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Low-Dose Human Chorionic Gonadotropin Maintains Intratesticular Testosterone in Normal Men with Testosterone-Induced Gonadotropin Suppression

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In previous studies of testicular biopsy tissue from healthy men, intratesticular testosterone (ITT) has been shown to be much higher than serum testosterone (T), suggesting that high ITT is needed relative to serum T for normal spermatogenesis in men. However, the quantitative relationship between ITT and spermatogenesis is not known. To begin to address this issue experimentally, we determined the dose-response relationship between human chorionic gonadotropin (hCG) and ITT to ascertain the minimum dose needed to maintain ITT in the normal range. Twenty-nine men with normal reproductive physiology were randomized to receive 200 mg T enanthate weekly in combination with either saline placebo or 125, 250, or 500 IU hCG every other day for 3 wk. ITT was assessed in testicular fluid obtained by percutaneous fine needle aspiration at baseline and at the end of treatment. Baseline serum T (14.1 nmol/liter) was 1.2% of ITT (1174 nmol/liter). LH and FSH were profoundly suppressed to 5% and 3% of baseline, respectively, and ITT was suppressed by 94% (1234 to 72 nmol/liter) in the T enanthate/placebo group. ITT increased linearly with increasing hCG dose (*P* < 0.001). Posttreatment ITT was 25% less than baseline in the 125 IU hCG group, 7% less than baseline in the 250 IU hCG group, and 26% greater than baseline in the 500 IU hCG group. These results demonstrate that relatively low dose hCG maintains ITT within the normal range in healthy men with gonadotropin suppression. Extensions of this study will allow determination of the ITT concentration threshold required to maintain spermatogenesis in man. (*J Clin Endocrinol Metab* 90: 2595–2602, 2005)

TESTOSTERONE (T) PRODUCTION and spermatogenesis are the two primary functions of the testis in man. Normal testicular function is dependent on the intratesticular activity of the pituitary gonadotropins, LH and FSH. LH stimulates Leydig cells to produce T within the testis. Intratesticular T (ITT) is an absolute prerequisite for normal spermatogenesis. FSH is also vital for normal testicular function and is necessary for quantitatively normal spermatogenesis in man (1, 2). Specifically, FSH is thought to play an important role early in spermatogenesis during spermatogonial maturation as well as late in the process during spermiation (3). The relative roles of intratesticular androgens and FSH are not fully understood in man.

Control of the intratesticular hormonal environment is in large part regulated through negative feedback of T at the level of the hypothalamus and the pituitary (4). Exogenous T has been shown to dramatically suppress gonadotropin release when administered at supraphysiological as well as physiological doses (5, 6). Administration of T alone has been shown to reduce sperm production in the majority of men to levels acceptable for contraception (7, 8). Gonadotropin withdrawal has also been shown to dramatically reduce ITT, which, in turn, decreases sperm production (9, 10). However, suppression of spermatogenesis is not uniform, and why some men are nonresponders is not clear. Possibilities include incomplete gonadotropin suppression, particularly with regard to FSH as well as inconsistencies in ITT suppression (5, 11, 12). The failure to uniformly suppress sperm production must be addressed in order to develop an effective male hormonal contraceptive.

Greater knowledge of the intratesticular hormonal environment necessary for normal spermatogenesis would contribute to the development of safe, effective, reversible male hormonal contraceptives. Investigation of the intratesticular hormonal environment has been hampered by the lack of safe, effective, and reliable methods for repeated sampling of the intratesticular environment. ITT has been measured in testicular biopsy tissue in cross-sectional studies. Percutaneous aspiration of testicular fluid allows for repeated sampling of the intratesticular environment for the measurement of T as well as other hormones.

The purpose of this study was 1) to evaluate ITT before and after gonadotropin withdrawal with exogenous T adminis-

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Abbreviations: DHT, Dihydrotestosterone; FNA, fine needle aspiration; hCG, human chorionic gonadotropin; ITT, intratesticular testosterone; LNG, levonorgestrel; T, testosterone; TE, testosterone enanthate.

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tration in a dose known to induce azoospermia, and 2) to evaluate ITT levels in response to graded doses of human chorionic gonadotropin (hCG) for selective replacement of LH activity. Determining the dose-response relationship between hCG and ITT will allow future studies of the dose-response relationship of ITT and spermatogenesis. Greater understanding of the effect of hCG on ITT will facilitate the design of future studies of the intratesticular hormonal microenvironment in relation to spermatogenesis with a focus on the ability to achieve uniform azoospermia for the ultimate goal of developing a successful male contraceptive.

Subjects and Methods

Subjects

The institutional review board for clinical investigation of University of Washington approved this study protocol before the initiation of this study. The study was conducted in the General Clinical Research Center facility at University of Washington Medical Center. Written informed consent was obtained from subjects before the screening evaluation.

Healthy men, aged 18–45 yr, with normal reproductive physiology were recruited for the study. Normal reproductive physiology was defined as normal physical exam, including testicular exam with Prader orchidometer; normal serum T, LH, and FSH; and sperm count more than 20 million/ml after 48 h of ejaculatory abstinence. Subjects had to be healthy as determined by medical history, physical examination, and clinical laboratory tests within normal limits. Exclusion criteria included chronic medical or mental illness, previous or current ethanol abuse, anabolic steroid use, abnormal screening laboratory tests, single functioning testicle in the scrotum, abnormal reproductive physiology as defined above, and participation in a hormonal contraceptive study within the past 6 months. Twenty-nine healthy men with a mean age of 24 yr (range, 18–41 yr) were randomly assigned to one of four treatment groups. All men were treated with T enanthate (TE), 200 mg im weekly, for rapid gonadotropin suppression in conjunction with a variable dose of hCG, delivered sc every other day for 3 wk: 0 (saline placebo), 125, 250, or 500 IU hCG. The placebo group served as the control group.

