ANDROLOGICAL CARE IN TRANSGENDER WOMEN

Perspectives for (new) life after transition

Iris de Nie

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ISBN	978-94-6458-047-1
Cover design by	Publiss, and Frederique van Domburg
Inner layout and design by	Publiss | www.publiss.nl
Printed by	Ridderprint | www.ridderprint.nl

Financial support for printing of this thesis was kindly provided by the Amsterdam Reproduction & Development (AR&D) research institute, ABN AMRO, and ChipSoft.

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VRIJE UNIVERSITEIT

ANDROLOGICAL CARE IN TRANSGENDER WOMEN

Perspectives for (new) life after transition

ACADEMISCH PROEFSCHRIFT
ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. C.M. van Praag,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op vrijdag 11 maart 2022 om 11.45 uur
in een bijeenkomst van de universiteit,
De Boelelaan 1105

door
Iris de Nie
geboren te Naarden

promotoren:	prof.dr. M. den Heijer prof.dr. J.A.F. Huirne
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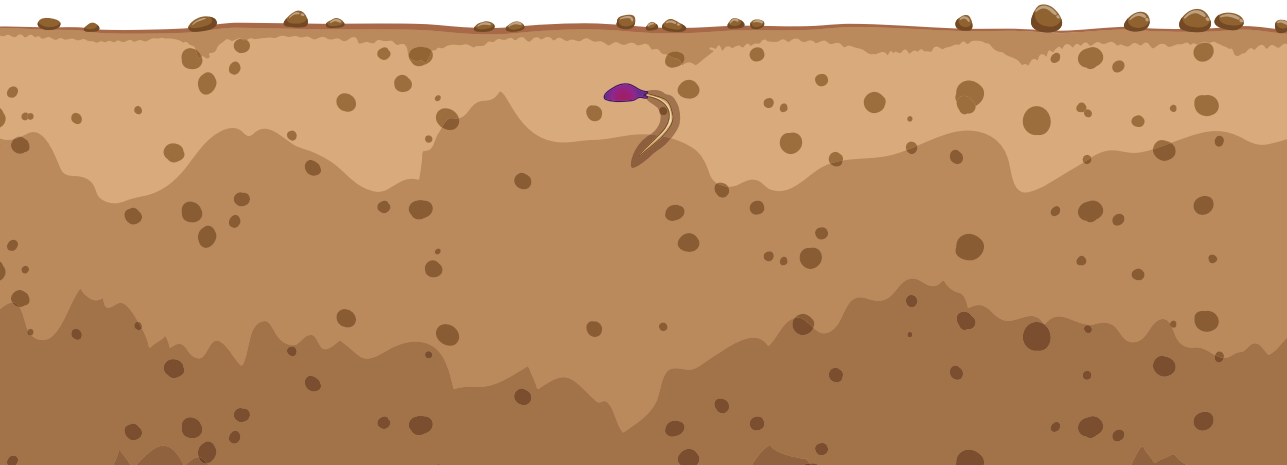


CHAPTER 1

GENERAL INTRODUCTION AND THESIS OUTLINE

GENERAL INTRODUCTION

In this thesis, research is presented on the topic 'Andrological care in transgender women'. Andrology is a medical discipline concerned with the anatomy, functions, and disorders of the male genital tract. Transgender women are people assigned male at birth, with a female gender identity. In this chapter we will first give some information about gender dysphoria and gender-affirming treatment for transgender women. Then, we will focus on the influence of gender-affirming treatment on the genital tract of transgender women and discuss the implications for fertility and cancer risk. For this purpose, we will also briefly explain the hypothalamic–pituitary–gonadal axis with particular focus on spermatogenesis and semen quality, and describe several types of cancers of the male genital tract and the role of sex steroids in the development of these malignancies. Lastly, we will describe the aims and content discussed in the different chapters.

Gender dysphoria and gender-affirming treatment

Gender dysphoria refers to the distress experienced by people with an incongruence between the sex assigned at birth and the experienced or identified gender. People assigned male at birth with a female gender identity are referred to as trans(gender) women, whereas birth-assigned males who identify as man are referred to as cis(gender) men. Transgender people may choose to start gender-affirming treatment to align physical characteristics with the identified gender, consisting of hormonal treatment and surgery.[1] For people presenting during adolescence, the first step of the medical treatment consists of puberty suppression with gonadotropin-releasing hormone agonists (GnRHa) in order to alleviate the distress caused by the development of secondary sex characteristics induced by puberty. When adolescents reach the age of 16 years, and have been on puberty suppressive therapy for at least six months, treatment is supplemented with gender-affirming hormones. Gender-affirming hormonal treatment (GAHT) for trans women involves androgen deprivation therapy on the one hand, and estrogen supplementation on the other hand.[2] The aim of this treatment is to lower serum testosterone to concentrations below 2 nmol/L and to elevate serum estradiol to concentrations between 200 to 600 pmol/L, in order to achieve feminization. Expected effects of this treatment include breast development, fat redistribution and a decrease in (facial) hair growth (Figure 1).[3] In addition, trans women of 18 years or older can opt for gender-affirming surgery (GAS) which can involve facial feminization, breast-augmentation, and bilateral orchiectomy often combined with vaginoplasty.[1] It has been shown that gender-affirming treatment effectively alleviates the experienced distress and increases quality of life, but even though it is generally considered safe, there may be risks and side effects, such as thromboembolic events or the development of sex hormone-related cancers (e.g. breast cancer).[4,5] Gender-affirming treatment also substantially affects the male genital tract but before that topic will be discussed, first some information on the hypothalamic–pituitary–gonadal axis, spermatogenesis and several types of cancers of the male genital tract will be given.

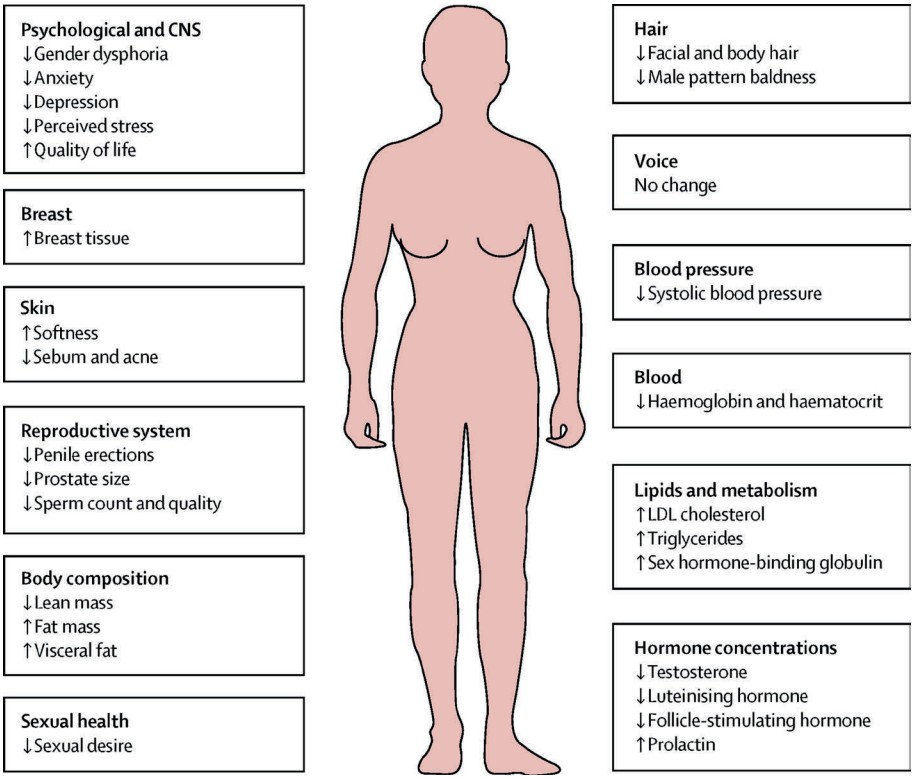


Figure 1. Effect of gender-affirming hormonal treatment in transgender women. Re-used from Tangpricha et al[3], with permission from Elsevier.

The hypothalamic–pituitary–gonadal axis and puberty in birth-assigned males

The hypothalamic–pituitary–gonadal axis describes the system in which 3 endocrine glands (i.e. the hypothalamus, the pituitary gland, and the gonads) act in concert through secretion of hormones (Figure 2). The hypothalamus is located in the brain and secretes gonadotropin-releasing hormone (GnRH) which stimulates the pituitary gland to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH). These hormones travel through the blood stream to, in their turn, stimulate the gonads to secrete sex steroids. In birth-assigned males, LH stimulates Leydig cells to produce testosterone. FSH affects - independently and in concert with testosterone - the proliferation, maturation and function of the supporting Sertoli cells that play a vital role in the maintenance of differentiating germ cells. Sertoli cells also secrete Inhibin B that, together with testosterone, acts as a negative feedback mechanism on the hypothalamus and pituitary gland. The hypothalamic–pituitary–gonadal axis is activated by high GnRH pulsing and initiates the development of secondary sex characteristics, which marks the onset of puberty.

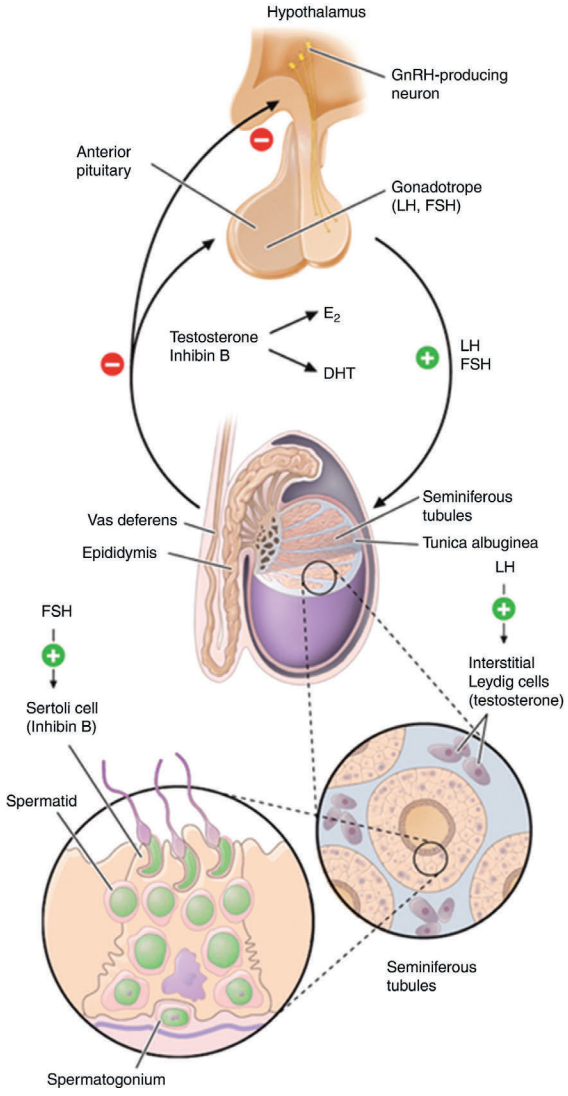
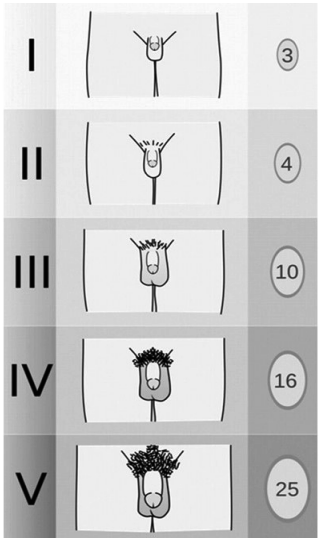


Figure 2. The hypothalamic–pituitary–gonadal axis in birth-assigned males. E_2, 17β-estradiol; DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone. Re-used from Dwyer et al[6], with permission from Springer Nature.

Pubertal stages are classified using the Tanner scale, which is an objective classification system that is used to track the development of secondary sex characteristics (Figure 3). Tanner stage 1 corresponds with the pre-pubertal stage. In birth-assigned males, testicular enlargement is the first physical manifestation of puberty and is often accompanied by a thinning of the scrotal skin. When reaching Tanner stage 3, birth-assigned males will have increased muscle growth, more noticeable penile and testicular growth, nocturnal ejaculations and the onset of voice lowering. These characteristics will develop further in Tanner stage 4, and Tanner stage 5 corresponds with the post-pubertal stage.



Tanner stage 1
Pre-pubertal stage, testicular volume ~3ml

Tanner stage 2
Testicular enlargement, thinning of scrotal skin, testicular volume ~4ml

Tanner stage 3
More noticeable penile and testicular growth, nocturnal ejaculation, onset of voice lowering, testicular volume ~10ml

Tanner stage 4
Further penile and testicular growth, darkening of scrotal skin, testicular volume ~16ml

Tanner stage 5
Adult genitalia, testicular volume ~25ml

Figure 3. Tanner staging in birth-assigned males. Adjusted from Engebretsen et al[7], with permission from BMJ Publishing Group Ltd.

Spermatogenesis and semen quality

From Tanner stage 3 onwards, intratesticular testosterone concentrations are generally high enough for spermatogenesis. Spermatogenesis is the process by which germ cells differentiate into spermatozoa (Figure 4). Spermatogonial stem cells, also called spermatogonia, are located in the outer wall of the seminiferous tubules in the testicles, and their mitotic division produces two types of cells. Type A spermatogonia replenish the spermatogonial pool and Type B spermatogonia differentiate into primary spermatocytes. These primary spermatocytes then undergo meiotic division I and differentiate into secondary spermatocytes, which in their turn go through meiotic division II and become spermatids. Subsequently, the final stage of spermatogenesis - called spermiogenesis - takes place which sees the maturation and elongation of spermatids into mature spermatozoa. Sertoli cells are located between the developing germ cells in the seminiferous tubules, and their function is to nourish these cells by secreting different substances that enhance spermatogenesis. The process of differentiation from spematogonia into spermatozoa generally takes 10 weeks, and starts in the outer wall and moves deeper into the tubule onto the inner wall (Figure 4).[8] As a result, mature spermatozoa are released in the lumen of the seminiferous tubules and are then transported to the epididymis in testicular fluid, with the aid of peristaltic contraction. Spermatozoa acquire their motility in the epididymis, and their ability to bind oocytes from an enzyme called fertilization promoting peptide, which is produced in the prostate.

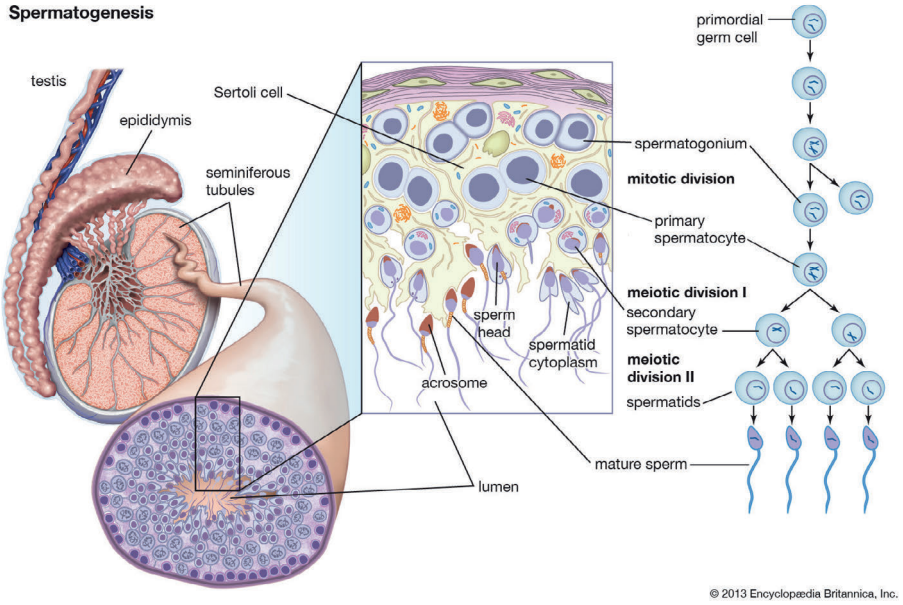


Figure 4. Spermatogenesis. By courtesy of Encyclopædia Britannica, Inc., copyright 2011; used with permission.

It is estimated that healthy birth-assigned males produce around 45 million spermatozoa per testicle per day.[9] To determine the semen quality of ejaculated samples, different parameters are used: volume of the semen sample, sperm concentration per milliliter, percentage of progressive motility, total sperm number per ejaculate and total motile sperm count. Total sperm number is calculated by multiplying the sample volume with the sperm concentration. Total motile sperm count (TMSC) is calculated by multiplying the sample volume, sperm concentration and the percentage of progressive motility, divided by 100%. The World Health Organization (WHO) assessed semen quality in the general population and determined reference values for human semen, based on their data (Table 1).[10,11] Since TMSC is derived from the absolute value of three other semen parameters, no reference value for TMSC is determined by the WHO. However, TMSC is a commonly used parameter in clinical practice and previous studies found that TMSC is a better indicator for the severity of male factor infertility than the WHO classification system. In addition, it has been shown that chances for spontaneous pregnancy are significantly lower in couples with a TMSC below 5 million compared to those with a TMSC above 5 million.[12,13]

Table 1. World Health Organization (WHO) data on human semen quality[10,11]

	Volume (ml)	Sperm concentration (10^6/ml)	Progressive motility (%)	Total sperm number (10^6/ejaculate)	Total motile sperm count (10^6/ejaculate)
Semen quality in general population of unscreened men (median, IQR)	3.2 (2.2-4.2)	64 (36-100)	57 (49-65)	196 (101-336)	113 (54–201)
Lower reference limit for human semen	1.5	15	32	39	N/A

Cancers of the male genital tract

The male genital tract compromises the penis, testes, epididymes, vas deferens, seminal vesicles, and the prostate. The most common cancers within the male genital tract, in order from least to most frequent, are penile, testicular and prostate cancer. In this thesis we will only focus on testicular and prostate cancer because of the potential influence of sex steroids on the development of these malignancies, as opposed to penile cancer which is mainly associated with being infected with Human Papilloma Virus.[14]

Testicular cancer mainly occurs in young people; the incidence in the Netherlands is 9.5 per 100.000 men, with a peak incidence of 32.4 per 100.000 men in those between 30-34 years old.[15] Testicular cancers can roughly be divided into sex cord or gonadal stromal tumors and germ-cell tumors, of which the latter most commonly occur. Germ-cell tumors are further classified as seminoma, non-seminoma and mixed germ-cell tumors. Prognosis, depending on histology, location of the primary tumor and metastases, and serum tumor marker levels, is generally better for seminoma compared to non-seminoma.[16] Although the incidence has increased over the past 40 years in most countries, the etiology of testicular cancer and the reasons for this rise remain unclear. Established risk factors for testicular cancer are a history of cryptorchidism, a low sperm count, presence of a contralateral testis tumor or a positive family history among first-grade relatives for testicular cancer.[16] Some theories also suggest that a relative excess of exogenous estrogens during pre- or post-natal life (e.g. diethylstilbestrol, pesticides) may play a causal role in the development of testicular cancer.[17-19]

Prostate cancer is the second most common cancer in men worldwide, accounting for approximately 15 per cent of all new cancers with incidence mainly dependent on age.[20,21] Other risk factors include genetic factors such as a positive family history and an African ethnicity.[21] It has been assumed that sex hormones, and androgens in particular, are involved in the pathogenesis of prostate cancer, because of the physiological dependency of prostate cells on androgens for functioning and proliferation.[22,23] In metastasized or advanced prostate cancer, androgen deprivation therapy is used to slow the progression of the disease.[24] However, a large meta-analysis showed no association between endogenous serum testosterone levels and prostate cancer incidence nor did it show an increased prostate cancer risk in hypogonadal men using testosterone replacement therapy.[25] There is currently very limited data available about a potential preventive effect of long-term androgen deprivation on the occurrence of prostate cancer.

Influence of gender-affirming treatment on the male genital tract

As mentioned above, under the influence of GAHT substantial changes occur in sexual function and the male genital tract. Trans women often experience a decrease in sexual desire as well as an increase in the time being comfortable without sexual activity, after initiating GAHT.[26] Furthermore, the low levels of serum testosterone result in an increased rate of erectile dysfunction and a decreased volume of the prostate and testes. Another consequence

of gender-affirming hormone use is a decrease or even an absence of spermatogenesis, resulting in an impaired reproductive function.[2] Since it has been shown that prolonged exposure to GAHT leads to spermatogenic involution, a decreased diameter of seminiferous tubules, and increased peritubular hyalinization, these effects may be irreversible.[27,28] After bilateral orchiectomy or vaginoplasty reproductive loss is certainly permanent.[29]

Fertility and family building

Although gender-affirming treatment significantly improves quality of life, the loss of reproductive function is an unwanted consequence for those with a (future) desire for children. In many countries, including the Netherlands, there were until recently strict transgender laws in place which required sterilization in order to be able to change gender on official documents and in population registries.[30] Therefore, almost all trans women visiting our gender identity clinic underwent this procedure until 2014. Loss of fertility was considered the price to pay for transition and options for fertility preservation were not discussed on a structural basis. Very little is known about how transgender people experienced the 'choice' of having to give up their fertility to be able to transition, and how they feel about living with the consequence of infertility. Especially in people who started their transition when they were younger than 21 years, when fertility or a future desire to have children may not have been in their scope of vision.

Fortunately, many countries now abandoned laws that required sterilization for legal gender recognition and therefore, fertility and future family building can now be openly discussed with transgender people.[30] Over the years, a general consensus has been developed on the need to provide counselling about the effect of the medical transition on fertility and the currently available options for fertility preservation.[1] The currently available options for fertility preservation in trans women include cryopreservation of ejaculated spermatozoa, or spermatozoa harvested from testicular tissue, or through vibratory- or electrostimulation.[31] Cryopreservation of ejaculated sperm is the most recommended option for fertility preservation, as it is least invasive and provides the best semen quality. Once cryopreserved sperm has been stored, assisted reproductive techniques enable trans women to have genetically related children with their female partner or a surrogate. Which technique is optimal, is determined by the post-thaw semen parameters: semen of good quality can be used for minimally invasive and inexpensive intrauterine insemination (IUI), while semen of low quality requires a more invasive and expensive technique such as in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI).[32]

Although data on semen characteristics in trans women show a high incidence of impaired semen quality, these studies report on relatively low number of people.[33-35] The etiology could not be identified in these studies due to the small sample size and a lack of endocrine laboratory results and complete clinical data on certain lifestyle factors known to influence semen quality, such as age, obesity and cigarette smoking.[36-38] With knowledge of the factors negatively influencing semen parameters in trans women it could be possible to optimize counseling about how to improve lifestyle before fertility preservation and hereby help trans women in their wish to parent genetically related offspring by cryopreserving semen suitable for the most patient-friendly strategy.

However, not for everybody fertility preservation is available prior to the start of gender-affirming treatment. Due to limited capacity of gender identity clinics, waiting lists for access to gender-affirming care are often long. Therefore, many transgender people seek alternative routes and even start using illicitly obtained hormones. Besides, some trans women choose to keep the male gonads and discontinue gender-affirming medication when an active desire for children emerges. Very little is known about the long-term effects of hormone treatment on gonadal function and gametes, and therefore the feasibility of fertility preservation in trans women who already started GAHT remains uncertain. Theoretically, serum testosterone concentrations will increase after cessation of GAHT which will provide for adequate circumstances for restoration of spermatogenesis, but data on semen quality after prolonged GAHT and outcomes of children born from these gametes are limited.[39]

For some trans women, semen cryopreservation prior to, or after discontinuation of, GAHT is not a realistic option, such as for people who start medical transition in an early pubertal stage (Tanner stage 2) when they do not have complete spermatogenesis yet. In this group, choices regarding fertility may pose a difficult dilemma as they often feel an extreme pressure to transition early in order to prevent irreversible development of secondary sex characteristics, such as lowering of the voice and facial hair growth. Therefore, cryopreservation of germ cells harvested from testicular tissue that is removed during GAS may be their only option for genetically related offspring. How, and if, these germ cells can be used for procreation depends on their maturation phase. Mature spermatozoa can directly be used for assisted reproductive techniques. The use of immature germ cells, conversely, relies on the possibility of *in vitro* spermatogenesis. Unfortunately, complete *in vitro* spermatogenesis has only been successfully demonstrated in mouse models, and still unsuccessful in human.[40] However, if *in vitro* spermatogenesis becomes available in the future, cryopreservation of immature germ cells might be a promising option for fertility preservation in those who started their medical transition in early pubertal stage. Currently, limited data is available on the effect of GnRHa and GAHT on testicular histology and the most advanced germ cell type that can be harvested form testicular tissue obtained at time of GAS. Moreover, previous studies that have been conducted on this topic showed contrasting results, ranging from a complete absence of germ cells to full spermatogenesis.[29]

Risk for cancer of the male genital tract during gender-affirming hormone use

Where limited research is published on the effect of GAHT on testicular histology and spermatogenesis, even less is reported about the influence on the occurrence of testicular cancer in trans women using GAHT, and only a few cases have been described.[41-45] As mentioned previously, exposure to exogenous estrogens is suggested to play a stimulating role in testicular cancer development. Since the abolition of transgender laws that required sterilization for legal gender recognition, an increasing number of people with non-binary identities or less need to confirm to binary cis presentation choose to keep their male gonads. As a consequence, in the future we might be faced with a growing population of young trans women using GAHT, who are still at risk for testicular cancer.

Another biologically male organ in trans women that is potentially at risk for cancer, is the prostate. The prostate is not removed during genital GAS because of the potential significant complications, such as incontinence. Therefore, trans women remain at risk for prostatic diseases after this procedure. As mentioned previously, it has been assumed that androgen deprivation therapy might have a preventive effect on the development of prostate cancer. However, androgen deprivation therapy in cis men is primarily used in patients diagnosed with advanced prostate cancer and only sporadically for other indications, such as to control sex impulses in patients with severe paraphilias.[46] Therefore, there are currently very limited data available about the influence of long-term androgen deprivation on the occurrence of prostate cancer.

THESIS OUTLINE

In this thesis we investigate two major topics. The first part focusses on fertility and family building, and in the second part we assess the risk for cancer of the male genital tract during gender-affirming hormone use.

The first part of this thesis will start with an evaluation of the experiences of transgender people, who are currently of adult reproductive age (± 30 years), with starting gender-affirming treatment as adolescent before 2014 and, as a consequence, the inevitable decision to give up the possibility of genetic parenthood. In **chapter 2** we will present their reflection on themes such as: how they experience their infertility in the present, how they experienced the topic fertility and the desire to have children during their transition, how they experienced the 'choice' of having to give up their fertility to be able to transition, if they -retrospectively- felt the need for reproductive counselling and fertility preservation and how they currently experience their gender identity in relation to their desire for children.

Since the abolition of transgender laws that required sterilization for legal gender recognition, fertility preservation is now thoroughly discussed and offered to transgender people. In **chapter 3**, we assess semen quality at time of fertility preservation before the initiation of GAHT in a large cohort of trans women, evaluate the adequacy for the different types of reproductive techniques and identify life style factors influencing semen quality.

