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Stimulation of Sperm Production by Human Luteinizing Hormone in Gonadotropin-Suppressed Normal Men*

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ABSTRACT. The relative roles of FSH and LH in the control of human spermatogenesis are not well established. We previously reported that supraphysiological doses of hCG can stimulate sperm production in gonadotropin-suppressed normal men despite prepubertal FSH levels. To determine whether more nearly physiological levels of human LH (hLH) also can stimulate spermatogenesis when FSH levels are suppressed, we administered hLH to normal men whose endogenous gonadotropin levels and sperm production were suppressed by exogenous testosterone enanthate (T). After a 3-month control period, 11 normal men received 200 mg T, im, weekly to suppress LH and FSH. T administration alone was continued for 3–4 months until 3 successive sperm concentrations (performed twice monthly) revealed azoospermia or severe oligospermia (sperm concentrations, <4 million/ml). Then, while continuing T, 4 of the 11 men (experimental subjects) simultaneously received 1100 IU hLH, sc, daily for 4–6 months to replace LH activity, leaving FSH activity suppressed. The effect on sperm production of the selective FSH deficiency produced by hLH plus T administration was determined. The remaining 7 men (control subjects) continued to receive T alone at the same dosage, without gonadotropin replacement, for an additional 6 months.

In the four experimental subjects, sperm concentrations increased significantly from 0.7 ± 0.7 million/ml (mean ± SEM) during T treatment alone to 19 ± 4 million/ml during hLH plus T administration ($P < 0.001$). However, none of the men achieved sperm concentrations consistently in their own pretreatment range. Sperm motilities and morphologies were normal in all four subjects by the end of hLH plus T administration. In contrast, sperm concentrations in the seven control subjects remained suppressed (<3 million/ml) throughout the entire period of prolonged T administration alone.

Serum LH bioactivity, determined monthly by *in vitro* mouse Leydig cell bioassay in all four experimental subjects, was markedly suppressed during T administration alone (120 ± 10 ng/ml) compared to that during the control period (390 ± 20 ng/ml; $P < 0.001$). With the addition of hLH to T, LH bioactivity returned to control levels (400 ± 40 ng/ml; P = NS compared to control value). Serum FSH levels determined monthly by RIA were reduced from 98 ± 12 ng/ml during the control period to undetectable levels (<25 ng/ml) during the T alone and the hLH plus T periods ($P < 0.01$). Urinary FSH excretion was in the normal adult range at the end of the control period (353 ± 117 mIU/h) and was markedly suppressed to prepubertal levels with T alone (62 ± 14 mIU/h) and hLH plus T (48 ± 17 mIU/h).

We conclude that dosages of hLH that result in LH levels in the physiological range can reinitiate sperm production in gonadotropin-suppressed normal men despite markedly suppressed FSH levels. Therefore, normal levels of FSH are not an absolute requirement for the stimulation of spermatogenesis after short term gonadotropin suppression. Since hLH was not able to return sperm counts to fully normal levels during selective FSH deficiency, we hypothesize that normal levels of FSH may be necessary for quantitatively normal spermatogenesis in man. (*J Clin Endocrinol Metab* **55**: 882, 1984)

THE SPECIFIC roles played by the pituitary gonadotropins LH and FSH in regulating normal spermatogenesis in man are poorly understood. According to current general concepts, LH is thought to be important in both the initiation and maintenance of spermatogenesis by stimulating intratesticular testosterone (T) production (1–3). FSH is believed to be required for the maturation of spermatids (spermiogenesis) at the time of initiation of sperm production (1–3), but its role in the maintenance of spermatogenesis is unclear.

Previous studies in man investigating the hormonal requirements for spermatogenesis have been difficult to interpret. These studies have suffered from uncertainties of the purity of the gonadotropin preparations used in replacement therapy of hypogonadotropic men and of the degree of endogenous gonadotropin deficiency present in these men. With the development of very sensitive and specific assays to assess the gonadotropin status of individuals and the availability of highly purified gonad-

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otropin preparations, the specific roles of LH and FSH in the control of human spermatogenesis can now be assessed with greater certainty.

