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Variability in Sperm Suppression during Testosterone Administration to Adult Monkeys Is Related to Follicle Stimulating Hormone Suppression and Not to Intratesticular Androgens

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Sex steroid-based male contraceptive regimens do not induce consistent azoospermia. The reason for this variable response is obscure. We used normal adult male monkeys, *Macaca fascicularis* (n = 9) as a model of testosterone (T)-induced gonadotropin suppression to understand the basis for variability in spermatogenic suppression during hormonal contraception. As observed in men, T administration to these monkeys induced azoospermia in some animals and variable degrees of spermatogenic suppression in others. Based on their sperm counts, we divided these animals into two groups: azoospermic (azoo; n = 4) and nonazoospermic (nonazoo; n = 5) groups. Sperm density, testis volumes, and serum T, bioassayable LH (bioLH), immunoassayable FSH (immunoFSH), bioassayable FSH (bioFSH), and inhibin B were examined every 2 wk during the control period, 20 wk of T administration using SILASTIC brand (Dow Corning Corp.) implants, and recovery. Testes were biopsied for estimation of intratesticular T, dihydrotestosterone, and 5α-androstane-3α,17β-diol. Serum T levels increased 1.5- to 2-fold, leading to decreased bioLH levels (48% of control) and intratesticular T levels (15% of control); neither LH nor intratesticular T levels differed between the azoo and nonazoo groups. In contrast, serum levels of FSH, by both bio- and immunoassay, during T administration were significantly lower in the azoo than in the nonazoo group. These results suggest that the degree of suppression of spermatogenesis is closely related to the degree of suppression of FSH levels and not to the levels of intratesticular androgens or to serum LH. These results imply that FSH plays a key role in supporting spermatogenesis in monkeys in this experimental regimen and suggest that maximal suppression of FSH may be essential to ensure consistent azoospermia in men during hormonal contraception. (*J Clin Endocrinol Metab* 87: 3399–3406, 2002)

ADMINISTRATION OF exogenous testosterone (T), either alone or in combination with other agents, produces profound suppression of spermatogenesis by inhibition of pituitary gonadotropins and depletion of testosterone within the testis (1–5). This approach is currently being investigated as a method of hormonal contraception for men. A consistent problem associated with T-based contraceptive regimens is that azoospermia is achieved in 40–90% of men, but a varying degree of spermatogenic suppression is seen within and between various ethnic groups (6, 7). The reason for this variation remains unknown, but it is a critical question in designing and developing future hormonal contraceptives for men.

A possible mechanism for such variation could be differences in residual testicular steroidogenesis (8); it remains controversial whether FSH levels in blood are important (5, 9) or not (10, 11) in the maintenance of sperm production in some men. There is a lack of evidence about the potential role of intratesticular androgens in the maintenance of spermatogenesis during hormonal contraceptive regimens. Also, there are very limited data showing the site(s) of inhibition of germ cell development during hormonal suppression.

The present study used nonhuman primates to investigate the relative importance of serum FSH and intratesticular androgens in maintaining low levels of spermatogenesis during gonadotropin suppression. In particular, we wished to assess whether variability in sperm production is due to differences in FSH levels or in the levels of intratesticular androgens. With respect to sites of inhibition of germ cell development, we have recently published data revealing that during T-induced gonadotropin suppression, azoospermic monkeys have more profound inhibition of early germ cell development than nonazoospermic monkeys (12). Data from these studies in monkeys will not only improve our understanding of hormonal control of primate spermatogenesis, but also help in optimizing the efficacy and acceptability of future hormone-based contraceptive regimens for men.

Materials and Methods

Animals

Nine normal adult male crab-eating macaques (*Macaca fascicularis*), ranging in age from 5–12 yr (7.89 ± 0.94) and in weight from 5–9 kg (6.18 ± 0.41) and with sperm counts of approximately 60.7 ± 17 millions/ejaculate, were used for the study. Animals were housed in the Washington Regional Primate Research Center at the University of

Abbreviations: Adiol, 5α-Androstane-3α,17β-diol; bioFSH, bioassayable FSH; bioLH, bioassayable LH; immunoFSH, immunoassayable FSH; DHT, dihydrotestosterone; T, testosterone; TB, testosterone buciclate.

Washington under defined environmental conditions (25.5 ± 1 C; 65% relative humidity; lights on at 0600 h, off at 1800 h). In addition to monkey chow, animals received fresh fruits, vitamins, and iron injections. Water was provided *ad libitum*. The monkeys employed in this study have been described in our previous report (12). All procedures were performed in accordance with the Endocrine Society Guidelines for the Care and Use of Experimental Animals (13) and were reviewed and approved by the Primate Center and the institutional animal care committee at University of Washington. Animals were sedated with ketamine HCl (10 mg/kg, im; Ames, Fort Dodge, IA) anesthesia for blood draws, electroejaculations, and insertion and removal of T implants. Diazepam (1 mg/kg, im; Elkins-Sinn, Inc., Cherry Hill, NJ), was given before ketamine HCl to all monkeys for the electroejaculation procedure.

Drug administration

T (4-androsten-17β-ol-one; Sigma, St. Louis, MO) was administered via SILASTIC brand implants (Dow Corning Corp., Midland, MI; 5.5 cm long; dimensions: inside diameter, 0.33 cm; outside diameter, 0.46 cm) for 20 wk. The implants were sterilized with ethylene oxide and were rinsed in sterile saline; two implants were inserted sc in the midscapular region of each animal (14). The study had three phases: control (4 wk), treatment (20 wk), and recovery (16 wk).

Parameters examined

Body weights, testicular volumes, and sperm counts. Body weight, testis volumes, and sperm counts were measured every 2 wk during control, treatment, and recovery periods. The values represented as treatment wk 0 are the mean of two values obtained during the control period. Testis volumes were measured using an orchidometer. To avoid any interobserver variation during the study, the same person measured testis volume each time. Seminal fluid was collected by rectal probe electroejaculation. Total sperm counts were determined in both exudate and coagulum using a hemocytometer and are expressed as spermatozoa $\times 10^6$/ejaculate (14). The criteria for azoospermia and nonazoospermia were taken from the WHO manual for semen analysis (15).