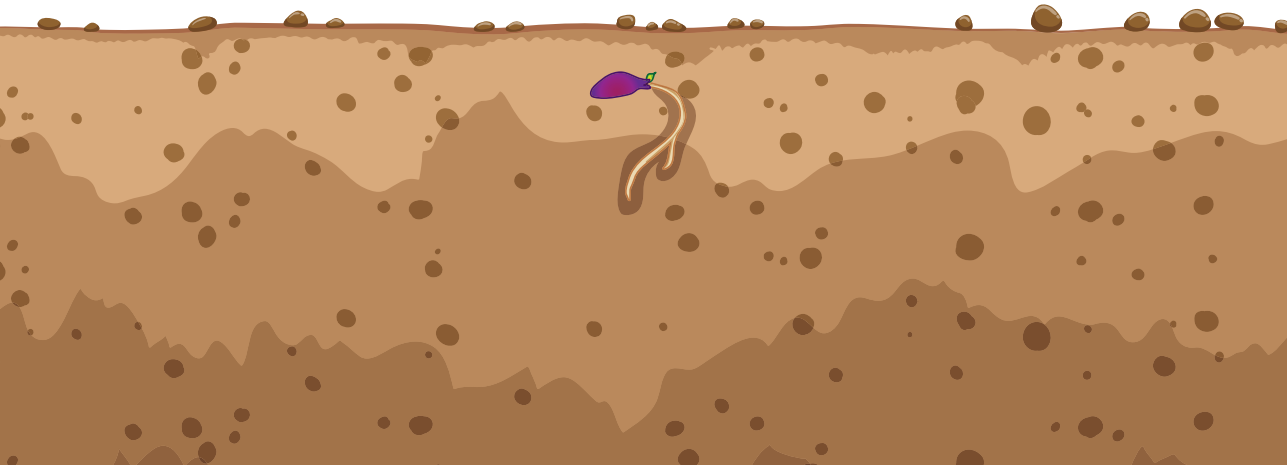
In addition, the aim of **chapter 4** is to evaluate the influence of habitual behavior, more typically observed in trans women, on semen quality by obtaining data on the habit of bringing the testicles from the scrotal position into the inguinal canal ('tucking'), wearing tight undergarments, and ejaculation frequency.

In **chapter 5** we will investigate the restoration of spermatogenesis in trans women who decide to temporarily discontinue GAHT for fertility preservation or for an attempt to naturally conceive with their female partner. We will present data on semen quality after gender-affirming hormone use and the duration to restore complete spermatogenesis.

Subsequently, in **chapter 6** we will assess if trans women can cryopreserve germ cells, obtained from their orchiectomy specimen at time of GAS, for fertility preservation, after having used puberty suppression and/or hormonal treatment. We will compare outcome between different Tanner stages at time of the start of medical treatment and hereby specifically evaluate options for those who start their medical transition in early puberty.

In the second part of this thesis, we focus on testicular cancer risk (**chapter 7**) and prostate cancer risk (**chapter 8**) in trans women using GAHT, by comparing the incidence of both cancers in our cohort with the incidence in the general population. Hereby, we will assess the influence of sex steroids on the development of cancer in the male genital tract and explore the need for regular screening.

Lastly, **chapter 9** of this thesis provides a summary of the main findings, a general discussion and an elaboration on perspectives for (new) life after transition.



CHAPTER 2

REFLECTING ON THE IMPORTANCE OF FAMILY BUILDING AND FERTILITY PRESERVATION: TRANSGENDER PEOPLE'S EXPERIENCES WITH STARTING GENDER-AFFIRMING TREATMENT AS ADOLESCENT

I de Nie*, JD Asseler*, M Arnoldussen, S Baas, ALC de Vries, JAF Huirne, TD Steensma, M den Heijer, NM van Mello

*these authors contributed equally

Submitted

ABSTRACT

Objective: To investigate how adults of reproductive age (± 30 years), who started gender-affirming treatment during adolescence, reflect on their reproductive wishes.

Materials and methods: Gender diverse adolescents who presented at our gender identity clinic since 1989 and commenced medical treatment at least 9 years ago were recruited for participation. Data were collected through an online survey, and a subsequent telephonic interview to validate the provided answers in the survey.

Results: The cohort consisted of 89 participants (66 trans masculine and 23 trans feminine people) with a mean age of 32.4 years (range 25.5-51.2) at time of the study, and 15.6 years (range 11.5-20.6) at time of start of medical treatment. All participants initiated medical treatment before 2014, when laws requiring sterilization for legal gender recognition were still in place, and only 30% of participants reported to have received information about fertility preservation which none of them pursued. In addition, 96% of participants underwent gonadectomy and thus became permanently infertile, which was troublesome for 27%. With today's knowledge, 44% of trans masculine and 35% of trans feminine people would pursue fertility preservation. The percentage of participants with a (future) desire for children increased from 34% at start of medical treatment to 56% at time of this study, of whom 23% had currently started a family.

Conclusion: These results implicate that views and desires regarding fertility and family building may change over time. Therefore, we would recommend health care providers to counsel every transgender adolescent about the effect of medical treatment on fertility and the options for fertility preservation. Furthermore, it is of utmost importance to repeat fertility counseling in time and at each step of the transition process, and create a safe environment in which adolescents are able to change their mind regarding pursuing fertility preservation.

INTRODUCTION

Within the last two decades, gender-affirming care for transgender youth has become widely available.[47] One of the topical debates in adolescent transgender care concerns the difficulty of making decisions regarding fertility at an early age, since medical treatment for gender dysphoria negatively affects reproductive function. As we are aware of the diversity, in this paper people assigned female at birth with a male gender identity are referred to as trans masculine, whereas people assigned male at birth with a female gender identity are referred to as trans feminine.

In the late 1980s, our center was the first worldwide to start with medical treatment for transgender adolescents, often referred to as the Dutch approach.[48] Initially, this treatment was solely offered to adolescents aged 16 years or older and consisted of gender-affirming hormones (androgens for trans masculine people, estrogens and anti-androgens for trans feminine people). Since 2000, gender-affirming hormone treatment (GAHT) of transgender adolescents can be preceded by a phase of puberty suppression, through administration of gonadotropin-releasing hormone agonist (GnRHa) when they have entered early puberty (from Tanner Stage 2 onwards).[49] The rationale of treatment with GnRHa is to prevent secondary sex characteristics development (e.g. lowering of voice, facial hair growth, breast growth, menstrual cycle) and hereby give adolescents time to explore options and to live in the experienced gender before making a decision to proceed with treatments that may be irreversible.[1]

Although early medical intervention has proven its effectiveness, an important limitation of both puberty suppression and gender-affirming hormone treatment (GAHT) is that they negatively affect reproductive function, as they inhibit gamete maturation.[50] After genital gender-affirming surgery (gGAS) including gonadectomy, reproductive loss is permanent. Until 2014, similar to many other countries, strict transgender laws were in place in the Netherlands which required sterilization for legal gender recognition. Since the abolition of this law, health care providers are strongly recommended to counsel transgender adolescents about the options for fertility preservation prior to initiating puberty suppression and GAHT, as well as before undergoing gGAS.[1,51]

For trans masculine people, currently available options for fertility preservation include oocyte vitrification and embryo cryopreservation. At our center, vitrification of oocytes can be performed in people of 16 years or older, either prior to commencing testosterone treatment or after 3 months of discontinuation. Since this procedure requires ovarian hyperstimulation with monitoring via transvaginal ultrasound and an ovum pickup procedure, it often causes large amounts of distress.[52] Embryo cryopreservation is only recommended in people with a stable long-term relationship and may, therefore, not be suitable for adolescents. Some trans masculine people also consider becoming pregnant as an option. However, this is only considered in a small number of people and it may cause major distress due to gender dysphoria.[53]

For trans feminine people, currently available options for fertility preservation include cryopreservation of ejaculated spermatozoa, or harvested from testicular tissue, or through vibratory-/electrostimulation. These options require complete spermatogenesis which only develops during puberty, under the influence of increasing intratesticular testosterone levels. Due to the start of medical transition in early puberty, spermatogenesis is often not yet present. Other barriers for fertility preservation in trans feminine people are that semen cryopreservation requires masturbation which may be stressful for some, and that testicular sperm extraction is an invasive procedure which requires anaesthesia and surgery.[54]

Decision making about fertility can be very difficult for adolescents since their early age and subsequently intellectual, emotional, and social immaturity may impede prediction of future desires regarding fertility and family planning. Besides, for a lot of people the desire of having children is a life-phase bound subject which may develop later in life. Recent studies found that up to 80% of transgender youth expressed an interest in having a family, but the need for biologically related children was questioned and only a small percentage reported to be frustrated if biological parenthood would not be feasible.[55,56] However, health care providers often worry that transgender adolescent's views regarding parenthood might change over time, and also many adolescents acknowledge that this may happen.[56-59] Coming of reproductive age, combined with improved body satisfaction and mental health, might result in an improved capability to establish romantic relationships and consider future family building.

In transgender adolescents, data on the potential change in wishes and desires regarding fertility, family building and the importance of biological parenthood, when coming of reproductive age, are lacking. Hereby, the long-term consequences of acquired infertility in transgender adolescents who have now reached adulthood remain still unknown. Therefore, this study aims to investigate how adults, who started gender-affirming treatment during adolescence, reflect on their reproductive wishes in the past and how this influenced their choices regarding family building in the present. With this knowledge, we aim to improve fertility counselling for transgender adolescents and their families and appropriately support them in making these difficult decisions.

METHODS

Study population

Gender diverse adolescents who presented at the Center of Expertise on Gender Dysphoria in Amsterdam between 1989 and 2000 and were treated with GAHT, were recruited for participation. As well as gender diverse adolescents who commenced medical treatment with gonadotropin-releasing hormone agonist (GnRHa) prior to GAHT, at least 9 years ago.

Between December 2019 and June 2021, eligible individuals were approached through mail with information about the study and an invitation to participate. Those who were interested in participation were informed in more detail via telephone, before obtaining written informed consent. People were not eligible for inclusion if they did not start medical

treatment, if they were above the age of 21 years at initiation of medical treatment, or if they were diagnosed with a disorder of sex development.

Study design and data collection

Participants were asked to complete an online survey about how they experienced their medical treatment, and on psychological functioning, experienced gender dysphoria, sexuality and fertility. For the current study, only demographic data and the results of fertility questionnaires were used. The other data will be published in upcoming papers.

The fertility questionnaire was developed by a multidisciplinary team and focused on different themes, such as fertility counseling at initiation of medical treatment, decision-making about fertility preservation, current feelings about infertility, and a potential desire to have children. All participants initiated medical treatment before 2014, when strict transgender laws were still in place which required a sterilization for legal gender recognition in the Netherlands. Therefore, the questionnaire was divided in a 'past' and 'present' section, to make a clear distinction between how participants experienced their decision-making process regarding fertility at initiation of treatment within the possibilities that were available at that time, and how they reflect on these decisions and possibilities with today's knowledge. The questionnaire consisted of approximately 25 multiple choice questions of which the majority contained the option to add an open text format answer in case none of the pre-defined responses were applicable. In addition, some open-ended questions gave the respondent the option to formulate their own answers and expand on them. After completion of the survey, a telephonic interview was conducted for further clarification of ambiguous answers and to give participants the possibility to elaborate on topics that were not specifically mentioned in the survey. The telephonic interview was written down in a summarized form.

Statistical analysis

For each participant, information obtained from the telephonic interview was used to validate the provided answers in the survey and, if applicable, data were supplemented or corrected. Subsequently, data from the surveys were analyzed using STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA) and presented as numbers with percentages. Baseline characteristics of the study population are expressed as mean with standard deviation (SD) when normally distributed, and as median with interquartile range (IQR) when non-normally distributed. With use of ATLAS.ti version 9 (ALTAS.ti Scientific Software Development GmbH for Windows, Germany), information from the open-ended questions was coded and interpreted by two of the researchers. The codes that emerged were further thematically organized in larger categories. In the presentation of the data, the emerged themes will be presented supported by illustrative quotes. To check for accuracy, quotes were translated and retranslated.

The study protocol was assessed by the Ethics Review Board of the VU University Medical Centre Amsterdam and it was concluded that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study.

RESULTS

Description of study population

A total of 207 individuals were approached for participation in this study. After obtaining informed consent, surveys were sent out to 114 participants of whom 92 completed their survey. Of these people, three initiated medical treatment above the age of 21 years and were therefore excluded. As a result, 89 participants were eligible for inclusion in the study cohort, which then consisted of 23 trans feminine and 66 trans masculine people. Characteristics of the total study cohort, and for trans feminine and masculine people separately, are shown in Table 1. Participants had a mean age of 32.4 years (SD 6.6, range 25.5-51.2) at time of the study, and 15.6 years (SD 2.2, range 11.5-20.6) at time of start of medical treatment. Of the total cohort, 82% received GnRHa prior to commencing GAHT. Educational level was relatively high (38% finished higher education), and more than 75% of the study population reported and income level above the poverty line. The majority of both trans masculine people and trans feminine people reported a heterosexual sexual orientation, but less than half of the study population reported to be in a relationship at time of the study. Only trans masculine people reported to have children, including biological, step-, foster, and adopted children.

Table 1. Baseline characteristics of study cohort

	Total (n=89)	Trans feminine (n=23)	Trans masculine (n=66)
Age at time of study	32.4 (SD 6.6, range 25.5-51.2)	31.9 (SD 5.5, range 25.6-44.5)	32.5 (SD 6.9, range 25.5-51.2)
Age at time of start medical treatment	15.6 (SD 2.2, range 11.5-20.6)	15.5 (SD 1.9, range 12.9-19.6)	15.7 (SD 2.3, range 11.5-20.6)
Used GnRHa prior to gender-affirming hormones - % (n)	82% (73)	87% (20)	80% (53)
Educational level			
Elementary education	2% (2)	0%	3% (2)
Primary vocational education (LBO)	1% (1)	4% (1)	0%
Pre-vocational secondary education (VMBO/MAVO)	14% (12)	13% (3)	14% (9)
Secondary vocational education (MBO)	39% (35)	39% (9)	39% (26)
General secondary/pre-university education (HAVO/ VWO)	6% (5)	9% (2)	5% (3)
Higher professional education (HBO)	21% (19)	26% (6)	20% (13)
University education (WO)	17% (15)	9% (2)	20% (13)
Level of income~			
Below poverty line	4% (4)	0%	6% (4)
On poverty line	18% (16)	22% (5)	17% (11)
Above poverty line	78% (69)	78% (18)	77% (51)
Civil status			
Single	60% (53)	65% (15)	58% (38)
In a relationship	40% (36)	35% (8)	42% (28)

	Total (n=89)	Trans feminine (n=23)	Trans masculine (n=66)
Sexual orientation*			
Heterosexual		70% (16)	62% (41)
Homosexual/lesbian		4% (1)	9% (6)
Bisexual		18% (4)	22% (15)
Asexual		0% (0)	2% (1)
Not indicated		4% (1)	3% (2)
Other		4% (1)	2% (1)
Children (biological/step-/foster/adoption)	23% (20)	0% (0)	30% (20)

~Poverty line is 971 euros/month (single); 1330 euros/month (cohabiting); 1620, 1830, 2000 euros/month (couple with 1, 2, 3 children); 1300, 1470, 1710 euros/month (single-parent family with 1, 2, 3 children).
*heterosexual: attracted to opposite gender, homosexual/lesbian: attracted to same gender, bisexual: attracted to opposite and same gender, asexual: not attracted to others.

Information about effect of treatment on fertility and options for fertility preservation

61% of participants reported to have received information about the effect of medical treatment for gender dysphoria on their fertility, 16% did not receive this information and 23% did not remember. However, only 30% of participants received information about the options for fertility preservation, whereas 44% of those who did not, would have liked to receive this information. Although 34% had a strong desire to have children at time of starting medical treatment (Figure 1), none of the participants pursued fertility preservation. The most reported reasons for not freezing eggs or sperm were it not being an option (27%) or not knowing about this option (25%), not feeling the need to do so (20%), not wanting to discontinue treatment (16%), or finding the procedure too burdensome (17%) or too expensive (6%). Nine participants (10%) mentioned not wanting a child from gametes that are not in line with their gender identity, a trans woman explained it as follows:

> *"I would not biologically want to be the father of my children. I think I would always have to think about that when I would look at my children. And I wouldn't want my children finding out and what a disappointment that must be for them."*

Capacity of making decisions and reflecting on infertility through treatment

When asked whether participants felt that they were old enough to make decisions about fertility at the start of medical treatment, 51% responded positive and 11% did not know. Nonetheless, 21% of participants felt that they were not old enough and indicated that they could only oversee these decisions when reaching a median age of 18 year (IQR 18-22).

Of the study cohort, 96% underwent gonadectomy and thus became permanently infertile. At that moment this was troublesome for 11% of participants, while at time of this study this was troublesome for 27% of participants. The most reported reasons for finding it

troublesome to have become infertile at time of this study were wanting genetically related children (74%) and feeling it is an emotional topic which is difficult to handle (35%). One participant reported:

> *"I would love to raise a child. However, with my past, the chances of adoption are nil. This makes the chances of me ever raising a child incredibly low, much to my regret."*

People who did not find it troublesome to have become infertile mentioned not having a desire for children (21%), not wanting genetically related children (21%), and finding medical treatment for gender dysphoria more important than staying fertile (67%) as most important reasons. Other mentioned reasons for not finding the loss of fertility troublesome, were being happy to be rid of their biological reproductive organs and/or not wanting children with their biological reproductive organs, one trans man explained this as follows:

> *"For me, getting pregnant myself or passing on female genes was never an option. I did not attach any emotional value to my uterus or ovaries, nor did I feel that my gonadectomy was the actual moment I became infertile. To be honest, I never saw having children as an option because I was not a woman, but I also wasn't able to make sperm".*

Also, infertility being a logical consequence of decisions made in the past and not having had other options, were mentioned as reasons for being at peace with the acquired infertility.

With today's knowledge, 14% of trans masculine and 17% of trans feminine people would not have chosen to undergo gonadectomy. In addition, 44% of trans masculine and 35% of trans feminine people would pursue fertility preservation, respectively 23% and 30% reported not to know if they would choose fertility preservation. Of all trans feminine people, 39% would prefer to freeze surgically obtained spermatozoa over ejaculated sperm because masturbation would be too burdensome for them.

Current desires regarding fertility and family building

As shown in Figure 1, 56% of participants reported to currently have a desire for children, to desire children in the future, or to have children. Figure 2 shows how participants would prefer to fulfill their desire to have children. Whereas 32% of trans masculine people would prefer to use a sperm donor, none of the trans feminine would consider this an option to fulfill their desire for children. A total of 20 trans masculine people reported to currently have children of whom one person did not undergo gGAS and was therefore able to become pregnant and have a biological child. Among the other 19 people, 13 had children with a female partner and a sperm donor, 6 had stepchildren, and 2 people reported to have children through adoption or foster care.

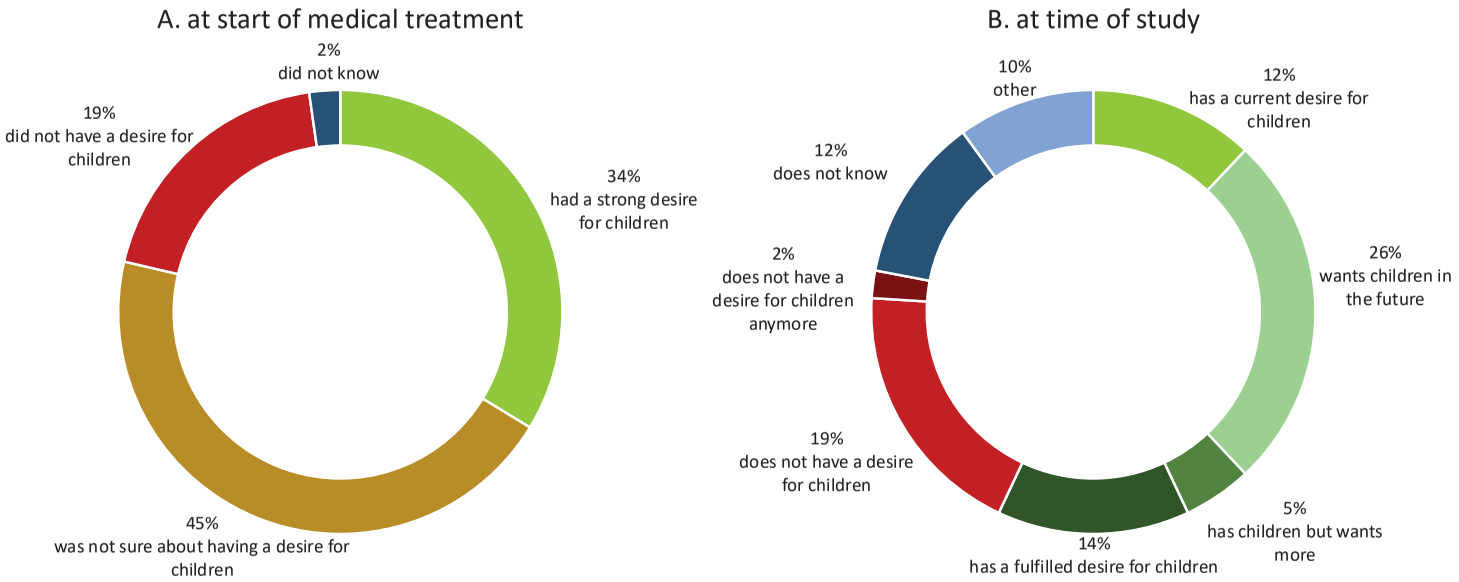


Figure 1. Desire for children (n=89). At time of start medical treatment (A) and at time of this study (B).

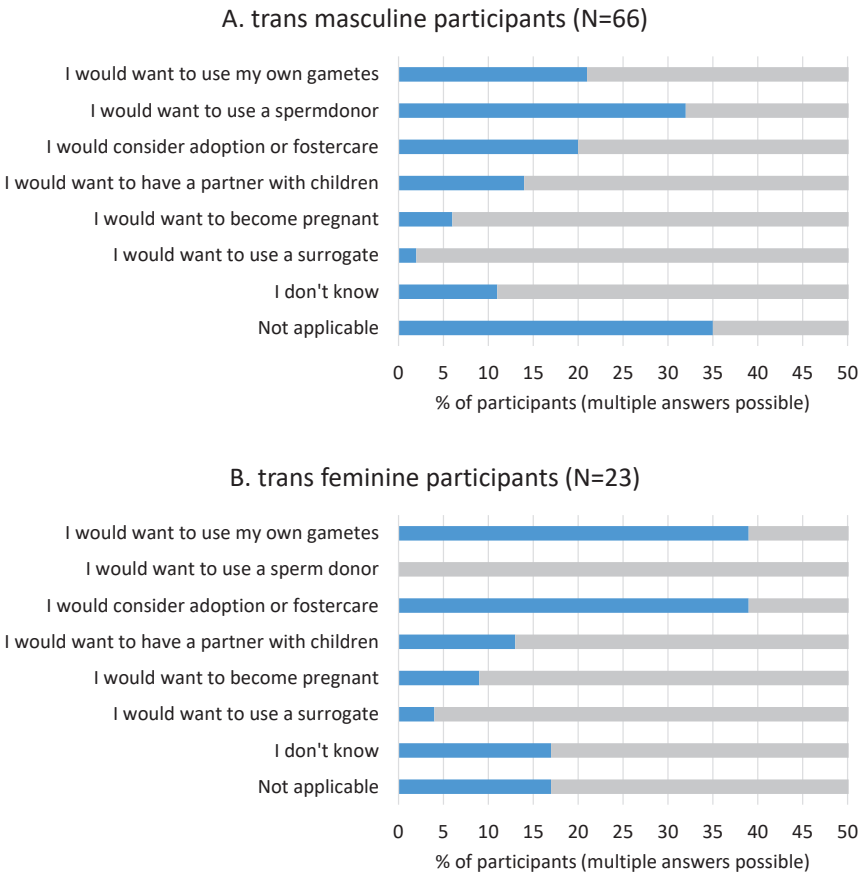


Figure 2. Preferred methods for fulfilling a desire for children. For trans masculine people (A) and trans feminine people (B).

Only 19% of participants reported not to have a desire for children, and 2% desired children in the past but not anymore (Figure 1). Besides just not wanting to have children, participants mentioned not being able to have a genetic bond with their child (9%), and the lack of a partner to start a family with (12%), as most important reasons for not desiring children. When asked how participants felt about not having children, 42% indicated to not have problems with this at all and 31% indicated to struggle with this in varying degrees (Figure 3).

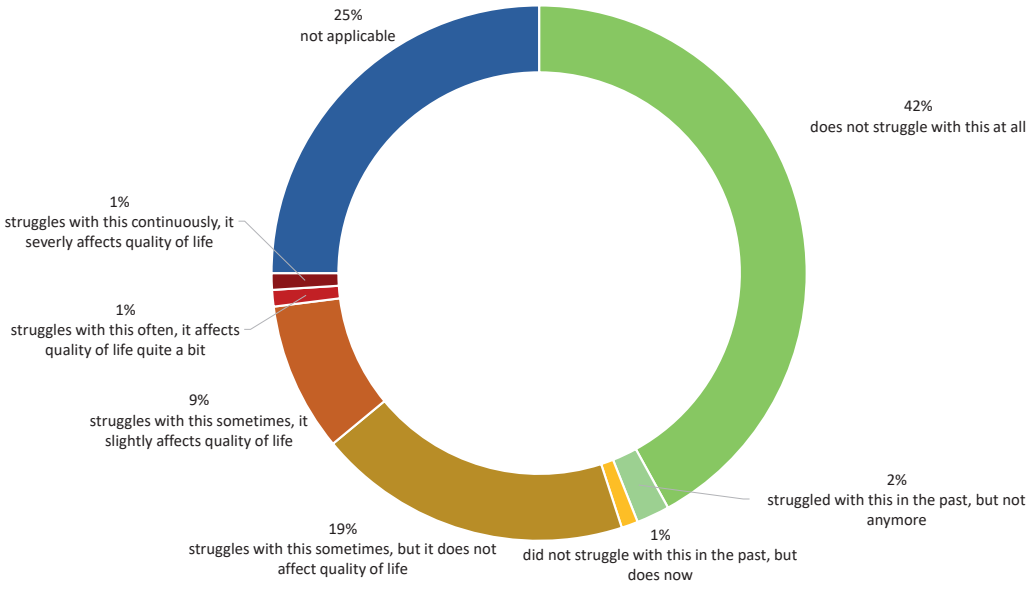


Figure 3. Feelings regarding not having children (N=89)

Advices for trans youth regarding fertility

Lastly, participants were asked what advice they would give to adolescents, who will shortly start medical treatment, concerning fertility and fertility preservation. The most often mentioned advice was to pursue fertility preservation to keep the option for genetically related children open (34%). Furthermore, 12% recommended adolescents to be well educated about fertility, the options for fertility preservation, and the consequences of their decisions regarding fertility. Some participants mentioned the importance of involving family, friend or peers in making choices regarding fertility, whereas others recommended to solely focus on their own feelings. Also, 17% of participants advised to carefully consider all forms of parenthood, and 13% warned adolescents that their views regarding parenthood might change over time:

> *"For 20 years I was firmly convinced that I never wanted a partner or children. Now, I have been married for 5 years, and we are in the process of having children. If someone had told me this in the first 20 years of my life, I would not have believed it at all. In retrospect, I would have liked to make other choices, but at that time I was not even able to consider them. Therefore, my advice is: procrastinate and play it safe whenever possible!"*

DISCUSSION

This is the first follow-up study reporting on reflections of transgender adults of reproductive age, who started their medical treatment as adolescents, on the consequences of gender-affirming treatment for their fertility and the possibilities of starting a family. Since all participants initiated medical treatment before 2014, at that time laws requiring sterilization for legal gender recognition were still in place. Many participants reported they did not receive proper fertility counseling and none of them pursued fertility preservation. In addition, almost all participants underwent gonadectomy and thus became permanently infertile. Approximately 1 out of 4 participants found becoming infertile troublesome, and stated that they were not able to make decisions regarding fertility and future family building during adolescence. Moreover, 1 out of 6 would, in retrospect, rather not have undergone gonadectomy and more than one third of the study population would have chosen to pursue fertility preservation. The percentage of participants with a (future) desire for children changed from 34% at start of medical treatment to 56% at time of this study, and 23% had already started a family. These results implicate that views and desires regarding fertility and family building may change over time, which may be related with improved body satisfaction and mental health, matured intellectual and emotional cognitions, social status, and coming of reproductive age when starting a family becomes a topical theme.

