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FERTILITY PRESERVATION

Oocyte retrieval outcomes among adolescent transgender males

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Abstract

Purpose To compare fertility preservation (FP) outcomes among adolescent transgender males with those of cisgender females.
Methods This retrospective cohort study included nine adolescent transgender males and 39 adolescent cisgender females who underwent FP between January 2017–April 2019 and September 2013–April 2019, respectively. The transgender males were referred before initiating testosterone, and the cisgender females were referred due to cancer diagnosis before starting anticancer treatment. Statistical analyses compared assisted reproductive technology (ART) data and FP outcomes between two groups.
Results Basal FSH levels (5.4 ± 1.7 mIU/mL) and AFC (19.8 ± 5.6) of all transgender males were normal compared with standard references. The mean age of transgender males and cisgender females was similar (16.4 ± 1.1 vs 15.5 ± 1.3 years, respectively, $P = 0.064$). The amount of FSH used for stimulation was significantly lower among the former compared with the latter (2416 ± 1041 IU vs 4372 ± 1877 IU, $P < 0.001$), but the duration of stimulation was similar (12.6 ± 4.0 and 10.1 ± 2.8 days, $P = 0.086$). Peak estradiol level was significantly higher among transgender males compared with cisgender females (3073 ± 2637 pg/mL vs 1269 ± 975 pg/mL, respectively, $P = 0.018$), but there were no significant differences in number of retrieved oocytes between the two groups (30.6 ± 12.8 vs 22 ± 13.2, $P = 0.091$), number of MII oocytes (25.6 ± 12.9 vs 18.8 ± 11.2, $P = 0.134$), or maturity rates (81.5 ± 10.0% vs 85.4 ± 14.6%, $P = 0.261$).
Conclusions Adolescent transgender males have an excellent response to ovulation stimulation before initiating testosterone treatment. Oocyte cryopreservation is, therefore, a feasible and effective way for them to preserve their fertility for future biological parenting.

Keywords Transgender males · Cisgender females · Assisted reproductive technology · Fertility preservation · Oocyte cryopreservation

Introduction

Transgender men are individuals who identify themselves as males but were assigned female sex at birth [1]. Cisgender individuals have a gender identity congruent with or the same as their sex assigned at birth. Gender dysphoria (GD) is defined as significant distress and social impairment caused by the feeling of discrepancy between one's assigned sex at birth and gender identity [2–4]. Gender-affirming hormone (GAH), i.e., testosterone in transgender males, is indicated to alleviate GD [1]. Increasing numbers of adolescents have been seeking healthcare services to support medical transition [5]. The endocrine treatment of transgender adolescents with GD consists of two phases, starting with pubertal suppression with gonadotropin-releasing hormone analogs (GnRHa) during the early stages of puberty (Tanner stage ≥ 2) followed by the addition of GAH from around ~ 14–16 years of age [6]. Pubertal development is halted during the first phase, and adolescents can further explore their gender identity and prepare for the next phase. The administration of hormones will then cause the development of the physical characteristics that affirm their gender identity, such as the induction of masculine characteristics by testosterone among those assigned female sex at birth.

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The effect of testosterone on fertility is inconclusive. Several studies have shown histologic and functional changes in ovaries [7–9] and decreased ovarian reserve [10], while others have not observed those adverse effects [11, 12]. Therefore, the World Professional Association for Transgender Health (WPATH) [13], the Endocrine Society [6], and the American Society for Reproductive Medicine (ASRM) [14] recommend that transgender persons should be encouraged to consider fertility preservation (FP) before starting GAH treatment. Medical FP is widely used by female oncology patients who wish to preserve fertility before undergoing anticancer therapy (chemotherapy/radiation) [15]. Options for preserving fertility in postmenarchal birth-assigned females similarly include oocyte and embryo cryopreservation [16, 17].

