Effects of Estrogen on Spermatogenesis in Transgender Women

Da David Jiang, Erica Swenson, Malachi Mason, Kevin R. Turner, Daniel D. Dugi, Jason C. Hedges, and Sarah L. Hecht

OBJECTIVE
To characterize spermatogenesis in the estrogenized transgender patient.

MATERIALS AND METHODS
This is a retrospective, single-center, cross-sectional study. Seventy-two transgender women underwent gender-affirming orchiectomy between May 2015 and January 2017. All were on long-term (>1 year) cross-sex hormonal therapy prior to orchiectomy. Patient data were obtained via chart review. Histologic analysis was performed by a pathology resident under the supervision of a genitourinary pathologist. The main outcome is histologic presence of germ cells and presence of spermatids (a proxy for preserved spermatogenesis) in orchiectomy specimens.

RESULTS
There were 141 pathologic specimens available for analysis. Germ cells were present in 114 out of 141 (81%) testicles. Spermatids were present in 57 (40%) testicles. Presence of germ cells was associated with older age (43 vs 35 years, \( P = .007 \)) and increased testicular weight (28.6 g vs 19.3 g, \( P < .001 \)). Presence of spermatids was associated with increased weight (31.5 g vs 23.3 g, \( P < .001 \)) and volume (20.3 mL vs 12.6 mL, \( P < .001 \)). There was a linear correlation between testis volume and preserved spermatogenesis (Pearson’s \( r = 0.448 \), \( P < .001 \)).

CONCLUSION
Despite long-term hormone therapy, the majority (80%) of transgender women have germ cells present in the testicle. Spermatogenesis is preserved in approximately 40% of these individuals. Duration of hormonal therapy did not affect the degree of preservation of germ cells or spermatogenesis but starting hormonal treatment at a younger age may be associated with decreased germ cells in the testicle. Volume of testicles predict presence of preserved spermatogenesis.


Little is known about fertility preservation in transgender women who have been on long-term hormone therapy. To assume that transgender patients are disinterested in producing biological offspring is misguided; multiple studies have shown the importance of fertility preservation to transgender people and is worthy of further study. A survey of 121 transgender women revealed that nearly half desire children, though nearly all stated that sterility was not an important reason to delay their transition.1 A more recent study from Germany revealed similar findings with 70% of testicular women on hormonal treatment expressing a desire for children in the future.2 Although 46% of subjects stated they would consider adoption, 11% reported a preference for biological offspring.2 A recently published international clinical guideline spearheaded by The Endocrine Society recommended discussing semen cryopreservation prior to the initiation of hormone therapy for transgender women.3 With earlier transition and pubertal suppression gaining popularity, this may not be an option for some patients.

Until recently, estrogen was thought to lead invariably to azoospermia. Data supporting this belief are largely anecdotal or come from small series.4 From our experience, many transgender women have little to no ejaculate, thus semen analysis is not a viable measure of sperm production and researchers must turn to histologic examination of the testicle. It is currently unknown whether the effects of estrogenization on spermatogenesis are reversible.3,4 It is also unknown whether the loss of spermatogenesis is complete, universal, related to demographic factors, or dependent on estrogen regimen or timing of estrogen exposure. Clarifying these variables and their relation to spermatogenesis may elucidate who is a candidate for currently available assisted reproductive techniques, and/or may lead to development of new technologies.

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Here, we aim to characterize spermatogenesis in the estrogenized testes of a large cohort of transgender women. Additionally, we hope to identify factors associated with presence of germ cells and preserved spermatogenesis.

MATERIALS AND METHODS
This is an IRB-approved retrospective cross-sectional study. Subjects included all transgender patients undergoing gender-affirming orchiectomy, either alone or as a part of vaginoplasty at Oregon Health and Science University from May 2015 to January 2017. All subjects (n = 72) were taking cross-sex hormones for at least 1 year prior to orchiectomy. Subjects undergoing orchiectomy alone (n = 50) did not stop estrogen prior to surgery but those who had orchiectomy with vaginoplasty (n = 22) were asked to stop estrogen 2 weeks before surgery due to risks of thromboembolic events. No patients were asked to stop spironolactone or progesterone. Demographic data were obtained via chart review.

