Testosterone to Estradiol Ratios in Fertile and Subfertile Men: A Large Cohort Analysis

Evan J. Panken, Solomon Hayon, Daniel R. Greenberg, Sai Kaushik SR Kumar, Robert E. Brannigan, and Joshua A. Halpern

OBJECTIVE To validate the established normal testosterone to estradiol ratio and characterize the distribution of testosterone to estradiol ratios in a large cohort of fertile and subfertile men.

MATERIALS AND METHODS Retrospective review of adult men (≥18 years of age) presenting for fertility evaluation between 2002 and 2021 who underwent evaluation by a reproductive urologist, had 2 separate semen analyses and had hormonal testing within 6 months of their index semen analysis. Men were dichotomized into fertile and subfertile groups based on total motile sperm count on 2 semen analyses. The subfertile cohort included men with a total motile sperm count < 20 million on both semen analyses. The main outcome measures were serum testosterone, serum estradiol, and serum testosterone to estradiol ratio.

RESULTS Among 816 men, 651 (79.8%) were classified as fertile and 165 (20.2%) as subfertile. Median testosterone (ng/dL) to estradiol (pg/mL) ratios were similar between the groups (14.48 vs 15.00, P = .5). The 20th percentile testosterone to estradiol ratio for the fertile group was 9.77.

CONCLUSION This is the largest study to date characterizing testosterone to estradiol ratios in men presenting for fertility evaluation. We validated the 10/1 ratio that was previously established as the 20th percentile for fertile men. We found no difference in testosterone to estradiol ratios between fertile and subfertile men defined by total motile sperm count, highlighting the need for further investigation to better define the cohort of men with infertility who could benefit from aromatase inhibitor therapy. UROLOGY xx: xxx–xxx, xxxx.

Infertility affects 1 out of 6 couples, and although the role of male factor infertility is difficult to quantify, prior studies have shown that male factor infertility is implicated in up to 40% and solely responsible for 10%-20% of infertile couples.[1,2] The evaluation and management of male factor infertility has progressed significantly in recent years, but the optimal evaluation and management strategy remains controversial due to many confounders and the lack of clinical trials.[3]

The American Urologic Association (AUA) and American Society of Reproductive Medicine (ASRM) guidelines recommend an endocrine evaluation in the assessment of infertility in men when clinical evidence suggests an underlying endocrinopathy. However, some experts advocate for an endocrine evaluation in all male infertility patients.[4] When an endocrinopathy is identified, it can be targeted with medications including aromatase inhibitors (AIs), human chorionic gonadotropin (hCG), and selective estrogen receptor modulators (SERMs).[5]

The delicate equilibrium of testosterone (T) and estradiol (E) levels both systemically and in the testicular micro-environment are thought to have a significant effect on spermatogenesis, although the exact mechanisms remain unknown.[6-8] A landmark study by Pavlovich et al in 2001 supported the conclusion that some men with infertility have a treatable endocrinopathy represented by a low T/E ratio.[9] This study defined a normal T/E ratio as 10/1 based on the 20th percentile distribution of a reference group of 40 fertile men.[9] The authors also studied a cohort of men with severe male factor infertility and characterized semen parameter improvement with AI treatment in men with a T/E ratio less than 10/1.[9] This established normal ratio of 10/1 that has been carried forward in subsequent literature and trials. While the first of its kind, the study was limited by the small number of participants and the broad inclusion

From the Department of Urology, Northwestern Memorial Hospital, Chicago, IL; the Department of Urology, Medical University of South Carolina, Charleston, SC; and the Posterity Health, Centennial, CO

Address correspondence to: Evan J. Panken, M.D., Northwestern Memorial Hospital, Department of Urology, 675 N St Clair St 20th Floor, Suite 150, Chicago, IL 60611. E-mail: evan.panken@northwestern.edu

Submitted: August 28, 2024, accepted (with revisions): November 5, 2024

https://doi.org/10.1016/j.urology.2024.11.004
0090-4295

criteria for the infertile group including soft small testes, increased follicle-stimulating hormone (FSH), or abnormal semen analysis (SA).

