Journal Pre-proof

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J.D. Asseler , J. Knieriem , J.A.F. Huirne , M. Goddijn , M.O. Verhoeven , N.M. van Mello

PII: S1472-6483(23)00162-1
DOI: https://doi.org/10.1016/j.rbmo.2023.03.007
Reference: RBMO 3196

To appear in: *Reproductive BioMedicine Online*

Received date: 6 December 2022
Revised date: 17 February 2023
Accepted date: 8 March 2023

Please cite this article as: J.D. Asseler , J. Knieriem , J.A.F. Huirne , M. Goddijn , M.O. Verhoeven , N.M. van Mello , Outcomes of oocyte vitrification in trans masculine persons, *Reproductive BioMedicine Online* (2023), doi: https://doi.org/10.1016/j.rbmo.2023.03.007

Highlights

1. ***Trans masculine persons show a normal response to COH for oocyte vitrification***
2. ***Prior testosterone use does not negatively impact treatment outcomes***
3. ***Hormone injections were identified as the most burdensome part of treatment***
4. Even though treatment was burdensome, patients were satisfied with the outcomes***.***

Outcomes of oocyte vitrification in trans masculine persons

Authors:

J.D. Asseler*[1,3,4]; J. Knieriem[1] ; J.A.F. Huirne[1,4]; M. Goddijn[2,4]; M.O. Verhoeven[1,4]; N.M. van Mello[1,3,4]

Affiliations

[1] Amsterdam UMC, location Vrije Universiteit Amsterdam, Department of Obstetrics and Gynaecology, De Boelelaan 1117, Amsterdam, the Netherlands

[2] Amsterdam UMC location University of Amsterdam, Department of Obstetrics and Gynecology, Meibergdreef 9, Amsterdam, The Netherlands

[3] Amsterdam UMC, Centre of Expertise on Gender Dysphoria, Amsterdam, The Netherlands

[4] Amsterdam Reproduction and Development research institute, Amsterdam, The Netherlands

*** Corresponding author:**

J.D Asseler, M.D.

E-mail: j.d.asseler@amsterdamumc.nl

ORCHID: 0000-0002-3823-529X

Postal address:

Center of Expertise on Gender Dysphoria (poli N)

Amsterdam UMC, location VUmc.

Antwoordnummer 7700, 1000SN, The Netherlands.

Authors roles:

J.A. and N.M. conceived and designed the study. J.A and J.K. contributed to acquisition of the data. J.A. contributed to the analysis and J.A., J.H., M.G., M.V. and N.M. contributed to the interpretation of the data. All authors made contributions to drafting and revising the

article critically for important intellectual content. All authors approved the final version of the article to be published.

Disclosure Statement:

M.G. and M.V. declare that their department (reproductive medicine) received research and educational grants from Guerbet, Merck and Ferring (all location VUmc), outside the scope of the submitted work. J.H. received grants from ZonMw, Samsung and PlantTec MedicalGmbH outside the scope of the submitted work. J.A., J.K., N.M. have no conflict of interest to disclose.

Data sharing statement:

The data underlying this article will be shared on reasonable request to the corresponding author.

Abstract

Research Question: What are the outcomes of oocyte vitrification treatment in trans masculine individuals (TMI's) prior to, and after testosterone use? And how did TMI's experience oocyte vitrification treatment?

Design: This retrospective cohort study was performed at the Amsterdam UMC in the Netherlands between January 2017 and June 2021. TMI's who had completed an oocyte vitrification treatment were consecutively approached for participation. Informed consent was provided by 24 persons. Participants (n=7) who initiated testosterone therapy were advised 3 months cessation prior to stimulation. Demographic characteristics and oocyte vitrification treatment data were retrieved from medical records. Evaluation of the treatment was collected via an online questionnaire.

Results: The median age of participants was 22.3 years (IQR 21.1 – 26.0) and mean BMI was 23.0 kg/m2 (SD 3.2). Following controlled ovarian hyperstimulation, a mean of 20 oocytes (SD 7) were retrieved and a mean of 17 oocytes (SD 6) could be vitrified. Aside from

a lower cumulative FSH dose, there were no significant differences between the prior testosterone users and testosterone naïve TMI's.. The overall satisfaction of oocyte vitrification treatment in participants was high. Hormone injections were considered the most strenuous part of treatment by 29% of participants, closely followed by ovum pick-up(25%).

