

Association of body mass index, age, and cigarette smoking with serum testosterone levels in cycling women undergoing in vitro fertilization

Robert L. Barbieri, M.D.,^a Pat M. Sluss, M.D.,^b Robert D. Powers, Ph.D.,^c
Patricia M. McShane, M.D.,^d Allison Vitonis, B.A.,^a Elizabeth Ginsburg, M.D.,^a and
Daniel C. Cramer, M.D., Sc.D.^a

^aOb-Gyn Epidemiology Center, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, Boston; ^bReproductive Endocrine Unit, Massachusetts General Hospital, Boston; ^cBoston IVF, Waltham; and ^dReproductive Science Center, Lexington, Massachusetts

Objective: [1] To examine the effects of body mass index (BMI), age, cigarette smoking, cause of infertility, and use of oral contraceptives on baseline serum testosterone (T), and [2] to examine associations between baseline serum T and IVF outcomes such as pre-hCG serum E₂, number of oocytes retrieved, oocyte fertilization rate, and pregnancy outcome in regularly cycling women.

Design: Prospective, cohort study.

Setting: Three IVF programs in eastern Massachusetts.

Patient(s): Four hundred twenty-five regularly cycling women planning to undergo IVF. Women with polycystic ovary syndrome, ovulatory infertility, or irregular cycles were excluded from this study.

Intervention(s): Collection of epidemiological data and baseline serum in women undergoing IVF.

Main Outcome Measure(s): Baseline serum total T, sex hormone binding globulin (SHBG), and calculation of free androgen index.

Result(s): Body mass index >26 kg/m² was associated with a significant increase in serum T ($P<.01$) and free androgen index ($P<.0001$). Serum T decreased significantly throughout the fourth decade of life ($P<.03$). A history of cigarette smoking >10 pack years was associated with increased serum T ($P<.01$). A diagnosis of endometriosis was associated with decreased serum T. Serum T correlated positively with pre-hCG serum E₂ and number of oocytes retrieved. However, serum T did not significantly influence fertilization or pregnancy rates.

Conclusion(s): In cycling infertile women, increasing BMI and cigarette smoking are associated with increased serum T. Advancing age and endometriosis are associated with decreased serum T. (Fertil Steril® 2005;83:302–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: Testosterone, SHBG, IVF, BMI, age, cigarette smoking, endometriosis, pregnancy

In premenopausal cycling women, circulating T is derived from direct secretion by the ovary and adrenal and from conversion of precursors, such as androstenedione to T. Testosterone circulates in 3 forms: free, bound to albumin, and bound to sex hormone binding globulin (SHBG). The free and albumin-bound fractions are believed to be bioavailable. The T bound to SHBG is thought to be unavailable for action in the periphery. Consequently, the free androgen index (FAI) (also called the free testosterone index by some authors because it is the ratio of T to SHBG) may provide more information about bioavailable T than that provided by total T or SHBG values alone.

In cycling premenopausal women with infertility, the determinants of circulating T have not been fully characterized. Testosterone is seldom routinely measured as part of a clin-

ical IVF work-up except in women with hyperandrogenism and/or oligomenorrhea, such as women with polycystic ovary syndrome. In this study, serum T and SHBG were measured in a sample of regularly cycling premenopausal women with infertility undergoing IVF in order to determine whether largely physiologic levels influenced IVF outcome. In addition, the epidemiologic correlates of basal circulating T, SHBG, and FAI were studied.

MATERIALS AND METHODS

Subject Population

Couples having IVF were enrolled into this study at 3 IVF programs in Eastern Massachusetts: Boston IVF, the Brigham and Women's Hospital IVF Program, and the Reproductive Science Center of Boston under a protocol approved by the Human Subjects Research Committee of the Brigham and Women's Hospital. Subjects were enrolled in two phases: Study 1, 1994 to 1998; and Study 2, 1999 to 2002. The samples were collected in two phases due to a transition in grant funding. Epidemiologic information and life exposures were derived from questionnaires that the

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Reprint requests: Robert L. Barbieri, M.D., Ob-Gyn Epidemiology Center, Department of Obstetrics, Gynecology and Reproductive Biology, Brigham and Women's Hospital, 221 Longwood Avenue, Boston, Massachusetts 02115 (FAX: 617-277-1440; E-mail: rbarbieri@partners.org).

subjects completed. Research staff abstracted the clinical records.

