

# Chronic Human Chorionic Gonadotropin Administration in Normal Men: Evidence that Follicle-Stimulating Hormone Is Necessary for the Maintenance of Quantitatively Normal Spermatogenesis in Man\*

ALVIN M. MATSUMOTO†, ANTHONY E. KARPAS, AND WILLIAM J. BREMNER

*Endocrinology Section, Veterans Administration Medical Center; Population Center for Research in Reproduction; and the Department of Medicine, University of Washington School of Medicine, Seattle, Washington 98108*

**ABSTRACT.** The role of FSH in the maintenance of spermatogenesis in man is poorly understood. To determine whether normal serum levels of FSH are necessary for the maintenance of quantitatively normal spermatogenesis, we first studied the effect on sperm production of selective FSH deficiency induced by chronic administration of hCG in normal men. Then, we determined the effect of FSH replacement in some of these men.

After a 3-month control period, eight normal men (aged 30–39 yr) received 5000 IU hCG, im, twice weekly for 7 months. Then while continuing the same dosage of hCG, subjects simultaneously received 200 mg testosterone enanthate (T), im, weekly for an additional 6 months. hCG administration alone resulted in partial suppression of the mean sperm concentration from  $88 \pm 24$  ( $\pm$ SEM) million/ml during the control period to  $22 \pm 7$  million/ml during the last 4 months of hCG treatment ( $P < 0.001$  compared to control values). With the addition of T to hCG, sperm counts remained suppressed to the same degree. Except for one man who became azoospermic while receiving hCG plus T, sperm motilities and morphologies remained normal in all subjects throughout the entire study. During both the hCG alone and hCG plus T periods, serum FSH levels were undetectable ( $<25$  ng/ml), and urinary FSH levels were comparable to those in prepubertal children and hypogonadotropic hypogonadal adults.

We replaced FSH activity in four of the eight men in whom prolonged selective FSH deficiency and partial suppression of

sperm production were induced by hCG administration. Immediately after the period of hCG plus T administration, T was stopped in four men who continued to receive hCG alone (5000 IU, im, twice weekly) for 3 months. Then, while continuing the same dosage of hCG, these men received 100 IU human FSH, sc, daily ( $n = 2$ ) or 75 IU human menopausal gonadotropin, sc, daily ( $n = 2$ ) for 5–8 months. During the second period of hCG administration alone, serum FSH levels were undetectable ( $<25$  ng/ml), and sperm concentrations were suppressed ( $34 \pm 13$  million/ml) compared to the control values for these four men ( $125 \pm 39$  million/ml;  $P < 0.001$ ). With the addition of FSH to hCG, FSH levels increased ( $213 \pm 72$  ng/ml) and sperm concentrations rose significantly, reaching a mean of  $103 \pm 30$  million/ml ( $P < 0.03$  compared to hCG alone).

In summary, we found that prolonged selective FSH deficiency induced by hCG results in partial suppression of spermatogenesis and that FSH replacement results in stimulation of sperm production nearly to control levels. These results imply that the suppressive effects of hCG on spermatogenesis are not due to testicular desensitization or to the high estradiol levels produced by hCG, but are due to the low levels of FSH induced by hCG administration. Our findings provide strong evidence that normal levels of FSH are not an absolute requirement for the maintenance of sperm production, but are necessary for the maintenance of quantitatively normal spermatogenesis in man. (*J Clin Endocrinol Metab* 62: 1184, 1986)

**T**HE HORMONAL regulation of spermatogenesis in man is poorly understood. By stimulating intratesticular testosterone (T) production, LH is thought to

play an important role in both the initiation and maintenance of sperm production (1, 2). FSH is believed to be required for spermatid maturation (spermiogenesis) during the initiation of spermatogenesis at the time of puberty (1, 2). However, the role of FSH in the maintenance of sperm production in adults is unclear.

We have reported that spermatogenesis can be stimulated by selective administration of LH in normal men whose gonadotropin and sperm production were suppressed by exogenous T administration (3–5). Stimulation of sperm production occurred despite undetectable serum FSH levels and prepubertal levels of urinary FSH excretion. These results demonstrated that normal levels

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Address requests for reprints to: Dr. Alvin M. Matsumoto, Veterans Administration Medical Center (111), 1660 South Columbian Way, Seattle, Washington 98108.

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of FSH are not an absolute requirement for reinitiating spermatogenesis in gonadotropin-suppressed normal men.

In the present study, we determined whether normal serum levels of FSH were necessary to maintain sperm production in normal men. hCG administration in normal men results in profound suppression of serum FSH levels (6), which is selective since hCG contains almost exclusively LH-like bioactivity (7). In this study, we produced selective deficiency of FSH levels in normal men by chronic hCG administration followed by simultaneous administration of hCG and T. Then, FSH was replaced in these men by administration of human FSH (hFSH) or human menopausal gonadotropin (hMG) during continuous hCG administration. The effects on sperm production of the prolonged selective FSH deficiency followed by FSH replacement were assessed.