Although some studies have been conducted on reproductive wishes and parenting intentions in transgender youth and adolescents, none of these studies focused on how people reflect on these topics years later, when they actually reached adult reproductive age. What these studies did show however, is that the rates of transgender adolescents expressing a desire for parenthood are much higher than the rates of people pursuing fertility preservation. Whereas, 48 to 80% expresses an interest in having children someday.[55-57,60], reported fertility preservation rates differ from 0 to 7% in trans masculine adolescents and 9 to 62% in trans feminine adolescents.[54,61-64] In line with these findings, 34% of the people in our study cohort reported that they had a desire for children when they initiated medical treatment in adolescence but nobody pursued fertility preservation. It must be noted however, that in the period that our participants started medical treatment, options for fertility preservation were not widely available and only 1 out of 3 people received information about these options. Therefore, it is essential that all transgender adolescents receive proper fertility counseling prior to initiation of medical treatment, regardless of their age, and that the topic is discussed recurrently prior to subsequent steps in the medical transition. Furthermore, it may be valuable to involve caregivers in fertility counseling, especially in young adolescents.

Other reported reasons for not pursuing fertility preservation were the costs of the procedure, not wanting to masturbate or undergo medical procedures, and a conflict between their gender identity and their biological gametes. These barriers are similar to what was reported in other study populations.[56,65] For example, Persky et al. found that 58% of transgender youth stated that the idea of having children with their biological gametes conflicted with their affirmed gender, and that for 47% financial considerations played a role in not pursuing fertility preservation.[66] For health care providers it is important to carefully assess potential barriers for fertility preservation in each individual, and tailor

the provided counseling and offered procedures to their specific needs and abilities (e.g. cryopreservation of surgically obtained spermatozoa to avoid masturbation, or an ovum pick up procedure under general anesthesia).

Many transgender adolescents with a desire for children do not find it important to have biological children; Kerman et al. reported that of participants who wanted a family, 84% were excited by the idea of adopting children.[56] While it was previously found that trans masculine people consider adoption more frequently than trans feminine people (78% vs 54%), we observed the opposite (20% vs 39%).[60] On the other hand, using a sperm donor to fulfill a desire for children was only considered by trans masculine people in our study cohort. In addition, only trans masculine people started a family, of whom 95% did not have biological children. These observations might be explained by the fact that the majority of participants reported to have a heterosexual sexual orientation, meaning that partners of trans masculine people are most likely able to become pregnant, whereas partners of trans feminine people are not.

Another reason for the low rate of fertility preservation among transgender adolescents might be due to an inability to oversee the importance of future fertility. Adolescents often experience severe distress of pubertal development due to their gender dysphoria, and may therefore be preoccupied with starting puberty suppression as soon as possible.[67] Especially in trans feminine adolescents, the equipoise of commencing medical treatment to avoid progression of puberty, and delaying treatment to enable semen cryopreservation as only option for biological children may be stressful, as puberty is accompanied by irreversible and often unwanted physical changes such as a lowering of the voice and facial hair growth. In our study cohort, many participants stated that they felt that medical treatment for gender dysphoria was more important than staying fertile. These findings are in line with the results of a study by Chiniara et al., which reported that 58% of trans masculine and 64% of trans feminine people ranked having children as their least important priority, while their highest life priorities were being in good health, do well in school/work and have close friends. When asked to envision what their ranking of these same priorities would be 10 years from now, having children was still the lowest ranked priority.[55] However, other studies found that up to 48% of transgender adolescents acknowledged that their views regarding parenthood might change over time, and they referenced their age and self-assessed maturity as they discussed future fertility decisions.[56,57] This was confirmed by participants in the current study, who felt that they were in retrospect too young to make decisions about fertility, who started to find it troublesome to have become infertile over the years, and who developed a desire for children although they did not expect to do so. Therefore, health care providers should warn adolescents that views on future family building might change over time and create a safe environment in which adolescents are able to change their mind regarding pursuing fertility preservation.

Since this study reports on the first transgender adults who started medical treatment during adolescence worldwide, the major strengths of this study are the unique study cohort and, consequently, the obtained insights on how transgender adults of reproductive age look back on choices made regarding fertility during adolescence approximately

15 years ago. Hereby, we were able to explore how views on family building and the importance of biological parenthood might change over time.

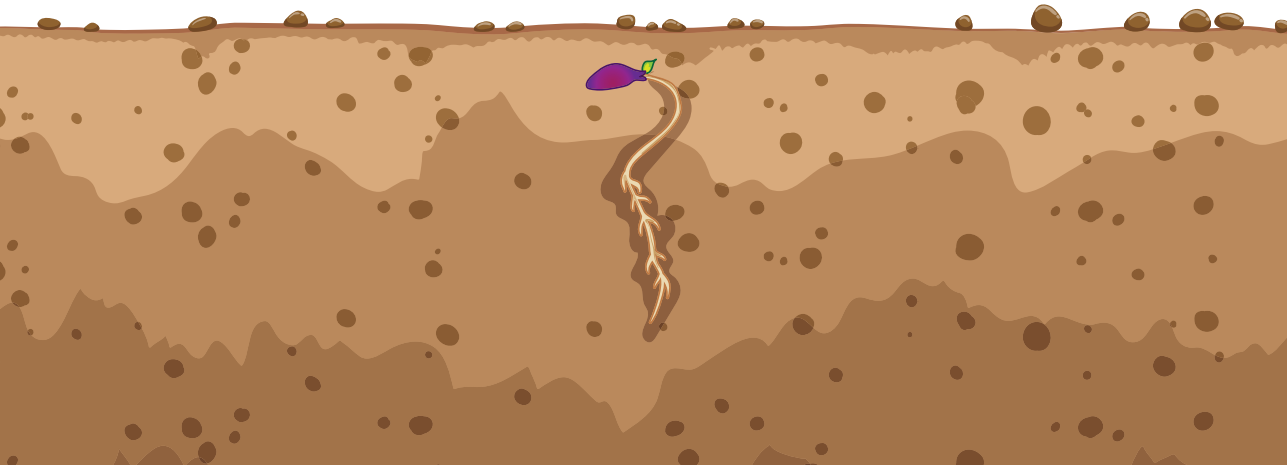
However, the design of this study has some limitations. Since people reflect on a period in which fertility counseling was not offered on a structural basis, results of this study are not directly translatable to current practice. Nowadays, a general consensus exists on the need to inform all people initiating medical treatment for gender dysphoria about its effect on fertility, and to offer fertility preservation options.[68] In our gender identity clinic, at present all transgender adolescents are therefore counseled by a fertility specialist prior to the start with puberty suppression as well as before initiation of GAHT. To assess how this affects decisional conflict and regret we will need to repeat this study in 10 to 15 years, although in the meantime clinical practice might have changed again. Another factor that has to be taken into account when interpreting the responses of participants is the influence of choice-supportive bias, which is the tendency to retroactively ascribe positive attributes to an option one has selected and to demote the forgone options.[69] This may be a useful coping strategy to be at peace with decisions made in the past and to avoid feelings of regret. Because of this, the percentage of people reporting to find it difficult to have become infertile, and to would have want to pursue fertility preservation or keep their gonads, might be lower. Conversely, the percentage of people recommending transgender adolescents to pursue fertility preservation might be relatively high due to a lack of knowledge about what to options for fertility preservation exactly entail and how invasive they can be. Furthermore, participants were asked to reflect on feelings and choices they made more than 10 years ago. Therefore, it may be possible that not all aspects were accurately remembered.

For future studies, it would be worthwhile to explore this topic more in-depth using semi-structured interviews and to longitudinally assess feelings about fertility with validated questionnaires.

CONCLUSION

Overall, this study provides valuable insights on transgender people's experiences with starting gender-affirming treatment as adolescent and their feelings regarding fertility and family building. Since the participants started medical treatment in a period when sterilization was required for legal gender recognition, the vast majority (96%) became permanently infertile due to this treatment. Although many report to be at peace with this and to have found other ways to start a family, a substantial part does experience their infertility as troublesome and reports that, with today's knowledge, they would have chosen fertility preservation or keep their reproductive organs. Moreover, the majority of participants developed a desire to have children, of whom more than 30% would have preferred to use their own gametes to fulfill this desire.

Based on these result we would recommend health care providers to counsel every transgender adolescent about the effect of medical treatment on fertility and the options for fertility preservation, even in the absence of a desire for children upon initiation of treatment. Furthermore, it is of utmost importance to discuss the possibility that views on future family building might change over time, repeat fertility counseling in time and at each step of the transition process, and create a safe environment in which adolescents are able to change their mind regarding pursuing fertility preservation.



CHAPTER 3

IMPAIRED SEMEN QUALITY IN TRANS WOMEN: PREVALENCE AND DETERMINANTS

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Published in Human Reproduction; July 2020, 35(7):1529–1536

ABSTRACT

Study question: What is the semen quality in trans women at time of fertility preservation, prior to the start of gender-affirming hormone treatment?

Summary answer: Before the start of gender-affirming hormone treatment semen quality in trans women was already strongly decreased compared to the general population.

What is known already: Hormone treatment for trans women (birth-assigned males, female gender identity) consists of anti-androgens combined with estrogens in order to achieve feminization and it is accompanied by a loss of reproductive capability. Trans women can opt for semen cryopreservation prior to their medical transition to retain the possibility to parent genetically related offspring. Post-thaw semen parameters determine which ART can be used. Knowledge of semen quality and the factors negatively influencing semen parameters in trans women are important to improve semen quality before fertility preservation.

Study design, size, duration: A retrospective cohort study was performed between 1972 and 2017. In total, 260 trans women were included for this study. Due to the study design, there was no loss to follow-up nor attrition.

Participants/materials, setting, methods: We studied the quality of the preserved semen in trans women, prior to their medical transition, who visited our gender clinic. Semen parameters were collected, as well as data on age, alcohol consumption, smoking, cannabis use, BMI, previous use of estrogens or anti-androgens and endocrine laboratory results. Semen parameters were categorized using reference values for human semen of the World Health Organization (WHO) and compared with data from the general population. Logistic regression analyses were performed to analyze the extent to which factors known to have a negative impact on semen quality in the general population explained the impaired semen quality in the cohort.

Main results and the role of chance: The cohort consisted of 260 trans women between the age of 16 and 52 years. Semen quality in trans women was significantly decreased compared to WHO data from the general population. In total, 21 trans women had an azoospermia and median semen parameters for the remaining trans women and the general population, respectively, were as follows: volume 2.7 ml and 3.2 ml ($p<0.05$), sperm concentration 40 million/ml and 64 million/ml ($p<0.05$), total sperm number 103 million and 196 million ($p<0.05$), and progressive motility 41% and 57% ($p<0.05$). Smoking (odds ratio (OR) 2.35 (95%CI 1.06-5.21)) and a higher age at time of fertility preservation (OR 1.04 (95%CI 1.00-1.08)) were found to correlate with an impaired progressive motility. Twelve trans women reported to have used anti-androgens and estrogens, and all had discontinued for at least three 3 months prior to the first attempt for semen cryopreservation. No correlation was found between previous gender-affirming hormone use and decreased semen parameters. The median post-thaw total motile sperm count was 1.0 million per vial (inter-quartile

range 0.1-3.1) and in only 26.4% of thawed semen samples was the quality adequate for a minimally invasive IUI.

Limitations, reasons for caution: Limitations include the retrospective design and insufficient data on transgender specific factors, such as bringing the testes into the inguinal position (tucking), wearing tight underwear and low masturbation frequency.

Wider implications of the findings: Semen quality in trans women was decreased compared to the general population, which could not be explained by known risk factors, such as BMI, alcohol consumption, cannabis use, gender-affirming hormone use or abnormal endocrine laboratory results. Although a negative impact of smoking was observed, it was insufficient to explain the overall decreased semen quality in this cohort. Since low pre-freeze semen quality results in an even lower post-thaw semen quality, the majority of trans women and their female partner or surrogate may need an invasive and burdensome treatment to establish a pregnancy.

INTRODUCTION

Gender dysphoria refers to the distress that results from a conflict between a person's assigned gender at birth and one's gender identity.[70] People assigned male at birth who identify as male are defined as cis men, and birth-assigned males who identify as female are defined as trans women. In the Netherlands, the prevalence of gender dysphoria in birth-assigned males is approximately 1 in 2,800.[71] Transgender people may start medical treatment to align their physical characteristics with their gender identity, including gender-affirming hormone treatment and gender-affirming surgery. Hormone treatment for trans women consists of anti-androgens combined with estrogens in order to achieve feminization. However, hormone treatment is accompanied by a loss of reproductive capability, and while spermatogenesis might restore after discontinuation of prolonged treatment with anti-androgens and estrogens, it has not been well studied.[29,72] After gender affirming surgery, involving penectomy and bilateral orchiectomy combined with vaginoplasty, reproductive loss will certainly be permanent.

Many trans women desire to start a family and parent genetically related offspring, just like many other people of reproductive age. A recent study among trans women showed that 69.9% had an interest in having children in the future and 76.6% considered fertility preservation before starting a medical transition.[73] For several years, scientific medical societies in the field of transgender health have emphasized the need to inform about the effect of the medical transition on fertility and the currently available options for fertility preservation.[1,2] The equipoise of commencing medical transition, and fertility preservation as the only option for biological children, may be stressful.

In trans women, the option for fertility preservation is semen cryopreservation. In case of azoospermia or other anatomic variations or emotional concerns, testicular sperm extraction (TESE) is possible but not always successful. Once cryopreserved sperm has been stored, ART enable trans women to have genetically related children with their female partner or via a surrogate. Which technique is optimal is determined by the post-thaw semen parameters: semen of good quality can be used for minimally invasive and inexpensive IUI, while semen of low quality requires a more invasive and expensive technique such as IVF or ICSI.[32]

Although data on semen characteristics in trans women show a high incidence of impaired semen quality, these studies report on a relatively low number of people.[33-35] The etiology could not be identified in these studies due to the small sample size and a lack of endocrine laboratory results and complete clinical data on certain lifestyle factors known to influence semen quality, such as age, obesity and cigarette smoking.[36-38]

With knowledge of the factors negatively influencing semen parameters in trans women it could be possible to optimize counseling in order to improve semen quality before fertility preservation. The purpose of this study is to assess semen quality in our large cohort of trans women, to evaluate semen adequacy for the different types of ART and to identify life style factors influencing semen quality.

MATERIAL AND METHODS

Patient selection

All trans women seen at the gender identity clinic in the VU University Medical Center (VUmc) between 1972 and 2017 who provided at least one semen sample for cryopreservation before 2018 were identified. People who were under 16 years of age at the time of semen cryopreservation were excluded. This resulted in 260 trans women for the present analyses who, in total, provided 748 semen samples stored in 11 different fertility laboratories in The Netherlands.

Study design and clinical data selection

The medical charts from included trans women were used to obtain data about their medical history, medication use, prior gender-affirming hormone use, alcohol consumption, smoking, cannabis use, BMI and semen characteristics. For information on BMI, alcohol consumption, smoking and cannabis use in the general Dutch population of similar age, data was obtained from the online database of Statistics Netherlands.[74]

The study protocol was assessed by the Ethical Review Board of the VU University Medical Center Amsterdam. It was concluded that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study, and necessity for informed consent was waived because of the retrospective design and the large study population.

Laboratory tests

Endocrine laboratory data were collected from the hospital registries of VUmc where clinical data, obtained during regular patient care, is stored. The laboratory results included serum concentrations of testosterone, estradiol, LH, and FSH. Counseling and referral for fertility preservation took place before commencing hormonal treatment, thus results from blood obtained by venipuncture at the initiation of hormone treatment were used. Dates of sperm banking were compared with dates of initiation of hormone treatment, accepting an interval of fewer than 120 days for reliable coupling of semen parameters to endocrine data.

Semen quality

Semen cryopreservation was performed in ISO 15189 accredited fertility laboratories in the Netherlands, all following the Dutch guideline for sperm banks.[75]

All trans women were asked to provide semen samples via masturbation after 2 to 5 days of abstinence. The vast majority of trans women provided at least two semen samples for cryopreservation. Semen characteristics were manually assessed at time of semen cryopreservation, prior to the freezing process. Samples were kept at 37 °C before analysis. The semen parameters measured were semen volume, sperm concentration and sperm motility. Volume was determined using a wide-bore volumetric pipette. To assess sperm concentration and motility, a Makler counting chamber (Sefi-Medical Instruments LTD, Haifa, Israel) and phase contrast microscope optics (200-400x) were used. Pre-freeze

and post-thaw sperm motility was classified using a three-category scheme: progressive motile, motile, and immotile.

Subsequently, ejaculates were diluted 1:1 and mixed thoroughly for at least 10 minutes with medium containing glycerol or egg yolk (TYB, Irvine Scientific) as cryoprotectant and put in 0.3 or 0.5 mL vials. Vials were put upright just above liquid nitrogen for gradual crystallization in nitrogen vapor**.** After total crystallization straws were stored in vapor phase nitrogen tanks. After 24 to 72 hours, one vial was thawed and a semen analysis was performed, as described above. Some fertility laboratories assessed post-thaw semen quality of all semen samples, some assessed only one of the provided semen samples, and in two centers no assessment of post-thaw quality was performed.

The choice of the most appropriate ART (IUI, IVF, or ICSI) is often determined by the total motile sperm count (TMSC) per vial since it reflects sperm concentration and motility, as well as the effects of sperm processing. It is difficult to establish the most optimal cut-off values for the different types of reproductive techniques since a successful treatment is also dependent on other factors, such as the woman's fertility, and therefore, recommended thresholds vary in literature.[76,77] In this study, suitability for the most appropriate ART was determined using the following cut-off values: TMSC >2 million is suitable for IUI, TMSC >1 million and <2 million is suitable for IVF, and TMSC <1 million is suitable for ICSI.

Statistical analysis

Descriptive analyses were conducted to assess the distributions of semen parameters and patient characteristics, normally distributed data are presented as mean with SD, and non-normally distributed data as median with interquartile range (IQR). Qualitative data are presented as number with percentages. For trans women who preserved multiple semen samples, the collective semen parameters were averaged and the means were used for statistical analysis.[78,79] Semen quality was categorized in the following descriptive diagnoses, using reference values of the World Health Organization (WHO) for human semen: oligozoospermia (reduced sperm count), asthenozoospermia (reduced sperm motility), oligoasthenozoospermia (reduced sperm count and motility), azoospermia (no sperm in the ejaculate) or normozoospermia (normal semen parameters).[11] Post-thaw semen quality was assessed by calculating the TMSC per vial. Since volumes of vials differed between fertility laboratories a correction was performed to enable accurate comparison.

Wilcoxon signed-rank tests were performed on non-normally distributed semen parameters to compare with data from the general population of unscreened men.[10] Azoospermic trans women were excluded from analysis of sperm concentration, total sperm number and percentage progressive motility.

Pre-determined factors (i.e. age at time of semen cryopreservation, alcohol consumption, smoking, cannabis use, BMI, previous feminizing hormone use, a history of inguinal hernia repair or cryptorchidism, and a history of depression and anxiety) were used in logistic regression analyses to assess their impact on semen quality in our cohort. Semen parameters

were dichotomized using WHO reference values to be able to compare impaired semen quality with normal semen quality.[10] Odds ratios (OR) with 95% CI were calculated.

STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA) was used for statistical analyses.

RESULTS

Description of study population

Our cohort consisted of 260 trans women who provided at least one semen sample for cryopreservation between August 1991 and December 2017. The median age at time of semen cryopreservation was 24.0 years (IQR 20.0-29.5). Alcohol consumption, smoking status, cannabis use and BMI were comparable to the general Dutch population and no abnormalities were found in the endocrine laboratory results (Table 1).[74] The medical history of 13 trans women was positive for inguinal hernia repair or cryptorchidism. Anxiety or depression was reported in 34 trans women. In total, 12 trans women reported to have used anti-androgens and estrogens, and all had discontinued for at least 3 months prior to the first attempt for semen cryopreservation. The median number of semen samples provided per person was 3.0 (IQR 2.5-3.0), and in total 748 semen samples were included for analyses.

Table 1. Baseline characteristics of study cohort (n=260 trans women)

	n	Mean (SD) or Median (IQR)	Percentage (n)	Dutch reference values*
Age at time of fertility preservation (years)	260	24 (IQR 20.0-29.5)		
Testosterone (nmol/L)	181	19.1 (SD 6.7)		9 – 30
Estradiol (pmol/L)	179	87.4 (SD 24.7)		12 – 177
LH (U/L)	177	3.5 (SD 2.1)		1 – 8.4
FSH (U/L)	18	4.1 (SD 4.0)		1 – 10.5
BMI (kg/m^2)	200	22.7 (SD 3.8)		
Underweight: <18.5			9.0 % (18)	3.9 %
Normal weight: 18.5-25.0			65.5 % (131)	63.6 %
Overweight: >25.0			25.5 % (51)	32.5 %
Alcohol	214			
Non drinker			44.4 % (95)	14.6 %
Current drinker			55.6 % (119)	85.4 %
Units/week	100	2.0 (IQR 1.0-4.0)		9.1
Smoking	121			
Never			52.9 % (64)	57.1 %
Previous smoker			9.9 % (12)	12.7 %
Current smoker			37.2 % (45)	30.2 %
Cig/day	45	10.0 (IQR 5.0-15.0)		6.9
Cannabis	170			
Never			84.1 % (143)	
Weekly			5.9 % (10)	9.9 %
Sporadically			10.0 % (17)	19.0 %
Previous hormone use	260			
Yes			4.6 % (12)	
No			95.4 % (248)	
History of anxiety or depression	260			
Yes			13.1 % (34)	
No			86.9 % (226)	
History of inguinal hernia or cryptorchidism	260			
Yes			5.0 % (13)	
No			95.0 % (247)	

* data on BMI, alcohol consumption, smoking and cannabis use are obtained from the online database of Statistics Netherlands[74].

SD: standard deviation, IQR: interquartile range.

Evaluation of semen variables and their determinants

The median values of all semen parameters in our cohort (volume 2.7 ml (IQR 1.9-3.6), sperm concentration 40 million/ml (IQR 13-58.7), total sperm number 103 million (IQR 26.9-182.2) and progressive motility 41% (IQR 26.7-53)) were significantly lower than the WHO data on semen quality in the general population of unscreened men (Figure 1).[10]

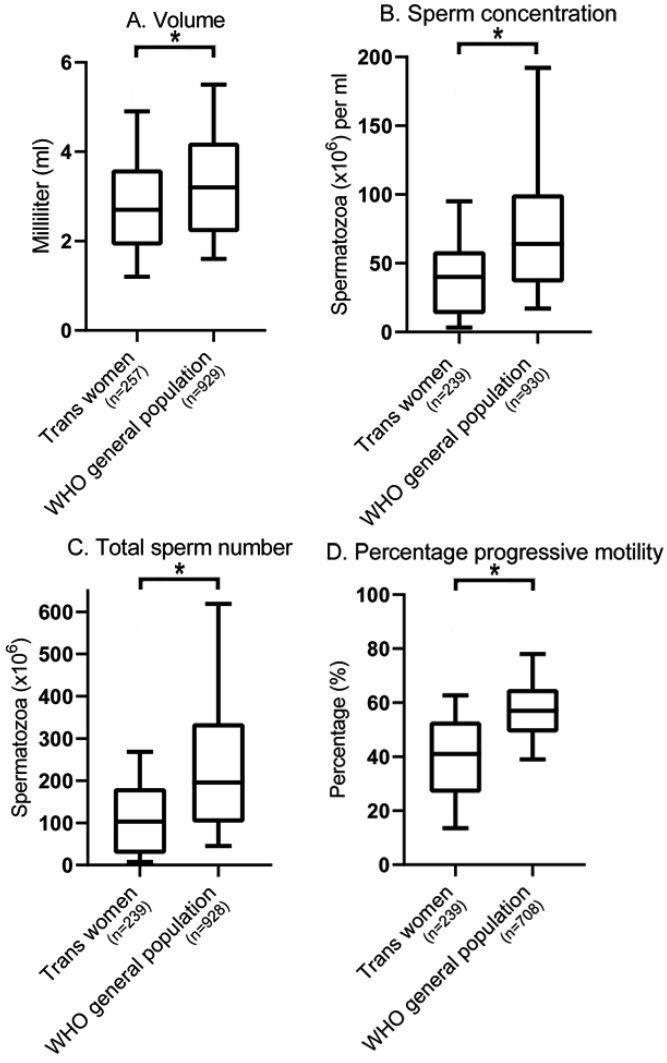


Figure 1. Box and whisker plots of semen analysis data. The data are semen volume, sperm concentration, total sperm number and percentage progressive motility from trans women compared with World Health Organization (WHO) values for unscreened men from the general population. The boxes represent the quartiles and the lines within them are the medians; the whiskers extend from the 10th to the 90th centiles.

* p-value <0.05 using Wilcoxon signed-rank tests, for panel B,C,and D azoospermic trans women were excluded from analysis.

When applying WHO semen criteria, a substantial percentage of the study population did not meet the reference value for semen volume (<1.5 ml, 18.1%), total sperm number (<39 million, 35.8%), sperm concentration (<15 million/ml, 33.5%) and progressive motility (<32%, 36.9%). In Figure 2, the classification of semen quality in our cohort is demonstrated using the descriptive diagnosis nomenclature.

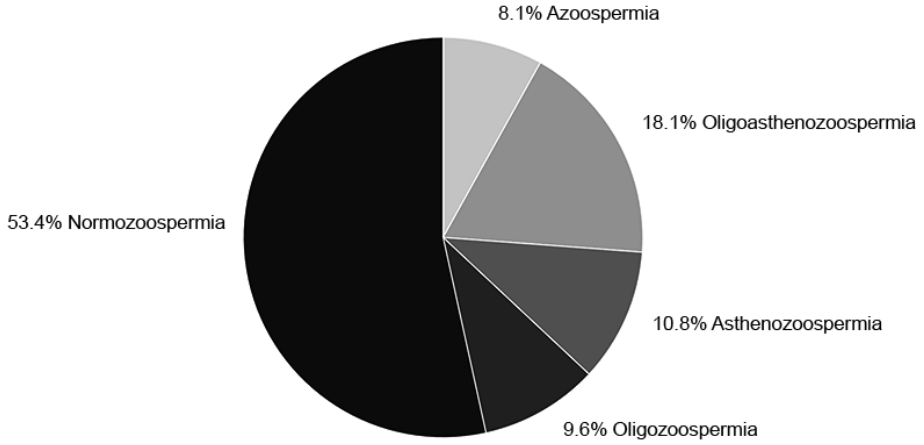


Figure 2. Classification of semen quality in the study sample of 260 trans women. The pie chart presents the descriptive diagnoses of trans women according to World Health Organization (WHO) reference values for human semen.

For 228 trans women a post-thaw semen quality was assessed; the median TMSC was 1.0 million per vial (IQR 0.1-3.1). In only 26.4% of the post-thawed samples was the semen quality adequate for IUI, 13.4% was suitable for IVF and 60.2% required ICSI.

In total, 21 trans women had an azoospermia. Three of these individuals reported to have used gender affirming hormones and stopped taking these 3 months prior to the first attempt of semen cryopreservation. Even 6 months after discontinuation of hormone treatment they still remained azoospermic. Seven hormone naïve trans women elected to undergo TESE, which resulted in cryopreserved spermatozoa in only three cases. Endocrine laboratory results for the azoospermic individuals were all in the normal range, except for FSH concentrations which were only available for three trans women but was elevated in one case (median 6.8 U/L, range 5.4-15.7 U/L).

Logistic regression analyses showed no effect of BMI, alcohol consumption or cannabis use on the semen parameters. Smoking was found to correlate with a progressive motility below 32% (OR 2.35 95%CI 1.06-5.21) but within smokers no relation between the number of smoked cigarettes per day and semen parameters was found. A higher age at time of fertility preservation also correlated with an impaired progressive motility (OR 1.04 95%CI 1.00-1.08). The decreased semen quality in our cohort could not be explained by abnormal endocrine laboratory results, previous gender-affirming hormone use, a history of cryptorchidism or inguinal hernia repair, and a history of anxiety or depression. Data for all logistic regression analyses are shown in Table 2.