Couples seeking their first IVF treatment at one of the participating clinics who did not require donor eggs or donor semen were eligible. Of approximately 4,150 eligible couples approached, 3,171 (76%) initially agreed to participate, of whom 2,691 completed their baseline questionnaire and consent for a final participation rate of 65%. In the study reported in this paper, the inclusion criteria were [1] a serum specimen was obtained prior to initiating an IVF cycle and [2] the outcome of the IVF cycle was known, resulting in a sample of 509 women.

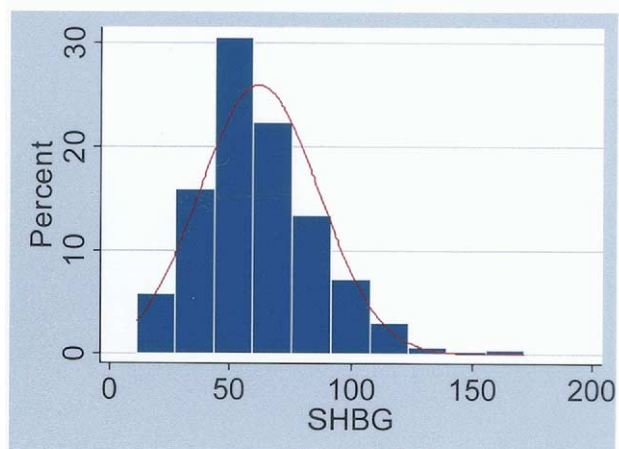
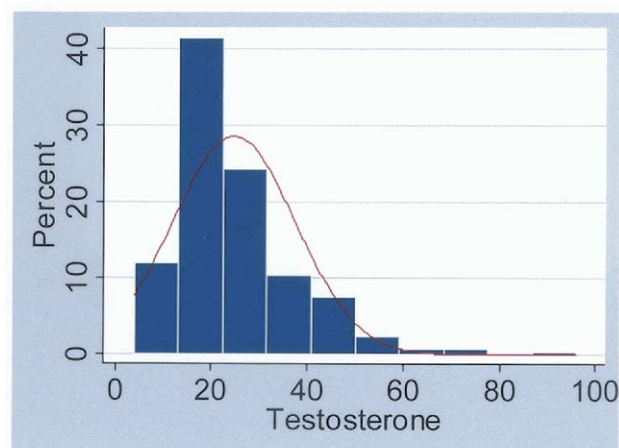
Subjects reported characteristics of their current menstrual cycles on a self-administered questionnaire. Regular menstrual cycles were defined as less than 10 days variation between shortest and longest cycles. Exclusion criteria included [1] a diagnosis of polycystic ovary syndrome (PCOS) ($n = 28$), [2] history of irregular menstrual cycles as self-reported by the patient ($n = 18$), and [3] primary diagnosis of ovulatory infertility ($n = 38$). This yielded 425 women for this study. Regarding cycle outcomes, we focused on first cycle results because this would yield the outcomes closest in time to when the pretreatment blood had been drawn. The serum specimens from the women prior to initiating an IVF cycle were obtained either during days 1 to 5 of the menstrual cycle (menses specimen) or at another time in the cycle (nonmenses specimen). All specimens were stored at -80°C until assayed.

Measurement of the serum T and SHBG was performed in the laboratory of Dr. Sluss, who was blinded to the outcome of the IVF cycle. Serum T was measured directly using a solid-phase immunoassay (Coat-a-Count; DPC, Los Angeles, CA). The analytical sensitivity was 4.0 ng/dL (0.14 nmol/L). The intra-assay precision was $<10\%$ and the inter-assay precision $<12\%$. Sex hormone binding globulin was measured using a fully automated system (Immulite; DPC). The method is a solid-phase two-site chemiluminescent enzyme immunometric assay. The analytical sensitivity was 1 nmol/L. The intra-assay precision was $<7\%$ and the inter-assay precision $<8\%$. Free androgen index was calculated by multiplying serum T in nmol/L by 100 and dividing by the SHBG concentration in nmol/L (1).