## Materials and Methods

### Subjects

Ten normal men, aged 30–39 yr, were recruited by newspaper advertisement and volunteered to participate in this study. All 10 were studied during a period of 19–23 months. However, after completion of the study, examination of injection records and hormone levels revealed poor compliance with the experimental protocol in 2 men, both of whom missed several hCG injections. Therefore, only the results from the 8 men who demonstrated excellent compliance with the experimental protocol are reported.

All subjects had a normal medical history and physical examination and normal complete blood count, coagulation time, 12-panel blood chemistry battery, and urinalysis. Six seminal fluid analyses, collected from each subject over 3 months, were normal (*i.e.* sperm concentration >20 million/ml, sperm motility >50%, and sperm morphology demonstrating >60% oval forms). In addition, normalcy of reproductive hormone status was established in all subjects by normal basal serum LH, FSH, T, and estradiol ( $E_2$ ) levels; normal LH and FSH secretory patterns on blood sampling every 20 min for 6 h; and normal LH and FSH responses to LHRH (0.2  $\mu$ g/min, iv, for 4 h).

### Experimental protocol

**Part I. Control period:** All men (subjects 1–8) underwent a 3-month period of control observations during which no hormones were administered. Baseline clinical status, gonadotropin and sex steroid levels, and seminal fluid parameters were established during this period, as described below.

**First hCG alone period:** After the control period, each subject received 5000 IU hCG (Profasi, Serono Laboratories, Inc., Braintree, MA), im, twice weekly for 7 months.

**hCG plus T period:** After the hCG alone period, while continuing the same dosage of hCG, each subject simultaneously received 200 mg T enanthate (Delatestryl, E. R. Squibb and Sons, Princeton, NJ), im, weekly. The combination of hCG and

T injections was continued for 6 months.

**Recovery period:** After the hCG plus T period, all hormone administration was discontinued in four subjects (subjects 5–8). Three of these men entered a posttreatment recovery period lasting 3–6 months, until three successive sperm concentrations returned to the subject's own pretreatment control range; one subject left the study at the end of the hCG plus T period. The remaining four subjects (subjects 1–4) continued to receive hCG alone (see *Part II*).

**Part II. Second hCG alone period:** Immediately after the period of combined hCG and T administration, subjects 1–4 continued to receive 5000 IU hCG, im, twice weekly alone for 3 months.

**hCG plus FSH period:** While continuing the same dosage of hCG, FSH activity was then replaced for an additional 5 months. In two men (subjects 1 and 2), FSH activity was replaced by administering 100 IU hFSH, sc, daily. The hFSH used was a highly purified preparation (LER 1577; lot 4) that was kindly provided by the National Pituitary Agency (Baltimore, MD). This preparation contained less than 1% LH activity in the ovarian ascorbic acid depletion, ventral prostate weight, and *in vitro* mouse Leydig cell bioassays. We have used this preparation in previous studies (8). Because of the limited availability of this purified hFSH preparation, the remaining subjects (subjects 3 and 4) received 75 IU hMG (Pergonal, Serono Laboratories), sc, daily to replace FSH activity during hCG administration. To determine the effect on sperm production of a longer period of FSH administration, two subjects continued to receive FSH replacement during hCG administration for an additional 2 (subject 3) and 3 months (subject 2).

**Third hCG alone period:** After the hCG plus FSH period, FSH injections were discontinued in two subjects (subjects 1 and 4), and hCG alone was continued for an additional 3 months. The resuppression of sperm counts with continuation of hCG alone after stopping FSH demonstrated that any rise in sperm counts during the hCG plus FSH period was due to FSH administration and not to a decline in the suppressive effect of hCG with time.

**Recovery period:** After the hCG plus FSH period (in subjects 2 and 3) and the third hCG alone period (in subjects 1 and 4), all subjects were followed for 3–6 months until three successive sperm counts returned to the subject's own mean control level.

The experimental protocol was reviewed and approved by the Human Subjects Review Committee at the University of Washington and the Research and Development Committee of the Seattle V.A. Medical Center. After a full explanation of the purpose and extent of the study, informed consent was obtained in all subjects.

**Hormone administration.** All hCG and 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 technique for self-administering daily FSH injections into the abdominal sc tissue. Lyophilized hFSH or hMG was diluted in bacteriostatic normal saline by the investigators (100 IU hFSH or 75 IU hMG/ml injection) on a monthly basis. Subjects received a monthly supply of diluted hFSH or hMG and were instructed to keep the diluted hormone refrigerated until injected. Each subject kept a personal injection record, which was reviewed

monthly by one of the investigators.

**Measurements and clinical observations:** Throughout the entire study, each subject submitted seminal fluid specimens twice monthly. These specimens were obtained by masturbation after 2 days of abstinence from ejaculation. At monthly intervals, one of the investigators interviewed each of the subjects concerning his general health and performed a physical examination. A venous blood sample and urine specimen were obtained at each monthly visit for measurement of routine hematological and blood chemistry studies and urinalyses.

