# Stimulation of Sperm Production by Human Chorionic Gonadotropin after Prolonged Gonadotropin Suppression in Normal Men

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**The precise hormonal milieu required for quantitatively normal spermatogenesis in man is unclear. The authors previously have shown that both supraphysiologic dosages of human chorionic gonadotropin (hCG) and physiologic dosages of human luteinizing hormone (hLH) can reinitiate sperm production in short-term (four months) gonadotropin-suppressed normal men who have prepubertal FSH levels. To determine whether normal FSH levels were necessary to stimulate sperm production after a prolonged period of gonadotropin and testicular suppression, the authors administered hCG to four normal men whose endogenous gonadotropin levels and sperm production were suppressed by prolonged exogenous testosterone (T) administration. After a 3-month control period, all subjects received 200 mg of T enanthate intramuscularly (im) each week to suppress LH and FSH for a total of 9 months and until successive sperm concentrations (performed twice monthly) revealed azoospermia or severe oligozoospermia (mean sperm concentration $< 3 \times 10^6$ spermatozoa/ml) for 6 months. Then, while continuing the same dosage of T enanthate, all four men simultaneously received 5000 IU of hCG im three times weekly for 6 months, replacing LH-like activity and leaving FSH activity suppressed. The effect on sperm production of the selective FSH deficiency produced by hCG plus T administration after the period of prolonged gonadotropin suppression was determined.**

**Exogenous T administration resulted in severe suppression of sperm concentrations from $79 \pm 7 \times 10^6$ spermatozoa/ml (mean ± SEM) during the control period to $0.8 \pm 0.5 \times 10^6$/ml after 12 weeks of T treatment. With the addition of hCG to T, sperm concentrations increased significantly in all four subjects, reaching a mean of $24 \pm 4 \times 10^6$ spermatozoa/ml after 12 weeks of hCG plus T administration. However, no subject achieved sperm concentrations consistently in his own control range during this period. Sperm morphology and motility were consistently normal in all men during hCG plus T administration.**

**Throughout the entire 9 months of prolonged exogenous T administration alone and 6 months of hCG plus T treatment, serum FSH was reduced to undetectable levels (< 25 ng/ml). Urinary FSH excretion was in the normal adult range during the control period (238 ± 29 mIU/h). FSH excretion was markedly suppressed to the ranges found in prepubertal children and adults with hypogonadotropic hypogonadism during the periods of prolonged T suppression (44 ± 15 mIU/h) and hCG plus T (38 ± 6 mIU/h) administration.**

**The authors conclude that sperm production can be reinitiated by hCG after prolonged gonadotropin and testicular suppression, despite markedly suppressed FSH levels. Normal levels of FSH are not an absolute requirement for reinitiation of sperm production in gonadotropin-suppressed men. However, since neither supraphysiologic dosages of hCG nor physiologic dosages of hLH are able to return sperm counts to fully normal levels during selective FSH deficiency, it is hypothesized that normal levels of both FSH and LH are necessary for quantitatively normal spermatogenesis in man.**

**Key words: spermatogenesis, hCG, gonadotropins, FSH, testosterone.**

**J Androl 1985; 6:137-143.**

The precise hormonal milieu necessary for quantitatively normal spermatogenesis in man is unclear. The pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), are clearly important regulators of sperm production. However, the specific roles of LH and FSH in controlling normal human sperm production are poorly

Supported by NIH Grant P50-HD-12629 and Veterans Administration Medical Research Funds.

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Dr. Matsumoto is a recipient of a Research Associate Career Development Award of the Veterans Administration.

Submitted for publication October 1, 1984; revised version received November 21, 1984; accepted for publication November 27, 1984.

understood. LH is thought to be necessary for the initiation and maintenance of spermatogenesis by stimulating intratesticular testosterone (T) production (Steinberger, 1971). FSH is believed to be required for spermatid maturation (spermiogenesis) during the initiation of sperm production, while its role in the maintenance of spermatogenesis is unclear (Steinberger, 1971).