Orchiectomy specimens were weighed, measured in 3 dimensions, fixed in formalin, and sectioned. Representative tissue sections were embedded in paraffin, cut (5 μm histologic sections) and stained with hematoxylin and eosin.

Histologic analysis was performed by a pathology resident under the supervision of a genitourinary pathologist. Qualitative grading was performed similar to that described by Koni et al in their histologic analysis of undescended testicles removed after puberty (Supplemental Fig. 1). Primary outcomes included complete absence of germ cells, presence of germ cells without spermatids and, presence of spermatids. Testicular volume was estimated from pathologic testicular dimensions, using the equation length × width × height × 0.71 mL. Univariate analysis for presence of germ cells and spermatids in the specimen was performed using the independent t-test (age, BMI, duration of hormones, weight of testis, and volume of testis) and the chi-squared test (race). The race variable was converted to a binary format (1 white, 0 non-white). The testis volumes were then grouped into categorical variables (<10 mL, 10-14.9 mL, 15-19.9 mL, 20-24.9 mL, ≥25 mL). Pearson’s correlation coefficient was used to assess correlation between testis volume and presence of germ cells or spermatids.

RESULTS
There were 141 pathologic specimens from 72 patients for histologic analysis (Table 1). Median age was 39 [IQR 30-53], most patients were white (88%), median BMI was 26.6 [IQR 23.2-31.4], the median duration of hormone use was just over 3 years (39 months [IQR 24-65]). The median weight and volume of the gonads were 26.4 grams [IQR 23.3-33.0] and 14.2 mL [IQR 10.2-20.2], respectively. Germ cells were present in 114 (87.8%) of the specimens. Spermatids were identified in 57 (40.4%) of the gonads.

The univariate analysis for presence of germ cells can be found in Table 2. Patients with germ cells present were slightly older than those without germ cells in the specimens (43 vs 35 years, P = .007). Germ cell presence correlated with increased testicular weight (29 g vs 19 g, P < .001) but was not statistically significant for volume (16.3 mL vs 13.3 mL, P = .264). There were no statistically significant associations between spermatogenesis and race, BMI, or duration of hormone therapy.

The analysis for presence of spermatids can be found in Table 3. Age was not statistically significantly different between the (2) groups (41.9 vs 40.4, P = .502). The weight of the testis was higher in those containing spermatids (31.5 g vs 23.3 g, P < .001). The volume of the testis was higher in those with spermatids (20.3 vs 12.6, P < .001). Patient race, BMI, and duration on hormone replacement were not statically significantly different between those with or without spermatids.

The data were further analyzed to detect a linear association between volume of the testis and presence of germ cells using Pearson’s correlation for significance (Supplemental Fig. 2). For testes less than 10 mL in volume, there was a 74% chance of detecting germ cells and this rose to 92% for testes ≥25 mL with a mild statistically insignificant positive correlation, r = 0.156, P = .065. There was a statistically significant positive Pearson’s correlation between volume of testes and preserved spermatogenesis (r = 0.448, P < .001). Of testes <10 mL, 9% contained spermatids and this increased to 85% for testes ≥25 mL (Fig. 1).

DISCUSSION
Our study finds that despite long-term cross-sex hormone therapy, germ cells persist in the vast majority of transgender women and over one third of these women have preserved spermatogenesis. This is in contrast to early research suggesting that estrogenization invariably leads to testicular atrophy and sterility, but is consistent with more recent studies showing that some patients do maintain spermatogenesis. In 1992, Lubbert et al investigated the effects of estrogen on hormonal and semen parameters in a single transgender patient. For this subject, administration of ethinyl estradiol led to progressive suppression of gonadotropins, testosterone, and sperm counts. A 1988 study of orchiectomy specimens of 11 transgender women

