0021-972X/04/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 89(1):142–149

doi: 10.1210/jc.2003-030616

Relationship between Serum Gonadotropins and Spermatogenic Suppression in Men Undergoing Steroidal Contraceptive Treatment

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This study aimed to establish whether the degree of suppression of serum FSH and LH was related to sperm concentration in three testosterone (T) plus progestin contraceptive regimens. We measured serum FSH and LH using a modified, highly sensitive immunofluorometric assay in samples obtained from three published studies using T enanthate (TE; 100 and 200 mg weekly) plus daily oral doses of cyproterone acetate (CPA; 5–100 mg), levonogestrel (LNG; 150–500 μg), or desogestrel (DSG; 150–300 μg). Overall, men with sperm concentrations below 0.1 million/ml had significantly lower gonadotropin levels (serum FSH, ~0.12 IU/liter; serum LH, ~0.05 IU/liter) than oligospermic men (sperm concentrations, 0.1–5 million/ml; serum FSH, 0.23–0.5 IU/liter; serum LH, 0.05–0.56 IU/liter), but the relationship was weak, indicating the possible existence of other determinants. Multivariate logistic regression was used to identify the influence of candidate predictors of spermatogenic effects of the T plus progestin regimens. In the LNG and DSG studies, the marked suppression of serum LH to less than 5% of baseline values (<0.15 IU/liter) was a consistent and highly significant predictor of sperm concentration (reduced to 2–7% that seen at higher LH levels) and the likelihood of its suppression below 1 million/ml (a proposed threshold for contraceptive efficacy). Serum FSH was not a significant independent predictor. The use of DSG and CPA (but not LNG) was a significant independent predictor of sperm suppression, and regimens that contained 200 mg TE weekly caused less spermatogenic suppression than 100 mg TE weekly. These findings suggest that T-progestin contraceptive regimens suppress sperm concentration by gonadotropin-dependent and -independent mechanisms. The suppression of serum LH is a major predictor of the suppression of sperm concentration suppression in the LNG and DSG treatment studies. On the other hand, the greater spermatogenic suppression in regimens containing DSG or CPA suggests that these progestins have additional actions to suppress spermatogenesis via a gonadotropin-independent mechanism(s) (*J Clin Endocrinol Metab* 89: 142–149, 2004)

THE ADMINISTRATION TO normal men of testosterone (T) alone (1, 2) or with other agents, such as progestin (3–6) or GnRH analogs (7–9), profoundly reduces pituitary gonadotropin secretion and sperm output. Gonadotropin suppression has long been held to be the major mechanism of T, T plus progestin, or T plus GnRH analog regimens. The withdrawal of FSH action from Sertoli cells and the reduction in testicular T levels resulting from LH withdrawal from Leydig cells markedly suppress spermatogenesis (10). The consequent reduction in sperm concentrations provides the potential for an effective and reversible male contraceptive (3–6, 11–15).

To ensure contraceptive efficacy, the attainment of azoospermia continues to be the goal of hormonal contraceptive regimens. Only about 70% of Caucasian men are rendered azoospermic, and 95% achieve sperm concentrations of 3 million/ml or less by T treatment alone (1, 2). Combined T plus progestin regimens appear more effective in achieving either azoospermia or the sperm concentration threshold of less than 1 million/ml, which is likely to provide satisfactory contraceptive efficacy (2). In about 5% of men, sperm concentrations fail to suppress to below 1 million/ml in response to T-based contraceptive treatments for reasons that are unclear.

The key role for FSH in spermatogonial development has been established in all species. In monkeys, we (16, 17) and others (18) have found that FSH levels correlate with spermatogonial development and that the failure to adequately suppress FSH is a potential reason for contraceptive failure. The importance of adequate FSH suppression in man has also been emphasized (19). Given the critical role of testicular T in spermatogenesis, the complete suppression of LH is also essential for effective and uniform spermatogenic suppression. Incomplete suppression of gonadotropins by suboptimal doses of T alone (20) or in combination with progestin (12) results in less complete and uniform spermatogenic suppression.

The hypothesis that the failure to fully suppress serum gonadotropins (*i.e.* to levels below ~5% of baseline) leads to inadequate spermatogenic suppression in a contraceptive

Abbreviations: CPA, Cyproterone acetate; DSG, desogesterel; LNG, levonogesterel; T, testosterone; TE, testosterone enanthate.

