RBMO

ARTICLE

Similar fertilization rates and preimplantation embryo development among testosterone-treated transgender men and cisgender women

BIOGRAPHY

Tal Israeli received her BSc in science from Tel-Aviv University, Israel, where she is currently completing an MD. Her thesis about protein CLIC5 interactants was awarded the Best Thesis Award 2021 and is awaiting publication in the *Israel Medical Association Journal*. Tal has a special interest in reproductive endocrinology, infertility and fertility preservation.

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KEY MESSAGE

Testosterone exposure is not associated with poorer fertilization rates or impaired early embryo development. Embryo cryopreservation is a feasible way for transgender men to preserve their fertility for future biological parenting.

ABSTRACT

Research question: What are the effects of testosterone treatment on oocyte fertilization and preimplantation embryo development among transgender men who have undergone fertility preservation?

Design: A retrospective study was undertaken in a university-affiliated tertiary hospital between April 2016 and November 2021. Embryos were divided into three groups by source: 210 embryos from 7 testosterone-exposed transgender men, 135 from 10 cisgender women who cryopreserved embryos, and 276 from 24 cisgender women who underwent fertility treatment. Statistical analyses compared assisted reproductive technology outcomes between the group of transgender men and both groups of cisgender women. Morphokinetic and morphological parameters were compared between the embryos derived from these three groups.

Results: The transgender men (30.2 ± 3.5 years of age) were significantly younger than the cisgender women who cryopreserved embryos (35.1 ± 1.8 years; $P = 0.005$) and the cisgender women who underwent fertility treatment (33.8 ± 3.2 years; $P = 0.017$). After adjusting for participant age, the fertilization rate was comparable between the transgender men and both groups of cisgender women ($P = 0.391$ and 0.659). There were no significant differences between the transgender men and the cisgender women who preserved fertility in terms of number of cryopreserved embryos (7.2 ± 5.1 and 3.5 ± 2.6; $P = 0.473$) or the distribution of embryo age at cryopreservation ($P = 0.576$). All morphokinetic parameters evaluated by time-lapse imaging, as well as the morphological characteristics, were comparable for the embryos in all three groups.

Conclusions: Testosterone exposure among transgender men has no adverse impact upon fertilization rates or preimplantation embryo development and quality.

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Declaration: The authors report no financial or commercial conflicts of interest.

KEY WORDS

Fertility preservation
Preimplantation embryo development
Testosterone
Time-lapse imaging
Transgender men

INTRODUCTION

Transgender men are assigned female sex at birth but identify as men. This mismatch might induce significant discomfort and distress, a condition known as gender dysphoria. Gender-affirming hormone (GAH) therapy for transgender men includes exogenous testosterone administration that induces 'masculine' physical traits, suppresses 'feminine' ones and reportedly relieves gender dysphoria (*Coleman et al., 2012*). The potential risks and adverse effects of gender-affirming testosterone are well known (*T'Sjoen et al., 2019*); however, the consequences for fertility of long-term exposure of the ovaries to testosterone are not fully understood.

Similar to transgender men treated with testosterone, there is a hyperandrogenic environment in women with polycystic ovarian syndrome (PCOS). As detailed in Feigerlová and colleagues' review (*Feigerlová et al., 2019*), several studies have demonstrated PCOS-related functional and morphological changes in the ovaries of transgender men after testosterone treatment while other studies have reported no or other changes in the ovaries, which is inconsistent with PCOS morphology. Correspondingly, there is no consensus about the effect of testosterone on anti-Müllerian hormone (AMH) concentrations. AMH is a glycoprotein secreted by small antral follicles. Its concentration is strongly correlated with the antral follicle count (AFC), and it serves as a clinically useful marker of functional ovarian reserve. Its values are elevated in PCOS and reduced when the number of developing follicles is decreased (*Iwase et al., 2018*).

Reported data on AMH concentrations among transgender men treated with testosterone are conflicting. One study demonstrated that a relatively short-term testosterone treatment resulted in a strong suppression of AMH secretion (*Caanen et al., 2015*), while others have not shown this (*Yaish et al., 2021*). At the oocyte level, mitotic spindle patterns appear normal with a normal complement of chromosomes in maturate oocytes obtained from the in-vitro maturation of ovaries exposed to testosterone in transgender men who have undergone oophorectomy (*Lierman et al., 2017*). However, the achievement of metaphase II oocytes is not equivalent to the ability to achieve successful fertilization. Indeed, when oocytes were collected under testosterone treatment in transgender men, the ovarian tissue oocytes that had been maturated *in vitro* resulted in a very low rate of successful embryo development, probably because of low maturation and fertilization rates, frequent aberrant cleavage patterns and a low rate of development to blastocyst stage (*Lierman et al., 2021*).

