Follicle-stimulating Hormone and Human Spermatogenesis

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ABSTRACT The role of follicle-stimulating hormone (FSH) in the control of spermatogenesis is not well established in any species, including man. We studied the effect of an experimentally-induced, selective FSH deficiency on sperm production in normal men. After a 3-mo control period, five normal men received testosterone enanthate (T) 200 mg i. m. weekly to suppress luteinizing hormone (LH) and FSH, until three successive sperm counts revealed azoospermia or severe oligospermia (sperm counts <3 million/ml). Then, while continuing T, human chorionic gonadotropin (hCG) 5,000 IU i. m. three times weekly was administered simultaneously to replace LH activity, leaving FSH activity suppressed. The effect of the selective FSH deficiency produced by hCG plus T administration on sperm production was determined.

Sperm counts (performed twice monthly throughout the study) were markedly suppressed during T administration alone (1.0±1.0 million/ml mean±SE, compared with 106±28 million/ml during the control period, $P < 0.001$). With the addition of hCG to T, sperm counts returned toward normal (46±16 million/ml, $P < 0.001$ compared with T alone). In two subjects, sperm counts during hCG plus T returned into the individual's control range. Sperm motility and morphology were consistently normal in all men during hCG plus T.

Serum FSH levels by RIA were normal (110±10 ng/ml) in the control period and were suppressed to undetectable levels (<25 ng/ml) in the T alone and hCG plus T periods. Urinary FSH excretion was markedly suppressed in the T alone (60±15 mIU/h-2nd IRP, $P < 0.01$) and hCG plus T (37±9 mIU/h, $P < 0.01$) periods compared with the control period (334±78 mIU/h).

We conclude that spermatogenesis as assessed by sperm counts, motilities, and morphologies may be reinitiated and maintained at normal levels in men with undetectable blood FSH levels and urinary excretion of FSH less than that of prepubertal children. This conclusion implies that, although FSH may exert effects on human testicular function, maintenance of normal spermatogenesis and reinitiation of sperm production after short-term suppression by exogenous steroids can occur in spite of nearly absent FSH stimulation.

INTRODUCTION

It is clearly established in man, as in other mammalian species, that normal spermatogenesis requires the stimulatory actions of pituitary gonadotropins (1, 2). Both luteinizing hormone (LH)[1] and follicle-stimulating hormone (FSH) exert effects on testicular function, but the specific role played by each in controlling spermatogenesis is unclear.

Soon after gonadotropins were partially purified, in the 1930s, the concept was proposed that LH stimulates testosterone production and FSH controls spermatogenesis (3). More recent work in animals has generally supported this concept. It has been demonstrated that LH binds specifically to Leydig cells, where it stimulates cyclic AMP accumulation and the conversion of cholesterol to pregnenolone, leading to increased formation of testosterone, the major testicular steroid product (4). LH has not been demonstrated to bind to cells within the seminiferous tubules nor to

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[1] *Abbreviations used in this paper*: FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; RIA, radioimmunoassay.

exert direct biochemical effects there. FSH binds to Sertoli cells and spermatogonia (5) within the seminiferous tubules. Binding of FSH to Sertoli cells is followed by cyclic AMP accumulation, protein kinase activation, and androgen binding protein production (6). FSH also stimulates the conversion of testosterone to estradiol by Sertoli cells (7).

However, the effects of FSH are peculiar in that they can be demonstrated only in prepubertal animals or in adults following hypophysectomy (1, 6, 7). In intact rats, for example, effects of FSH administration can be demonstrated in 21-d-old animals, but not in 80-d-old animals (6). Similarly, studies in immature rats using antibodies of FSH, specifically neutralizing its biological activity, have demonstrated decreases in testicular size and androgen binding protein production (8). Analogous studies in adult rats have demonstrated no effect on testicular function (9). Such data have raised the possibility that FSH may be important only during initiation of spermatogenesis at the time of puberty or reinitiation of sperm production following hypophysectomy and not be important in the maintenance of normal spermatogenesis in adults.

The use of antibodies to FSH in adult male nonhuman primates has yielded results different from those found in rats. Either passive or active (10 induction of neutralizing antibodies to FSH in normal adult monkeys has been reported to lead to decreases in sperm production and fertility, with no detectable effect on LH and testosterone levels. The reasons for the differences between the antibody studies in monkeys and rats are not clear and the results have been ascribed to species differences in the biological role of FSH.

The role of FSH in the control of human testicular function is also unclear. Studies of gonadotropin replacement in patients with hypogonadotropic disease have suggested that FSH is usually (11), but not always (1, 12), required for the initiation of spermatogenesis. The interpretation of such studies has been difficult because of uncertainties as to the purity of the hormone preparations administered and the degree of hormone deficiency in the patients. No agent is presently available for experimental use that is capable of producing a selective deficiency in FSH production (13) so that the effect of this deficiency might be studied in man. A few men have been reported to have selective deficiencies of FSH, generally associated with defective spermatogenesis (14). Some of these reports have been unconvincing due to inadequacies in demonstrating the FSH deficiency and none has shown return to normal sperm production with the selective replacement of FSH.

We resolved to study the effect of an experimentally-induced selective deficiency of FSH in normal men. We administered testosterone (T) to normal men, suppressing both LH and FSH levels until three successive sperm counts revealed azoospermia or severe oligospermia. Then, while T injections were continued, human chorionic gonadotropin (hCG) injections were added. In this way, a selective deficiency of blood FSH levels was produced since hCG contains almost exclusively LH-like bioactivity (15). Levels of LH and FSH were carefully monitored by immunoassay of blood and urine and by bioassay of blood. The effect of this FSH-deficiency state on sperm production was assessed.