Serum FSH and LH levels were measured in monthly blood samples throughout the study. Serum T and E<sub>2</sub> levels were determined in the last monthly blood sample of the control, hCG alone, hCG plus T, and hCG plus FSH periods for each subject. During hormone administration, these monthly blood samples were drawn immediately before scheduled injections of hCG, hCG plus T, or hCG plus FSH. At the end of the control, first hCG alone, and hCG plus T periods, 6-h urine aliquots were collected for measurement of FSH levels.

**Hormone assays.** The methodologies for the RIA for serum FSH and LH were described previously (3). The tracer used in the LH RIA was purified hCG (supplied courtesy of Dr. C. Alvin Paulsen) radioiodinated with <sup>125</sup>I using chloramine-T (9). Otherwise, both FSH and LH RIAs employed reagents distributed by the National Pituitary Agency and LER 907 as the reference standard. The sensitivity of the FSH RIA was 25 ng/ml, and the intra- and interassay coefficients of variation were 7.3% and 9.7%, respectively. The sensitivity of the LH RIA was 6 ng/ml, and the intra- and interassay variabilities were 5.5% and 8.4%, respectively. Assay results were calculated using the computer program of Burger *et al.* (10).

The RIA for urinary FSH was performed at 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 was measured by RIA as described previously (11) using the Second International Reference Preparation of hMG as the reference standard.

The RIA for T and E<sub>2</sub> used reagents provided by the WHO Matched Reagent Programme (12). The methodologies were detailed previously (13). The assay sensitivities were 0.1 ng/ml for the T RIA and 12 pg/ml for the E<sub>2</sub> RIA. The intra- and interassay coefficients of variation were 5.1% and 9.8%, respectively, for the T RIA and 8.2% and 8.8%, respectively, for the E<sub>2</sub> RIA. In addition, measurement of T and E<sub>2</sub> levels in pooled samples provided monthly by the WHO External Quality Control Program were consistently very close to mean values obtained in other laboratories.

All samples from individual subjects were analyzed in the same assay.

**Seminal fluid analysis.** Seminal fluid analyses were performed by the Seminal Fluid Core Laboratory of the Population Center for Research in Reproduction, University of Washington (under the direction of Dr. C. Alvin Paulsen). Sperm concentrations were determined using a Coulter counter (Coulter Electronics, Inc., Hialeah, FL). Concentrations below 15 million/ml were confirmed by direct determination using a hemocytometer. These methods were described previously (14). 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 (15).

**Statistical analysis.** Mean sperm concentrations during the control period and after the initial 3 months until the end of each of the hCG alone, hCG plus T, and hCG plus FSH periods were determined for each subject. Sperm concentrations after 3 months of hCG, hCG plus T, and hCG plus FSH treatments were chosen to eliminate any transition effects of changing sperm concentrations during the first 3 months of each period. To normalize the distribution of sperm concentrations, log transformation was employed before statistical analysis. The mean sperm concentrations from each study period were compared using Student's paired *t* test.

Mean monthly serum FSH and LH levels during the control, hCG alone, hCG plus T, and hCG plus FSH periods were determined for each subject. These data as well as the serum T and E<sub>2</sub> levels and urinary FSH excretion measured at the end of each study period were compared using Student's paired *t* test.

## Results

### Seminal Fluid Analysis

**Part I.** The mean sperm concentration for all subjects during the 3-month control period was  $88 \pm 24$  ( $\pm$ SEM) million/ml. After the control period, administration of hCG (5000 IU, im, twice weekly) resulted in suppression of sperm production in all subjects (Figs. 1 and 2). Sperm concentrations were reduced to  $22 \pm 7$  million/ml during the last 4 months of hCG administration alone ( $P < 0.001$  compared to control values; Fig. 1). Suppression of sperm production was highly variable between subjects (Fig. 2). Sperm concentrations were suppressed below 10 million/ml in three men, but no individual became azoospermic. Sperm concentrations were reduced to 7–56% (mean, 27%) of control values during hCG administration alone (Fig. 2). Sperm motility and morphology were consistently normal in all subjects during the first hCG alone period.

While continuing the same dosage of hCG, the subjects then simultaneously received T enanthate (200 mg, im, weekly). During the hCG plus T period, sperm concentrations remained suppressed ( $26 \pm 7$  million/ml during the last 3 months of hCG plus T) and did not differ significantly from those during the first hCG alone period (Fig. 1). Sperm concentrations remained reduced below 10 million/ml in the same three men who were oligospermic during the first hCG alone period; one subject became azoospermic by the end of the hCG plus T period. Sperm concentrations ranged from 0.1–63% (mean, 33%) of control values during simultaneous administration of hCG and T (Fig. 2). Except for the individual who became azoospermic, sperm motility and morphology remained