Blood was drawn at baseline and weekly during the 3-wk treatment phase. Serum T, LH, and FSH and sperm count were repeated 3 months after the start of the study to check for return to normal baseline status. Serum was separated by centrifugation and stored at −70 C until assays for serum T, LH, FSH, and hCG levels were done at the end of the study. Testicular fluid was obtained through percutaneous aspiration, as described below, at baseline and after 3 wk of treatment with TE and hCG on d 21 (13). The first dose of hCG was given immediately after the first percutaneous fine needle aspirate of testicular fluid on d 0, and the last dose of hCG was delivered on d 20 of the study, the day before the second percutaneous fine needle aspirate of testicular fluid on d 21.

Percutaneous testicular fluid aspiration

Testicular fluid was sampled by percutaneous fine needle aspiration (FNA) at baseline and after 3 wk of treatment with TE and hCG. The testicular fluid collected by blind FNA is a mixture of interstitial fluid and seminiferous tubule fluid, although it is probably predominantly seminiferous tubule fluid because seminiferous tubules form the bulk of testicular volume. While developing this procedure, testicular fluid samples were analyzed microscopically to confirm the presence of sperm and, hence, seminiferous tubule fluid (13).

Subjects were placed in the supine position and appropriately draped. The skin over the spermatic cord was cleansed with alcohol on both sides. A cord block was then performed bilaterally with 1% buffered lidocaine injected around the spermatic cord. The skin overlying the anterior-superior portion of the testes was then cleansed with alcohol. A 19-gauge butterfly needle with tubing was attached to a 5-cc syringe via a three-way stopcock and inserted percutaneously into the anterior superior portion of the testicle. Negative pressure was created in the syringe with the three-way stopcock. The needle was held in place until an adequate amount of testicular fluid (>5 μl) was withdrawn into the tubing. The tubing was then clamped with a hemostat, and the needle was withdrawn to eliminate reflux of fluid from within the tunica albuginea. The tubing with testicular fluid sample was immediately placed on ice. The aspirate procedure was then repeated on the contralateral testicle. Testicular fluid samples were withdrawn from the butterfly tubing, immediately placed on ice, and centrifuged at 300 × *g*. Supernatant fluid was stored at −70 C. Right and left testicular fluid samples were pooled for ITT measurement. Two of the 29 individuals had insufficient testicular fluid from the second aspiration procedure for ITT measurement: one in the 125-IU hCG group and one in the 250-IU hCG group.

Serum hormones

Serum LH and FSH were measured by immunofluorometric assay (Delfia, Wallac, Inc., Turku, Finland). All samples from a single individual were run in the same assay to eliminate differences due to interassay variation. The sensitivity of the LH assay was 0.019 IU/liter, and the intra- and interassay coefficients of variation for a midrange pooled value of 1.2 IU/liter were 3.2 and 12.5%, respectively. The sensitivity of the FSH assay was 0.016 IU/liter, with intra- and interassay coefficients of variation of 2.9 and 6.1%, respectively, for a midrange pooled value of 0.96 IU/liter. hCG had a cross-reactivity of 0.02% in the LH assay and was undetectable in the FSH assay.

The serum T assay was a solid phase RIA (Coat-A-Count total T assay, Diagnostic Products Corp., Los Angeles, CA), with a lower limit of detection of 0.14 nmol/liter. The interassay coefficient of variation was 4.9%, and the intraassay coefficient of variation was 8.2% for a midrange pooled value of 13.2 nmol/liter.

Serum hCG was measured with a time-resolved immunofluorometric assay (Delfia, Wallac, Inc.) with sensitivity of 0.5 IU/liter. Inter- and intraassay coefficients of variation were 3.2 and 4.1%, respectively, for a midrange pooled value of 72.8 IU/liter. The cross-reactivity with LH was less than 0.5%, 0.02% with FSH, and 0.08% with TSH.

All serum hormone assays were run in the same laboratory at University of Washington. Samples from the same individual were run simultaneously, and all assays were performed in duplicate.

ITT

Intratesticular fluid T was measured with a well-validated RIA (13–18). For this assay, samples were extracted with diethyl ether, followed by measurement of T by RIA. [1,2,6,7,16,17-N-^{3}H]T (specific activity, 140.9 Ci/mmol) was obtained from NEN Life Science Products (Wilmington, DE). Rabbit T antiserum was obtained from ICN Biomedicals, Inc. (Costa Mesa, CA). The sensitivity and intra- and interassay coefficients of variation of the RIAs for T were 10 pg/tube, 11.2%, and 9.6%, respectively. All samples for a given individual were assayed simultaneously, and all samples were assayed in the same laboratory at Johns Hopkins University. There were no apparent matrix effects in the intratesticular fluid assay. There was some minimal cross-reactivity (<5%) of the antibody with dihydrotestosterone (DHT). Because DHT constitutes a very small percentage (~1–2%) of the androgen present within the testes (9), the amount of DHT potentially measured with the assay in these samples was very small (<1%).

Semen analysis

Semen samples were assessed for volume, then analyzed for total sperm count and sperm concentration. Sperm counts were determined by computer assisted semen analysis (Hamilton-Thorn IVOS, Beverly, MA). Semen analysis and sperm counts were not an end point in this study due to the 3-wk study timeframe.

Statistical analysis

All hormone data were log-transformed for statistical analyses, then back-transformed for ease of presentation. Data are presented as the mean ± SEM, except where noted otherwise. ANOVA was used to detect treatment effects within and across groups over time as well as across groups over single time points. Significant changes over time within groups were analyzed for change from baseline at individual time points with paired *t* tests. The Mann-Whitney rank-sum test was used for comparisons of ITT between groups. Simple and multiple linear regression analyses were used to examine the relationship between hormones and ITT. StatView version 5.0.1 (SAS Institute, Cary, NC) and Stata version 6.0 (StataCorp LP, College Station, TX) statistical software were used for analyses. α was set at 0.05.

Results

The mean age of the subjects was 24 ± 1.3 yr. There were no significant differences between groups at baseline in age, body mass index; sperm count; serum T, LH, or FSH; or ITT (Table 1). All 29 participants completed the study. The FNA procedure was well tolerated by all participants without significant discomfort. There were no significant adverse events during the study.

