The Relative Roles of Follicle-Stimulating Hormone and Luteinizing Hormone in Maintaining Spermatogonial Maturation and Spermiation in Normal Men

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Context: Male hormonal contraception via gonadotropin and intratesticular androgen withdrawal disrupts spermatogenesis at two principal sites: 1) spermatogonial maturation, and 2) spermiation.

Objective: The objective of this study was to explore the relative dependence of each stage of germ cell development on FSH and LH/intratesticular androgen action.


Interventions: Subjects (n = 6/group) were assigned to 6 wk of 1) testosterone (T) implant (4 × 200 mg sc once)+ depot medroxy progesterone acetate (DMPA; 150 mg im once); 2) T implant+DMPA+FSH (300 IU sc twice weekly); and 3) T implant+DMPA+human chorionic gonadotropin (hCG; 1000 IU sc twice weekly as an LH substitute). Men then underwent a vasectomy and testicular biopsy with previously reported control data used for comparison.

Results: T+DMPA alone significantly suppressed type B spermatagonia, preleptotene through to pachytene spermatocytes, and round spermatids from control (P < 0.05). All germ cell subtypes were maintained at control levels by either FSH or LH activity, except pachytene spermatocytes, which were found to be lower in the hCG vs. FSH (P < 0.01) and control groups (P < 0.05).

Conclusions: FSH and LH maintained spermatogenesis independently in this gonadotropin-suppressed model. Compared with LH, FSH showed better maintenance of pachytene spermatocyte number, whereas improved conversion to round spermatids was suggested with hCG treatment. Future contraceptive treatment strategies must consider independent regulation of spermatogenesis by both FSH and LH/intratesticular androgens for maximum efficacy. (J Clin Endocrinol Metab 91: 3962–3969, 2006)

In man, both gonadotropins are required to reinitiate and maintain quantitatively normal sperm production after experimentally induced oligoazoospermia (1–4). However, the relative roles of FSH and LH/intratesticular androgens on germ cell development remain unclear. Male hormonal contraceptive regimens using androgens, alone or combined with a progestin, achieve similar conception rates in young men. After treatment with T, 200 mg im weekly, and the induction of oligoazoospermia, hCG (5000 IU im three times weekly) (2, 4) or pituitary-derived LH (1100 IU sc daily) (17) was found to restore sperm concentration partially. In a similar study, suppression of serum LH and selective FSH replacement (100 IU sc daily) also partially restored sperm concentration (1). In such studies, the complete elimination of one or other gonadotropin cannot be assured, but such data support the contention that both FSH and LH/intratesticular androgens are needed for quantitatively normal spermatogenesis to occur.

It is recognized that both FSH and LH are necessary for the initiation of spermatogenesis (14) and the establishment of fertility in men with hypogonadotropic hypogonadism (15). However, once initiated, human chorionic gonadotropin (hCG; as an LH substitute) alone can maintain spermatogenesis, albeit at a submaximal rate over the longer term (16). In contrast, the administration of FSH and testosterone (T) to men with hypogonadotropic hypogonadism is unable to induce or maintain spermatogenesis, with sperm concentrations falling to a mean of 0.3 ± 0.1 million/ml at 3 months and azoospermia by 6 months (15).

Additional data on the relative roles of FSH and LH in spermatogenesis come from a series of studies using gonadotropin withdrawal followed by selective reintiation in normal young men. After treatment with T, 200 mg im weekly, and the induction of oligoazoospermia, hCG (5000 IU im three times weekly) (2, 4) or pituitary-derived LH (1100 IU sc daily) (17) was found to restore sperm concentration partially. In a similar study, suppression of serum LH and selective FSH replacement (100 IU sc daily) also partially restored sperm concentration (1). In such studies, the complete elimination of one or other gonadotropin cannot be assured, but such data support the contention that both FSH and LH/intratesticular androgens are needed for quantitatively normal spermatogenesis to occur.