Despite the recommendations to consider fertility preservation (FP) before starting the GAH treatment, the recently published FP utilization rates in transgender individuals were low (2–4%) [18–23]. Several studies explored the factors affecting fertility decision-making among transgender people [19, 20, 22, 24–26] and identified the major barriers to FP as being the lack of professional information, cost, invasiveness of procedures, and desire not to delay medical transition. Segev-Becker et al. recently reported [27] that 6.5% of pubertal transgender males referred to our institute completed FP prior to the initiation of hormonal treatment. Those authors suggested that the difference between the FP percentages can be the result of comprehensive fertility counseling given to their patients in addition to cultural differences.

Several biological factors for transgenderism have been suggested, and some of them are associated with fertility and different responses to ovarian stimulation. Female-to-male gender was associated with specific polymorphisms of the estrogen receptor α (ERα) and estrogen receptor β (ERβ) sex hormone receptors [28, 29]. Polymorphism of the ERα and ERβ genes was associated with risk of female infertility [30, 31], and polymorphism of the ERα and ERβ genes led to worse or better assisted reproductive technology (ART) outcomes according to the specific polymorphism [31–33]. High levels of prenatal testosterone in natal females play a role in the etiology of GD [34, 35], with females exposed to elevated prenatal testosterone having been reported to exhibit reduced fertility [36]. In addition, there is evidence that a hyperandrogenic intrauterine environment results in development of polycystic ovary syndrome (PCOS) in adult life [37, 38]. PCOS is the most common cause of anovulatory infertility, and hyper-response to ovarian stimulation is a well-known characteristic among patients diagnosed with PCOS [39]. Moreover, there are data that suggest a higher incidence of PCOS amongst transgender men [40–43]. An interesting question is whether the FP outcomes of transgender individuals before they had started GAH is different from their cisgender counterparts.

In the current study, adolescent cisgender females with cancer referred for FP before starting anticancer therapy comprised the control group. Choosing cancer patients as a control group for comparing the outcomes of ovarian stimulation is controversial due to the concern that the malignant disease negatively affects the ovarian response. Some reports have suggested a lower response to ovarian stimulation in cancer patients when compared with various types of controls [44–46], although others could not confirm that finding [47–53]. However, a recent meta-analysis comprising ten case-controlled studies indicated that a cancer diagnosis is not associated with reduced response to ovarian stimulation, including the number of total oocytes retrieved, number of mature oocytes, fertilization rate, and 2 pronuclei embryos [54]. Other groups [55, 56] that have recently published studies related to ovarian structure and function of transgender men have used cancer patients as a control group. Based on these data and with no other option of forming an age-matched control group, we chose cancer patients to form the control group.

To date, there are limited published studies on the fertility potential of adolescent transgender males [55, 57–59]. To the best of our knowledge, this is the first study to examine the fertility preservation outcomes of transgender males seeking FP prior to the initiation of GAH therapy in comparison to adolescent cisgender females with cancer referred for FP before starting anticancer therapy.

Materials and methods

Study population and participant recruitment

This retrospective study was performed at the IVF Unit, Fertility Institute in Tel Aviv Sourasky Medical Center and IVF and the Infertility Unit, Helen Schneider Hospital for Women, Rabin Medical Center, both tertiary university-affiliated medical centers. The medical records of 11 adolescent transgender males (age range 13–18 years) and 39 adolescent cisgender females (age range 13-18 years) with cancer who preserved fertility between January 2017 and April 2019 and September 2013 and April 2019, respectively, were reviewed. All of the transgender adolescents were referred from the Gender Dysphoria Clinic at Dana-Dwek Children's Hospital, Tel Aviv Medical Center, after they were evaluated by a community mental health professional and were diagnosed with GD according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition 302.85 (DSM 5) criteria. All of the adolescent cisgender females were referred for FP due to a cancer diagnosis. This group included 17 patients with Hodgkin's lymphoma, 5 with Ewing's sarcoma, 5 with Wilms' tumor, 3 with osteosarcoma, 2 with acute myeloid leukemia, and one patient from each of the following

cancers: rhabdomyosarcoma, myelodysplastic syndrome, uterine papillary serous carcinoma, acute lymphoblastic leukemia, germ cell tumor, ependymoma, and synovial sarcoma. At the time of referral to FP, all participants were postmenarchal, and all the adolescent transgender males were at Tanner stage 5 of puberty and had regular menstrual cycles. A regular menstrual cycle was defined when the interval between bleeding periods was in the range of 21–35 days.