To our knowledge, there have not been further studies that have reassessed the established normal T/E ratio. There have been calls to avoid utilizing the 10/1 ratio as a cutoff in the absence of further research efforts validating the definition of a low T/E ratio.[10] Given that the AUA and ASRM guidelines discuss the option of utilizing AI treatment in infertile men, we believe it is critical to reassess the previously described T/E threshold with a larger cohort.[5] We sought to characterize the T/E ratio distribution in a larger, contemporary cohort and to compare T/E ratios among fertile and subfertile men presenting for fertility evaluation.

MATERIALS AND METHODS

We retrospectively reviewed our institutional electronic database to identify adult men (≥18 years of age) presenting for fertility evaluation between 2002 and 2021. We included all patients who underwent evaluation by a reproductive urologist, had 2 separate semen analyses, and had hormonal testing within 6 months of their index SA. We queried our database to obtain demographic information, clinical variables, hormonal testing, and semen parameters. Baseline patient demographic information included age and self-identified race. Clinical variables included body mass index (BMI) and presence or absence of varicocele on physical exam. Hormonal testing included serum testosterone, FSH, luteinizing hormone (LH), estradiol, and T/E ratio. Semen parameters included semen volume, sperm concentration, sperm motility, and total motile sperm count. We dichotomized men into fertile and subfertile groups based on total motile sperm count (TMSC). Men were classified into the subfertile group if they had a TMSC < 20 million on both SA.[11] Men who had one SA with a TMSC < 20 million and the other with a TMSC > 20 million were included in the fertile group. Men with azoospermia, semen volume < 1.0 cc, men with only 1 SA, men without serum hormonal testing within 6 months from index SA, and men on hormonal medications (including exogenous testosterone, AIs, hCG, or SERMs) were excluded. We did not exclude men with a history of cryptorchidism or genetic abnormalities. The primary outcome was serum T/E ratio.

The statistical analysis was performed using R (Version 4.2.0). Categorical variables are presented as n (%) and were analyzed using Pearson's chi-squared test. Continuous variables are presented as median (interquartile range) and were analyzed using the Welch Two Sample t-test. All tests of significance were 2-sided, and a P-value of < .05 was deemed statistically significant. This study was approved by the Institutional Review Board (study number: 00208030).

RESULTS

A total of 816 men were identified that met the inclusion criteria, of which 651 (79.8%) were characterized as fertile and 165 (20.2%) as subfertile based on TMSC. Table 1 shows the characteristics of the 2 groups. There was no statistical difference in age between the fertile and subfertile cohorts (35.33 (32.18, 38.80) vs 35.43 (32.34, 39.44), P = .8). BMI was lower in the fertile versus subfertile cohorts (26.00 (23.89, 29.00) kg/m^2 vs 27.26 (24.39, 30.44) kg/m^2, P = .003), and there was a lower prevalence of varicocele in the fertile versus subfertile cohorts (25% vs 33%, P = .023).

Analysis of serum hormone values showed FSH (mIU/mL) was significantly lower in the fertile versus subfertile cohorts (4.00 (2.80, 5.50) vs 6.60 (4.40, 10.40), P < .001), whereas median testosterone level (ng/dL) was similar between the fertile and subfertile cohorts (341.00 (278.00, 408.50) vs 357.00 (298.00, 442.00), P = .056). Likewise, there was no significant difference in estradiol levels (pg/dL) between the fertile and subfertile cohorts (24.00 (19.00, 30.00) vs 24.00 (19.00, 31.00), P = .8).

The T/E ratio was similar in the fertile and subfertile cohorts (14.48 (10.85, 19.57) vs 15.00 (10.96, 19.73), P = .5). Table 2 and Figure 1 show the distribution of T/E ratios by percentile for both cohorts. The 20th percentile

Table 1. Baseline characteristics and semen parameters of fertile and subfertile men presenting for initial fertility evaluation.