Serum hormones. Blood samples were collected from the femoral vein every 2 wk during the study. Samples were drawn between 0900–1200 h, refrigerated overnight at 4 C, and centrifuged at 1500 × *g* for 15 min, and the sera were separated and stored at −20 C until hormone analysis. T was measured in duplicate by Delfia immunofluorometric assay (Wallac, Inc., Turku, Finland). The detection limit of the assay was 0.5 nmol/liter. The intra- and interassay coefficients of variation were 5% and 10%, respectively. Immunoassayable FSH (immunoFSH) was measured by the method described by Ramaswamy *et al.* (16) using new homologous RIA reagents supplied by the National Hormone and Pituitary Program. Recombinant cynomolgus FSH (NICHHD Rec-MoFSH-RP-1, AFP 6940A) was used as a reference preparation. The minimal detectable dose was 0.06 ng/ml. The intra- and interassay coefficients of variation were less than 5% and 8%, respectively. The bioFSH levels were determined by an *in vitro* FSH bioassay that uses a granulosa tumor cell line from ovarian tumor of transgenic mice expressing inhibin α-promoter/simian virus 40-TAg fusion gene. This cell line was stably transfected with recombinant human FSH receptor and was used to develop *in vitro* bioassay for FSH. The FSH response was measured based on cAMP production by the granulosa cells (17). The recombinant human FSH (biopotency: 1 mg = 10,000 IU; Gonal-F, Serono, Randolph, MA) was used as the standard. This standard is 1.8 times more potent than recombinant cynolomolgus monkey FSH (NICHHD, Rec. MoFSH-RP-1, AFP 6940A). The detection limit of the assay was 0.08 IU/liter, and the intra- and interassay coefficients of variation were 8% and 13%, respectively. Postmenopausal and castrated monkey sera exhibited parallel dose-response curves. This assay system is highly specific for FSH, and no significant increase in cAMP was discerned with hCG, LH, TSH, GH, and PRL. Inhibin B was assayed by a two-site ELISA using reagents from Serotec's kit (Oxford, UK) for inhibin B (18). The intra- and interassay coefficients of variation were 9.2% and 7.9%, respectively. The bioassayable LH (bioLH) levels were measured using a modification of procedures described by van Damme *et al.* (19) and Dufau *et al.* (20). This assay quantitates the LH-dependent secretion of T from dispersed mouse Leydig cells in the primary culture and has been shown to be suitable for monkey blood (21). The detection limit of the assay was 0.03 μg/ml. The intra- and interassay coefficients of variation were 12% and 15%, respectively.

Testis biopsies. Open testicular biopsies were performed alternatively on the right and left testes in each animal under aseptic conditions. Ketamine/xylazine (70 mg/ml ketamine and 6 mg/ml xylazine) anesthesia was given at a dose of 0.1 ml/kg body weight. All animals were biopsied 4 wk before the treatment (control); at treatment wk 2, 14, and 20; and at 12 wk posttreatment. Approximately 300–500 mg tissue were removed and then divided in half. One half was fixed for 5–6 h in Bouin's fluid for histology and quantitation of germ cell number (12); the other half was snap-frozen in liquid nitrogen for the measurement of testicular androgens.

Estimation of testicular androgens. Androgens [T, dihydrotestosterone (DHT), and 5α-androstane-3α,17β-diol (Adiol)] were extracted from the testicular tissue (70–220 mg tissue/monkey), separated by HPLC, and quantified by RIA as previously described (22). Briefly, HPLC-purified samples were resuspended in assay buffer [0.1% (wt/vol) gelatin in 0.1 M PBS (0.154 M NaCl, pH 7.4)]. The primary antibody, Cox 0457 (Sirosera, Sydney, Australia), was diluted 1:400,000 in 1:800 normal sheep serum, and tracer was iodinated histamine-T (10,000 cpm/100 μl). Standard curves were constructed using T (1.0–250 pg/tube), DHT (1.0–250 pg/tube), or Adiol (3.9–1000 pg/tube). The assay (final volume 400 μl/tube) was incubated overnight at 4 C, after which second antibody (100 μl donkey antisheep IgG diluted 1:20 in assay buffer) was added and incubated at room temperature for 30 min. Antigen-antibody complexes were precipitated by incubation (4 C, 30 min) with 1 ml 6% polyethylene glycol 6000 and centrifuged (4,000 × *g*, 4 C, 30 min), and pellets were consolidated by the addition of 1 ml 5% (wt/vol) potato starch followed by recentrifugation. To follow steroid recoveries throughout processing, 9,000–12,000 cpm radiolabeled [^{3}H]T ([1,2,6,7,17-^{3}H]T; 121 Ci/mmol), [^{3}H]DHT ([1,2,3,4,5,6-^{3}H]DHT; 110 Ci/mmol; NEN Life Science Products, Boston, MA), and [^{3}H]Adiol were added to each tube after homogenization. [1,2,3,4,5,6-^{3}H]Adiol was prepared in-house from [1,2,3,4,5,6-^{3}H]DHT using 3α-hydroxysteroid dehydrogenase (Sigma, St. Louis, MO) with purification of [^{3}H]Adiol by HPLC. The specific activities of all three tritiated tracers were determined by RIA (22) and were shown to be 66–110 Ci/mmol. The final potencies of T, DHT, and Adiol in testicular samples were corrected for the added mass of the tritiated tracers. Recoveries of added steroid ^{3}H-labeled tracers in monkey assays at the final RIA stage were: T, 51.2% (SD, 4.5%; n = 45); DHT, 26.4% (SD, 6.0%; n = 45), and Adiol 42.5% (SD, 5.6%; n = 45). The within-assay variation was assessed from the coefficient of variation for the measurement of five samples from the same testis and was 17% for T and 18% for DHT. The between-assay variation in the respective assays was based on the repeated assay of a steroid stock and was between 12–15%. The sensitivity of the combined extraction, HPLC, and RIA components of the assay was calculated from the sensitivity of the RIA, the average recoveries of tritiated steroid, and the average tissue mass extracted in the assay. These values were 0.94 pmol/liter for T, 1.2 pmol/liter for DHT, and 2.9 pmol/liter for Adiol.