Understanding the effects of long-term testosterone exposure on fertility becomes more and more crucial as more transgender people present for GAH therapy at increasingly younger ages (*Kreukels et al., 2012*). Moreover, many of them express the desire to have biological children and would consider fertility preservation (*Auer et al., 2018*; *De Sutter et al., 2002*; *Wierckx et al., 2012*). Accordingly, several international organizations recommend a discussion about fertility preservation before GAH therapy or surgery (*Deutsch and Feldman, 2013*; *De Wert et al., 2014*; *Ethics Committee of the ASRM, 2015*; *Hembree et al., 2017*). Furthermore, they advise fertility preservation before any exposure to GAH and recommend stopping GAH therapy at least 3 months before the preservation procedure in transgender people who have already started to take hormones (*Deutsch and Feldman, 2013*; *De Wert et al., 2014*; *Ethics Committee of the ASRM, 2015*; *Hembree et al., 2017*).

Feasible fertility preservation options for post-pubertal transgender men include oocyte and embryo cryopreservation (*De Roo et al., 2016*; *Johnson and Finlayson, 2016*), both of which require assisted reproductive technology (ART) that includes hormonal ovarian stimulation followed by oocyte retrieval. Ovarian stimulation outcomes between transgender men who had already initiated hormonal transition with the use of testosterone and matched cisgender women were reported as being comparable. Overall, there were no significant differences in the peak oestradiol concentrations, the number of retrieved oocytes, the number of mature oocytes and the maturity rate of the oocytes between the two groups (*Adeleye et al., 2019*; *Amir et al., 2020*; *Leung et al., 2019*).

The currently limited available data suggest that, despite testosterone treatment, the ovarian reserve and in-vivo oocyte maturation are preserved among transgender men. However, the fertility potential in terms of fertilization and early embryo development from in-vivo-maturated oocytes previously exposed to testosterone has not yet been explored. The present study aimed to assess the impact of testosterone exposure on fertilization and preimplantation embryo development from in-vivo-maturated oocytes collected during fertility preservation in transgender men. The study was a retrospective analysis of IVF cycles conducted at the authors' clinic using time-lapse technology, in which several morphokinetic parameters were compared between a group of testosterone-treated transgender men and two groups of cisgender women. In parallel, embryo static morphology was also retrospectively assessed and compared between these groups.

MATERIALS AND METHODS

Ethical approval

This study was approved on 23 May 2021 by the ethics committee (Helsinki) of the Tel Aviv Sourasky Medical Center (TASMC; no. 0257-21-TLV).

Study population and participant recruitment

This retrospective study was performed between April 2016 and November 2021 at the IVF Unit, Fertility Institute of TASMC, a tertiary university-affiliated medical centre. Thirty-four cisgender women and seven transgender men who underwent conventional IVF cycles were included. All of the transgender men were referred from the endocrinology clinic of the medical centre after they had been evaluated by a community mental health professional and diagnosed as having gender dysphoria according to the *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition 302.85 criteria, and all had embarked upon androgen therapy before initiating the IVF cycles (range 14–156 months). Cisgender women who underwent IVF for social fertility preservation (n = 10) or infertility (n = 24) were chosen as control groups. To prevent any influence of infertility factors on the outcome of IVF, the analysis was limited to data from women who underwent IVF due to unexplained infertility (n = 20, 83%) and mechanical factor infertility (n = 4, 17%).

Data collection

All relevant data were collected from the TASMC computerized database. The data recorded in the electronic charts included the following: clinical details (age, body mass index, marital status, number of children, thyroid-stimulating hormone concentrations, prolactin concentrations, serum total testosterone concentration, sperm origin and previous testosterone treatment), fertility potential details (FSH concentration and AFC), ovarian stimulation details (ovarian stimulation protocol, ovarian stimulation duration, total FSH dose and peak serum oestradiol concentration), outcome (number of retrieved oocytes) and specific IVF outcomes (the number of 2 pronuclei embryos, number of embryos that were frozen, and day each embryo was frozen). All of the transgender men stopped testosterone injections for at least 3 months before referral for fertility preservation (the time of discontinuation of testosterone ranging between 4 and10 months), and menses subsequently resumed in all the participants who had regular menstrual cycles. A regular menstrual cycle was defined as one where the interval between bleeding periods was in the range of 21–35 days.