In vivo animal models suggest that spermatogonial mat-
uration is largely FSH dependent (18–21); spermiogenesis appears to be reliant on LH/intratesticular T (22, 23), whereas spermiation requires both FSH and LH/intratesticular T (24). Mice solely lacking FSH action [receptor (25) or β-subunit (26)] are able to complete spermatogenesis, albeit at a reduced level. In contrast, the loss of both FSH and LH action in the GnRH-deficient mouse (27) or of LH action at a reduced level. In contrast, the loss of both FSH and LH in the LH receptor knockout mouse appears to be due to LH-independent androgen action (31). Complete androgen receptor knockout mice (32) also do not progress past the pachytene spermatocyte stage, with the Sertoli cell-specific androgen receptor knockout model showing only occasional round spermatids (33, 34). Such data illustrate that germ cell development in the mouse can be completed in the absence of FSH but not androgen action.

In contrast, men with LH β-subunit mutations (35, 36) may produce low numbers of sperm, but there are no data on germ cell development nor intratesticular androgen levels, leaving open the possibility of LH-independent androgen secretion/action as observed in the LH receptor knockout mouse. Data from the few reported men with FSH β-subunit (37) or receptor (38) mutations indicate that most patients have severe oligospermia but others have higher sperm densities, suggesting that, although FSH may not be a prerequisite for completion of spermatogenesis, it may be of relatively greater importance in human compared with murine spermatogenesis.

The aim of this study was to establish whether exogenous FSH and LH are independently able to support spermatogenesis and specifically whether they differ in their support of germ cell progression. To this end, we performed a prospective, randomized, three-arm, 6-wk study using a “suppress and replace” model. Endogenous gonadotropins were suppressed using a combined androgen/progestin male hormone contraceptive regimen together with their selective maintenance via administration of recombinant human (rh) FSH or hCG as an LH substitute. End points included reproductive hormone levels, sperm concentration, stereological evaluation of germ cell number, and testicular steroid measurement.

Subjects and Methods

Subjects

Eighteen men were recruited through media advertisement to participate in this study. The study protocol was approved by the Southern Health Human Research Ethics Committee, and all subjects gave written informed consent before screening. Men underwent medical interview, physical examination, and biochemical investigations. Subjects were required to fulfill each of the following criteria: 1) age 21–45 yr; 2) normal physical findings and normal testicular volumes; 3) two normal semen analyses according to World Health Organization criteria (39); 4) normal serum FSH, LH, T, SHBG, and estradiol (E2); and 5) normal liver and renal function and complete blood count. Men with a past history of hypertension; significant cardiovascular, thromboembolic, renal, hepatic, prostatic, and testicular disease; or infertility were excluded. Men with a past history of drug abuse or who were taking significant prescribed medications were also excluded, as were men who were subject to testing for steroid usage in competitive sports.

Study design

This research study was divided into three phases: a 4-wk screening phase, a 6-wk treatment phase, and a 4-wk recovery period. After screening, men were randomly assigned to one of the following three treatment groups (n = 6/group): 1) T implant, 800 mg sc once (4 × 200 mg; Organon, Lane Cove, Australia) + depot medroxyprogesterone acetate (DMPA), 150 mg im once (2 ml @ 150 mg/ml; Pfizer, West Ryde, Australia); 2) T implant, 800 mg sc once, + DMPA, 150 mg Im once, + FSH, 300 IU sc twice weekly (Puregon; Organon); 3) T implant, 800 mg sc once, + DMPA, 150 mg im once, + hCG, 1000 IU sc twice weekly (Pregnyl; Organon). Blood for reproductive hormone analysis was drawn weekly at screening and before gonadotropin administration and vasectomy. Gonadotropin administration was performed by the investigators on d 0 of each week, with men in groups 2 and 3 educated to self administer either FSH (300 IU) or hCG (1000 IU sc) on d 4. Men were required to provide weekly semen samples with a minimum of 2 d abstinence before collection.

Assays

Serum FSH and LH were measured using sensitive immunofluorometric assays (40) (Delfia, hFSH kit no. A0710201; and hLH spec kit no. A031-101, Perkin-Elmer, Wallac Inc., Turku, Finland). The sensitivities of the FSH and LH assays were 0.01 and 0.015 IU/liter with an interassay variation of 13.4% and 15.3%, respectively. The dose ranges of the standards used in the respective assays were 0.015–10 IU/liter for FSH and 0.01–10 IU/liter for LH.

Serum hCG was measured in an automated immunoassay (Beckman Coulter Inc., Chaska, MN) with an assay sensitivity of 1.0 IU/liter and an interassay variation across the working range of 2.3–9.5%.

