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Timing of testosterone discontinuation and assisted reproductive technology outcomes in transgender patients: a cohort study

Mohammad Albar, MD, FRCSC, Alex Koziarz, MD, MSc, Eileen McMahon, NP, Crystal Chan, MD, MSc, FRCSC, Kimberly Liu, MD, MSL, FRCSC

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Running title: Stopping testosterone for IVF cycles

Article title: Timing of testosterone discontinuation and assisted reproductive technology outcomes in transgender patients: a cohort study

Mohammad Albar[1], MD, FRCSC; Alex Koziarz[2], MD, MSc; Eileen McMahon[3,4], NP; Crystal Chan[5], MD, MSc, FRCSC; Kimberly Liu[6], MD, MSL, FRCSC

1. Department of Obstetrics and Gynaecology, University of King Abdulaziz, Jeddah, Saudi Arabia.
2. Department of Radiology, McMaster University, Hamilton, Ontario, Canada.
3. Hannam Fertility Centre, Toronto, Ontario, Canada.
4. Bloomberg Faculty of Nursing, University of Toronto, Ontario, Canada.
5. Markham Fertility Centre, Toronto, Ontario, Canada.
6. Mount Sinai Fertility, Toronto, Ontario, Canada.

Correspondence:
Kimberly Liu, MD, FRCSC, MSL
Reproductive Endocrinology & Infertility
Associate Professor
Medical Director
Mount Sinai Fertility
University of Toronto
Phone: 416-586-4686
Email: Kimberly.Liu@sinaihealth.ca

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Capsule: In a retrospective cohort study, was no association detected between timing of testosterone cessation and number of oocytes in transgender patients.

ABSTRACT

Objective: To determine if there is an association between the timing of testosterone discontinuation and ART outcomes.

Design: Retrospective cohort study.

Setting: Single academic center.

Patients: We included consecutive transgender patients seeking fertility preservation between October 2019 to April 2021. Patients who identified as transgender on androgens for >1 month on presentation were included.

Interventions: None.

Main outcome measures: A linear regression model was used to evaluate the effect of testosterone discontinuation duration on the number of mature oocytes retrieved.

Results: Eighteen patients (mean age 27.7 [SD 5.2] years, mean BMI 27.3 [SD 4.6] kg/m^2, mean AMH 27.2 [SD 11.8], median AFC 20 [interquartile range (IQR) 14-32]) were included in the analysis. No patient underwent transition-related surgery (eg: oophorectomy, hysterectomy). None of the patients were previously pregnant. Mean time on testosterone was 44 (SD 29.6) months. Median time off testosterone until start of ovarian stimulation was 7.7 weeks (IQR 4.3-20.7) weeks. All patients underwent oocyte cryopreservation, except one who had embryo cryopreservation. Median total number of oocytes was 11 (IQR 7-14). Median number of mature oocytes was 7.5 (IQR 5-12) oocytes. The univariate regression model evaluating duration of time off testosterone prior to ART demonstrated no significant association with the outcome of mature oocytes (regression coefficient 0.19, 95% CI –0.13 to 0.50, p=0.226).

Conclusion: In a retrospective analysis of transgender patients recently on testosterone undergoing ART, there was no association detected between timing of testosterone cessation and number of mature oocytes.

Key words: ART; IVF; transgender.

Introduction

The transgender population has been a marginalized and understudied population in medicine (1-3). Nearly half of transgender patients reported a desire to have children. Of the transgender patients with ovaries, 37% would consider freezing oocyte if that option were available (4). However, a considerable proportion of transgender patients decline proceeding with fertility preservation due to the need for testosterone discontinuation (5).

There are variable reported durations in the literature on when to discontinue testosterone before starting the fertility preservation cycle. Presently, no guidelines specify the duration or necessity of testosterone discontinuation. Therefore, counseling transgender patients who have already started testosterone therapy is challenging. In a series of 16 transgender men reporting a mean testosterone discontinuation time of 4.5 months, most patients resumed menses before starting assisted reproductive technology (ART). A matched retrospective cohort study reported that transgender patients with a history of testosterone therapy were able to have cycle outcomes similar to that of cisgender women (6). Several observational studies have described oocyte cryopreservation among transgender men before initiation or after discontinuation of androgen therapy (6-11).