METHODS

Subjects. Five normal men aged 25–40 yr were studied over a period of 10–22 mo. Normality was established by complete medical histories, physical examinations, and measurement of routine hematological variables, blood chemistries, and urinalyses. In addition, six seminal fluid analyses collected over 3 mo were normal[2] as were basal LH, FSH, and T levels. Normality of the gonadotropins was confirmed by 20-min sampling for 6 h and by responsiveness to 4 h infusions of luteinizing hormone releasing hormone (LHRH) (see below).

The experimental protocol was approved by the Human Subjects Review Committee of the University of Washington. Informed consent was obtained from volunteers who agreed to participate after being provided with a full explanation of the purpose and extent of the study.

Experimental design. The first 3 mo of the study constituted a control period during which observations and measurements (see below) were performed in each subject, but no hormones were administered. After the control period, testosterone enanthate (Delatestryl, E. R. Squibb and Sons, Princeton, N. J.) administration was begun (200 mg, i. m. weekly). The injections of T alone were continued until three successive seminal fluid analyses (obtained every 2 wk) revealed sperm counts <5 million/ml. At this point, while the injections of T were continued, administration of hCG (Profasi, Serono Laboratories, Inc., Braintree, Mass.), 5,000 IU i. m. three times weekly, was added. The combination of hCG and T injections was continued in all five men until three successive sperm counts were within the individual's control range or a minimum of 17 wk.

At this time, to demonstrate that the increases in sperm counts found were due to hCG and not to a decline in the suppressive effect of testosterone, hCG injections were stopped in two subjects and T alone was continued until sperm counts were again suppressed to very low levels. Then T was discontinued and the two subjects entered a posttreatment control period lasting until three successive sperm counts were within the subject's control range.

In one subject, following the hCG plus T phase of the study, T injections were stopped and hCG was maintained for 6 mo to assess the effect of hCG alone on the normal human testis. This subject also underwent posttreatment observation until three successive sperm counts were within his control range. The remaining two subjects left the study at the end of the hCG plus T phase. All injections were administered by the investigators or their nursing assistants. Injection records allowed assessment of each subject's compliance with the experimental protocol.

[2] Sperm count >20 million/ml, motilities >50% and morphology demonstrating >60% normal oval forms (16).

During each month of the study, each subject submitted two seminal fluid specimens, obtained by masturbation after 2 d of abstention from ejaculation. In addition, one of the investigators interviewed each of the subjects monthly concerning his general health and sexual function and performed a brief physical examination. A venous blood sample and a urine sample were obtained at each visit for measurement of routine hematological and blood chemical variables and urinalyses. In addition, serum levels of LH, FSH, and T were determined. During the experimental periods, blood sampling for hormone measurement was performed immediately before the injections of T or of hCG and T.

Near the end of each of the three phases of the study (control, T alone and hCG plus T administration) each subject underwent 2 d of intensive study. On the first of these days, an indwelling venous cannula was placed in an arm vein and blood samples were obtained every 20 min for 6 h for measurement of LH and FSH levels. During this same 6 h, urine was collected for measurement of FSH. On the second day, an LHRH infusion (0.2 μg/min for 4 h) was administered to each subject through a cannula in an arm vein. Venous blood samples were obtained from a cannula in the opposite arm for measurement of LH and FSH levels. Three samples were obtained at 15-min intervals before the infusion began; sampling continued at 15-min intervals during the initial 120 min of the infusion, after which sampling was at 30-min intervals until the end of LHRH administration. Both the repeated sampling study and the LHRH infusion were begun between 0800 and 1000 h.

Radioimmunoassays (RIA). The RIA for serum FSH (17) used reagents distributed by the National Pituitary Agency. The reference standard was LER 907, a partially purified preparation of human pituitary gonadotropin. The hormone used for iodination (18) was HS-1 and the first antibody was rabbit anti-human FSH, batch No. 5. Assay results were calculated using the computer program of Burger et al. (19). Sensitivity was 25 ng/ml; intra-and interassay variability were 7.3 and 9.7%, respectively.

The RIA for serum LH used a standard (LER 907) and a first antibody (anti-human LH, batch 2) supplied by the National Pituitary Agency. The tracer was hCG purified in this laboratory and radioiodinated with ^{125}I using chloramine T (18). The sensitivity of this assay was 6 ng/ml; the intraassay variability was 5.5% and the interassay variability was 8.4%.

Testosterone was measured by radioimmunoassay, using reagents supplied by the World Health Organization Matched Reagent Programme (20). The sensitivity was <10 pg/tube (0.1 ng/ml). The intraassay coefficient of variation was 5.1% and the interassay coefficient of variation was 9.8%.

The RIA for urine FSH was performed courtesy of Dr. Richard J. Santen. Aliquots of urine (80 ml) were precipitated with acetone, centrifuged, and resuspended in RIA buffer (21). FSH was then measured by RIA with the Second International Reference Preparation of Human Menopausal Gonadotropin (2nd IRP-hMG) used as the reference standard.