Conclusions: There is no difference in response to controlled ovarian hyperstimulation for oocyte vitrification treatment between the prior testosterone users and testosterone naïve TMI's. The questionnaire identifies hormone injections as the most burdensome aspect of the oocyte vitrification treatment. We can use this information to improve gender sensitive fertility counselling and fertility treatment strategies.

Key words

Cryopreservation, Transgender, OHSS, Oocyte vitrification, Fertility Preservation

Introduction

A desire for biological children is not related to ones gender identity.However, some gender affirming treatments provided for gender incongruence may lead to infertility. Gender affirming treatment may consist of gender affirming hormone therapy (GAHT) and/or surgeries. Transgender men and gender diverse (TGD) persons assigned female at birth (we will use the term trans masculine individuals (TMI's) from here onward), may start testosterone to aid their transition. Gender affirming surgeries (GAS) may include a mastectomy, hysterectomy, oophorectomy, colpectomy, metoidioplasty and/or phalloplasty *(Wiepjes et al., 2018)*. Some gender affirming treatments have permanent consequences for fertility. Specifically, a hysterectomy annuls the option for future pregnancy and an oophorectomy annuls the option for future use of oocytes if these are not secured prior to this surgery. Since testosterone is categorized as teratogenic and usually leads to an anovulatory state and amenorrhea in its trans masculine users, the negative effect of exogenous testosterone on fertility is often hypothesized. So far, studies on fertility in TMI's seem reassuring, describing successful ART (oocyte cryopreservation, fertilization and live birth

rates) *(Leung et al., 2019)* as well as natural conception*(Light et al., 2014)* after using testosterone but these are case series or small sample size cohort studies.

Since the desire for genetic offspring may already exist or develop over time, it is vital healthcare professionals provide information on fertility preservation (FP) prior to gender affirming treatments with negative fertility consequences *(Coleman et al., 2022)*. FP for TMI's currently includes oocyte vitrification. Oocyte vitrification treatment is considered a burdensome treatment for cis gender (i.e. non TGD) women but may be even more burdensome for TMI's due to their experienced gender dysphoria *(Armuand et al., 2017)*. Gender dysphoria is broadly defined as discomfort or distress that is caused by a discrepancy between a person's gender identity and that person's sex assigned at birth (and the associated gender role and/or primary and secondary sex characteristics)*(Coleman et al., 2022)*.

TGD specific drawbacks of oocyte vitrification treatment are rising serum estradiol (E2) levels, postponing or discontinuation testosterone, internal ultrasounds/examination and bleeding periods. Other than being a burdensome and a potentially dysphoria triggering treatment, oocyte vitrification is a medical intervention bearing an – although low – risk of side effects and complications. The ovum pick-up (OPU) comes with pain, a risk of blood loss or infection. Furthermore, a high response to hormonal treatment, usually in younger patients, may also result in an ovarian hyperstimulation syndrome (OHSS)*(Klemetti et al., 2005)*.

Even though oocyte vitrification, IVF and ICSI treatments have been performed and studied for several years in cis gender women, outcomes of oocyte vitrification treatment in TMI's and especially their experiences undergoing said treatment remain under-explored.

Our objective is to describe the outcomes of oocyte vitrification treatment of TMI's and report on their experiences reflecting on this treatment at our center. This data may identify trans

masculine specific healthcare needs so we can improve gender sensitive fertility counselling and fertility treatment strategies.

Materials and methods

Study Design and Population

This single center, retrospective cohort study was performed by the Center of Expertise on Gender and the Center for reproductive medicine both located at the Amsterdam UMC, location VUmc, Amsterdam, the Netherlands.

All TMI's, assigned female at birth, referred to the center of reproductive medicine at the Amsterdam UMC for FP and who underwent oocyte vitrification between January 2017 and June 2021 were approached for participation in the study. Demographic characteristics on treatment data and complications were collected from medical records. OHSS was classified into three categories: mild, moderate and severe as described by Golan et al*(Golan and Weissman, 2009)*. Evaluation of the treatment was assessed by an online survey.