Compliance with TE injections was 100%. Of a total of 319 hCG/placebo sc injections in 29 participants over a 3-wk period, three were missed giving an overall drug compliance rate of 99.1%. The three missed sc injections were in three different participants, one in the placebo group and two in the 250-IU hCG group. The latter two individuals both missed a single 250-IU hCG dose early in the 3-wk treatment phase on d 5. Therefore, it is unlikely that the missed hCG doses significantly affected the intratesticular fluid T concentrations measured on d 21.

Serum hCG

Serum hCG showed a dose-dependent increase during the 3-wk treatment phase among groups receiving hCG, with undetectable levels in the placebo group (Fig. 1). hCG was administered every other day, whereas serum hCG levels were measured once per week. Blood samples on d 7 and 21 were drawn about 24 h after hCG administration (note similar serum levels on d 7 and 21), whereas the blood samples drawn on d 14 were taken approximately 48 h after the last hCG dose and therefore represent a trough serum level during treatment. The lower serum hCG levels observed on d 14 in all three treatment groups were presumably due to the longer interval between the last hCG dose and the blood sample drawn on d 14 *vs.* d 7 and 21.

Serum T

The mean serum T concentration for all volunteers before treatment was 14.1 ± 1.1 nmol/liter (Table 1). Serum T was significantly elevated from baseline in all four groups by d 7 ($P < 0.0001$). The lowest hCG dose group had serum T levels similar to those in the placebo group, whereas higher serum levels were achieved in the two highest hCG groups, 250 and 500 IU (Fig. 2).

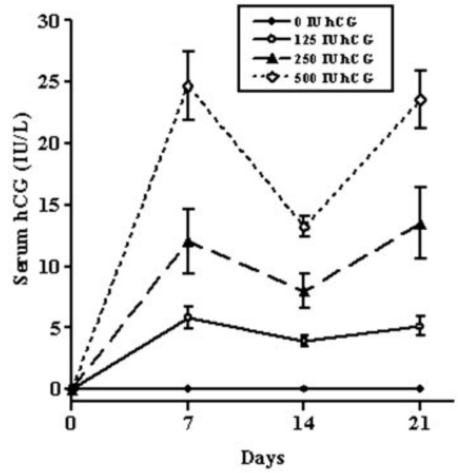

FIG. 1. Serum hCG during the treatment phase by group. Values are the means + SEM (*bars*). The placebo group (●) had undetectable serum hCG. The graph depicts the increasing serum hCG levels achieved with increasing hCG dose. Serum hCG levels were measured 1 d after the last hCG dose on d 7 and 21 of the treatment phase and on 2 d after the last hCG dose on d 14 of the treatment phase.

Serum gonadotropins

Serum LH and FSH were suppressed to less than 1.0 IU/liter after 3 wk of treatment with hCG and TE. There were no differences in LH and FSH between treatment groups at baseline or at any time point during the treatment phase (Fig. 3). Mean LH was suppressed from 4.11 ± 0.37 to 0.21 ± 0.03 IU/liter (5% of baseline) on d 21. Mean FSH was suppressed from 2.83 ± 0.37 to 0.09 ± 0.01 IU/liter (3% of baseline) on d 21. Gonadotropin levels returned to normal baseline levels in all participants at the end of the recovery phase (data not shown).

ITT

The mean weight of testicular fluid obtained from percutaneous aspiration was 18 ± 2 mg (range, 1–188 mg). The mean baseline ITT concentration for all 29 participants before treatment was 1174 ± 79 nmol/liter (range, 60–2080 nmol/liter; Fig. 4A). TE administration combined with placebo hCG (saline) suppressed ITT 94%, from a mean of 1234 ± 179 nmol/liter at baseline (range, 653-2080 nmol/liter) to 72 ± 10 nmol/liter (range, 42–112 nmol/liter) after 3 wk of treatment ($P < 0.001$). Intratesticular fluid T decreased in all seven subjects administered placebo hCG.

Simple linear regression showed a linear increase in ITT

TABLE 1. Baseline characteristics of 29 participants and by treatment group

	Baseline (n = 29)	hCG treatment group			
		0 IU (n = 7)	125 IU (n = 8)	250 IU (n = 7)	500 IU (n = 7)
Age (yr)	24 ± 1.3	21 ± 0.8	27 ± 2.5	25 ± 2.3	25 ± 3.4
BMI (kg/m^2)	25 ± 0.6	26 ± 0.7	26 ± 2.3	24 ± 3.0	24 ± 2.2
Sperm count (millions/ml)	110 ± 12	107 ± 34	94 ± 26	132 ± 11	111 ± 15
LH (IU/liter)	4.11 ± 0.37	3.57 ± 0.35	4.59 ± 0.91	4.04 ± 0.96	4.16 ± 0.68
FSH (IU/liter)	2.83 ± 0.37	2.54 ± 0.55	3.91 ± 0.99	1.81 ± 0.35	2.91 ± 0.74
Serum T (nmol/liter)	14.1 ± 1.1	14.8 ± 1.3	12.3 ± 2.2	15.3 ± 1.5	14.2 ± 3.4
ITT (nmol/liter)	1174 ± 79	1234 ± 179	969 ± 145	1402 ± 125	1120 ± 163

Values are the mean ± SEM. BMI, Body mass index.

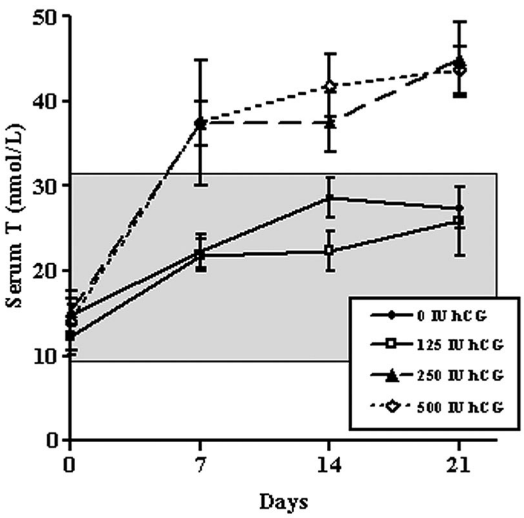

FIG. 2. Serum T during the treatment phase by group. Values are the mean + SEM (*bars*). The *shaded box* represents the normal reference range of serum T in healthy men for this assay. Serum T increased from baseline in all four groups in response to TE (200 mg, im, weekly; $P < 0.05$) and remained elevated during the treatment phase. The two higher hCG dose groups (250 and 500 IU, sc, every other day) had serum T levels above the normal range during the treatment phase.

