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Research Article

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In the four men who received the high dosage hFSH regimen, sperm counts were markedly suppressed during T administration alone (0.3±0.2 million/cm^3, mean±SE, compared with 94±12 million/cm^3 during the control period). Serum LH bioactivity (determined by in vitro mouse Leydig cell assay) was suppressd (140±7 ng/ml compared with 375±65 ng/ml during control period) and FSH levels (by radioimmunoassay) were reduced to undetectable levels (<25 ng/ml, compared with 98±21 ng/ml during control period) during T alone. With the addition of 100 IU hFSH s.c. daily to T, sperm counts increased significantly in […]

Reinitiation of Sperm Production in Gonadotropin-suppressed Normal Men by Administration of Follicle-stimulating Hormone

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ABSTRACT The specific roles of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in controlling human spermatogenesis are poorly understood. We studied the effect of an experimentally induced, selective LH deficiency on sperm production in normal men. After a 3-mo control period, five men received 200 mg testosterone enanthate (T) i.m./wk to suppress LH, FSH, and sperm counts. Then, while continuing T at the same dosage, human FSH (hFSH) was administered simultaneously to replace FSH activity, leaving LH activity suppressed. Four men received 100 IU hFSH s.c. daily plus T (high dosage hFSH) for 13–14 wk, while one man received 50 IU hFSH s.c. daily plus T (low dosage hFSH) for 5 mo. The effect on sperm production of the selective LH deficiency produced by hFSH plus T administration was assessed.

In the four men who received the high dosage hFSH regimen, sperm counts were markedly suppressed during T administration alone (0.3±0.2 million/cm^3, mean±SE, compared with 94±12 million/cm^3 during the control period). Serum LH bioactivity (determined by in vitro mouse Leydig cell assay) was suppressd (140±7 ng/ml compared with 375±65 ng/ml during control period) and FSH levels (by radioimmunoassay) were reduced to undetectable levels (<25 ng/ml, compared with 98±21 ng/ml during control period) during T alone. With the addition of 100 IU hFSH s.c. daily to T, sperm counts increased significantly in all subjects (33±7 million/cm^3, $P < 0.02$ compared with T alone). However, no subject consistently achieved sperm counts within his control range. Sperm morphology and motility were normal in all four men and in vitro sperm penetration of hamster ova was normal in the two men tested during the hFSH-plus-T period. During high-dosage hFSH administration, serum FSH levels increased to 273±44 ng/ml (just above the normal range for FSH, 30–230 ng/ml). Serum LH bioactivity was not significantly changed compared with the T-alone period (147±9 ng/ml). After the hFSH-plus-T period, all four men continued to receive T alone after hFSH was stopped. Sperm counts were again severely suppressed (0.2±0.1 million/cm^3), demonstrating the dependence of sperm production on hFSH administration.

Serum T and estradiol (E_2) levels increased two- to threefold during T administration alone compared with the control period. Both T and E_2 levels remained unchanged with the addition of hFSH to T, confirming the lack of significant LH activity in the hFSH preparation.

In the one man who received low dosage hFSH treatment, sperm counts were reduced to severely oligospermic levels, serum FSH was suppressed to undetectable levels, and serum LH bioactivity was markedly lowered during the T-alone period. With the addition of 50 IU hFSH s.c. daily to T, sperm counts increased, to a mean of 11±3 million/cm^3. During this period, serum FSH levels increased to a mean of 105±11 ng/ml (slightly above this man's control range and within the normal adult range), while LH bioactivity remain suppressed. After hFSH was stopped and T alone was continued, sperm counts were again severely reduced to azoospermic levels.

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We conclude that FSH alone is sufficient to reinitiate sperm production in man during gonadotropin suppression induced by exogenous T administration. FSH may stimulate sperm production in this setting by increasing intratesticular T through androgen-binding protein production or by increasing the sensitivity of the spermatogenic response to the intratesticular T present during exogenous T administration.

INTRODUCTION

The hormonal milieu necessary for normal spermatogenesis in man is poorly understood. It is well established that sperm production in man requires the trophic influences of the pituitary gonadotropins (1, 2). However, the specific roles played by luteinizing hormone (LH)[1] and follicle-stimulating hormone (FSH) in controlling spermatogenesis are unclear.

According to current concepts, LH, through its stimulation of testosterone (T) production, plays a crucial role in the initiation and maintenance of sperm production (1, 2). LH binds specifically to the Leydig cells of the testis and stimulates high, intratesticular levels of T, which are thought to be necessary for spermatogenesis (3). FSH is thought to be necessary for the initiation of the spermatogenic process (1, 2). FSH binds to Sertoli cells and spermatogonia, stimulates production of androgen-binding protein (ABP), and is believed to be responsible for the maturation of spermatids (spermiogenesis) during the initiation of spermatogenesis (3–5). The role of FSH in the maintenance of sperm production remains unclear.

These general concepts of the hormonal regulation of spermatogenesis have been based largely on studies in experimental animals, primarily in the rat. However, comparison of studies in several species suggests that there are species differences in the endocrine control of spermatogenesis. For example, administration of highly specific, neutralizing antibodies to FSH has no effect on testicular function in adult male rats (6). In contrast, either passive administration or active induction of neutralizing antibodies to FSH in adult male monkeys has been reported to decrease sperm production and fertility (7–11). High-dosage T administration has been demonstrated to restore and maintain spermatogenesis in hypophysectomized male monkeys and rats (1, 12–14). However, exogenous T administration, at a dosage sufficient to induce normal intratesticular T concentrations, was unable to restore normal spermatogenesis in hypophysectomized rams (15).