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

study context has been explored to some degree, although no correlations have been found. Handelsman *et al.* (21) found no relationship between the nadir serum gonadotropin levels and the occurrence of azoo- and oligospermia in men participating in WHO trials, although there were differences in baseline serum FSH levels. Similarly, Wallace *et al.* (22) could not attribute an azoo- *vs.* oligospermic response to T alone to differences in the extent of gonadotropin suppression. Recently, two studies have reported that gonadotropin levels are higher in men failing to become azoospermic in response to treatment (23, 24). Certainly, no clear threshold level below which either FSH or LH must be suppressed has been identified.

In exploring the potential relationship between gonadotropin levels and spermatogenesis, there are two key unresolved issues. First, the lower limits for biological effects of FSH and intratesticular T are not known. In GnRH-immunized rats, even when intratesticular T levels were markedly reduced (~1–3% baseline), further inhibition of spermatogenesis was seen when an androgen receptor antagonist was coadministered (25), implying that low levels of androgens (due to LH-dependent or -independent secretion) still have a biological effect in the presence of undetectable serum immunoreactive LH. Similarly for FSH, the persistence of FSH levels below 5% of the baseline might have a significant effect on Sertoli cell function, although this is a harder issue to establish with *in vivo* models. The minimal threshold for intratesticular FSH and T levels might differ both between individual men and between ethnic groups. Such a hypothesis is exceedingly difficult to pursue in clinical studies.

The gonadotropin assay methods applied in previous clinical contraceptive studies have lacked sensitivity to confidently define gonadotropin levels below approximately 5% of baseline levels. Such assays have reported FSH and LH sensitivities of 0.02–0.05 IU/liter for both assays, respectively, but the extrapolation of the standard curves below the lower point on the standard curve and the lack of detailed assay performance data below this point have clouded the interpretation of these data. In essence, the term undetectable has been ill defined, and the kit manufacturers have not stated these sensitivity values.

To examine the relationship between sperm concentration and serum gonadotropin levels, serum samples were obtained from a series of studies in which T was combined with three different progestins. We used new, highly sensitive gonadotropin assays to test the hypothesis that profound suppression of gonadotropins (<5% of baseline) results in uniform suppression of sperm concentration to below 0.1 million/ml and the corollary that less complete spermatogenic suppression is due to incomplete suppression of circulating gonadotropins.

Materials and Methods

Study design

The clinical studies investigated in this report are detailed in Table 1 and involved the administration of T enanthate (TE; 100 and 200 mg weekly) and daily oral doses of either cyproterone acetate (CPA; 5–100 mg) (11, 13, 23) for 3–4 months, levonogesterel (LNG; 150–500 μg) (3, 14), and desogesterel (DSG; 150–300 μg) (15) for 5–6 months. All studies were performed with the approval of the relevant institutional review boards, and all subjects gave their informed consent. Sera from these study centers were sent frozen in a dry liquid nitrogen shipper to Melbourne for LH and FSH assays. Other serum hormone data and sperm concentrations pertaining to these studies are detailed in the relevant publications. As these studies included several sperm concentrations at the end of treatment, and men quite often showed azoospermia in one sample and the occasional sperm only seen in the centrifuged ejaculate in another, for the purpose of analyses these men were grouped as below 0.1 million/ml. For all statistical calculations in this study, a previously reported azoospermic value was entered as 0.05 million/ml. The use of such a value recognizes the intrinsic difficulty of differentiating true azoospermia from exceedingly low sperm concentrations when evaluating the sample using WHO methods.

TABLE 1. Characteristics of the three contraceptive trials of TE plus progestins: LNG, DSG, and CPA

No. of subjects	TE (mg/wk)	Progestin	mg/d	Time points analyzed (months)	Ref.
5	100			0,3,4	11
9	100	CPA	5	0,3,4	23
7	200	CPA	5	0,3,4	23
5	100	CPA	12.5	0,3,4	13
5	100	CPA	25	0,3,4	13
5	100	CPA	25 bid	0,3,4	11
5	100	CPA	50 bid	0,3,4	11
18	100				3
18	100	LNG	0.125	0,5,6	14
15	100	LNG	0.250	0,5,6	14
17	100	LNG	0.500	0,5,6	3
7	50	DSG	0.15	0,5,6	15
7	100	DSG	0.15	0,5,6	15
8	100	DSG	0.30	0,5,6	15

bid, Twice daily.