Table 2. Effect of patient-related factors on semen parameters

	Semen volume <1.5 ml OR (95% CI)	Sperm concentration <15x10^6 OR (95% CI)	Total sperm number <39x10^6 OR (95% CI)	Progressive motility <32% OR(95% CI)
Age at time of fertility preservation - years	0.96 (0.91-1.01)	1.03 (0.99-1.07)	1.00 (0.97-1.04)	1.04 (1.00-1.08)*
BMI – kg/m^2	1.01 (0.93-1.11)	1.05 (0.97-1.13)	1.04 (0.97-1.12)	1.04 (0.97-1.12)
Alcohol - yes/no	0.96 (0.47-1.89)	0.59 (0.34-1.04)	0.71 (0.41-1.24)	0.85 (0.49-1.48)
Smoking - yes/no	2.21 (0.95-5.17)	1.33 (0.62-2.82)	2.01 (0.95-4.27)	2.35 (1.06-5.21)*
Cigarettes per day (in smokers)	1.09 (0.98-1.22)	1.03 (0.94-1.14)	1.02 (0.93-1.13)	1.11 (1.00-1.24)
Cannabis use - yes/no	∞	0.80 (0.36-1.91)	0.79 (0.34-1.85)	0.40 (1.15-1.04)
Previous hormone use - yes/no	1.55 (0.40-5.94)	2.94 (0.91-9.55)	2.64 (0.81-8.56)	2.50 (0.77-8.11)
History of anxiety or depression - yes/no	1.16 (0.47-2.83)	1.83 (0.89-3.76)	1.41 (0.69-2.91)	0.88 (0.41-1.85)
History of inguinal hernia repair or cryptorchidism - yes/no	0.85 (0.17-3.81)	0.58 (0.16-2.17)	0.52 (0.14-1.95)	1.07 (0.34-3.37)

* p-value <0.05 using logistic regression analyses.

At time of writing, six trans women in our cohort had used their cryopreserved semen a median 6 years (range 2-18 years) after cryopreservation. One individual decided to donate two vials to a befriended couple. The other five trans women successfully used their semen with their female partner. Two couples conceived through IUI, one couple through IVF and the other two couples through ICSI.

DISCUSSION

As far as we are aware, this is the largest cohort study showing an impaired semen quality in trans women at time of fertility preservation. In only 26.4% of the post-thawed samples was semen quality considered adequate for IUI. Although smoking and a higher age did affect progressive motility, it did not provide an explanation for the overall reduced quality in this cohort. Therefore, these results suggest the existence of one or more transgender specific factors that negatively influence semen quality.

A high percentage of trans women had semen parameters below the WHO reference values when compared with a study on semen quality of young men from the general population, i.e. a sperm concentration below 15 million/ml (33.5% versus 17.5%) and progressive motility below 32% (36.9% versus 14.4%).[80] Furthermore, we observed a higher incidence of azoospermia (8.1% versus 0.8%) compared to the study of Hart et al.

Our findings are in line with previous studies on semen quality in trans women, which also showed a high incidence of azoospermia, oligozoospermia and asthenozoospermia.[33-35] Only one of these studies collected data on personal behavior, feelings of depression, anxiety and stress, as well as serum hormone concentrations. However, none of the characteristics were able to explain the reduced semen quality in trans women.[35] Despite the significantly larger sample size, our cohort study demonstrates similar observations.

Many trans women desire to start a family and prefer to use their gametes for this purpose.[73] This is in direct conflict with their desire for medical transition, as anti-androgens, estrogens and gender-affirming surgery impair their reproductive function. Semen cryopreservation enables trans women to parent genetically related offspring later in life and they are strongly recommended to cryopreserve semen prior to hormone treatment as spermatogenesis might not restore, or only partially, after discontinuation. Furthermore, discontinuation of hormone treatment can be quite stressful for trans women due to the returning effects of testosterone. Therefore, trans women have to reflect on their reproductive wishes at a young age and early in the transition process.

Early banking of sperm enables trans women to have future reproductive options. However, a decreased semen quality limits the number of ART that can be used. A high post-thaw TMSC is preferred because semen can be used for IUI and the insemination can take place in the natural cycle of a cis woman. Therefore, it is an uncomplicated and noninvasive technique, with minimal monitoring and risks. IVF or ICSI, however, may have serious consequences and risks for the cis woman undergoing this treatment as it involves controlled ovarian stimulation and an oocyte retrieval procedure.[81] In our cohort, more than 70% of the thawed samples were only suitable for IVF or ICSI. Previous studies already demonstrated a high variation in the cryosurvival of semen, mainly depending on pre-freeze motility and total sperm count.[82-84] Although, because of the cryopreservation process, post-thaw motility is always decreased compared to pre-freeze values, it has been shown that higher pre-freeze sperm counts result in an increased potential to withstand cryopreservation. Conversely, lower pre-freeze semen quality results in an even lower post-thaw motility, underlining the importance of improving semen quality by improving certain lifestyle factors prior to the fertility preservation process.[83]

The question arises of how we can counsel trans women to take the appropriate action to optimally cryopreserve their sperm. Multiple studies, performed in cis men, established the negative effect of a higher age, obesity, cigarette smoking and high levels of alcohol intake on semen quality.[36-38,85] In our cohort, some effects of these lifestyle factors were observed; smoking and a higher age correlated with an impaired progressive motility. However, as shown in Table 1, compared with data from the general Dutch population of similar age, our cohort consisted of generally healthier individuals in terms of BMI, alcohol intake, smoking and cannabis use.[74]

Other factors associated with a lower semen quality are psychological stress, depression and anxiety: previous studies showed a significantly negative effect of these factors on sperm concentration, motility and morphology.[86,87] Trans women desiring a medical transition might experience more stress compared to cis men but our data did not show a decreased semen quality in trans women who reported suffering from anxiety or depression. Hypothetically, semen quality in trans women is affected by transgender specific factors, such as a low frequency of masturbation, gender-affirming hormone use, keeping the genitals tight against the body or even bringing the testicles into the inguinal canal (tucking).[88-91] Trans women with previous gender-affirming hormone use in our

cohort had discontinued their medication for at least 3 months, and no negative effect on the semen parameters was observed. Lifestyle factors, such as tucking and wearing tight-fitting underwear, might increase scrotal temperatures which is associated with an impaired semen quality.[92] In 1985 a study was performed to evaluate the influence of tucking on semen quality in order to provide a contraceptive method in cis men. They found an inhibition of spermatogenesis after 3 months of daily tucking and semen parameters were lowest after 6 months.[89] Unfortunately, we were not able to reproduce this negative influence of tucking since it was not recorded in the medical files. Taking all these factors into account, we were not able to provide a clear explanation for the impaired semen quality in our cohort.

The major strengths of our study are the large cohort size, which is much bigger than previous studies on this topic, since approximately 95% of all transgender people in the Netherlands are treated in our center, and the evaluation of potential influencing factors on semen quality obtained through access to the medical files.

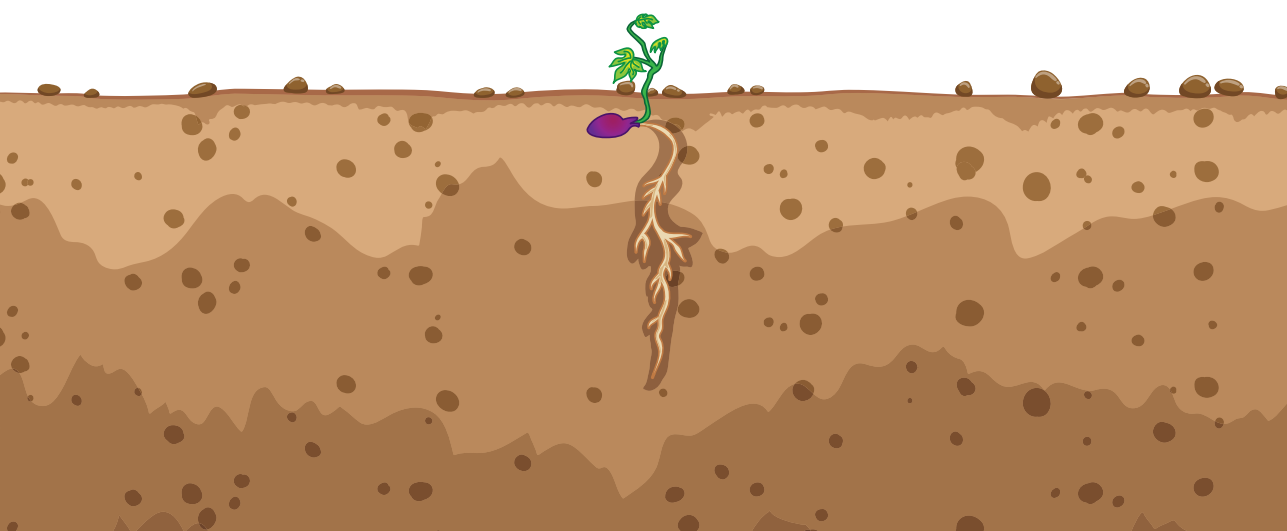
However, owing to the retrospective design of the study, information on transgender specific life style factors was only available for a few individuals and, for example, did not include the frequency and the duration of tucking or when tucking was last performed. As a result, we were unable to demonstrate that these habits might explain the observed reduced semen quality. Also, the retrospective design may lead to an underestimation of the prevalence of anxiety and depression in our cohort. Another limitation of the study is the absence of data on semen morphology as this was not recorded during the process of semen cryopreservation. Furthermore, although abstinence time was advised to be 2-5 days, exact abstinence time was not recorded and we were therefore unable the correct for this factor.

For future studies it would be worthwhile to prospectively obtain data on endocrine laboratory results and detailed information about lifestyle factors at time of fertility preservation in order to adequately determine the etiology of the impaired semen quality in trans women. Furthermore, more knowledge of the influence of gender-affirming hormone treatment on spermatogenesis and its restoration after discontinuation might help to establish the optimal strategy for trans women with a desire for genetically related offspring.

Conclusion

Fertility preservation has now become widely available in the Netherlands for most trans women and enables them to parent genetically related children after medical transition. Our findings show a high frequency of impaired semen parameters compared to the general population. Since low pre-freeze semen quality results in an even lower post-thaw semen quality, the majority of trans women and their female partner or surrogate may need an invasive and burdensome treatment to establish a pregnancy.

This cohort study shows that patient-related factors associated with a decreased semen quality in the general population, do not seem to be responsible for the impaired quality in trans women. We have commenced a prospective cohort study on how transgender specific factors, such as tucking, affect semen quality in trans women in order to optimize counselling. With this knowledge we aim to help trans women in their wish to parent genetically related offspring by cryopreserving semen that will be suitable for the least burdensome strategy.



CHAPTER 4

A COHORT STUDY ON FACTORS IMPAIRING SEMEN QUALITY IN TRANSGENDER WOMEN

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Published in American Journal of Obstetrics and Gynecology; October 2021 [article in press]

ABSTRACT

Background: Transgender women (birth-assigned males, female gender identity) can choose to cryopreserve semen prior to their medical transition, to retain the possibility to parent genetically related offspring later in life. Our previous retrospective study showed that semen quality in transgender women is decreased compared to the general population. The etiology of this impaired semen quality remains largely unknown, but might be related to habitual behavior more typically observed in transgender women, e.g. the desire to hide their testicles due to genital dysphoria. Therefore, we decided to conduct a consecutive study with prospectively obtained data on behavior and lifestyle in transgender women.

Objective: To study the influence of a low ejaculation frequency, wearing tight undergarment, and bringing the testes in the inguinal position (tucking), on semen quality in transgender women at time of fertility preservation.

Study design: In this cohort study, transgender women were included between May 2018 and September 2020, at time of fertility counseling, prior to the start of hormonal treatment. Data were collected on demographics, lifestyle factors, medical history, endocrine laboratory results, and semen parameters. Semen parameters were categorized using reference values for human semen of the World Health Organization and compared with semen quality in the general population. Odds ratios (OR) with 95% confidence intervals (95% CI) were calculated using multivariable logistic regression analysis to assess the impact of tucking, wearing tight undergarment, and a low ejaculation frequency on semen quality, correcting for potential confounders.

Results: Overall, 113 transgender women were included. Median semen parameters were significantly decreased compared to the general population. Crude logistic regression analyses showed an association between always wearing tight undergarment (OR 3.06, 95% CI 1.11-8.49), and extensive tucking (OR 6.09, 95% CI1.54-24.01), on having a total motile sperm count below 5 million. Multivariable analyses showed that the association with tucking was independent of demographic factors, lifestyle factors and medical history (OR 7.95, 95% CI 1.66-37.99). However, this was not the case for the association with always wearing tight undergarment (OR 2.89, 95% CI 0.95-8.82). Ejaculation frequency did not influence total motile sperm count.

Conclusions: Behavioral factors, including wearing tight undergarment and extensive tucking, may contribute to the lower semen quality in transgender women. These results enable optimization of fertility counseling on how to adjust lifestyle before pursuing semen cryopreservation.

INTRODUCTION

Many young people with gender dysphoria – distress caused by an incongruence between the sex assigned at birth and the gender identity – seek medical treatment to align their physical characteristics with the identified gender.[70] This medical treatment involves gender-affirming hormonal treatment (GAHT), often followed by gender-affirming surgery (GAS).[93] For transgender women – people assigned male at birth with a female gender identity – GAHT generally consists of a combination of antiandrogens and estrogens.[2] After at least one year of GAHT, transgender women may choose to undergo GAS which may involve bilateral orchiectomy often combined with vaginoplasty.[93]

Although this treatment significantly improves quality of life, the loss of reproductive function is an often unwanted consequence.[60,73,94] The use of gender-affirming hormones severely impairs semen quality which might not, or only partially, recover after cessation of this medication.[39,95] After bilateral orchiectomy, reproductive loss is permanent. Therefore, it is important that (future) desire for children and the options for fertility preservation are discussed and offered prior to the start of gender-affirming treatment.

Cryopreservation of ejaculated spermatozoa is preferred, as it is physically noninvasive and provides the best semen quality. However, our previous retrospective study showed that even before initiating GAHT, semen quality in transgender women is decreased compared to cisgender men – birth assigned males who also identify as male – and in a substantial percentage of semen samples, quality is lower than WHO reference values for human semen.[10,11,96] Therefore, it is worth identifying lifestyle interventions which may improve the pre-freeze quality. Previously we were not able to provide an explanation for the impaired semen quality in transgender women. Factors known to have a negative influence in the general population like smoking, alcohol abuse or obesity, were not responsible for the overall decreased semen quality in our cohort.[96] However, semen quality might also be affected by habitual behavior more typically observed in transgender women, such as the desire to hide their testicles. Scrotal hyperthermia, as a result of wearing tight undergarment or bringing the testicles into the inguinal canal (tucking), may negatively influence sperm motility and concentration.[88-92] Furthermore, genital dysphoria may result in a low frequency of ejaculation which may negatively impact sperm motility. Although these behavioral factors are often assumed to be explanatory for the impaired semen quality, their frequency and effect on semen quality in transgender women have never been studied.

The purpose of the current study is to obtain data on all factors with a potential negative impact on semen quality in transgender women and further validate the findings from our previous study.

MATERIALS AND METHODS

Study design and patient selection

A cohort study was conducted at the Center of Expertise on Gender Dysphoria of Amsterdam UMC. Transgender women above the age of 16 years, who were referred to a fertility specialist and decided to pursue fertility preservation between May 2018 and September 2020, were asked for participation. The Ethical Review Board of the VU University Medical Center Amsterdam concluded that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study. The study protocol was registered in the Netherlands Trial Register with registration number NTR7185.

Data collection

During the first visit with a fertility specialist, information was collected on carefully selected factors with a potential negative impact on semen quality, based on literature assessing the influence of lifestyle on semen quality in cisgender men, and behavioral factors more typically observed in transgender women. Data included demographics, lifestyle factors, medical history, tucking, wearing of tight undergarment, and ejaculation frequency. Blood samples were taken for serum testosterone and FSH concentrations. Subsequently, participants were referred to a regional fertility clinic for semen cryopreservation. Semen parameters were collected after completion of the semen cryopreservation process.

Laboratory tests

Semen cryopreservation was performed in ISO 15189 accredited fertility laboratories, following the Dutch guideline for sperm banks.[75] Transgender women were advised to collect a sperm sample after 2-5 days of ejaculatory abstinence. Prior to the freezing process, semen characteristics (semen volume, sperm concentration and sperm motility) were manually assessed by qualified laboratory technicians. Volume was determined using a wide-bore volumetric pipette, and sperm concentration and motility were assessed using a Makler counting chamber (Sefi-Medical Instruments LTD, Haifa, Israel) and phase contrast microscope optics (200–400x). Pre-freeze and post-thaw sperm motility were classified using a three-category scheme: progressive motile, motile and immotile. Total motile sperm count (TMSC) was calculated by multiplying the sample volume (ml), sperm concentration per ml and the progressive motility.

To prepare the samples for cryopreservation, ejaculates were diluted 1:1 and mixed thoroughly for at least 10 min with medium containing glycerol or egg yolk (TYB, Irvine Scientific) as cryoprotectant and put in 0.3 or 0.5 ml vials. Vials were put upright just above liquid nitrogen for gradual crystallization in nitrogen vapor. After total crystallization, straws were stored in vapor phase nitrogen tanks. Half of the fertility laboratories also assessed post-thaw quality by thawing a vial after 24–72 hours and assessing the semen characteristics again. Some of these laboratories assessed post-thaw quality of all semen samples, others assessed only one of the provided semen samples. The predicted most appropriate reproductive technique was determined by the post-thaw TMSC per vial. In

this study, the following cutoff values were used: TMSC >2million is suitable for intra uterine insemination (IUI), TMSC >1and <2 million is suitable for in vitro fertilization (IVF) and TMSC <1 million is suitable for intra cytoplasmatic sperm injection (ICSI).

Serum hormone levels were assessed in the laboratory for Endocrinology of Amsterdam UMC.

Statistical analysis

Baseline characteristics 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. Since participants cryopreserved multiple semen samples, the collective semen parameters were averaged and their means were used for statistical analysis. Semen characteristics from our cohort were compared with data from the general population of unscreened men, using Wilcoxon signed rank tests.[10] By using the reference values for human semen as determined by the World Health Organization (WHO), semen quality was categorized in the following descriptive diagnoses: normozoospermia, asthenozoospermia, oligozoospermia, oligoasthenozoospermia, or azoospermia.[11] Azoospermic transgender women were excluded from analysis of sperm concentration, total sperm number, and percentage progressive motility. For post-thaw semen quality, TMSC was calculated per vial volume of 0.5 mL and categorized as described above.

Since data on semen quality were strongly right-skewed, logistic regression analyses were used to assess the influence of factors known to have a negative impact on semen quality in cisgender men, (i.e. age at time of semen cryopreservation, alcohol consumption, smoking, cannabis use, BMI, a history of urological problems, and a history of depression and anxiety), as well as factors that may specifically impact semen quality in transgender women (i.e. previous feminizing hormone use, ejaculation frequency, wearing of tight undergarment, and tucking).[36-38,85,87] Semen quality was expressed in five semen parameters (semen volume, sperm concentration, total sperm number, progressive motility, and TMSC), of which TMSC was used as main outcome measurement since it takes the absolute value of three semen parameters into consideration simultaneously. Based on results from a previous study on indicators for the severity of male factor infertility, TMSC was dichotomized using 5 million as cut-off value.[12,13] The other semen parameters were dichotomized using WHO reference values (semen volume <5 ml, sperm concentration <15 million per ml, sperm motility <32%, and total sperm number <39 million).[10]

Ejaculation frequency, wearing tight undergarment, and tucking were each divided in three categories: low (<6x/month), average (6-12x/month), and high (>12x/month) ejaculation frequency; never, sometimes, and always wearing tight undergarment; never, sometimes (1-8x/month), and often (>8x/month) tucking. Firstly, crude analyses were performed, then multivariable logistic regression analyses were conducted, correcting for the other determinants. Both crude and corrected odds ratios (OR) with 95% confidence intervals (95% CI) are presented. STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA) was used for statistical analyses.

RESULTS

Description of the study cohort

In total, 113 transgender women were included. Baseline characteristics are shown in Table 1. Serum testosterone and FSH concentrations were in the normal range, and alcohol consumption, smoking status, cannabis use and BMI were comparable with the general Dutch population of similar age.[74] Six transgender women reported to have used self-obtained gender-affirming hormones but discontinued this treatment a median 3.8 months (IQR 2.5-6.0) prior to semen cryopreservation. In our cohort 40 transgender women (35.4%) suffered from anxiety or depression and 24 (21.2%) reported previous urological problems, among which orchiopexy (n=5), testicular torsion (n=1) or epididymo-orchitis (n=1). In total, 46 transgender women (40.7%) reported to be solely attracted to women, 25 (22.2%) were solely attracted to men, and 40 (35.3%) were attracted to both women and men.

Table 1. Characteristics of study cohort (n=113)

	Percentage (n)	Mean (SD) or Median (IQR)	Dutch reference values`
Age at time of fertility counseling (years)		24.1 (SD 5.8)	
FSH (U/L)^		3.5 (SD 3.3)	1.0-10.5
Testosterone (nmol/L)~		17.2 (SD 6.6)	9.0-30.0
BMI (kg/m^2)		23.3 (SD 5.4)	
Underweight: <18.5	15.9% (18)		4.5%
Normal weight: 18.5-25.0	56.6% (64)		63.8%
Overweight: >25.0	27.4% (31)		31.7%
Alcohol			
Non drinker	48.7% (55)		14.3%
Current drinker	51.3% (58)		85.7%
Units/week		1.0 (IQR 1.0-4.0)	9.8
Smoking			
Never	66.4% (75)		54.9%
Previous smoker	15.0% (17)		14.5%
Current smoker	18.6% (21)		30.6%
Cig/day		5.5 (3.0-10.0)	
Cannabis use			
Never	81.4% (92)		
Sporadically	6.2% (7)		18.8%
Weekly	12.4% (14)		12.5%
Previous hormone use			
Yes	5.3% (6)		
No	94.7% (107)		
History of urological problems*			
Yes	21.2% (24)		
No	78.8% (89)		
History of anxiety or depression			
Yes	35.4% (40)		
No	64.6% (73)		
Sexual dysfunction			
Yes	4.4% (5)		
No	95.6% (108)		

Data available for ^107 for ~109 transgender women

*Urological problems include genital/inguinal surgery (e.g. orchiopexy, inguinal hernia repair, varicocele repair), scrotal infection (e.g. epididymitis, orchitis) and testicular torsion.

`Data on BMI, alcohol consumption, smoking and cannabis use are obtained from the online database of Statistics Netherlands.[74]

SD: standard deviation, IQR: interquartile range.

Results of semen cryopreservation

The median number of semen samples provided per person was 2.9 (SD 0.8), and the total number of provided samples was 323, divided over 3101 vials. The median semen parameters in our cohort were as follows: volume 2.4 ml (IQR 1.5-3.5), sperm concentration 27.5 million/ml (IQR 13.0-51.7), total sperm number 66.9 million (IQR 20.6-126.8), percentage of progressive motility 50.7 (IQR 38.8-60.3), and total motile sperm count 32.8 million (IQR 6.6-73.3). Semen quality was significantly lower than reported by the WHO for the general population (Figure 1).[10]

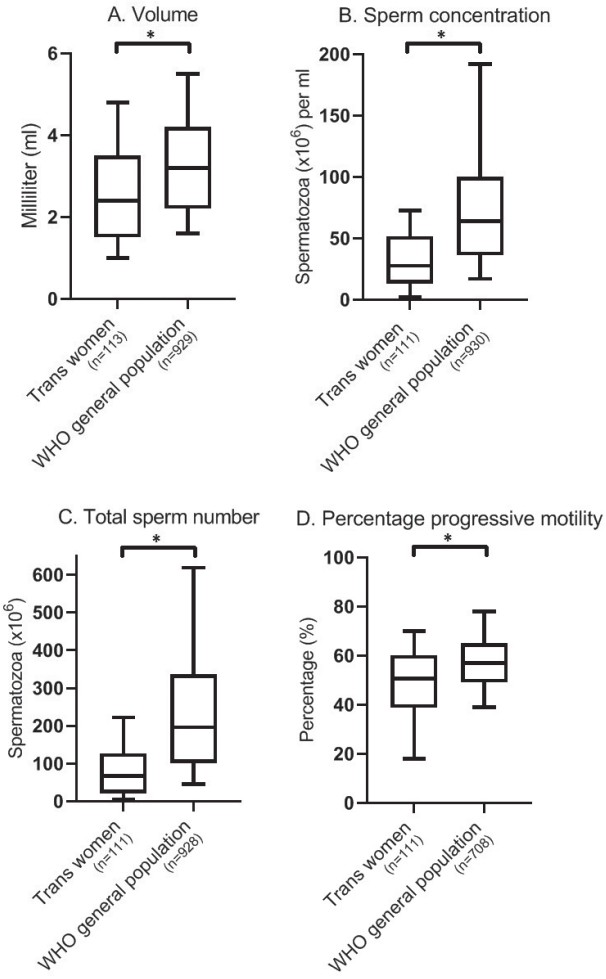

Figure 1. Box and whisker plots of semen analysis data. The data are semen volume, sperm concentration, total sperm number and percentage progressive motility from transgender women compared with World Health Organization (WHO) values for unscreened men from the general population. The boxes represent the quartiles and the lines within them are the medians; the whiskers extend from the 10th to the 90th centiles.

* p-value <0.05 using Wilcoxon signed-rank tests, for panel B, C, and D azoospermic transgender women were excluded from analysis.

Figure 2 shows the classification of semen quality in the study cohort according to the WHO reference values for human semen, the majority (60.2%) was classified as normozoospermia.[11] Post-thaw semen quality was available for 74 transgender women and the median TMSC was 0.5 million per vial (IQR 0.2-1.7). The majority of post-thawed samples was only suitable for invasive techniques, such as IVF (9.6%) or ICSI (67.1%), and in only 23.3% of samples the post-thaw quality was suitable for IUI. Characteristics of these 74 transgender women did not differ from the total cohort (data not shown).

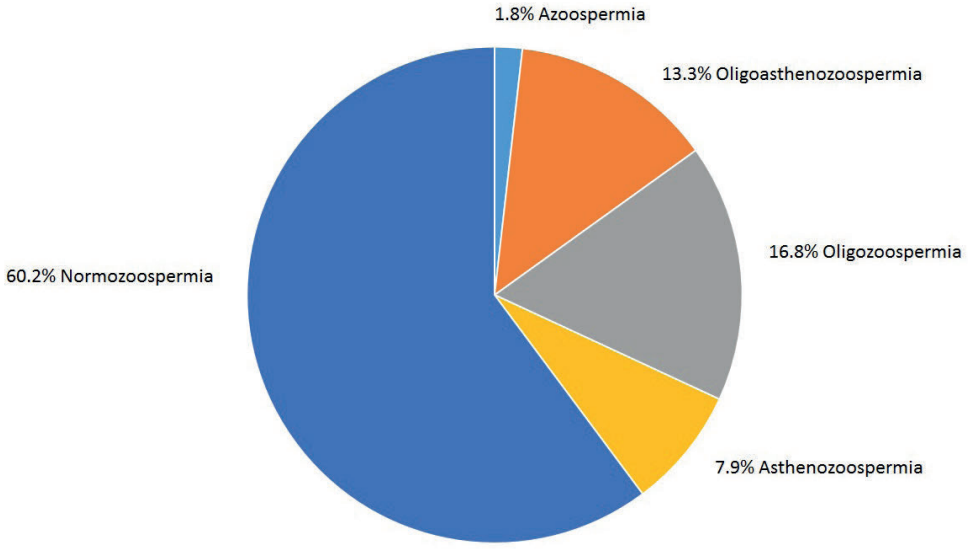

Figure 2. Classification of semen quality in the study sample of 113 transgender women. The pie chart presents the descriptive diagnoses of transgender women according to World Health Organization (WHO) reference values for human semen.