Serum T was measured by an automated chemiluminescent immunoassay (Chiron Diagnostics, East Walpole, MA) with a sensitivity of 0.3 nmol/liter and an interassay variation across the working range of 5.0–11.4%.

Serum SHBG was measured by an automated chemiluminescent immunoassay (catalog no. LKSH5; Diagnostic Products Corp., Los Angeles, CA) with a sensitivity of 2 nmol/liter and an interassay variation across the working range of 7.9–14.7%.

Serum E2 was measured by double-antibody competitive immunoassay (catalog no. KE2D5; Diagnostic Products Corp.) with a sensitivity of less than 20 pmol/liter and an interassay variation across the working range of 7.3–14.7%.

Testicular biopsies and stereological assessment

The testicular biopsies were performed under general anesthesia using a previously described technique (41). Tissue for stereological analysis was fixed in Bouin’s fluid and embedded in methacrylate, and 25-μm sections were cut and stained with hematoxylin and eosin and germ cell number (type A dark spermatogonia, type A pale spermatogonia, all type A spermatogonia, type B spermatogonia, preleptotene spermatocytes, leptotene to zygote spermatocytes, pachytene spermatocytes, steps 1–2 round spermatids, steps 3–6 elongating spermatids, and steps 7–8 elongated spermatids) was assessed using the optical disector approach as previously described (13).

Testicular steroids

Testicular steroids including T, dihydrotestosterone (DHT), 3αAdiol, and 3βAdiol were quantitated as previously described (13, 41). For 3αAdiol and 3βAdiol, all samples were assayed in the one assay, with intraassay variations (n = 3–4) from parallel fragments from the same testis sample of 10.0 and 18.1%, respectively. The intraassay variations for T and DHT were 15.4 and 8.0%, respectively, whereas interassay variations (n = 2) were 10.4–14.1% for T and 6.5–13.9% for DHT.

Control data

Previously reported control data on intratesticular steroid levels from five subjects were used for comparative analysis of intratesticular T, DHT, and 3αAdiol levels (13). Testis biopsies from the same five control subjects (13) were processed for stereology and were counted along with testis biopsies from the current study, providing control data for comparison.
of germ cell numbers. Baseline characteristics for the control group (13) and three treatment groups were assessed with no significant differences found.

**Statistical analyses**

Data are shown as mean ± SEM, n = 5–6/group for all parameters. Statistical comparisons were performed using Sigmastat (Systat, Point Richmond, CA). All data were log transformed before analysis, and the variances were analyzed for homogeneity. Nonparametric statistics were used when equal variance testing failed. Serum hormone and sperm concentration data were analyzed by repeated-measures ANOVA followed by Tukey test or, when normality testing failed, by Kruskal-Wallis one-way ANOVA on ranks followed by Dunn’s method for post hoc comparison.

**Baseline characteristics**

As outlined in Table 1, no baseline differences were detected between groups.

**Serum gonadotropin levels**

Serum FSH levels declined significantly at d 7–42 in the T+DMPA and T+DMPA+hCG groups, falling to nadir of 0.09 IU/liter (1.5% of baseline, d 35, \( P < 0.001 \)) and 0.03 IU/liter (0.8% of baseline, d 28, \( P < 0.001 \)), respectively (Fig. 1A). Although FSH levels appeared to be further suppressed in the T+DMPA+hCG group over T+DMPA alone, no significant differences were determined. Administration of FSH maintained levels at baseline and higher than that in the T+DMPA and T+DMPA+hCG treatment groups (d 7–42, \( P < 0.05 \)). Serum LH levels declined significantly from baseline in all groups at d 7–42, with no differences in LH levels between groups detected (Fig. 1B).

