm recovery

| Patient	AGE	Dx	Baseline T (ng/dL)	Pre TRT FSH (mIU/mL)	Pre TRT LH (mIU/mL)	Duration of HPG axis reset	Prior sperm retrieval	Pre mTESE FSH (mIU/mL)	Pre mTESE LH (mIU/mL)	Pre mTESE T (ng/dL)	Pre mTESE inhibin B (pg/mL)	Outcome of mTESE	iVF outcome
1	30	KS	150	21.8	25.9	10 Months	none	2.6	0.6	582	<10	+ SPERM; 3 sperm	1 failed FET
2	33	KS	120	56.6	54.5	8 weeks	(–) mTESE	1.1	Not avail	Not avail	<10	+ round spermatids; 3 seen	Not attempted
3	36	Idiopathic	128	18.8	13.7	4 months	None	Not avail	7.1	352	<7	+ sperm; 3/HPF	10 eggs fertilized, arrested at 8 cell stage

of options to improve the intratesticular testosterone before sperm search operations mixed with the potential for a detrimental effect of gonadotropins on Sertoli and Leydig cells. Emerging evidence has provided a potentially novel protocol for improving spermatogenesis in infertile men with hypergonadotrophic, hypogonadal NOA.

Among female infertility patients, controlled ovarian hyperstimulation (COH) protocols exist to improve the response rate of women who respond poorly to *in vitro* fertilization (IVF) hormonal regimens, including the administration of estradiol patches and manipulation of the HPG axis with gonadotropins (31). COH protocols incorporate transdermal estradiol and GnRH-a in the luteal phase, followed by the administration of gonadotropins in the follicular phase to optimize the follicular hormonal milieu and antral follicle responsiveness. This protocol addresses the idea that individual follicles may have increased innate FSH receptor sensitivity, allowing for an increased response to a low rise in FSH in the early luteal phase. Thus, these follicles may demonstrate greater responsiveness to exogenous gonadotropins in the subsequent follicular phase leading to heterogeneity and unsynchronized follicular maturation before oocyte retrieval (32). The COH protocol utilizes early FSH suppression with estradiol and GNRH-a to counteract the innate FSH receptor sensitivity of individual follicles allowing for more synchronized follicular maturation and decreased heterogeneity after hyperstimulation with gonadotropins in the follicular phase (33). Clinically, this resulted in a significantly reduced number of canceled IVF cycles and improved considerably the number of oocytes retrieved and embryos transferred (31).

A similar protocol can be employed in men via HPG axis suppression and reactivation in a more controlled, pulsatile manner. In 2018, Hu *et al.* (34) investigated the effects of suppressing endogenous gonadotropins with GNRHα (Goserelin) and replacing them with exogenous gonadotropins in idiopathic NOA men. A total of 35 men were enrolled who had failed testicular sperm extraction (TESE). There were 10 men in a control group and 25 men in a group that received 150 IU of human menopausal gonadotropins (hMG) twice weekly and 2,000 IU of HCG therapy once weekly after HPG suppression. Ultrasound assessed the testicular volume and hormone levels were followed. An inhibin rise was seen in 11 of 25 patients after 4 weeks on the gonadotropin therapy; however, this rise was sustained in only five patients at 20 weeks. Among these five men, one recovered sperm in their ejaculate at a concentration of 1.42×10^{6}/mL and another had sperm found on repeat TESE.

Additional evidence for HPG axis reset: an independent case series

Other case reports come from a review of the corresponding author's patients, including two patients with non-mosaic Klinefelter syndrome (47 XXY) and a third patient with idiopathic infertility who were all hypergonadotrophic, hypogonadal, and azoospermic on at least two semen analyses. In preparation for microsurgical testicular sperm extraction (microTESE), all three patients were symptomatic from their hypogonadism (*Table 1*). The two men with non-mosaic Klinefelter syndrome showed pre-treatment testosterone (ng/dL) and FSH (mIU/mL) levels
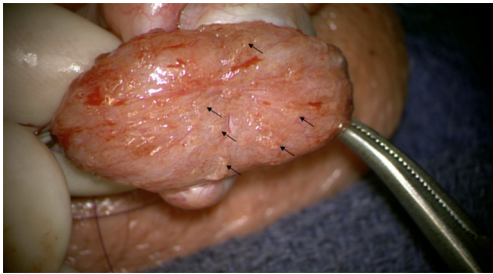
Figure 1 Microsurgical testicular sperm extraction in a male with 47XXY who underwent hypothalamic-pituitary-gonadal (HPG) axis reset. Black arrows indicate representative areas of dilated seminiferous tubules.

of 120–150 ng/dL and 21.8–56.6, respectively. Following the administration of TE 200 mg/mL per week and 3000 IU of HCG three times weekly for a minimum of 6 weeks, FSH levels for the 47XXY men decreased to 1.1–2.6 mIU/mL and testosterone levels rose to >300 ng/dL in both patients. The third patient's pre- and post-treatment LH (mIU/mL) levels decreased from 13.7 to 7.1. Pre-treatment FSH levels were 18.8 mIU/mL; however, post-treatment FSH levels were not available. All patients were found to have sperm on microsurgical TESE (*Figure 1*). *In vitro*, fertilization was attempted for one patient with Klinefelter syndrome which resulted in an unsuccessful fresh embryo transfer despite high grade embryos (blastocyst stage - 2AA). From this IVF cycle, 30 oocytes were retrieved, 2 high-quality embryos were formed, and one was transferred. The second high-quality embryo was a cryopreserved pending repeat transfer. No IVF cycles have been attempted for the second or third patient.

One explanation for the successful sperm retrieval in these patients is the suppression of gonadotropins to more physiologic levels (<10 mIU/mL). Perhaps a further reduction of gonadotropins to undetectable levels may not have yielded the same outcome.

While this case series of three patients is small, it is the first report of TRT used to enhance the fertility potential of hypergonadotrophic, hypogonadal NOA men who previously had no effective strategy for hormonal optimization before their sperm retrieval. The observations made permit expression of a hypothesis that needs further investigation and validation.

Conclusions

Keeping men on TRT fertile has proven to be difficult

for male fertility specialists. When the option of avoiding testosterone with alternative therapies, such as clomiphene citrate, aromatase inhibitors, and human chorionic gonadotropin, is not possible, some strategies can mitigate the negative impact of TRT on spermatogenesis. It is important to note that these therapies are not well established, possess limitations in their applicability, and carry side effects.

Novel methods of treating hypogonadal symptoms and keeping men fertile include the use of nasal testosterone and resetting the HPG axis with TRT and controlled administration of HCG. While preliminary results suggest that these methods may increase success for fertility treatments, more extensive research is needed to demonstrate the efficacy and safety of these therapies. The most recent evidence provides hope for the future of male fertility in patients that require TRT.

Footnote

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