Influence of determinants on semen quality

Logistic regression analyses showed no effect of age, BMI, smoking, cannabis use and medical history on the semen parameters. In addition, no significant association was found between previous hormone use and having impaired semen parameters. Drinking alcohol was associated with a normal semen volume, sperm concentration, total sperm number and total motile sperm count (Table 2).

Table 2. Effect of demographics, lifestyle factors, and medical history on semen parameters

	Semen volume <1.5 ml OR (95% CI)	Sperm concentration <15x10^6 OR (95% CI)	Total sperm number <39x10^6 OR (95% CI)	Progressive motility <32% OR(95% CI)	Total motile sperm count <5 x10^6 OR(95% CI)
Age at time of fertility preservation - years	0.95 (0.88-1.04)	0.97 (0.91-1.05)	0.99 (0.93-1.06)	1.05 (0.98-1.13)	1.01 (0.93-1.09)
BMI – kg/m^2	1.03 (0.95-1.11)	0.97 (0.89-1.05)	0.98 (0.91-1.05)	1.03 (0.95-1.11)	1.02 (0.94-1.10)
Alcohol - yes/no	0.40 (0.17-0.97)*	0.48 (0.21-1.07)*	0.36 (0.16-0.81)*	0.76 (0.32-1.84)	0.24 (0.09-0.67)*
Smoking - yes/no	1.57 (0.56-4.38)	1.41 (0.52-3.77)	2.35 (0.90-6.17)	0.50 (0.13-1.85)	0.56 (0.15-2.07)
Cigarettes per day	1.00 (0.89-1.13)	1.03 (0.93-1.15)	1.04 (0.94-1.16)	0.88 (0.73-1.08)	0.89 (0.74-1.08)
Cannabis use - yes/no	1.18 (0.41-3.41)	1.41 (0.52-3.77)	1.45 (0.55-3.82)	1.62 (0.55-4.77)	1.46 (0.25-8.44)
Previous hormone use - yes/no	1.46 (0.25-8.44)	2.24 (0.43-11.70)	1.86 (0.36-9.70)	0.66 (0.07-5.88)	1.9 (0.33-11.11)
History of anxiety or depression - yes/no	1.20 (0.50-2.89)	1.05 (0.46-2.39)	1.01 (0.45-2.28)	1.81 (0.74-4.41)	0.92 (0.35-2.39)
History of urological problems - yes/no	0.94 (0.33-2.66)	0.49 (0.17-1.45)	0.88 (0.34-2.27)	1.15 (0.40-3.28)	0.68 (0.21-2.22)

*p-value <0.05 using logistic regression analyses.

Ejaculation frequency, and the prevalence of wearing tight undergarment and tucking in our study cohort are presented in Table 3. Table 4 shows the effect of these factors on TMSC. The median ejaculation frequency in our cohort was 10 times a month (IQR 5.0-15.0). Logistic regression analysis showed a negative impact of a low ejaculation frequency on progressive motility, but it did not affect TMSC. Approximately half of the study population reported to never wear tight undergarment, the other half wears it sometimes (17.7%) or always (31.9%). Always wearing tight undergarment was associated with an impaired TMSC (OR 3.06, 95% CI 1.11-8.49), although this effect was no longer significant after adjustment for confounding factors. Tucking was performed by 26 of included transgender women (23.0%), a median 5.0 (IQR 1.0-30.0) times a month and with a median duration of 6.5 (IQR 5.0-8.0) hours per day. In total, 10 transgender women (8.9%) reported to perform tucking more than 8 times a month which was associated with a TMSC below 5 million (OR 6.09, 95% CI 1.54-24.01). This association did not change in the multivariable analysis (OR 7.95, 95% CI 1.66-37.99), and was mainly caused by a negative effect on sperm concentration. Table 5 demonstrates the effect of ejaculation frequency, wearing tight undergarment, and tucking on semen volume, sperm concentration, total sperm number, and progressive motility.

Table 3. Ejaculation frequency and prevalence of wearing tight undergarment and tucking in study cohort

	Percentage (n)	Median (IQR)
Ejaculation frequency		10.0 (IQR 5.0-15.0)
Low (<6x/month)	27.4% (31)	
Average (6-12x/month)	36.3% (41)	
High (>12x/month)	36.3% (41)	
Wearing tight undergarment		
Never	50.4% (57)	
Sometimes	17.7% (20)	
Always	31.9% (36)	
Tucking		
Never	77.0% (87)	
Sometimes (1-8x/month)	14.1% (16)	
Often (>8x/month)	8.9% (10)	
Frequency (per month)		5.0 (IQR 1.0-30.0)
Duration (hours per day)		6.5 (IQR 5.0-8.0)
Last time (days ago)		12.0 (IQR 1.0-30.0)

Table 4. Effect of ejaculation frequency, wearing tight undergarment and tucking on total motile sperm count

	Total motile sperm count <5 x10^6	
	Univariable analysis OR (95% CI)	**Multivariable analysis^ OR (95% CI)**
Ejaculation frequency (per month)		
Average (6-12x) vs low (<6x)	1.11 (0.37-3.33)	1.72 (0.49-6.05)
High (>12x) vs low (<6x)	0.73 (0.23-2.35)	0.78 (0.19-3.15)
Wearing tight undergarment		
Sometimes vs never	1.63 (0.43-6.19)	1.40 (0.32-6.06)
Always vs never	3.06 (1.11-8.49)*	2.89 (0.95-8.82)
Tucking		
Sometimes (1-8x) vs never	0.27 (0.03-2.19)	0.47 (0.05-4.17)
Often (>8) vs never	6.09 (1.54-24.01)*	7.95 (1.66-37.99)*

^Correction for demographic factors (age, BMI) + lifestyle factors (alcohol consumption, smoking, cannabis use) + medical history (previous hormone use, urological problems, anxiety and depression)

*p-value<0.05 using logistic regression analysis

Table 5. The effect of ejaculation frequency, wearing tight undergarment, and tucking on semen volume, sperm concentration, total sperm number and progressive motility

	Semen volume <1.5 ml OR (95% CI)	Sperm concentration <15x10^6 OR (95% CI)	Total sperm number <39x10^6 OR (95% CI)	Progressive motility <32% OR(95% CI)
Ejaculation frequency (per month)				
Univariable analysis				
Average (6-12x) vs low (<6x)	0.38 (0.13-1.11)	0.94 (0.35-2.51)	0.89 (0.34-2.29)	4.33 (1.11-16.89)*
High (>12x) vs low (<6x)	0.46 (0.16-1.30)	0.67 (0.24-1.83)	0.53 (0.19-1.42)	3.01 (0.75-12.06)
Multivariable analysis^				
Average (6-12x) vs low (<6x)	0.35 (0.11-1.10)	1.11 (0.38-3.22)	1.05 (0.37-3.01)	4.78 (1.11-20.48)*
High (>12x) vs low (<6x)	0.36 (0.11-1.21)	0.64 (0.19-2.07)	0.56 (0.17-1.81)	2.24 (0.48-10.55)
Wearing tight undergarment				
Univariable analysis				
Sometimes vs never	1.29 (0.42-4.01)	0.93 (0.29-3.01)	1.71 (0.59-5.00)	1.08 (0.26-4.55)
Always vs never	0.80 (0.30-2.14)	2.24 (0.93-5.42)	1.68 (0.70-4.02)	4.38 (1.61-11.88)
Multivariable analysis^				
Sometimes vs never	1.06 (0.32-3.53)	0.92 (0.27-3.17)	1.66 (0.51-5.37)	1.15 (0.26-5.10)
Always vs never	0.71 (0.24-2.03)	2.71 (1.01-7.24)*	2.01 (0.76-5.34)	3.63 (1.27-10.37)*
Tucking				
Univariable analysis				
Sometimes (<8x) vs never	0.37 (0.08-1.75)	0.78 (0.23-2.65)	0.24 (0.05-1.13)	0.51 (0.12-2.45)
Often (>8) vs never	1.11 (0.26-4.64)	3.52 (0.92-13.52)	2.53 (0.66-9.65)	3.58 (0.94-13.67)
Multivariable analysis^				
Sometimes (<8x) vs never	0.44 (0.08-2.24)	1.05 (0.28-3.90)	0.30 (0.06-1.55)	0.49 (0.10-2.47)
Often (>8) vs never	1.21 (0.25-6.02)	5.48 (1.18-25.59)*	3.39 (0.75-15.36)	2.76 (0.65-11.68)

^Correction for demographic factors (age, BMI) + lifestyle factors (alcohol consumption, smoking, cannabis use) + medical history (previous hormone use, urological problems, anxiety and depression)

*p-value<0.05 using logistic regression analysis

COMMENT

Principal findings

This current study showed once more that semen quality in transgender women is decreased compared to the general population, even prior to GAHT. A negative impact of always wearing tight undergarment and extensive tucking on TMSC was observed. The association with tucking was independent of demographic factors, lifestyle factors and medical history.

Results in the context of what is known

The median semen parameters observed in the current study are in line with previous studies and similar to the results of the large retrospective cohort study we performed in 2019.[34,35,96] Post-thaw semen quality was in only 23.3% of samples adequate for IUI. When comparing baseline characteristics of the current cohort with the retrospective cohort, data on demographics and lifestyle appear to be very similar and show a young, relatively healthy population. However, the prospectively obtained data on medical history show a high percentage of people with anxiety or depression, and previous urological

problems, which might have been underestimated in the retrospective cohort study.[96] When assessing the influence of these factors on the different semen parameters, none of the characteristics were able to explain the overall reduced semen quality in our cohort. Although drinking alcohol was associated with having a normal semen volume, sperm concentration, total sperm number and total motile sperm count, both the percentage of current drinkers in our cohort and the consumed units of alcohol per week, are lower than in the general population of similar age.[74]

In this study we collected detailed data on habitual behavior more typically observed in transgender women. Many transgender women socially transition before starting medical treatment, and combined with the long waiting period for genital GAS, transgender women may feel the need to hide their external genitalia in order to express themselves more feminine by wearing tight-fitting clothes. However, until now it remained unclear how often tucking is performed by transgender women and what the consequences are. In our cohort, 14.1% of transgender women reported to perform tucking sometimes whereas 8.9% does it often. A study from 1985 assessed the influence of tucking in a cohort of cisgender men and observed the most dramatic decline in TMSC with a drop of 98% after more than 6 consecutive months of daily tucking.[89] This is in line with our observation that extensive tucking is associated with a low TMSC in transgender women (OR 7.95, 95% CI 1.66-37.99) and confirms the significant negative influence on semen quality.

Another way to hide the external genitalia is wearing tight undergarment which may increase scrotal temperatures. The scrotal temperature is regulated by blood flow and the position of the testicles - towards and away from the body - through a collaboration between the cremaster muscle and the dartos muscle. Wearing tight undergarment may disrupt this system. Half of the transgender women in this study reported to regularly wear female underwear, stockings or special transgender underwear to press the genitals tight against the body. In cisgender men several studies have been conducted to evaluate the influence of tight undergarment on semen quality with varying results.[91,97,98] In our cohort, we found a negative impact of always wearing tight undergarment on TMSC, which is similar to what was observed in two other studies on this subject.[91,97] However, the odds ratio was slightly lower for the corrected model (OR 2.79, IQR 0.92-8.43) than for the crude analysis (OR 3.06, IQR 1.11-8.49).

Lastly, we assessed ejaculation frequency in transgender women and hypothesized that it might be low due to genital dysphoria and hereby impair progressive motility. Indeed, when comparing those with the lowest ejaculation frequency with those with an average ejaculation frequency in our cohort, a negative impact on progressive motility was observed. However, no association with TMSC was found. Previously conducted studies in cisgender men suggest that daily ejaculation, resulting in a short abstinence time, may cause a decline in semen volume and sperm concentration but at the same time improves progressive motility.[99,100] Reversely, studies assessing the effect of abstaining from ejaculation for 11-21 days showed a significant decrease in the percentage progressive motility and normal morphology but an increase in semen volume and sperm

concentration.[101,102] In this way, ejaculation frequency and abstinence time will most likely not have an impact TMSC, as TMSC takes the absolute value of three semen parameters into consideration simultaneously.

Clinical implications

The impaired pre-freeze semen quality in our cohort has implications for future use of cryopreserved semen, since freezing and thawing further decreases sperm motility. We observed a large proportion of samples with low post-thaw TMSC which has consequences for many transgender women, since 40.7% of our cohort reported to be solely attracted to women and 35.3% were attracted to both women and men. Therefore, the future use of cryopreserved semen with a female partner is a realistic scenario and implicates that the majority will need an invasive and burdensome IVF or ICSI treatment to start a family.

The results of our study provide a possibility to optimize fertility counseling by encouraging transgender women to minimize wearing tight undergarment and tucking before pursuing semen cryopreservation. However, since only a minority of transgender women reported to perform these habits, these results do not explain the overall decreased semen quality in transgender women and part of the etiology remains unknown.

Research implications

For future research it would be worthwhile to further assess the impact of GAHT on spermatogenesis and especially its restoration after cessation of this treatment, in a longitudinal manner. Hereby, we might be able to establish an optimal strategy and explore the feasibility of natural conception for transgender women with a female partner and a desire for genetically related offspring.

Strengths and limitations

The major strength of this study is the prospective design which enabled us to collect detailed information on habitual behavior more typically observed in transgender women, including the frequency and duration of tucking, as well as information about all other relevant factors (i.e. demographics, lifestyle and medical history). As a result, we were able to accurately assess the influence of ejaculation frequency, wearing tight undergarment, and tucking on semen quality, while correcting for potential confounders. A limitation of this study is that some results are based on self-reported behavior and lifestyle which can lead to bias because of its subjective nature. However, no viable alternative is available. Furthermore, since this is the first study reporting on ejaculation frequency and tucking frequency in transgender women, it was not possible to use reference values from previous reports. Therefore, the stratification of these factors was based on clinical relevance.

Conclusions

Transgender women can choose to cryopreserve their semen prior to starting GAHT, in order to retain the possibility to parent genetically related offspring later in life. However, semen quality in transgender women is often already impaired at time of fertility preservation and the majority of cryopreserved samples is only suitable for invasive ART to establish a pregnancy. With this cohort study, we are the first to report a negative impact of wearing tight undergarment and tucking on semen quality in transgender women. These results enable optimization of fertility counseling on how to adjust lifestyle before pursuing semen cryopreservation to improve future reproductive options.

CHAPTER 5

SUCCESSFUL RESTORATION OF SPERMATOGENESIS FOLLOWING GENDER-AFFIRMING HORMONE THERAPY IN TRANSGENDER WOMEN

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Submitted

ABSTRACT

Importance: Increasing numbers of transgender individuals are presenting for gender-affirming medical care. For transgender individuals assigned male at birth, gender-affirming hormone therapy (GAHT) generally consists of estrogen combined with an anti-androgen and will promote feminization but also impair spermatogenesis. Clinical guidelines therefore suggest sperm cryopreservation prior to GAHT based on the dogma that GAHT causes irreversible azoospermia and infertility. However, it is unknown if spermatogenesis can recover if GAHT is stopped.

Objective: To determine if the loss of spermatogenesis in transgender women using gender-affirming hormonal treatment can be reversed after hormonal treatment is ceased.

Design: Case series.

Setting: Sexual health clinic in Coffs Harbour, Australia, and gender identity clinic in Amsterdam, the Netherlands.

Participant: Nine transgender women (mean age 26.1 years) who had been on GAHT for >6 months (median 36 months) before interrupting treatment for reproductive purposes.

Intervention: Longitudinal follow up to assess semen quality after cessation of hormonal treatment.

Main outcome measure: Presence of spermatozoa in semen analysis.

Results: Spermatozoa were successfully identified in all nine participants after cessation of GAHT. Six individuals already had spermatozoa present on their initial semen analyses taken 3-27 months after stopping hormones; the other three showed initial azoospermia but eventually had spermatozoa identified 8-17 months after hormone cessation. Of the four individuals who attempted to conceive with their partners, three successfully did so 4-40 months after stopping treatment.

Conclusion and relevance: Taken together, these findings suggest that the inhibitory effect of GAHT on spermatogenesis can be reversed, and have important implications for clinical practice.

INTRODUCTION

Increasing numbers of transgender, gender diverse, and non-binary (henceforth, trans) people are seeking hormonal intervention as part of gender-affirming medical care. For trans individuals assigned male at birth, gender-affirming hormone therapy (GAHT) typically consists of estrogen combined with an anti-androgen. Such treatment promotes feminization, but can also cause unwanted effects, including impaired spermatogenesis.[3] Specifically, multiple studies have observed that most trans women who have received GAHT do not produce mature sperm at the time of gender-affirming surgery.[27,28,103-107] Some of these studies reported relatively normal spermatogenesis in a variable minority of trans women (e.g. 11-40%), but Vereecke et al. found that none of the 97 individuals they studied had complete spermatogenesis at gonadectomy.[103,106,107]

Current international clinical guidelines therefore recommend that trans women "should be informed about sperm preservation options and encouraged to consider banking their sperm prior to hormone therapy".[93] Such advice would appear sensible, especially in light of prominent claims from leaders in the transgender health field that GAHT "eventually results in irreversible infertility".[3] However, to know if GAHT actually causes "irreversible infertility" requires the longitudinal study of patients and a determination *whether loss of spermatogenesis can be reversed after GAHT is ceased*. To our knowledge, no such studies have been previously reported. To address this important gap, we therefore identified trans individuals assigned male at birth, each of whom stopped GAHT for reproductive purposes, and assessed their subsequent ability to produce sperm.

PARTICIPANTS AND METHODS

Trans women attending either a sexual health clinic in Coffs Harbour, Australia, or the Center of Expertise on Gender Dysphoria at the Amsterdam UMC, Netherlands, who wished to temporarily stop GAHT for reproductive purposes were identified, and informed consent to participate in this study was obtained. Relevant clinical data were extracted from the medical records and included: confirmed gender incongruence, age at commencement of GAHT, type of estrogen and anti-androgen therapy, duration and dose, reasons for stopping GAHT, subsequent semen analysis results, and reproductive outcomes. Any patients on GAHT for <6 months were excluded from the study.

RESULTS

In total, nine trans women were included who met our study criteria (Table 1). Four individuals stopped GAHT to conceive with their current partners; the remaining five wished to bank sperm to conceive in the future. Their mean age was 26.1 years, and median duration on GAHT was 36 months (range 6-216 months). Seven individuals had been on oral estradiol (median dose 4mg, range 2-4mg), while the other two had been on topical estradiol, and all had used estrogen in combination with an anti-androgen (spironolactone or cyproterone acetate).

Viable spermatozoa were found in all nine individuals after cessation of GAHT (Table 1). Six had sperm present on their initial semen analyses taken 3-27 months after stopping hormones; two others had no sperm on their initial semen analyses, but eventually had sperm identified 8-10 months after hormone cessation; the remaining individual did not have any sperm identified in serial semen analyses up to 13 months after ceasing GAHT, but eventually had spermatozoa successfully extracted via testicular biopsy after 17 months.

Table 1. Clinical characteristics of nine trans women who stopped GAHT and their subsequent semen analyses

	Patient 1	**Patient 2**	**Patient 3**	**Patient 4**
Initial presentation to gender clinic				
Location	**The Netherlands**	**The Netherlands**	**The Netherlands**	**The Netherlands**
Age at first presentation to gender clinic	26	18	25	28
Age at diagnosis of Gender Dysphoria	28	21	25	28
Medical history	left testicular torsion	androgenetic alopecia	n/a	commenced hormone treatment on own initiative after purchasing online
Psychiatric history	n/a	bipolar disorder	n/a	n/a
Concurrent medication	n/a	finasteride (for alopecia)	n/a	n/a
Baseline serum hormone levels				
Estradiol (pmol/L)	123	72	82	705
Testosterone (nmol/L)	27	19	15	<0.5
LH (IU/L)	2.8	3.6	2.4	<0.1
FSH (IU/L)	n.d.	n.d.	n.d.	
Fertility history and previous counseling				
Previous children	nil	nil	nil	nil
Reproductive desires prior to commencing oestrogen	wish for fertility preservation, wants children in the future.	used illicitly obtained hormones, no prior fertility counseling	not discussed	used illicitly obtained hormones, no prior fertility counseling
Prior fertility preservation	semen cryopreservation: 43 vials suitable for ICSI	none	none	none
Oestrogen therapy				
Age started	28	18	23	24
Agent	estradiol patches	estradiol valerate	estradiol valerate	estradiol valerate
Starting dose	100mcg/24 hours twice a week	4 mg/day	2 mg/twice a day	2 mg/twice a a day

Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
The Netherlands	**The Netherlands**	**Australia**	**The Netherlands**	**The Netherlands**
26	32	28	26	27
27	33	28	27	29
started hormone treatment in Lebanon	n/a	n/a	circumcision	used oral contraceptives for 2 months in 2012
depression; multiple suicide attempts	n/a	schizophreniform psychosis and depression but not active at time of gender dysphoria diagnosis	personality disorder NOS	ADHD
paroxetine 20 mg/ day	n/a	n/a	n/a	n/a
n.d.	95	122	127	124
n.d.	21	14.8	29	33
n.d.	5.2	4.2	5.1	9
n.d.	n.d.	n.d.	n.d.	n.d.
nil	nil	nil	nil	nil
used illicitly obtained hormones, no prior fertility counseling	no prior fertility counseling	fertility counselling was provided; not wanting children, planning to adopt; fertility preservation declined	fertility counselling was provided; declined fertility preservation	fertility counselling was provided; declined fertility preservation
none	none	none	none	none
24	33	28	27	30
estradiol valerate	estradiol valerate	estrodial valerate	estradiol valerate	estradiol valerate
2 mg/twice a day	2 mg/twice a day	4mg/day	2 mg/twice a day	2 mg/twice a day

	Patient 1	Patient 2	Patient 3	Patient 4
Dose schedule	n/a	n/a	switched to estradiol patches 100 mcg/24 hours twice a week	switched to estradiol patches 100 mcg/24 hours twice a week
Age stopped	31	18	41	32
Dose at cessation	100 mcg/24 hours twice a week	2 mg/day	estrogel 0.06% 1.25 mg twice a day	estradiol valerate 2 mg /twice a day
Duration of oestrogen therapy (months)	22	6	216	99
Anti-androgen therapy				
Age started	28	18	23	24
Agent	cyproterone acetate	spironolactone	cyproterone acetate	cyproterone acetate
Starting dose	25mg/day	100 mg/day; 1 mg/day	100 mg/day	50 mg/day
Dose schedule	switched to 10 mg/day after 15 months	n/a	switched to 50 mg/day after 24 months	
Age stopped	31	18	41	32
Dose at cessation	10 mg/day	100 mg/day; 1 mg/day	50 mg/day	50 mg/day
Duration on anti-androgen (months)	17	6	216	93
Gender affirming surgery	None	None	breast augmentation	breast augmentation
Physical response to hormone treatment				
Physical changes	breast development, decreased muscle strength	unknown	unknown	unknown
Subsequent serum hormone levels - timing	20 months after commencement of estradiol	n.d.	204 months after commencement of estradiol	93 months after commencement of hormones
Estradiol (pmol/L)	641	n.d.	120	286
Testosterone (nmol/L)	0.4	n.d.	0.9	0.2
LH (IU/L)	0.1	n.d.	0.3	<0.1
FSH (IU/L)	n/d	n.d.	n.d.	n.d.

Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
decreased to 2 mg/ day		n/a	temporarily used a decreased dose of 2mg/ day	
27	36	29	33	33
estradiol valerate 2 mg/day	estradiol valerate 2 mg/twice a day	4mg/day	estradiol valerate 2 mg/ twice a day	estradiol valerate 2mg/ twice a day
36	36	14	56 (period of cessation in 2014)	36
24	33	28	27	29
spironolactone	cyproterone acetate	cyproterone acetate	cyproterone acetate	cyproterone acetate
100 mg/day	50 mg/day	25 mg/day	50 mg/twice a day	50 mg/day
	decrease dose to 12,5 mg/day	Switched to 25 mg/ alternate days after 6 months	dose decreased to 20 mg/day	
27	36	29	33	33
spirinolactone 100 mg/day	cyproterone acetate 12.5 mg/day	25 mg/alternate days	20mg/day	50 mg/day
36	36	14	56 (period of cessation in 2014)	40
none	none	none	none	none
none	breast development, decreased facial hair growth	Tanner stage 3-4 breast development	breast development	decreased muscle strength, decreased body hair growth, softer skin, breast development
n.d.	15 months after starting hormones	13 months after commencement of oestrogen	24 months after re-initiation of HT	36 months after commencement estradiol, 40 months after commencement anti-androgens
n.d.	246	556	320	154
n.d.	4.4	0.9	0.7	0.4
n.d.	1.1	0.2	1.2	<0.1
n.d.	n.d.	n.d.	n.d.	n.d.

	Patient 1	Patient 2	Patient 3	Patient 4
Cessation of anti-androgens/ oestrogen				
Rationale	wants to conceive with new partner	wants to cryopreserve semen to keep all options open	wants to conceive with partner (trans male)	wants to cryopreserve semen to keep all options open, partner (cis male) has active child wish
Physical changes after cessation	increased hair growth and transpiration, higher volume of ejaculate	unknown	none, no hot flushes, no increased hair growth	acne
Subsequent serum hormone levels - timing	3 months after cessation	n.d.	n.d.	n.d.
Estradiol (pmol/L)	82	n.d.	n.d.	n.d.
Testosterone (nmol/L)	17.0	n.d.	n.d.	n.d.
LH (IU/L)	3.1	n.d.	n.d.	n.d.
FSH (IU/L)	9.0	n.d.	n.d.	n.d.
Time after stopping oestrogen (months)	3	3	27**	4
Volume (mL)	1.2	4.5*	2.1*	3.5*
Sperm concentration (million/mL)	3.3	48*	77.7*	0.2
Sperm motility (%)	20	69*	51*	19
Time after stopping oestrogen (months)	7	3	n/a	n/a
Volume (mL)	1.3	5*	n/a	n/a
Sperm concentration (million/mL)	3.8	55*	n/a	n/a
Sperm motility (%)	41*	55*	n/a	n/a
Time after stopping oestrogen (months)	8	3	n/a	n/a
Volume (mL)	4.8*	6*	n/a	n/a
Sperm concentration (million/mL)	20.2*	53*	n/a	n/a
Sperm motility (%)	48*	75*	n/a	n/a
Time after stopping oestrogen (months)	n/a	n/a	n/a	n/a
Volume (mL)	n/a	n/a	n/a	n/a
Sperm concentration (million/mL)	n/a	n/a	n/a	n/a
Sperm motility (%)	n/a	n/a	n/a	n/a

Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
prefers genetically related offspring over adoption	wants to conceive with female partner	wants to conceive with new partner	wants to cryopreserve semen to keep all options open	strong parental desire
increased hair loss/ baldness	increased facial hair growth	breast atrophy, testicular volume increase	higher volume of ejaculation, increased sexual function, increased muscle strength	increased hair growth (body and face)
3 months after cessation	22 months after cessation	6 months after cessation	7 months after cessation	13 months after cessation
n.d.	n.d.	164	n.d.	n.d.
18	11	18.8	21	16
n.d.	n.d.	5.2	9.2	11
3.3	6.2	17.2	19	23
3	7	1	4	7
1.3	3.4*	0.2	unknown	1
1.2	31.3*	0	0	0
17	40*	nil	0	0
3	22	8	7	7
1.7*	1.6*	1	unknown	2.5*
0.8	22.3*	<2	0	0
49*	63*	6	0	0
3	22	9	10	13
0.9	2.5*	2*	3.2*	3.2*
0.4	7.1	<2	9	0
56*	13	50*	44*	0
n/a	22	n/a	11	n/a
n/a	2.2*	n/a	2.7*	n/a
n/a	21*	n/a	11.8	n/a
n/a	43*	n/a	36*	n/a

	Patient 1	Patient 2	Patient 3	Patient 4
	cis-gender female partner got pregnant 4 months after cessation of hormone treatment through natural conception		trans male partner got pregnant 40 months after cessation of hormone treatment, through natural conception	

* indicates that semen parameters were above the normal WHO reference range for semen volume (1.5mL), sperm concentration (15 million/mL) or progressive motility (32%).[3]

** Patient 3's first semen analysis was significantly delayed due to a move across continents, during which she was unable to be with her partner. They were eventually reunited after which the semen analysis was performed.