Ovarian stimulation, fertilization, embryo culture and embryo transfer

Ovarian stimulation was carried out using the gonadotrophin-releasing hormone (GnRH) antagonist, short GnRH agonist and long GnRH agonist protocols (*Amir et al., 2019*). Ovulation was triggered with 250 µg of choriogonadotropin-alpha (Ovitrelle; Serono, Switzerland), 0.2 mg of triptorelin (Decapeptyl; Ferring Pharmaceuticals; Kiel, Germany) or a combination of the two when at least three follicles had achieved a diameter of 18 mm. Ovum retrieval was performed 36 h later, and the embryologists determined the total number of oocytes retrieved per cycle.

All of the embryos in this study were fertilized by conventional IVF. They were all incubated in an integrated EmbryoScope time-lapse monitoring system (UnisenseFertiliTech A/S, Vitrolife, Denmark) from the time of fertilization until transference or cryopreservation. Either embryo transfer or cryopreservation was carried out 2–6 days following oocyte retrieval. The developmental stage at which the embryos were frozen for each of the patients was determined according to a set of clinical and laboratory considerations including the patient's age, the number of fertilized eggs, the number of embryos, the quality of the embryos in previous cycles and the overall quality of the embryos. Data on frozen embryos were compared only between the transgender men and cisgender women who underwent embryo cryopreservation (embryo transfer was performed in most of the cisgender women who underwent IVF due to infertility and therefore these comparisons were not carried out).

Time-lapse monitoring of embryo morphokinetics and morphology assessment

Use of time-lapse imaging is standard practice in the authors' IVF laboratory in addition to a morphological assessment of all embryos. Embryo scoring and selection by means of time-lapse monitoring were performed by analysing the time-lapse images of each embryo with software developed specifically for image analysis (EmbryoViewer workstation; UnisenseFertilitech A/S). Embryo morphology and developmental events were recorded in order to demonstrate the precise timing of the observed cell divisions in relation to the timing of fertilization, specifically the time of pronuclei fading (tPNf) and time of cleavage to a 2-blastomere embryo (t2), a 3-blastomere embryo (t3), a 4-blastomere embryo (t4), etc. up to an 8-blastomere (t8) embryo. The time point t8 was the last assessed parameter even for embryos that were further cultured to be transferred or frozen on day 5. The other analysed parameters were the lengths of the second and third-cell cycles (cc2 and cc3, respectively), and the synchrony in the division from 3 to 4 cells (s2) and from 5 to 8 cells (s3). All the assessments of the embryos were performed by senior embryologists. Scores were allocated to day 3 embryos by means of the KIDScore algorithm (*Petersen et al., 2016*). Conventional morphology of the embryos was studied on day 3, taking into account the number of blastomeres, the symmetry among blastomeres and the degree of fragmentation. The embryos were scored from grade 1 (high quality) to grade 4 (poor quality) in accordance with these parameters (*Racowsky et al., 2009*; *Racowsky et al., 2010*).

Statistical analysis

Data were analysed using SPSS version 25.0 (SPSS Inc., USA). They were summarized as mean ± SD, mean ± SE or number of responders (percentage) according to the variables. The comparison of continuous variables between groups was made using a Independent Samples T-Test. The effect of the participant's age on the other variables was tested with a Pearson's correlation. Significance was tested using a t-test, Mann–Whitney *U*-test, chi-squared test or Fisher's exact test, as appropriate. Analysis of variance was used to control for participant age when a significant correlation was found. The effect sizes were calculated by means of Cohen's D. The effect of testosterone treatment on morphokinetic parameters was assessed by a mixed-model analysis. A *P*-value of <0.05 was considered significant.

RESULTS

Clinical characteristics of the study participants

Eight transgender men cryopreserved embryos in the authors' unit during the study period, and seven of them were included in this study (one had undergone fertilization by means of intracytoplasmic sperm injection and was therefore excluded from the analysis). The control groups comprised 34 cisgender women, of whom 10 were fertile and had cryopreserved embryos and 24 had undergone fertility treatment.