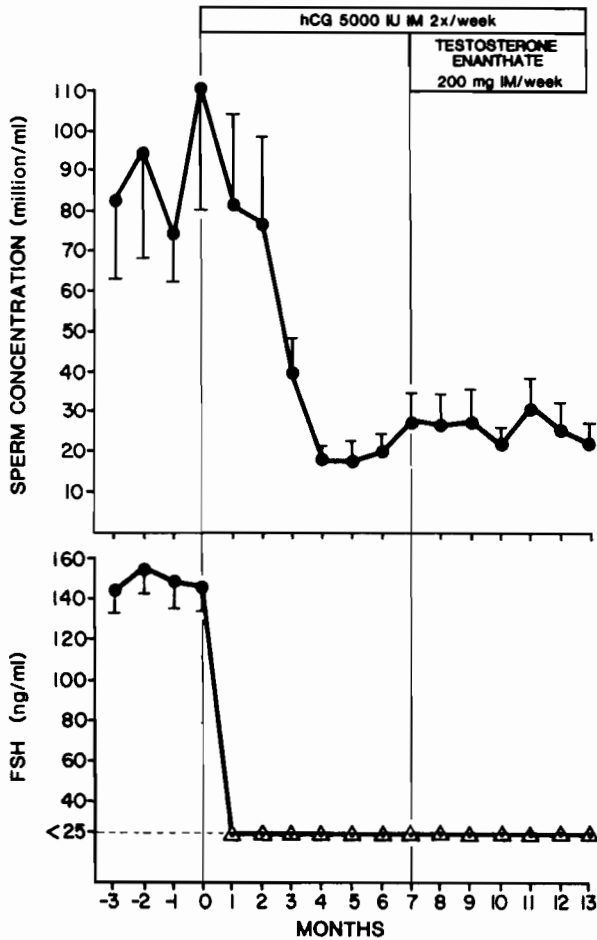


FIG. 1. Mean ( $\pm$ SEM) monthly sperm concentrations and serum FSH levels in eight normal men during the control, first hCG alone, and hCG plus T periods of the study (part I). Administration of hCG alone resulted in reduction of serum FSH to undetectable levels ( $\Delta$ ) and partial suppression of sperm concentrations. With the addition of T to hCG, FSH levels remained undetectable, and sperm concentrations remained suppressed to the same degree. ---, The limit of detectability of the FSH RIA.

normal throughout the hCG plus T period.

After the hCG plus T period, three subjects had seminal fluid collections continued after discontinuation of hormone administration. In these men, sperm concentrations returned to their own control ranges within 3-6 months.

*Part II.* Immediately after the hCG plus T period, subjects 1-4 continued to receive hCG alone (5000 IU, im, twice weekly) for 3 months. Sperm concentrations (Fig. 3) during this second period of hCG alone were significantly suppressed in all four subjects (mean  $\pm$  SEM, 34  $\pm$  13 million/ml) compared to their control values (125  $\pm$  39 million/ml;  $P < 0.001$ ). Only one man (subject 2) had a sperm concentration below 10 million/ml during the second period of hCG administration alone; this subject also had two azoospermic sperm counts (Table

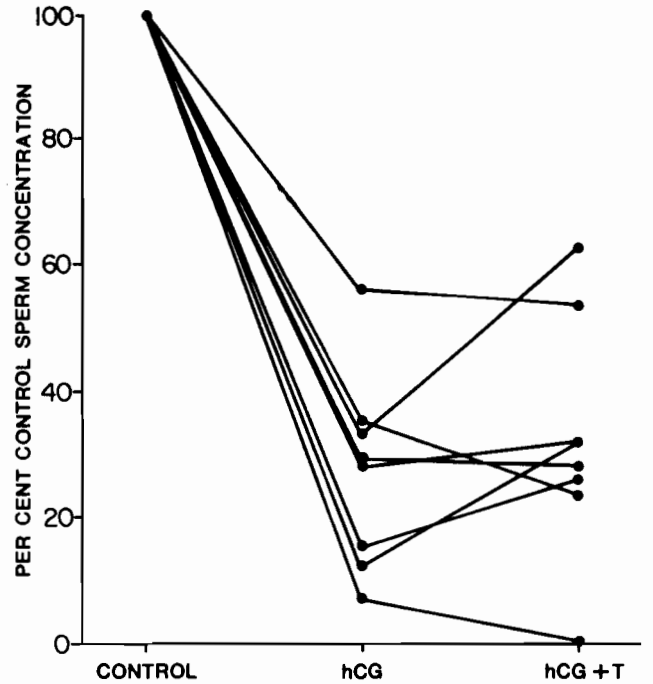


FIG. 2. Individual mean sperm concentrations during the first hCG alone and hCG plus T periods of study (part I). Each point represents the mean sperm concentration, expressed as a percentage of the mean control values (100%), for an individual subject after the initial 3 months of hCG alone or hCG plus T.

TABLE 1. Individual mean sperm concentrations and ranges (million per ml; part II)

Subject no.	Control	Second hCG alone	hCG plus FSH
1	63 $\pm$ 13 (43-113)	21 $\pm$ 3 (13-29)	84 $\pm$ 4 (80-107)
2	76 $\pm$ 8 (48-98)	2.7 $\pm$ 0.9 (0-6.8)	36 $\pm$ 15 (12-80)
3	234 $\pm$ 34 (113-358)	57 $\pm$ 9 (38-81)	113 $\pm$ 29 (53-194)
4	125 $\pm$ 10 (83-147)	55 $\pm$ 12 (29-65)	179 $\pm$ 34 (99-247)

Values are the means  $\pm$  SEM (ranges in parentheses) of sperm concentrations in the control period, second hCG alone period, and during the last 2 months of the hCG plus FSH period for each subject.