There was no obvious relationship between the duration of GAHT and timing of when spermatozoa were first identified (Figure 1). Four individuals recorded at least one normal semen analysis; the other four had either low volume, sperm concentration and/or sperm motility. Three of the four patients who stopped GAHT to naturally conceive with their current partners successfully did so after 4, 20 and 40 months.

Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
	cis-gender female partner got pregnant 20 months after cessation of hormone treatment			after failing to identify any mature sperm on repeated semen specimens, further physical examination and investigations were performed. Examination revealed a normal penis, right testicle volume of 2 ml, left testicle volume of 3 ml and normal consistency on both sides without any abnormalities of the epididymis or vas deferens. Scrotal ultrasound revealed: right testicle volume of 2.04 ml, left testicle volume 3.66 ml, normal testicular structure and vascularisation, normal epididymis. Karyotype was 46XY with no Y chromosome abnormalities seen. testicular sperm extraction was subsquently performed. Complete spermatogenesis was sporadically encountered in both testicles, and mature spermatozoa were harvested 17 months after stopping hormones.

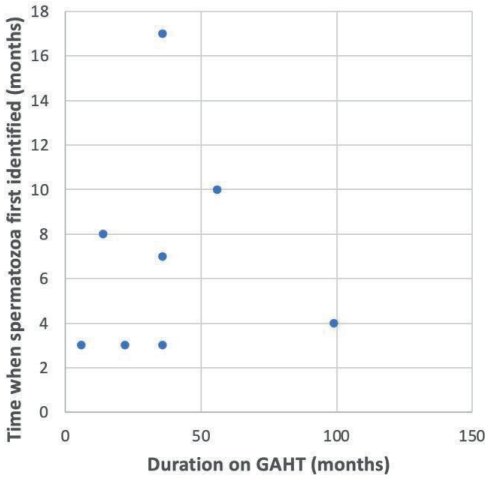


Figure 1. Relationship between GAHT duration and time when spermatozoa first identified after stopping GAHT.
Note: Patient 3 was excluded from this analysis given that the first semen analysis was significantly delayed (see Table 1).

DISCUSSION

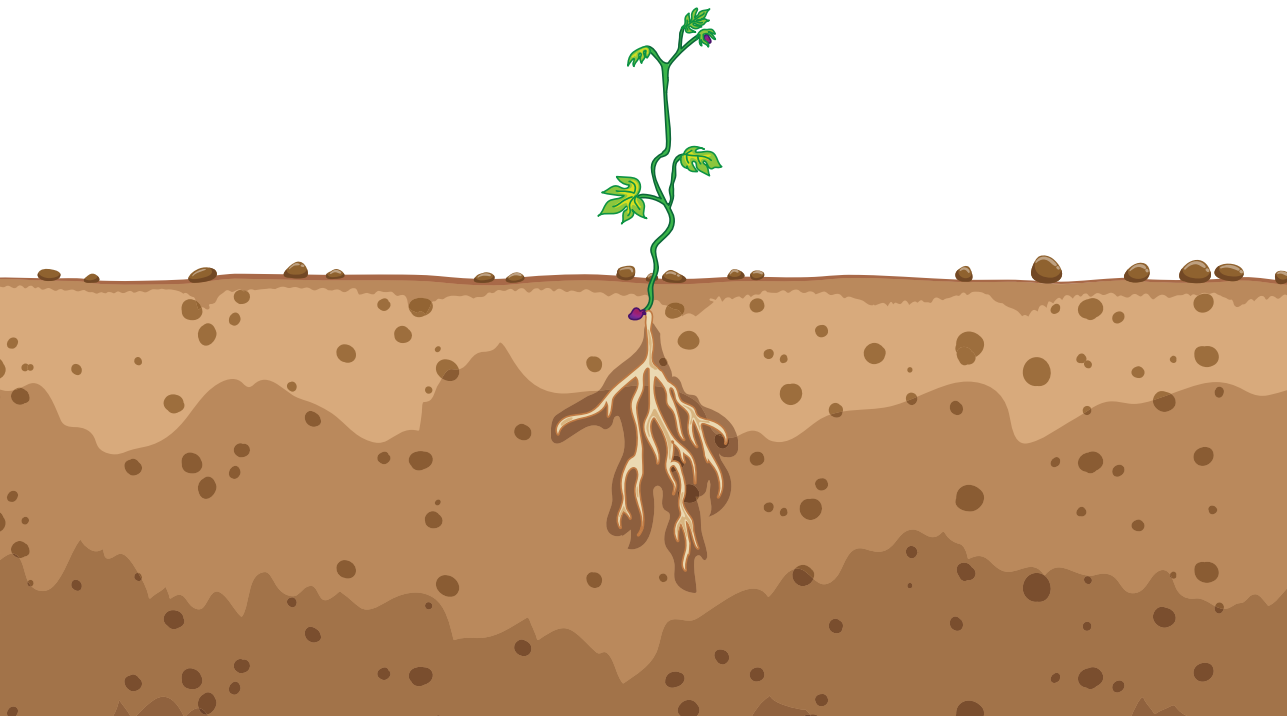
The prevailing dogma in transgender health is that GAHT in trans women "eventually results in irreversible infertility".[3] This helps to fuel controversy over provision of GAHT, especially to adolescents, and is reflected in arguments to support legal efforts to restrict young people's access to gender-affirming care.[108]

While there are numerous reports that GAHT impairs spermatogenesis, current evidence indirectly suggests this impact may be transient. For example, Alford et al. recently described a trans woman who – prior to gender-affirming genital surgery and following a course of FSH and clomiphene – successfully cryopreserved sperm from semen specimens collected 6-10 weeks after GAHT cessation.[109] Similarly, Adeleye et al. reported that three trans women who stopped GAHT all had mature sperm present on semen analysis 3-6 months later.[39] However, for all four of these trans women, it is unclear to what extent their spermatogenesis was impaired by GAHT in the first place. As noted earlier, up to 40% of trans women have evidence of normal spermatogenesis at the time of gonadectomy despite GAHT.[107] Thus, it is conceivable that spermatogenesis in each of these four trans women was never actually impaired, and knowing whether GAHT reversibly affected their spermatogenesis is not possible.

Our observation that six trans women had sperm identified from their initial semen analyses after stopping GAHT provides further indirect evidence that GAHT-induced impairment of spermatogenesis can be reversible. After all, the probability that at least one of these six patients had completely stopped producing mature sperm due to GAHT is very high ($1-0.4^6 = 99.6\%$), indicating that spermatogenesis is likely to have recovered

in some of these individuals. More noteworthy though is our observation that three trans women who had no sperm on their initial semen collections subsequently produced sperm afterwards. This is, to our knowledge, the first conclusive evidence that spermatogenesis can recover following GAHT cessation.

In summary, our data strongly suggest that the negative impact of GAHT on spermatogenesis can be reversed and cast into serious doubt previous claims that such treatment inevitably leads to permanent infertility. Our findings also have important implications for fertility counselling in transgender health. For example, many trans women receiving GAHT who still have their gonads believe that they are permanently infertile, as do their clinicians. Our results will help such individuals make more informed reproductive choices moving forwards. Similarly, for trans women wanting to commence GAHT in the future, our findings may influence their decision-making regarding fertility preservation (e.g., some may be less inclined to freeze their sperm, knowing that they may be able to produce sperm should they stop GAHT later on). Nonetheless, we would still recommend sperm cryopreservation prior to GAHT for anyone who might want to be a genetic parent in the future. After all, recovery of spermatogenesis took many months in some cases, during which time testosterone levels increased and are likely to have had negative physical and psychological consequences. Moreover, invasive testicular sperm extraction was required in one case, and >55% (5/9) of individuals had impaired semen quality after stopping GAHT.



CHAPTER 6

HISTOLOGICAL STUDY ON THE INFLUENCE OF PUBERTY SUPPRESSION AND HORMONAL TREATMENT ON DEVELOPING GERM CELLS IN TRANSGENDER WOMEN

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Published in Human Reproduction; November 2021 [article in press]

ABSTRACT

Study question: Can transgender women cryopreserve germ cells obtained from their orchiectomy specimen for fertility preservation, after having used puberty suppression and/or hormonal treatment?

Summary answer: In the vast majority of transgender women there were still immature germ cells present in the orchiectomy specimen, and in 4.7% of transgender women - who all initiated medical treatment in Tanner stage 4 or higher - mature spermatozoa were found, which would enable cryopreservation of spermatozoa or testicular tissue after having used puberty suppression and/or hormonal treatment.

What is known already: Gender affirming treatment (i.e. puberty suppression, hormonal treatment, and subsequent orchiectomy) impairs reproductive function in transgender women. Although semen cryopreservation is generally offered during the transition process, this option is not feasible for all transgender women (e.g. due to incomplete spermatogenesis when initiating treatment in early puberty, in case of inability to masturbate, or when temporary cessation of hormonal treatment is too disruptive). Harvesting mature spermatozoa, or testicular tissue harboring immature germ cells, from orchiectomy specimens obtained during genital gender-affirming surgery (gGAS) might give this group a chance of having biological children later in life. Previous studies on spermatogenesis in orchiectomy specimens showed conflicting results, ranging from complete absence of germ cells to full spermatogenesis, and did not involve transgender women who initiated medical treatment in early- or late-puberty.

Study design, size, duration: Histological and immunohistochemical analyses were performed on orchiectomy specimens from 214 transgender women who underwent gGAS between 2006 and 2018. Six subgroups were identified, depending on pubertal stage at initiation of medical treatment (Tanner stage 2-3, Tanner stage 4-5, adult), and whether hormonal treatment was continued or temporarily stopped prior to gGAS in each of these groups.

Participants/materials, setting, methods: All transgender women used a combination of estrogens and testosterone suppressing therapy. Orchiectomy specimen sections were stained with Mayer's haematoxylin and eosin and histologically analyzed to assess the Johnsen score and the ratio of most advanced germ cell types in at least 50 seminiferous tubular cross sections. Subsequently, immunohistochemistry was used to validate these findings using spermatogonia, spermatocytes, or spermatids markers (MAGE-A3/4, γH2AX, Acrosin, respectively). Possibilities for fertility preservation were defined as: preservation of spermatozoa, preservation of spermatogonial stem cells, or no possibilities (in case no germ cells were found). Outcomes were compared between subgroups and logistic regression analyses were used to assess the association between the duration of hormonal treatment and the possibilities for fertility preservation.

Main results and the role of chance: Mature spermatozoa were encountered in 4.7% of orchiectomy specimens, all from transgender women who had initiated medical treatment in Tanner stage 4 or higher. 88.3% of the study sample only contained immature germ cells (round spermatids, spermatocytes, or spermatogonia, as most advanced germ cell type). In 7.0% a complete absence of germ cells was observed, all these samples were from transgender women who had initiated medical treatment in adulthood. Cessation of hormonal treatment prior to gGAS did not affect the presence of germ cells or their maturation stage, nor was there an effect of the duration of hormonal treatment prior to gGAS.

Limitations, reasons for caution: Since data on serum hormone levels on the day of gGAS were not available, we were unable to verify if the transgender women who were asked to temporarily stop hormonal treatment four weeks prior to surgery actually did so, and if people with full spermatogenesis were compliant to treatment.

Wider implications of the findings: There may still be options for fertility preservation in orchiectomy specimens obtained during gGAS since a small percentage of transgender women had full spermatogenesis, which could enable cryopreservation of mature spermatozoa via a testicular sperm extraction procedure. Furthermore, the vast majority still had immature germ cells, which could enable cryopreservation of testicular tissue harboring spermatogonial stem cells. If maturation techniques like *in vitro* spermatogenesis become available in the future, harvesting germ cells from orchiectomy specimens might be a promising option for those who are otherwise unable to have biological children.

INTRODUCTION

Gender dysphoria refers to the distress experienced by people with an incongruence between their sex assigned at birth and their gender identity.[70] People assigned male at birth with a female gender identity are referred to as transgender women.

Many transgender women seek medical treatment to avoid (further) masculinization and induce feminization, and hereby align their physical characteristics with their gender identity. The preferred treatment protocol depends on the person's age at time of start of medical treatment. For adolescents (<18 years), treatment can be initiated when a person reaches puberty (Tanner stage 2 or higher, determined by the development of secondary sex characteristics). It aims to suppress further pubertal development by administration of a gonadotropin-releasing hormone agonist (GnRHa) which reversibly inhibits the production of sex hormones. Hereby, adolescents have more time to explore options and to live in the experienced gender before deciding whether or not to proceed with additional, sometimes irreversible, treatments. At the age of approximately 16 years, treatment can be supplemented with estrogens to induce the development of female secondary sex characteristics.[2] For transgender women presenting at adult age (≥18 years), treatment usually does not consist of a phase of hormone suppression only, but immediately involves a combination of anti-androgens and estrogens, to achieve feminization. The combination of testosterone suppressing therapy and estrogen supplementation is referred to as gender affirming hormonal treatment (GAHT). Transgender women of 18 years or older who have used GAHT for at least one year, can opt for genital gender affirming surgery (gGAS) if no surgical contra-indications are present. gGAS may comprise vaginoplasty, gender confirming vulvoplasty or bilateral orchiectomy, depending on the desires of the individual.[110]

The use of testosterone suppressing therapy results in a severely impaired reproductive function, since spermatogenesis - the differentiation of spermatogonial stem cells into spermatozoa - requires adequate levels of intratesticular testosterone.[39] This reproductive loss is permanent after gGAS. Although gender affirming treatment significantly improves quality of life, reproductive loss may be an unwanted consequence.[60,73,94] Therefore, it is important that (future) desire for biological children and the options for fertility preservation are discussed and offered prior to the start of medical treatment.[2]

The currently available option for fertility preservation in transgender women is cryopreservation of spermatozoa from a semen sample, obtained through ejaculation. Cryopreservation of surgically obtained spermatozoa through testicular sperm extraction (TESE) may serve as an alternative for those who are unable to ejaculate or in case of azoospermia.[111]

A complicating factor for contemporary fertility preservation in transgender female adolescents is the requirement of complete spermatogenesis, which only develops from Tanner stage 3 onwards, under the influence of increasing intratesticular testosterone

levels. If puberty suppression is started in Tanner stage 2, full spermatogenesis is usually not present yet and therefore preservation of spermatozoa is not possible.[54] The equipoise of commencing medical treatment to avoid progression of puberty and delaying treatment to enable semen cryopreservation as only option for biological children may be stressful, as puberty is accompanied by irreversible and often unwanted physical changes such as a lowering of the voice and facial hair growth. Severe genital dysphoria may pose another barrier for fertility preservation, since semen cryopreservation requires masturbation which is non-negotiable for some young transgender women.[54] In addition, TESE, the currently available alternative to obtain spermatozoa for cryopreservation requires invasive procedures including surgery and (general) anesthesia.

For transgender women, cryopreservation of germ cells harvested from testicular tissue obtained during gGAS may serve as an alternative to keep the option for genetically related offspring open. How these germ cells can be used for procreation depends on their maturation phase. Spermatozoa can directly be used for ART. However, the use of immature germ cells relies on the feasibility of maturation techniques outside the human body, such as *in vitro* spermatogenesis. Unfortunately, complete *in vitro* spermatogenesis has only been successfully demonstrated in mouse models and is still unsuccessful in humans.[40] If *in vitro* spermatogenesis becomes available in the future, cryopreservation of testicular tissue containing spermatogonial stem cells might be a promising option for fertility preservation in those who are otherwise unable to retain the possibility of having genetically related offspring.

Currently, limited data is available on the effect of GAHT on testicular histology and the most advanced germ cell type that can be harvested from testicular tissue obtained at time of gGAS. Previous studies conducted on this topic showed varying proportions of hyalinization of seminiferous tubules as well as conflicting results regarding spermatogenesis, ranging from a complete absence of germ cells to full spermatogenesis.[29,104] Moreover, none of these studies focused on people who initiated medical treatment in early puberty.

The primary aim of this study is to evaluate the influence of puberty suppression and/or GAHT on exocrine testicular function, by determining the most advanced germ cell type in orchiectomy specimens obtained during gGAS. We aim to compare the outcome between people who started medical treatment as adult (≥18 years) and those who started as adolescent in early-puberty (Tanner stage 2-3) or late-puberty (Tanner stage 4-5). In addition, we will assess the influence of discontinuation of medical treatment four weeks prior to gGAS in each of these groups, and the association between the duration of hormonal treatment and the possibilities for fertility preservation. Hereby, we will get insights in the options for fertility preservation in orchiectomy specimens obtained during gGAS after having used puberty suppression and/or hormonal treatment.

MATERIALS AND METHODS

Study population and clinical data collection

For this study, we used orchiectomy specimens of transgender women who underwent bilateral orchiectomy combined with vaginoplasty at the Center of Expertise on Gender Dysphoria of Amsterdam UMC between 2006 and 2019. All participants provided written permission for the use of their body material and clinical data for research purposes. The Ethical Review Board of the Amsterdam UMC, location VUMC provided approval for conducting this study (METC2014322).

A total of 788 transgender women were identified. Data on medical history, age and Tanner stage at start of medical treatment, documented hormone use, date of gGAS, alcohol consumption, smoking, drug use, BMI at time of gGAS, and last known serum hormone levels before gGAS, were collected from the medical files. Transgender women were categorized according to age and Tanner stage at initiation of medical treatment (Tanner stage 2-3, Tanner stage 4-5, or ≥18 years). Transgender women operated before 2017 discontinued GAHT four weeks prior to surgery, because of a presumed increased risk of perioperative thrombosis. As evidence suggested this risk is negligible, GAHT is continued in the perioperative period since July 2017. Six subgroups were created based on Tanner stage/age at start of medical treatment and continuation/discontinuation of GAHT prior to gGAS. People with an unknown age or Tanner stage at time of initiation of medical treatment were excluded. Other exclusion criteria were hard drug use, cryptorchidism, a medical history of receiving chemotherapy, or genetic disorders which can all possibly impair spermatogenesis. Lastly, since the vast majority used estrogens combined with either triptorelin, or cyproterone acetate, people who used estrogen monotherapy and those who used spironolactone as anti-androgenic treatment were excluded to create a homogeneous study population. A maximum number of 80 transgender women were enrolled per group as this was deemed sufficient to answer the study questions. A random sample was drawn from groups that exceeded 80 individuals using STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA). In total, 263 transgender women were selected for inclusion in the study cohort.

Testicular tissue preparation and analysis

Preparation for histology

Testicular tissue was obtained from the biobank of the Pathology department of Amsterdam UMC, where orchiectomy specimens, obtained during gGAS, were stored after histopathological analysis for clinical purposes. Upon arrival at the Pathology department, the orchiectomy specimens were fixed in 4% w/v paraformaldehyde and embedded in paraffin. For this study, seven slices of 5 μm thickness of one testicle were sectioned and mounted on microscope slides. From one slide of each specimen paraffin sections were deparaffinized and subsequently stained with Mayer's haematoxylin and eosin, and at least one other slide was used for immunohistochemistry to confirm germ cell subtypes.

Histological analysis

Histological examination was conducted using a bright field microscope (Olympus BX41, OM Digital Solutions Americas, Bethlehem, PA, USA). From each specimen, at least 50 seminiferous tubules per slide were analyzed to assess spermatogenesis by determining the most advanced germ cell type from each seminiferous tubular cross section based on their location within the tubule and nuclear morphology. The Modified Johnsen's scoring system was used to assign a score to each tubule, and per slide a mean Johnsen's score was calculated. The Modified Johnsen's scoring system involves a 10-point Likert scale where score 1 corresponds to complete sclerosis without recognizable seminiferous epithelium, and score 10 implies the presence of more than 10 elongated spermatids without immature and apoptotic cells in the lumen (Supplementary Table 1).[112]

After assessment of spermatogenesis, overall testicular histology was assessed including the presence of a lumen in the seminiferous tubules and rate of seminiferous tubule hyalinization. The lumen was categorized as open, half-open or absent. Hyalinization was defined as a hyaline area separating the peritubular layer from the basal membrane of the seminiferous tubule.

Preparation for immunohistochemistry

In order to validate our findings, a second slide of each specimen was analyzed using immunohistochemistry. The primary antibodies were chosen based on the most advanced germ cell type that was identified during histological analysis, or on uncertainty regarding the presence of a germ cell type.

For the detection of spermatogonia, slides were stained for spermatogonial marker MAGE-A3/A4 using mouse monoclonal Anti-Mage A3/A4 antibody (clone 57B; Merck Millipore, Germany). Endogenous peroxidase activity was inactivated with 0.3% H_2O_2/PBS for 10 min at room temperature in the dark. Non-specific binding sites were then blocked with Superblock (ScyTek Lab, USA) for 1 hour at room temperature in a humid slide box. Sections were subsequently incubated overnight at 4 °C with Anti-Mage A3/A4 antibody diluted 1:2000 in BrightDiluent (Immunologic, the Netherlands). The next day, all slides were washed three times with phosphate buffered saline (PBS) followed by 30 min incubation with Powervision goat-anti Mouse/Rabbit poly-horseradish peroxidase (DPVO110HRP, Immunologic, the Netherlands) secondary antibody at room temperature. After washing, the signal was visualized using Bright-DAB (3,3'-diaminobenzidine, Immunologic, the Netherlands) after which the sections were counterstained with Mayer's haematoxylin. Finally, after dehydration in increasing ethanol concentrations and xylene, the slides were encapsulated with glass coverslips using Entellan® (Merck Millipore, Germany) for further microscopic analysis.

For the detection of spermatocytes, slides were stained for γH2AX using mouse monoclonal Anti-phospho-Histone H2A.X (Merck Millipore, Germany) antibody. Antigen retrieval was carried out by boiling tissue sections in Tris-EDTA buffer (10mM Tris, 1mM EDTA, pH=9.0).

The buffer was first heated until boiling in the microwave for 3 min at maximum Watt. After cooling down for 2 min at room temperature, the buffer was heated again in the microwave for 12 min at minimum Watt. Non-specific binding sites were blocked with 5% BSA/PBS/0.5% Triton X-100. This was followed by overnight incubation at 4 °C with Anti-phospho-Histone H2A.X diluted 1:150 in 1% BSA/PBS/0.05% Tween. After incubation of the primary antibody, the same steps were performed as for the detection of spermatogonia with MAGE A3/A4.

For the detection of round spermatids and spermatozoa, slides were stained for the presence of their Acrosin cap using rabbit polyclonal Acrosin antibody (ThermoFisher, PA5-61804). Antigen retrieval was carried out by boiling tissue sections in 0.01 M sodium citrate buffer (tri-sodium citrate dihydrate $Na_3C_6H_5O_7.2H_2O$, pH 6.0). The buffer was first heated until boiling in the microwave for 3 min at maximum Watt. After cooling down for 2 min at room temperature, the buffer was heated again in the microwave for 10 min at minimum Watt. Subsequently, the buffer was cooled down for 10 min at room temperature and placed under running tap water. After these steps, a standard immunohistochemical preparation protocol was followed, as described above.

For all three antibodies, slides with testicular tissue from a prostate cancer patient with normal spermatogenesis served as a positive control. Negative controls were carried out by replacing the first antibody by isotype IgG (Supplementary Figure 1).

Immunohistochemical analysis

The immunohistochemically stained slides were examined using a bright field microscope (Olympus BX41) and assessed on the presence of the specifically targeted germ cell type. Outcome was then used to validate the Modified Johnsen's scoring of the histologically analyzed slide of that same specimen. Results from the immunohistochemically stained slides were preferred if there was a difference between the two.

Statistical analyses

After completion of histological and immunohistochemical analyses, results were linked to clinical data and descriptive analyses were conducted for the total cohort and the six subgroups. Data are presented as means (SD) when normally distributed, as medians with interquartile ranges (IQR) when non-normally distributed, or as numbers with percentage.

Progress of spermatogenesis, determined by the presence of the most advanced germ cell type per orchiectomy specimen, was used as main outcome measurement (no germ cells, spermatogonia, spermatocytes, round spermatids, or spermatozoa). Secondary outcome measurements included mean Johnsen score per orchiectomy specimen, the degree of hyalinization, and presence of a lumen.

To assess the possibilities for fertility preservation three categories were defined: preservation of spermatozoa; preservation of spermatogonial stem cells for those with

round spermatids, spermatocytes, or spermatogonia as most advanced germ cell type; and no possibilities for those with a complete absence of germ cells. Outcome was expressed as proportion with 95% confidence interval (95%CI) and compared between people who started medical treatment as an adult (>18 years) and those who started as adolescent in early-puberty (Tanner stage 2-3) or late-puberty (Tanner stage 4-5).[113] Since some categories contained no observations, we were not able to perform statistical tests. Therefore, differences between groups are shown in a figure. To assess the effect of cessation of GAHT prior to surgery, Fisher's exact tests were used to compare outcome within each pubertal stage at initiation of medical treatment. The significance level was set at $P<0.05$, and all tests were two-sided.

Lastly, logistic regression analyses were performed to assess the association between the duration of medical treatment and the possibility for preservation of spermatozoa, as well as the possibility for preservation of spermatogonial stem cells. Since the duration of medical treatment prior to gGAS, as well as progress of spermatogenesis both might be dependent on the age at start of medical treatment, a correction was performed for this factor. Odds ratios (OR) with 95%CI were calculated.

All statistical analyses were performed using STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA).

RESULTS

Initially, 263 transgender women were selected for inclusion in the study cohort. A total of 35 individuals were excluded when, upon preparation for analysis of the orchiectomy specimens, it became evident that for these transgender women no tissue was stored at the Pathology department of Amsterdam UMC. Another 14 transgender women were excluded because no testicular parenchyma was encountered on the prepared slides. Therefore, the final cohort consisted of 214 transgender women divided into 6 subgroups (Figure 1).

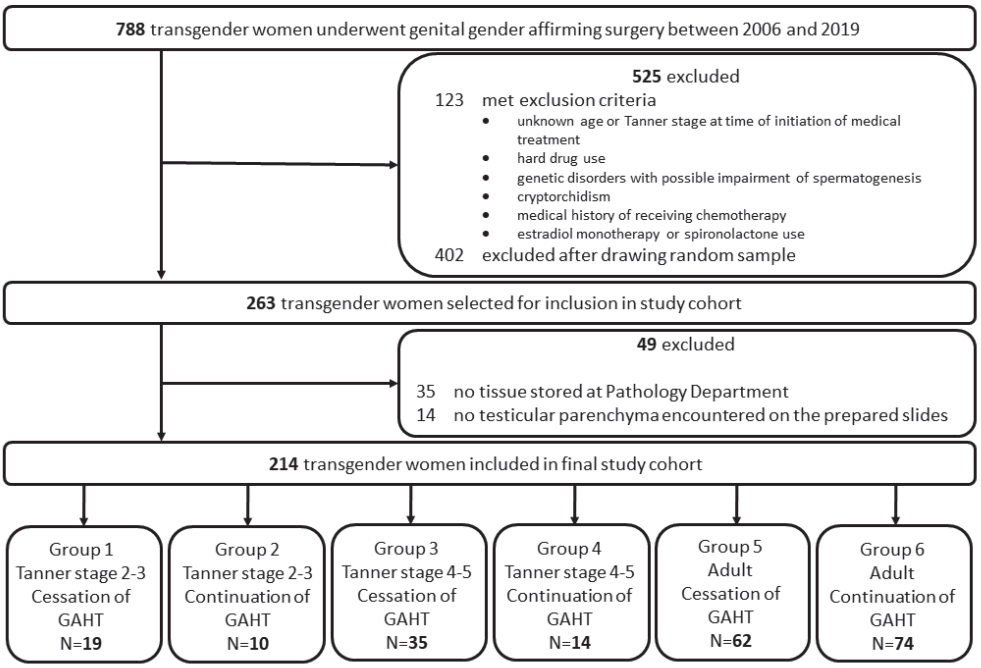


Figure 1. Study flowchart

Characteristics at time of gGAS are presented in Table 1. Mean age at gGAS was 29.6 years (SD 12.4) and was lower in people who started medical treatment in adolescence compared to those who started medical treatment in adulthood. Since adolescents started medical treatment with puberty suppressive therapy and had to wait until reaching the age of 18 years before being able to undergo gGAS, prior medical treatment duration was longer in the adolescent subgroups compared to those who initiated treatment at adult age. Different estradiol formulations were prescribed, including estradiol patches (50-150 µg/24 hours twice weekly), estradiol gel (0.75-3.0 mg daily) and oral estradiol valerate or hemihydrate (2-6 mg daily). Testosterone suppressing therapy consisted of triptorelin injections (3.75 mg i.m./s.c. every 4 weeks or 11.25 mg i.m. every 12 weeks) for those who initiated treatment as adolescent, and cyproterone acetate (25-100 mg daily) for those who initiated treatment as adult. The last known serum hormone levels, median 189 days (IQR 96-340) before gGAS, showed that testosterone was adequately suppressed (median 0.7 nmol/L, IQR 0.5-1.0) and estradiol levels were in the female range (median 193 pmol/L, IQR 120-307). Furthermore, LH and FSH levels were suppressed. In transgender women with a cessation of GAHT 4 weeks prior to gGAS, estradiol levels were lower, and testosterone and LH levels were higher, compared to those who continued GAHT until gGAS.