Data analysis

Animals were divided *post hoc* into azoospermic (azoo) and nonazoospermic (nonazoo) groups based on the response to T administration. Friedman ANOVA was used to test for significant changes in the measured variables over time. This analysis was performed separately on the data from two groups and on the data from nine animals combined. When significant differences over time were found, individual Wilcoxon signed-ranks tests were used to determine the time points at which values were significantly different from control. To test for differences between the azoo and nonazoo groups during the control, treatment, and recovery periods, a mean value over each period was calculated for each animal and analyzed by Mann-Whitney *U* test. Spearman's rank coefficient was used to determine the relationship between mean sperm counts and mean bioFSH levels (mean values were computed from samples collected during treatment wk 10–16, when stable spermatogenic suppression was achieved). Unless otherwise indicated,

summary statistics are presented as the mean ± SEM and the results of statistical tests are considered significant when $P \leq 0.05$.

Results

Spermatogenic suppression

During the entire study ejaculates were successfully obtained from all animals, and ejaculatory responses were unchanged. The values represented as treatment wk 0 are the mean of two values obtained during the control period. In response to T administration, sperm counts in all animals decreased significantly from the control value after 10 wk (Fig. 1A). All animals showed a maximal suppression of sperm counts between 10–16 wk of treatment, with six animals showing at least one azoospermic sample. The average

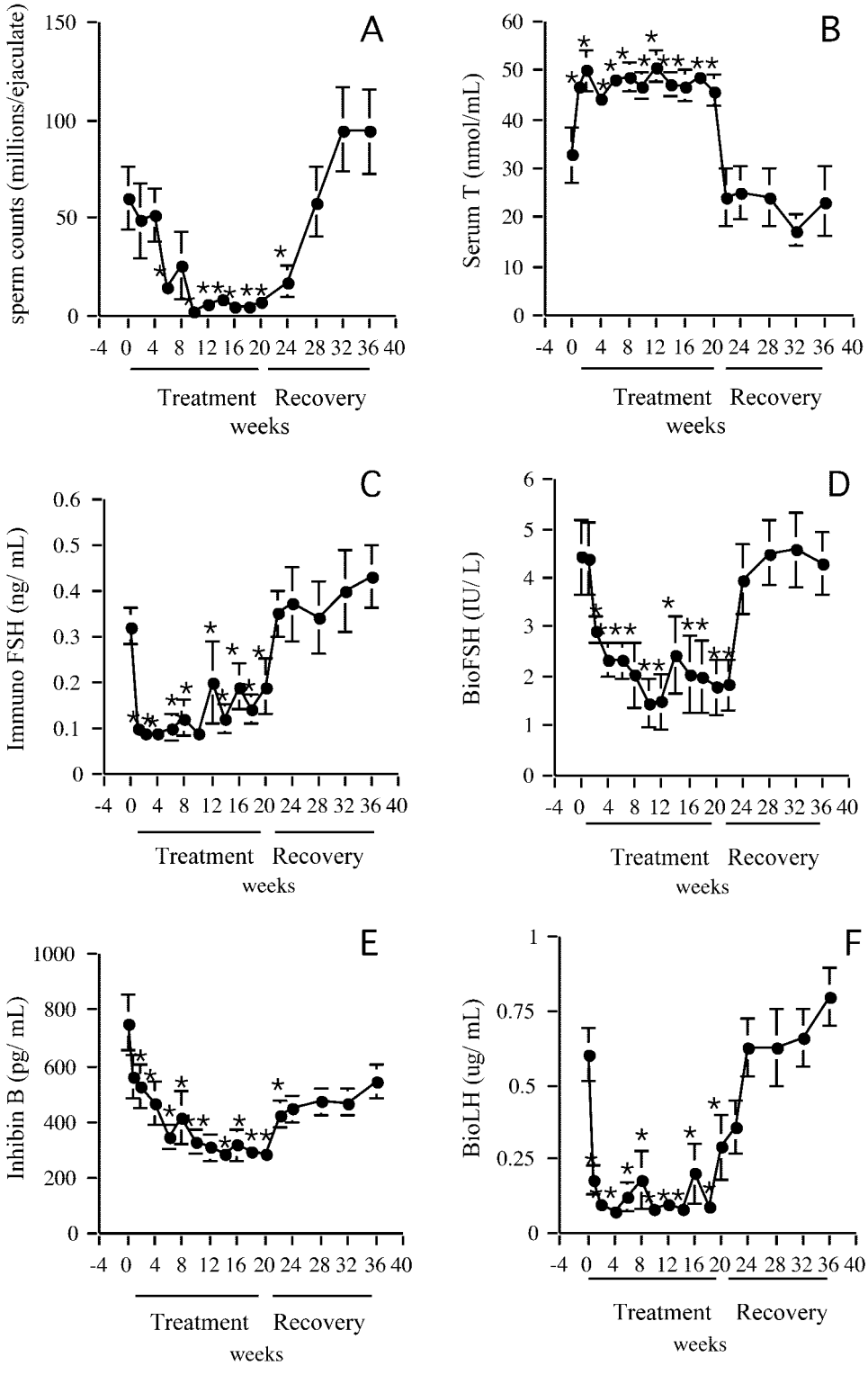

FIG. 1. Sperm counts and serum hormones in all animals (n = 9) during 20-wk T administration. A, Sperm counts; B, serum T; C, immunoFSH; D, bioFSH; E, inhibin B; F, bioLH. Values are expressed as mean ± SEM. *$P <$ 0.05, significant difference from control.

sperm counts were 5% of the control value at 10 wk, 8% of the control value at 16 wk, and 13% of the control value at 20 wk. During recovery, the sperm counts of all animals returned to control levels (Fig. 1A).

Body weight and testis volumes

During T administration there was no significant change in body weight. The average body weights (n = 9) before treatment and after 20 wk of T administration were 6.18 ± 0.41 and 6.4 ± 0.47 kg (P = NS), respectively. The mean testicular size (left plus right) during the control period was 26.5 ± 2.6 ml. During treatment, testes size was decreased significantly by 2 wk and was 44% of the control value by the end of treatment (n = 9; data not shown). A complete recovery in testis volume was observed after cessation of treatment.