We previously reported that selective replacement of LH activity with either supraphysiologic dosages of human chorionic gonadotropin (hCG) (Bremner et al, 1981) or physiologic dosages of human LH (hLH) (Matsumoto et al, 1984) could stimulate spermatogenesis in normal men whose gonadotropin and sperm production were suppressed by exogenous T administration. Both hCG- and hLH-induced stimulation of sperm production occurred despite undetectable serum FSH levels and prepubertal urinary FSH excretion. These results demonstrated that normal blood FSH levels were not an absolute requirement for stimulating spermatogenesis in gonadotropin-suppressed normal men.

In both of these previous studies, the length of time of gonadotropin suppression prior to selective replacement of LH activity was relatively short, that is, about four months (Bremner et al, 1981; Matsumoto et al, 1984). Studies in animals and men suggest that the requirement of FSH for reinitiation of spermatogenesis may be dependent on the length of time that the testes are deprived of gonadotropin stimulation (Steinberger, 1971). Therefore, with short periods of gonadotropin deficiency, sperm production may be maintained in the absence of normal FSH levels if adequate intratesticular T levels could be maintained, such as by hCG or LH administration alone. However, both LH and FSH were necessary to reinitiate spermatogenesis after a more prolonged period of hypogonadotropism (Steinberger, 1971).

In the present study, we determined whether normal blood levels of FSH were necessary to stimulate sperm production after a prolonged period of gonadotropin and testicular suppression. Exogenous T was administered to normal men to suppress endogenous gonadotropin levels and sperm production. T treatment alone was continued for a total of 9 months. After this period of prolonged gonadotropin deficiency and testicular suppression, hCG was administered along with testosterone to selectively replace LH activity, leaving FSH activity suppressed. The effect on sperm production of administering hCG alone, after a period of prolonged gonadotropin deprivation, was determined.

## Methods

### *Subjects*

Four normal men (subjects 1–4), aged 26 to 35 years, were recruited by newspaper advertisement and volunteered to participate in this study. All subjects had a normal medical history, physical examination, and screening laboratory studies (including complete blood count, coagulation times, 12-panel blood chemistry battery, and urinalysis). In addition, all men had six normal seminal fluid analyses, obtained over a 3-month period (ie, sperm concentration $> 20 \times 10^6$ spermatozoa/ml, sperm motility $> 50\%$, and sperm morphology demonstrating $> 60\%$ oval forms), normal basal LH, FSH, and T levels, normal LH and FSH secretory patterns on blood sampling every 20 minutes for 6 hours, and normal LH and FSH responses to LHRH (50 μg continuously infused intravenously over 4 hours).

### *Experimental Protocol*

The study 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 VA Medical Center. 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.

*Control Period.* The first 3 months of the study constituted a control period during which observations and measurements (as described below) were performed, but no hormones were administered.

*Prolonged T Suppression Period.* Following the control period, each subject received 200 mg of T enanthate (Delatestryl, E.R. Squibb and Sons, Princeton, NJ) im each week until successive sperm concentrations (performed twice monthly) were suppressed below $5 \times 10^6$ spermatozoa/ml for a total of 6 months. As suppression of sperm concentrations occurred by 3 months of exogenous T treatment in all subjects, the total length of T enanthate administration alone was 9 months.

*hCG Plus T Period.* After the prolonged T suppression period, while continuing the same dosage of T enanthate, each subject simultaneously received 5000 IU hCG (Profasi, Serono Laboratories, Inc., Braintree, MA) im three times weekly. The combination of hCG and T injections was continued for 6 months.

*T Resuppression Period.* To demonstrate that any increases in sperm concentrations observed during the hCG plus T period were due to hCG, and not a result of a decline in the suppressive effects of exogenous T, hCG injections were stopped in one subject (subject 4), and T administration alone was continued until three successive sperm concentrations were again suppressed below $5 \times 10^6$ spermatozoa/ml.

*Recovery Period.* Following the hCG plus T period in two subjects (subjects 1 and 3) and the T resuppression period in one subject (subject 4), all hormones were discontinued and these men entered a recovery period until three successive sperm concentrations returned to the subjects' own pretreatment control range. The remaining subject (subject 2) left the study at the end of the hCG plus T period.

All injections were administered by the investigators or

their nursing assistants. Injection records were kept to monitor compliance with the experimental protocol.