Table 1. Demographic data for the 72 subjects and histologic data for the 141 orchiectomy specimens

| Age (median, IQR) | 39 (30-53) |
| White race (n, %) | 115 (87.8%) |
| BMI (median, IQR) | 26.6 (23.2-31.4) |
| Months on hormones (median, IQR) | 39 (24-65) |
| Testis weight in grams (median, IQR) | 26.4 (23.3-33.0) |
| Testis volume (median, IQR) | 14.2 (10.2-20.2) |
| Basal membrane thickening (n, %) | 0 (12.8%) |
| Dystrophic calcifications (n, %) | 2 (1.4%) |
| Leydig cell hyperplasia (n, %) | 0 (0%) |
| Interstitial fibrosis (n, %) | 12 (8.5%) |
| Germ cells present (n, %) | 57 (40.4%) |
| Presence of Spermatid (n, %) | 57 (40.4%) |

BMI, body mass index; GCNIS, germ cell neoplasia in situ. Histologic data is based on modified Koni criteria, which derives from the histologic analysis of undescended testicles removed after puberty.
on long-term estrogen therapy describes atrophy of Leydig cells, fibroblastic proliferation in the testicle and altered or markedly diminished spermatogonia. All patients were on long-term but varied estrogen regimens.

More recently, 3 histopathologic studies have been published assessing spermatogenesis in the estrogenized testicle. The first, from Germany, studied 108 patients and found 24% had preserved spermatogenesis. Patients who stopped hormones 6 weeks prior to orchiectomy had a higher ratio of preserved spermatogenesis (45%). The duration of hormone therapy prior to surgery is unknown. Another study of 50 transgender women found a high percentage (80%) of specimens with maturation arrest at the level of the spermatogonia with only 20% with some spermatogenesis. Unfortunately the length of hormone therapy was unknown and the average age of these patients were younger than our cohort, which may have had an effect although the discrepancy is larger than expected.

### Table 2. Univariate analysis for presence of germ cells in the testis

<table>
<thead>
<tr>
<th></th>
<th>Germ Cells Present</th>
<th>Germ Cells Absent</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, 95% CI)</td>
<td>42.5 (39.9-43.2)</td>
<td>34.8 (31.5-38.1)</td>
<td>P = .007</td>
</tr>
<tr>
<td>White race (n, %)</td>
<td>95 (88.0%)</td>
<td>20 (87.0%)</td>
<td>P = .894</td>
</tr>
<tr>
<td>BMI (mean, 95% CI)</td>
<td>28.1 (26.9-29.3)</td>
<td>27.7 (24.6-30.7)</td>
<td>P = .780</td>
</tr>
<tr>
<td>Months on hormones (mean, 95% CI)</td>
<td>63.1 (50.8-75.5)</td>
<td>61.9 (30.3-93.5)</td>
<td>P = .934</td>
</tr>
<tr>
<td>Testis weight in grams (mean, 95% CI)</td>
<td>28.6 (27.2-30.3)</td>
<td>19.3 (13.9-24.6)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Testis volume (mean, 95% CI)</td>
<td>16.3 (14.8-17.8)</td>
<td>13.3 (11.1-15.4)</td>
<td>P = .064</td>
</tr>
</tbody>
</table>

BMI, body mass index.

Independent t test was used for age, BMI, duration on hormones, testis weight, and testis volume. Chi-squared was used for race. Significant P values (<.05) are highlighted in bold.

### Table 3. Univariate analysis for the presence of spermatids (preserved spermatogenesis) in the testis

<table>
<thead>
<tr>
<th></th>
<th>Spermatids Present</th>
<th>Spermatids Absent</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean, 95% CI)</td>
<td>41.9 (37.8-46.0)</td>
<td>40.4 (37.9-43.0)</td>
<td>P = .502</td>
</tr>
<tr>
<td>White race (n, %)</td>
<td>50 (90.9%)</td>
<td>65 (85.5%)</td>
<td>P = .353</td>
</tr>
<tr>
<td>BMI (mean, 95% CI)</td>
<td>28.4 (26.6-30.4)</td>
<td>27.7 (26.3-29.1)</td>
<td>P = .497</td>
</tr>
<tr>
<td>Months on hormones (mean, 95% CI)</td>
<td>51.7 (38.6-65.0)</td>
<td>70.4 (53.3-87.5)</td>
<td>P = .115</td>
</tr>
<tr>
<td>Testis weight in grams (mean, 95% CI)</td>
<td>31.5 (29.6-33.4)</td>
<td>23.3 (21.2-25.5)</td>
<td>P &lt; .001</td>
</tr>
<tr>
<td>Testis volume (mean, 95% CI)</td>
<td>20.3 (18.1-22.5)</td>
<td>12.6 (11.4-13.8)</td>
<td>P &lt; .001</td>
</tr>
</tbody>
</table>

BMI, body mass index.