1). With the exception of the two azoospermic counts, sperm motility and morphology were consistently normal in all subjects during the second hCG period.

While continuing the same dosage of hCG, the subjects then received FSH replacement with either 100 IU hFSH, sc, daily (subjects 1 and 2) or 75 IU hMG, sc, daily (subjects 3 and 4) in addition to hCG. With the addition of FSH to hCG, sperm concentrations increased significantly in all four subjects (Fig. 3), reaching a mean of 103  $\pm$  30 million/ml during the last 2 months of hCG plus FSH ( $P < 0.03$  compared to hCG alone). All subjects had at least two sperm concentrations within their own control range. The mean sperm concentrations during

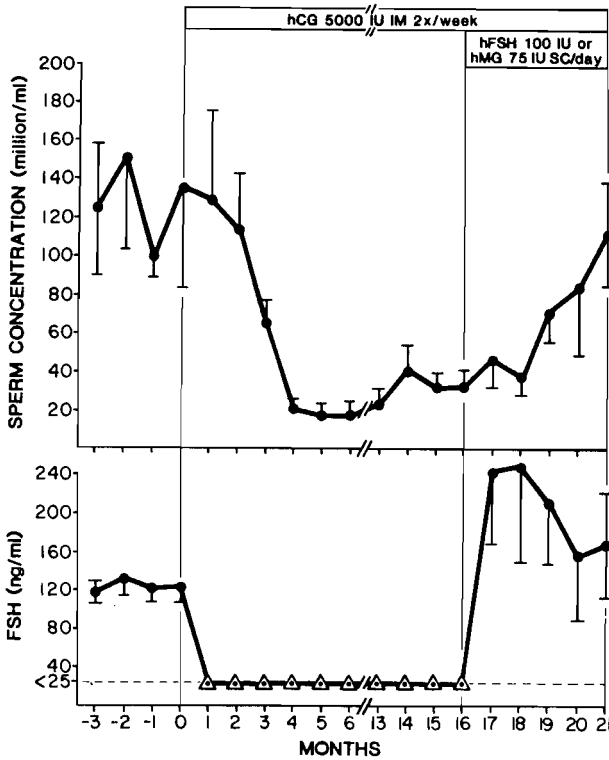


FIG. 3. Mean ( $\pm$ SEM) monthly sperm concentrations and serum FSH levels in four normal men (subjects 1-4) during the control and first and second hCG alone and hCG plus FSH periods (part II). Chronic hCG administration resulted in partial suppression of sperm production for 13 months and marked reduction of serum FSH to undetectable levels ( $\Delta$ ) for 16 months. The addition of FSH to hCG resulted in an increase in FSH levels and stimulation of sperm production to control levels. ---, The limit of detectability of the FSH RIA.

hCG plus FSH in two men (subjects 1 and 4) were above their own mean control values, while in the other two men (subjects 2 and 3), they were below their individual mean control levels (Table 1). In the latter two subjects, continuing FSH replacement during hCG administration for an additional 2-3 months failed to increase sperm concentrations further. Sperm motility and morphology were consistently normal in all four men during the hCG plus FSH period.

In the two subjects (subjects 1 and 4) who had hCG administration continued after the discontinuation of FSH replacement (third hCG alone period), sperm concentrations were again suppressed to levels similar to those before the addition of FSH (Fig. 4). Sperm concentrations in these men returned to control levels within 3-4 months after the discontinuation of hCG administration (Fig. 4).

After the hCG plus FSH period, the remaining two men (subjects 2 and 3) had all hormone administration discontinued. These men had return of sperm concentrations to control levels within 3-6 months.