### *Measurements and Clinical Observations*

Twice monthly seminal fluid analyses were performed in all subjects throughout the entire study. Seminal fluid 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 routine hematology and blood chemistry studies and urinalyses.

In all four subjects, serum LH and FSH levels were measured in monthly blood samples drawn throughout the study. Serum T and estradiol ($E_2$) levels were determined in the last monthly blood sample of the control, prolonged T suppression, and hCG plus T periods. During hormone administration, the monthly blood samples were obtained immediately before scheduled injections of T or hCG plus T. At the end of the control, prolonged T suppression, and hCG plus T periods, 6-hour urine samples were collected for measurement of FSH levels.

### *Radioimmunoassays (RIA)*

The methodologies for the serum FSH and LH radioimmunoassays have been described previously (Bremner et al, 1981). The tracer used in the serum LH RIA was purified hCG (supplied courtesy of Dr. C. Alvin Paulsen) radioiodinated with iodine-125 ($^{125}I$) using chloramine T (Greenwood et al, 1963). Otherwise, both assays employed reagents distributed by the National Pituitary Agency and LER 907 as the reference standard. The sensitivity of the serum 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 serum 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 (1972).

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 (Reiter et al, 1973), using the Second International Reference Preparation of Human Menopausal Gonadotropin as the reference standard.

The RIA for T and $E_2$ used reagents provided by the World Health Organization (WHO) Matched Reagents Programme. The methodologies have been detailed previously (Matsumoto et al, 1983a). The assay sensitivity was 0.1 ng/ml for T and 12 pg/ml for $E_2$. The intra- and interassay coefficients of variation were 5.1 and 9.8%, respectively, for T, and 8.2 and 8.8%, respectively, for $E_2$.

### *Seminal Fluid Analysis*

Sperm concentrations in seminal fluid samples were determined using a Coulter counter (Coulter Electronics, Inc., Hialeah, FL). Concentrations below $15 \times 10^6$ spermatozoa/ml were confirmed by direct determination using a hemocytometer. These methods have been described previously (Gordon et al, 1965). 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 the WHO criteria (Belsey et al, 1980). Seminal fluid analyses were performed in the Seminal Fluid Core Laboratory (Director: Dr. C. Alvin Paulsen) of the Population Center for Research in Reproduction, University of Washington.

### *Statistical Analysis*

Mean sperm concentrations during the control period, after the initial 12 weeks of prolonged T suppression, and after the initial 12-week period of hCG plus T, were determined for each subject. Sperm concentrations after 12 weeks of T suppression and hCG 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 following institution of hCG. 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, prolonged T suppression, and hCG plus T periods were determined for each subject. These data, as well as the serum T and $E_2$ levels and urinary FSH excretion measured at the end of each study period, were compared with Student's paired *t*-test.

## Results

### *Seminal Fluid Analysis*

The mean sperm concentration, seminal fluid volume, and total sperm count for all subjects during the 3-month control period were $79 \pm 7 \times 10^6$ spermatozoa/ml (mean ± SEM), 2.7 ± 0.4 ml, and $214 \pm 48 \times 10^6$ spermatozoa, respectively. Individual sperm concentrations for subjects 1–4 during this period averaged 70, 65, 87, and $94 \times 10^6$ spermatozoa/ml, respectively, with ranges of 45 to 106, 29 to 117, 52 to 149, and 70 to $122 \times 10^6$/ml, respectively.

Following the control period, exogenous T enanthate administration (200 mg im weekly) resulted in severe suppression of sperm production to $< 3 \times 10^6$ spermatozoa/ml by 3 months. Sperm concentrations remained suppressed below this level for 6 months in all subjects (Fig. 1). Sperm concentrations were reduced to $0.8 \pm 0.5 \times 10^6$ spermatozoa/ml after the initial 12 weeks of T administration ($P < 0.001$ compared with control values). Seminal fluid volume was unchanged (2.6 ± 0.4 ml) and total sperm count was reduced to $2 \pm 1 \times 10^6$ during this period. All four