The in vitro bioassay of LH-hCG was a modification (20) of the procedures described by van Damme et al. (22) and by Dufau et al. (23). This assay is based on the measurement of T production by dispersed Leydig cells from immature mice. The standard used was partially purified hCG obtained from Serono, Inc. (Profasi). Serial dilutions of serum samples containing high LH levels were shown to be parallel to the standard. The minimally detectable amount of LH was 5 μIU/tube. All samples were run in duplicate, frequently at two dilutions. The mean intra- and interassay coefficients of variation for pooled human sera were 14 and 24%, respectively.

Seminal fluid analysis. Measurement of spermatozoal concentrations in seminal fluid (sperm count) was performed as described previously (24). Most counts could be assessed accurately using the Coulter counter (Coulter Instruments, Inc., Hialeah, Fla.), but those <15 million/ml were confirmed using a hemocytometer. To normalize the distribution of sperm counts, log transformation was employed before statistical analysis. No significant changes in seminal volume occurred with hormonal treatment, so the sperm counts gave an accurate estimate of total sperm output in the ejaculate. Sperm motility and morphology were assessed as described by MacLeod (25).

Statistical analysis. Mean sperm counts during the control phase and during the last 6 wk of the T alone phase of the study were calculated for each subject. These data were compared, using Student's paired *t* test, to mean sperm counts during the 10th–30th wk of the hCG plus T phase of the study. The 10th–30th wk were chosen to eliminate the transition effects of gradually rising sperm counts in the first 9 wk after initiating hCG administration.

RESULTS

Following the 3-mo control period, administration of testosterone led to severe inhibition of sperm production (Fig. 1). Three subjects became azoospermic,

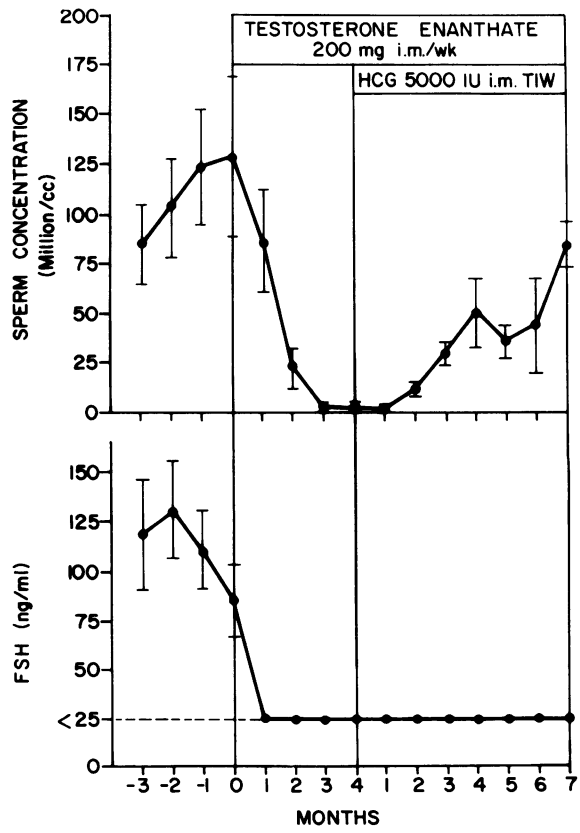

FIGURE 1 Monthly sperm concentrations and serum FSH data in five normal men during the control, testosterone administration alone, and hCG plus testosterone phases of the study (mean±SE). Note the increase in sperm concentration induced by hCG in spite of very low serum levels of FSH.

TABLE I
Urinary FSH Levels (mIU/h)

Control	Testosterone alone	hCG + testosterone	hCG alone	Prepubertal children*	Hypogonadotrophic hypogonadism*
(n = 5)	(n = 5)	(n = 5)	(n = 1)	(n = 11)	(n = 4)
334±78	60±15‡	37±9‡	45	77±12	54±14

* Data taken from Kulin and Santner (29).
‡ $P < 0.01$ compared to control using Student's paired t test.

whereas two consistently exhibited sperm counts of <3 million/ml. While the testosterone injections were continued, hCG was added (5,000 IU i.m., three times weekly). Sperm counts (Fig. 1) increased markedly during hCG administration ($P < 0.001$ compared with testosterone injections alone). In two subjects, sperm counts during hCG plus T injections returned into the normal control range for each man. In the other three men, although sperm counts increased markedly on hCG plus T, reaching mean levels of 12, 13, and 94 million/ml, they did not consistently reach the men's control ranges. Medication records revealed that the two men with the lowest counts did not receive all their scheduled hCG injections. Sperm motility (>50% motile sperm) and morphology (>60% normal oval forms) were consistently normal in all men during hCG plus T injections.

Serum FSH values (Fig. 1) were normal (111±10 ng/ml) in the control period and were suppressed to undetectable levels (<25 ng/ml) in the T alone and in the hCG plus T phases of the study. Urine FSH levels (Table I) revealed a marked decrease from control to very low levels during T alone, and hCG plus T injections. Excretion of FSH during hCG plus T administration was lower than that of adults with gonadotropin deficiency leading to hypogonadism and much lower than that of normal prepubertal children (Table I). In the man who was maintained on hCG alone, the urine FSH level remained markedly suppressed (Table I).

Serum T levels during T administration were increased significantly above control levels (Table II). When hCG was added, T increased further, to levels significantly above those when the subjects were receiving T alone (Table II). Serum LH-like bioactivity, as assessed by in vitro bioassay decreased significantly when the men were receiving T alone; these levels increased to values approximately six times greater than control when hCG injections were added (Table II).