**Semen analysis**

In the T+DMPA group, sperm concentration fell from baseline from d 28 onward (\( P < 0.001 \)) (Fig. 2). In the T+DMPA+FSH group, sperm concentration was decreased (mean concentration of 44 ± 27 million/ml) at d 42 (\( P = 0.003 \)). No fall in sperm concentration was seen in the T+DMPA+hCG group during the treatment period (mean sperm concentration at d 42, 49 ± 11 million/ml, \( P = 0.627 \)). Sperm concentration was found to be higher in the FSH and hCG groups compared with the T+DMPA group, d 28 and 35 (\( P < 0.05 \)) and at d 42 (hCG group alone, \( P < 0.05 \)). No differences were found between the T+DMPA+FSH and T+DMPA+hCG treatment groups at d 42. In the T+DMPA+FSH group, one subject had a d 42 sperm concentration of 152 million/ml, whereas the other four available subject sperm concentrations were less than 30 million/ml. In the T+DMPA+hCG group, four of the five available subject sperm concentrations were greater than 30 million/ml at d 42.

**T, SHBG, and E2**

No differences in T levels from baseline were detected in the T+DMPA treatment group, whereas there were higher levels found in the T+DMPA+FSH group at some time points (d 14–28 and 42, \( P < 0.05 \)) (Fig. 3A). In the T+DMPA+hCG group, T levels were higher than baseline, except on d 35 (198–263% of baseline, \( P < 0.05 \)) throughout.

**TABLE 1. Baseline characteristics of the subjects**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr)</th>
<th>Combined testes volume (ml)</th>
<th>Sperm concentration (106/ml)</th>
<th>Serum FSH (IU/liter)</th>
<th>Serum LH (IU/liter)</th>
<th>Serum T (nmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T+DMPA (n = 6)</td>
<td>39 ± 1.8</td>
<td>52 ± 5</td>
<td>115 ± 24</td>
<td>5.8 ± 1.3</td>
<td>3.4 ± 0.7</td>
<td>18 ± 3.3</td>
</tr>
<tr>
<td>T+DMPA+FSH (n = 6)</td>
<td>40 ± 1.5</td>
<td>49 ± 3</td>
<td>93 ± 29</td>
<td>4.7 ± 0.6</td>
<td>3.9 ± 0.4</td>
<td>16 ± 1.4</td>
</tr>
<tr>
<td>T+DMPA+hCG (n = 6)</td>
<td>35 ± 1.3</td>
<td>55 ± 3</td>
<td>87 ± 23</td>
<td>3.8 ± 0.5</td>
<td>4.7 ± 0.8</td>
<td>17 ± 2.0</td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>37 ± 1.7</td>
<td>44 ± 2</td>
<td>72 ± 20</td>
<td>2.8 ± 0.5</td>
<td>3.9 ± 1.1</td>
<td>17 ± 3.0</td>
</tr>
</tbody>
</table>

Results are shown as mean ± SEM, n = 5–6/group.
the study treatment period (Fig. 3A). Serum SHBG levels remained in the normal reference interval (13–71 nmol/liter), with no differences detected except a 77% of baseline decrease at d 21 (P = 0.021) in the T+DMPA+FSH group (data not shown). Serum E2 levels in the T+DMPA group and T+DMPA+FSH group remained in the normal range (25–130 pmol/liter). In the T+DMPA+hCG group, E2 levels were higher than baseline and the other two treatment groups (P < 0.05) throughout the treatment phase of the study (Fig. 3B).

Germ cell numbers

Germ cell data for control and treated men are expressed as number per Sertoli cell [a constant denominator with contraceptive treatment (42)] (Table 2) and as percent control as number per Sertoli cell [a constant denominator with contraceptive treatment (42)]. Serum levels of intratesticular T ranged from 5.15 to 223% of control, with higher levels from baseline in the T+DMPA group and T+DMPA+FSH group (P < 0.05). No decrease in any germ cell subtype was seen in the T+DMPA+FSH group, with only pachytene spermatocytes being reduced in the T+DMPA+hCG group compared with control (P < 0.05).

Between treatment groups, higher spermatocytes (preleptotene-pachytene) were seen in the T+DMPA+FSH group compared with the T+DMPA group alone (P < 0.05). Higher spermatocytes (preleptotene-zygotene) were also seen in the T+DMPA+hCG group compared with the T+DMPA group (P < 0.05). The only germ cell subtype found to be significantly different between the two gonadotropin-treated groups was pachytene spermatocytes, which were lower in the hCG-treated group compared with FSHTreated group (P < 0.01). Heterogenous patterns of germ cell suppression were seen between men within treatment groups similar to those previously described (41) (data not shown). No correlation between sperm concentration and any germ cell subtype within treatment groups was observed.