Data collection

All relevant data were collected from the computerized database of the two hospitals. The data in the electronic patient charts included the following: clinical details (age, body mass index [BMI], length of time since the first menstrual period, Tanner stage and type of cancer), fertility potential details [hormone profile and antral follicle count (AFC)], ART details [length of cycle, total follicle-stimulating hormone (FSH) dose, peak serum estradiol, peak serum progesterone, and type of ovulation trigger], and stimulation outcomes [number of retrieved oocytes, number of MII oocytes and maturation rate (derived from the number of MII oocytes/number of oocytes aspirated)]. In our study, none of the adolescent transgender subjects with two exceptions received any treatment prior to the FP. One of the exceptions was treated with GnRH analog for 8 months and stopped the treatment 10 months before FP (he started menstruating again after stopping the blocker), and the other was treated with norethisterone 10 mg/day and desogestrel 0.075 mg/d for 5 months and stopped the treatment immediately before FP. These two patients were excluded from the study. The adolescent cisgender females had not received any anticancer treatment prior to undergoing FP.

Ovarian stimulation and oocyte cryopreservation

Prior to FP, all of the participants under 18 years of age and their legal guardians signed informed assent and consent forms, respectively, that provided detailed information regarding the known potential side effects of treatment. Controlled ovarian stimulation was carried out by the GnRH antagonist protocol. Although the time period for inclusion of cisgender females (~ 6 years) is much longer than for transgender males (~ 2 years), no changes that could impact the results were made to the protocol between the two time periods. All cycles were initiated with menses in the transgender group, while 72% of the cycles were initiated with menses and 28% were started randomly in the cisgender group. Follicle follow-up for all participants was by transabdominal ultrasound because of their ages, and ovum retrieval was by transvaginal access. The stimulation was started with the administration of daily recombinant FSH [rFSH; Gonal F (Serono, Geneva, Switzerland) or Puregon (Organon, Oss, The Netherlands)] from day 2–3 of the cycle. GnRH antagonist (cetrorelix acetate 0.25 mg, Cetrotide®, Serono or Ganirelix, Orgalutran®, Merck and Co., Inc.) was started when the leading follicle was ≥ 12 mm, or the estradiol level was > 450 pg/ml, and it continued until the day of trigger administration. GnRH-α triptorelin 0.2 mg/d (Decapeptyl; Ferring, Kiel, Germany) or choriogonadotropin α 250 mcg (Ovitrelle; Serono, Geneva, Switzerland) or dual trigger (GnRH-α triptorelin 0.2 mg/d and choriogonadotropin α 250 mcg) were administered when at least three follicles achieved a diameter of 18 mm. Ovum pickup was performed 36 h later by transvaginal puncture with the participant under general anesthesia, after which the recovered oocytes were vitrified.

Statistical analysis

Data were analyzed using SPSS, version 21.0 (SPSS, Inc., Chicago, IL, USA). The data are summarized as mean ± SD or number of responders (percentage) according to the variables. Categorical data were analyzed with chi-square test, continuous variables compared between groups with Mann Whitney test. A P value of < 0.05 was considered significant. A multivariate linear regression analysis was performed to control for age, treatment duration and total FSH dose as confounders for the number of retrieved oocytes.

Results

The clinical data of nine transgender adolescents who participated in this study are presented in Table 1. Their mean age and BMI at referral to FP were16.4 years and 21.8 kg/m^2, respectively. The mean length of time since their first period was 4.2 years. The mean length of menstrual cycles was 29.4 days (median length 28 days; range 28–35 days). The hormonal profile, total testosterone, thyroid stimulating hormone (TSH), and prolactin levels of all of the adolescent transgender subjects were within the normal range: FSH (5.4 mIU/mL), luteinizing hormone (4.9 mIU/mL), estradiol (137 pg/mL), total testosterone (1.1 nmole/L), TSH (2.6 μIU/mL), and prolactin (286 mIU/L). The mean AFC was 19.8.