(Gender Affirming) Hormone Treatment

Oocyte vitrification is performed either prior to commencing gender affirming hormone treatment with testosterone or after three months of testosterone cessation. In testosterone TMI's users, the cessation of testosterone was carefully timed to prevent this period from being longer than absolutely necessary.

Following the European protocol*(Dekker et al., 2016)*, gender affirming hormone treatment consists of testosterone administration dermally (Androgel®, Besins International (25 or 50 mg, daily)) or via intramuscular injection (either Nebido®, Bayer (1 g, per 10–14 weeks) or Sustanon®, Aspen Pharma Trading Limited (250 mg per 2–4 weeks)). Prior to starting testosterone, adolescents may use puberty suppression treatment with gonadotropin-releasing hormone agonist (GnRHa), triptolin (Pamorelin®, Ipsen (11,25 mg per 12 weeks)) to prevent (further) development of secondary sex characteristics discordant with the experienced gender identity.

Since the often desired cessation of menses is not immediately, or sometimes ever, achieved whilst on testosterone, some TMI's use a form of progesterone-only cycle regulation like lynestrenol or norethisterone in combination with testosterone.

Controlled Ovarian Hyperstimulation

Oocyte vitrification at our center is performed in persons between the age of 16 and 39 years. The standard ovarian stimulation protocol in our center was a long agonist protocol. Patients received daily triptorelin (Decapeptyl®, Ferring) 0.1 mg subcutaneous, preceding the vitrification treatment from cycle day (cd) 21 in a natural cycle, or 1 week prior to stopping their cycle regulation, for pituitary down regulation. Stimulation was started on cd three by daily injection of recombinant follicle stimulation hormone (r-FSH) (Gonal-F®, Merck Serono) after a transvaginal ultrasound. The r-FSH dose was determined by antral follicle count (AFC) while also taking into account that TMI's would probably only want to undergo this treatment once. After 5 stimulation days the r-FSH dose could be adjusted according to ovarian response. Triptorelin and r-FSH was continued until day of trigger administration. Recombinant human chorionic gonadotropin (r-hCG) (Ovitrelle® Merck Serono) was used as a trigger when the major cohort of follicles reached a diameter of 18 mm. OPU was performed 36 hours later by transvaginal puncture with the patient under conscious sedation (Midazolam) and analgesia (Pethidine).

If patients used puberty suppression treatment with GnRHa, the ovarian stimulation protocol was started immediately after the depot triptorelin should be renewed, resulting in ultra-long agonist protocol. Patients who were already using other hormone treatment for cycle regulation were also classified as following an ultra-long agonist protocol.

Oocyte Vitrification Technique

Oocytes were denuded within two hours after oocyte retrieval. Following the nuclear maturity evaluation, only the metaphase I and II (MI and MII) oocytes were selected for immediate vitrification. Vitrification was performed according to the Cryotop® method, as described

elsewhere*(Kuwayama, 2007)*. All the vitrification solutions were obtained from Kitazato (Kitazato BioPharma Co. Ltd., Shizuoka, Japan).

Survey

A questionnaire, designed for this study, was developed by the research team based on (sub)themes described in three qualitative studies describing the experiences of TMI's during their fertility treatment*(Armuand et al., 2017, Bartholomaeus and Riggs, 2020, Birenbaum-Carmeli et al., 2020)* as well as anecdotal patient feedback on the oocyte vitrification treatment. In many qualitative studies evaluating assisted reproductive technology, treatment cost is often mentioned as a barrier to care. However, in the Netherlands gender dysphoria is considered a medical indication for FP and is therefore covered by insurance. Cost was therefore not included in the multiple choice options.

The questions focused on the personal experience of the patient during FP treatment. The questionnaire comprised of ten multiple-choice items, with an free text option 'other' added. When applicable, Likert scale items were used, ranging from very dissatisfied to very satisfied. At the end of the questionnaire, there was an open-ended item so the participant could further elaborate or introduce new items.

All questionnaires were administered via Castor's Electronic Data Capture (EDC) platform, a secure web-based application for electronic collection and management of research data. Castor complies with all applicable laws and regulations, including ICH E6 Good Clinical Practice (GCP), 21 CFR Part 11, EU Annex 11, General Data Protection Regulation (GDPR), HIPAA (US), ISO 9001 and ISO 27001.