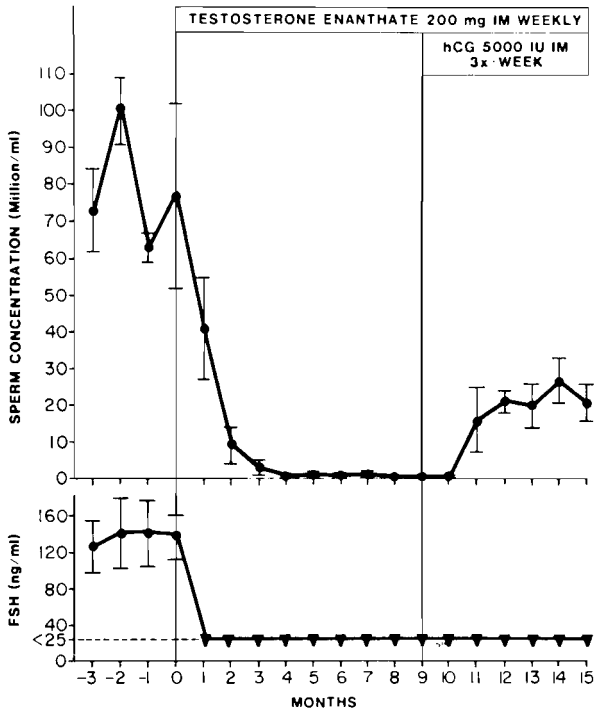


**Fig. 1.** Mean monthly sperm concentrations and FSH levels in four normal men during the control, prolonged T suppression, and hCG plus T periods of the study (mean ± SEM). Prolonged exogenous T administration markedly suppressed sperm concentrations to severely oligozoospermic levels for 6 months, and reduced serum FSH to undetectable levels (▼) for 9 months. Note the increase in sperm concentration with the addition of hCG to T, despite continued undetectable serum FSH levels. The limit of detectability in the FSH RIA is represented by -----.

subjects became azoospermic by the end of the prolonged T suppression period.

While continuing the same dosage of T, subjects then simultaneously received hCG (5000 IU im three times weekly). Sperm concentrations increased significantly in all subjects with addition of hCG to T (Fig. 1), reaching a mean of 25 ± 4 × $10^6$ spermatozoa/ml after 12 weeks of hCG administration ($P <$ 0.01 compared with T alone). The mean seminal fluid volume was unchanged (2.6 ± 0.2 ml) and the total sperm count increased to 65 ± 9 × $10^6$ during this period. Although sperm concentrations increased markedly in all four subjects during hCG plus T administration, no subject achieved sperm concentrations consistently within his own control range. Individual sperm concentrations for subjects 1–4 during hCG plus T averaged 35, 16, 30, and 20 × $10^6$ spermatozoa/ml, respectively, and the maximum sperm concentrations achieved during this period were 75, 31, 47, and 31 × $10^6$/ml, respectively. Sperm motility and morphology were normal consistently in all men during hCG plus T administration.

Following the hCG plus T period, subject 4 continued to receive T alone after hCG injections were discontinued. In this man, sperm concentrations were again severely suppressed to a mean of 3 × $10^6$/ml after 2 months of T administration alone. In the three subjects (subjects 1, 3, and 4) who had seminal fluid collections continued after discontinuation of hormonal treatment, sperm concentrations returned to their own control range within 5 months.

### *FSH Levels*

The mean serum FSH level during the control period was 136 ± 26 ng/ml (Fig. 1). Throughout the entire 9 months of the prolonged T suppression period, and the entire 6 months of the hCG plus T period, serum FSH was reduced to undetectable levels (< 25 ng/ml) in all four subjects ($P <$ 0.02 compared with control values). FSH levels remained undetectable during the T resuppression period in the man (subject 4) who continued to receive T after hCG was stopped.

Urinary FSH levels (Table 1) during the control period (238 ± 29 mIU/h) were within the normal adult range (190–1700 mIU/h). FSH excretion was

TABLE 1. Urinary FSH Levels (mIU/h)*

| Control† (n = 4) | Prolonged T† Suppression (n = 4) | hCG Plus T‡ (n = 4) | Prepubertal Children‡ (n = 11) | Hypogonadotropic Hypogonadism‡ (n = 4) |
|---|---|---|---|---|
| 238 ± 29 | 44 ± 15§ | 38 ± 6§ | 77 ± 12 | 54 ± 14 |

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

†Measured in 6-hour urine aliquots at the end of each study period.