The clinical characterizations of the entire cohort are detailed in TABLE 1. The seven transgender men (30.2 ± 3.5 years of age) were significantly younger than the 10 cisgender women who preserved fertility (35.1 ± 1.8 years; $P = 0.005$) and the cisgender women who underwent fertility treatment (33.8 ± 3.2 years; $P = 0.017$). No significant differences in the ovarian reserve markers, including FSH and AFC, were observed between the three groups. The serum total testosterone concentration was significantly higher among the transgender men (2.8 ± 2.9 nmol/l) compared with both groups of cisgender women (1.2 ± 0.5 nmol/l and 1.5 ± 0.4 nmol/l; $P = 0.045$ and 0.046, respectively). Among the transgender men, the mean testosterone exposure was 99.7 ± 49.2 months (range 14–156 months) and the mean time of discontinuation of testosterone prior to stimulation was 6.5 ± 2.1 months (range 4–10 months).

Ovarian stimulation outcomes

The ART data and outcomes of the three groups are summarized in TABLE 2. There

TABLE 1 COMPARISON OF CLINICAL PARAMETERS BETWEEN TRANSGENDER MEN AFTER TESTOSTERONE TREATMENT AND CISGENDER WOMEN

Characteristic	Group A Transgender men (*n* = 7)	Group B Cisgender women who underwent fertility preservation (*n* = 10)	Group C Cisgender women who underwent fertility treatment (*n* 24)	*P* value A versus B	*P* value A versus C
Age (years), mean (SD)	30.2 (3.5)	35.1 (1.8)	33.8 (3.2)	0.005	0.017
BMI (kg/m^2), mean (SD)	23.0 (2.4)	23.7 (3.9)	21.1 (2.2)	0.819	0.209
Marital status, *n* (%)				1.00	0.009
Single	7 (100)	10 (100)	10 (41.7)		
Married	0 (0)	0 (0)	14 (58.3)		
Children (*n*), mean (SD)	0 (0)	0 (0)	0.46 (0.5)	1.00	0.043
TSH (µIU/ml), mean (SD)	1.8 (0.7)	1.9 (0.7)	2.0 (1.0)	0.964	0.744
Prolactin (mIU/l), mean (SD)	308.8 (139.2)	279.2 (102.6)	301.1 (170.4)	0.876	0.988
Testosterone (nmol/l)	2.8 (2.9)	1.2 (0.5)	1.5 (0.4)	0.045	0.046
FSH (mIU/ml)	7.1 (3.2)	5.7 (2.4)	6.9 (1.9)	0.335	0.953
AFC (total *n*)	13.4 (5.9)	15.1 (6.3)	15.7 (6.6)	0.842	0.729
Sperm origin, *n* (%)				0.603	0.028
Partner	1 (14.3)	3 (30)	16 (66.7)		
Donor	6 (85.7)	7 (70)	8 (33.3)		
Testosterone therapy (months)				–	–
Time on testosterone (SD)	99.7 (49.2)	–	–		
Range	14-156	–	–		
Time off testosterone (SD)	6.5 (2.1)	–	–		
Range	4-10	–	–		

Values are presented as mean (SD) or *n* (%).

Standard reference ranges: TSH, 0.5–4.8 µIU/ml; prolactin, 108.78–557.13 mIU/l; testosterone, 0.48–1.85 nmol/l; FSH, 1–9.2 mIU/ml.

A *P*-value of <0.05 was considered significant.

AFC, antral follicle count; BMI, body mass index; TSH, thyroid-stimulating hormone.

was no difference in the mean number of FSH stimulation days between the transgender men (10.3 ± 1.2 days) and the cisgender women who cryopreserved embryos (11.6 ± 1.6 days; $P = 0.282$), and the cisgender women who underwent fertility treatment (9.8 ± 1.9 days; $P = 0.319$). There was no significant difference in the amount of FSH used for ovulation induction between the transgender men (2743.3 ± 783.7 mIU/ml) and the cisgender women who had undergone fertility preservation (3657.6 ± 1154.6 mIU/ml; $P = 0.178$); however, the amount of FSH used was significantly higher for the transgender men (2743.3 ± 783.7 mIU/ml) compared with the cisgender women who underwent fertility treatment (2029.8 ± 959.2 mIU/ml; $P = 0.024$). There was no difference in peak oestradiol concentrations between the transgender men and the cisgender women who cryopreserved embryos (2777.4 ± 1506.0 mIU/ml and 2557.5 ± 1965.6 mIU/ml, respectively; $P = 0.808$) or the cisgender infertile women (1785.6 ± 951.4 mIU/ml; $P = 0.083$).