The literature includes reports that transgender men demonstrate similar oocyte retrieval outcomes to cisgender women (6); however, there are no reports examining whether timing of testosterone cessation impacts fertility preservation outcomes. Therefore, we aimed to evaluate whether timing of testosterone cessation was associated with ART outcomes in transgender men presenting to a high volume fertility center.

Materials and Methods

This study is a retrospective cohort study of consecutive transgender men seeking fertility preservation at a single, high volume academic fertility clinic between October 2019 and April 2021. Institutional research ethics board approval was obtained. Transgender male patients taking androgens for at least one month were included in the study. Transgender patients who did not start hormone replacement therapy or stopped hormone replacement therapy for more than one year were excluded. Patients were followed until completion of their ART cycle. The primary analysis evaluated the association exists between the duration of testosterone cessation and the number of mature oocytes retrieved in transgender men.

All patients who had initiated testosterone therapy stopped before their fertility preservation cycle start. The standard recommendation at this clinic was discontinuation of testosterone at least 4 weeks prior to ovarian stimulation; however, some patients voluntarily stopped testosterone earlier. Resumption of menses was not required prior to starting the cycle. Gonadotropin dosing was determined by the treating physician based on patient factors such as age, weight, and ovarian reserve. Transvaginal ultrasound and bloodwork monitoring was initiated to monitor the ovarian response. GnRH antagonist was started between day 7 and 9 of the stimulation cycle once the patient met one of the following two criteria: a serum estradiol (E2) of greater than 2,000 pmol/L or a follicle measuring ≥14 mm. A daily subcutaneous injection of a GnRH antagonist (0.25 mg) was administered by the patient subcutaneously to prevent an endogenous surge in LH. Monitoring of the ovarian response continued until there were 3 or more follicles visualized by transvaginal ultrasound with a mean diameter of ≥17 mm. Ovulation was subsequently triggered with a subcutaneous injection of HCG or GnRH agonist depending on the managing IVF physician. If a GnRH agonist trigger was chosen, twelve hours after their trigger

medication the patient returned to the clinic for blood work including LH and progesterone. This was a routine blood test to confirm that an endogenous LH surge has occurred. Transvaginal ultrasound-guided oocyte retrieval was performed approximately 36 hours following the administration of the initial trigger.

Statistical analysis

Continuous variables were examined for normality using normality probability plots and the Shapiro-Wilk test (12). Normally distributed variables were reported as mean with standard deviation. Non-normally distributed variables were reported as median with interquartile range (IQR).

The primary outcome for this study was the association between testosterone discontinuation duration (weeks) and the number of mature oocytes. Other covariates evaluated as secondary outcomes included: patient age, anti-Müllerian hormone (AMH), antral follicle count (AFC), and duration on testosterone (weeks). All covariates were continuous variables. These models were also run for the outcome of the total number of oocytes. The total dataset was >99% complete; there was no missing data for covariates or outcomes used in the regression analyses. All patients completed follow-up necessary to obtain outcome data for their respective ART cycles. Statistical analysis was performed using R statistical software (version 4.1.2) (13).

Results

A total of 18 transgender men were included. Mean age at cycle start was 27.7 (SD 5.2, IQR 24 to 30) years. None of the patients were previously pregnant. None of the patients who pursued treatment had undergone transition-related surgery (e.g., oophorectomy or hysterectomy). Mean AMH was 27.2 (SD 11.8) pmol/L. One patient had an AMH of 7.1 pmol/L. Median AFC was 20

(IQR 14 to 32). All patients had previously been on testosterone treatment and had stopped prior to their fertility preservation cycle. Mean time on testosterone therapy was 44 (SD 29.6) months. Median time off testosterone until start of ovarian stimulation was 7.7 (IQR 4.3 to 20.7) weeks. There were no complications secondary to fertility preservation cycle, including no thromboembolic events. **Table 1** details the remaining demographics of included patients.