In regard to the process of spermiation, examination of steps 7–8 elongated spermatid numbers revealed no differences among treatment groups or compared with the control group. However, mean sperm concentrations derived from semen analyses collected on the day of testicular biopsy were significantly higher in the T+DMPA+hCG group compared with T+DMPA alone (P < 0.05), with a similar, although not significant, trend also noted in the T+DMPA+FSH group.

Testicular steroid levels

T+DMPA and T+DMPA+FSH treatments resulted in falls in intratesticular T (0.5–2% of control, P < 0.001), with the administration of hCG resulting in increased intratesticular T levels (262% of control, P = 0.006) (Table 3). T+DMPA and T+DMPA+hCG administration did not result in significant changes in intratesticular DHT levels (55% of control, P = 0.156; and 223% of control, P = 0.086, respectively), whereas levels in the T+DMPA+FSH group significantly
TABLE 2. Germ cell numbers expressed as number per Sertoli cell in testicular biopsies from men receiving no treatment (control) vs. 6 wk of selective gonadotropin maintenance (T + DMPA, T + DMPA + FSH, T + DMPA + hCG)

<table>
<thead>
<tr>
<th>Germ cells</th>
<th>Control (n = 5)</th>
<th>T + DMPA (n = 6)</th>
<th>T + DMPA + FSH (n = 6)</th>
<th>T + DMPA + hCG (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All A</td>
<td>1.060 ± 0.138</td>
<td>0.875 ± 0.086</td>
<td>0.878 ± 0.098</td>
<td>0.863 ± 0.103</td>
</tr>
<tr>
<td>A dark</td>
<td>0.690 ± 0.056</td>
<td>0.641 ± 0.054</td>
<td>0.636 ± 0.054</td>
<td>0.641 ± 0.089</td>
</tr>
<tr>
<td>A pale</td>
<td>0.370 ± 0.089</td>
<td>0.234 ± 0.044</td>
<td>0.242 ± 0.050</td>
<td>0.223 ± 0.040</td>
</tr>
<tr>
<td>B</td>
<td>0.017 ± 0.002</td>
<td>0.006 ± 0.001</td>
<td>0.013 ± 0.001</td>
<td>0.010 ± 0.002</td>
</tr>
<tr>
<td>Pl</td>
<td>0.064 ± 0.01</td>
<td>0.013 ± 0.003</td>
<td>0.045 ± 0.011</td>
<td>0.028 ± 0.004</td>
</tr>
<tr>
<td>L-Z</td>
<td>0.358 ± 0.036</td>
<td>0.085 ± 0.012</td>
<td>0.299 ± 0.040</td>
<td>0.172 ± 0.026</td>
</tr>
<tr>
<td>PS</td>
<td>1.234 ± 0.247</td>
<td>0.357 ± 0.058</td>
<td>1.128 ± 0.159</td>
<td>0.648 ± 0.148</td>
</tr>
<tr>
<td>rST</td>
<td>2.451 ± 0.242</td>
<td>1.433 ± 0.201</td>
<td>1.876 ± 0.270</td>
<td>1.807 ± 0.223</td>
</tr>
<tr>
<td>3–6 ST</td>
<td>1.505 ± 0.272</td>
<td>1.070 ± 0.080</td>
<td>1.005 ± 0.173</td>
<td>1.279 ± 0.154</td>
</tr>
<tr>
<td>7–8 ST</td>
<td>0.897 ± 0.169</td>
<td>0.597 ± 0.109</td>
<td>0.708 ± 0.127</td>
<td>0.974 ± 0.083</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n = 5–6/group. All A, all type A spermatagonia; A dark, type A dark spermatagonia; A pale, type A pale spermatagonia; B, type B spermatagonia; Pl, Preleptotene spermatocytes; L-Z, leptotene to zygotene spermatocytes; PS, pachytene spermatocytes; rST, steps 1–2 round spermatids; 3–6 ST, steps 3–6 elongating spermatids; 7–8 ST, steps 7–8 elongated spermatids.

***P < 0.05 compared with control.

b P < 0.05 compared with T + DMPA.

* P < 0.01 compared with T + DMPA + FSH.