Statistics Analyses

Statistical procedures were performed using SPSS version 20.0 (SPSS, Inc., Chicago, IL, USA). Baseline characteristics of the cohort are presented as mean with standard deviation (SD) when normally distributed, and as median with interquartile range (IQR) when non-normally distributed. Qualitative data are presented as number with percentage. Subgroup

analyses were performed for the persons having used testosterone prior to medical oocyte vitrification treatment. Unpaired T-testing was performed in normally distributed groups. In non-normally distributed groups, non-parametric Mann–Whitney U and Chi-square tests were performed where appropriate. A p value of 0.05 was considered statistically significant.

Ethical Approval

This study did not fall within the scope of the Dutch Medical Research with Human Subjects Law (WMO) and was reviewed and approved by the local Ethical Committee of the Amsterdam UMC, location VUmc (METC no. 2020.0635). Written informed consent was obtained from all participants.

Results

Participants

Between January 2017 and June 2021, 38 TMI's were referred to the center of reproductive medicine attached to the Center of Expertise on Gender at the Amsterdam UMC after fertility counseling. Following a second consultation at the fertility clinic, 30 persons underwent oocyte vitrification treatment and were approached for participation in the study. Five persons were unwilling to participate in the study, and one person could not be reached. Twenty-four persons provided informed consent and were included in the study (FIGURE 1).

The baseline characteristics of all participants are presented in TABLE 1. The median age and mean BMI of participants was 22.3 years (IQR 21.1 – 26.0) and 23.0 kg/m2 (SD 3.2), respectively. Seven persons were using testosterone, two persons were using puberty suppression (GnRH analogues) and seven persons were using other hormonal cycle regulation prior to their FP. Participants were otherwise healthy and did not use any other relevant medication.

Oocyte Vitrification Treatment

Details on the controlled ovarian hyperstimulation (COH) protocol are described in TABLE 1. The mean AFC on cd three was 27.2 (SD 10.3). Fifteen participants (62.5%) were treated

following a standard long agonist hormone protocol. Nine participants (37.5%) were treated following an ultra-long agonist protocol. Participants started on different doses of r-FSH, ranging from 100 IU to 225 IU with the most frequent dose being 225 IU (29.2%). Four participants' r-FSH dose was adjusted upwards during treatment.

The results of the oocyte vitrification treatment are described in TABLE 2. After a mean of 12 (SD 2) stimulation days, a trigger was administered. The mean highest serum E2 measured 2-3 days prior to OPU was 11062 pmol/L (SD 5385). There were no complications during OPU and a mean of 20 oocytes (SD 7) were found, of which 17 MII. A mean of 17 oocytes (MI and MII) (SD 6) were vitrified.

Five participants (21%) developed OHSS,three (13%) of which were classified as severe according to Golan et al*(Golan and Weissman, 2009)*. Two of which required hospital admission to administer adequate intravenous fluids and one of these persons also underwent an paracentesis during their hospital admission. One other person developed an ovarian torsion which required an emergency oophorectomy in another center. This person was already intending on an bilateral oophorectomy after completing oocyte vitrification treatment as part of their GAS.

Since participating in the study, one participant underwent a second oocyte vitrification treatment. To date, none of the TMI's have made a request to use their vitrified gametes for family building.

Prior Testosterone Use

Seven participants were on testosterone prior to COH and discontinued this for 3 months. When comparing participants on testosterone prior to oocyte vitrification treatment to testosterone naïve participants (n=7), no statistically significant differences were found in stimulation days, serum E2, number of oocytes retrieved and vitrified and complications during or post OPU. Cumulative r-FSH dose was significantly lower in prior testosterone users (p=0.03).