The ART data and outcomes of the adolescent transgender males (study group) and the cisgender females (control group) are summarized in Table 2. There was no significant difference in the mean age at referral to FP between the two groups (16.4 vs 15.5 years, respectively, $P = 0.064$). Although there was no difference in the mean number of FSH stimulation days between them (12.6 and 10.1 days, $P = 0.086$), the amount of FSH used was significantly lower in the former compared with the latter (2416 IU vs 4372 IU, $P < 0.001$). The peak estradiol level was significantly higher among the transgender males compared with the cisgender females (3073 pg/ml vs 1269 pg/ml, respectively, $P = 0.018$), but there were no significant differences between the two groups in the

Table 1 Clinical parameters of the adolescent transgender males

Subject	Age (y)	BMI (kg/m^2)	Length of time since the first period (y)	Length of menstrual cycle (d)	FSH (mIU/mL)	LH (mIU/mL)	E2 (pg/mL)	T (nmole/L)	TSH (μIU/mL)	Prolactin (mIU/L)	AFC (total number)
1	17	23.3	5	35	7.3	4.3	147	0.6	6.07	341	13
2	17	23.9	2	30	4.8	2.8	80	1.2	1.46	495	25
3	18	20.8	7	28	5.71	19.6	201	2.62	1.23	236	15
4	17	22.3	5	28	6.53	3.72	200	0.8	3.2	255	18
5	16	20	4	28	6.6	3	84	1.4	2.17	201	16
6	14	21.6	0.5	28	4.1	1.7	132	0.8	4.04	138	30
7	17	19	5	30	6.55	5.18	145	0.9	3.38	477	17
8	16	24.4	5	28	1.5	0.5	132	1	0.95	251	25
9	16	21.5	4	30	6.1	3.9	116	0.8	1.02	188	20
Mean (SD)	16.4 ± 1.1	21.8 ± 1.7	4.2 ± 1.9	29.4 ± 2.3	5.4 + 1.7	4.9 ± 5.6	137 ± 42	1.1 ± 0.6	2.6 ± 1.7	286 ± 125	19.8 ± 5.6

BMI body mass index, *FSH* follicle-stimulating hormone, *LH* luteinizing hormone, *E2* estradiol, *T* total testosterone, *TSH* thyroid stimulating hormone, *AFC* antral follicle count, *ND* no data, *SD* standard deviation

Standard reference ranges: FSH 1–9.2 mIU/mL; LH 0.4–11.7 mIU/mL; E2 34–170 pg/mL; T 0.48–1.85 nmole/L; TSH 0.5–4.8 μIU/mL; prolactin 108.78–557.13 mIU/L

number of oocytes retrieved (30.6 vs 22, $P = 0.091$), the number of MII oocytes (25.6 vs 18.8, $P = 0.134$), or the maturity rates (81.5% vs 85.4%, $P = 0.261$). On multivariate linear regression analysis, adjusting for age, treatment duration, and total FSH dose, there was no significant group difference in the number of retrieved oocytes (Table 3).

Discussion

There is growing interest to preserve fertility among adolescent transgender males, but there are currently limited published data on FP outcomes from ART in that selective group. Various studies have included adolescent transgender males in ART outcomes, but no studies have focused solely on this group [55, 57–59]. This study is the first to demonstrate that FP outcomes from ART among adolescent transgender males are comparable with those of adolescent cisgender females. The control population in the present study was comprised of adolescent cisgender females who were diagnosed with cancer and were referred for FP before initiating anticancer therapy which may impair fertility. Oocyte cryopreservation is a well-known method of FP in adolescent and young adult oncology patients [15]. It has been used as a viable method for FP in transgender individuals as well [16, 17]. Our data support the methodology of controlled ovarian hyperstimulation as a feasible means of FP in adolescent transgender males.