Median number of mature oocytes was 7.5 (IQR 5 to 12). Median number of total oocytes was 11 (IQR 7 to 14). One patient cryopreserved seven embryos. For this patient, 14 oocytes were retrieved: 12 were fertilized through conventional IVF using donor sperm. Of these, seven progressed to the embryo stage. A total of seven embryos were cryopreserved: five were cryopreserved on day five and two were cryopreserved on day 6. The remainder of patients underwent oocyte cryopreservation. The median total number of cryopreserved oocytes was 7.5 (IQR 5 to 12).

There was no significant association between testosterone discontinuation duration and number of mature oocytes (regression coefficient 0.19, 95% CI –0.13 to 0.50, p=0.226). AMH (p=0.039) and AFC (p=0.001) were associated with the number of mature oocytes. There was no statistical significance demonstrated between age and number of mature oocytes (p=0.059), and between age and total number of oocytes (p=0.058). There was no association detected between duration on testosterone and number of mature oocytes (p=0.339). Remaining regression outcomes are reported in **Table 3**. **Figure 1** plots the testosterone discontinuation duration and mature oocyte count with 95% confidence interval bands.

Discussion

Our study is among the largest cohort of transgender patients previously on testosterone therapy undergoing fertility preservation. Our analyses did not detect an association between testosterone discontinuation time and the number of oocytes obtained, as well as the number of mature oocytes. These results have important implications for transgender men who wish to undergo ART but are concerned of the potential undesirable effects of stopping testosterone therapy (14) or are concerned of the effects of testosterone on fertility outcomes (15).

Studies have reported that approximately 0.6% of the population in the United States (16) and 0.24% of the population in Canada (17) identify as trangender. Approximately 47% of transgender persons wish to have a child that is genetically related to them (18). Notably, 37% of transgender men with ovaries reported considering freezing oocytes if that option were available (4). Unfortunately, in addition to fertility preservation being a costly, time-consuming and invasive process (19-21), the effects of testosterone cessation can be psychologically stressful (14). A study found that even though 76% of transgender men and women had considered fertility preservation prior to transition, only 3% of transgender men had completed this process (22). The resumption of pelvic bleeding is one of the causes of distress for this patient population (14). A study of 41 transgender men examined the timing of resumption of pelvic bleeding following testosterone cessation: 8% of patients had resumption at <1 month following testosterone cessation; 24% had resumption at 1 month following cessation; 28% after 2 months; 16% after 3 months; 4% after 4-6 months (24). Twenty percent had no pelvic bleeding prior to pregnancy (24). The patients in our study were on testosterone for varying durations ranging from one month to eight years. Univariate analyses showed no association between the duration of testosterone prior to cycle start and oocyte yield. Since patients who discontinued testosterone for only one month did not have inferior results to those who stopped for more than one month, discontinuing testosterone for 1 month may be a

reasonable compromise to reduce the discomfort associated with the return of menses without compromising good oocyte yield. Future studies are needed to evaluate even shorter periods of testosterone discontinuation. A recent case report by Gale and colleagues reported a 20-year-old transgender man on testosterone treatment for 18 months who chose to continue treatment throughout the fertility preservation cycle (18). The patient had a robust outcome with 22 mature oocytes.