Gonadotropin assays

Serum FSH and LH were determined by our sensitive immunofluorometric assays as previously detailed (26). These procedures are adaptations of commercial FSH and LH (Delfia, Wallac, Turku, Finland) immunofluorometric assays resulting in increased sensitivities (LH, 0.005 IU/liter; FSH, 0.008 IU/liter in this study) in application to serum. Assay sensitivity was improved by optimizing incubation times and the inclusion of gonadotropin-free serum obtained by exhaustive immunoabsorption to reduce assay matrix effects in the assay observed with high concentrations of serum. The reproducibility of the assays as assessed from the coefficient of variation of the repeated measurement (n = 12–13) of two serum pools with low and high gonadotropin levels was: for FSH, 15.6% (0.178 ± 0.028 IU/liter) and 9% (4.07 ± 0.37 IU/liter), respectively; and for LH, 11.5% (0.145 ± 0.017 IU/liter) and 8.1% (3.42 ± 0.28 IU/liter), respectively. Undetectable values were assigned the limits of assay detection for statistical and graphical analyses. When serum samples are stored under these conditions, no significant change in gonadotropin immunoreactivity has been observed (our unpublished observation).

Statistics

A number of approaches were employed to analyze these data. Firstly, data were divided into three groups according to the degree of sperm suppression, azoospermia/extreme oligospermia (<0.1 million/ml), moderate-severe oligospermia (0.1-<5 million/ml), or mild oligospermia (>5 million/ml), and were compared using Student's *t* test. Secondly, given the interdependence of the variables to be assessed (T dosage, progestin usage, and gonadotropin levels), sperm concentration data were analyzed in two ways. 1) Sperm concentrations were divided above or below a nominated threshold of 1 or 3 million/ml. These threshold values were chosen because they have been proposed as upper limits for acceptable contraceptive efficacy (27). This outcome was then analyzed using logistic regression, with associations between predictors and the outcome expressed as odds ratios for achieving this threshold. 2) Regression analysis was performed, which allowed for interpretations

of the results as fold increases (or decreases) in sperm concentration when comparing subsets of predictors. In such analyses the comparison is made with reference to a selected group, which is presented as unity. The regression analysis was performed with the sperm concentration outcome on a transformed, but continuous, scale. The transformation used was the natural logarithm ($\log_e$). This transformation resulted in residuals of the right form (approximately normal and constant variance).

For the purpose of analysis, all predictors were divided as follows. 1) To assess the dose-dependent effects of T, we compared men receiving 100 *vs.* 200 mg weekly. 2) As different progestins were used, and their bioequivalence for spermatogenic suppression could not be assessed, we compared groups of men treated with T plus no progestin *vs.* men treated with T plus LNG, DSG, or CPA (regardless of progestin dosage). 3) For analysis of gonadotropin suppression, men were grouped according to whether their gonadotropin levels suppressed above or below

TABLE 2. A comparison of serum FSH and LH values in DSG- and LNG-treated groups after 5 and 6 months of treatment

	Pretreatment values	Months of treatment	Sperm concentration (million/ml)							
			<0.1	%	0.1–5		%	>5		%
Sperm conc. 10^6/ml	69.6 ± 38.9	5	0.05 ± 0.01	0.7	1.91 ± 1.07		2.7	13.8 ± 12.1		20
		6	0.05 ± 0.00	0.7	1.92 ± 1.27		2.8	18.5 ± 10.6		27
FSH (IU/liter)	2.50 ± 1.37	5	0.14 ± 0.23	5.6	0.23 ± 0.52	ns	9.2	0.39 ± 0.52	ns	16
		6	0.12 ± 0.20	4.8	0.50 ± 1.13	[a]	20	0.75 ± 1.03	[a]	30
LH (IU/liter)	3.51 ± 1.59	5	0.06 ± 0.21	1.7	0.05 ± 0.07	ns	1.4	0.77 ± 1.16	[a]	22
		6	0.05 ± 0.12	1.4	0.56 ± 1.29	[a]	16	1.08 ± 1.39	[a]	31

Subjects were divided according to whether they had azoospermia or were extremely oligospermic (sperm concentration, <0.1 million/ml, n = 58–59), moderate-severely oligospermic (sperm concentration, 0.1–5 million/ml, n = 6–10), or in the fertile range (>5 million/ml, n = 13). % refers to percentage of pretreatment values. Assay sensitivity (IU/liter), LH 0.005; FSH, 0.008.

[a] <0.1 million/ml group *vs.* other groups. ns, Not significant ($P < 0.001$).