Although no difference in the mean number of oocytes retrieved between the transgender men (21 ± 10.9) and the cisgender women who had undergone fertility preservation (10.3 ± 6.7; $P = 0.095$) was observed, the mean number of oocytes retrieved from the transgender men (21 ± 10.9) was significantly higher compared with the mean number of oocytes retrieved from the cisgender women who had undergone fertility treatment (11.5 ± 5.7; $P = 0.033$). There was no significant difference in the fertilization rate between the transgender men and each group of cisgender women ($P = 0.391$ and 0.659, respectively). Finally, no significant differences between the cryopreserved embryos of the transgender men and those of the cisgender women who preserved fertility were observed in terms of the number (7.2 ± 5.1 and 3.5 ± 2.6, respectively; $P = 0.473$) or the distribution of embryo age at cryopreservation ($P = 0.576$).

Morphokinetic and morphological characteristics

A total of 210 embryos of transgender men were compared morphokinetically with 135 embryos from cisgender women who cryopreserved fertility and 276 embryos from women who had undergone fertility treatment. Early embryonic development was recorded by time-lapse imaging. The mean timing of tPNf, t2–t8, cc2, cc3, s2 and s3 were not significantly different between the transgender group and the two cisgender groups (TABLE 3).

The KIDScore was calculated for 127 embryos from transgender men, 74 embryos from cisgender women who cryopreserved fertility, and 235 embryos from women who had undergone fertility treatment. All of these embryos were available for embryoscopic analysis at 66 h and were fit to be graded according to the model; in this, each

TABLE 2 COMPARISON OF OVARIAN STIMULATION DATA AND OUTCOMES BETWEEN TRANSGENDER MEN AFTER TESTOSTERONE TREATMENT AND CISGENDER WOMEN

Characteristic	Group A Retrieval cycles in transgender men (*n* = 10)	Group B Retrieval cycles in cisgender women whounderwent fertility preservation (*n* = 13)	Group C Retrieval cycles in cisgender women who underwent fertility treatment (*n* = 35)	*P*-value A versus B	*P*-value A versus C
Retrieval cycles per patient, mean	1.43	1.30	1.46	0.896	0.992
Ovarian stimulation protocol				0.603	0.129
GnRH antagonist	9 (90)	10 (76.9)	21 (60)		
Short GnRH agonist	1 (10)	2 15.4)	14 (40)		
Long GnRH agonist	–	1 (7.7)	–		
FSH stimulation days (*n*)	10.3 (1.2)	11.6 (1.6)	9.8 (1.9)	0.282	0.319
FSH total dose (mIU/ml)	2743.3 (783.7)	3657.6 (1154.6)	2029.8 (959.2)	0.178	0.024
Peak oestradiol (pg/ml)	2777.4 (1506.0)	2557.5 (1965.6)	1785.6 (951.4)	0.808	0.083
Final maturation trigger				0.604	<0.001
HCG	1 (10)	3 (23.1)	33 (94.3)		
GnRH agonist	9 (90)	10 (76.9)	1 (2.9)		
HCG + GnRH agonist	–	–	1 (2.9)		
Oocytes retrieved (*n*)	21 (10.9)	10.3 (6.7)	11.5 (5.7)	0.095	0.033
2PN embryos	11.9 (7.3)	5.8 (3.3)	6.6 (4.3)	0.324	0.167
Fertilization rate, % (SD)	56.5 (15.6)	61.8 (26.7)	57.6 (21.1)	0.391	0.659
Total *n* of cryopreserved embryos[a]	7.2 (5.1)	3.5 (2.6)	–	0.473	–
Day embryos were cryopreserved[a]				0.576	–
Day 2 or 3	4 (40)	8 (61.5)	–		
Day 5 or 6	4 (40)	3 (23.1))	–		
Day 3 and 5	2 (20)	2 (15.4)	–		

Values are presented as mean (SD) or *n* (%) unless indicated otherwise.

A *P*-value of <0.05 was considered significant.