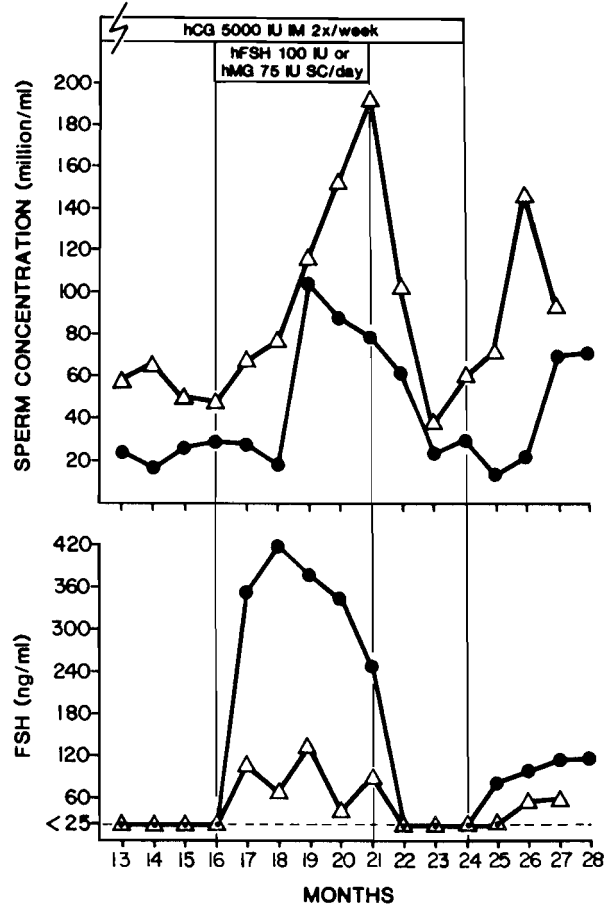


FIG. 4. Mean monthly sperm concentrations and serum FSH levels in two normal men during the second hCG alone, hCG plus FSH, third hCG alone, and recovery periods of the study (part II). Subject 1 ( $\bullet$ ) received hFSH, and subject 4 ( $\Delta$ ) received hMG during the hCG plus FSH period. Replacement of FSH during hCG administration resulted in stimulation of sperm concentrations and FSH levels compared to those during the previous period of hCG administration alone. Continuation of hCG after stopping FSH treatment resulted in reduction of FSH to undetectable levels ( $\Delta$ ) and suppression of sperm concentrations to levels similar to those in the previous period of hCG alone. Discontinuation of all hormone administration resulted in recovery of both sperm concentrations and FSH levels.

#### Gonadotropin levels

*Part I.* Serum FSH levels (Fig. 1) were normal during the control period ( $149 \pm 12$  ng/ml). Throughout the entire 7 months of the first period of hCG administration alone and 6 months of simultaneous hCG and T administration, serum FSH levels were undetectable ( $<25$  ng/ml) in all subjects ( $P < 0.001$  compared to control values).

Urinary FSH levels (Table 2) during the control period were within the normal adult range. FSH excretion was markedly suppressed during the hCG alone and hCG plus T periods, reaching levels typical of those in prepubertal children and hypogonadotropic hypogonadal adults (Table 2).

Serum LH levels were normal during the control period

TABLE 2. Urinary FSH levels (mIU per h; part I)

Control	First hCG alone	hCG plus T	Prepubertal children	Hypogonadotropic hypogonadism
(n = 8) <sup>a</sup>	(n = 8) <sup>a</sup>	(n = 8) <sup>a</sup>	(n = 11) <sup>b</sup>	(n = 4) <sup>b</sup>
533 ± 109	43 ± 9 <sup>c</sup>	38 ± 4 <sup>c</sup>	77 ± 12	54 ± 14

All values are the mean ± SEM. Analyses were performed by Core Endocrine Laboratory, Milton S. Hershey Medical Center. The normal adult range in this laboratory is 190–1700 mIU/h; the normal prepubertal range is 15–100 mIU/h.

<sup>a</sup> Measured in 6-h urine aliquots collected at the end of each study period.

<sup>b</sup> Data taken from the study of Kulin and Santner (21).

<sup>c</sup>  $P < 0.001$  compared to control.

(35 ± 4 ng/ml). During the first hCG alone and hCG plus T periods, serum LH levels were greater than 150 ng/ml due to cross-reactivity of hCG in the LH assay.

*Part II.* During the 3 months of the second period of hCG administration alone, serum FSH levels (Fig. 3) were also undetectable (<25 ng/ml). With the addition of FSH to hCG, serum FSH levels increased significantly in all four subjects (Fig. 3), reaching a mean of 213 ± 72 ng/ml ( $P < 0.05$  compared to hCG alone). Subjects 1 and 2, who received hFSH, had higher serum FSH levels (373 ± 16 and 297 ± 53 ng/ml, respectively) than subjects 3 and 4, who received hMG replacement (95 ± 8 and 86 ± 21 ng/ml, respectively).

In the two men (subjects 1 and 4) who continued to receive hCG for 3 months after FSH was stopped, serum FSH was again suppressed to undetectable levels throughout the entire third hCG alone period (Fig. 4). In the remaining two subjects (subjects 2 and 3), all hormone administration was stopped after the hCG plus FSH period. Serum FSH levels returned to their individual control levels by 3–4 months.

During the hCG alone and hCG plus FSH periods, serum LH levels were greater than 150 ng/ml because of cross-reactivity of hCG in the LH assay.

#### Steroid levels

*Part I.* Serum T levels (Table 3) during the first period of hCG administration alone increased significantly (~1.5-fold) above control levels. With the addition of T to hCG, serum T levels increased further, to levels significantly above those during the first hCG alone period (3-fold higher levels than control values).

Serum E<sub>2</sub> levels (Table 3) during the first period of hCG administration alone increased significantly (>3-fold) above control levels. With the addition of T to hCG, serum E<sub>2</sub> levels increased further, to levels significantly above those during the first hCG alone period (5-fold higher levels than control values).