Survey

The mean time between OPU and taking the questionnaire was 18.5 months (SD 13.2). All included participants (n=24) completed the questionnaire (response rate 100%). The strongest motivations to pursue FP was a strong desire for a future biological child in 46% and to ensure future options in 46%. Prior to starting their oocyte vitrification treatment, almost half of participants (46%) were most anxious for the internal examination. After completing their treatment however, 13% found the internal examination the most strenuous part of the treatment. Hormone injections were most frequently (29%) considered to be the most strenuous part of oocyte vitrification treatment, closely followed by the OPU chosen by 25% of participants. 25% and 8% of patients described the hormone injections and the OPU respectively, as the least strenuous part of the fertility treatment. The vast majority of participants were (very) satisfied with their treatment, number of frozen oocytes and provided care, see FIGURE 2. Only 17% of participants would not choose to pursue medical oocyte vitrification treatment again with the knowledge they have today. Furthermore, all participants would recommend other TMI's to pursue medical oocyte vitrification treatment if motivated. In all multiple choice questions, we added the option, 'other'; this option was not utilized by any of the participants.

Discussion

Our objective of this study was to describe the outcomes of oocyte vitrification treatment of TMI's and report on their experiences reflecting on this treatment at our center. In our cohort of 24 TMI's, we found a mean AFC of 27.2 follicles on cd 3, 20 oocytes were retrieved at OPU of which 17 oocytes could be vitrified. Aside from a lower cumulative r-FSH dose, there were no differences observed in TMI's who had previously used testosterone compared to testosterone naïve TMI's. We found a noticeable number patients developing OHSS (21%).. When evaluating the questionnaire responses, we found that the hormone injections were considered the most strenuous part of treatment by 29% of participants, closely followed by the OPU(25%). The overall satisfaction of treatment in participants was high.

Literature on oocyte vitrification outcomes in TMI's is limited. To date, only six case reports*(Cho et al., 2020, Gale et al., 2021, Greenwald et al., 2022, Martin et al., 2021, Resende et al., 2020, Wallace et al., 2014)*, five case series*(Broughton and Omurtag, 2017, Chen et al., 2018, Insogna et al., 2020, Maxwell et al., 2017, Stark and Mok-Lin, 2022)* and six retrospective cohort studies *(Adeleye et al., 2019, Amir et al., 2020, Amir et al., 2020, Barrett et al., 2022, Israeli et al., 2022, Leung et al., 2019)* have been published (see FIGURE 3).

Only four studies analyzed testosterone users versus testosterone naïve persons*(Adeleye et al., 2019, Amir et al., 2020, Barrett et al., 2022, Leung et al., 2019)*. Comparable to our study, no significant difference in oocyte yield between groups was reported. Furthermore, our study showed no difference in peak serum E2, similar to studies by Amir et al, Barret et al and Leung et al. The study by Adeleye et al*(Adeleye et al., 2019)* found a lower peak serum E2 in the prior testosterone users compared to testosterone naïve TMI's and cis gender women. This difference was attributed to two outliers in the transgender cohort with a diminished ovarian reserve. When repeating analysis without these 2 persons, the difference in peak serum E2 was no longer significant. In our subgroup analyses, prior testosterone users used a lower cumulative r-FSH dose compared to testosterone naïve TMI's. However, these results should be interpreted with caution since the sample size was small. No previously published studies analyzed cumulative r-FSH dose.

The high oocyte yield and OHSS rates in our cohort may be explained by three components. First, our participants were young and had a high AFC which are known predictors for developing OHSS *(Sun et al., 2021)*. Second, the careful consideration of caregivers to be more aggressive in r-FSH dose to maximize oocyte yield and prevent a second burdensome stimulation cycle. Third, the use of long-agonist COH protocols. Only recently, ESHRE guidelines have recommended considering antagonist COH protocols in TMI's *(Preservation et al., 2020)*. Using an antagonist stimulation protocol, as opposed to an agonist stimulation protocol, is beneficial since ovulation is triggered by a short endogenous luteinizing hormone

(LH) surge induced with a GnRH agonist instead of the prolonged exogenous LH action induced by the administration of r-hCG. In addition, the antagonist protocol requires a lower number of subcutaneous hormone injections which were classified as the most strenuous part of oocyte vitrification treatment by 29% of persons in our survey *(Lambalk et al., 2017, Toftager et al., 2016)*. Since completing analyses for this study, antagonist stimulation protocols have been implemented in clinical practice at our clinic for this subgroup of patients.