Table 1. Baseline characteristics at time of genital gender affirming surgery

	Total (n=214)	Adolescent Tanner stage 2-3 (n=29)		Adolescent Tanner stage 4-5 (n=49)		Adult (n=136)	
		Cessation of GAHT (n=19)	*Continuation of GAHT (n=10)*	*Cessation of GAHT (n=35)*	*Continuation of GAHT (n=14)*	*Cessation of GAHT (n=62)*	*Continuation of GAHT (n=74)*
Age (years) - Mean (SD)	29.6 (12.4)	19.0 (1.5)	19.6 (1.9)	19.7 (1.2)	19.3 (0.7)	34.5 (12.3)	36.2 (12.2)
Alcohol							
Drinker % (n)	44% (82)	43% (6)	30% (3)	60% (18)	21% (3)	56% (26)	35% (26)
Non-drinker % (n)	56% (106)	57% (8)	70% (7)	40% (12)	79% (11)	44% (20)	65% (48)
Unknown - n	26	5	0	5	0	16	0
Smoking							
Smoker % (n)	7% (12)	0%	0%	8% (2)	0%	22% (10)	0%
Non-smoker % (n)	93% (171)	100% (15)	100% (10)	92% (24)	100% (14)	78% (34)	100%
Unknown - n	31	4	0	9	0	18	0
Cannabis use							
Yes % (n)	3% (5)	0%	0%	4% (1)	7 (1)	6% (2)	1% (1)
No % (n)	97% (166)	100% (15)	100% (10)	96% (24)	93% (13)	94% (31)	99% (73)
Unknown - n	43	4	0	10	0	29	0
BMI (kg/m^2) - Mean (SD)	23.1 (3.3)	22.0 (3.3)	23.2 (2.8)	21.6 (3.6)	20.9 (3.6)	23.9 (2.9)	23.8 (3.0)
Mean duration of medical treatment (years)~ - (SD)	3.3 (2.0)	5.9 (1.4)	6.8 (1.3)	4.1 (1.8)	2.8 (0.6)	2.8 (1.9)	2.3 (1.2)
Testosterone suppression							
Triptorelin injections % (n)	36% (78)	100% (19)	100% (10)	100% (35)	100% (14)	0%	0%
Cyproterone acetate % (n)	64% (136)	0%	0%	0%	0%	100% (62)	100% (74)
Estrogen supplementation							
Transdermal formulation % (n)	25% (54)	11% (2)	10% (1)	0%	0%	40% (25)	35% (26)
Oral formulation % (n)	75% (160)	89% (17)	90% (9)	100% (35)	100% (14)	60% (37)	65% (48)
Serum hormone levels before gGAS – Median (IQR)^							
Testosterone (nmol/L)	0.7 (0.5-1.0)	1.0 (0.8-1.0)	0.6 (0.5-0.8)	1.0 (0.6-1.2)	0.6 (0.5-1.1)	0.7 (0.5-1.0)	0.5 (0.5-0.8)
Estradiol (pmol/L)	193 (120-307)	95 (43-332)	160 (141-392)	120 (82-220)	222 (100-281)	219 (130-282)	237 (151-341)
LH (U/L)	0.1 (0.1-0.3)	0.2 (0.1-0.4)	0.3 (0.2-0.5)	0.3 (0.2-0.4)	0.2 (0.2-0.4)	0.1 (0.1-0.3)	0.1 (0.1-0.1)
FSH (U/L)	0.2 (0.1-0.5)	0.2 (0.1-0.5)	0.4 (0.4-0.5)	0.2 (0.1-0.5)	-	0.3 (0.1-0.5)	0.8 (0.1-3.0)

~ including gonadotropin-releasing hormone agonist use, if applicable

^ data were available for 201 (testosterone and LH), 200 (estradiol), and 53 (FSH) transgender women, respectively.

In 10 transgender women (4.7%) some seminiferous tubules contained full spermatogenesis, all of whom had initiated medical treatment in Tanner stage 4 or higher and it occurred in both the group that had continued GAHT until gGAS and in the group that had discontinued four weeks prior to gGAS (Table 2, Figure 2E). Complete absence of germ cells was encountered in 15 transgender women (7.0%) (Figure 2A), all of whom had initiated medical treatment in adulthood. Also, mean Johnsen's scores were lowest in the adult cohort. In the subgroup of transgender women who initiated medical treatment in Tanner stage 2 or 3, all specimens showed immature germ cells of which spermatogonia were most commonly observed (60-79%) (Figure 2B, C, and D). Supplementary Table 2 shows the Modified Johnsen's score for each individual separately.

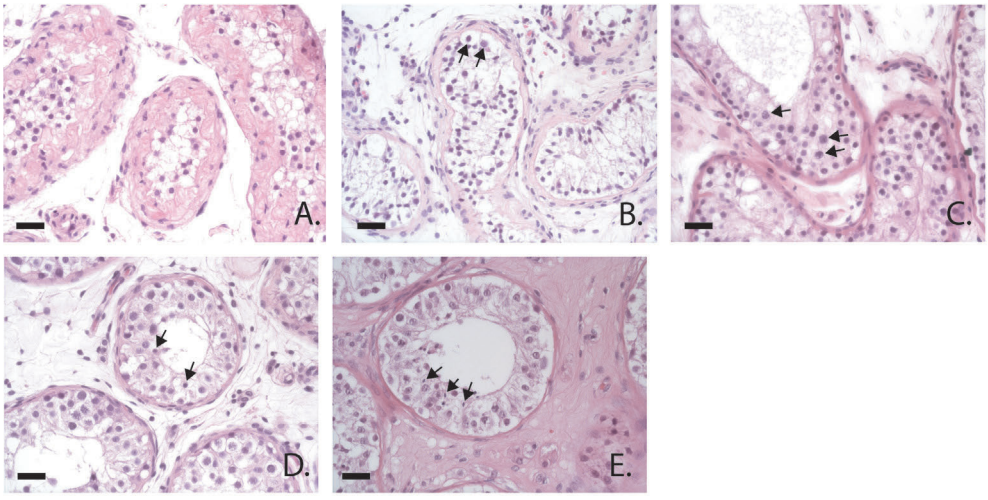


Figure 2. Orchiectomy specimens with their most advanced germ cell type. A. No germ cells present, B. Spermatogonia, C. Spermatocytes, D. Round spermatids, E. Spermatozoa. Arrows indicate most advanced germ cells. Bar represents 20µm.

Table 2. Results of histological and immunohistochemical analyses of orchiectomy specimens. Data are % (n) unless stated otherwise.

	Total (n=214)	**Adolescent Tanner stage 2-3 (n=29)**		**Adolescent Tanner stage 4-5 (n=49)**		**Adult (n=136)**	
		Cessation of GAHT (n=19)	*Continuation of GAHT (n=10)*	*Cessation of GAHT (n=35)*	*Continuation of GAHT (n=14)*	*Cessation of GAHT (n=62)*	*Continuation of GAHT (n=74)*
Spermatozoa	4.7 (10)	0 (0)	0 (0)	6 (2)	22 (3)	6 (4)	1 (1)
Round spermatids	0.5 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1)	0 (0)
Spermatocytes	21.5 (46)	21 (4)	40 (4)	31 (11)	14 (2)	23 (14)	15 (11)
Spermatogonia	66.3 (142)	79 (15)	60 (6)	63 (22)	64 (9)	61 (38)	70 (52)
No germ cells	7.0 (15)	0 (0)	0 (0)	0 (0)	0 (0)	8 (5)	14 (10)
Mean Johnsen's score - (SD)	2.5 (0.8)	2.6 (0.3)	2.7 (0.4)	2.8 (0.8)	3.2 (1.4)	2.5 (0.8)	2.3 (0.6)
Hyalinization	75.2 (161)	47 (9)	40 (4)	63 (22)	79 (11)	76 (47)	92 (68)
Lumen							
Open	8.4 (18)	0 (0)	0 (0)	3 (1)	22 (3)	18 (11)	4 (3)
Half open	25.2 (54)	26 (5)	10 (1)	31 (11)	14 (2)	26 (16)	26 (19)
Absent	66.4 (142)	74 (14)	90 (9)	66 (23)	64 (9)	56 (35)	70 (52)

Hyalinization of seminiferous tubules was observed in 161 orchiectomy specimens (75.2%) and was most common in the adult subgroup (Figure 3E and F). An open or half-open lumen of the seminiferous tubule was encountered in 8.4% and 25.2% of the orchiectomy specimens (Figure 3A and B), respectively. The complete absence of a lumen was most common in those who initiated treatment in Tanner stage 2 or 3 (Figure 3C).

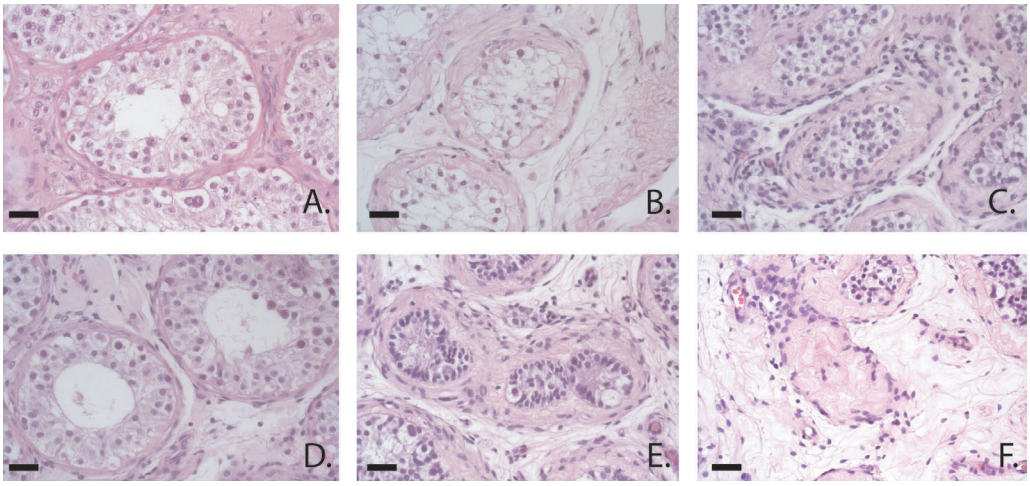


Figure 3. Different aspects of lumen and degrees of hyalinization of seminiferous tubules. A. Open lumen, B. Half-open lumen, C. Absent lumen, D. No hyalinization, E. Mild hyalinization, F. Severe hyalinization. Bar represents 20µm.

When comparing the options for fertility preservation, we found that for some transgender women it would still have been possible to harvest mature spermatozoa from testicular tissue obtained during gGAS (Figure 4). This was the case for 4% (95%CI 2-8) of the adult subgroup and 10% (95%CI 4-22) of adolescents in the Tanner stage 4-5 subgroup, compared to 0% in the Tanner stage 2-3 subgroup. For 100% of people in the Tanner stage 2-3 subgroup, 90% (95%CI 78-96) of people in the Tanner stage 4-5 subgroup and 85% (95%CI 78-90) of the adult subgroup, preservation of testicular tissue containing spermatogonial stem cells would have been their only option for fertility preservation. Furthermore, for 11% (95%CI 7-17) of the adult subgroup no options for fertility preservation would have been available, compared to 0% of the two adolescent subgroups. No statistically significant differences were found between those who had continued GAHT until gGAS and those with four weeks cessation of GAHT prior to gGAS.

Lastly, logistic regression analyses showed no association between the duration of GAHT and the possibility for preservation of spermatozoa (OR 0.75, 95%CI 0.47-1.18) or spermatogonial stem cells (OR 1.03, 95%CI 0.81-1.31).

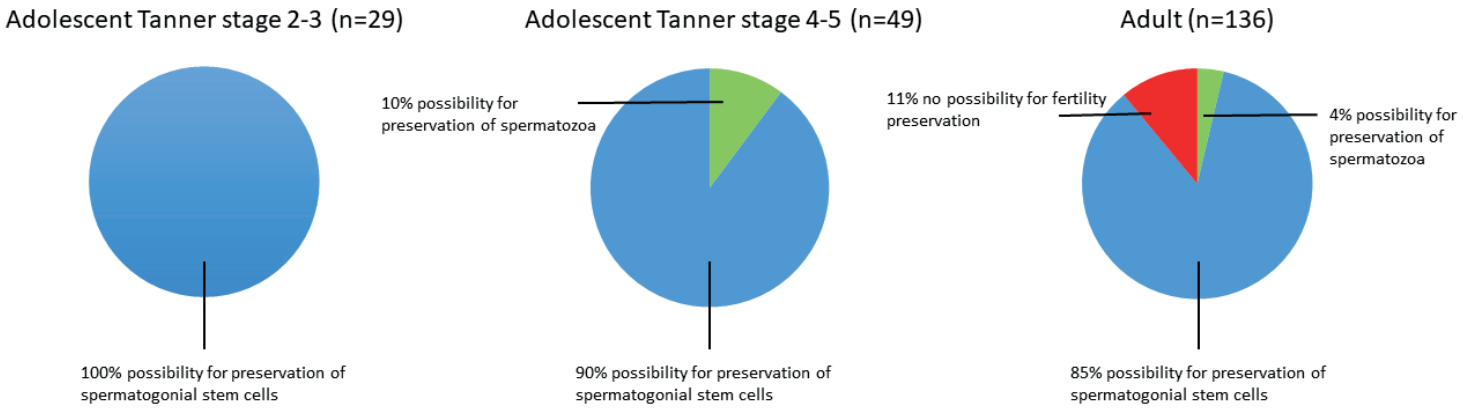


Figure 4. Comparison of the possibilities for fertility preservation between people who started medical treatment as adolescent in early-puberty (Tanner stage 2-3) or late-puberty (Tanner stage 4-5), and those who started as an adult (>18 years).

DISCUSSION

The results of our study imply that there may be options for fertility preservation for transgender women who are unable to pursue semen cryopreservation, by using testicular tissue from orchiectomy specimens obtained during gGAS. In a small percentage of transgender women who initiated medical treatment in Tanner stage 4 or higher, complete spermatogenesis was observed in the orchiectomy specimen. For this group, it would theoretically be possible to perform TESE and cryopreserve the harvested spermatozoa from this specimen. Furthermore, the vast majority of transgender women still had immature germ cells in their orchiectomy specimen. This is the first study to report on people who initiated medical treatment in Tanner stage 2-3, and it was found that in 100% of their orchiectomy specimens immature germ cells were present. If maturation techniques like *in vitro* spermatogenesis become available in the future, cryopreservation of testicular tissue containing spermatogonial stem cells might be a promising option for this group to retain the possibility to have biological children. A complete absence of germ cells was only observed in transgender women who commenced GAHT as adult. Cessation of GAHT prior to gGAS did not affect the possibilities for fertility preservation, neither was there an effect of the duration of GAHT prior to gGAS.

Although some previous studies have been conducted on the influence of GAHT on spermatogenesis and testicular architecture, this is the first study taking age and pubertal stage at time of initiation of medical treatment into account. Between 1970 and 1990, several small studies were conducted reporting on 4 to 11 transgender women per study.[28,114-118] Therefore, no strong conclusions could be drawn, but results showed high proportions of tubular hyalinization and reduced spermatogenesis in all transgender women. The first large cohort study on this topic was performed in 2015 and assessed orchiectomy specimens of 108 transgender women from three clinics with different pre-operative treatment protocols (6 weeks, 2 weeks, or no discontinuation of GAHT prior to gGAS).[27] Their results on testicular histology and spermatogenic state were highly heterogeneous and did not show a relation with treatment strategy. Remarkably, a high number of transgender women (24% of their study cohort) had complete spermatogenesis at time of gGAS. This finding was confirmed by Jiang et al. who even observed complete spermatogenesis in 40% of the 72 included transgender women.[107] However, several other recent studies found lower percentages of complete spermatogenesis ranging from 0 to 11% of the study cohort.[103,104,106,119] It must be noted that hormonal and pre-operative treatment protocols vary considerably within, and between, the different studies conducted on this topic. Therefore, for the current study it was decided to only include transgender women who used estradiol in combination with testosterone suppressing therapy (triptorelin when initiated in adolescence, cyproterone acetate when initiated in adulthood), and to report results for those who continued GAHT until gGAS separate from those who discontinued four weeks prior to gGAS.

Since a study performed by Vereecke et al. also adhered strict in- and exclusion criteria that are similar to those in our adult subgroup, their results allow for the most accurate comparison.[103] In addition, their method of analysis using immunohistochemistry to

determine the most advanced germ cell type is similar to our study. In their cohort of 97 transgender women, 12.4% had a complete absence of germ cells which is in line with the observed 11% in our cohort. However, none of their orchiectomy specimens showed complete spermatogenesis, as opposed to 4% of orchiectomy specimens in the adult subgroup of our cohort. Vereecke et al. also assessed the relationship between serum hormone levels and spermatogenic state in their cohort. They found that higher serum testosterone levels were associated with more advanced maturation, and higher serum estradiol levels were associated with a lower number of spermatogonia. However, the hormone levels were not measured on the day of gGAS, but at the last visit in the outpatient clinic 91.0 (57.5–152.5) days before surgery.[103] In contrast, Schneider et al. did collect serum and intratesticular testosterone levels on the day of gGAS but did not find an obvious correlation with spermatogenic state.[27] In our gender identity clinic, hormone levels are not determined on the day of gGAS and laboratory results from the last visit in the outpatient clinic likely do not adequately reflect hormonal status during gGAS because of the preoperative cessation of GAHT 4 weeks prior to surgery. It was therefore decided not to assess this relationship in our cohort.

An interesting observation in the current study is that testicular histology and spermatogenesis seemed more negatively affected by GAHT in the adult subgroup compared to the adolescent subgroups, despite the lower mean duration of medical treatment in the former prior to gGAS. A higher percentage of hyalinization of the seminiferous tubules was observed in the adult subgroup, as well as a complete absence of germ cells in 15 orchiectomy specimens. The difference between the adult subgroup and the adolescent subgroups might be explained by age, lifestyle (a higher percentage of smokers and alcohol drinkers), higher dosages of estradiol, or the use of cyproterone acetate instead of GnRHa as testosterone suppressing therapy. Whereas GnRHa only leads to inhibition of gonadotropin secretion, cyproterone acetate also has progestative effects and acts as a direct antagonist of the androgen receptor. It hereby inhibits the influence of androgens on the androgen-dependent organs, among which the testes. The latter might have more profound and irreversible effects on testicular tissue. Because of unwanted side-effects of cyproterone acetate (e.g. increased risk for meningioma), transgender women commencing GAHT in our clinic above the age of 18 years now receive GnRHa as testosterone suppressing therapy instead of cyproterone acetate. The potential consequence of irreversible infertility might be an extra reason to not prescribe cyproterone acetate anymore. In a future study, it would be interesting to assess if differences in testicular histology and spermatogenesis between adults and adolescents are still observed when they both receive GnRHa as testosterone suppressing therapy.

Cessation of GAHT prior to gGAS did not affect the possibilities for fertility preservation. In our study, the pre-operative cessation of GAHT involved a period of four weeks, whereas the differentiation of spermatogonial stem cells into spermatozoa generally takes 10 to 12 weeks.[8] Therefore, the period of cessation was most likely not long enough to influence the options for fertility preservation. If transgender women would be willing to discontinue GAHT for at least 12 weeks prior to gGAS, this might positively influence the chances of finding mature spermatozoa in the orchiectomy specimen. Moreover, they could even

consider an attempt for cryopreservation of spermatozoa from a semen sample, obtained through ejaculation. However, it is unknown if spermatogenesis can recover if GAHT is stopped and how much time is needed for this purpose. Furthermore, it should not be underestimated that cessation of GAHT will result in increased testosterone levels which is likely to have negative physical and psychological consequences, and that masturbation is often not an option in transgender women due to severe genital dysphoria. A disadvantage of spermatozoa that are harvested from testicular tissue, is that they are not suitable for a minimally invasive and inexpensive IUI and can only be used for ICSI.[32] In addition, such ICSI treatments using surgically obtained spermatozoa are not always successful, since the cumulative ongoing pregnancy rate per cycle has been reported to be 22.8% and the live birth rate 22.3%.[120] Therefore, cryopreservation of a semen sample prior to initiation of GAHT remains the preferred method of fertility preservation in transgender women and harvesting germ cells from orchiectomy specimens might only be considered an alternative in those for whom this is not an option.

The lumina of the seminiferous tubules in those who initiated medical treatment in Tanner stage 2-3 were all either half-open, or absent. This observation might be explained by the immaturity of testicular tissue in early puberty, since an open lumen develops parallel to the development of spermatogenesis under the influence of increasing intratesticular testosterone levels. The fact that germ cells were encountered in all orchiectomy specimens from transgender women who initiated medical treatment as adolescent, is reassuring. Decision making about fertility can be very difficult for adolescents since their intellectual, emotional, and social immaturity may impede assessment and prediction of future desires regarding fertility and family planning. A recent study among transgender youth showed that 67% of young transgender women expressed a desire for future parenthood, but only 7% indicated to be frustrated if biological parenthood would not be feasible.[55] Another study, however, reported that 48% of transgender adolescents acknowledged that their desires regarding parenthood might change over time.[57] Reduced levels of gender dysphoria and improved mental health might result in an improved capability to establish romantic relationships and consider future family building. Our observation that immature germ cells remain present in testicular tissue during GAHT suggest that transgender adolescents still have potential options for fertility preservation after initiation of treatment by cryopreserving testicular tissue from orchiectomy specimen obtained during gGAS.

Cryopreservation of testicular tissue containing spermatogonial stem cells is mostly offered to pre-pubertal boys with cancer, prior to undergoing gonadotoxic therapies such as chemo- and radiotherapy, but some clinics also offer this option to transgender adolescents.[63] In the absence of complete spermatogenesis, the purpose of spermatogonial stem cell preservation in cisgender adolescents is to transplant these cells back into the testes years later, via injection into the rete testis space that is contiguous with all seminiferous tubules. Spermatogonial stem cells have the potential to colonize the testicular niche and regenerate spermatogenesis.[121] However, re-transplantation is not a feasible option for transgender women, as they will most likely use lifelong GAHT and many will undergo bilateral orchiectomy. Therefore, spermatogonial stem cell preservation will only be a viable method for fertility preservation in transgender women when other

options for maturation become available, such as de novo testicular morphogenesis or *in vitro* spermatogenesis. Although these techniques are successful in animal models, they are still experimental and far from the clinical realm.[122] Continuing research in this area will hopefully make these techniques available so that transgender adolescents, who are otherwise unable to have genetically-related children, will be able to retain this possibility by cryopreserving testicular tissue containing spermatogonial stem cells. Furthermore, future research should focus on how GAHT influences the quality of germ cells and the safety of using cells harvested from orchiectomy specimens, for reproductive techniques. Lastly, it is important to examine how transgender women feel about fertility preservation options in orchiectomy specimens obtained during gGAS.

A limitation of this study is the lack of data on serum hormone levels on the day of gGAS. We were therefore unable to verify if the transgender women who were asked to temporarily stop hormonal treatment four weeks prior to surgery actually did so, and if people with complete spermatogenesis were compliant to treatment. However, the last known serum testosterone levels before gGAS were suppressed in all participants. Furthermore, despite our efforts to create a homogeneous study population, by excluding people who used estrogen monotherapy and those who used spironolactone as anti-androgenic treatment, participants still used varying formulations of estrogens and switched between different formulations over time. We were therefore unable to assess if different estrogen formulations have different effects on testicular histology and spermatogenesis. Strengths of our study include the large sample size of 214 transgender women, and the creation of six subgroups to allow for comparison between different pre-operative protocols before gGAS and pubertal stage at initiation of medical treatment. Hereby, this study provides novel information about the influence of starting medical treatment in early puberty on testicular function, and its consequences for the possibilities for fertility preservation at time of gGAS. This is relevant because we are seeing a global increase of the number of referrals of adolescents to gender identity clinics.[123,124] At the same time, there is increasing controversy over the provision of GAHT to adolescents, with the negative effect on fertility often cited as an argument for limiting adolescents' access to gender-affirming care.[108] Our observation that the spermatogonial stem cell pool is still intact in people who initiated GAHT during adolescence is therefore valuable information in this debate.

CONCLUSION

Counseling of transgender women about the effect of medical treatment on fertility and the currently available options for fertility preservation remains essential. However, for some transgender women with a wish for fertility preservation, there are barriers that prevent the use of semen cryopreservation. For example some initiate medical treatment in early puberty before the development of complete spermatogenesis, some are unable to masturbate, and some feel that a temporary cessation of GAHT would be too psychologically and physically disruptive. The results of this study show that there may still be options for fertility preservation using orchiectomy specimens obtained during gGAS. In a small percentage of transgender women who initiated medical treatment in Tanner stage 4 or higher, spermatozoa could have been harvested from the orchiectomy specimen at time of gGAS. In addition, the vast majority (> 85%) of transgender women in our cohort could still opt for cryopreservation of testicular tissue harboring spermatogonial stem cells. A complete absence of germ cells was only observed in a small number (7%) of transgender women in our cohort, who all commenced GAHT as adult. The possibilities for fertility preservation seem irrespective of pre-operative cessation of GAHT and the duration of GAHT prior to gGAS.

Initiation of medical treatment in early-pubertal adolescents (Tanner stage 2-3) limits the ability to retrieve mature spermatozoa that can directly be used for assisted reproductive techniques. However, if maturation techniques like *in vitro* spermatogenesis become available in the future, harvesting germ cells from orchiectomy specimens might be a promising option for those who are otherwise unable to have biological children.