The specific role of LH and FSH and the precise interrelationship between gonadotropins and intratesticular T in the control of human spermatogenesis are unclear. Studies of gonadotropin replacement in hypogonadotropic patients have been difficult to interpret because of uncertainties as to the purity of the gonadotropin preparations used and to the degree of gonadotropin deficiency present in these patients. Furthermore, no agent presently available for experimental use can produce a selective deficiency of LH or FSH in man. We have previously reported that human chorionic gonadotropin (hCG) can reinitiate sperm production in gonadotropin-suppressed normal men, despite undetectable blood FSH levels and urinary excretion of FSH less than that of prepubertal children (16). Our results demonstrated that normal serum levels of FSH are not an absolute requirement for sperm production in man.

Despite the general belief that high concentrations of intratesticular T are necessary to maintain normal spermatogenesis, the actual amount of T necessary in the seminiferous tubule to maintain normal sperm production and fertility are not well defined. In fact, there is accumulating evidence suggesting that high intratesticular T levels are not always necessary for spermatogenesis. In a recent study (17), adult male rats were treated with T propionate at dosages that moderately lowered serum FSH, reduced serum LH to undetectable levels, and suppressed intratesticular T levels 30-fold. Despite the markedly reduced intratesticular T concentrations, complete spermatogenesis was maintained. Similarly, other investigators were able to maintain spermatogenesis and fertility in hypophysectomized adult male rats despite four- to sevenfold reductions in testicular T concentrations (18). In man, patients with the fertile eunuch syndrome have isolated LH deficiency, which results in low serum T levels and eunuchoidism. Despite absent LH activity, these men have been reported to initiate and maintain spermatogenesis without treatment (19–21). In all of these instances, sperm production is maintained in the presence of low LH activity and normal or slightly suppressed FSH activity. These data suggest that normal levels of LH activity are not absolutely necessary for spermatogenesis.

In the present study, we resolved to determine the effect on sperm production of an experimentally induced selective LH deficiency in normal men. We administered exogenous T to normal men to suppress endogenous LH and FSH activity and to reduce sperm production to severely oligospermic or azoospermic levels. Then, while T was continued, highly purified human FSH (hFSH) was administered simultaneously to replace normal FSH activity, leaving LH activity

[1] *Abbreviations used in this paper:* ABP, androgen-binding protein; E_2, estradiol; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; hFSH, human FSH; LH, luteinizing hormone; T, testosterone.

suppressed. LH levels determined by bioassay, FSH levels determined by radioimmunoassay, and sperm concentrations were carefully monitored throughout the entire study. The effect on sperm production of FSH replacement alone, in the setting of suppressed LH activity, was assessed.

METHODS

Subjects

Five normal men, aged 23–42 yr, were recruited by newspaper advertisement and volunteered to participate in this study. They were studied over a period of 12 to 16 mo. The study protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington. Informed consent was obtained from each volunteer after a thorough explanation of the purpose and design of the study.

The following criteria were used to establish normality in the subjects: (*a*) all subjects had a normal medical history, physical examination, complete blood count, coagulation times, 12-panel chemistry battery, and urinalysis; (*b*) for each subject, six seminal fluid analyses, obtained over a 3-mo period were normal (i.e., sperm concentration > 20 million/cm^3, sperm motility > 50%, and > 60% oval forms); and (*c*) all subjects had normal basal T, LH, and FSH levels, normal LH and FSH secretory patterns on blood sampling every 20 min for 6 h, and normal LH and FSH responsiveness to a 4-h, continuous, intravenous infusion of 50 μg LH-releasing hormone.

Study design

Control period. Each subject underwent a 3-mo period of control observation, during which no hormones were administered. During this time, clinical observations, hormonal measurements, and seminal fluid analyses were performed at regular intervals as described below.

Initial T-alone period. After the control period, each subject began receiving 200 mg i.m. of testosterone enanthate (T) (Delatestryl, E. R. Squibb and Sons, Princeton, NJ) weekly. T alone was administered until three successive sperm counts (performed twice monthly) became <5 million/cm^3.

hFSH-plus-T period. After this initial T-alone period, while continuing T at the same dosage, four of the five subjects simultaneously received 100 IU hFSH s.c. daily for a period of 13 to 14 wk to replace FSH activity (high-dosage hFSH treatment). The remaining subject received 50 IU hFSH s.c. daily in addition to continued T administration for 5 mo (low-dosage hFSH treatment). The hFSH preparation used in these selective replacement studies (LER 1577, Lot No. 4) was kindly provided by the National Pituitary Agency, Baltimore, MD. This preparation contained <1% LH activity in the ovarian ascorbic acid depletion and ventral prostate weight bioassays, reported by the National Pituitary Agency, as well as in the in vitro mouse Leydig cell bioassay performed in our laboratory. However, this hFSH preparation contained significant amounts (17%) of immunoreactive, nonbioactive LH-like material. As a result, monitoring of LH activity during the study required the measurement of LH bioactivity using the in vitro mouse Leydig cell bioassay (described below) instead of LH by radioimmunoassay (RIA). On the other hand, close correspondence of FSH measured by RIA and bioassay permitted the use of RIA to monitor FSH activity during the study.