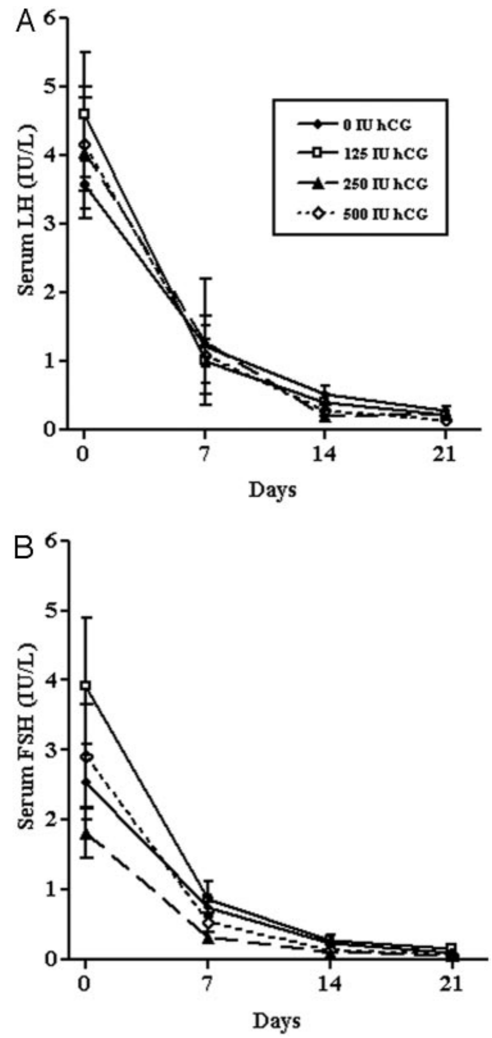

FIG. 3. A, Serum LH during the treatment phase by group. Values are the mean + SEM (*bars*). There was no significant difference in baseline or treatment phase LH levels between groups. LH was suppressed to 5% of baseline by d 21 of the treatment phase. B, Serum FSH during the treatment phase by group. Values are the mean + SEM (*bars*). There was no significant difference in baseline or treatment phase FSH levels between groups. Mean FSH was suppressed to 3% of baseline by d 21 of the treatment phase.

with increasing hCG dose ($P < 0.001$) as well as increasing serum hCG ($P < 0.001$; Fig. 4B). ITT decreased 25% in the 125-IU hCG/TE group from a mean of 969 ± 145 nmol/liter (range, 60–1418 nmol/liter) to 726 ± 144 nmol/liter (range, 97–1180 nmol/liter) after treatment, but was not statistically different from baseline ($P > 0.05$). ITT decreased from baseline in five of these individuals, but increased in two; one subject in this group did not have sufficient fluid volume for analysis at the second aspiration.

ITT decreased by 7% in the TE/250-IU hCG group, from a mean of 1402 ± 125 nmol/liter (range, 1089–1984 nmol/liter) to 1306 ± 316 (range, 608-2948 nmol/liter) after treatment, but was not statistically different from baseline ($P > 0.05$). ITT decreased in three individuals and increased in three individuals from baseline in this group; one subject in this group did not have sufficient fluid volume for analysis at the second aspiration.

ITT increased 26% in the TE/500-IU hCG group from a mean of 1120 ± 163 nmol/liter (range, 383-1766 nmol/liter) to 1409 ± 286 nmol/liter (range, 109-2267 nmol/liter) after treatment, but was not statistically different from baseline ($P > 0.05$). ITT increased from baseline in five individuals and decreased in two individuals in this group.

All three hCG/TE groups had posttreatment ITT levels statistically significantly higher than the posttreatment ITT level in the placebo hCG/TE group ($P < 0.01$).

Serum hCG and ITT (regression analysis)

After 3 wk of treatment with hCG/TE, serum hCG showed a positive linear relationship with intratesticular fluid T. hCG was a statistically significant determinant of intratesticular fluid T when analyzed by hCG dose group ($P < 0.001$) or by serum hCG level ($P < 0.001$; Fig. 4B).

Serum LH, FSH and ITT (regression analysis)

After 3 wk of treatment with TE and hCG, serum LH was not predictive of ITT ($P = 0.6$). Serum FSH was negatively correlated with ITT ($P = 0.02$). However, neither posttreatment serum FSH ($P = 0.41$) nor serum LH ($P = 0.93$) was a predictor of ITT in the presence of serum hCG ($P < 0.001$) in multiple linear regression modeling.

Serum T and ITT

Comparisons of serum and intratesticular T must be made with caution because T levels were measured by two different RIAs (described above), one for serum T and one for intratesticular fluid T. The mean baseline ITT concentration for all 29 participants before treatment (1174 ± 79 nmol/liter) was approximately 80-fold higher than that of serum T (14.1 ± 1.1 nmol/liter; Fig. 5). Although serum T increased