‡Data taken from Kulin and Santner (1977).

§$P <$ 0.01 compared with control.

markedly suppressed during the prolonged T suppression period (44 ± 15 mIU/h) and the hCG plus T period (38 ± 6 mIU/h), reaching levels in the ranges found in prepubertal children (15–100 mIU/h) and hypogonadotropic hypogonadal adults (Table 1).

*LH Levels*

During prolonged T administration alone, serum LH levels were suppressed to very low levels (10 ± 3 ng/ml) compared with control levels (30 ± 4 ng/ml, $P < 0.01$). In two men (subjects 3 and 4), serum LH levels were undetectable (< 6 ng/ml) during the prolonged T suppression period. During hCG plus T administration, the LH assay yielded values > 200 ng/ml due to the cross-reactivity of hCG. In subject 4, serum LH was again reduced to undetectable levels during the T resuppression period.

*T and $E_2$ Levels*

Both serum T and $E_2$ levels during prolonged administration of T alone were increased significantly above control levels (Table 2). When hCG was added to T, both T and $E_2$ levels increased further to levels significantly above those found when the subjects were receiving T alone (Table 2).

*Clinical Observations*

With the exception of subject 1, all subjects remained in good health throughout the entire study and no adverse effects of either T or hCG were observed. As we have reported elsewhere (Sandblom et al, 1983), subject 1 developed obstructive sleep apnea syndrome with excessive daytime somnolence and erythrocytosis (hematocrit greater than 55%) related to T administration. Hematocrits increased slightly in all the remaining subjects, but no one else developed a hematocrit greater than 54%. Other hematology and coagulation studies, blood chemistries, and urinalyses remained unchanged throughout the study. In all subjects, palpable breast tissue and testicular size remained within 1 cm of pretreatment measurements during hormone treatment. Injection records revealed excellent compliance with the experimental protocol.

TABLE 2. Serum T and $E_2$ Levels*

| | Control | Prolonged T Suppression | hCG Plus T |
|---|---|---|---|
| Serum T (ng/ml)† | 5.0 ± 0.6 | 12.2 ± 0.5‡ | 18.1 ± 2.2§ |
| Serum $E_2$ (pg/ml)† | 34 ± 4 | 99 ± 22‖ | 153 ± 11§ |

*Measured by monthly blood samples at the end of each study period.
†All values are mean ± SEM for four subjects.
‡$P < 0.001$ compared with control.
§$P < 0.05$ compared with prolonged T suppression.
‖$P < 0.05$ compared with control.

## Discussion

Our results demonstrate that spermatogenesis can be reinitiated by hCG after prolonged gonadotropin and testicular suppression, despite prepubertal FSH levels. In a setting of prolonged (9 months) and marked suppression of gonadotropin and sperm production induced by exogenous T, selective replacement of LH-like activity by hCG administration significantly stimulated spermatogenesis in all four subjects, as assessed by sperm concentrations, motilities, and morphologies. Reinitiation of sperm production occurred despite undetectable serum FSH levels and urinary FSH excretion comparable to that of prepubertal children and adults with gonadotropin deficiency leading to hypogonadism. Three of the four subjects achieved mean sperm concentrations within the normal adult range after 12 weeks of hCG plus T administration. Although two men produced at least one sperm concentration in their own control range, none of their mean sperm concentrations fell within their respective pretreatment ranges. In the one subject who continued to receive T alone after discontinuation of hCG, sperm concentrations were again suppressed to severely oligozoospermic levels. This result confirms our previous findings (Bremner et al, 1981; Matsumoto et al, 1983b, 1984) that the suppressive effect of exogenous T does not decline with prolonged therapy, and demonstrates that the stimulation of sperm production was due to hCG.