Blood samples obtained at 20-min intervals for 6 h in each of the three phases of the study revealed normal levels of LH and FSH in the control period, and consistently undetectable levels of FSH in all men during the T alone and hCG plus T phases of the study (an example shown in Fig. 2). Evidence of episodic LH secretion was noted in the control period in all subjects. During T administration, LH (measured by RIA) was suppressed to very low levels (14±5 ng/ml) compared with the control period (38±8 ng/ml), $P < 0.001$. During hCG plus T administration, FSH levels remained undetectable while the LH assay yielded values >200 ng/ml due to the cross-reactivity of hCG.

Gonadotropin responses to the 4-h LHRH infusions (Fig. 3) in the control period revealed the normal biphasic pattern of LH increase and monophasic FSH increase (26). During both T administration alone and during hCG plus T, FSH levels were undetectable throughout the LHRH infusion in four of the five subjects. One man exhibited a slight FSH response in

TABLE II
Serum Levels of Testosterone and LH-like Bioactivity

	Control	Testosterone alone	hCG + testosterone	hCG alone
	(n = 5)	(n = 5)	(n = 5)	(n = 1)
Serum testosterone, *ng/ml*	6.0±1.1	13.3±0.7*	18.4±0.8‡	8.0
Serum LH-like bioactivity, *mIU/ml*	52.4±11.3	9.7±2.4§	313±52§	314

* $P < 0.001$ compared with control.
‡ $P < 0.001$ compared with T alone.
§ $P < 0.01$ compared with control.

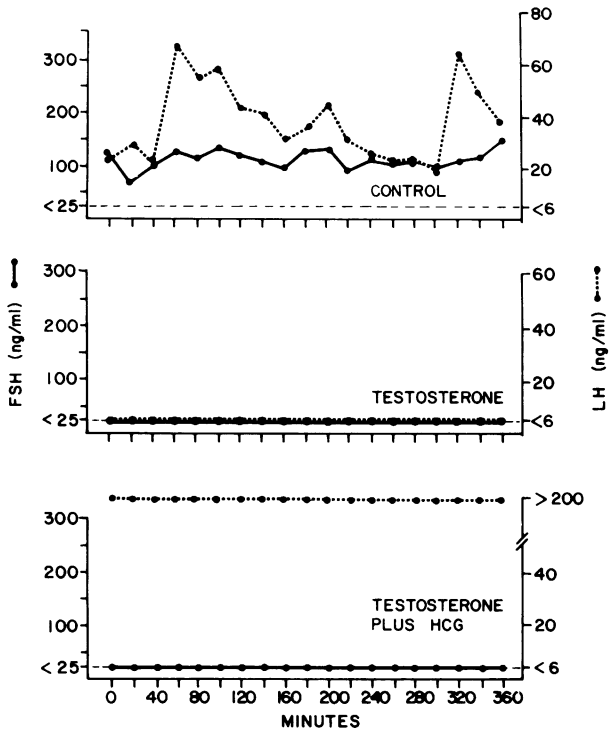

FIGURE 2 Serum LH and FSH levels measured at 20-min intervals for 6 h in a representative subject during the control, testosterone alone, and hCG plus testosterone phases of the study. Both LH and FSH were markedly suppressed by testosterone alone. FSH suppression continued during the hCG plus T phase of the study, while the apparent "LH" levels were high due to cross reactivity of hCG in the LH assay.

both of these periods. No detectable increase in LH during LHRH administration was found in three subjects on T alone, while small increases were noted in the other two volunteers.

Following the hCG plus T phase of the study, in which all men participated, two men received only T injections for 2.5 and 4.0 mo. Sperm counts during the T injections alone returned to azoospermic or severely oligospermic levels (data from one subject in Fig. 4). After stopping the T administration, sperm counts in these men returned to normal values. Serum FSH levels (Fig. 4) were undetectable throughout the T alone, hCG plus T and second T alone phases of the study. Urinary FSH values were suppressed below those of prepubertal children during the T alone and hCG plus T phases of the study (Fig. 4).

In another subject (Fig. 5), following the hCG plus T phase of the study, T injections were stopped and hCG alone was continued for 6 mo. Serum FSH values (Fig. 5) were suppressed from normal during the control period to undetectable levels during the T alone, hCG plus T and hCG alone phases of the study. Urinary FSH levels (Fig. 5) were suppressed to below those of prepubertal children during the T alone, hCG plus T, and hCG alone phases of the study. Sperm counts in this man (Fig. 5) were severely suppressed on T alone, but returned to normal on hCG plus T and remained normal on hCG alone and in the posttreatment control phase.