Table 2 Comparison of ovarian stimulation cycles of adolescent transgender males and adolescent cisgender females

Characteristic	Male transgender adolescents ($N = 9$)	Cisgender females ($N = 39$)	*P* value
Age (y)			0.064
Mean (SD)	16.4 (1.1)	15.5 (1.3)	
Range	(14–18)	(13–18)	
Ovarian stimulation duration (days)	12.6 (4.0)	10.1 (2.8)	0.086
FSH total dose (mIU/mL)	2416 (1041)	4372 (1877)	< 0.001
Peak E2 (pg/mL)	3073 (2637)	1269 (975)	0.018
Peak progesterone (ng/mL)	1 (0.57)	1.2 (0.7)	0.697
Oocytes retrieved (*n*)	30.6 (12. 8)	22 (13.2)	0.091
MII oocytes (*n*)	25.6 (12.9)	18.8 (11.2)	0.134
Maturity rate (%)	81.5 (10.0)	85.4 (14.6)	0.261

FSH follicle-stimulating hormone, *E2* estradiol, *SD* standard deviation

Table 3 Multivariate linear regression analysis for the number of retrieved oocytes

Variable	Standardized coefficient	*P* value
Adolescent transgender males vs. adolescent cisgender females	− 0.161	0.071
Ovarian stimulation duration (days)	0.321	0.110
FSH total dose (mIU/mL)	− 0.690	0.002

Serum FSH and AFC are well-established markers of ovarian reserve [60]. Unfortunately, given the urgency of initiating anticancer treatment in young females with cancer, these data were not collected in our control group. However, comparisons of FSH levels and AFC of transgender males to reference or previously reported values in childhood and adolescence [60–62] indicated that their ovarian reserve is preserved. Basal total testosterone levels in adolescent transgender males were within the normal range for cisgender females [63, 64], indicating that the patients were not taking "black market" testosterone and did not demonstrate androgen excess (a feature of PCOS that has been associated with transgender men [40–43]).

Although the duration of hormonal stimulation did not differ between the two groups, significantly lower total doses of gonadotropins had been used in the stimulation cycles of the transgender males. One reason for this difference may be because an oocyte cryopreservation cycle was "one-shot deal" before chemotherapy or radiation therapy for most of the cancer patients, and therefore aggressive stimulation was intentional in order to optimize egg yield for a single cycle. Gonadotropins were given with caution to transgender males, however, for fear of ovarian hyperstimulation. Although the stimulation doses were significantly higher for the cisgender females, the peak estradiol levels were significantly lower in that group compared with the transgender males. The finding of lower peak estradiol levels in the cancer patients compared with healthy controls has been attributed to the use of letrozole for ovarian stimulation in the cancer group [54]. However, letrozole was not administered in our series. The significance of this finding is especially interesting given the similar number of retrieved oocytes and mature oocytes as main parameters of the ovarian response. Because the granulosa cells are the main source of estradiol, reduced estradiol production may represent an early sign of the possible negative effect of the cancer state on granulosa-cell performance. However, our results are consistent with those of others [49], who found low peak estradiol levels (without letrozole treatment) with no other effects on ART outcomes in cancer patients. All cycles in the transgender participants were initiated with menses, while almost one third of the cycles were initiated randomly in the oncology patients. Importantly, the random start ovarian stimulation results in oocyte yield outcomes were similar to those achieved with standard start protocols (the mean numbers of retrieved oocytes were 22.1 ± 13.3 vs 21.9 ± 13.4, and the mean numbers of mature oocytes were 19.5 ± 11.8 vs 18.6 ± 11.2, respectively).