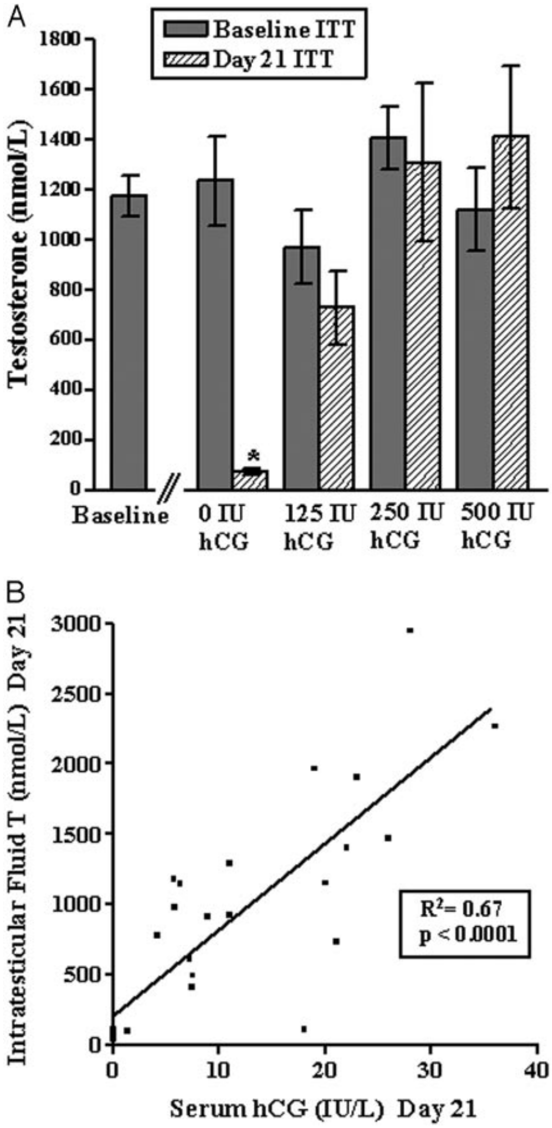

FIG. 4. A, Intratesticular fluid T levels at baseline and after 3 wk of therapy with TE (200 mg, im, weekly) plus saline placebo or hCG (125, 250, or 500 IU) sc every other day. ■, Mean baseline ITT levels; ▨, mean posttreatment ITT levels on d 21. The first column is the mean baseline ITT for all 29 participants before the start of TE/hCG treatment. The remaining four pairs of columns show baseline and posttreatment ITT by hCG dose group. There was no difference in baseline ITT among the four treatment groups. Posttreatment ITT levels differed (by ANOVA, $P < 0.001$). *, Posttreatment ITT was significantly lower than baseline ITT in the TE and placebo hCG group ($P < 0.001$). Posttreatment ITT was not statistically different from baseline values in the three hCG dose groups (125, 250, and 500 IU). Values are the mean + SEM (*bars*). All three hCG dose groups had posttreatment ITT levels that were significantly higher than the posttreatment ITT level in the TE plus placebo group. B, Serum hCG demonstrates a positive linear relationship with ITT after 3 wk of treatment with TE and hCG by simple linear regression ($P < 0.0001$). The data presented are raw data. Simple linear regression performed with log-transformed data yielded $r^2 = 0.76$ ($P < 0.0001$). Serum hCG remained the only statistically significant predictor of ITT in multiple linear regression models including serum T, LH, and FSH.

from baseline in all groups ($P < 0.05$), ITT remained significantly higher than serum T in all four groups after treatment ($P < 0.05$; Fig. 5). After 3 wk of TE/hCG treatment, serum T

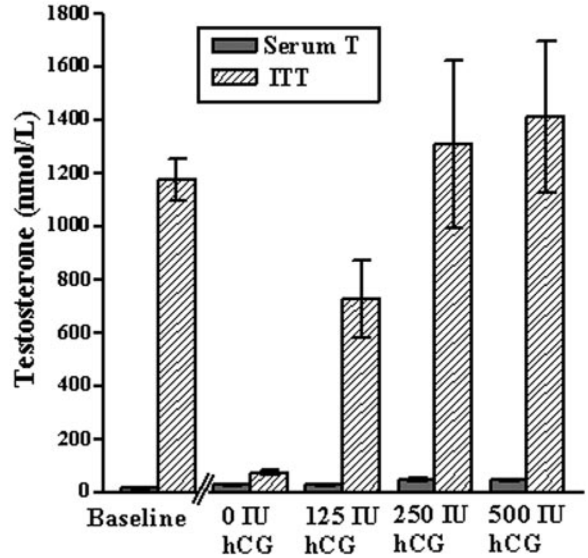

FIG. 5. Comparison of serum T and ITT levels at the end of the treatment phase. ■, Serum T; ▨, ITT. Values are mean with SEM bars. The first pair of columns to the *left* show the mean baseline serum T and ITT levels for all 29 participants. The four pairs of columns to the *right* show the mean serum T and ITT levels for the four treatment groups on d 21 after 3 wk of TE plus saline placebo or hCG treatment. Serum T increased in all four treatment groups from baseline ($P <$ 0.05), but remained lower than posttreatment ITT levels in all four groups ($P < 0.05$). Although posttreatment ITT was higher than serum T levels in all four groups, the high ITT to serum T gradient seen at baseline (84×) was not maintained during the treatment phase. There was a 2- to 3-fold gradient in the placebo group, a 28-fold gradient in the 125-IU hCG group, a 29-fold gradient in the 250-IU hCG group, and 32-fold gradient in the 500-IU hCG group. Comparisons of serum T and ITT must be made with caution, given that T levels were measured by two different RIAs, one for serum samples and one for intratesticular fluid (see *Subjects and Methods* for assay details).

showed a positive linear relationship with ITT ($P = 0.002$). However, hCG dose appeared to be the more significant determinant of ITT. In a multiple linear regression model, serum T ($P = 0.7$) became an insignificant predictor of ITT in the presence of hCG ($P < 0.001$).

Discussion

A significant intratesticular fluid to serum T gradient was observed in this group of young normal men at baseline. In this study, serum T was 1.2% of ITT, an 84-fold gradient. A similar testicular to serum gradient has been reported in studies of testicular biopsy tissue in the 1970s (19) as well as more recently (9, 13). However, the absolute ITT levels reported in testicular homogenates are higher than the ITT levels found in the testicular fluid aspirates in this study. This difference is probably the result of the release of cellular T stores in testicular homogenates compared with secreted T in fluid aspirates obtained with minimal cellular disruption. Normal intratesticular fluid T concentrations were maintained by low doses of hCG (125, 250, and 500 IU every other day for 3 wk) in men with gonadotropin suppression from exogenous T. Presumably, normal ITT levels within the testis should support normal spermatogenesis.