Mean and standard deviations for T, SHBG, and FAI were examined in subjects categorized by various baseline characteristics as well as categorical outcomes. Differences among women by concentration of T, SHBG, and FAI for particular characteristics were assessed by analysis of variance followed by the Bonferroni test to assess pairwise differences. Pearson correlations were calculated when the hormone values were compared with continuous outcomes such as E_2 level during treatment, the number of oocytes retrieved, and the fertilization rate. The fertilization rate was defined as the number of oocytes with two pronuclei after insemination divided by the number of oocytes inseminated and was restricted to the 369 women who had a least one

FIGURE 1

Frequency distribution of baseline serum total T (ng/dL) and SHBG (nmol/L) in regularly cycling infertile women preparing to undergo IVF.



Barbieri. Impact of BMI and age on T. *Fertil Steril* 2005.

oocyte inseminated. To adjust for potential confounding factors, generalized linear or unconditional logistic regression modeling was used depending on whether the outcome variable was continuous or dichotomous. To account for skew present in many reproductive analytes, log transformation of the hormonal variables was used for significance testing, though arithmetic means and standard deviations are presented.

RESULTS

Figure 1 presents the distribution of serum total T and SHBG for the subjects.

The overall mean serum T was 24.7 ng/dL. Serum T on the arithmetic scale was positively skewed and normalized on the log scale. About 96% of the women had values less

TABLE 1**Mean hormone levels by characteristics of subjects or specimens.**

	Number	Testosterone (ng/dL), mean (SD)	SHBG (nmol/L), mean (SD)	FAI, mean (SD)
Age (y)				
<30	29	31.2 (16.1)	61.9 (27.8)	2.0 (1.4)
30–34	139	25.1 (13.6)	63.1 (25.5)	1.6 (1.2)
35–39	174	23.9 (12.2)	60.5 (24.1)	1.6 (1.2)
>39	83	23.6 (11.0)	62.7 (23.7)	1.5 (1.1)
<i>P</i> value		.03	.74	.16
BMI				
<20.4	87	21.9 (10.2)	68.2 (24.7)	1.3 (0.8)
20.4–21.6	89	24.4 (14.4)	69.1 (25.1)	1.3 (0.8)
21.7–23.4	82	24.1 (12.5)	63.5 (22.2)	1.5 (1.0)
23.5–26.2	88	24.8 (13.4)	59.7 (22.2)	1.6 (1.1)
≥26.3	76	28.5 (12.8)	47.9 (23.5)	2.6 (1.7)
<i>P</i> value		.01	<.0001	<.0001
Primary diagnosis				
Male	153	26.0 (14.0)	59.1 (23.6)	1.8 (1.4)
Tubal	115	26.2 (14.0)	58.4 (24.9)	1.8 (1.2)
Endometriosis	65	21.8 (10.3)	65.5 (23.3)	1.3 (0.8)
Unexplained/other	88	23.0 (10.3)	68.1 (25.7)	1.3 (0.6)
<i>P</i> value		.06	.005	.0003
Study				
1	258	23.9 (12.7)	60.1 (23.0)	1.6 (1.1)
2	167	25.9 (13.0)	64.5 (26.8)	1.7 (1.3)
<i>P</i> value		.06	.22	.52
Specimen				
Menstrually-timed	184	23.1 (11.1)	61.7 (27.0)	1.6 (1.2)
Other	241	26.0 (13.9)	62.0 (22.8)	1.7 (1.1)
<i>P</i> value		.04	.40	.30
OC use				
None	99	25.9 (13.7)	58.9 (23.1)	1.8 (1.4)
1–35 months	99	23.7 (10.8)	63.5 (26.1)	1.5 (1.1)
36–71 months	92	23.3 (12.0)	59.4 (22.2)	1.6 (1.2)
≥72 months	121	26.1 (14.4)	64.4 (25.2)	1.6 (1.1)
<i>P</i> value		.28	.27	.35
Years smoked				
0	257	24.0 (12.9)	64.1 (26.1)	1.6 (1.2)
1–10	83	22.8 (10.7)	57.4 (21.0)	1.6 (0.9)
>10	79	28.8 (14.0)	59.0 (22.7)	2.0 (1.4)
<i>P</i> value		.01	.17	.01
Glass of alcohol per week				
0	227	25.6 (13.9)	61.9 (26.0)	1.7 (1.3)
1–3	93	22.8 (10.6)	61.8 (23.3)	1.5 (1.1)
>3	105	24.4 (12.2)	62.0 (23.1)	1.5 (0.9)
<i>P</i> value		.34	.85	.35