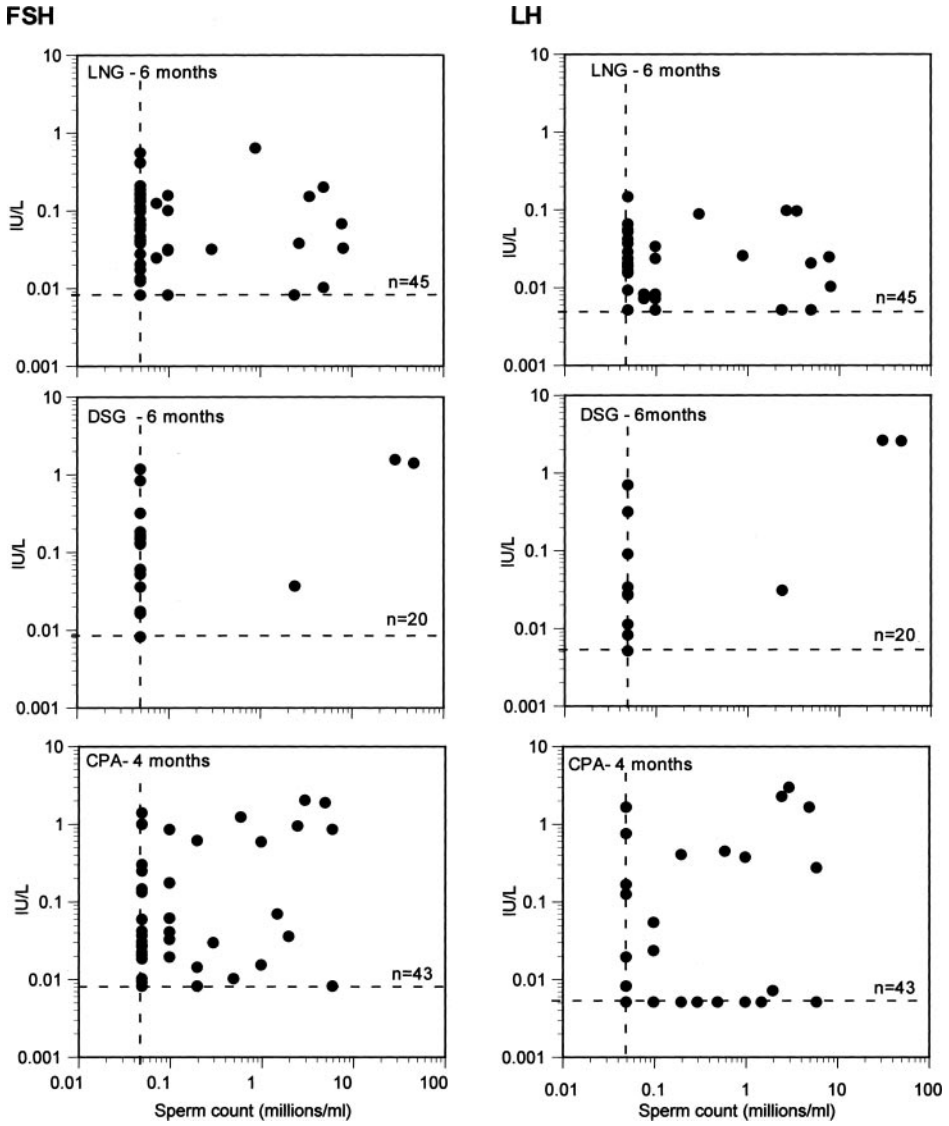

FIG. 1. Regression analysis shows poor correlation between suppression of gonadotropin levels after treatment and suppression of spermatogenesis. Scatterplots comparing sperm concentration *vs.* serum FSH or LH levels after treatment in three contraceptive studies involving T plus a progestin (LNG, DSG, or CPA).

5% of the mean baseline value of the whole group (for serum LH, 0.15 IU/liter; FSH, 0.11 IU/liter). This 5% baseline value, although arbitrary, was chosen to provide a basis for comparing the effects of low *vs.* high residual concentrations of serum gonadotropins.

Results

Serum FSH and LH were markedly suppressed (≤5% of baseline) in men with sperm concentrations below 0.1 million/ml (Table 2). Higher serum gonadotropin values were observed during treatment in moderate-severe oligospermic (sperm concentration, 0.1–5 million/ml) and mildly oligospermic men (>5 million/ml), although these increases were not always significant. Serum gonadotropin levels were detectable in the majority of azoospermic men. For example, after 5 months of T plus progestin treatment (LNG and DSG studies), serum LH was detectable in 63% (38 of 61) and serum FSH was detectable in 85% (49 of 58) of men with azoospermia. After 4 months of T plus CPA treatment, serum LH was detectable in 24% (5 of 21) and serum FSH was detectable in 91% (19 of 21) of men with azoospermia.