*Part II.* During both the second hCG alone and hCG

TABLE 3. Serum T and E<sub>2</sub> levels

	Control	First hCG alone	hCG plus T
<b>Part I (n = 8)</b>			
Serum T (ng/ml)	5.3 ± 0.2	8.5 ± 1.6 <sup>a</sup>	15.8 ± 1.7 <sup>b</sup>
Serum E <sub>2</sub> (pg/ml)	29 ± 4	95 ± 14 <sup>c</sup>	144 ± 18 <sup>d</sup>
		Second hCG alone	hCG plus FSH
<b>Part II (n = 4)</b>			
Serum T (ng/ml)	5.2 ± 0.2	10.3 ± 2.1 <sup>a</sup>	11.7 ± 1.2 <sup>a</sup>
Serum E <sub>2</sub> (pg/ml)	38 ± 14	92 ± 31 <sup>a</sup>	103 ± 36 <sup>a</sup>

All values are the mean ± SEM, measured in blood samples at the end of each study period.

<sup>a</sup>  $P < 0.05$  compared to control.

<sup>b</sup>  $P < 0.05$  compared to hCG alone.

<sup>c</sup>  $P < 0.05$  compared to hCG alone.

<sup>d</sup>  $P < 0.01$  compared to hCG alone.

plus FSH periods, serum T levels in all four subjects (Table 3) increased significantly (~2-fold) above those during their control period. Serum E<sub>2</sub> levels (Table 3) were also significantly increased (~3-fold) above control levels during the second hCG alone and hCG plus FSH periods.

#### Clinical observations

All subjects remained in good general health throughout the entire study. Five of the eight subjects developed an increase in palpable breast tissue (measured circumferentially from the areola) of 2–3 cm bilaterally during hCG treatment alone. Breast enlargement was associated with tenderness in three men and expressible serous discharge in one individual. In these five men, breast size remained stable or regressed, and symptoms resolved completely in several weeks despite continued hCG therapy. None of the subjects developed breast enlargement or symptomatology during the simultaneous administration of hCG and T or during subsequent periods of hCG alone and hCG plus FSH. Mild truncal acne developed in four subjects during hCG and T administration and in one subject during the second hCG alone period. Testicular size (measured by calipers) remained within 1 cm of pretreatment measurements in all subjects during hormonal treatment. Hematocrit increased slightly in all subjects, but no one developed a hematocrit above 52%. Routine blood chemistries and urinalyses were normal throughout the entire study.

#### Discussion

Our results demonstrate that chronic hCG administration in normal men resulted in marked reduction of FSH secretion to prepubertal levels and partial suppression of

sperm production. Furthermore, in these men, replacement of FSH activity stimulated serum production nearly to control levels.

Undetectable serum FSH levels and urinary FSH excretion comparable to those in prepubertal children and adults with hypogonadotropic hypogonadism were achieved in all subjects for the entire 13–16 months of hCG and combined hCG plus T administration. In this setting of prolonged severely suppressed FSH levels, all eight subjects had significant, but not complete, suppression of spermatogenesis. Only three subjects had sperm concentrations below 10 million/ml during treatment, and only one individual became azoospermic during combined hCG and T administration. Except for the one individual who became azoospermic, sperm motilities and morphologies remained normal in all subjects throughout the entire study.

In the setting of prolonged selective FSH deficiency, replacement of FSH activity in four subjects resulted in significant stimulation of sperm production. All four subjects had at least two sperm concentrations during hCG plus FSH that were within their own control range. However, two men (subjects 2 and 3) had mean sperm concentrations during the last 2 months of hCG and FSH administration that were below their mean control sperm concentrations. To determine whether the relatively short duration (5 months) of the hCG plus FSH period may have contributed to the failure of complete normalization of sperm production, FSH replacement was extended for 2–3 months in these two men. More prolonged FSH replacement did not result in any further increases in sperm concentrations. Therefore, it is unlikely that the duration of FSH replacement contributed to the failure of full normalization of sperm counts in these men.

The lack of complete return of sperm production in subjects 2 and 3 could not be explained by the FSH preparation used to replace FSH activity or the serum FSH levels achieved during FSH replacement. Subject 2 received hFSH replacement and had higher FSH levels ( $297 \pm 53$  ng/ml) during the hCG plus FSH period compared to those in his control period ( $145 \pm 7$  ng/ml). Subject 3 received hMG, which resulted in lower FSH levels ( $95 \pm 8$  ng/ml) compared to those in his control period ( $150 \pm 3$  ng/ml). Injection records and serum FSH levels revealed excellent compliance with the experimental protocol. However, since the subjects injected themselves with FSH and were responsible for keeping their own injection records, it is possible that undocumented irregular FSH administration may have contributed to the failure of complete normalization of sperm production in the two subjects.

There is evidence that high levels of  $E_2$  stimulated by hCG may adversely affect testicular responses to gonad-

otropins (16–19). Serum  $E_2$  levels during the hCG plus FSH period were approximately 3-fold higher than control values. Despite these very high  $E_2$  levels, sperm production was stimulated significantly and nearly to control levels with FSH replacement. However, we cannot eliminate the possibility that high  $E_2$  levels may have contributed to the failure of complete normalization of sperm production in the two men whose mean sperm concentrations during the hCG plus FSH period were below those of their own control period.