FIG. 4. Changes in germ cell number over the 6-wk treatment phase in men (n = 6/group) randomized to receive: 1) T implant, 800 mg sc, + DMPA, 150 mg im once; 2) T implant + DMPA + FSH, 300 IU sc twice weekly; or 3) T implant + DMPA + hCG, 1000 IU sc twice weekly. Data are expressed as a percentage of that seen in control subjects (n = 5). Germ cell subtypes are depicted along the x-axis as follows: type A dark spermatagonia (Ad); type A pale spermatagonia (Ap); all type A spermatagonia (All A); type B spermatagonia (B); preleptotene spermatocytes (Pl); leptotene to zygotene spermatocytes (L-Z); pachytene spermatocytes (PS); steps 1–2 round spermatids (rST); steps 3–6 elongating spermatids (3–6 ST); steps 7–8 elongated spermatids (7–8 ST); sperm concentration in the ejaculate on the day of testicular biopsy (sperm). T + DMPA treatment suppressed type B spermatagonia through to rSTs (P < 0.05). All germ cell subtypes were maintained similar to control in the FSH- and hCG-treated groups except PS in the T + DMPA + hCG group (P < 0.05). PS were significantly lower in the T + DMPA + hCG compared with T + DMPA + FSH group (P < 0.01). Logarithmic second y-axis depicting sperm concentration in the ejaculate on the day of testicular biopsy (sperm) with sperm concentrations of 0 million/ml assigned a value of 0.001. No differences between treatment groups 7–8 ST were determined. However, significantly higher sperm concentrations in the T + DMPA + hCG group compared with T + DMPA group (P < 0.05) were noted, with a similar, although not significant, trend also found in the T + DMPA + FSH group.

This study has shown that FSH and LH independently support spermatogenesis in a gonadotropin-suppressed human model. Using stereological approaches to assess germ cell development, the effect of selective gonadotropin maintenance in men receiving an androgen-progestin hormonal contraceptive regimen was examined. In the absence of gonadotropin supplementation, a similar reduction in sperm concentration and germ cell number (from type B spermatagonia through to round spermatids) to that previously reported (13, 41) was noted. FSH treatment delayed a fall in sperm concentration until d 42, whereas hCG treatment prevented any decline. All germ cell subtypes were maintained by either gonadotropin, except for pachytene spermatocytes, which were lower in the hCG-treated group. The data suggest that FSH is more efficient than hCG in supporting pachytene spermatocyte populations in this experimental paradigm. Also, the conversion of pachytene spermatocytes to round spermatids, a process associated with completion of the second meiotic division, appeared to be more efficient in the hCG-treated group despite reduced numbers of pachytene spermatocytes.

In rodents and monkeys, spermatogonial maturation is decreased (27% of control, P < 0.01). Levels of αAdiol differed significantly from control in only the T + DMPA + FSH treatment group (13% of control, P = 0.011). A positive correlation was found between intratesticular T levels and sperm concentration in the ejaculate on the day of testicular biopsy (sperm) with sperm concentrations of 0 million/ml assigned a value of 0.001. No differences between treatment groups 7–8 ST were determined. However, significantly higher sperm concentrations in the T + DMPA + hCG group compared with T + DMPA group (P < 0.05) were noted, with a similar, although not significant, trend also found in the T + DMPA + FSH group.

Analysis of 3βAdiol levels was also completed using an alternative control group of men (n = 7) (41) because no control 3βAdiol levels were available from the original study (13). 3βAdiol levels were reduced to a mean concentration of 12 ± 2 nmol/liter in the T + DMPA and 6 ± 2 nmol/liter in the T + DMPA + FSH group (4–7% of control, P < 0.001), with T + DMPA + hCG (54 ± 300 nmol/L) resulting in a 345% increase compared with control that was not significant due to large variance within the treatment group.

Recovery and adverse events

 Gonadotropin and T levels were followed until recovery. All treatments were well tolerated, with no serious adverse events.