A likely consequence of our high oocytes yield, may be the high occurrence of OHSS in our cohort. OHSS is a syndrome comprising significant ovarian enlargement, high concentration of estradiol, and a fluid and protein shift from the intravascular compartment to other compartments. This shift may lead to hemoconcentration, decreased perfusion of vital organs, and thromboembolic events. Although self-limiting, some patients may require hospitalization for fluid administration and/or paracentesis of fluid*(Blumenfeld, 2018)*. In previously published research on oocyte vitrification treatment in the trans masculine population, one case report describes a mild case of OHSS*(Gale et al., 2021)* and one retrospective cohort of 20 patients describing four mild/moderate cases of OHSS*(Barrett et al., 2022)*.

Several parts of oocyte vitrification treatment were considered to be the most burdensome by our study participants: hormone injections, OPU, menses, internal examination and recovery. This data can be used to better prepare patients during fertility counseling so they are more prepared. Another strategy to lessen the burden for TMI's is to perform OPU under deep sedation since this reduces a patients anxiety and pain and improves patient satisfaction compared to light/moderate sedation*(Hoshijima et al., 2021)*.

Strengths and Limitations

This is one of the largest studies describing oocyte vitrification outcomes in TMI's, and the first in the Netherlands. This study confirms previous findings that TMI's respond well to COH for FP and have a normal number of oocytes retrieved and vitrified. This strengthens to

the idea that TMI's are good candidates for oocyte vitrification treatment for FP. This study also shows no difference between the prior testosterone users and testosterone naïve TMI's. Another strength of this study is the addition of the survey. These results present a more complete overview of all outcomes, including subjective outcomes, of oocyte vitrification treatment in TMI's.

A limitation of this study is the small sample size, especially when performing sub analyses in prior testosterone users versus testosterone naïve TMI's. Another limitation is the fact that we do not know the fertilisability of the vitrified material since none of the participants have chosen to fertilize their oocytes for embryo cryopreservation or to pursue conception yet. Furthermore, recall bias could have played a role when answering the questionnaire many months after OPU with a mean of 18.5 months and a large distribution (SD 13.2).

Conclusion

Our study adds to the limited data available for TMI's undergoing oocyte vitrification for FP. Our results are in line with previous published research; a normal anteral follicle count on cycle day 3; a normal response to oocyte vitrification treatment; no clinically relevant differences between the prior testosterone users and testosterone naïve TMI's. Our survey identified the hormone injections as the most burdensome part of treatment and concluded that even though the treatment was burdensome, most patients were satisfied with their treatment and outcome. If necessary, participants would undergo oocyte vitrification again and would also recommend it to others. Further research is necessary to determine the effect of long-term testosterone on the reproductive function of the oocytes of TMI's.

Acknowledgements

The authors especially want to thank all patients for their participation in the study.

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Figure Captions

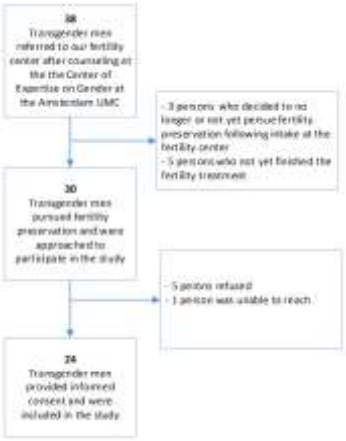

FIGURE 1. Recruitment flowchart of participants

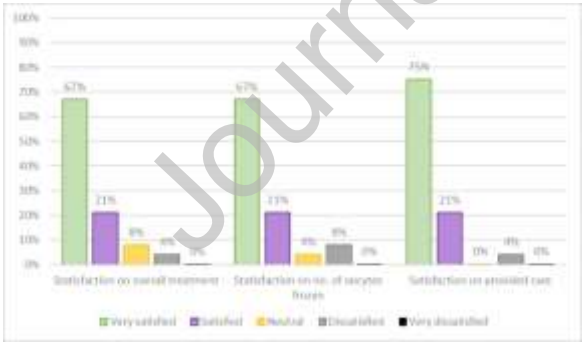

FIGURE 2. Patient satisfaction

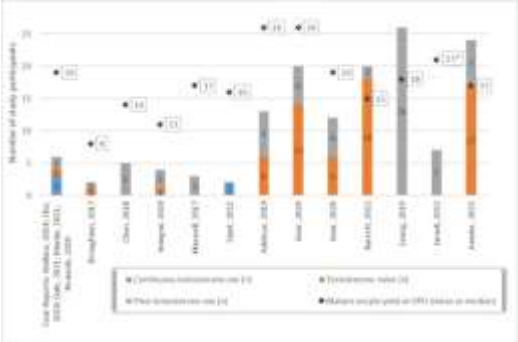

FIGURE 3. Overview of previously published data on oocyte vitrification treatment in trans masculine people, including this current study. Presented are mean/median mature oocyte yield at ovum pick-up (OPU) and study participants, subdivided by testosterone use. Case reports are pooled together. *Mature oocyte yield unknown, number of retrieved oocytes at OPU reported.