Serum hormones

The serum T level during the control period was 32.7 ± 5.76 nmol/ml (n = 9). After insertion of T implants, serum T levels increased significantly from the control value after 1 wk of T administration. By treatment wk 2, serum T levels reached a peak of 50.01 ± 4.16 nmol/ml. Serum T levels were maintained in that range until removal of implants (Fig. 1B). The mean bio- and immunoFSH levels during control were 4.42 ± 0.74 IU/liter and 0.32 ± 0.04 ng/ml (n = 9), respectively. During T administration, immunoFSH decreased significantly after 1 wk, and a significant decrease in bioFSH levels was observed after 2 wk of T administration. A maximal suppression of both immuno- as well as bioFSH was observed between 10 and 20 wk of T administration. During that time, bioFSH levels were decreased in the range of 30–40%, and immunoFSH levels were decreased to 30–60% of the control value (Fig. 1, C and D). The inhibin B level during the control period was 752 ± 106 pg/ml (n = 9) and decreased significantly after 2 wk of T-induced gonadotropin suppression. Nadir inhibin B levels were observed between 10 and 20 wk; during that time, inhibin B levels were approximately 40% of the control value (Fig. 1E). The mean bioLH levels during the control period was 0.6 ± 0.09 μg/ml. BioLH levels decreased significantly after 1 wk and remained in the range of 10–30% of the control value through wk 18 of treatment. At wk 20, the bioLH levels slightly rebounded and were 48% of the control value (Fig. 1F). All hormones returned to the control level during the 16-wk recovery period (Fig. 1).

Intratesticular androgens

Intratesticular T, DHT, and Adiol were measured using testis biopsies taken during control, treatment, and recovery periods. The mean intratesticular T levels during the control period were 859 ± 327 nmol/liter testis (n = 9). After 2 wk of treatment, intratesticular T levels dropped significantly to 15% of the control value and remained in that range until the end of treatment (Fig. 2A). The mean DHT level was 3.59 ± 0.99 nmol/liter (n = 9) during the control period and did not change significantly during T administration (Fig. 2B). The average Adiol level during the control period was 8.44 ± 2.35 nmol/liter (n = 9) and remained unchanged during treatment (data not shown).

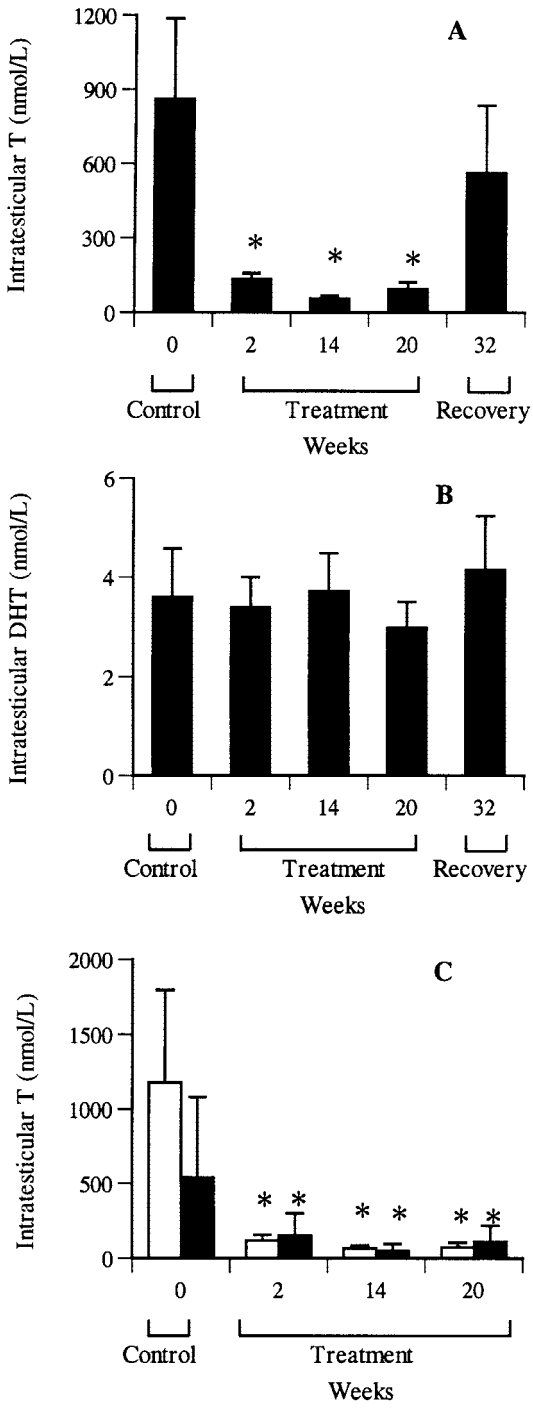

FIG. 2. Effect of T administration on the intratesticular androgens during control, treatment, and recovery. A, intratesticular T (n = 9); B, intratesticular DHT (n = 9); C, intratesticular T in the azoo and nonazoo group. □, Azoo group (n = 4); ■, nonazoo group (n = 5). Values are expressed as mean ± SEM. *$P < 0.05$, significant difference from control.

Correlation among bioLH, bioFSH, inhibin B, intratesticular T, and sperm counts

As we wished to understand the basis for the mechanisms underlying inconsistent azoospermia, a correlation was sought between nadir sperm counts in each animal (occur-

ring between wk 10–16 of T administration) and endocrine markers, including intratesticular T analyzed from the testicular biopsy performed at 14 wk. A mean value of the four serum samples collected between wk 10–16 was determined for each animal. No correlation was found between sperm counts and either bioLH levels (r = 0.15; P = NS), intratesticular T (r = 0.14; P = NS), or inhibin B (r = 0.28; P = NS; data not shown). However, a significant correlation (r = 0.73; $P < 0.05$) was observed between nadir sperm counts and bioFSH levels (Fig. 3).