Independent t test was used for age, BMI, duration on hormones, testis weight, and testis volume. Chi-squared test was used for race. Significant P values (<.05) are highlighted in bold.

**Figure 1.** The presence of spermatids in the testis increases markedly with increasing testicular volume. The positive trend between testicular volume and presence of spermatids was statistically significant on Pearson’s correlation coefficient, $r = 0.448$, $P < .001$. (Color version available online.)
largest study comes from Thailand; they examined orchiectomy specimens from 173 transgender women on long-term hormone therapy, and found normal spermatogenesis in 11% of patients; some full spermatogenesis was detected in 37% of patients. All patients were on long-term hormone therapy and all held their hormones for 4 weeks prior to surgery. These histologic findings are comparable to what we found in our American patients with 40% having some full spermatogenesis. In this study, as in ours, duration of hormone therapy was not correlated with presence of germ cells \( (P = .934) \) or spermatogenesis \( (P = .115) \). Interestingly, as in our study, older patients in this study seemed to have a slightly higher rate of preserved germ cells. One hypothesis is that earlier age at initiation of hormones may have a more detrimental effect on spermatogenesis than the actual length of the hormone use. Subanalysis of the Thai study data reveals that the average starting age of hormonal therapy for those with germ cells was 37 years compared to 30 years for those without germ cells present \( (P = .012) \).

Our study is encouraging in that we find comparably high rates of preserved spermatogenesis without holding hormone therapy preoperatively. While there is evidence to suggest that stopping hormones leads to recovery of testicular function we do not yet understand how to predict recovery or how long to hold estrogen therapy. Furthermore, in our anecdotal experience with the patients in this study, and hundreds more, patients loathe to discontinue hormone therapy. Satisfactory feminization takes a long time to achieve and is rapidly reversed when hormones are withheld.

Our most immediately clinically applicable finding is that increased testicular size reliably predicts increased rates of preserved spermatogenesis. When stratified by volume, 9% of testes <10 mL had detectable spermatogenesis compared to 58%-65% of testes between 15 mL and 24.9 mL and 85% of testis ≥25 mL (Fig. 1). Essentially, smaller testes were associated with lower likelihood of finding sperm on histology. We believe this is a clinically useful observation. To our knowledge, this is the first published report of this finding in the literature. We expect that as the field of transgender fertility preservation matures, testicular size will be a useful data point for counseling patients.

It is still unclear which patients will have preserved spermatogenesis prior to initiating therapy. We agree with others that prior to gender transition patients should be counseled to expect infertility and be presented with the option to cryopreserve sperm. Given that early initiation of hormone therapy is correlated with decreased spermatogenesis, and with pubertal suppression gaining popularity, sperm banking may not be an option for many patients. Indeed, it seems transgender women who cryopreserve have poor semen parameters. This emphasizes the need to further investigate fertility preservation in the estrogenized patient.

Histologically observed preservation of spermatogenesis does not equate to fertility, however this is a promising first avenue of study. While spermatids from transgender women have not been used for intracytoplasmic sperm injection in transgender women, this method of assisted reproductive technology has been successfully used in the cisgender population for the past 2 decades. While we did not offer testicular sperm extraction (TESE) for our patients at the time of orchiectomy, moving forward, one could perhaps consider TESE at the time of orchiectomy, similar to the onco-TESE procedure in the setting of an orchiectomy for testicular cancer. For those with only immature germ cells, future scientific investigation may include germ cell harvest and in vitro spermatogenesis. Such research would also applicable to infertile men with spermatogenic arrest and/or oncofertility, a rapidly expanding area of interest within the field of male fertility preservation.