Endogenous FSH levels were severely suppressed both during administration of T alone and during hCG plus T treatment in all of our subjects. Serum FSH levels were undetectable in a very sensitive radioimmunoassay that reliably differentiates the lower end of the normal adult male range from values in hypogonadotropic patients (Bremner et al, 1977). Urinary FSH excretion has been demonstrated to be more sensitive than serum assays for detecting very low levels of FSH (Kulin and Santner, 1977). During hCG plus T administration, urinary FSH levels in our subjects were in the prepubertal range. These small amounts of FSH were not sufficient by themselves to stimulate spermatogenesis, as similar amounts of FSH were present during the prolonged T suppression period when sperm counts were in the severely oligozoospermic or azoospermic range.

Serum LH levels were also markedly reduced during the prolonged T suppression period in all subjects. Because of cross-reactivity of hCG in the LH assay used in this study, LH activity could not be assessed during hCG plus T administration. Although LH bioactivity was not determined in the study, it is clear that the dosage of hCG used exceeded physiologic levels. In a previous study, administration of the same dosage of hCG to T-suppressed normal men resulted in LH bioactivity (as assessed by an *in vitro* mouse Leydig cell bioassay) approximately six times that found during the control period (Bremner et al, 1981). In addition to the supraphysiologic levels that were produced by hCG in the present study, the pattern of LH activity produced by injections of hCG three times per week did not mimic the pulsatile fluctuation of LH levels that normally occurs every 90 to 120 minutes in man (Santen and Bardin, 1973). Despite the unphysiologic pattern of LH activity, sperm production was significantly stimulated by hCG. Whether this abnormal pattern of LH stimulation may have contributed to the lack of quantitatively normal spermatogenesis in our subjects is not known.

It is likely that the mechanism underlying the stimulatory effect of hCG on spermatogenesis relates to its ability to stimulate intratesticular T production. High intratesticular T levels are known to be important for the initiation and maintenance of sperm production (Steinberger, 1971). High dosage T administration has been shown to be able to stimulate spermatogenesis in prepubertal and hypophysectomized animals (Steinberger, 1971). Recently, Marshall et al have demonstrated stimulation of spermatogenesis in stalk-sectioned (1983) and prepubertal (1984) monkeys by high dosages of exogenous T, presumably in the presence of very low FSH levels. Analogous to the men in our study, these monkeys did not achieve quantitatively normal sperm production.

Administration of exogenous T enanthate alone in our men resulted in significant increases of serum T levels. Despite the high serum T levels induced by exogenous T administration, Morse et al (1973) have shown that intratesticular T levels are reduced severely as a result of endogenous gonadotropin suppression. With the addition of hCG to T and the stimulation of testicular steroidogenesis, T levels increased even further in all subjects, and serum $E_2$ levels were approximately five times greater than control values. These results confirm our previous findings (Matsumoto et al, 1983a), and demonstrate that hCG can stimulate T and sperm production in long-term gonadotropin-suppressed men despite very high $E_2$ levels. It is possible that the high $E_2$ levels stimulated by hCG in our subjects may have contributed to their failure to achieve fully normal sperm production. In this regard, Sherins and Clark (1983) have demonstrated significant stimulation of spermatogenesis with the addition of testolactone (an agent that inhibits the aromatization of T to $E_2$) to hCG in hypogonadotropic hypogonadal men who failed to stimulate sperm production with hCG treatment alone.

We have demonstrated that sperm production in normal men can be reinitiated by supraphysiologic dosages of hCG (Bremner et al, 1981) or physiologic dosages of hLH (Matsumoto et al, 1984) after short-term gonadotropin suppression induced by exogenous T. The present study extends our findings by demonstrating that hCG can also stimulate spermatogenesis after a prolonged period of gonadotropin suppression. Together, our findings demonstrate that normal levels of FSH are not absolutely required for the reinitiation of sperm production in man. However, in none of our studies has fully normal sperm production been achieved by selective replacement with hCG or hLH administered singly. Therefore, it is likely that normal levels of both FSH and LH are necessary for quantitatively normal spermatogenesis in man.

## Acknowledgments

The authors appreciate the excellent technical assistance of Lorraine Shen, Patricia Gosciewski, Florida Flor, Elaine Rost, Connie Pete, Patricia Payne, and Judy Tsoi; and the assistance of Larry Mix in preparing this manuscript. We also appreciate the generous gifts of reagents for the FSH and LH RIA from the National Pituitary Agency and the reagents for the T and $E_2$ RIA from the World Health Organization.

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