We previously reported that spermatogenesis can be reinitiated by hCG in normal men whose gonadotropin and sperm production were suppressed by exogenous T (4). This hCG-induced stimulation of spermatogenesis occurred despite undetectable serum levels of FSH and urinary FSH excretion in the prepubertal range. These results demonstrated that a normal level of FSH is not an absolute requirement for reinitiation of spermatogenesis after short term gonadotropin suppression in man. However, the dosages of hCG used in these studies were clearly supraphysiological, resulting in approximately 6-fold higher LH bioactivity compared to control values (4). We questioned whether the pharmacological dosages of hCG used could have masked an important physiological role of FSH in spermatogenesis. In this regard, Sherins *et al.* (5) reported that only very small amounts of FSH activity are required to stimulate spermatogenesis in hypogonadotropic men receiving pharmacological dosages of hCG. In addition, hCG has intrinsic FSH-like activity, about 1/1000th as potent as its LH-like activity (6). Although it is unlikely that this small amount of FSH-like activity of hCG contributed significantly to the stimulation of spermatogenesis in our previous study, replacement of LH activity at more physiological levels would minimize such a contribution.

In the present study, we determined whether sperm production could be reinitiated in gonadotropin-suppressed normal men by more nearly physiological dosages of human LH (hLH). Exogenous T was administered to normal men to suppress endogenous gonadotropin levels and sperm production. Then, while continuing T, hLH was administered to selectively replace LH activity, leaving FSH levels suppressed. The dosage of hLH was chosen, on the basis of preliminary studies, to result in serum LH bioactivity that fluctuated within the normal adult physiological range with sc administration.

Materials and Methods

Subjects

The experimental protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington and the Research and Development Committee of the Seattle V.A. Medical Center. Eleven normal men, aged 22–42 yr, were recruited by newspaper advertisement and volunteered to participate in the study. After thorough explanation of the purpose and design of the study, informed consent was obtained from all of the men. All subjects had a normal medical history, physical examination, complete blood count, coagulation times, 12-test blood chemistry battery, and urinalysis. Six seminal fluid analyses, obtained over 3 months, were normal in all men (*i.e.* sperm concentration above 20 million/ml, sperm motility above 50%, and more than 60% oval forms). All subjects had normal basal serum LH, FSH, and T levels, normal LH and FSH secretory patterns on blood sampling every 20 min for 6 h, and normal LH and FSH responsiveness to 50 μg GnRH continuously infused iv for 4 h.

Experimental design

Control period. Each subject underwent a 3-month period of control observations, during which no hormones were administered. Baseline clinical status, gonadotropin and T levels, and seminal fluid parameters were established during this phase, as described below.

T suppression period. All subjects then received 200 mg T enanthate (Delatestryl, Squibb and Sons, Princeton, NJ), im, weekly until three successive sperm concentrations (performed twice monthly) were below 5 million/ml. This occurred after 3–4 months of exogenous T therapy in all subjects.

Experimental period. After the T suppression period, while continuing the same dosage of T enanthate, four men (experimental subjects 1–4) simultaneously received 1100 IU hLH, sc, daily for a period of 4–6 months to selectively replace LH activity. The hLH preparation used in this study was LER 1549 (batch A-3), kindly provided by the National Pituitary Agency (NPA; Baltimore, MD). This preparation was reported by the NPA to contain less than 0.2% FSH activity in the rat ovarian augmentation bioassay for FSH.

To demonstrate that any increases in sperm concentrations during the administration of hLH plus T were due to an effect of hLH administration and not the result of a decline in the suppressive effects of exogenous T, the remaining seven men (subjects 5–11) served as control subjects. After the T suppression period, these subjects continued to receive T enanthate alone at the same dosage for an additional 6 months (*i.e.* total of 9 months altogether).

Recovery period. After the experimental period, all hormones were discontinued, and two experimental (subjects 1 and 4) and four control (subjects 5–8) subjects entered a posttreatment recovery period until three successive sperm concentrations returned into the subjects' own pretreatment control range. The remaining five subjects left the study at the end of the experimental period.

Hormone administration

To assure gonadotropin suppression, all T enanthate injections were administered by the investigators or their nursing assistants. Injection records were kept to monitor compliance with the study protocol. The majority of the daily hLH injections were self-administered by the subjects after careful instruction on the techniques of injection into the abdominal sc tissue. The remainder of injections were given by the investigators or their assistants. Lyophilized hLH was diluted in bacteriostatic normal saline by the investigators (1100 IU hLH/ml) on a monthly basis. Experimental subjects were given a monthly supply of diluted hLH, which they were instructed to keep refrigerated until injected. Each subject kept a personal

injection record, which was reviewed monthly by one of the investigators.