A regression analysis of sperm concentration against either serum FSH or LH for the three contraceptive trails showed poor correlations over a wide range of responses (Fig. 1). A regression of serum FSH and LH across a wide range of normal-suppressed values showed an excellent correlation up until about 5% of control values (0.1 IU/liter; r = 0.84; $P < 0.001$; Fig. 2). However, at lower markedly suppressed levels, FSH did not continue to fall, whereas LH values declined toward the limit of assay detection.

These data suggest that additional factors, such as effects of T dose and progestin type and dose, are important in regulating either sperm concentrations or gonadotropin levels. To explore the effects of T dose and progestin type on suppression of sperm concentration, we performed multiple linear regression analyses.

LNG and DSG studies

In both studies LH was a major predictor of sperm concentration after both 5 and 6 months of treatment. In DSG and LNG groups, LH values below 5% of the baseline (<0.15 IU/liter) were associated with sperm concentrations 2–7% of those seen in men in whom serum LH levels remained greater than 0.15 IU/liter. FSH showed a significant association in the univariate analysis, but not in the multivariate analysis (Table 3).

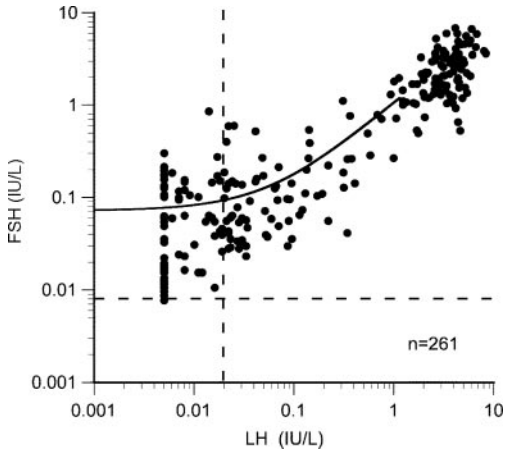

FIG. 2. Scatterplot comparing treatment serum FSH *vs.* LH levels in two contraceptive studies involving T plus a progestin (LNG or DSG). A curved line of best fit is presented. The correlation coefficient covering the range 0.1–10 IU/liter LH was 0.84.

The use of LNG was not a significant predictor of sperm concentration, although this did approach significance at 5 months of treatment, whereas the inclusion of DSG was associated with a significant inhibitory effect (5- to 6-fold) on sperm concentration independent of the serum gonadotropin level.

The outcome of a sperm concentration below 1 million/ml showed similar trends (Table 4). Serum LH at levels less than 5% of the control values showed significant odds ratios of 14- to 56-fold for suppression to severe oligospermia, but lower ratios (1- to 10-fold), some of which were significant, were seen with FSH. Odds ratios for LNG usage (*i.e.* 0.5–2) were not significant, but the corresponding ratios for DSG were (*i.e.* 8–14).

CPA studies

A dose of 100 mg TE weekly suppressed spermatogenesis significantly (8- to 12-fold) more than 200 mg TE weekly. The addition of CPA significantly reduced sperm concentration (4- to 18-fold) more at either 3 or 4 months of treatment. Neither serum LH nor FSH was a significant predictors of sperm concentration (Table 3).

Similar trends were observed when sperm concentrations were dichotomized as being above or below a nominated threshold (<1 million/ml), with significant changes attributed to TE dose and CPA usage, but not to serum LH or FSH levels (Table 4).

Discussion

These data show that, in general, men whose sperm concentrations are markedly reduced (<1 million/ml) have lower gonadotropin levels than men showing less spermatogenic suppression. Such an observation has been made previously in smaller groups of men (23, 24), but not invariably. We observed that for most men, there is a threshold for both serum LH (<2% of baseline) and FSH levels (<5% of baseline) within the detectable range below which azoo- or extreme oligospermia uniformly occur. However, there were a number of men in whom this gonadotropin hypothesis failed to explain the degree of spermatogenic suppression. Our analysis suggests that gonadotropin-independent mechanisms are important for inducing uniform suppression of spermatogenesis in male hormonal contraceptive regimens.