Second T-alone period. After the hFSH-plus-T period, hFSH injections were stopped in all five subjects and T alone was continued until three successive sperm counts were again suppressed to <5 million/cm^3. The resuppression of sperm counts with continued administration of T alone after discontinuation of hFSH was used to demonstrate that any rise in sperm counts observed during the hFSH-plus-T period was due to hFSH administration and not to a decline in the suppressive effect of exogenous T with time.

Recovery period. After the second T-alone period, T was then discontinued and two subjects entered a posttreatment control period until three successive sperm counts returned to the subject's own control range. The remaining three subjects left the study at the end of the second T-alone period.

Hormone administration

All T injections were administered by the investigators or their nursing assistants and records were kept to assess compliance with the study protocol. All subjects were carefully instructed on the techniques of self-administering hFSH injections into the abdominal subcutaneous tissue. Most of the daily hFSH injections were self-administered by the subjects and the remainder were performed by the investigators or their assistants. Lyophilized hFSH was diluted in bacteriostatic normal saline by the investigators (50 or 100 IU hFSH/cm^3) on a monthly basis. Subjects received a monthly supply of diluted hFSH and were instructed to keep the hFSH refrigerated until injected. Each subject kept a personal injection record which was reviewed monthly by one of the investigators.

Measurements and clinical observations

During each month of the study, subjects submitted two seminal fluid specimens, obtained by masturbation after two days of abstinence from ejaculation. In addition, at monthly intervals, each subject was interviewed by one of the investigators concerning general health and a brief physical examination was performed. A venous blood sample and urine sample was obtained at each monthly visit for measurement of routine hematological and blood chemical studies and urinalyses. In addition, random serum LH, FSH, and T levels were determined monthly. During the treatment periods, these monthly blood samples were obtained immediately before scheduled injections of T or of hFSH plus T. Serum estradiol (E_2)level was measured on the last monthly blood sample of the control, initial T-alone, and hFSH-plus-T periods for each subject. At the end of these study periods, 6-h urine samples were collected for measurement of FSH levels.

Near the end of the hFSH-plus-T period, the four subjects on high dosage hFSH treatment (100 IU/d) had serial blood sampling performed between two injections of hFSH. This sampling was done to determine the extent of fluctuations of FSH and LH levels between hFSH injections that would not be detected in the monthly blood samples. Subjects were studied beginning between 0800 and 0900, 24 h after their last hFSH injection. Serial blood sampling was performed through an indwelling venous cannula. After drawing an initial blood sample (0 h), each subject was given 100 IU hFSH s.c. in the abdominal subcutaneous tissue. Subsequently, blood samples were obtained every hour for the

subsequent 8 h and then 24 h after the hFSH injection. Serum FSH levels were measured on each blood sample. Serum LH bioactivity was determined on the 0-, 2-, 4-, 6-, 8-, and 24-h blood samples. In two subjects, an in vitro sperm penetration assay was performed at the end of the hFSH-plus-T period.

Hormone assays

FSH RIA. The RIA for serum FSH has been described previously (16). The reagents used were distributed by the National Pituitary Agency. The reference standard used was LER 907. The tracer was HS-1 radioiodinated with ^{125}I using chloramine T (22). The first antibody was rabbit anti-human FSH, batch No. 5. The assay results were calculated with the computer program of Burger et al. (23). The sensitivity of this assay was 25 ng/ml. The intraassay variability was 7.3% and the interassay variability was 9.7%.

The RIA for urinary FSH was performed by the Core Endocrine Laboratory, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, PA. 80-ml aliquots of urine were precipitated with acetone, centrifuged, and resuspended in RIA buffer (24). FSH was then measured by RIA, with the Second International Reference Preparation of Human Menopausal Gonadotropin as the reference standard.

LH bioassay. The in vitro bioassay of LH was a modification (16, 25) of the procedures described by Van Damme et al. (26) and Dufau et al. (27). This assay is based on the measurement of T production from dispersed Leydig cells isolated from immature Swiss Webster mice (5–7 wk of age). The reference standard used as LER 907. All samples were run in duplicate at a volume of 10 μl. The minimal detectable amount of LH activity was 125 ng/ml. The mean intraassay and interassay coefficients of variation for pooled human sera were 14 and 24%, respectively.

T and E_2 RIA. The RIA for T and E_2 used reagents provided by the World Health Organization Matched Reagent Programme (28). The antisera were raised in rabbits against bovine serum albumin conjugates of T and E_2-17β. Antitestosterone antiserum exhibited cross-reactivity of 14% with 5α-dihydrotestosterone, 6% with 5α-androstanediol and <2% with other steroids tested. Anti-E_2 antiserum exhibited 17% cross-reactivity with estrone. The T assay was preceded by ether extraction and the E_2 assay was preceded by ether extraction and celite chromatography, using 40% ethyl acetate eluant. In both assays, separation of bound from free hormone was accomplished by dextran-coated charcoal separation. The assay sensitivity was 10 pg/tube (0.1 ng/ml) for T and 6 pg/tube (12 pg/ml) for E_2. The intraassay and interassay variabilities were 5.1 and 9.8%, respectively, for T and 8.2 and 8.8%, respectively, for E_2.