Measurements and clinical observations

All subjects submitted twice monthly seminal fluid specimens throughout the entire study. These specimens were obtained by masturbation after 2 days of abstinence from ejaculation. At monthly intervals, each subject had a history and physical examination performed by one of the investigators. A venous blood sample and urine specimen were obtained at each monthly visit for measurement of routine hematological and blood chemistry studies and urinalyses.

In the four experimental subjects, serum LH, FSH, and T levels were determined monthly. During hormone administration, these monthly blood samples were drawn immediately before scheduled injections of T or hLH plus T. In addition, at the end of the control period, the T suppression period, and the hLH plus T period, 6-h urine samples were collected for measurement of FSH levels. Near the end of hLH plus T treatment, serial blood sampling was performed between two daily injections of hLH to determine the extent of fluctuations of LH and FSH levels that would not be detected in the monthly blood samples. After drawing an initial blood sample, each subject was given his usual daily injection of 1100 IU hLH, sc. Then, blood samples were obtained 8 h (in preliminary studies, the time of peak LH levels after sc injection of hLH in hypogonadotropic men) and 24 h after the hLH injection. Serum LH and FSH levels were measured in each sample.

Hormone assays

Serum FSH levels were determined by RIA using methodology described previously (4). Reagents used in this RIA were distributed by the NPA, and the reference standard used was LER 907. Assay results were calculated with the computer program of Burger *et al.* (7). The sensitivity of this assay was 25 ng/ml, and the intra- and interassay coefficients of variation were 7.3% and 9.7%, respectively.

The RIA for urinary FSH was performed by the Core Endocrine Laboratory, Milton S. Hershey Medical Center, Pennsylvania State University (Hershey, PA). Eighty-milliliter aliquots of urine were precipitated with acetone, centrifuged, and resuspended in assay buffer. FSH then was measured by RIA, as described previously (8), using the Second International Reference Preparation of human menopausal gonadotropin as the reference standard.

Serum LH levels were measured by an *in vitro* bioassay using a modification (9) of procedures described by VanDamme *et al.* (10) and Dufau *et al.* (11). In this assay, T production is measured from dispersed Leydig cells isolated from immature Swiss Webster mice (aged 5–7 weeks). The reference standard used was LER 907. All samples were run in duplicate at a volume of 10 μl, and the minimal detectable amount of LH activity was 100 ng/ml. The mean intra- and interassay variabilities for pooled human sera were 14% and 24%, respectively.

The RIA for T used reagents provided by the WHO Matched Reagent Programme (12). The T assay was preceded by ether extraction, and separation of bound from free hormone was accomplished by dextran-coated charcoal separation. The anti-T antiserum exhibited cross-reactivities of 14% with 5α-dihydrotestosterone, 6% with 5α-androstanediol, and less than 2% with all other steroids tested. The assay activity was 0.1 ng/ml, and the intra- and interassay variabilities were 5.1% and 9.8%, respectively.

Samples from individual subjects were analyzed in the same assay.

Seminal fluid analysis

Sperm concentrations in seminal fluid samples were determined using a Coulter counter (Coulter Electronics, Inc., Hialeah, FL), and concentrations below 15 million/ml were confirmed by direct determination using a hemocytometer. These methods were described previously (13). Since no significant changes in seminal fluid volume occurred throughout the study, sperm concentrations gave an accurate assessment of total sperm output in the ejaculate. Sperm motility and morphology were assessed using WHO criteria (14).

Statistical analysis

Mean sperm concentrations during the control period, after the initial 12 weeks of the T suppression period, and after the initial 12 weeks of the experimental period were calculated for each subject. Sperm concentrations after 12 weeks of T suppression and hLH plus T were chosen to eliminate the transition effects of gradually falling sperm concentrations during the initial 12 weeks of T treatment and the gradually rising sperm concentrations during the first 12 weeks after institution of hLH. To normalize the distribution of sperm concentrations, log transformation was employed before statistical analysis. The mean sperm concentrations from each of the study periods were then compared using Student's paired t test.

Mean monthly serum LH, FSH, and T levels during the control and T suppression periods and during the hLH plus T administration were determined in the four experimental subjects. These data as well as urinary FSH excretion measured at the end of each study period were compared with Student's paired t test.