Variable	Fertile (TMSC > 20 M) N = 651 *Median (IQR)*	Subfertile (TMSC < 20 M) N = 165 *Median (IQR)*	*P*-value
Age, years	35.33 (32.18, 38.80)	35.43 (32.34, 39.44)	.8
BMI, kg/m^2	26.00 (23.89, 29.00)	27.26 (24.39, 30.44)	.003
FSH, mIU/mL	4.00 (2.80, 5.50)	6.60 (4.40, 10.40)	< .001
Testosterone, ng/dL	341.0 (278.0, 408.5)	357.0 (298.0, 442.0)	.056
Estradiol, pg/mL	24.00 (19.00, 30.00)	24.00 (19.00, 31.00)	.8
Varicocele	160 (25%)	55 (33%)	.023
TMSC #1, Million	80.32 (42.05, 135.9)	4.05 (1.64, 8.38)	< .001
TMSC #2, Million	78.12 (45.48, 129.7)	4.98 (1.73, 8.68)	< .001
T/E Ratio	14.48 (10.85, 19.57)	15.00 (10.96, 19.73)	.5

BMI, body mass index; FSH, follicle-stimulating hormone; IQR, interquartile range; TMSC, total motile sperm count; T/E, testosterone/estradiol

Table 2. Distribution of testosterone to estradiol ratio (T/E) by fertility status.

	5th Percentile	10th Percentile	20th Percentile	25th Percentile	50th Percentile	75th Percentile	90th Percentile	95th Percentile
Fertile (n = 651)	5.98	7.27	9.77	10.8	14.5	19.6	23.8	27.6
Subfertile (n = 165)	5.95	7.97	9.95	11.0	15.0	19.7	23.9	26.8

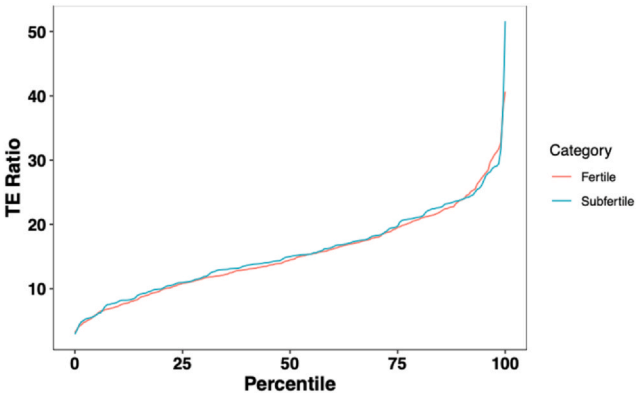

Figure 1. Distribution of testosterone to estradiol ratio (T/E) by percentile among fertile and subfertile men.

for the fertile and subfertile cohorts were 9.77 and 9.95, respectively.

DISCUSSION

In this retrospective study, we characterized the T/E ratio in a large cohort of fertile and subfertile men determined by TMSC. In our cohort of 165 subfertile men, there was no difference in estradiol levels, testosterone levels, or in the T/E ratio compared to the 651 fertile men. In both groups, the 20th percentile T/E ratio was approximately 10/1.

Our study was notable for multiple interesting findings. First, the overall distribution of T/E ratio across the fertile cohort was highly consistent with the original findings from Pavlovich et al.[9] Despite the small sample size of that study and the associated limitations in drawing conclusions regarding a normal distribution of parameters, the originally reported 20th percentile value was validated in our significantly larger cohort.

Second, we found there was no difference in the T/E ratio between fertile and subfertile men as classified by TMSC. This contrasts with the results from Pavlovich et al which found a significant difference in the T/E ratio between their fertile and infertile cohorts.[9] This is likely attributable to multiple differences in study design. In addition to the difference in cohort size between the 2 studies, there was a significant difference in the cohort characteristics and inclusion criteria. We defined our cohorts strictly according to TMSC, which has been shown to have improved prognostic value for pregnancy outcomes when compared to WHO semen analysis parameters and is a criteria that is easily reproducible in clinical practice.[11,12] In contrast, the criteria for infertility in the Pavlovich et al study were broad, including men with soft small testes, increased FSH, or abnormal SA, which was not clearly defined. Additionally, the current study excluded azoospermic men whereas the original study included 43 azoospermic men. As such, the difference in T/E ratio may be most pronounced, and most clinically relevant, for azoospermic men. The lack of difference in T/E ratio between fertile

and non-azoospermic subfertile men in the current study raises the possibility that T/E ratio is less clinically relevant for this cohort.