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than 50 ng/dL, and 64% had values less than 25 ng/dL. Table 1 presents the mean levels of T, SHBG, and FAI by characteristics of subjects or specimens. Advancing age was associated with a significant decrease in T ($P=.03$), but not SHBG or FAI. The serum T for women <30 years and >39

years were 31.2 (SD 16.1) and 23.6 (SD 11.0), respectively ($P<.04$). Subjects were divided into quintiles based on body mass index (BMI), and increasing BMI was associated with increasing serum T ($P<.01$) and FAI ($P<.0001$), and decreasing SHBG ($P<.0001$). The serum T for women with a

TABLE 2**Correlation coefficients among hormones continuous IVF outcomes in first cycle.**

	SHBG	FAI	Pre-hCG E ₂	Oocytes retrieved	Fertilization rate ^{a,b}
Testosterone					
N	424	424	412	403	376
Correlation	0.014	0.762	0.104	0.138	0.064
P value	.78	<.0001	.03	.006	.22
SHBG					
N		424	413	404	377
Correlation		-0.637	0.111	-0.028	0.006
P value		<.0001	.02	.57	.91
FAI					
N			412	403	376
Correlation			0.009	0.124	0.044
P value			.86	.01	.39
E ₂					
N				395	373
Correlation				0.64	-0.032
P value				<.0001	.53
Oocytes retrieved					
N					377
Correlation					-0.048
P value					.35

^aBased on log transformed hormone values.

^bDefined as number of oocytes with 2 pronuclei after insemination divided by the number of oocytes inseminated.

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BMI of 20.4 to 21.6 versus a BMI >26.3 were 24.4 (SD 14.4) and 28.5 (SD 12.8), respectively. Testosterone levels measured in menstrually timed specimens tended to be lower than that measured in the nonmenstrually timed specimens ($P=.04$).

Primary infertility diagnosis had a borderline significant association with serum T and a significant association with SHBG and FAI (Table 1). These differences largely related to lower T and FAI among women with a primary diagnosis of endometriosis and unexplained infertility. Logistic regression analysis adjusting for age, BMI, and smoking and applied to women with a primary diagnosis of endometriosis or unexplained infertility confirmed that women in the former (endometriosis) group but not the latter had a significantly lower ($P<.02$) FAI than women with a diagnosis of male factor or tubal infertility (data not shown). A history of cigarette smoking, >10 pack years, was also significantly associated with increased T and FAI. Past oral contraceptive use and current alcohol consumption did not significantly influence the measured analytes.

Table 2 shows the correlation coefficients between T, SHBG, and FAI and several quantitative variables during the IVF cycle including E₂ (pre-hCG), number of oocytes retrieved, and the fertilization rate. Both serum T and SHBG

were positively correlated with pre-hCG E₂. Serum T and FAI, but not SHBG, were positively correlated with the number of oocytes retrieved.