Heterogeneity in response to T administration (comparison of azoo vs. nonazoo groups)

In response to T administration there was a clear heterogeneity in the pattern of sperm suppression. Some animals achieved stable azoospermia, whereas in other animals spermatogenesis continued, albeit at a low rate. As our main aim was to understand the basis of variability in sperm suppression during T-based hormonal contraception, we divided animals (n = 9) into two subgroups. Animals who achieved stable suppression of sperm counts to azoospermia and who had at least two consecutive azoospermic samples during the last 10 wk of the treatment period (*i.e.* when nadir suppression had occurred) were classified as azoospermic (azoo group; n = 4). Those animals who were never consistently azoospermic in the last 10 wk of treatment and who had variable degrees of sperm suppression were classified as nonazoospermic (nonazoo group; n = 5). The sperm counts in the nonazoo group ranged between 1–40% of the control. There was no significant difference in mean sperm counts during the control period between animals that attained azoospermia and those that remained nonazoospermic (81.10 ± 10.26 *vs.* $53.18 \pm 27.37 \times 10^6$/ ejaculate; P = NS; Fig. 4A). In both groups sperm counts were maximally suppressed between 10 and 16 wk of treatment.

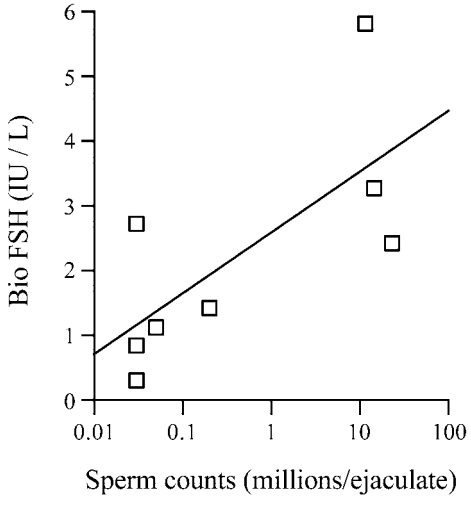

FIG. 3. Plot illustrating the relationship between bioFSH levels and sperm counts. Each point represents the mean of four samples collected from wk 10–16. The *line* indicates the least-squares best-fit regression. The Spearman rank coefficient was rho = 0.73 ($P < 0.05$). (Data from one animal that had sperm counts of zero for all four samples were omitted from the plot, but were included in the correlation calculation.)

Comparison of serum and testicular hormones did not show any significant differences in the levels of intratesticular T, serum T, or bioLH between the azoo and nonazoo groups during control, treatment, or recovery periods (Figs. 2C and 4, B and D). Although inhibin B levels decreased more rapidly from the control value during the initial 4 wk of treatment in the azoo group, no significant difference was observed at either time point between the two groups during treatment. Toward the end of treatment, inhibin B levels in the azoo group were 38% of the control value and approximately 42% of the control value in the nonazoo group (Fig. 4C).

There were clear differences in the degree of FSH suppression between the two subgroups. BioFSH levels during the control period were 4.37 ± 0.83 and 4.4 ± 0.7 IU/liter (P = NS) in the azoo and nonazoo groups, respectively. During treatment, bioFSH levels decreased significantly from the control level in the azoo group and were 26% of the control value at the end of treatment. In contrast, the nonazoo group showed an insignificant decrease in bioFSH levels, which was maintained at 65% of the control level through the end of treatment. The two subgroups were significantly different from each other between treatment wk 6 and 20 (Fig. 4E). The immunoFSH levels between the azoo and nonazoo groups were 0.275 ± 0.05 *vs.* 0.339 ± 0.08 ng/ml, respectively (P = NS). In response to T administration, immunoFSH levels decreased significantly from the control level in both groups; however, the levels were more profoundly suppressed in the azoo group than in the nonazoo group. The two groups were significantly different from each other at 20 wk of treatment (Fig. 4F). In general, the azoo group showed a more marked suppression of FSH than the nonazoo group.

Discussion

The present study was undertaken as a part of the male contraceptive program investigating the possible reason(s) for variable suppression of spermatogenesis in men during hormonal contraception. This primate model proved to be excellent for the study of male contraception, showing azoospermia in some animals and variable degrees of spermatogenic suppression in others. We observed that the variability in spermatogenic suppression during 20 wk of exogenous T administration is associated with differences in the degree of FSH suppression and is not associated with differences in the intratesticular androgens or serum LH levels. Data from this study point to the critical role of FSH in the maintenance of spermatogenesis.

To understand the reason for the heterogeneity in sperm suppression during hormonal contraception, we divided animals into azoospermic and nonazoospermic subgroups. We did not see any difference in serum T either during the control period or 20 wk of T administration. Therefore, the variability in spermatogenic suppression cannot be explained by differences in serum T. However, comparison of bioFSH and immunoFSH levels between the two groups showed that FSH levels were more markedly suppressed in the azoo group compared with the nonazoo group. These results suggest that residual FSH levels play a key role in supporting spermatogenesis in monkeys. Although a severe