To identify potential predictors of the degree of spermatogenic suppression, logistic regression and odds ratio analyses were used to identify independent (multivariate analysis) or dependent (univariate analysis) factors. We have identified three independent predictors of the degree of sperm suppression and the likelihood of achieving a sperm concentration below 1 million/ml, the threshold below which contraceptive efficacy seems likely (2, 28, 29). These factors were 1) the dosage of T, 2) the suppression of serum LH below 5% of control levels, and 3) the use of progestin at any dose. On the other hand, the suppression of serum FSH below 5% of the control values (or below assay detection;

TABLE 3. Regression analysis: influence of candidate predictors (TE dose, progestin usage, FSH, LH) on sperm concentration in three T plus progestin regimens

		5 months of treatment			6 months of treatment		
		n	Univariate	Multivariate	n	Univariate	Multivariate
LNG							
LNG (μg)	0	35	3.71 (1.3–10.6)	2.33 (0.97–5.6)	49	1.49 (0.38–5.83)	0.63 (0.21–1.91)
	150–500	31	1	1	14	1	1
FSH (IU/liter)	<0.11	47	0.18 (0.06–0.55)	0.61 (0.21–1.74)	35	0.17 (0.06–0.49)	0.51 (0.18–1.4)
	>0.11	19	1	1	28	1	1
LH (IU/liter)	<0.15	55	0.03 (0.01–0.09)	*0.05 (0.01–0.17)*	53	0.02 (0.01–0.07)	*0.03 (0.01–0.12)*
	>0.15	11	1	1	10	1	1
DSG							
TE (mg)	50	7	1	1	5	1	1
	100	32	0.91 (0.10–8.1)	0.77 (0.13–4.6)	32	0.45 (0.03–6.33)	2.15 (0.29–16.0)
DSG (μg)	0	17	15.8 (3.8–65.3)	*9.1 (2.3–36.9)*	29	14.2 (1.9–106)	5.3 (0.99–28.2)
	150–300	22	1	1	8	1	1
FSH (IU/liter)	<0.11	23	0.17 (0.03–0.83)	0.43 (0.11–1.75)	13	0.08 (0.01–0.41)	0.58 (0.11–3.11)
	>0.11	16	1	1	24	1	1
LH (IU/liter)	<0.15	29	0.02 (0.005–0.09)	*0.07 (0.01–0.36)*	24	0.02 (0.01–0.09)	*0.04 (0.01–0.20)*
	>0.15	10	1	1	13	1	1
		3 months of treatment			4 months of treatment		
		n	Univariate	Multivariate	n	Univariate	Multivariate
CPA							
TE (mg)	100	34	1	1	34	1	1
	200	7	8.59 (2.04–36.1)	*11.9 (3.24–43.4)*	7	8.3 (2.6–26.4)	*9.66 (3.40–27.5)*
CPA (mg)	0	5	9.57 (1.78–51.6)	*17.8 (3.67–88.2)*	5	4.07 (0.93–17.9)	*9.28 (2.65–32.5)*
	5–100	36	1	1	36	1	1
FSH (IU/liter)	<0.11	34	1.53 (0.31–7.49)	1.59 (0.36–6.95)	29	1.36 (0.45–4.11)	0.87 (0.31–2.47)
	>0.11	7	1	1	12	1	1
LH (IU/liter)	<0.15	37	0.68 (0.09–5.11)	1.05 (0.15–7.42)	36	2.68 (0.59–12.2)	3.90 (0.89–17.1)
	>0.15	4	1	1	5	1	1

The rationale for this series of comparisons is outlined in *Materials and Methods*. For example, in the CPA studies at 3 months, the use of 200 mg T weekly was independently (multivariate analysis) associated with an 11.9-fold higher sperm concentration than the 100-mg dose. In the LNG studies, a serum LH level below 0.15 IU/liter was associated with sperm concentration 5% that of subjects with higher LH levels. In the CPA and DSG studies, the absence of progestin was independently associated with sperm concentrations 17.8- and 9.1-fold higher, respectively. Data are presented as mean and 95% confidence limits. Significant associations (CI not overlapping unity) values are shown in *italics*.

data not shown) was not independently predictive of sperm suppression. This is not to say that substantial suppression of FSH is not important, but when gonadotropin levels are within the low range, it is LH (and, by implication, intratesticular T) that is the main determinant of more complete spermatogenic suppression.

We acknowledge that the number of subjects in the three studies is limited and that their time courses were variable. As a result, larger confirmatory studies would be desirable. However, these studies are unusual because incremental doses of T and/or the various progestins were available, enabling us to undertake a useful logistic regression analysis.

In both the LNG and DSG studies, LH was a strong and independent predictor of sperm concentration, whereas FSH has a dependent role, presumably through its close correlation with LH at higher LH/FSH serum levels. When serum LH levels were below 0.15 IU/liter (5% of the control), sperm concentrations were only 2–7% of those seen when serum LH levels were above this threshold. Through our use of highly sensitive gonadotropin assays, we have determined that there is a dissociation between the degree of suppression of serum FSH and LH levels below 0.1 IU/liter. Continued secretion of low levels of FSH probably occurs via GnRH-independent mechanisms, as the absence of serum LH (at least to <0.4% baseline) suggests the abolition of GnRH stimulation. There is evidence that there is GnRH-independent expression of FSH β-subunit. For example, in rats, T exhibits such a stimulatory effect on pituitary FSH release (30). It is possible that a similar, if less marked, process is seen in men.