Seminal fluid analysis

Sperm concentrations in seminal fluid samples were determined by Coulter counter (Coulter Electronics Inc., Hialeah, FL) and concentrations < 15 million/cm^3 were confirmed by direct determination using a hemocytometer. These methods have been described previously (29). Since no significant changes in seminal fluid volume occurred with hormonal treatment, sperm concentrations gave an accurate assessment of total sperm output in the ejaculate. Sperm morphology and motility were assessed as described by MacLeod (30).

In vitro sperm penetration assay

The in vitro sperm penetration assay was performed in the Reproductive Genetics Laboratory of the Department of Obstetrics and Gynecology, University of Washington, courtesy of Dr. Morton A. Stenchever and Ms. Dianne Smith. The methodology has been described previously (31). This assay is based on the ability of human sperm to penetrate zona pellucida-free hamster ova in vitro.

Statistical analysis

As sperm counts are not normally distributed, log transformation of sperm concentrations was employed to normalize these data before statistical analysis. Mean sperm concentrations during the control period, after the initial 8 wk of T alone, and after initial 8 wk of hFSH plus T were calculated for each subject. Sperm concentrations after 8 wks of T alone and hFSH plus T were chosen to eliminate the transition effects of gradually falling sperm counts during the initial 8 wk of T treatment and the gradually rising sperm counts during the first 8 wk after starting hFSH. These data were then compared with Student's paired t test.

Mean hormone levels were determined for monthly blood samples during each study period for each subject. These data, as well as the urinary FSH levels during each period, were compared with Student's paired t test.

RESULTS

High-dosage hFSH treatment (four subjects). After the 3-mo control period, exogenous T administration (200 mg i.m. weekly) resulted in marked suppression of sperm production (Fig. 1). Sperm counts after 2 mo of initial T administration alone were reduced to 0.3±0.2 million/cm^3 (mean±SEM) compared with 94±12 million/cm^3 during the control period. Two subjects became azoospermic, while the remaining two subjects had sperm counts consistently suppressed to <2 million/cm^3.

While T injections were continued, all subjects received hFSH (100 IU s.c. daily) simultaneously with T. Sperm counts (Fig. 1) increased significantly with the addition of hFSH to T, reaching a mean of 33±7 million/cm^3 after 2 mo of hFSH plus T ($P < 0.02$ compared with T alone). Although sperm concentrations increased markedly on hFSH plus T, they did not consistently reach the individuals' control ranges. The mean sperm concentrations achieved after 3 mo of hFSH-plus-T treatment were 34, 43, 29, and 9 million/cm^3. The maximum sperm concentrations achieved on hFSH plus T were 88, 51, 35, and 13 million/cm^3. Sperm motility and morphology were consistently normal in all four men during hFSH-plus-T injections. In two of the four subjects, an in vitro sperm penetration assay was performed at the end of the hFSH-plus-T period. In both of these men, in vitro sperm penetration was normal, with 23 and 15% of hamster ova penetrated.

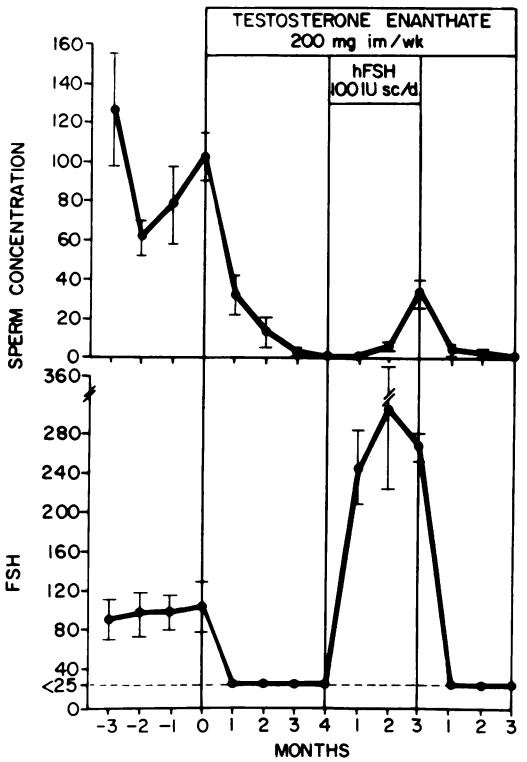

FIGURE 1 Mean monthly sperm concentrations (million per cubic centimeter) and serum FSH levels (in nanograms per milliliter) in four normal men during the control, initial T-alone, hFSH-plus-T, and second T-alone periods of the study (mean±SE). Exogenous T administration markedly suppresses sperm concentrations to severely oligospermic levels and serum FSH to undetectable levels. Note hFSH replacement at a slightly supraphysiological dosage increases sperm concentration.

After the hFSH-plus-T period, all four men continued to receive T alone after hFSH injections were stopped. Sperm counts were again severely suppressed in all subjects, reaching a mean of 0.2±0.1 million/cm^3 after 2 mo of T treatment alone (Fig. 1). Two subjects became azoospermic, whereas two subjects had sperm counts suppressed to <0.7 million/cm^3 after 2 mo of T alone. In two subjects, seminal fluid collections continued after T injections were terminated. Both men demonstrated return of sperm counts into their own control range within 5 mo.