A similar intratesticular to serum T gradient is seen in the rat (17). Rat models of spermatogenesis have shown a testis to serum T gradient with 100-fold higher T levels within the testis (17). The high ITT levels are in excess of the ITT concentration needed to support normal spermatogenesis; ITT can be reduced to 20% of normal levels without impacting normal spermatogenesis in the rat (16). However, below this threshold there is a direct quantitative relationship between ITT and sperm production. High doses of exogenous T can restore spermatogenesis in the rat (20–23). Additionally, replacing ITT by injecting microspheres containing T directly into the rat testis restored ITT levels as well as spermatogenesis to normal (24). In the rat, the high ITT levels have been shown to exceed the ITT level necessary for normal spermatogenesis. The threshold ITT concentration necessary for normal spermatogenesis in the rat is more than twice the normal serum T concentration (16). Similar studies in man have been limited by the inability to reliably assess the intratesticular microenvironment repeatedly. Studies relying on testicular biopsy have been cross-sectional in design, with the comparison of ITT levels across individuals who have undergone various hormonal manipulations. This study design is biased by the high variability in ITT between individuals. Percutaneous aspiration of testicular fluid has allowed us to perform a longitudinal study, with repeated assessment of the intratesticular hormonal environment in men, which allows for the serial assessment of ITT in response to hormonal manipulation.

Previous studies have shown that weekly administration of either 200 or 300 mg T, im, maximally suppresses gonadotropin secretion (6); moreover, these doses of T inhibit gonadotropin secretion within 2–3 d of administration (25). As expected, we observed that serum gonadotropin levels were significantly reduced by exogenous T in this study. Gonadotropin suppression without hCG administration caused dramatic reductions in ITT (94%) from baseline in the TE and placebo hCG group. Exogenous TE (200 mg weekly) has also been shown to reduce sperm production to azoospermic levels in approximately 70% of Caucasian men (7, 8). Spermatogenesis was not assessed in this 3-wk study, but in a previous study of normal men (n = 7) with gonadotropin suppression induced with 6 months of T and a progestin, levonorgestrel (LNG), intratesticular fluid T was suppressed 98% from baseline (15). Intratesticular fluid T levels in these men after 6 months of TE plus LNG treatment were similar to their baseline serum T levels. In this group of seven men, ITT levels suppressed to levels approximating their baseline serum T levels were coincident with suppressed spermatogenesis. The addition of progestins to exogenous T has been shown to enhance gonadotropin suppression and azoospermia in a greater proportion of men (26–28) than T alone. The ITT levels (13 nmol/liter) in this small study were lower than the ITT levels in the TE/placebo hCG group in the current study (72 nmol/liter). The lower ITT levels may relate to the longer treatment phase (6 months *vs.* 3 wk), the additive effect of LNG to LH suppression, or other inhibitory effects of progestins within the testis. Although this low ITT level (13 nmol/liter) appeared to be insufficient to maintain spermatogenesis, the minimum ITT concentration required for normal spermatogenesis in men is unknown.

The quantitative use of hCG to selectively replace LH activity within the testis would allow for manipulation of the intratesticular androgenic environment, thereby enabling a study of the quantitative relationship between ITT and spermatogenesis. In this study, hCG increased the ITT concentration, presumably through stimulation of Leydig cell steroidogenesis. The dose of hCG required to maintain baseline ITT concentrations in men with maximal gonadotropin suppression is significantly lower than that historically used in the treatment of infertility due to hypogonadotropic hypogonadism.

A review of the literature reveals a broad range of relatively high doses of gonadotropin replacement using hCG ranging from 1250 IU three times weekly to 3000 IU twice weekly (29–32). Even higher doses of hCG (5000 IU, three times per week) have been shown to be safe in experimental models of gonadotropin withdrawal (33, 34). Regimens of 2000 IU administered im two or three times weekly have been used with hCG dose adjustment according to serum T levels with a goal of normal physiological serum T levels (32, 35, 36). This approach is based on the assumption that if normal serum T levels were established by hCG administration, ITT concentrations would be sufficient to support normal spermatogenesis. However, ITT was never directly assessed in these studies. The minimum hCG dose needed to restore ITT to levels sufficient for initiating and maintaining spermatogenesis is not known.

All three hCG groups in this study (125, 250, and 500 IU, given every other day) maintained ITT at levels statistically indistinguishable from baseline. These doses are 10–20% of the doses commonly used in male infertility treatment (1250–2000 IU, two or three times weekly). Endocrinologists and andrologists have been aware that the doses of hCG traditionally used to treat certain types of infertility are supraphysiological and may expose patients to high levels of T and estradiol, with the consequent risk of clinically significant gynecomastia (37). The ability to prescribe hCG doses at lower levels to target normal serum and ITT and normal spermatogenesis would be useful for this patient population.

However, men rendered hypogonadotropic with exogenous T administration are different from men with infertility due to hypogonadotropic hypogonadism in two important ways. First, the study participants started with normal gonadotropin levels and were treated with high dose TE to induce gonadotropin withdrawal at the same time they were treated with hCG with the aim of maintaining ITT. In contrast, hypogonadotropic infertile men are treated with either T replacement or hCG for fertility, but not both simultaneously. The weekly administration of TE raised serum T levels significantly in all groups and may have resulted in higher ITT concentrations than would have been observed in a patient with hypogonadotropic hypogonadism receiving hCG therapy alone. Second, in the clinical setting, ITT production and spermatogenesis have to be induced after a prolonged period of gonadotropin deficiency. Therefore, the low-dose hCG used in this study may not normalize ITT in hypogonadotropic infertile men. However, lower hCG doses than those traditionally used may be sufficient to restore spermatogenesis.