Previous studies in man have raised the possibility that exogenously administered androgens might act directly on the seminiferous epithelium to support spermatogenesis in a gonadotropin-depleted environment and that this effect ought be considered in the design of effective contraceptives (31–33). In this study we observed that 100 mg TE weekly suppressed spermatogenesis significantly (8- to 12-fold) more than 200 mg TE weekly. In recently reporting a subset of these CPA subjects (23), we also speculated that the use of this higher dose of T might induce or maintain low grade spermatogenesis in normal men. Our multivariate analysis corroborates this hypothesis. This effect might be due to a direct stimulatory action of T on the seminiferous epithelium as seen in rodents (34). Such an effect is difficult to directly demonstrate in man, although high dose T does support spermatogenesis in other primate models, such as the hypophysectomized monkey (35).

The pharmacokinetic profile of TE is clearly suboptimal in providing wide excursions in serum T and often excessive peak levels. As a result, this formulation is not favored for

TABLE 4. Odds ratios for achieving a sperm concentration below 1 million sperm/ml for three T plus progestin regimens

		5 months				6 months			
		N	Y	Univariate	Multivariate	N	Y	Univariate	Multivariate
LNG									
LNG (μg)	0	14	21	1	1	14	35	1	1
	≥150	8	23	1.92 (0.67–5.56)	1.39 (0.40–4.76)	3	11	1.47 (0.35–6.25)	0.51 (0.10–2.56)
FSH (IU/liter)	<0.11	12	35	3.24 (1.06–9.9)	1.18 (0.27–5.15)	5	30	4.5 (1.4–15)	1.3 (0.27–6.65)
	>0.11	10	9	1	1	12	16	1	1
LH (IU/liter)	<0.15	12	43	35.8 (4.2–309)	*31.0 (3.2–301)*	8	45	50.5 (5.6–456)	*51.4 (4.5–590)*
	>0.15	10	1	1	1	9	1	1	1
DSG									
TE (mg)	50	3	4	1	1	2	3	1	1
	100	12	20	1.25 (0.24–6.57)	1.64 (0.07–40.1)	10	22	1.47 (0.21–10.2)	0.21 (0.01–4.1)
DSG (μg)	0	11	6	1	1	12	17	[a]	[a]
	≥150	4	18	8.3 (1.58–33.3)	*14.3 (1.11–200)*	0	8		
FSH (IU/liter)	<0.11	6	17	3.6 (0.94–14.2)	3.3 (0.25–45.4)	1	12	10.2 (1.1–90)	1.1 (0.06–21.1)
	>0.11	9	7	1	1	11	13	1	1
LH (IU/liter)	<0.15	6	23	34.5 (3.6–328)	14.3 (0.92–221)	2	22	36.7 (5.3–255)	*55.7 (3.2–976)*
	>0.15	9	1	1	1	10	3	1	1

		3 months				4 months			
		N	Y	Univariate	Multivariate	N	Y	Univariate	Multivariate
CPA									
TE (mg)	100	4	30	1	1	2	32	1	
	200	4	3	0.10 (0.02–0.62)	*0.03 (0.002–0.37)*	4	3	0.05 (0.01–0.37)	[b]
CPA (mg)	0	3	2	1	1	2	3	1	
	5–100	5	31	9.09 (1.2–100)	*100 (2.6–1000)*	4	32	5.3 (0.68–50)	[b]
FSH (IU/liter)	<0.11	7	27	0.64 (0.07–6.25)	0.43 (0.01–13.0)	5	24	0.44 (0.05–4.19)	
	>0.11	1	6	1.00	1.00	1	11	1.00	[b]
LH (IU/liter)	<0.15	7	30	1.43 (0.13–15.9)	0.53 (0.01–20.8)	6	30		
	>0.15	1	3	1.00	1.00	0	5	[a]	[b]

The rationale for this series of comparisons is outlined in *Materials and Methods*. For example, in the CPA studies at 3 months, the use of 200 mg T weekly was independently (multivariant analysis) associated with only 3% the probability of achieving this threshold than the 100-mg dose. In the LNG studies, a serum LH level below 0.15 IU/liter was associated with 31-fold higher prospect of achieving the threshold. In the CPA and DSG studies, the use progestin was independently associated with 100 and 14-fold higher prospects of achieving the threshold, respectively. The number of men reaching (Y) or not reaching (N) the threshold values are shown for each group and parameter. Data are mean and 95% confidence limits. Significant association (CI not overlapping unity) values are shown in *italics*. For details of abbreviations see Table 1.