Discussion

This study has shown that FSH and LH independently support spermatogenesis in a gonadotropin-suppressed human model. Using stereological approaches to assess germ cell development, the effect of selective gonadotropin maintenance in men receiving an androgen-progestin hormonal contraceptive regimen was examined. In the absence of gonadotropin supplementation, a similar reduction in sperm concentration and germ cell number (from type B spermatagonia through to round spermatids) to that previously reported (13, 41) was noted. FSH treatment delayed a fall in sperm concentration until d 42, whereas hCG treatment prevented any decline. All germ cell subtypes were maintained by either gonadotropin, except for pachytene spermatocytes, which were lower in the hCG-treated group. The data suggest that FSH is more efficient than hCG in supporting pachytene spermatocyte populations in this experimental paradigm. Also, the conversion of pachytene spermatocytes to round spermatids, a process associated with completion of the second meiotic division, appeared to be more efficient in the hCG-treated group despite reduced numbers of pachytene spermatocytes.
largely reliant on FSH, whereas the later stages of spermatogenesis, including completion of meiosis, spermiogenesis, and spermiation, are thought to be more dependent on intratesticular androgens (12, 43). To the extent possible, this study of limited subject number has now shown that FSH or LH/intratesticular androgens can similarly maintain germ cell development. Low levels of serum FSH (1–2% control) and LH/intratesticular androgens can similarly maintain germ cell development. Low levels of serum FSH (1–2% control) and LH (<0.4% control), and intratesticular T (<2% control), persist after combined androgen-progestin treatment, but it is unclear whether such levels are biologically active. LH-independent Leydig cell androgen secretion (31) may exist in man and may thereby lie beyond the reach of existing male hormonal contraceptive regimes. One could speculate whether synergistic effects may occur between an observed residual intratesticular T of 26 nmol in the FSH-treated group, or between serum FSH levels of 0.04 IU/liter in the intratesticular T-replete hCG group. Also of consideration is the role of other nonsteroidal hCG-stimulated Leydig cell products, such as INSL3, which may provide spermatogenic support via reduced germ cell apoptosis (44).

In the present study, spermatogonial and preleptotene spermatocytes in the FSH- and hCG-treated groups were not different. Data in rats have shown the withdrawal of FSH via passive immunoneutralization results in decreased spermatogonial number (75% of control) (45, 46). Similarly, Bonnet monkeys (Macaca radiata) immunized against the FSH receptor demonstrate reduced spermatogonial to spermatocyte conversion (47). LH suppression has not been shown to affect spermatogonial maturation in rats significantly (48), with data in monkeys lacking. Despite previous data suggesting a relatively greater role for FSH than LH/intratesticular androgens in the process of spermatogonial maturation in rodents and monkeys, the current study findings suggest that, at least in the short term, LH/intratesticular androgens can maintain spermatogonia and their progression into spermatocytes.

The present study supports that FSH and/or LH/intratesticular androgens are able to support spermatocyte development. However, FSH appears potentially more effective than LH/intratesticular androgens in supporting pachytene spermatocytes, given the reduction in their number in the hCG-treated group. This is consistent with animal data that has shown that FSH can partially maintain spermatocyte number in hypophysectomized (49–51) and GnRH antagonist-treated rats (52) and monkeys (21). It is generally accepted that both FSH and intratesticular androgens can support spermatocyte survival in rodents (53). However, FSH appears more effective than intratesticular androgens in the current experimental paradigm.

Either gonadotropin was able to support spermiogenesis to a similar degree, although analysis was limited due to the 6-wk study timeframe. Interestingly, round spermatid numbers were not different in the FSH- and hCG-treated groups, despite the latter having reduced pachytene spermatocyte numbers, possibly indicating increased efficiency of conversion between these two germ cell subtypes in the presence of maintained intratesticular androgens. This proposition is supported by studies in mice with Sertoli cell-specific ablation of androgen receptors (SCARKO mice) in which androgen has been shown to be essential for the completion of meiosis and the production of haploid spermatids (34).

Spermiation was supported by either gonadotropin in a similar way to that seen in rats (24). However, hCG may be more effective in supporting this process, given that only the hCG-treated group maintained sperm concentration at baseline, despite similar stage 7–8 elongated spermatid numbers across both treatment groups. Furthermore, although FSH treatment was able to maintain sperm concentrations similarly to hCG, only one man in the FSH-treated group maintained a sperm concentration above 30 million/ml, compared with four in the hCG-treated group.