There were no significant differences in the number of retrieved oocytes, the number of mature oocytes, and the maturity rate of the oocytes between the two groups. A case series published recently demonstrated good ART outcomes among 3 adolescent transgender males and 1 adult transgender man who underwent FP [58]. A similar trend for similar or favorable ART outcomes was recently reported in adult transgender males who had not been exposed to testosterone [55, 59]. Leung et al. [59] were the first to show a higher number of oocytes retrieved in transgender males compared with cisgender females. Unlike our finding, they did not observe any significant difference in peak estradiol level between the two groups, but like us, they found no difference in the percentage of mature oocytes between the two groups. Adeleye et al. [55] observed similar ovarian stimulation outcomes, including cycle length, peak estradiol level, peak estradiol per oocyte, oocytes retrieved, mature oocytes, and maturity rate, between transgender males with no testosterone exposure and cisgender females. The control group in both of those studies consisted of infertile women, a factor which could have influenced the results. Indeed, Leung et al [59] mentioned that their control group was problematic because of the infertility factor and noted that a better comparison group would be oncology-fertility patients, as those in the current work. As mentioned in the introduction, a recent meta-analysis concluded that cancer diagnosis is not associated with reduced response to ovarian stimulation [54]. In our study, low ovarian stimulation outcomes were mostly among Wilms' tumor patients. Four of the five female adolescents who were diagnosed with Wilms' tumor displayed a poor response to ovarian stimulation. Wilms' tumor is associated with mutations in the tumor suppressor gene, WT1 [65]. In mice, Wt1 plays a key role in follicular development, and WT1-mutant mice have smaller ovaries and significantly fewer follicles [66], which is very similar to premature ovarian failure in human patients. The WT1 gene plays a critical role in folliculogenesis in humans, and mutations in this gene were found to be associated with premature ovarian failure [67]. We are not aware of any studies on responses to ovarian stimulation in Wilms' tumor patients before anticancer treatment. Importantly, the exclusion

of this subgroup from the current study did not affect the trend of results or the conclusions. Further studies are needed to evaluate the association between Wilms tumor and poor ovarian response.

The results of the current study indicate that the FP outcomes from ART among adolescent transgender males are comparable to those of adolescent cisgender females. Although we did not examine ER polymorphism in our small cohort, the ovarian stimulation response did not differ between transgender males and cisgender females. An evaluation for PCOS according to the Rotterdam criteria [68] was not performed, and therefore we cannot conclude the incidence of PCOS among the transgender males who participated in our study; in addition, our sample size was small, and more studies are needed to examine that subject in greater depth. We are aware that the ultimate endpoint for FP analyses in transgender individuals is a live birth rate, and we believe that this study is a good first step.

The present study has several limitations. First, it is retrospective in design. Second, it includes a relatively small sample size. Most of the adolescent transgender individuals nationwide are referred to the Fertility Institute in Tel Aviv Sourasky Medical Center because it is part of a national center for transgender health medicine, and many adolescent females with cancer are referred to the IVF and Infertility Unit in the Rabin Medical Center because it is one of the main centers for the treatment of children and youth with cancer. This collaboration enabled the acquisition of adequate numbers of participants in both groups. Third, our control population, consists of oncology patients, may not be representative of the general population of healthy adolescent cisgender females. The underlying disease itself may be associated with low ovarian reserve and diminished fertility preservation outcomes. Fourth, the absence of many baseline characteristics, including BMI, Tanner stage, menstrual history, hormonal profile, and AFC, for cisgender females could have affected the interpretation of the results. Further studies that include this information are desirable. Fifth, in our study, ER polymorphism was not examined. Based on the rationale that the ART outcomes may be affected by ER polymorphism, this information is missing. Lastly, PCOS was suggested to be in a higher incidence among transgender men, and therefore affects ovarian stimulation response. In the current study, we did not evaluate PCOS among the participants, a major factor that may contribute to the higher egg yield.

In conclusion, with the greater acceptance of the field of reproductive and parenting among transgender people, the age of transgender patients seeking gender-affirming treatments is declining. The effects of GAH have not been clearly defined, and the knowledge about fertility preservation outcomes needs to expand. To date, there are limited data on the induction of ovulation among adolescent transgender males, and the findings of this study comprise one of the steps in expanding the understanding of FP outcomes in that select population. Healthcare providers can more confidently assure the patients and their parents that the oocyte yield is adequate and comparable with that of their cisgender counterparts, and recommend the option of FP. Finally, our data indicate that an antagonist-based protocol for ovarian stimulation triggered by a GnRH agonist for oocytes maturation is a feasible means of ART in this population.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study was approved by the ethics committee (Helsinki) of the Tel Aviv Medical Center (#0647-19-TLV) and the institutional and review board of the Rabin Medical Center (#0757-19-RMC).

Statement of informed consent Informed consent was obtained from all individual participants included in the study.

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