Serum FSH levels (Fig. 1), normal during the control period (98±21 ng/ml). were suppressed to undetectable levels (<25 ng/ml) during the initial T-alone period. With the addition of 100 IU hFSH s.c. daily to T, serum FSH levels increased to a mean of 273±44 ng/ml, just above the upper limits of the normal range for FSH levels (30–230 ng/ml). After discontinuation of hFSH injections, continued T injections alone again suppressed FSH levels to <25 ng/ml, the limit of detectability of FSH in our assay. Urinary FSH levels (Table I) were within the normal adult range during the control period (308±12 mIU/h, normal adult range 190–1,700 mIU/h). With T administration alone, urinary FSH levels were suppressed to prepubertal levels (38±4 mIU/h, prepubertal range 15–100 mIU/h). At the end of the hFSH-plus-T period, urinary FSH excretion was increased to the upper portion of the normal adult range (1,143±524 mIU/h).

Serum LH bioactivity (Fig. 2) was markedly suppressed during initial T administration alone (140±7 ng/ml) compared with the control period (373±65 ng/ml, $P < 0.03$). With the addition of hFSH injections to T administration, serum LH bioactivity was not significantly changed compared with the initial T-alone period (147±9 ng/ml). LH bioactivity remained unchanged after hFSH injections were stopped during the second T alone period (153±5 ng/ml).

Serum T levels (Fig. 2) increased from 6.5±0.4 ng/ml during the control period to 13.7±1.9 ng/ml during the initial T-alone period. T levels during the hFSH-plus-T period (13.1±3.1 ng/ml) and the second T-alone period (11.0±2.2 ng/ml) were not significantly different from the initial T-alone period.

Near the end of the hFSH-plus-T period, blood samples were obtained hourly for 8 and 24 h after a subcutaneous injection of 100 IU hFSH in all four subjects. Serum FSH levels rose slightly, while serum LH bioactivity remained unchanged between hFSH injections (Fig. 3).

Serum E_2 levels (Table I) increased significantly from 31±6 pg/ml at the end of the control period to 84±18 pg/ml at the end of the initial T-alone period ($P < 0.05$). E_2 levels remained statistically unchanged during the FSH-plus-T period compared with the T-alone period (63±7 pg/ml).

Low-dosage hFSH treatment (one subject, data not shown). After a 3-mo control period, T administration

TABLE I
Urinary FSH and Serum E_2 Levels

	Control	T alone	FSH plus T
Urinary FSH (*mIU/h*)*	308±102	38±4‡	1,143±524§
Serum E_2 (*pg/ml*)‖	31±6	84±18‡	63±7

* Measured on 6-h urine aliquots at the end of each study period. Normal adult range, 190–1,700 mIU/h; Normal prepubertal range, 15–100 mIU/h.
‡ $P < 0.05$, compared with control.
§ $P < 0.05$, compared with T alone.
‖ Measured on monthly samples at the end of each study period.

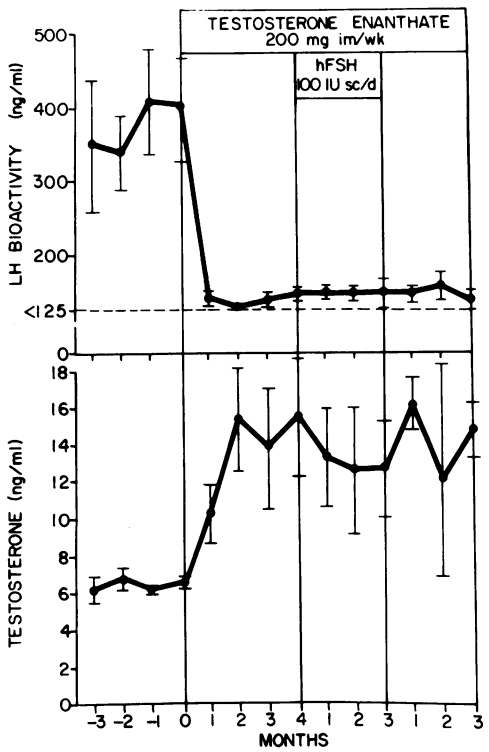

FIGURE 2 Mean monthly serum LH bioactivity and T levels in four normal men during the control, initial T-alone, hFSH-plus-T, and second T-alone periods of the study (mean±SE). Exogenous T administration raises serum T levels and markedly suppresses serum LH bioactivity. Note serum T levels and LH bioactivity do not change with the addition of hFSH to T.