Results

Seminal fluid parameters

After the 3-month control period, exogenous T enanthate administration (200 mg, im, weekly) resulted in a marked suppression of sperm production to less than 5 million/ml after 3–4 months in all subjects. In the four experimental subjects, sperm concentrations were reduced to 0.7 ± 0.7 million/ml (mean ± SEM) after the initial 12 weeks of T administration, compared with 98 ± 7 million/ml during the control period. Three of the four experimental subjects (subjects 1–3) became azoospermic, while the remaining man (subject 4) had sperm concentrations consistently below 4 million/ml. While continuing exogenous T at the same dosage, the experimental subjects simultaneously received hLH (1100 IU,

sc, daily) for 4–6 months (for 4 months in subject 3, for 5 months in subject 2, and for 6 months in subjects 1 and 4). Sperm concentrations increased significantly in all subjects with the addition of hLH to T (Fig. 1), reaching a mean of 19 ± 4 million/ml after 3 months of hLH plus T administration ($P < 0.001$ compared to T alone). Although sperm concentrations increased during hLH plus T therapy, they did not consistently reach the individuals' control ranges in any subject. The means of the last three sperm concentrations during the hLH plus T period for subjects 1–4 were 36, 34, 8, and 15 million/ml, compared to mean sperm concentrations during the control period of 117, 89, 101, and 84 million/ml, respectively, and ranges of sperm concentrations during the control period of 55–173, 25–126, 40–143, and 25–180 million/ml, respectively. The maximum sperm concentrations achieved during the hLH plus T period were 64, 80, 19, and 29 million/ml, for subjects 1–4, respectively. Three men (subjects 1, 2, and 4) achieved at least one sperm concentration within their control range during hLH plus T administration. Sperm motility and morphology at the end of the hLH plus T period were normal in all four experimental subjects.

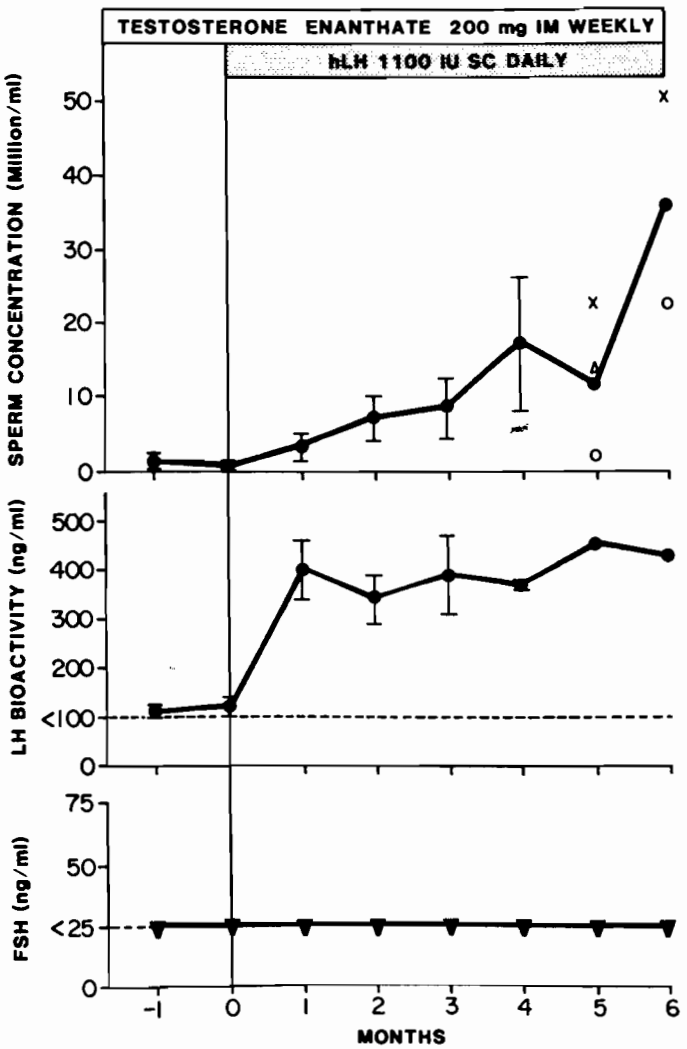

FIG. 1. Mean monthly sperm concentration (*upper panel*), serum LH bioactivity (middle panel), and serum FSH level (*lower panel*) in subjects 1–4 during the last 2 months of the T suppression period and the first 4 months of the hLH plus T period (mean ± SEM). In the last 2 months of the hLH plus T period, mean monthly sperm concentrations are presented for each subject remaining in the study. X, △, and ○, Mean monthly sperm concentrations for subjects 1, 2, and 4. Sperm concentrations and serum LH bioactivity are severely suppressed, and serum FSH levels are undetectable (▼) during T suppression. The addition of hLH to T treatment increases LH bioactivity into the physiological range and stimulates sperm production, despite continued undetectable FSH levels. ---, The limits of detectability of the LH and FSH assays.