All subjects remained in good general health with no change in sexual function as assessed by questionnaire throughout the study. Except for the development of mild acne during T administration in three of the five subjects, there were no adverse effects of T or hCG treatment. No significant changes in amount of palpable breast tissue (within 1 cm of control measurements laterally from the areola) or testicular size (within 1 cm of control measured by calipers) occurred during any of the hormonal treatments. Routine hematological studies, blood chemistries and urinalyses were essentially unchanged during the study. No

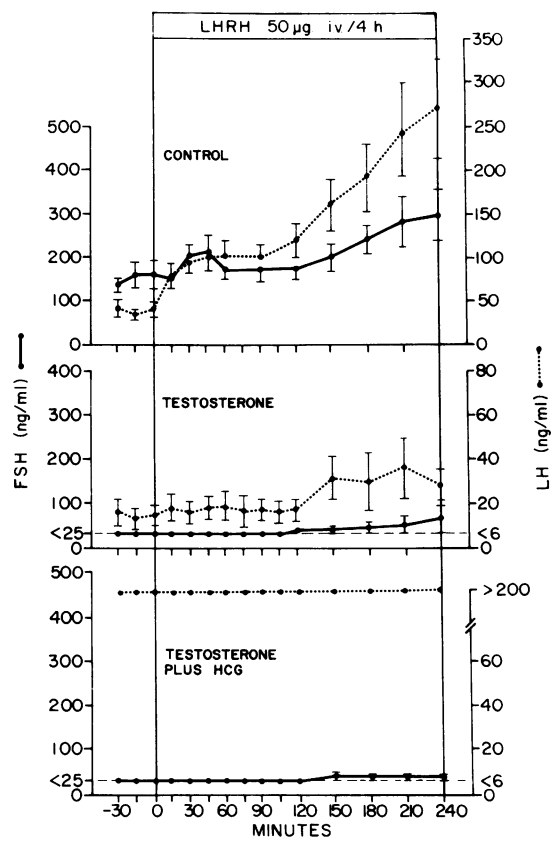

FIGURE 3 Serum LH and FSH levels in five normal men during 4-h constant LHRH infusions (mean±SE). The response of FSH to LHRH was markedly suppressed during both the testosterone and the hCG plus testosterone phases of the study. LH responsiveness was also suppressed by testosterone and was not determined during hCG plus T.

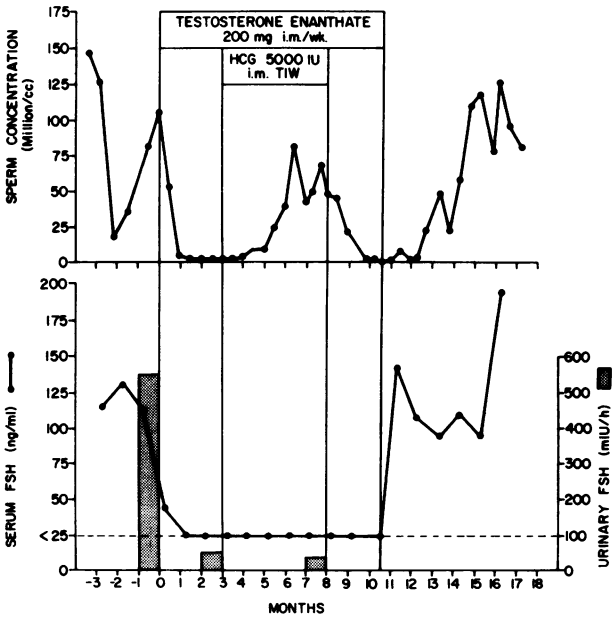

FIGURE 4 Sperm concentrations and serum and urinary FSH levels in one of two men in whom testosterone injections were continued beyond the end of the hCG plus testosterone phase of the study. FSH excretion rates were measured on 6-h urine aliquots obtained near the end of each study period. Note the stimulation of sperm production by hCG in spite of the very low FSH levels and the resuppression of sperm counts by testosterone after stopping hCG administration. In addition, sperm counts and serum FSH levels returned to normal after stopping all hormone administration.

subject developed significant erythrocytosis (hematocrits all <51).

DISCUSSION

Our results demonstrate that spermatogenesis as assessed by sperm counts, motilities, and morphologies may be reinitiated and maintained at normal levels in men with undetectable FSH levels in blood, and urinary excretion of FSH that is less than that of prepubertal children. Two of our five subjects demonstrated sperm counts, motilities, and morphologies that were indistinguishable from their own control values during the period that they were receiving hCG plus T and had undetectable serum FSH levels. The other three subjects reinitiated spermatogenesis in spite of undetectable serum FSH levels, maintained normal sperm motilities and morphologies and demonstrated mean sperm counts within the normal adult male range. However, they did not achieve mean counts within their own control range during the time of hCG plus T administration in this study. In two of these subjects, irregular administration of hCG may have been important in their failure to achieve complete normalization of sperm counts. The limited duration of the hCG plus T phase of this study may also have contributed to the failure to achieve full normalization of sperm counts. Human spermatogenesis requires 74±5 d to produce mature spermatozoa from immature spermatogonia (27). It may be that longer hCG stimulation would have allowed a more complete recruitment of germ cell production in these men.

That endogenous FSH production was severely depressed during the administration of hCG plus T was demonstrated clearly in several ways. Serum levels of FSH were undetectable in a radioimmunoassay that is sufficiently sensitive to differentiate reliably between the low end of the normal adult range and the values found in prepubertal boys or hypogonadotropic subjects (28). Serum levels of FSH in this assay were always undetectable in basal sampling in the men during hCG plus T administration in this study, even when sampling was at 20-min intervals for 6 h. Infusion of LHRH demonstrated that even with 4 h of stimulation, four of five subjects failed to increase their FSH levels into the detectable range, whereas the fifth man had a slight increase late in the infusion. Measurement of the excretion of FSH in urine has been shown to be a more sensitive method than serum analysis for detecting low levels of FSH production (29). Using this urinary assay, FSH production in the men during hCG plus T administration was demonstrated to be lower than that of hypogonadotropic hypogonadal