Tables

TABLE 1. Patient characteristics and their controlled ovarian hyperstimulation treatment

	Subjects N = 24
Age (years) at OPU	22.3 (21.1 – 26.0)
BMI (kg/m^2)	23.0 (3.2)
Prior GnRHa use	3 (12.5%)
Prior testosterone use	7 (29.2%)
Time on testosterone (months)	23.9 (15.2 – 30.3)
Current hormone use	
GnRHa	2 (8.3%)
Progesterone (Orgametril®)	4 (16.7%)
Progesterone only contraceptive pill (Cerazette®)	2 (8.3%)
Contraceptive pill	1 (4.2%)
Prior GAS	
Mastectomy	8 (33.3%)
Hysterectomy	0 (0%)
Colpectomy	0 (0%)

Other	0 (0%)
AFC on cycle day 3	27.2 (10.3)
Stimulation protocol	
Long agonist	15 (62.5%)
Ultra-long agonist	9 (37.5%)
r-FSH start dose	
100 IU	1 (4.2%)
112.5 IU	2 (8.3%)
125 IU	6 (25.0%)
150 IU	4 (16.7%)
187.5 IU	1 (4.2%)
200 IU	3 (12.5%)
225 IU	6 (29.2%)

Data are expressed in mean (SD), median (interquartile range) and numbers (%)

Abbreviations used: OPU = Ovum Pick-Up, BMI = Body Mass Index, GnRH-a = Gonadotrophin-Releasing Hormone agonists, GAS = Gender Affirming Surgery, AFC = Antral Follicle Count, r-FSH = recombinant Follicle Stimulating Hormone

TABLE 2. Result of oocyte vitrification treatment

	All subjects n = 24	Testosterone naïve n = 17	Prior testosterone use n = 7	P value
Stimulation days	12 (2)	12 (11 – 13)	11 (2)	*p=0.23
Cumulative r-FSH dose	1878 (554)	2034 (469)	1498 (595)	^p=0.03
Max serum E2 (pmol/L)	11062 (5385)	10205 (5622)	13145 (4444)	^p=0.23
Complications during OPU	0 (0%)	-	-	-
Number of oocytes retrieved	20 (7)	19 (7)	24 (6)	*p=0.19
Number of oocytes frozen	17 (6)	16 (6)	20 (5)	*p=0.25
Complications following OPU				
OHSS	5 (21%)	3 (18%)	2 (29%)	

Infection	0 (0%)	-	-	~p=0.61
Other	1 (4%)	-	1 (14%)	
OHSS classification				
Mild – Moderate	2 (8%)	2 (12%)	-	~p=0.40
Severe	3 (13%)	1 (6%)	2 (29%)	

Data are expressed in mean (SD), median (interquartile range) and numbers (%)

* Mann-Whitney-U test, ^ Unpaired T test, ~ Chi square test

Abbreviations used: r-FSH = recombinant Follicle Stimulating Hormone, E2 = Estradiol, OPU = Ovum Pick-Up, OHSS = Ovarian Hyperstimulation Syndrome

Vitea lead author

Joyce Asseler is PhD-student at the AmsterdamUMC researching the safety of testosterone and fertility in gender diverse persons. Furthermore, she is a M.D. at the Centre of Expertise on Gender Dysphoria at the Amsterdam UMC providing gynaecological care and fertility counselling for gender diverse persons.

Key Message

Our study shows a normal response to oocyte vitrification treatment and no clinically significantly differences between prior testosterone users testosterone naïve participants. Our survey identified the hormone injections as the most burdensome part of treatment and concluded that even though the treatment was burdensome, most patients were satisfied with their treatment and outcome.