We recognize multiple limits to our study. The study is performed at a single center. It is retrospective in nature with no matched controls. The specific estrogen regimens varied widely among patients and serum hormonal levels were not monitored by our institution. Lastly, we emphasize that histologic examination of the testicle is only a preliminary avenue of study. We caution against over interpreting the findings; they are descriptive only, and not yet proven to be predictive of or translatable to actual fertility.

CONCLUSION

Despite being on long-term hormone therapy, the majority (80%) of transgender women have persistent germ cells in their testicles, and over one third have preserved spermatogenesis. Larger testicular size reliably predicts preservation of spermatogenesis, and this may be used for preoperative fertility counseling. While these findings should lend optimism to the pursuit of fertility preservation in transgender women, future study is needed to characterize factors predictive of preserved spermatogenesis, reversibility of spermatogenic suppression, and whether the extant spermatids are viable for use in assisted reproductive technologies.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urology.2019.06.034.

References


**EDITORIAL COMMENT**

This article highlights the importance of fertility preservation prior to gender affirming surgery which ideally should be done prior to initiation of hormone therapy. This is critical to the preoperative care of the transgender person who wishes to undergo surgery. The World Professional Association for Transgender Health recommends discussion of fertility prior to the initiation of treatment and prior to any fertility affecting surgery. Estrogen appears to have an incomplete deleterious effect on spermatogenesis. Adeleye et al reported that patients on estrogen therapy still had low levels of sperm in their ejaculate (mean total motile 0.2 million/cc). The authors of the current study here report that of the 72 patients on hormone therapy for a median of 39 months who underwent bilateral orchiectomy with or without vaginoplasty, 81% still had germ cells present and 40% had evidence of elongated spermatids present on histologic evaluation. There was no statistically significant difference in age or time on hormone therapy in presence of some full sperm maturation noted on histologic evaluation. The most important factor identified in this paper was that in testes less than 10 mL, only 9% had elongated spermatids, and this increased to 85% in testes >25 mL.

So why do some patients on estrogen and other androgens such as spironolactone still have spermatogenesis, while others do not? It may be a function of compliance of estrogen therapy, dosage of estrogen or effects of the androgens. Schneider et al reviewed their data in a similar population. They evaluated 3 groups based on discontinuation of estrogen at 2 weeks, 6 weeks prior to procedure or not at all. There was no significant difference in spermatogenesis in the different groups despite higher serum and intratesticular testosterone levels in those that stopped the estrogen. The important take home message here is that despite estrogen therapy, some transgender females will continue to undergo spermatogenesis. Size matters; as smaller testes had a much lower chance of finding spermatogenesis than normal sized testes which is not the case in most cases of nonobstructive azoospermia. One must think of these patients to be similar to oncologic patients where this is the last time that biologic sperm will be available to them to cryopreserve. Even at the late stage of gender affirming surgery, testis sperm extraction ex vivo at time of orchiectomy should be offered to all patients undergoing bilateral orchiectomy with or without vaginoplasty to preserve sperm for future use.

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**AUTHOR REPLY**

The search for reliable fertility preservation options for transgender women is ongoing. It is not clear which patients on long-term estrogen and other androgens have preserved spermatogenesis. Our research may lead to hope for these individuals as we found evidence of spermatogenesis in 40% of orchietomy specimens. Our data suggest that patients with preserved testicular size are the best candidates for successful sperm retrieval, though all patients should be counseled that such efforts at fertility preservation remain investigational. We agree that patients undergoing orchiectomy as part of gender-affirming surgery should be offered ex vivo sperm extraction.

Ideally, cryopreservation of semen should be done prior to initiation of hormone therapy; however, with the increasing popularity of pubertal suppression for transgender children, this may become a vanishing option. The current Holy Grail of fertility preservation research lies in the successful culture and differentiation of spermatogonial stem cells. This is an active area of basic science research with meaningful recent advancements in the animal world. In vitro culture of human spermatogonia has proven challenging, as has in vivo maturation of human spermatogonia in xenografted cryopreserved immature testicular tissue. Despite these challenges, if cryopreserved human tissue can be matured later to produce viable sperm, this would not only impact...
transgender fertility preservation but also pediatric oncofertility and multiple instances of nonobstructive azoospermia.

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