[a] Data on cryopreserved embryos were compared only between transgender men and cisgender women who underwent embryo cryopreservation (embryo transfer was performed in most cisgender women who underwent IVF due to infertility and therefore these comparisons were not done).

2PN, 2 pronuclei; GnRH, gonadotrophin-releasing hormone; HCG, human chorionic gonadotrophin.

embryo receives a score between 1 and 5 (1 indicating the lowest potential for pregnancy and 5 indicating the highest). The mean scores were similar for embryos from the transgender men and from the cisgender women who preserved fertility and the cisgender women who had undergone fertility treatment (3.5 ± 0.3 versus 3.4 ± 0.3 and 3.0 ± 0.1, *P* = 0.896 and 0.229, respectively; TABLE 3). However, a significant group difference between the proportion of embryos graded either 4 or 5 or 2 or less was observed (*P* = 0.006 and 0.015; TABLE 4).

To complete the embryo development assessment, a conventional morphological evaluation was performed on day 3. The number of blastomeres, symmetry among blastomeres and degree of fragmentation were examined, and each embryo received a score between 1 and 4 (1 indicating the highest potential for pregnancy and 4 indicating the lowest). The mean scores were similar for embryos from the transgender men and both groups of cisgender women (2.1 ± 0.2 versus 2.2 ± 0.2 and 2 ± 0.1; *P* = 0.761 and 0.666, respectively; TABLE 3). No significant group difference between the proportion of embryos graded 1 or 2, or those graded 3 or more was observed (*P* = 0.405 and 0.073; TABLE 4). Finally, no significant group difference was found in the percentage of embryos whose development was stopped before day 3 (8.1% versus 8.1% and 11.6%; *P* = 0.406 and 0.993; TABLE 3).

DISCUSSION

To the best of the authors' knowledge, this is the first study to demonstrate that oocytes from transgender men exposed to long-term testosterone treatment were fertilized and cleaved to the two-cell stage to the same extent as those of two control groups of cisgender women. Furthermore, the total analysis of 621 embryos (210 from transgender men and 411 from cisgender women) indicates that the development and quality of embryos from testosterone-exposed transgender men were comparable to those of embryos from cisgender women.

The group of transgender men was compared with two groups of cisgender women, one consisting of fertile women who cryopreserved embryos and the other including women who underwent IVF for fertility treatment. The duration of hormonal stimulation was similar in all three groups, but the total doses of gonadotrophins were significantly higher in the transgender men compared with the cisgender women who underwent fertility treatment. The administration of high gonadotrophin doses is reportedly acceptable for achieving adequate

TABLE 3 COMPARISON OF MORPHOKINETIC PARAMETERS OF EMBRYOS FROM TRANSGENDER MEN AFTER TESTOSTERONE TREATMENT AND FROM CISGENDER WOMEN

Parameter	Group A Embryos derived from transgender men (*n* = 210)	Group B Embryos derived from cisgender women who underwent fertility preservation (*n* = 135)	Group C Embryos derived from cisgender women who underwent fertility treatment (*n* = 276)	*P*-value A versus B	*P*-value A versus C
tPNf	26.4 (1.0)	27.9 (0.9)	27.9 (0.6)	0.366	0.276
t2	29.9 (1.2)	32.2 (1.1)	31.2 (0.6)	0.197	0.396
t3	39.1 (1.3)	41.5 (1.2)	40.2 (0.7)	0.209	0.501
t4	41.2 (1.4)	43.9 (1.4)	43.1 (0.8)	0.222	0.280
t5	52.5 (1.7)	53.9 (1.6)	52.5 (0.9)	0.570	0.977
t6	55.9 (2.2)	56.8 (2.1)	56.4 (1.2)	0.785	0.869
t7	58.9 (2.5)	59.1 (2.3)	59.6 (1.4)	0.948	0.799
t8	61.2 (2.6)	63.5 (2.5)	63.3 (1.5)	0.561	0.509
cc2	9.4 (0.5)	9.7 (0.6)	9.2 (0.3)	0.793	0.763
cc3	13.7 (0.9)	12.1 (1.0)	12.6 (0.5)	0.305	0.379
s2	2.3 (0.6)	2.3 (0.7)	2.9 (0.4)	0.959	0.495
s3	9.7 (1.8)	9.5 (1.9)	11.3 (1.1)	0.937	0.486
KIDScore[a]	3.5 (0.3)	3.4 (0.3)	3.0 (0.1)	0.896	0.229
Morphological score[a]	2.1 (0.2)	2.2 (0.2)	2.0 (0.1)	0.761	0.666
Number of embryos whose development was stopped before day 3 (%)	17 (8.1)	11 (8.1)	32 (11.6)	0.406	0.993

Values are presented as mean (SE) unless otherwise stated.