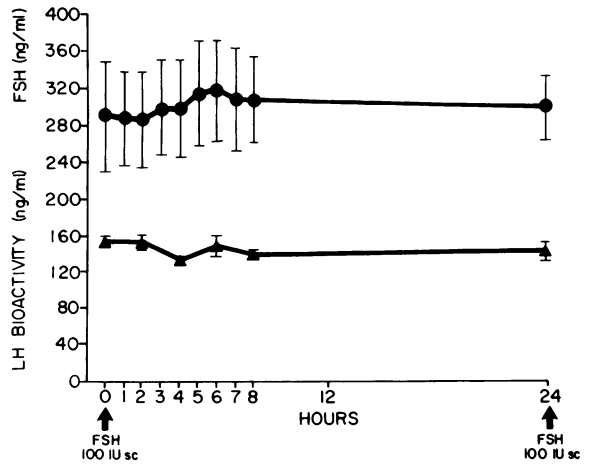

FIGURE 3 Mean serum FSH levels (●) and LH bioactivity (▲) between daily subcutaneous injections of hFSH in four normal men during the hFSH-plus-T period of study (mean±SEM). Note the slight increase in serum FSH levels after a subcutaneous injection of hFSH. Serum LH bioactivity is unchanged between hFSH injections.

alone (200 mg i.m. weekly) markedly suppressed sperm production in this subject to severely oligospermic levels. The mean sperm concentration during the last 2 mo of the T-alone period was 0.2±0.1 million/cm^3 compared with 117±18 million/cm^3 during the control period. Then, while T injections were continued, hFSH (50 IU s.c. daily) was added. Sperm counts increased with the addition of hFSH to T, reaching a mean of 11±3 million/cm^3 after 2 mo of hFSH plus T. The maximum sperm concentration achieved during hFSH plus T in this subject was 17.4 million/cm^3. Sperm motility and morphology were normal at the end of the hFSH-plus-T period. After hFSH was stopped, T alone was continued at the same dosage. Sperm counts were again severely suppressed, and this man became azoospermic after 2 mo of T administration alone.

Serum FSH levels were suppressed from normal levels (75±4 ng/ml) during the control period to undetectable levels (<25 ng/ml) during the initial T-alone period. With the addition of 50 IU hFSH s.c. daily, serum FSH levels increased to a mean of 105±11 ng/ml, slightly above this man's control range for FSH and within the normal adult range. After discontinuation of hFSH and continuation of T alone, serum FSH levels were again suppressed to below detectable levels (<25 ng/ml). Serum LH bioactivity was markedly suppressed during initial T administration alone (133±5 ng/ml) compared with control (429±50 ng/ml). With the addition of hFSH to T, LH bioactivity remained suppressed, unchanged from the initial T-alone period (147±8 ng/ml). Serum LH bioactivity remained unchanged after hFSH injections were discontinued (142±2 ng/ml).

Serum T levels increased from 4.6±0.4 ng/ml during the control period to 15.8±1.1 ng/ml during the initial T-alone period. T levels during the hFSH-plus-T period (16.9±2.0 ng/ml) and the second T-alone period (16.2±1.0 ng/ml) were unchanged compared with the initial T-alone period.

Review of the injection records from all subjects revealed very good compliance with the study protocol. No T injections were missed and only an occasional hFSH injection was missed.

All subjects remained in good general health throughout the study. Mild truncal acne developed in three of the five subjects during T administration. Three subjects experienced a mild aching or burning sensation at the site of subcutaneous hFSH injection. This resolved spontaneously without treatment and did not interrupt hFSH treatment in any subject. Otherwise, no adverse effects of either T or hFSH treatment were observed. No significant changes in palpable

breast tissue (within 1 cm of control measurements circumferentially from the areola) or testicular size (within 1 cm of control, measured by calipers) occurred during any of the hormonal treatments. Routine hematologic studies, blood coagulation parameters, blood chemistries, and urinalyses remained essentially unchanged during the study. Hematocrit increased slightly (2–3%) in all subjects, but no subject developed significant erythrocytosis (hematocrits all <53%).

DISCUSSION

Our results demonstrate that in a setting of markedly suppressed gonadotropin levels induced by T, exogenous FSH administration alone can reinitiate sperm production in normal men. Exogenous T administration in our subjects resulted in a severe reduction in LH and FSH levels. As a result of this endogenous gonadotropin suppression, sperm production was severely suppressed in all of our subjects during the initial T-alone period. These results confirm our earlier findings (16) and those of other investigators (32) on the effect of administration of exogenous T to normal men. In this setting of T-induced endogenous hypogonadotropism, our subjects received highly purified hFSH to replace FSH alone, leaving LH levels suppressed. Four subjects received a moderately high dosage hFSH replacement (100 IU s.c. daily). All four men demonstrated significant stimulation of sperm production on hFSH plus T. Three of the four subjects attained mean sperm counts within the normal adult male range and achieved at least one sperm count in their control range. The remaining subject, who was azoospermic during the initial T alone period, demonstrated a definite rise in sperm counts with the addition of hFSH to T. However, his mean sperm concentration remained in the oligospermic range. None of the subjects achieved sperm counts consistently within his own control range.

Careful examination of injection records kept by the subjects revealed very good compliance with the daily hFSH injection protocol. Only an occasional missed hFSH injection was noted in our subjects. However, as the subjects injected themselves with hFSH and were responsible for keeping their own injection records, the accuracy and reliability of these records could not be assessed with certainty. Since T injections were given by the investigators, it is possible that unrecorded, irregular hFSH administration may have contributed to the lack of complete return of sperm production during the hFSH-plus-T period. The relatively short duration of hFSH-plus-T period may also have contributed to the failure of complete normalization of sperm counts. The duration of hFSH administration was relatively short because of the relative lack of availability of purified hFSH for experimental use. Production of mature spermatozoa from immature spermatogonia requires 74±5 d in man (33). It is possible that more complete return of spermatogogenesis might have occurred if the duration of hFSH treatment had been longer.