[a] Could not be calculated as sperm concentration for all subjects in these groups were below 1 million/ml or below 0.15 IU/liter.

[b] Where multivariate model could not converge.

ongoing contraceptive studies, but nonetheless, our TE data provide valuable insight in direct T effects on spermatogenesis in man. The recent use of more physiological T regimens in male contraception (such as using longer acting im T formulations or implants) has been largely motivated by the need to reduce side-effects, but our analysis suggests that lower dose T regimens might be essential for uniform spermatogenic suppression.

The interaction between the administered T acting directly on the testis and serum gonadotropin levels has long been considered. For example, the addition of GnRH agonists to T was not found useful, perhaps due to the initial flare of gonadotropin secretion and the return of FSH levels over the longer term (36). On the other hand, GnRH antagonists appear promising in maintaining gonadotropin suppression, but did not augment spermatogenic suppression when combined with 200 mg TE weekly, leading to speculation that this high dose of T might have acted locally (33). Such work points to the avoidance of excessive T exposure and the maximal and sustained suppression of gonadotropins.

In addition to suppression of gonadotropins, progestins may suppress sperm concentration by nongonadotropic mechanisms. Differences were observed among the three progestins as independent predictors of sperm concentration. CPA and DSG showed marked gonadotropin-independent inhibitory effects, whereas LNG showed a smaller and largely insignificant effect. The relatively low power of the study might account for these differences. There are several explanations based on published observations that might account for these progestin effects. Progestins inhibit 5α-reductase activity (37) and thereby reduce the intratesticular level of dihydrotestosterone that might maintain spermatogenesis when LH levels are suppressed (10). We recently published androgen levels in the human testis during T plus progestin contraception and found that, despite a 98% decline in T levels, 5α-reduced androgen levels were little changed (38). The effect of 5α-reductase inhibition on spermatogenesis in the contraceptive setting (low LH and markedly lowered intratesticular T) has not been well addressed in previous small pilot studies using finasteride, which has little activity against the type 1 isoenzyme prevalent in the testis (28, 39). Up-regulation of the type 1 5α-reductase enzyme is seen in the rat when LH levels are very low (40), and this phenomenon might also occur in men. Inhibition of this enzyme pathway might therefore be relevant to sperm suppression. Second, progestins inhibit 17α-hydroxysteroid de-

hydrogenase (41, 42), a key step in androgen biosynthesis, and thereby reduce androgen action on the epithelium. This hypothesis has not been tested in men undergoing hormonal contraceptive treatments. Finally, it is possible that progestin have direct testicular effects, because progesterone receptors have been reported in the Leydig cells (43).

Progestins and their metabolites have different possible mechanisms of action on the gonadal axis. For example, DSG exhibits higher 5α-reductase inhibitory activity than LNG (37). Our findings with CPA are interesting because there is a dramatic gonadotropin-independent inhibitory effect of CPA on sperm concentration. As previously proposed, CPA might act as an antiandrogen in the testis (13). Based on our analysis, the term progestin, which is classically defined based on endometrial effects, ought not be used to imply a uniform class effect on spermatogenesis. Of course other processes may contribute to the nonuniformity of suppression by hormonal contraception, such as heterogeneity of the CAG length of the androgen receptor as has been suggested (44, 45).

In conclusion, we suggest that T-progestin contraceptive regimens suppress sperm concentration by gonadotropin-dependent and -independent mechanisms. The suppression of serum LH, but not FSH, is a major predictor of sperm concentration suppression in the LNG and DSG treatment studies. In addition, 100 mg T weekly results in greater suppression of spermatogenesis than 200 mg T weekly. Our findings emphasize that the complete suppression of LH and intratesticular T levels is crucial for a uniformly effective male hormonal contraceptive. Finally, our data suggest that some progestins (*e.g.* DSG and CPA) suppress spermatogenesis by a gonadotropin-independent mechanism(s). We suggest that alternative strategies could be devised to increase the efficacy of male androgen-based contraceptives by exploiting the apparent differences in progestins in gonadotropin-dependent and -independent inhibition of spermatogenesis.

Acknowledgments

Received April 8, 2003. Accepted October 15, 2003.

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This work was supported by CONRAD USA (Grant CIG-01-68) and the Australian National Health and Medical Research Council (Program Grant 983212).

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