In our study, most patients were on testosterone hormone therapy for more than 12 months, with no negative impact on the baseline ovarian reserve given a mean AMH of 27.2 pmol/L. Most patients had normal baseline E2 and FSH levels, with average to above-average ovarian reserve results for a given age. Several case reports and case series have reported similar normal ovarian reserve parameters and ovarian responses (6-11,23). In the present study, the average total dose of gonadotropins used was 2284 IU and the median number of total oocytes was 11 (mature oocytes 7.5). Leung and colleagues matched cisgender and transgender patients across several variables including age and AMH, with transgender patients receiving significantly higher doses of gonadotropins (4155 vs 2707, p=0.002) (6). The mean number of oocytes retrieved per patient was 18.6, of which 77% were mature oocytes (6). Notably, the mean number of gonadotropins used in cisgender patients (2707 IU) was similar to amount of gonadotropins used in our study in transgender patients (2284 IU). Leung and colleagues explained that the higher doses used are because of the mindset that this is a "one-shot deal" due to the intent of maximizing oocyte yield given the transition-related and financial costs of performing a cycle. Accordingly, higher gonadotropin doses may be needed in transgender patients to obtain a similar number of oocytes as in cisgender patients. Another larger study showed similar number of retrieved oocytes compared to our results (25). Law and colleagues performed a population-based cohort study of

221,221 treatment cycles in cisgender patients (25): in the 18-29 year-old group, 36.1% had 4-9 oocytes retrieved, 28.5% had 10-14 oocytes retrieved, and 15.7% had 15-19 oocytes retrieved. This right-skewed distribution with the majority (64.6%) of patients having between 4-14 oocytes retrieved is similar to the right-skewed distribution in our study of transgender patients whereby 72.2% had between 4-14 oocytes retrieved. Future studies evaluating ART outcomes between transgender and cisgender patients may consider differences in populations, gonadotropin dosage, or other cycle factors should differences in oocyte yield be demonstrated. Should future studies support higher gonadotropin dosages explaining this discrepancy, higher doses of gonadotropins may be considered to maximize the success of a single cycle.

Part of the lack of consensus on timing of testosterone cessation may be secondary to an incomplete understanding of the effects of androgen therapy on the ovarian environment. A study by Caanen and colleagues reported a reduction in AMH levels (median 3.5 to 0.3 μg/L, $p<0.0001$) in 22 female-to-male transgender patients with a mean age of 22.4 years (24). The authors conclude that androgens are important in the regulation of AMH (26). Leung and colleagues performed a matched cohort study and found that after matched on age, BMI, and AMH, transgender men showed similar number of oocytes compared to cisgender women (6). These patients discontinued testosterone for a mean of 4 months (6). The transgender patients had a mean AMH of 24.3 (SD 13.6) pmol/L and mean age of 28.3 (SD 6.7) (6). In our study, the mean AMH was 27.3 pmol/L, but the median total oocytes was 11 and median mature oocytes was 7.5. As the transgender population remains to be investigated in large studies, several hypotheses can explain these results: transgender patients may inherently have lower oocyte yield, the effect of using testosterone prior to cycle may reduce the number of retrieved oocytes, ART cycles may require optimization (eg,

higher gonadotropin dosage), or there is no true difference and the observed difference is secondary to selection bias.

Our study had several strengths. Our centre is one that aims to reduce the period of testosterone discontinuation and the start of stimulation to 4 weeks. This period is shorter than other discontinuation periods (27), and accordingly permits evaluating patients with both short and long discontinuation periods. In addition, our primary analysis attempted to evaluate whether a relationship exists between testosterone discontinuation duration and oocyte yield. This analysis is the first step towards ultimately identifying a minimum evidence-based threshold for when to discontinue testosterone in this population. Nevertheless, our study contains limitations. First, our sample size is low, so the lack of association in our primary outcome may be due to an unpowered sample. Unfortunately, there always remains a probability of an outcome being the negative due to insufficient statistical power. With limited knowledge of true effect size in trangender patients, determining the statistical power of an analysis is difficult. We aimed to minimize the impact of our low sample size by only evaluating a single covariate in the model. Future studies including patients from several centers are needed to validate our findings with higher statistical power. Second, our study was retrospective, which increases the risk for selection bias. Third, there was clinical heterogeneity such as differences in trigger (HCG versus lupron) which may have differences in effect on oocyte maturity. Fourth, pregnancy success rates were not assessed in this study, so the pragmatic question of the impact of testosterone discontinuation on oocyte quality remains to be completely evaluated. Finally, the transgender population is a diverse population, likely requiring larger studies to capture the diversity of patients interested in pursuing assisted reproductive technology. Larger studies would increase the clinical generalizability of our results and also allow for subgroup analyses that may predict differences in ART outcomes.