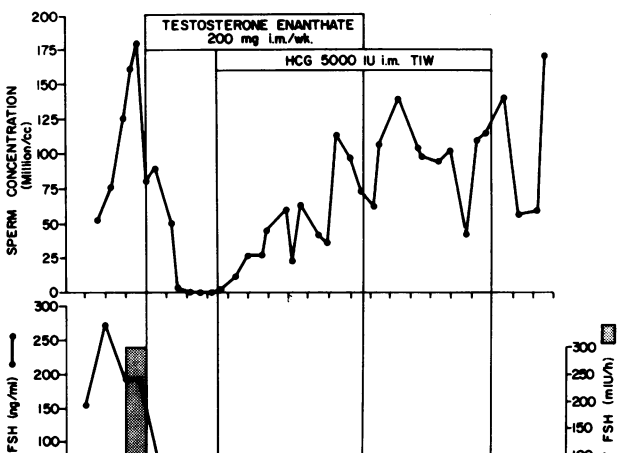

FIGURE 5 Sperm concentrations and serum and urinary FSH levels in one man in whom hCG injections were continued beyond the end of the hCG plus T phase of the study. FSH excretion rates were measured on 6-h urine aliquots obtained near the end of each study period. Note that during administration of hCG alone, sperm counts remained within the subject's control range in spite of very low serum and urinary levels of FSH.

adults and much lower than that of normal prepubertal children. From these results, it is clear that sperm production in man can be reinitiated and maintained in spite of nearly absent FSH stimulation.

In two men (data from one subject in Fig. 4), stopping the administration of hCG after the hCG plus T phase of the study and continuing only the T injections led to suppression of sperm production equally as complete as when T was first administered alone. These results demonstrated clearly that the reinitiation and maintenance of sperm production were due to the hCG injections and not to a decline in the suppressive effect of T with time.

In one man (Fig. 5), continuing hCG alone after the hCG plus T phase of the study led to maintenance of normal sperm production for 6 mo. This maintenance of normal sperm production occurred in spite of serum FSH levels that were consistently undetectable during the time on hCG alone and urinary FSH levels that were suppressed to levels below those of prepubertal children.

While our results demonstrate clearly that FSH levels were very low during the time spermatogenesis was reinitiated and maintained by hCG, an FSH effect was not completely absent. Very low levels of endogenous FSH were detectable in the urinary assay during hCG administration (Table I). That this amount of endogenous FSH is insufficient by itself to maintain spermatogenesis was shown by the fact that during the T alone phase of the study, similar amounts of endogenous FSH were present and sperm counts were very low.

Another source for a slight amount of FSH-like bioactivity was within the hCG molecule itself (15, 30). The FSH-like activity of hCG has been demonstrated by a variety of techniques to be ~1/1000 of the LH-like activity in this molecule (15, 30). It is unlikely that this very small amount of FSH-like activity was of significance in the present study. We demonstrated that the amount of LH bioactivity in the blood of the men receiving hCG injections was approximately six times that found during the control period. This does not approach the thousandfold increase required to produce an FSH-like effect. It is of interest that the LH molecule also has intrinsic FSH-like bioactivity ~1/1,000 as potent as its LH activity (15).

Early work using hCG in three men (5,000 IU i. m., three times weekly) suggested that it suppressed spermatogenesis (31). The mechanism for this effect was thought to be stimulation of androgen and estrogen production from the testis, leading to inhibition of pituitary FSH production (31), although serum levels of androgens, estrogens and FSH could not be measured at that time. Heller et al. (32) have reported a stimulatory effect of hCG (4,000 IU i. m., every 2nd d) on spermatogenesis in men whose sperm production was suppressed by T. These workers did not measure serum FSH levels and assumed that these levels remained normal and stimulated spermatogenesis (32). More recent work (33) has demonstrated that hCG administration to men with functional testes leads to marked suppression of endogenous FSH production, a result confirmed by our present studies. In a careful study, Sherins (33) demonstrated that hCG administration (1,000 IU i. m., every 2nd d) to men with idiopathic oligospermia led to suppression of serum FSH values to undetectable levels, but had no effect on sperm production.

Our results agree with those of Heller et al. (32) in that hCG administration to men whose sperm production is suppressed by exogenous T led to reinitiation and maintenance of spermatogenesis. However, since we have measured FSH production in this situation and found it to be severely suppressed, our interpretation of the results differs from that of Heller; endogenous FSH does not seem to be responsible for stimulating spermatogenesis during hCG administration. Our results in one man maintained on hCG alone (Fig. 5) are consistent with those of Sherins (33); hCG alone suppressed endogenous FSH production but led to no change in sperm counts. In addition, we found no evidence that hCG, administered chronically in this moderately supraphysiological dosage, led to testicular regression as has been reported with high dosage hCG and LH-releasing hormone administration to animals (34, 35). It may be that a higher dosage of hCG is required to demonstrate this effect in men.

It is known from a variety of previous studies that the high concentrations of testosterone found normally within the testis are of major importance in initiating and maintaining normal sperm production (1, 2). High levels of intratesticular testosterone are known to be able to maintain and possibly initiate spermatogenesis even in hypophysectomized or prepubertal animals (1, 2). hCG is known to stimulate and maintain high levels of intratesticular testosterone (36). It is likely that a major mechanism for the stimulatory effect of hCG on spermatogenesis found in this study is the ability of hCG to increase intratesticular testosterone concentrations. The lack of a direct relationship between the level of testosterone in peripheral blood and spermatogenesis was confirmed in the T alone phase of the study; blood testosterone levels were more than doubled and spermatogenesis was severely inhibited. Almost certainly, intratesticular testosterone levels at this time were markedly suppressed (37). Associated with the restimulation of intratesticular testosterone levels and spermatogenesis by the addition of hCG, peripheral testosterone levels increased further.