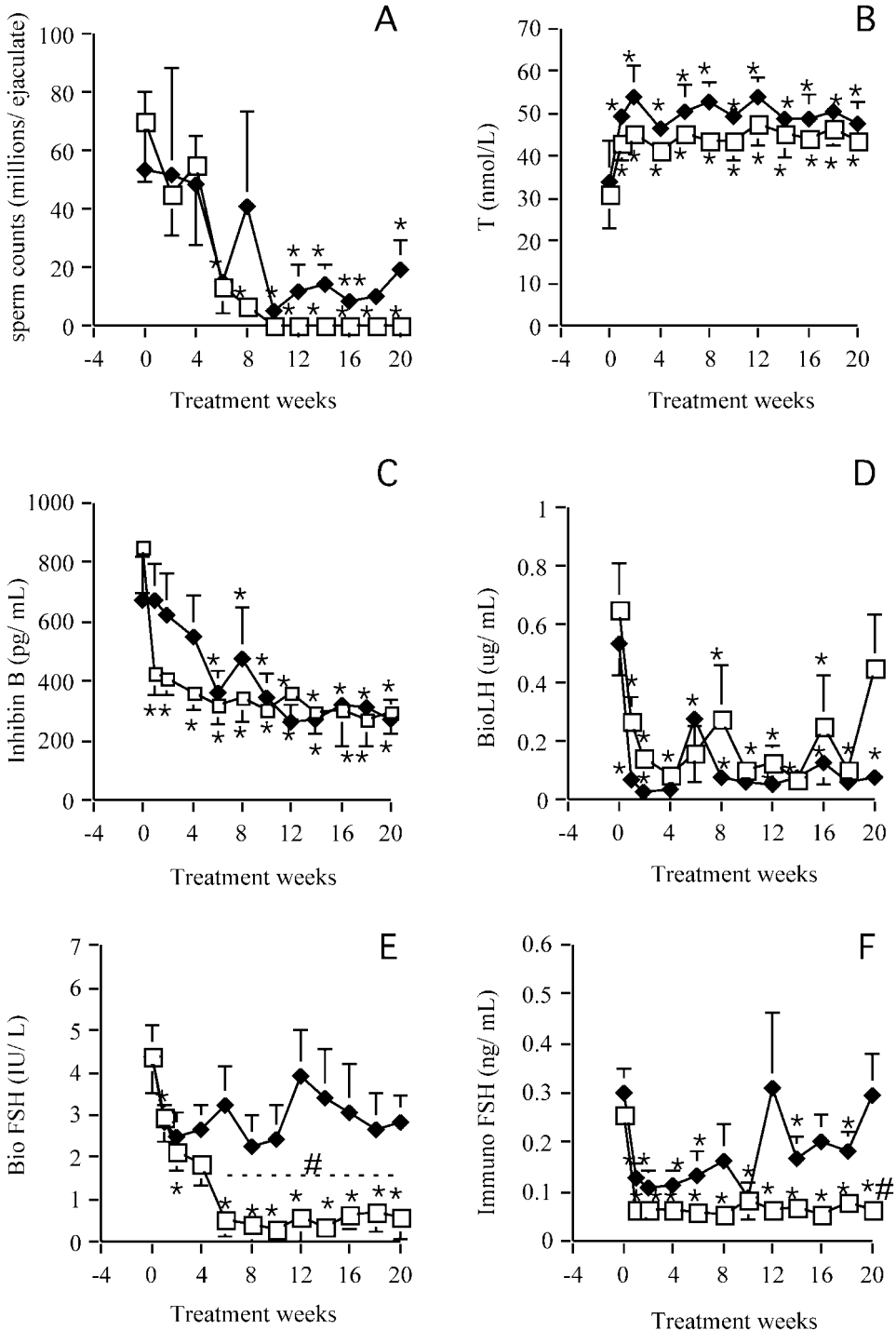

FIG. 4. Sperm counts and serum hormones in the azoo and the nonazoo group. A, Sperm counts; B, serum T; C, inhibin B; D, bioLH; E, bioFSH; F, immunoFSH. *Open symbols*, Azoo group (n = 4); *closed symbols*, non-azoo group (n = 5). Values are expressed as mean ± SEM $*P < 0.05$, significant difference from control; $\#P < 0.05$, significant difference between the azoo and the nonazoo group.

reduction in bioLH levels was observed, there were no significant differences in the rate or the extent of suppression of bioLH between groups.

To further clarify which hormone is important in maintaining spermatogenesis, we sought a correlation between serum LH, FSH, inhibin B, T, and intratesticular androgens and sperm counts during treatment wk 10–16, when sperm suppression reached nadir levels. We found a significant correlation only between sperm counts and bioFSH levels. This suggests that FSH is the factor responsible for maintaining spermatogenesis in these monkeys. These results are consistent with those of earlier studies using other experimental paradigms in monkeys, which demonstrated that FSH is a prime regulator of spermatogenesis in this species. For example, administration of FSH to either GnRH antagonist-treated (23) or normal (24) macaques fully maintained spermatogonial numbers with a considerable increase in spermatocytes and spermatids. Other studies in monkeys showed that active or passive immunization against FSH results in pronounced reduction in testis size and sperm

counts (25, 26). In a study in hypophysectomized monkeys it was demonstrated that T alone maintains complete spermatogenesis; however, spermatogonial proliferation was impaired in the absence of FSH (27).

In a study similar to ours using the long-acting testosterone ester, testosterone buciclate (TB), Weinbauer *et al.* (28) concluded that FSH plays a key regulatory role in this contraceptive model. The researchers observed that the transient escape of FSH suppression, unaccompanied by a rise in serum LH, resulted in the restoration of sperm numbers. These results in monkeys are further strengthened by recent contraceptive studies in men indicating that differences in the degree of FSH suppression may contribute to the degree of spermatogenic suppression (5, 9). In contrast, other contraceptive studies (1, 10) in normal men showed no significant difference in the degree of FSH and LH suppression between azoospermic and oligozoospermic men.

These results led us to investigate further whether FSH or other intrinsic differences are responsible for causing a variable degree of spermatogenic suppression during hormonal contraception. To understand the biological basis of this variation, we examined testicular tissue from these animals. First, we studied changes in germ cell development after exogenous T administration. We observed that the lower circulating FSH levels in azoospermic animals were associated with a significant decrease in the conversion of Ap to B spermatogonia, leading to a lower number of B spermatogonia and later germ cell types per Sertoli cell compared with the nonazoo group (12). These results suggest that the degree of FSH suppression may be critical in determining the extent of spermatogenic inhibition produced. Further, in support of a role for FSH in the maintenance of human sperm production during T treatment, it was demonstrated that FSH administration partially restored sperm production in men whose spermatogenesis was suppressed by T administration (29–31). Also, studies in monkeys showed that combined administration of pure FSH and GnRH antagonist was able to maintain the number of spermatogonia and, in part, round spermatids, whereas in animals treated with GnRH antagonist only, the number of germ cells decreased much faster and more markedly (24). These observations suggest that the degree of FSH suppression may be critical in determining the extent of spermatogenic inhibition produced.