In addition to the significant rise in sperm counts during the hFSH-plus-T period, all four subjects achieved normal sperm motility (>50%) and morphology (>60% oval forms) by the end of the hFSH-plus-T period. In the two subjects tested, in vitro sperm penetration of hamster ova was normal during hFSH plus T. These results suggest that the functional capacity of the ejaculated sperm was normal in the men receiving hFSH.

In the setting of suppressed gonadotropin and sperm production induced by exogenous T administration, one subject received a lower dosage of hFSH replacement (50 IU s.c. daily). This subject also demonstrated a significant rise in sperm counts on hFSH replacement alone. Even though he continued to receive hFSH for a longer duration (5 mo) than the subjects who received the higher dosage hFSH (13–14 wk), sperm counts remained in the oligospermic range by the end of the hFSH-plus-T period.

After the period of hFSH-plus-T administration, hFSH was stopped in all five subjects, while T was continued at the same dosage. Sperm counts were again severely suppressed in all subjects. These results clearly demonstrate that reinitiation of sperm production during the hFSH-plus-T period was due to hFSH administration and not to a decline in the suppressive effect of exogenous T with time.

As we have shown previously (16), serum FSH levels were suppressed to undetectable levels and urinary FSH levels were in the prepubertal range during exogenous T administration. In four subjects, administration of 100 IU hFSH s.c. daily (high dosage hFSH) resulted in FSH levels only slightly above the normal range for FSH. However, these levels of FSH were nearly three times those measured in the control period. Serum FSH levels between daily injections of hFSH remained relatively constant, presumably as a result of relatively slow release of hFSH from a subcutaneous depot and the long half-life of FSH in blood. Therefore, monthly FSH levels reflected the general FSH activity throughout the hFSH-plus-T period. Furthermore, the reinitiation of sperm production during high-dosage hFSH injections occurred in the setting of slightly supraphysiological FSH stimulation and may have represented a pharmacological rather than a physiological effect of FSH. On the other hand, the one subject who received a lower dosage of hFSH (50 IU s.c. daily) demonstrated FSH levels during hFSH plus T that were clearly within the normal range for

FSH and comparable to those in his control period. In this man, sperm production was reinitiated with hFSH replacement that resulted in serum FSH levels well within the physiological range.

Serum LH bioactivity as assessed by an in vitro mouse Leydig cell assay was severely suppressed during exogenous T administration alone and remained suppressed at similar levels during hFSH-plus-T administration. Serum T and E_2 levels were increased to similar levels both during the T alone and during the hFSH-plus-T periods. It is well established that LH stimulates T and E_2 production in vivo in man (2). The fact that serum T and E_2 levels did not significantly change with addition of hFSH to T confirms the in vitro assessment of serum LH bioactivity. Both of these findings confirm the LH bioassay determinations (by ventral prostate weight and ovarian ascorbic acid depletion) reported by the National Pituitary Agency and clearly demonstrate the lack of significant LH activity contaminating the hFSH preparation used in these studies. Although it is remotely possible that the radioimmunoassayable LH contamination of the hFSH preparation might have played a role in restoring sperm production, such a role would have to involve LH activity that was not detectable by the aforementioned bioassays. Therefore, the rise in sperm counts observed during the hFSH-plus-T period resulted from FSH replacement alone, in a setting of selective LH deficiency (as assessed by in vitro and in vivo bioassay).

Previous studies of gonadotropin replacement in experimental animals (primarily the rat) as well as in man have not shown that FSH replacement alone in hypogonadotropic animals could initiate, reinitiate, or maintain spermatogenesis (1, 34–38). Both LH and FSH activity or, in certain instances, LH activity alone have been required for initiation and maintenance of sperm production in these gonadotropin replacement studies (1, 34–39). Similarly, selective removal of LH activity in adult rats and rabbits by active or passive immunization results in marked suppression of spermatogenesis (40, 41). These findings support the concept that LH activity is necessary for spermatogenesis. Previous studies in animals have also demonstrated that the high concentrations of T normally found within the testis are important in the initiation and maintenance of normal sperm production. Administration of large doses of exogenous T, which maintain high intratesticular T levels, have been shown to maintain and initiate spermatogenesis in hypophysectomized or prepubertal animals (1, 12–14, 42). In man, spermatogenesis has been demonstrated in the testes of a prepubertal boy bearing a Leydig cell tumor which produced high, local intratesticular androgen concentrations (43). Exogenous T administration to normal men has been shown to reduce serum LH levels, resulting in a marked reduction in intratesticular T concentrations (despite increased serum T levels) and suppression of spermatogenesis (44). These data have led to the general concept that LH activity, by stimulating testicular Leydig cells and maintaining high intratesticular T concentrations, is required for normal sperm production.