A generalized linear model of the predictors of the number of oocytes retrieved demonstrated that T but not BMI was associated with a significantly increased number of oocytes retrieved (Table 3). In that model, advancing age and years

TABLE 3**Generalized linear model for number of oocytes retrieved adjusted for log transformed testosterone, age, BMI, years smoked, and specimen type (menstrually timed or other).**

Variable	Estimate	Probability
Testosterone	2.166	.02
Age	-0.478	<.0001
BMI	0.047	.64
Years smoked	-0.151	.02
Specimen type	-0.085	.92

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TABLE 4**Mean hormone levels by first cycle pregnancy outcomes.**

	Number	Testosterone (ng/dL), mean (SD)	SHBG (nmol/L), mean (SD)	FAI, mean (SD)
Clinical pregnancy				
Yes	126	25.6 (13.3)	62.5 (26.5)	1.8 (1.4)
No	299	24.3 (12.6)	61.6 (23.9)	1.6 (1.1)
<i>P</i> value		.22	.90	.38
Detailed outcome				
Cancelled cycles	41	24.0 (10.3)	55.2 (18.8)	1.6 (0.8)
Failed fertilization	18	21.5 (13.7)	62.7 (31.1)	1.4 (0.8)
Failed implantation	239	24.6 (13.0)	62.6 (24.0)	1.6 (1.1)
SAB	15	28.1 (21.6)	79.9 (33.4)	1.3 (1.0)
Liveborn	108	25.4 (12.0)	60.4 (24.8)	1.8 (1.4)
<i>P</i> value		.46	.07	.29
Fertilization rate				
<50%	259	24.9 (13.2)	63.4 (25.2)	1.6 (1.2)
≥50%	118	24.7 (13.0)	61.0 (25.1)	1.7 (1.3)
<i>P</i> value		.84	.32	.62

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of cigarette smoking were associated with a decreased number of oocytes retrieved. Neither T nor SHBG were significantly correlated with fertilization rate. Finally, Table 4 shows that there were no significant differences in serum T or SHBG when categorized by various pregnancy outcomes.

DISCUSSION

Most studies of serum T levels in infertile women have focused on women with irregular cycles and stigmata consistent with PCOS. However, the epidemiologic correlates of serum T, SHBG, and FAI have not been extensively studied in regularly cycling infertile premenopausal women. In this study women with irregular menstrual cycles or a known diagnosis of PCOS were excluded, and the circulating T of the subjects was well within the physiologic range. The following variables were found to significantly influence T, SHBG, or FAI: BMI, age, smoking, and primary infertility diagnosis. In addition, T, SHBG, or FAI correlated with quantitative (but not qualitative) measures of IVF outcome, including E₂ and oocytes retrieved.

We found that T and FAI were positively and SHBG negatively correlated with BMI. It should be noted that FAI is calculated from a ratio of T and SHBG. Consequently, FAI increases as serum T increases or serum SHBG decreases. Studies have consistently demonstrated that increasing BMI is associated with increased serum T and decreased SHBG in both cycling and noncycling premenopausal women, perimenopausal women, and postmenopausal women. For example, in a large cohort (n = 1,526) of premenopausal women (mean age 31 years), increasing BMI

was associated with increased serum T and decreased SHBG in both normal ovulatory women and oligo-ovulatory women (2). In the Study of Women's Health Across the Nation (SWAN), with a large cohort (n = 2,930) of perimenopausal women (mean age 46 years), increasing BMI was associated with increased serum total T and decreased SHBG (3). In a meta-analysis of hormone findings from case-control studies of postmenopausal women with and without breast cancer, serum T and SHBG levels were reported for over 624 women with breast cancer and 1,669 controls. In this meta-analysis, increasing BMI was associated with increased serum T and decreased SHBG in both the cases and the controls (4).

The biological factors that account for correlations between BMI, T, and SHBG are not fully understood. The observation that increased BMI is associated with decreased SHBG is likely caused, in part, by an effect of relative hyperinsulinemia on suppression of hepatic SHBG production (5). The mechanisms that subservise the relationship between BMI and serum T are less clear. In regularly cycling women, the majority of circulating T arises from peripheral conversion of androstenedione (50%), with direct contributions from the ovary (25%) and adrenal (25%). It is unclear whether this association is due to decreased metabolism of T, increased peripheral conversion from androstenedione, greater androstenedione secretion, or increased T secretion from the ovary and/or adrenal. Obesity is associated with hyperinsulinemia, and hyperinsulinemia can stimulate ovarian androgen production (6). Consequently, obesity may cause both a decrease in serum SHBG and an increase in

serum T through effects on central metabolism, including the induction of insulin resistance and hyperinsulinemia. In our data, the effect appears to be most pronounced with a BMI above 26.3, suggesting that a primary prevention strategy of maintaining BMI below 26 may reduce the risk of women becoming hyperandrogenic.