The effect of TE 200 mg, im, weekly alone on ITT was demonstrated in the placebo hCG group. After 3 wk of therapy with TE alone, the ITT concentration (72 nmol/liter) was approximately 2.5-fold higher than the serum T concentration (27 nmol/liter). After 3 wk, LH was significantly suppressed (5% of baseline). Presumably, intratesticular production of T by Leydig cells was markedly reduced in the absence of LH activity. The serum T level at this time was high normal at 27 nmol/liter, but it is unclear how a peripheral source of T would concentrate in the testis to result in a concentration higher than that found in serum. The higher ITT relative to serum T after 3 wk of TE alone in this study cannot be explained on the basis of androgen-binding proteins, because in man the concentration of SHBG in the human testis and serum are not significantly different (13). Both LH and FSH were suppressed significantly from baseline (5 and 3%, respectively), but not to undetectable levels. It is possible the low levels of gonadotropin activity are responsible for persistent low levels of ITT and/or spermatogenesis. However, serum LH ($P = 0.93$) was not a statistically significant determinant of ITT in the presence of serum hCG ($P < 0.001$) in a multiple linear regression model (overall model, $P < 0.0001$). It is possible that the Leydig cells were producing small amounts of T at 3 wk despite LH withdrawal. Spermatogenesis has been shown to be present in the LH receptor knockout mouse despite absent LH activity and very low ITT levels. Low-level residual ITT and FSH activity is believed to be responsible for spermatogenesis in this mouse model (38). It is possible that other factors become physiologically relevant in the low T intratesticular microenvironment in humans, such as low level FSH activity or increased DHT activity due to up-regulation of the enzyme 5α-reductase (9). However, although posttreatment serum FSH was negatively linearly associated with ITT in simple linear regression analysis, serum FSH ($P = 0.41$) was not a significant predictor of ITT in the presence of serum hCG ($P < 0.001$) in a multiple linear regression model (overall model, $P < 0.0001$).

Studies of the intratesticular hormonal environment can be undertaken if we can develop a model in which we can reliably control the ITT concentration. Clamping the pituitary with exogenous hormones and/or GnRH receptor analogs (39–41) allows for the selective replacement of gonadotropins to determine the relative contributions of intratesticular androgens and FSH in normal spermatogenesis. Clearly, low-dose hCG can restore ITT to normal levels in men with gonadotropin suppression from exogenous T administration. Although there was no statistically significant difference in ITT among the 125-, 250-, and 500-IU hCG dose groups in pairwise comparisons, there was a linear increase in ITT with increasing hCG dose, which was statistically significant by simple linear regression ($P < 0.001$). The 250-IU dose group had posttreatment ITT levels closest to their baseline levels of all the groups, with a posttreatment ITT 7% lower than their baseline ITT. However, the two higher hCG groups (250 and 500 IU) had higher serum T during TE/hCG treatment than the TE/placebo group or the TE/125-IU hCG group. The ITT concentration achieved with TE/125-IU hCG (group 2) was also not statistically different from baseline, and this group had lower serum T than the two higher hCG dose groups. The contribution of serum T to ITT levels is not clear, and these results must be interpreted with caution, given that different immunoassays were used to measure T in serum and intratesticular fluid.

In summary, assessment of the testicular hormonal environment through percutaneous fluid aspiration has shown a similar testis to serum T gradient as previous testicular biopsy studies in men and rats. Additionally, low doses of hCG maintain baseline levels of ITT in men with gonadotropin withdrawal from exogenous T administration. Lower doses of hCG may be as effective in treating male infertility due to hypogonadotropism as the higher doses used historically. Selective replacement of LH activity with low-dose hCG, as demonstrated in this study, will allow the design of future studies investigating the relative roles of intratesticular androgens and FSH in the control of human spermatogenesis. Such work will be applicable to the goal of developing uniformly effective male contraception.

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References

1. **Matsumoto AM, Bremner WJ** 1989 Endocrine control of human spermatogenesis. J Steroid Biochem 33:789–790
2. **Matsumoto AM, Karpas AE, Bremner WJ** 1986 Chronic human chorionic gonadotropin administration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man. J Clin Endocrinol Metab 62:1184–1192
3. **McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Kretser DM, Pratis K, Robertson DM** 2002 Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Recent Prog Horm Res 57: 149–179
4. **Sheckter CB, Matsumoto AM, Bremner WJ** 1989 Testosterone administration inhibits gonadotropin secretion by an effect directly on the human pituitary. J Clin Endocrinol Metab 68:397–401
5. **Anderson RA, Wallace AM, Wu FC** 1996 Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive study. III. Higher 5α-reductase activity in oligozoospermic men administered supraphysiological doses of testosterone. J Clin Endocrinol Metab 81:902–908
6. **Matsumoto AM** 1990 Effects of chronic testosterone administration in normal men: safety and efficacy of high dosage testosterone and parallel dose-dependent suppression of luteinizing hormone, follicle-stimulating hormone, and sperm production. J Clin Endocrinol Metab 70:282–287
7. **WHO** 1990 Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet 336:955–959
8. **WHO** 1996 Contraceptive efficacy of testosterone-induced azoospermia and oligospermia in normal men. Fertil Steril 65:821–829
9. **McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, de Kretser DM, Robertson DM** 2002 Effects of testosterone plus medroxyprogesterone acetate on semen quality, reproductive hormones, and germ cell populations in normal young men. J Clin Endocrinol Metab 87:546–556
10. **McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, de Pratis K, Robertson DM** 2002 Hormonal regulation of spermatogenesis in primates and man: insights for development of the male hormonal contraceptive. J Androl 23:149–162
11. **Wallace EM, Gow SM, Wu FC** 1993 Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive

study. I. Plasma luteinizing hormone, follicle stimulating hormone, testosterone, estradiol, and inhibin concentrations. J Clin Endocrinol Metab 77:290–293
12. **Amory JK, Anawalt BD, Bremner WJ, Matsumoto AM** 2001 Daily testosterone and gonadotropin levels are similar in azoospermic and nonazoospermic normal men administered weekly testosterone: implications for male contraceptive development. J Androl 22:1053–1060
13. **Jarow JP, Chen H, Rosner TW, Trentacoste S, Zirkin BR** 2001 Assessment of the androgen environment within the human testis: minimally invasive method to obtain intratesticular fluid. J Androl 22:640–645
14. **Chen H, Chandrashekar V, Zirkin BR** 1994 Can spermatogenesis be maintained quantitatively in intact adult rats with exogenously administered dihydrotestosterone? J Androl 15:132–138
15. **Coviello AD, Herbst KL, Amory JK, Anawalt BD, Yan X, Brown TR, Wright WW, Bremner WJ, Matsumoto AM, Jarow JP** 2004 Intratesticular testosterone concentrations comparable with serum levels are not sufficient to maintain normal sperm production in men receiving a hormonal contraceptive regimen. J Androl 25:931–938
16. **Zirkin BR, Santulli R, Awoniyi CA, Ewing LL** 1989 Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. Endocrinology 124:3043–3049
17. **Turner TT, Jones CE, Howards SS, Ewing LL, Zegeye B, Gunsalus GL** 1984 On the androgen microenvironment of maturing spermatozoa. Endocrinology 115:1925–1932
18. **Schanbacher BD, Ewing LL** 1975 Simultaneous determination of testosterone, 5α-androstan-17β-ol-3-one, 5α-androstane-3α,17β-diol and 5α-androstane-3β,17β-diol in plasma of adult male rabbits by radioimmunoassay. Endocrinology 97:787–792
19. **Morse HC, Horike N, Rowley MJ, Heller CG** 1973 Testosterone concentrations in testes of normal men: effects of testosterone propionate administration. J Clin Endocrinol Metab 37:882–886
20. **Awoniyi CA, Sprando RL, Santulli R, Chandrashekar V, Ewing LL, Zirkin BR** 1990 Restoration of spermatogenesis by exogenously administered testosterone in rats made azoospermic by hypophysectomy or withdrawal of luteinizing hormone alone. Endocrinology 127:177–184
21. **Awoniyi CA, Santulli R, Sprando RL, Ewing LL, Zirkin BR** 1989 Restoration of advanced spermatogenic cells in the experimentally regressed rat testis: quantitative relationship to testosterone concentration within the testis. Endocrinology 124:1217–1223
22. **Awoniyi CA, Santulli R, Chandrashekar V, Schanbacher BD, Zirkin BR** 1989 Quantitative restoration of advanced spermatogenic cells in adult male rats made azoospermic by active immunization against luteinizing hormone or gonadotropin-releasing hormone. Endocrinology 125:1303–1309
23. **Zirkin BR, Santulli R, Strandberg JD, Wright WW, Ewing LL** 1993 Testicular steroidogenesis in the aging brown Norway rat. J Androl 14:118–123
24. **Turner TT, Howards SS, Gleavy JL** 1990 On the maintenance of male fertility in the absence of native testosterone secretion: site-directed hormonal therapy in the rat. Fertil Steril 54:149–156
25. **Schulte-Beerbuhl M, Nieschlag E** 1980 Comparison of testosterone, dihydrotestosterone, luteinizing hormone, and follicle-stimulating hormone in serum after injection of testosterone enanthate of testosterone cypionate. Fertil Steril 33:201–203
26. **Anawalt BD, Bebb RA, Bremner WJ, Matsumoto AM** 1999 A lower dosage levonorgestrel and testosterone combination effectively suppresses spermatogenesis and circulating gonadotropin levels with fewer metabolic effects than higher dosage combinations. J Androl 20:407–414
27. **Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM** 1996 Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. J Clin Endocrinol Metab 81:757–762
28. **Meriggiola MC, Bremner WJ** 1997 Progestin-androgen combination regimens for male contraception. J Androl 18:240–244
29. **Burris AS, Clark RV, Vantman DJ, Sherins RJ** 1988 A low sperm concentration does not preclude fertility in men with isolated hypogonadotropic hypogonadism after gonadotropin therapy. Fertil Steril 50:343–347
30. **Johnsen SG** 1978 Maintenance of spermatogenesis induced by HMG treatment by means of continuous HCG treatment in hypogonadotrophic men. Acta Endocrinol (Copenh) 89:763–769
31. **Kliesch S, Behre HM, Nieschlag E** 1995 Recombinant human follicle-stimulating hormone and human chorionic gonadotropin for induction of spermatogenesis in a hypogonadotropic male. Fertil Steril 63:1326–1328
32. **Liu PY, Turner L, Rushford D, McDonald J, Baker HW, Conway AJ, Handelsman DJ** 1999 Efficacy and safety of recombinant human follicle stimulating hormone (Gonal-F) with urinary human chorionic gonadotrophin for induction of spermatogenesis and fertility in gonadotrophin-deficient men. Hum Reprod 14:1540–1545
33. **Matsumoto AM, Bremner WJ** 1985 Stimulation of sperm production by human chorionic gonadotropin after prolonged gonadotropin suppression in normal men. J Androl 6:137–143
34. **Matsumoto AM, Paulsen CA, Hopper BR, Rebar RW, Bremner WJ** 1983 Human chorionic gonadotropin and testicular function: stimulation of testosterone, testosterone precursors, and sperm production despite high estradiol levels. J Clin Endocrinol Metab 56:720–728
35. **Finkel DM, Phillips JL, Snyder PJ** 1985 Stimulation of spermatogenesis by gonadotropins in men with hypogonadotropic hypogonadism. N Engl J Med 313:651–655
36. **Liu L, Banks SM, Barnes KM, Sherins RJ** 1988 Two-year comparison of testicular responses to pulsatile gonadotropin-releasing hormone and exogenous gonadotropins from the inception of therapy in men with isolated hypogonadotropic hypogonadism. J Clin Endocrinol Metab 67:1140–1145
37. **Zitzmann M, Nieschlag E** 2000 Hormone substitution in male hypogonadism. Mol Cell Endocrinol 161:73–88
38. **Zhang F-P, Pakarainen T, Poutanen M, Toppari J, Huhtaniemi I** 2003 The low-gonadotropin-independent constitutive production of testicular testosterone is sufficient to maintain spermatogenesis. Proc Natl Acad Sci USA 100:13692–13697
39. **Bagatell CJ, McLachlan RI, de Kretser DM, Burger HG, Vale WW, Rivier JE, Bremner WJ** 1989 A comparison of the suppressive effects of testosterone and a potent new gonadotropin-releasing hormone antagonist on gonadotropin and inhibin levels in normal men. J Clin Endocrinol Metab 69:43–48
40. **Bagatell CJ, Matsumoto AM, Christensen RB, Rivier JE, Bremner WJ** 1993 Comparison of a gonadotropin releasing-hormone antagonist plus testosterone (T) versus T alone as potential male contraceptive regimens. J Clin Endocrinol Metab 77:427–432
41. **Bagatell CJ, Rivier JE, Bremner WJ** 1995 Dose effects of the gonadotropin-releasing hormone antagonist, Nal-Glu, combined with testosterone enanthate on gonadotropin levels in normal men. Fertil Steril 64:139–145

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