A *P*-value of <0.05 was considered significant.

[a] Morphokinetic data were obtained from all embryos produced, while KIDScore values and morphological scores were assigned to day 3 embryos.

cc2, cc3, length (hours) of the second and third cell cycles; s2, s3, synchrony (hours) in the division from 3 to 4 and from 5 to 8 cells; t2, t3, t4, t5, t6, t7, t8, time (hours) between fertilization and pronuclei fading, 2-, 3-, 4-, 5-, 6-, 7- and 8-cell stages; tPNf, pronuclei fading.

TABLE 4 DISTRIBUTION OF KIDSCORE AND MORPHOLOGY SCORE OF TRANSGENDER MEN AFTER TESTOSTERONE TREATMENT AND OF CISGENDER WOMEN

Embryo category	Group A Embryos derived from transgender men	Group B Embryos derived from cisgender women who cryopreserved embryos	Group C Embryos derived from cisgender women who underwent fertility treatment	*P*-value A versus B	*P*-value A versus C
KIDScore					
Number of embryos	127	74	235	0.006	0.015
1 (low quality)	18 (14.2)	7 (9.5)	53 (22.6)		
2	25 (19.7)	31 (41.9)	51 (21.7)		
3	3 (2.4)	0 (0)	20 (8.5)		
4	31 (24.4)	9 (12.2)	42 (17.9)		
5 (high quality)	50 (39.4)	27 (36.5)	69 (29.4)		
Morphology score					
Number of embryos	129	79	238	0.405	0.073
4 (low quality)	24 (18.6)	13 (16.5)	24 (10.1)		
3	33 (25.6)	26 (32.9)	74 (31.1)		
2	8 (6.2)	8 (10.1)	24 (10.1)		
1 (high quality)	64 (49.6)	32 (40.5)	116 (48.7)		

Values are presented as *n* (%).

KIDScore values and morphological scores were assigned to day 3 embryos.

A *P*-value of <0.05 was considered significant.

numbers of oocytes in freeze-all cycles (*Mizrachi et al., 2020*). As expected, the number of retrieved oocytes was comparable between the two groups of individuals who preserved fertility and significantly higher in the transgender men compared with the cisgender women with fertility problems. Similar to the findings of the current study, there is a fair amount of evidence that transgender men exposed to testosterone can provide mature oocytes comparable to what is seen in cisgender women (*Adeleye et al., 2019*; *Amir et al., 2020*; *Leung et al., 2019*,). In addition, human oocytes collected from the ovarian cortex of testosterone-exposed transgender men have demonstrated a normal meiotic spindle structure after in-vitro maturation (*Lierman et al., 2017*).

There are several lines of evidence relating to the oocyte fertilization of transgender men who have previously been treated with testosterone (*Adeleye et al., 2019*; *Amir et al., 2020*; *Leung et al., 2019*). In the current study, fertilization rates were comparable in all three study groups. However, it is still unknown how past testosterone treatment might affect early embryo development. Various patterns of embryo cleavage have been related to different success rates for embryo implantation, clinical pregnancy and live birth (*Adamson et al., 2016*; *Meseguer et al., 2011*; *Pribenszky et al., 2017*). Using time-lapse imaging, the current study found that the morphokinetic parameters of embryos derived from testosterone-treated transgender men were comparable to those of embryos from cisgender women.

Three studies by other groups have explored the influence of testosterone therapy on early embryo development, but none of them examined it among in-vivo human maturated oocytes. Rothenberg and colleagues (*Rothenberg et al., 2019*) reported impaired fertilization in oocytes retrieved from female mice on active treatment with testosterone, but blastocyst progression was normal in those that did become fertilized. Bartels and co-workers (*Bartels et al., 2021*) found that although ovaries from testosterone-treated mice were significantly smaller than those of non-treated mice, they contained normal numbers of follicles and responded to gonadotrophin stimulation by the ovulation of similar numbers of eggs that fertilized and cleaved *in vitro*. Lierman and collaborators (*Lierman et al., 2021*) recently explored the developmental capacity of in-vitro-maturated oocytes originating from cumulus complexes found during ovarian tissue preparation at the moment of gender-confirmation surgery and during testosterone treatment for fertility preservation in transgender men. Those authors demonstrated a very low rate of successful embryo development as reflected by low maturation and fertilization rates, frequent aberrant cleavage patterns and low rates of development to the blastocyst stage. However, ovarian tissue oocyte in-vitro maturation for fertility preservation is still an experimental technique and not amenable to a comparison with the standard IVF used in the current study.