Conclusions

In a sample of 18 transgender patients undergoing fertility preservation, there was no association between testosterone discontinuation duration and ART including the number of mature oocytes. Transmale patients who have already started hormone therapy and who are interested in minimizing their time off of T may therefore still consider fertility preservation. Larger studies are needed to confirm these findings.

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Table legends

Table 1: Characteristics of patients

Table 2: *In-vitro* fertilization outcomes

Table 3: Simple unadjusted regression analyses for total oocytes and mature oocytes

Figure legends

Figure 1: Plot of testosterone discontinuation duration versus mature oocytes with 95% confidence interval band, demonstrating no association

Table 1: Characteristics of patients

Characteristic	Value*
Age	27.7 (5.2) years
BMI (n=16)	27.3 (4.6) kg/m^2
Smoking	
Never	13 (72.2%)
Present	4 (22.2%)
Past	1 (5.6%)
AMH	27.2 (11.8) pmol/L
AFC	20 (14 to 32)
Partner sex (n=17)	
Female	11 (64.7%)
Male	1 (5.9%)
No partner	5 (29.4%)
Time on testosterone therapy	191 (129) weeks
Time off testosterone therapy prior to IVF	7.7 (4.3 to 20.7) weeks
Resumed bleeding prior to stimulation	10 (55.6%)
Estradiol at trigger	4695 (3135 to 6430) pmol/L
Progesterone at trigger	2.7 (1.5) nmol/L
Luteal phase priming	
None	15 (83.3%)
Estrace	1 (5.6%)
OCP	2 (11.1%)
Recombinant FSH dose	210.4 (52.6) IU
Total gonadotropin dose per patient	2284 (858) IU
FSH only protocol	13 (72.2%)
FSH + LH protocol	5 (27.8%)
Days of stimulation	10.6 (1.5) days
Trigger	
hCG	14 (77.8%)
GnRH Agonist	4 (22.2%)
Endometrial lining at time of trigger	9.1 (3.3) mm

*Continuous variables reported as mean (SD) or median (interquartile range).
All variables have complete data unless otherwise specified.
BMI: body mass index; PCOS: polycystic ovarian syndrome; AMH: anti-Müllerian hormone; AFC: antral follicle count; IVF: in-vitro fertilization; OCP: oral contraceptive pill; FSH: follicle-stimulating hormone; LH: luteinizing home; hCG: human chorionic gonadotropin.

Table 2: *In-vitro* fertilization outcomes

Outcome	**Value***
Follicles over 11 mm at time of trigger	15.8 (6.2)
Follicles 16 to 22 mm at time of trigger	8 (6 to 10)
Total oocytes	11 (7 to 14)
Mature oocytes	7.5 (5 to 12)

*Continuous variables reported as mean (SD) or median (interquartile range).

Table 3: Simple unadjusted regression analyses for mature oocytes and total oocytes

Outcome	Covariates	Regression coefficient (95% CI)	P-value
Mature oocytes	Age	–0.62 (–1.26 to –0.03)	0.059
	AMH	0.29 (0.02 to 0.57)	0.039
	AFC	0.43 (0.20 to 0.67)	0.001
	Duration on testosterone	–0.01 (–0.04 to 0.02)	0.339
	Testosterone discontinuation duration	0.19 (–0.13 to 0.50)	0.226
Total oocytes	Age	–0.74 (–1.51 to –0.03)	0.058
	AMH	0.34 (0.003 to 0.68)	0.048
	AFC	0.58 (0.33 to 0.83)	0.0002
	Duration on testosterone	–0.01 (–0.05 to 0.02)	0.434
	Testosterone discontinuation duration	0.24 (–0.13 to 0.62)	0.188

CI: confidence interval; AMH: anti-Müllerian hormone; AFC: antral follicle count.