Similar to the experimental subjects, exogenous T administration resulted in marked suppression of sperm concentrations in all seven control subjects (data not shown). In these men, sperm concentrations after the initial 12 weeks of T administration were reduced to 2 ± 1 million/ml, compared to 65 ± 9 million/ml during the control period. Four of the seven control subjects became azoospermic, while the remaining men had sperm concentrations consistently below 3 million/ml after 3–4 months of T treatment. In contrast to the men who received hLH and T, sperm concentrations remained suppressed in all control subjects to less than 3 million/ml (0.9 ± 0.5 million/ml) throughout the entire experimental period. By the end of this period of prolonged T treatment, all seven control subjects were azoospermic.

The six men (two experimental and four control subjects) who had seminal fluid collections continued after discontinuation of hormonal therapy achieved three successive sperm concentrations within their own control range within 6 months. The means of the last three sperm concentrations for the two experimental subjects during the recovery period were 174 and 36 million/ml for subjects 1 and 4, respectively.

LH bioactivity

In the four experimental subjects, serum LH bioactivity (Fig. 1) was markedly suppressed during the T suppression period (120 ± 10 ng/ml) compared to that during the control period (390 ± 20 ng/ml; $P < 0.001$). With the addition of hLH injections to T treatment, LH bioactivity returned to pretreatment control levels in all of these men (400 ± 40 ng/ml; P = NS compared to control values).

Serial sampling between two hLH injections near the end of the hLH plus T period revealed a 2-fold increase in serum LH bioactivity from 330 ± 50 ng/ml immediately before hLH injection to 750 ± 140 ng/ml (at the upper limit of the normal adult range) 8 h after injection.

LH bioactivity returned to preinjection values (400 ± 40 ng/ml) 24 h after LH injection.

FSH levels

Serum FSH (Fig. 1) was reduced to undetectable levels (<25 ng/ml) during the T suppression period compared to that during the control period (98 ± 12 ng/ml; $P < 0.01$). With the addition of hLH to T treatment, FSH levels remained below 25 ng/ml throughout the entire experimental period. Urinary FSH levels (Table 1) were within the normal adult range during the control period and were significantly reduced to prepubertal levels during the T suppression period. With the addition of hLH to T treatment, urinary FSH excretion remained suppressed in the prepubertal range.

Serum FSH levels were consistently below 25 ng/ml throughout serial sampling between two hLH injections performed near the end of the hLH plus T period.

T levels

Serum T levels (Table 1) increased 2-fold from the control period to the T suppression period. With the addition of hLH to T treatment, serum T levels increased significantly compared to those during the T suppression phase.

Clinical observations

All subjects remained in good health throughout the entire study. Mild truncal acne developed in three control subjects during T administration. Otherwise, no adverse effects of either T or hLH treatment were observed. Palpable breast tissue and testicular size remained within 1 cm of pretreatment measurements during hormonal therapy. Hematocrit increased slightly in all subjects, but no one developed a hematocrit above 54%. Routine hematological and coagulation studies, blood chemistries, and urinalyses were unchanged throughout the study. Examination of the monthly injection records revealed excellent compliance with the daily hLH injection regimen. Only occasional hLH injections were missed.

Discussion

Our results demonstrate that spermatogenesis can be reinitiated in gonadotropin-suppressed normal men by doses of hLH that produce LH levels within the physiological range. This stimulation of spermatogenesis occurs despite prepubertal FSH levels. In a setting of markedly suppressed gonadotropin and sperm production induced by exogenous T, all four experimental subjects demonstrated significant stimulation of spermatogenesis, as assessed by sperm concentrations, motilities, and morphologies with selective replacement of hLH for 4–6 months. Reinitiation of sperm production occurred despite undetectable serum FSH levels and prepubertal range urinary FSH excretion. Seven control subjects received exogenous T at the same dosage for an additional 6 months after initial T suppression. In these men, sperm concentrations remained severely suppressed throughout the entire treatment period. These results demonstrate that the suppressive effect of T does not dissipate with prolonged therapy and imply that the rise n sperm concentrations that occurred in the experimenal subjects was an effect of hLH.