Second, we wanted to investigate whether there is a difference in the intratesticular androgens that may cause variable suppression of spermatogenesis. Our results showed that during T implant administration, intratesticular T concentrations decreased in both subgroups and were not statistically different. These results demonstrate that differences in intratesticular T levels were not responsible for differences in sperm production between the azoo and the nonazoo groups. A similar extent of suppression of intratesticular T levels was observed in GnRH antagonist-treated (32, 33) or hypophysectomized (34) cynomolgus monkeys where testicular T levels did not fall below approximately 20% of control despite a reduction in serum T to less than 5% of the control level. In contrast, recent results reported by Weinbauer *et al.* (28) showed that there was no significant decrease in intratesticular T levels (despite undetectable bioLH levels) in normal adult monkeys treated with TB. It was interesting to note in the Weinbauer *et al.* study (28) that the higher dose of TB, which produced a 2-fold increase in serum T, did not produce higher intratesticular androgen levels, but provoked a more profound reduction in testis size and sperm counts. The basis of the persistence of relatively high levels of intratesticular T after gonadotropin withdrawal in their study is unclear and differs markedly from our own results and those from other studies of induced gonadotropin deficiency (32–34).

DHT is an active metabolite of T within the seminiferous tubule (35) and is a more potent androgen than T (36). In support of a role of DHT in heterogeneous suppression of sperm counts in men undergoing contraception, Anderson *et al.* (37) reported a significant increase in the production of 5α-reduced androgens after prolonged T enanthate treatment in men rendered oligospermic, but not in those who became azoospermic. The researchers speculated that DHT may permit the maintenance of low levels of spermatogenesis in men remaining oligospermic on T enanthate treatment. In the present study we assessed whether there is any difference in testicular DHT levels between animals who became azoospermic and those who remained nonazoospermic. We did not see a significant decrease in testicular DHT levels from the control level between the azoo and nonazoo groups. A possible explanation for unchanged DHT levels despite a 70% reduction in its substrate T could be that 5α-reductase is up-regulated during androgen withdrawal in the primate testis. Preliminary data in rats indicate that testicular 5α-reductase activity is up-regulated during androgen deficiency (38); however, it is not known whether this applies to monkeys during T administration.

In summary, administration of exogenous T to normal adult male macaque monkeys proved an excellent model for examining the variability in spermatogenic suppression during hormonal contraception. We observed in this model that variability in spermatogenic suppression is not associated with differences in residual intratesticular androgens, LH, or inhibin B levels, but, rather, is associated with differences in the degree of FSH suppression between azoo and nonazoospermic animals. These results suggest that FSH is a key factor in the maintenance of primate spermatogenesis in this setting. An implication of these results is that in human male contraceptive studies, greater attempts should be made to assure full suppression of FSH secretion.