In our study, serum T levels appeared to decrease with age even over the relatively narrow age range studied. Very few studies have examined the effects of age on serum T and SHBG in premenopausal women. In the Michigan Bone Health Study, increasing age from <34 years to >41 years was associated with a significant decline in serum T (7). One possible explanation for the decrease in serum T with aging is that LH stimulation of ovarian androgen secretion begins to decline during the decade of the 30s (8). Interestingly, this effect occurs before a decline in ovarian estrogen secretion, possibly owing to the fact that a compensatory increase in FSH with ovarian aging at 35 to 45 years of age maintains ovarian estrogen secretion but does not maintain ovarian androgen secretion (9). Of note, Burger and colleagues in a longitudinal study of 172 women from 45 to 55 years of age, followed for 7 years through a natural menopause, did not observe a decrease in serum T, but did observe a decrease in serum SHBG. This resulted in an increase in FAI during this interval. The investigators speculated that decreases in E₂ during the transition to menopause may account for the decrease in serum SHBG and increase in FAI (10).

Consistent with our findings, other studies have reported that serum T is increased in premenopausal smokers compared to nonsmokers. For example, in both the SWAN study (3) and the Michigan Bone Health Study (7), serum T was reported to be increased in women who were current smokers compared to never smokers. In women undergoing IVF, cigarette smokers appear to have increased concentrations of T (11). Smoking-associated increases in circulating androgens appears to be present in postmenopausal women as well. In one study of postmenopausal women, cigarette smoking was associated with a significant increase in androstenedione and T (12). In another study of postmenopausal women, cigarette smoking was associated with a significant increase in serum DHEAS and androstenedione, but not T (13). Three possible mechanisms for this effect are: [1] nicotine increases pituitary release of ACTH, thereby causing increased adrenal androgen secretion (14); [2] components of cigarette smoke, including cotinine, block the adrenal 21-hydroxylase enzyme, thereby increasing adrenal androgen production (15); and [3] smoking slows metabolism of androgens in the liver (16).

Our study did not detect an association between moderate consumption of alcohol and serum T nor was one found in the Michigan Bone Health Study (7). Conversely, the SWAN study (3) found that alcohol consumption at levels above the median were associated with an increase in T when compared to nondrinkers. Additional studies would appear necessary.

The effect of serum T, within the normal range, on IVF outcome in regularly cycling women has not been extensively studied. In a study of regularly cycling women (17), baseline serum T was significantly lower (37 ng/dL) in women who became pregnant than in women who did not become pregnant (52 ng/dL). A smaller study found similar results (18). In our cohort of regularly cycling women there was a significant positive relationship between serum T and both pre-hCG E₂ and number of oocytes retrieved; but no significant relationship between serum T and the fertilization rate was found, nor any difference in the serum T level between women who became pregnant and those who did not conceive based on first cycle results. Again, additional studies will be necessary to resolve these differences.

We found that a primary infertility diagnosis of endometriosis (and to a lesser extent unexplained infertility) was associated with a decreased serum FAI. A complex interaction among genetic, immunologic, reproductive, and hormonal factors is thought to cause endometriosis. Few other studies have reported on serum T, SHBG, and FAI in women with and without endometriosis. However, given that the growth of endometriosis appears to be stimulated by estrogen (19) and inhibited by free androgens (20), it is biologically plausible that some women may be at increased risk for developing endometriosis because of a low serum free androgen concentration or a low ratio of free T to E₂. Alternatively, the presence of endometriosis in infertile patients may signal ovarian dysfunction that is associated with a decreased secretion of T. The observation that the diagnosis of endometriosis was associated with low FAI is provocative and requires further study. Prediagnostic specimens from a prospective cohort of women that includes some who later developed endometriosis would be ideal.

In conclusion, while our study suggests no value to the routine measurement of T or SHBG in IVF patients without PCOS, it points to a number of interesting physiologic determinants of androgen measures in women and the broader role of androgen in reproductive physiology and normal aging.

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