Although hCG was capable of reinitiating spermatogenesis in this study, and hCG alone stimulates sperm production in some patients with deficiencies in LH and FSH production (12), other patients have required the addition of FSH to hCG to initiate spermatogenesis (11). Interestingly, FSH alone has never been reported to initiate or maintain spermatogenesis in man (1, 2). The reason for the apparent requirement for FSH stimulation in addition to hCG in some situations and not others is unclear. Similarly, the reason for the apparent requirement for FSH to allow normal reinitiation of sperm production in the breeding season in monkeys (10) while FSH was not required for normal testicular function in rats (9) is not clearly understood. A possible explanation for some of these disparate results is that the length of time that the testis is deprived of gonadotropin stimulation before hCG or LH is administered may be important. In prepubertal animals or adults that have been gonadotropin deficient for a sufficiently long time, both LH and FSH may be necessary to reinitiate spermatogenesis. In animals or men that have been gonadotropin deficient for only a short time, hCG (or LH) alone may be sufficient for normal sperm production. We are presently investigating whether suppression of LH and FSH for 8 to 9 mo in men before adding gonadotropin replacement will allow us to demonstrate a requirement for FSH stimulation.

Although our work has demonstrated that hCG can stimulate human spermatogenesis in the absence of significant levels of FSH, we do not conclude that FSH has no role in this process. As described above, the dosage of hCG used or the time of suppression of endogenous gonadotropins prior to adding hCG in this study may have been important in masking an effect of FSH. A closer approximation of serum LH-like bioactivity to that found normally or a longer deprivation of gonadotropin stimulation prior to adding LH-like activity may reveal an important role for FSH in normal testicular function in man.

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REFERENCES

1. Steinberger, E. 1971. Hormonal control of spermatogenesis. *Physiol. Rev.* **51:** 1–22.
2. diZerega, G. S., and R. J. Sherins. 1980. Endocrine control of adult testicular function. *In* The Testis, H. Burger, D. deKretser, editors. Raven Press, New York. p. 127–140.
3. Greep, R. O., and H. L. Fevold. 1937. The spermatogenic and secretory function of the gonads of hypophysectomized adult rats treated with pituitary FSH and LH. *Endocrinology.* **21:** 611–618.
4. Dufau, M., T. Tsuruhara, K. A. Horner, E. J. Podesta, and K. J. Catt. 1977. Intermediate role of adenosine cyclic monophosphate and protein kinase during gonadotrophin induced steroidogenesis in testicular interstitial cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 3419–3423.
5. Orth, J., and A. K. Christensen. 1978. Autoradiographic localization of specifically bound ^{125}I-labelled follicle-stimulating hormone on spermatogonia of the rat testis. *Endocrinology.* **103:** 1944–1951.
6. Means, A. R., J. L. Fakundig, C. Huckins, D. J. Tindall, and R. Vitale. 1976. Follicle stimulating hormone, the Sertoli cell and spermatogenesis. *Rec. Prog. Horm. Res.* **32:** 477–525.
7. Dorrington, J. H., and D. T. Armstrong. 1974. FSH stimulates estradiol-17β synthesis in cultured Sertoli cells. *Proc. Natl. Acad. Sci. U. S. A.* **72:** 2677–2681.
8. Madhwa Raj, H. G., and M. Dym. 1976. The effects of selective withdrawal of FSH or LH on spermatogenesis in the immature rat. *Biol. Reprod.* **14:** 489–494.
9. Dym, M., H. G. Madhwa Raj, Y. C. Lin, H. E. Chemes, N. J. Kotite, S. N. Nayfeh, and F. S. French. 1979. Is FSH required for maintenance of spermatogenesis in adult rats? *J. Reprod. Fert. Suppl.* **26:** 175–181.
10. Wickings, E. J., and E. Nieschlag. 1980. Suppression of spermatogenesis over two years in rhesus monkeys actively immunized with follicle-stimulating hormone. *Fertil. Steril.* **34:** 269–274.
11. Mancini, R. E., A. C. Seigver, and A. Perez Lioret. 1969. Effect of gonadotropins on the recovery of spermatogenesis in hypophysectomized patients. *J. Clin. Endocrinol. Metab.* **29:** 467–478.
12. Johnsen, S. G. 1978. Maintenance of spermatogenesis induced by hMG treatment by means of continuous hCG treatment in hypoponadotrophic men. *Acta Endocrinol.* **89:** 763–769.
13. Bremner, W., and D. M. deKretser. 1976. The prospects for new, reversible male contraceptives. *N. Engl. J. Med.* **295:** 1111–1117.
14. Maroulis, G. B., A. F. Parlow, and J. R. Marshall. 1977. Isolated follicle-stimulating hormone deficiency in man. *Fertil. Steril.* **28:** 818–822.
15. Siris, E. S., B. C. Nisula, K. J. Catt, K. Horner, S. Birken, R. E. Canfield, and G. T. Ross. 1978. New evidence for intrinsic follicle-stimulating hormone-like activity in human chorionic gonadotropin and luteinizing hormone. *Endocrinology.* **102:** 1356–1361.
16. MacLoed, J. 1971. Human male infertility. *Obstetrical and Gynecological Survey.* **26:** 335–351.
17. Midgley, A. R. 1967. Radioimmunoassay for human follicle-stimulating hormone. *J. Clin. Endocrinol. Metab.* **27:** 295–299.
18. Greenwood, F. C., W. M. Hunter, J. S. Glover. 1963. The preparation of ^{131}I-labelled human GH of high specific radioactivity. *Biochem. J.* **89:** 114–123.
19. Burger, H. G., V. W. K. Lee, and G. C. Rennie. 1972. A generalized computer program for the treatment of data