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References

1. **Wallace EM, Gow SM, Wu FCW** 1993 Comparison between testosterone enanthate-induced azoospermia and oligospermia in a male contraceptive study. I. Plasma luteinizing hormone, follicle stimulating hormone, testosterone, estradiol and inhibin concentrations. J Clin Endocrinol Metab 77:290–293
2. **Bebb RA, Anawalt BD, Christensen RB, Paulsen CA, Bremner WJ, Matsumoto AM** 1996 Combined administration of levonorgestrel and testosterone induces more rapid and effective suppression of spermatogenesis than testosterone alone: a promising male contraceptive approach. J Clin Endocrinol Metab 81:757–762
3. **Amory JK, Bremner WJ** 1998 The use of testosterone as a male contraceptive. Baillieres Clin Endocrinol Metab 12:471–484
4. **Swerdloff RS, Bagatell CJ, Wang C, Anawalt BD, Berman N, Steiner B, Bremner WJ** 1998 Suppression of spermatogenesis in man induced by Nal-Glu gonadotropin releasing hormone antagonist and testosterone enanthate (TE) is maintained by TE alone. J Clin Endocrinol Metab 83:3527–3533
5. **Büchter D, von Eckardstein S, von Eckardstein A, Kamischke A, Simoni M, Behre HM, Nieschlag E** 1999 Clinical trial of transdermal testosterone and oral levonorgestrel for male contraception. J Clin Endocrinol Metab 84:1244–1249
6. **WHO** 1990 Contraceptive efficacy of testosterone-induced azoospermia in normal men. Lancet 336–955-959
7. **WHO** 1996 Contraceptive efficacy of testosterone-induced azoospermia and oligozoospermia in normal men. Fertil Steril 65:821–829
8. **Anderson RA, Wallace AM, Kicman AT, Wu FCW** 1997 Comparison between testosterone enanthate-induced azoospermia and oligospermia in a male contraceptive study. IV. Suppression of endogenous testicular and adrenal androgens. Hum Reprod 12:1657–1662
9. **Behre HM, Baus S, Kliesch S, Keck C, Simoni M, Nieschlag E** 1995 Potential of testosterone buciclate for male contraception: endocrine differences between responders and non-responders. J Clin Endocrinol Metab 80:2394–2403
10. **Handelsman DJ, Conway AJ, Boylan LM** 1992 Suppression of human spermatogenesis by testosterone implants. J Clin Endocrinol Metab 175:1326–1332
11. **Anderson RA, Wu FCW** 1996 Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive study. II. Pharmacokinetics and pharmacodynamics of once weekly administration of testosterone enanthate. J Clin Endocrinol Metab 81:896–901
12. **O'Donnell L, Narula A, Balourdos G, Gu YQ, Wreford NG, Robertson DM, Bremner WJ, McLachlan RI** 2001 Impairment of spermatogonial development and spermiation following testosterone-induced gonadotropin suppression in adult monkeys (*Macaca fascicularis*). J Clin Endocrinol Metab 86:1814–1822
13. **The Endocrine Society** 1997 Guidelines for the care and use of experimental animals. J Clin Endocrinol Metab 82:40A
14. **Bremner WJ, Bagatell CJ, Steiner RA** 1991 Gonadotropin-releasing hormone antagonist plus testosterone: a potential male contraceptive. J Clin Endocrinol Metab 73:465–469
15. **WHO** 1992 Laboratory manual for the examination of human semen and semen-cervical mucus interaction, 3rd Ed. Cambridge: Cambridge University Press
16. **Ramaswamy S, Pohl CR, McNeilly AS, Winters SJ, Plant TM** 1998 The time course of follicle-stimulating hormone suppression by recombinant human inhibin A in the adult male rhesus monkey (*Macaca mulatta*). Endocrinology 139:3409–3415
17. **Narula A, Ryhänen P, Manna PR, Huhtaniemi IT, Bremner WJ,** Development of an *in vitro* bioassay for FSH using a cell line transfected with human FSH receptor. 32nd Annual Meeting of the Society for Study of Reproduction, Pullman, WA, 2000, p 117
18. **Groome NP, Illingworth PJ, O'Brien M, Pal R, Rodger FE, Mather JP, McNeilly AS** 1996 Measurement of dimeric inhibin B throughout the human menstrual cycle. J Clin Endocrinol Metab 81:1401–1405
19. **van Damme MP, Robertson DM, Diczfalusy E** 1974 An improved in vitro bioassay method for measuring luteinizing hormone (LH) activity using mouse Leydig cell preparations. Acta Endocrinol (Copenh) 77:655–671
20. **Dufau ML, Mendelson CR, Catt KJ** 1974 A highly sensitive *in vitro* bioassay for luteinizing hormone and chorionic gonadotropin: testosterone production by dispersed Leydig cells. J Clin Endocrinol Metab 39:610–613
21. **Cummings DE, Kumar N, Bardin CW, Sundaram K, Bremner WJ** 1998 Prostate-sparing effects in primates of the potent androgen MENT: a potential alternative to testosterone androgen replacement and male contraception. J Clin Endocrinol Metab 83:4212–4919
22. **O'Donnell L, Stanton PG, Wreford NG, Robertson DM, McLachlan RI** 1996 Inhibition of 5α-reductase activity impairs testosterone-dependent restoration of spermiogenesis in adult rats. Endocrinology 137:2703–2710
23. **Weinbauer GF, Behre HM, Fingscheidt U, Nieschlag E** 1991 Human follicle-stimulating hormone exerts a stimulatory effect on spermatogenesis, testicular size, and serum inhibin levels in the gonadotropin releasing hormone antagonist-treated non-human primate (*Macaca fascicularis*). Endocrinology 133: 439–445
24. **van Alphen MAA, van de Kant HJG, de Rooij DG** 1988 Follicle stimulating hormone stimulates spermatogenesis in the adult monkey. Endocrinology 123:1449–1455
25. **Srinath BR, Wickings EJ, Witting C, Nieschlag E** 1983 Active immunization with follicle stimulating hormone for fertility control: a 4.5 year study in male rhesus monkeys. Fertil Steril 40:110–117
26. **Moudgal NR, Ravindranath N, Murthy GS, Dighe RR, Aravindam GR, Martin F** 1992 Long-term contraceptive efficacy of vaccine of ovine follicle-stimulating hormone in male bonnet monkeys (*Macaca radiata*). J Reprod Fertil 96:91–102
27. **Marshall GR, Zorub DS, Plant TM** 1995 Follicle-stimulating hormone amplifies the population of differentiated spermatogonia in the hypophysectomized testosterone-replaced adult rhesus monkey (*Macaca mulatta*). Endocrinology 136:3504–3511
28. **Weinbauer GF, Schlatt S, Walter V, Nieschlag E** 2001 Testosterone-induced inhibition of spermatogenesis is more likely related to suppression of FSH than to testicular androgen levels in the cynomologus monkey model (*Macaca fascicularis*). J Endocrinol 168:25–38
29. **Matsumoto AM, Karpas AE, Paulsen CA, Bremner WJ** 1983 Reinitiation of sperm production in gonadotropin-suppressed normal men by administration of follicle-stimulating hormone. J Clin Invest 72:1005–1015
30. **Bremner WJ, Matsumoto AM, Sussman AM, Paulsen CA** 1981 Follicle stimulating hormone and human spermatogenesis. J Clin Invest 68:1044–1049
31. **Matsumoto AM, Karpas AE and Bremner WJ** 1986 Chronic human chorionic gonadotropin administration in normal men: evidence that follicle-stimulating hormone is necessary for the maintenance of quantitatively normal spermatogenesis in man. J Clin Endocrinol Metab 62:1184–1192
32. **Weinbauer GF, Göckeler E, Nieschlag E** 1988 Testosterone prevents complete suppression of spermatogenesis in the gonadotropin-releasing hormone antagonist-treated non-human primate (*Macaca fascicularis*). J Clin Endocrinol Metab. 67:284–290
33. **Zhengwei Y, Wreford NG, Schlatt S, Weinbauer GF, Nieschlag E, McLachlan RI** 1998 Acute and specific impairment of spermatogonial development by GnRH antagonist-induced gonadotropin withdrawal in the adult macaque (*Macaca fascicularis*). J Reprod Fertil 112:139–147
34. **Marshall GR, Jockenhövel F, Lüdecke D, Nieschlag E** 1986 Maintenance of complete but quantitatively reduced spermatogenesis in hypophysectomized monkeys by testosterone alone. Acta Endocrinol (Copenh) 113:424–431
35. **Rivarola MA, Podestá EJ, Chemes HE, Aguilar D** 1973 In vitro metabolism of testosterone by whole human testis isolated seminiferous tubules and interstitial tissue. J Clin Endocrinol Metab 37:454–460
36. **Zhou Z, Lane MV, Kemppainen JA, French FS, Wilson EM** 1995 Specificity of ligand-dependent androgen receptor stabilization: receptor domain interactions influence ligand dissociation and receptor stability, Mol Endocrinol 9:208–218
37. **Anderson RA, Wallace AM, Wu FCW** 1996 Comparison between testosterone enanthate induced azoospermia and oligospermia in a male contraceptive study. III. Higher 5α-reductase activity in oligozoospermic men administered supraphysiological doses of testosterone. J Clin Endocrinol Metab 81:902–908
38. **Pratis K, O'Donnell L, McLachlan RI, Robertson DM,** Hormonal regulation of type 1 and type 2 5α-reductase activity in rat testis. Proc of the 81st Annual Meeting of The Endocrine Society, San Diego, CA, 1999, p 254