Two experimental subjects (subjects 1 and 2) achieved nean sperm concentrations in the normal adult range during the hLH plus T period, while the remaining two men had mean concentrations in the oligospermic range. None of the subjects demonstrated sperm concentrations consistently within his own control range, although three men (subjects 1, 2, and 4) did achieve at least one sperm concentration within their control range. Self-injection records revealed good compliance with the daily hLH regimen. However, it is possible that unreported, irregular hLH administration could have contributed to the failure to achieve complete return of spermatogenesis. Because of limited availability of highly purified hLH for experimental use, the total duration of hLH treatment was limited to 4–6 months. In man, complete spermatogenesis requires approximately 75 days (15). Therefore, it is possible that the relatively short duration of a hLH treatment may have also contributed to the failure of complete normalization of sperm concentrations.

During hLH administration, mean serum LH bioactivity, measured in monthly blood samples and serial sampling between hLH injections, was documented to be within the physiological normal adult range. Therefore, it is unlikely that the small amount of FSH-like activity intrinsically present in the LH molecule (16) contributed significantly to the stimulation of spermatogenesis. However, it is well established that serum LH levels fluctuate normally in an episodic or pulsatile manner, with discrete pulses of LH occurring every 90–120 min in man (17). The pattern of LH levels produced in this study by a single daily sc injection of hLH did not mimic the physiological pulsatile pattern in man. Despite the nonpulsatile pattern of LH levels, sperm production was significantly stimulated. Whether administration of hLH

TABLE 1. Urinary FSH and serum T levels

	Control	T suppression	hLH plus T
Urinary FSH (mIU/h)[a]	353 ± 117	62 ± 14[b]	48 ± 17[b]
Serum T (ng/ml)[c]	5.5 ± 0.4	10.6 ± 0.2[d]	11.7 ± 0.1[e]

All values are the mean ± SEM for four subjects.

[a] Measured on 6 h urine aliquots at the end of each study period. Normal adult range, 190–1700 mIU/h; normal prepubertal range, 15–100 mIU/h.

[b] $P < 0.05$ compared to control.

[c] Measured on monthly blood samples, drawn immediately before scheduled injections of T or hLH plus T.

[d] $P < 0.001$ compared to control.

[e] $P < 0.01$ compared to T suppression.

in a pattern that more closely mimicked the normal pulsatile pattern of LH secretion would result in complete return of spermatogenesis is not known.

Endogenous FSH levels were severely suppressed during the administration of hLH plus T in all of our subjects. Serum FSH levels were undetectable in a very sensitive RIA that reliably detects values within the normal adult range and differentiates between values found in normal men and levels that are below the normal range (18). In addition, urinary FSH excretion, which is more sensitive than serum assays for detecting very low levels of FSH secretion (19), was in the range found in prepubertal children during hLH plus T. These small amounts of FSH detectable in the urine were insufficient by themselves to stimulate spermatogenesis, as similar amounts of endogenous FSH were present during the T suppression phase when sperm concentrations were very low.

Although our present results demonstrate that doses of hLH that result in LH levels in the physiological range can stimulate sperm production in the absence of significant FSH levels, we do not conclude that FSH has no role in spermatogenesis. In the present study, as well as in our previous studies (4, 20), suppression of gonadotropin and sperm production before instituting selective gonadotropin replacement was relatively short term (3–5 months). It is possible that normal levels of FSH may be necessary to reinitiate spermatogenesis after more prolonged gonadotropin deficiency.

In a previous study, we found that hFSH can stimulate spermatogenesis in gonadotropin-suppressed normal men, demonstrating that normal levels of LH were not absolutely required for stimulation of sperm production (20). We have also shown that normal levels of FSH were not absolutely required for reinitiation of spermatogenesis (Ref. 4 and the present study). However, in none of these studies has selective gonadotropin replacement with hFSH, hCG, or hLH administered singly fully normalized sperm production. Therefore, although it is possible to demonstrate a stimulatory role for either hFSH or hLH alone, neither gonadotropin by itself is sufficient to induce quantitatively normal sperm production. It is likely that normal levels of both LH and FSH are required for quantitatively normal spermatogenesis in man. The finding that each gonadotropin in the near absence of the other is capable of partially stimulating sperm production implies that selective gonadotropin suppression will not be an effective technique for male contraceptive development.

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