Despite similarities in the overall distribution of T/E ratios between fertile and subfertile men, prior studies have shown that specific groups of subfertile men may benefit from treatment with an AI. In men with impaired semen parameters, AI therapy increases serum testosterone and decreases serum estradiol, thus raising the T/E ratio, while also having a positive effect on semen parameters.[9,10,13-16] In general, testosterone deficiency is often implicated in men with oligospermia, and there is an established correlation between semen parameters and low serum testosterone levels.[5,17] Likewise, the role of estradiol in the feedback pathways of the hypothalamic-pituitary-gonadal axis has been well established and may impact semen parameters. As such, it remains unclear whether the benefit of AI therapy is specifically driven by changes in serum testosterone, serum estradiol, T/E ratio, or some combination thereof.

Given the potential benefits of AI therapy, criteria are needed to determine the optimal candidates for treatment. Certainly, men with high estradiol levels and symptomatic hyperestrogenism warrant treatment. However, among asymptomatic men, the parameters for treatment remain unknown. While the current study validates the ratio of 10/1 as the 20th percentile of both fertile and subfertile populations, it is unclear whether this is a reasonable clinical cutoff for treatment initiation. The lower bound of the normal range for a variety of other laboratory parameters is typically established at a lower percentile of the normal distribution. For example, WHO reference ranges utilize the 5th percentile of fertile men as the lower bound for all bulk semen parameters. We found that the 5th percentile for T/E ratio was approximately 6/1, and it is possible that this may be a more reasonable clinical cutoff for treatment initiation. In our study, 42 patients would be eligible for AI treatment initiation if this 6/1 ratio (5th percentile) was used as a cutoff, compared to 171 patients if the 10/1 ratio (20th percentile) was used. Naelitz et al described other predictors (T:LH ratio > 100) of spermatogenic response to AI treatment, and further, larger studies are needed to establish reasonable criteria for AI treatment in the asymptomatic patient.[18]

Our study addresses a gap in the literature by characterizing the distribution of T/E ratio by fertility status in a large cohort of men presenting for fertility evaluation. The strengths of our study include the large number of men included in both groups, utilizing TMSC as our definition of subfertility, which is reproducible and applicable to management of male patients presenting for fertility evaluation.[5] Likewise, the exclusion of men with azoospermia further generalizes the prior findings of Pavlovich et al to a cohort of non-azoospermic subfertile men. Our study is limited by potential selection bias due to retrospective study design and the lack of longitudinal data on AI treatment and subsequent treatment outcomes. Nonetheless, this is the largest cohort to date, and the current descriptive study provides a data-informed basis for T/E ratio and AI treatment criteria in future, prospective studies of AI therapy in subfertile men.

CONCLUSION

In this retrospective study, we characterized the distribution of T/E ratio in a large cohort of men presenting for fertility evaluation. We validated the 10/1 ratio that was previously established as the 20th percentile for fertile men, and we found no difference in T/E ratio between fertile and subfertile men defined by TMSC. Additional studies are needed to better define the cohort of men with infertility who could benefit from the use of AI therapy, and the distribution of T/E ratios characterized herein can inform future clinical trial design and possibly clinical practice.

Ethical Declarations

This study was approved by the Institutional Review Board (study number: 00208030).

Disclosures

The authors declare that they have no relevant financial interests.

Data Availability Statement

The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Joshua A Halpern: Writing—review and editing, Supervision, Methodology, Conceptualization. Robert E Brannigan: Writing—review and editing, Conceptualization. Sai Kaushik SR Kumar: Writing—review and editing, Formal analysis, Data curation. Daniel R Greenberg: Writing—review and editing, Conceptualization. Solomon Hayon: Writing—review and editing, Data curation, Conceptualization. Evan J Panken: Writing—original draft, Investigation, Conceptualization.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

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