from competitive protein-binding assays including radioimmunoassays. *J. Lab. Clin. Med.* **80:** 302–312.
20. Steiner, R. A., A. P. Peterson, J. Y. L. Yu, H. Conner, M. Gilbert, B. ter Penning, and W. J. Bremner. 1980. Ultradian luteinizing hormone and testosterone rhythms in the adult male monkey, *Macaca fascicularis*. *Endocrinology* **107:** 1489–1493.
21. Reiter, E. O., H. E. Kulin, and S. M. Hamwood. 1973. Preparation of urine containing small amounts of FSH and LH for radioimmunoassay: comparison of the kaolin-acetone and acetone extraction techniques. *J. Clin. Endocrinol Metab.* **36:** 661–665.
22. Van Damme, M. P., D. M. Robertson, and E. Diczfalusy. 1974. An improved *in vitro* bioassay method for measuring luteinizing hormone (LH) activity using mouse Leydig cell preparations. *Acta Endocrinol.* **77:** 655–671.
23. Dufau, M. L., G. D. Hodgen, A. L. Goodman, and K. J. Catt. 1977. Bioassays of circulating luteinizing hormone in the rhesus monkey: comparison with radioimmunoassay during physiological changes. *Endocrinology.* **100:** 1557–1565.
24. Gordon, D. L., D. L. Moore, T. Thorslund, and C. A. Paulsen. 1965. The determination of size and concentration of human sperm with an electronic particle counter. *J. Lab. Clin. Med.* **65:** 506–512.
25. MacLeod, J. 1970. The significance of deviations in human sperm morphology. *In* The Human Testis. E. Rosemberg and C. A. Paulsen, editors. Plenum Publishing Corp., New York. p. 481–492.
26. Bremner, W. J., and C. A. Paulsen. 1974. Two pools of luteinizing hormone in the human pituitary: evidence from constant administration of luteinizing hormone-releasing hormone. *J. Clin. Endocrinol. Metab.* **39:** 811–815.
27. Heller, C. G., and Y. Clermont. 1964. Kinetics of the germinal epithelium in man. *Rec. Prog. Horm. Res.* **20:** 545–575.
28. Bremner, W. J., N. N. Fernando, and C. A. Paulsen. 1977. The effect of luteinizing hormone releasing hormone in hypogonadotrophic ennuchoidism. *Acta Endocrinol.* **86:** 1–14.
29. Kulin, H., and S. Santner. 1977. Timed urinary gonadotropin measurements in normal infants, children and adults, and in patients with disorders of sexual maturation. *J. Pediatr.* **90:** 760–765.
30. Northcutt, R. C., and A. Albert. 1970. Follicle-stimulating activity of human chorionic gonadotropin: further evidence for non-identity with follicle stimulating hormone. *J. Clin. Endocrinol. Metab.* **31:** 91–95.
31. Maddock, W. A., and W. O. Nelson. 1952. The effects of chorionic gonadotropin in adult men: increased estrogen and 17-ketosteroid excretion, gynecomastia, Leydig cell stimulation and seminiferous tubule damage. *J. Clin. Endocrinol. Metab.* **12:** 985–1014.
32. Heller, C. G., H. C. Morse, M. Su, and M. J. Rowley. 1970. The role of FSH, ICSH and endogenous testosterone during testicular suppression by exogenous testosterone in normal men. *In* The Human Testis. E. Rosemberg and C. A. Paulsen, editors. Plenum Publishing Corp., New York. p. 249–257.
33. Sherins, R. J. 1974. Clinical aspects of treatment of male infertility with gonadotropins: testicular response of some men given hCG with and without Pergonal. *In* Proceedings of Serono Symposium on Male Infertility and Sterility. R. E. Mancini and L. Martini, editors. Academic Presss, Inc., New York **5:** 545–565.
34. Pelletier, G., L. Cusan, and F. Labrie. 1979. Inhibition of spermatogenesis in the rat by treatment with human chorionic gonadotropin (hCG). *Proc. Am. Soc. Androl.* Abstract 37.
35. Labrie, F., C. Auclair, L. Cusan, P. A. Kelly, G. Pelletier, and L. Ferland. 1978. Inhibitory effect of LHRH and its agonists on testicular gonadotropin receptors and spermatogenesis in the rat. *Int. J. Androl. Suppl.* **2:** 303–318.
36. Lindner, H. R. 1961. Androgens and related compounds in the spermatic vein blood of domestic animals. I. Neutral steroids secreted by the bull testis. *J. Endocrinol.* **23:** 139–159.
37. Morse, H. C., N. Horike, M. J. Rowley, and C. G. Heller. 1973. Testosterone concentrations of normal men: effects of testosterone propionate administration. *J. Clin. Endocrinol. Metab.* **37:** 882–886.