As with previous studies, the combination of T + DMPA resulted in a profound fall in intratesticular T levels to 1–2% baseline (13, 41, 54). In this study, a dose of hCG 1000 IU sc twice weekly was chosen based on recently reported testicular needle aspiration data (55), with the aim of maintaining high-normal intratesticular T levels. However, substantially higher intratesticular T levels (262% of control) were achieved than those seen in that study in which a minimum total weekly dose of 750–1000 IU hCG was required to maintain baseline intratesticular T and a dose of 1500–2000 IU weekly resulted in a 26% increase over baseline (55). Our data suggest that lower doses of hCG may be necessary to better mimic physiological intratesticular T levels. However, based on rodent data (43), it is unlikely that there would be any difference in spermatogenic outcome between normal and the modestly elevated intratesticular T levels seen in this study.

Intratesticular 5α reduced androgen levels did not fall to the same extent as intratesticular T, with T + DMPA supporting the up-regulation of 5α reductase in the face of reduced intratesticular T (41, 56). Although FSH did not maintain intratesticular DHT and intratesticular 5α Adiol levels similar to that of control, there were no significant differences between the T + DMPA and T + DMPA + FSH groups or ratios of intratesticular T:intratesticular DHT. Previous work in rodents has shown a positive role for FSH in the regulation of LH and intratesticular DHT. Previous work in rodents has shown a positive role for FSH in the regulation of type 1, 5α reductase (57), a finding not supported by the

### TABLE 3. Intratesticular steroid levels in men undergoing no treatment (control) vs. 6 wk of selective gonadotropin maintenance (T + DMPA, T + DMPA + FSH, T + DMPA + hCG)

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 5)</th>
<th>T + DMPA (n = 6)</th>
<th>T + DMPA + FSH (n = 6)</th>
<th>T + DMPA + hCG (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T (nmol/liter)</td>
<td>2230 ± 180</td>
<td>43 ± 11*</td>
<td>27 ± 3*</td>
<td>5850 ± 450*</td>
</tr>
<tr>
<td>DHT (nmol/liter)</td>
<td>22 ± 4</td>
<td>12 ± 3*</td>
<td>6.0 ± 0.3*</td>
<td>49 ± 26</td>
</tr>
<tr>
<td>3αAdiol (nmol/liter)</td>
<td>24 ± 5</td>
<td>25 ± 20</td>
<td>3 ± 3*</td>
<td>33 ± 7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, n = 5–6/group.

* P < 0.01 compared with control.

a,b P < 0.05 compared with T + DMPA + hCG.
current study data, which suggested reduced 5α reductase activity in the FSH-treated group.

In conclusion, the stereological assessment of germ cell number after short-term selective maintenance of FSH or LH/intratesticular T showed that either gonadotropin was able to support all stages of the spermatogenic process including spermatogonial maturation, meiosis, spermiogenesis, and spermatocytosis. Both FSH and hCG were able to maintain germ cell numbers at that of control with pachytenic spermatocytes, the only germ cell subtype to differ significantly between treatment groups (lower with hCG), underscoring the role of FSH in maintenance of spermatocyte number. In contrast, no differences in round spermatid number were found, potentially highlighting the importance of intratesticular androgens in the completion of meiosis. This study also demonstrates that relatively small doses of hCG are required to maintain physiological intratesticular T and may have implications for dose adjustment in the treatment of men with secondary testicular failure. These data characterize the dual gonadotropic dependence of human spermatogenesis and accentuate the need for male hormonal contraceptive strategies to withdraw FSH and LH/intratesticular androgens maximally to initiate and maintain spermatogenic suppression.

Acknowledgments

The authors acknowledge the excellent assistance of Fiona McLean for intratesticular steroid assays, G. A. Balurados for germ cell counting, Enid Pruyers for serum hormone measurements, and Clinical Research Nurses Ms. Joanne McKenzie and Ms. Elise Forbes.

Received May 26, 2006. Accepted July 31, 2006.

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This study was funded by the National Health and Medical Research Council (NH&MRC) of Australia, Program Grant no. 241100, NH&MRC Post Graduate Scholarship Grant ID 241031 (to K.L.M.), and Research Fellowships 169020 (to R.I.M.) and 169201 (to D.M.R.).

The authors have nothing to disclose.

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JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.