After the period of simultaneous hCG and FSH administrations, two subjects continued to receive hCG alone after FSH replacement was stopped. Serum FSH levels were again reduced to undetectable levels, and sperm concentrations in these men were again suppressed to levels similar to those during the prior period of hCG administration alone (before the addition of FSH replacement). These results demonstrate that the stimulation and maintenance of sperm production during the hCG plus FSH period were due to FSH replacement and not to a decline in the suppressive effect of hCG with time.

Endogenous FSH levels were markedly suppressed in all subjects during the administration of both hCG alone and hCG in combination with T, as determined by an assay that differentiates between values found in normal men and levels below the normal range (20). Measurement of FSH excretion in a timed urine specimen has been reported to be more sensitive than serum measurements for detecting very low levels of FSH activity (21). During the periods of both hCG treatment alone and hCG plus T administration, urinary FSH excretion was reduced to levels comparable to those in prepubertal children and hypogonadotropic hypogonadal adults.

Our findings of quantitative reduction in sperm production in normal men with selective FSH deficiency induced by chronic hCG administration are in agreement with several previous studies by other investigators. Studies of hCG administration to normal men or men with idiopathic oligospermia have demonstrated severe reduction of serum FSH levels (6, 22, 23). In some of these studies, as in ours, FSH suppression induced by hCG resulted in a reduction of sperm counts, although not azoospermia (18, 23). In other studies, sperm counts were not affected by chronic hCG administration (22). Either passive or active induction of neutralizing antibodies to FSH in normal adult male monkeys has been reported to reduce sperm production and fertility, with no detectable effect on LH or T levels (24–26). However, in none of these immunization studies was complete suppression of spermatogenesis achieved. A few reports have appeared describing men with selective deficiency of FSH, generally associated with decreased spermatogenesis (27–29). Generally, these reports are unconvinc-

ing due to inadequacies in the demonstration of FSH deficiency and failure to study the effect of FSH therapy.

In previous studies, we demonstrated that sperm production can be stimulated in gonadotropin-suppressed normal men by selective replacement of LH-like activity (3-5). Stimulation of spermatogenesis occurred in these studies despite prepubertal FSH levels. This stimulatory effect on spermatogenesis was found with the administration of either supraphysiological (3, 5) or near-physiological LH activity (4) and after either short (3, 4) or long (5) periods of gonadotropin suppression. However, in none of our previous studies was fully normal sperm production (*i.e.* restoration to control levels) achieved with selective replacement of LH activity alone.

In the present study, we found that sperm production was suppressed when selective FSH deficiency was induced by chronic hCG administration. However, the degree of spermatogenic suppression was not complete. In fact, the sperm concentrations during hCG alone and hCG plus T periods in this study were similar to those achieved by selective replacement of LH activity in gonadotropin-suppressed normal men in our previous studies (3-5). Thus, a significant degree of sperm production can be maintained, despite very low FSH levels. Furthermore, in normal men with selective FSH deficiency and suppressed spermatogenesis induced by hCG administration, FSH replacement stimulated sperm production nearly to control levels. These findings provide strong evidence for an important role of normal FSH levels in the maintenance of quantitatively normal spermatogenesis in man.

Although FSH levels were very low during the administration of hCG and hCG plus T, FSH activity was not completely absent. Urinary FSH excretion was detectable, albeit at very low levels, during both of these treatment periods. Another source of slight FSH-like bioactivity was the hCG itself (7, 30). hCG has a very small amount of intrinsic FSH-like bioactivity, approximately 1/1000th that of its LH-like activity (7, 30). Although LH bioactivity in serum was not measured in this study, it is clear from the high sex steroid levels stimulated by hCG that the dosage of hCG was supraphysiological. In a previous study using a higher dosage of hCG (5000 IU, three times weekly), serum LH bioactivity during hCG administration was approximately 6 times the control value (3). This 6-fold increase in LH bioactivity did not approach the 1000-fold increase necessary to produce significant FSH-like activity. Therefore, it is unlikely that the lower dosage of hCG used in the present study contributed a significant amount of FSH-like bioactivity.

Five of the eight subjects in this study developed significant increases in palpable breast tissue after hCG administration alone. With continued hCG administration, breast tissue remained unchanged or decreased in

size. In marked contrast, no significant changes in palpable breast tissue occurred in any of our previous studies in which T was administered with hCG to normal men (3-5). It is possible that the relatively higher ratio of serum E<sub>2</sub> to T levels that occurred during the administration of hCG alone compared to that during simultaneous hCG and T administration may have predisposed our subjects to develop gynecomastia.

In conclusion, our findings demonstrate that normal levels of FSH are not an absolute requirement for the maintenance of spermatogenesis, but are necessary for the maintenance of quantitatively normal sperm production in man. The finding that selective suppression of FSH activity results in incomplete suppression of sperm production implies that such a strategy will not be an effective technique for male contraceptive development.

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