Despite the generally well accepted concept that high intratesticular T levels are required for spermatogenesis, the actual amount of T required by the seminiferous tubules to maintain spermatogenesis is not known. In fact, data from some previous studies suggest that high intratesticular T concentrations may not be an absolute requirement for sperm production. Cunningham and Huckins (17) chronically administered T propionate (100 μg/100 g body wt s.c. daily) to adult male rats. This treatment resulted in a drastic reduction in intratesticular T levels, a marked suppression of serum LH to undetectable levels, but only a partial suppression of serum FSH levels. Despite undetectable LH levels and a 30-fold reduction in intratesticular T levels, complete spermatogenesis as assessed by histology persisted. Schanbacher (15) administered various dosages of T by subdermal silastic implants and injections to mature breeding rams. At dosages of exogenous T that suppressed serum LH to undetectable levels, markedly reduced rete testis T levels, and only partially reduced serum FSH levels, sperm production persisted, although at a markedly suppressed level.

In man, patients with the fertile eunuch syndrome are reported to have isolated deficiency of LH resulting in low serum T levels and eunuchoidal features (19–21). As a result of inadequate LH stimulation, these patients presumably also have low intratesticular T levels. Despite low LH and T levels, spermatogenesis is preserved, although it may be quantitatively subnormal (19–21). FSH levels in the fertile eunuch syndrome are reported to be normal (19–21). Recently, there have been reports of persistent spermatogenesis in men with hypogonadotropic hypogonadism treated with T(45) and primary Leydig cell failure (46), both settings in which intratesticular T levels are presumably lower than normal.

The existence of the fertile eunuch syndrome and the above reports of maintained spermatogenesis in states of low intratesticular T in man and animals, all suggest that normal LH activity and high intratesticular T levels may not always be necessary for sperm production to occur. Our results agree with these findings. The experimental state of selective LH deficiency created in our subjects is analogous to the gonadotropin status of patients with the fertile eunuch syndrome. The results of our study demonstrate clearly that normal LH activity is not an absolute requirement for

reinitiation of sperm production after short-term gonadotropin suppression. Although LH activity was very low during the time when spermatogenesis was reinitiated by hFSH, it was not completely absent and LH bioactivity was clearly detectable during hFSH administration. That this amount of LH activity was insufficient by itself to maintain sperm production was demonstrated by the fact that during the T alone periods, similar LH bioactivity was present and yet sperm counts were markedly suppressed.

Although our work has demonstrated that spermatogenesis can be reinitiated by FSH alone in a setting of very low LH activity, we do not conclude that LH has no role in this process. Sperm production in our subjects was quantitatively subnormal. It is possible that normal LH activity is required for quantitatively normal spermatogenesis and that sperm counts in our subjects would have returned consistently into the individual's control range had we replaced normal LH activity. Furthermore, the paradigm used in our study was one of relatively short-term gonadotropin suppression and it is possible that with a longer period of gonadotropin suppression, FSH alone might not have stimulated sperm production.

The mechanism or mechanisms by which spermatogenesis is facilitated in our subjects despite very low LH activity (and presumably low intratesticular T levels) is not known. FSH has been demonstrated to increase Leydig cell LH receptors (47, 48), stimulate certain testicular steroidogenic enzymes (49), and augment T secretion stimulated by LH (47, 48, 50) in immature hypophysectomized rats. Enhancement of LH-stimulated T secretion by FSH has been suggested in a preliminary report of gonadotropin therapy of hypogonadotropic men (39). If hFSH administration in our subjects had resulted in an enhancement of the very low levels of LH-stimulated T production by the testis, the increased T production should have been reflected in the serum T levels. Serum T levels did not change with the administration of hFSH. Therefore, it is unlikely that FSH-mediated augmentation of intratesticular T production contributed significantly to the stimulation of spermatogenesis induced by FSH. It is still possible that FSH may have facilitated LH stimulation of a product other than T that in turn resulted in stimulation of sperm production. FSH has been shown to stimulate production of ABP by the Sertoli cell in immature rats (3, 4, 51). ABP is a high-affinity binding protein for T (3, 4, 51). Therefore, it is possible that FSH may stimulate sperm production despite very low LH activity by stimulating ABP production, thereby increasing ambient intratesticular T concentration. Finally, Yasuda and Johnson (52) administered various dosages of T propionate alone and in combination with FSH to adult rats. These investigators demonstrated a synergism between T and FSH on testis weight and spermatogenesis. Therefore, it is also possible that FSH may sensitize or facilitate the spermatogenic response to the intratesticular T present during exogenous T administration.

In a previous study, we showed that normal levels of FSH are not an absolute requirement for reinitiation of sperm production after short-term gonadotropin suppression (16). In the present study, we have demonstrated that normal levels of LH are also not absolutely required for reinitiation of spermatogenesis. However, in neither of these studies has selective hFSH or hCG replacement returned sperm production fully to normal levels in all men studied. We feel that although it is possible to demonstrate a stimulatory role for either hFSH or human LH alone on human spermatogenesis, neither gonadotropin may be sufficient by itself to induce quantitatively normal sperm production in all men. It is likely that normal levels of both human LH and hFSH are necessary to maintain quantitatively normal spermatogenesis.

Since each gonadotropin, in the near absence of the other, is capable of at least a partial stimulatory effect on sperm production, it seems very unlikely that selective suppression of either gonadotropin alone would be an effective method of suppressing sperm production to the extent necessary to cause infertility. Therefore, our results do not lend support to the concept that selective gonadotropin suppression might be an effective technique for male contraceptive development.

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