Several studies of transgender men have shown PCOS-related functional and morphological changes in their ovaries after testosterone treatment (*Futterweit and Deligdisch, 1986*; *Loverro et al., 2016*; *Pache et al., 1991*; *Spinder et al., 1989*). These changes were attributed to a hyperandrogenic environment found both in women with PCOS and in testosterone-exposed transgender men. Previous studies that explored the association between PCOS and early embryo morphokinetics reported conflicting findings. Some observed significant delays in some morphokinetic parameters (*Tabibnejad et al., 2019*; *Wissing et al., 2014*), while others found no differences in those parameters (*Sundvall et al., 2015*, *Tam et al., 2019*). Interestingly, the transgender men in the current study displayed slightly increased testosterone concentrations. It is not, however, possible to reach any firm conclusions about the incidence of PCOS in these transgender individuals because they did not undergo a full evaluation according to the Rotterdam criteria (*Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group, 2004*).

Congenital adrenal hyperplasia (CAH) is another condition involving endogenous hyperandrogenism. Interestingly, individuals with CAH are reportedly more prone to have gender dysphoria (*Dessens et al., 2005*). Rates of infertility and miscarriage risk among women with CAH are higher than those in the general population (*Stikkelbroeck et al., 2003*). However, it is difficult to isolate the effect of testosterone on fertility from other confounding factors related to fertility in women with CAH (including high progesterone concentrations, endometrial defects, previous genital surgery and neuroendocrine factors).

All of the transgender men included in the current study stated that they did not intend to carry the pregnancy, and that the embryos would be transferred to another country for surrogacy, which was prohibited in the authors' country at the time of data collection. To date, four of them have sent the embryos abroad, and in all four cases pregnancies have been achieved using surrogates. Two surrogates have given birth, one to a singleton and the other to twins. Two additional surrogates are pregnant, one with a twin pregnancy at week 20 and the other in the first trimester of pregnancy.

Several limitations of the present study bear mention. First, it is retrospective in design. Second, the findings must be taken with extreme caution in light of the very small number of participants. However, this is an inherent limitation due to the small number of transgender individuals who access fertility care. The authors believe that the important takeaway message from this paper is that transgender men do not perform more poorly than cisgender women in the area reported. Third, the control groups were composed of women who were significantly older than the study group and of women who were receiving fertility treatment, both of which are factors that could have influenced the results. A better comparison group would have been fertile women of the same age as the study group. Fourth, the standard practice is to freeze embryos at the blastocyst stage. In this study, embryos were frozen at different stages of development and therefore the total number of frozen embryos is less interpretable because all the embryos do not have a similar implantation potential (day 3 embryos perform more poorly than day 5 embryos, etc). However, when compared with the cisgender group, more embryos from the transgender group were frozen at the blastocyst stage, which strengthens the main conclusion that transgender men do not perform more poorly than cisgender women on the study's measures. Finally, all of the transgender men in this study discontinued testosterone treatment before starting ovarian stimulation,

and stopping hormonal therapy might cause considerable anguish and gender dysphoria (*Baram et al., 2019*; *Voultsos et al., 2021*). Therefore, further studies that include participants who do not stop exposure to testosterone before fertility preservation are needed.

In conclusion, this study demonstrates that transgender men have a fertilization rate comparable to that of cisgender women even after previous long-term exposure to testosterone. In addition, preimplantation embryo development is not impaired after chronic testosterone therapy. Therefore, embryo cryopreservation can be considered an effective way for transgender men to preserve their fertility for future biological parenting. Further studies are needed to examine whether testosterone treatment should be discontinued before ovarian stimulation among transgender men and, if so, for how long. It would also be interesting to explore the pregnancy outcomes among transgender men who have undergone fertility preservation.

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Received 12 February 2022; received in revised form 13 April 2022; accepted 26 April 2022.