Supplementary Table 1. The Modified Johnsen's scoring system

Johnsen score	Characteristics
1	no recognizable seminiferous epithelium (sclerosis)
2	Sertoli cell only (SCO), absence of germ cells
3	only the presence of spermatogonia alongside Sertoli cells, but no spermatocytes
4	presence of 1-10 spermatocytes, but no spermatids
5	presence of ≥10 spermatocytes, but no spermatids
6	presence of 1-10 round spermatids, but no elongated spermatids
7	presence ≥10 round spermatids, but no elongated spermatids
8	presence of 1-10 elongated spermatids
9	presence of ≥10 elongated spermatids, but a disorganized epithelium with released immature and apoptotic cells in the lumen
10	presence of ≥10 elongated spermatids without immature and apoptotic cells in lumen

Supplementary Table 2. The Modified Johnsen's score for each individual

ID	Number of seminiferous tubules with corresponding JS										Mean JS
	JS1	JS2	JS3	JS4	JS5	JS6	JS7	JS8	JS9	JS10	
1	0	13	27	10	0	0	0	0	0	0	2.94
2	0	5	31	15	0	0	0	0	0	0	3.2
3	6	42	6	0	0	0	0	0	0	0	1.96
4	0	36	14	0	0	0	0	0	0	0	2.28
5	0	41	9	0	0	0	0	0	0	0	2.18
6	0	39	12	0	0	0	0	0	0	0	2.24
8	5	32	13	0	0	0	0	0	0	0	2.16
9	4	24	21	1	0	0	0	0	0	0	2.38
10	19	29	3	0	0	0	0	0	0	0	1.72
11	0	25	21	4	0	0	0	0	0	0	2.58
14	1	24	24	4	0	0	0	0	0	0	2.54
15	32	18	0	0	0	0	0	0	0	0	1.36
16	4	40	6	0	0	0	0	0	0	0	2.04
17	7	44	0	0	0	0	0	0	0	0	1.86
18	23	18	11	0	0	0	0	0	0	0	1.77
19	0	24	30	0	0	0	0	0	0	0	2.56
20	21	29	0	0	0	0	0	0	0	0	1.58
21	0	16	35	0	0	0	0	0	0	0	2.69
22	4	29	19	0	0	0	0	0	0	0	2.29
23	0	38	13	0	0	0	0	0	0	0	2.26
24	9	38	2	0	0	0	0	0	0	0	1.86
25	0	0	38	12	0	0	0	0	0	0	3.24
26	0	1	10	33	6	2	0	0	0	0	3.96
27	4	20	23	3	0	0	0	0	0	0	2.5
28	8	29	14	0	0	0	0	0	0	0	2.12
29	0	17	31	3	0	0	0	0	0	0	2.73
30	0	1	6	16	17	5	4	1	0	0	4.7
31	0	1	16	28	6	0	0	0	0	0	3.76
32	0	31	5	14	0	0	0	0	0	0	2.66
33	1	16	28	6	0	0	0	0	0	0	2.76
34	8	26	15	1	0	0	0	0	0	0	2.18
35	5	36	11	0	0	0	0	0	0	0	2.12
37	0	5	18	22	4	0	0	1	0	0	3.6
38	0	9	43	2	0	0	0	0	0	0	2.87
39	2	20	30	0	0	0	0	0	0	0	2.54
40	6	37	6	1	0	0	0	0	0	0	2.04
41	0	40	10	1	0	0	0	0	0	0	2.24

ID	Number of seminiferous tubules with corresponding JS										Mean JS
	JS1	JS2	JS3	JS4	JS5	JS6	JS7	JS8	JS9	JS10	
42	0	37	14	0	0	0	0	0	0	0	2.28
45	2	41	9	0	0	0	0	0	0	0	2.13
46	0	42	4	4	0	0	0	0	0	0	2.24
47	4	25	23	0	0	0	0	0	0	0	2.37
48	0	12	40	0	0	0	0	0	0	0	2.77
49	2	9	40	0	0	0	0	0	0	0	2.75
51	2	2	49	0	0	0	0	0	0	0	2.89
52	2	8	41	0	0	0	0	0	0	0	2.76
53	1	3	48	0	0	0	0	0	0	0	2.9
55	50	0	0	0	0	0	0	0	0	0	1
56	16	34	0	0	0	0	0	0	0	0	1.68
57	2	7	43	0	0	0	0	0	0	0	2.79
58	1	44	8	0	0	0	0	0	0	0	2.13
59	6	17	28	0	0	0	0	0	0	0	2.43
60	12	35	4	0	0	0	0	0	0	0	1.84
62	2	32	12	5	0	0	0	0	0	0	2.39
63	12	38	0	0	0	0	0	0	0	0	1.76
65	4	26	19	1	0	0	0	0	0	0	2.34
66	5	12	35	0	0	0	0	0	0	0	2.58
67	8	42	0	0	0	0	0	0	0	0	1.84
68	5	35	11	0	0	0	0	0	0	0	2.12
69	2	37	11	0	0	0	0	0	0	0	2.18
70	2	50	1	0	0	0	0	0	0	0	1.98
71	2	26	16	5	0	0	0	0	0	0	2.49
72	1	15	31	3	0	0	0	0	0	0	2.72
73	0	10	34	6	0	0	0	0	0	0	2.92
74	0	9	15	26	0	0	0	0	0	0	3.34
75	9	41	6	0	0	0	0	0	0	0	1.95
76	1	46	3	0	0	0	0	0	0	0	2.04
77	0	30	13	8	0	0	0	0	0	0	2.57
78	1	27	16	1	0	0	0	0	0	0	2.38
79	18	32	0	0	0	0	0	0	0	0	1.64
80	2	31	19	0	0	0	0	0	0	0	2.33
81	1	5	40	4	0	0	0	0	0	0	2.94
82	7	8	33	3	0	0	0	0	0	0	2.63
83	9	43	0	0	0	0	0	0	0	0	1.83
84	1	33	17	0	0	0	0	0	0	0	2.31
85	0	7	22	11	3	8	4	3	0	0	4.12

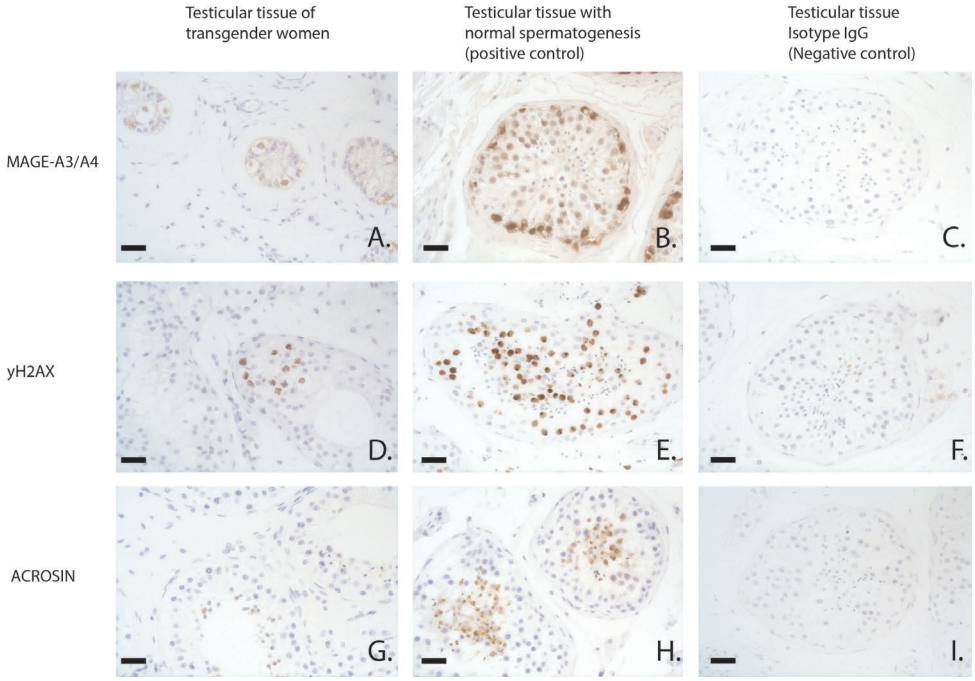
ID	Number of seminiferous tubules with corresponding JS										Mean JS
	JS1	JS2	JS3	JS4	JS5	JS6	JS7	JS8	JS9	JS10	
86	0	35	14	2	0	0	0	0	0	0	2.35
87	16	20	14	0	0	0	0	0	0	0	1.96
88	0	6	43	3	0	0	0	0	0	0	2.94
89	0	21	5	25	2	0	0	0	0	0	3.15
91	0	4	14	19	8	9	0	1	0	0	4.15
92	2	12	38	0	0	0	0	0	0	0	2.69
93	10	45	1	0	0	0	0	0	0	0	1.84
94	1	6	44	0	0	0	0	0	0	0	2.84
95	2	3	45	0	0	0	0	0	0	0	2.86
96	4	5	43	0	0	0	0	0	0	0	2.75
97	9	41	2	0	0	0	0	0	0	0	1.87
100	7	10	35	0	0	0	0	0	0	0	2.54
101	16	34	0	0	0	0	0	0	0	0	1.68
102	1	6	44	0	0	0	0	0	0	0	2.69
103	1	4	46	0	0	0	0	0	0	0	2.88
104	4	44	9	0	0	0	0	0	0	0	2.09
105	35	6	20	0	0	0	0	0	0	0	1.75
106	3	6	43	0	0	0	0	0	0	0	2.77
110	0	3	47	0	0	0	0	0	0	0	2.94
112	3	3	45	0	0	0	0	0	0	0	2.82
113	12	39	3	0	0	0	0	0	0	0	1.83
114	3	4	44	0	0	0	0	0	0	0	2.8
115	1	5	45	0	0	0	0	0	0	0	2.86
117	4	23	25	2	0	0	0	0	0	0	2.46
118	50	0	0	0	0	0	0	0	0	0	1
119	0	2	49	0	0	0	0	0	0	0	2.96
121	0	0	4	6	4	12	8	18	0	0	6.31
122	2	37	10	1	0	0	0	0	0	0	2.2
124	1	22	27	1	0	0	0	0	0	0	2.55
125	4	6	45	0	0	0	0	0	0	0	2.75
126	4	47	3	0	0	0	0	0	0	0	1.98
127	8	21	25	0	0	0	0	0	0	0	2.31
128	2	4	47	0	0	0	0	0	0	0	2.85
129	2	11	39	1	0	0	0	0	0	0	2.74
130	2	2	48	0	0	0	0	0	0	0	2.88
131	4	46	6	0	0	0	0	0	0	0	2.04
132	36	13	1	0	0	0	0	0	0	0	1.3
133	5	43	3	0	0	0	0	0	0	0	1.96

ID	Number of seminiferous tubules with corresponding JS										Mean JS
	JS1	JS2	JS3	JS4	JS5	JS6	JS7	JS8	JS9	JS10	
134	2	9	40	0	0	0	0	0	0	0	2.75
135	0	2	49	0	0	0	0	0	0	0	2.96
136	0	0	7	10	1	9	7	17	0	0	5.98
138	2	27	32	0	0	0	0	0	0	0	2.49
139	0	2	10	30	9	0	0	0	0	0	3.9
140	1	4	46	0	0	0	0	0	0	0	2.88
141	1	22	28	0	0	0	0	0	0	0	2.17
142	8	42	1	0	0	0	0	0	0	0	1.86
143	3	31	16	0	0	0	0	0	0	0	2.26
144	0	0	9	28	1	12	1	3	0	0	4.57
146	9	29	14	0	0	0	0	0	0	0	2.1
147	4	34	12	0	0	0	0	0	0	0	2.16
148	2	44	4	0	0	0	0	0	0	0	2.04
149	0	13	39	0	0	0	0	0	0	0	2.75
150	50	0	0	0	0	0	0	0	0	0	1
151	4	7	40	0	0	0	0	0	0	0	2.7
152	1	3	46	0	0	0	0	0	0	0	2.9
153	0	2	6	5	4	6	7	15	5	0	6.2
154	2	45	3	0	0	0	0	0	0	0	2.02
155	3	5	43	0	0	0	0	0	0	0	2.78
156	4	19	30	0	0	0	0	0	0	0	2.49
157	46	6	3	0	0	0	0	0	0	0	1.22
158	0	3	48	0	0	0	0	0	0	0	2.94
159	0	4	47	0	0	0	0	0	0	0	2.92
160	6	44	4	0	0	0	0	0	0	0	1.96
161	0	27	24	0	0	0	0	0	0	0	2.47
162	0	2	49	0	0	0	0	0	0	0	2.96
163	3	18	28	2	0	0	0	0	0	0	2.57
164	3	24	25	0	0	0	0	0	0	0	2.62
165	2	7	42	0	0	0	0	0	0	0	2.78
166	0	17	27	6	0	0	0	0	0	0	2.78
168	0	0	0	9	7	10	3	17	4	0	6.48
169	5	9	38	0	0	0	0	0	0	0	2.63
170	10	14	30	0	0	0	0	0	0	0	2.37
171	4	29	21	0	0	0	0	0	0	0	2.31
172	2	33	17	0	0	0	0	0	0	0	2.29
173	2	41	11	0	0	0	0	0	0	0	2.17
175	2	31	16	4	0	0	0	0	0	0	2.42

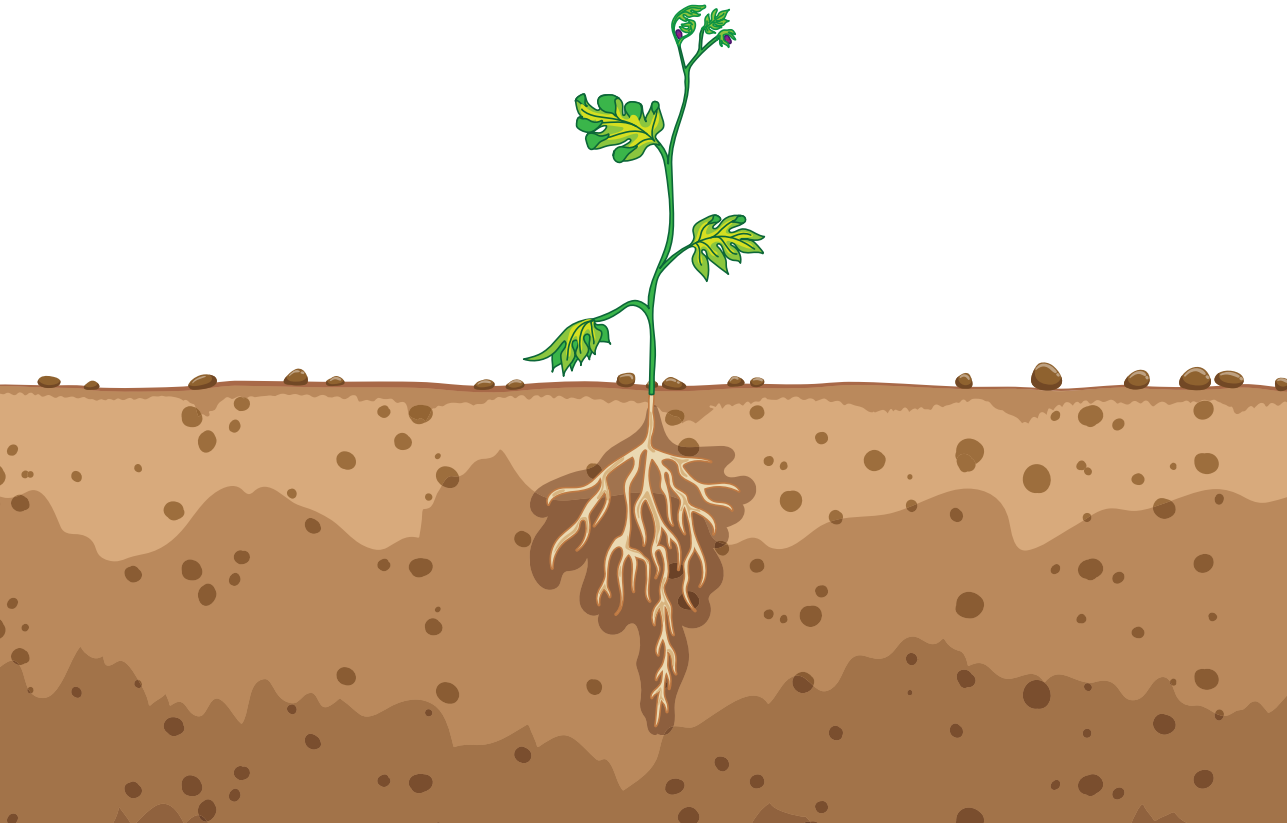
ID	Number of seminiferous tubules with corresponding JS										Mean JS
	JS1	JS2	JS3	JS4	JS5	JS6	JS7	JS8	JS9	JS10	
176	4	6	42	0	0	0	0	0	0	0	2.73
177	4	31	18	0	0	0	0	0	0	0	2.26
178	2	2	47	0	0	0	0	0	0	0	2.88
179	1	5	45	0	0	0	0	0	0	0	2.86
182	2	8	37	4	0	0	0	0	0	0	2.84
183	3	28	22	0	0	0	0	0	0	0	2.36
184	6	42	5	0	0	0	0	0	0	0	1.98
186	2	27	21	6	0	0	0	0	0	0	2.55
187	2	11	39	0	0	0	0	0	0	0	2.71
188	2	39	16	0	0	0	0	0	0	0	2.25
190	43	7	0	0	0	0	0	0	0	0	1.14
192	0	10	27	15	3	0	0	0	0	0	3.02
194	4	10	38	0	0	0	0	0	0	0	2.65
195	37	11	2	0	0	0	0	0	0	0	1.3
197	17	18	11	5	0	0	0	0	0	0	2.12
198	5	9	40	0	0	0	0	0	0	0	2.65
199	3	44	4	0	0	0	0	0	0	0	2.02
201	2	7	42	0	0	0	0	0	0	0	2.78
203	0	9	42	0	0	0	0	0	0	0	2.82
204	9	38	4	0	0	0	0	0	0	0	1.9
205	16	47	0	0	0	0	0	0	0	0	1.74
206	17	31	11	0	0	0	0	0	0	0	1.9
207	0	1	16	14	8	3	1	7	0	1	4.65
208	2	38	16	0	0	0	0	0	0	0	2.25
210	3	13	36	0	0	0	0	0	0	0	2.63
211	0	6	46	0	0	0	0	0	0	0	2.88
213	21	33	0	0	0	0	0	0	0	0	1.61
214	2	6	43	0	0	0	0	0	0	0	2.8
215	3	11	42	0	0	0	0	0	0	0	2.7
216	0	6	46	0	0	0	0	0	0	0	2.88
217	3	23	24	0	0	0	0	0	0	0	2.42
218	0	3	49	0	0	0	0	0	0	0	2.94
219	1	7	42	0	0	0	0	0	0	0	2.82
220	2	6	43	0	0	0	0	0	0	0	2.8
221	0	44	8	0	0	0	0	0	0	0	2.15
222	0	32	20	0	0	0	0	0	0	0	2.38
223	0	5	46	0	0	0	0	0	0	0	2.9
224	6	31	16	0	0	0	0	0	0	0	2.19

ID	Number of seminiferous tubules with corresponding JS										Mean JS
	JS1	JS2	JS3	JS4	JS5	JS6	JS7	JS8	JS9	JS10	
225	2	2	47	0	0	0	0	0	0	0	2.88
226	0	3	47	0	0	0	0	0	0	0	2.94
228	2	11	37	0	0	0	0	0	0	0	2.7
229	0	12	29	9	0	0	0	0	0	0	2.94
230	0	12	31	7	0	0	0	0	0	0	2.9
231	4	46	2	0	0	0	0	0	0	0	2.04
232	0	6	45	0	0	0	0	0	0	0	2.88
235	7	27	18	0	0	0	0	0	0	0	2.21
238	2	29	19	0	0	0	0	0	0	0	2.44
241	3	39	9	0	0	0	0	0	0	0	2.12
244	11	37	3	0	0	0	0	0	0	0	1.84
245	0	3	48	0	0	0	0	0	0	0	2.94
247	5	43	3	0	0	0	0	0	0	0	1.96
249	2	7	42	0	0	0	0	0	0	0	2.78
251	2	28	21	0	0	0	0	0	0	0	2.42
255	0	6	36	8	0	0	0	0	0	0	3.04
257	7	14	31	0	0	0	0	0	0	0	2.46
258	0	4	47	0	0	0	0	0	0	0	2.92
259	0	36	16	0	0	0	0	0	0	0	2.31
260	0	17	31	3	0	0	0	0	0	0	2.73
263	2	22	27	0	0	0	0	0	0	0	2.49
265	12	41	3	0	0	0	0	0	0	0	1.84
266	0	16	27	11	1	0	0	0	0	0	3
267	3	47	2	0	0	0	0	0	0	0	1.98
268	1	9	41	0	0	0	0	0	0	0	2.78

JS: Johnsen's score



Supplementary Figure 1. Immunohistochemical analyses. A. MAG-3A/A4 in transgender women, B. MAG-3A/A4 positive control, C. MAG-3A/A4 negative control, D. γH2AX in transgender women, E. γH2AX positive control, F. γH2AX negative control, G. Acrosin in transgender women H. Acrosin positive control, I. Acrosin negative control. Bar represents 20μm.



CHAPTER 7

INCIDENCE OF TESTICULAR CANCER IN TRANS WOMEN USING GENDER-AFFIRMING HORMONAL TREATMENT: A NATIONWIDE COHORT STUDY

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Published in BJU International; August 2021 [article in press]

ABSTRACT

Objective: To assess the incidence of testicular cancer in trans women (male sex assigned at birth, female gender identity) using gender-affirming hormonal treatment.

Materials and Methods: Data of trans women starting hormonal treatment at our gender identity clinic between 1972 and 2017 were linked to the national pathology database to obtain testicular cancer diagnoses. The standardized incidence ratio (SIR) was calculated using the number of observed testicular cancer cases in our cohort and the number of expected cases based on age-specific Dutch incidence rates. Subgroup analyses were performed in testicular tissues send for histopathological analysis at time of bilateral orchiectomy, and when follow-up time exceeded 5 years.

Results: The cohort consisted of 3,026 trans women with a median follow-up time of 2.3 years (interquartile range 1.6-3.7). Two testicular cancer cases were identified whilst 2.4 cases were expected (SIR 0.8, 95% CI 0.1-2.8). In addition, one testicular cancer case was encountered in an orchiectomy specimen (0.1%). In the 523 trans women with a follow-up time exceeding 5 years (median 8.9 years, interquartile range 6.4-13.9), no testicular cancer was observed.

Conclusion: Testicular cancer risk in trans women is similar to the risk in cis men. The testicular cancer cases occurred within the first 5 years after commencing hormonal treatment, and the percentage of cases encountered at time of bilateral orchiectomy was low. Since no testicular cancer was observed in trans women with a long follow-up time, long-term hormonal treatment does not seem to increase testicular cancer risk.

INTRODUCTION

Testicular cancer mainly occurs in young people; the incidence in the Netherlands is 9.5 per 100.000 men, with a peak incidence of 32.4 per 100.000 men in those between 30-34 years old.[15] Testicular cancers can roughly be divided into sex cord or gonadal stromal tumors and germ-cell tumors, of which the latter most commonly occur. Germ-cell tumors are further classified as seminoma, non-seminoma and mixed germ-cell tumors. Prognosis, depending on histology, location of the primary tumor and metastases, and serum tumor marker levels, is generally better for seminoma compared to non-seminoma.[16] Although the incidence has increased over the past 40 years in most countries, the etiology of testicular cancer and the reasons for this rise remain unclear. Established risk factors for testicular cancer are a history of cryptorchidism, a low sperm count, presence of a contralateral testis tumor or a positive family history among first-grade relatives for testicular cancer.[16] Some theories also suggest that a relative excess of exogenous estrogens during pre- or post-natal life (e.g. diethylstilbestrol, pesticides) may play a causal role in the development of testicular cancer.[17-19] It is hypothesized that, following endocrine disruption, some of the primordial germ cells lose track of their normal development and become premalignant cells that may develop into carcinoma in-situ cells, which in their turn may develop into a complete cancer.[18]

An increasing group of birth-assigned males with long-term exposure to exogenous estrogens are people with gender dysphoria. Gender dysphoria refers to the distress that results from a conflict between a person's assigned sex at birth and one's gender identity.[70] People assigned male at birth who also identify as male are referred to as cis men, whereas birth-assigned males who identify as female are referred to as trans women. Birth-assigned males who neither identify as male nor female fall under the umbrella term gender queer, non-binary or alternative gender. Birth-assigned males with gender dysphoria desiring to align their physical characteristics with their gender identity can choose to undergo medical treatment, consisting of gender-affirming hormonal treatment (GAHT) and gender-affirming surgery (GAS). The hormonal treatment protocol usually consists of antiandrogens, to suppress serum testosterone concentrations, combined with estrogens, to achieve feminization. For people presenting during adolescence (below the age of 18 years) treatment can be initiated when a person reaches puberty (Tanner stage 2 or higher), and aims to suppress pubertal development by administration of gonadotropin-releasing hormone agonist (GnRHa). After at least six months of puberty suppression and having reached the age of 16 years, treatment can be supplemented with estrogens. GAS can involve facial feminization, breast-augmentation, and bilateral orchiectomy often combined with vaginoplasty.[2] For the sake of clarity, we will refer to birth-assigned males seeking feminizing medical treatment as trans women.

Until 2014, a sterilization law was in place in the Netherlands, meaning that a gonadectomy was required for legal gender recognition. Therefore, almost all trans women visiting our gender identity clinic underwent this procedure until 2014. However, since this law has changed, an increasing number of people with non-binary identities or less need to confirm to binary cis presentation choose to keep their male gonads. As a consequence, in the future we might be faced with a growing population of young trans women using

GAHT, who are still at risk for testicular cancer. Several studies have been conducted on the influence of androgen deprivation, estradiol supplementation, or a combination of these two, on testicular tissue and showed incomplete spermatogenesis, a decreased diameter of seminiferous tubules, and increased peritubular hyalinization.[28,29,104,125] However, very little is known about the influence on the occurrence of testicular cancer and only a few cases of testicular cancer in trans women using GAHT have been reported.[41-45]

The primary aim of this study was to evaluate the incidence of testicular cancer in trans women using GAHT and, hereby, assess the safety of hormonal treatment in terms of testicular cancer risk. A secondary aim was to assess the outcome of histopathological analyses of orchiectomy specimens obtained during GAS.

METHODS

Study design and population

For this nationwide retrospective cohort study, we identified all people who visited the gender identity clinic of the Amsterdam UMC between 1972 and September 2017. Approximately 95% of all transgender people in the Netherlands visit our center for either psychological, endocrine, or surgical treatment. Since only trans women using GAHT were eligible for inclusion, people who never used GAHT, those who underwent bilateral orchiectomy prior to the start of GAHT, or those of whom the start date of GAHT was unknown, were excluded. Other exclusion criteria involved being under 18 years of age at the time of the study (2020), or having used female and male hormones alternatingly during the follow-up period. Lastly, since data were partially obtained from the Dutch national pathology database (PALGA), which covers histopathologic diagnoses nationwide since 1991, trans women were also excluded when their last visit to the gender identity clinic was before 1991.[126]

Hormonal treatment for trans women generally consists of a combination of antiandrogens and estrogens. The most commonly prescribed antiandrogen in this cohort was cyproterone acetate (10 to 100 mg daily) and only sporadically spironolactone (100 to 200 mg daily) was used. Different administration routes for estrogens exist, such as transdermal, oral and intramuscular formulations. The different types of estrogens prescribed in our center included estradiol patches (50 to 150 µg/24 hours twice weekly), estradiol gel (0.75 to 3.0 mg daily), estradiol valerate (2 to 6 mg daily), ethinyl estradiol (25 to 100 µg daily), conjugated estrogens (0.625 to 1.25 mg daily), estradiol implants (20 mg every 3 to 6 months), and estradiol injections (10 to 100 mg every 2 to 4 weeks). From 2001 onward, mainly estradiol patches, estradiol gel, or estradiol valerate were used. People who started hormonal treatment when they were younger than 18 years, often used GnRHa, namely triptorelin, prior to the start with estrogens, and continued this medication until orchiectomy.

The Ethical Review Board of the VU University Medical Center Amsterdam, concluded that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study.

Necessity for informed consent was waived because of the retrospective design, the large study population, and the risk of selection bias (e.g. excluding deceased trans women).

Data collection

Data on medical history (e.g. testicular cancer, cryptorchidism), age at start of GAHT, documented hormone use, endocrine laboratory results, date of bilateral orchiectomy, date of last visit to our clinic, and data on mortality were collected from the medical files of the participants. This database was linked to PALGA to obtain data regarding testicular cancer histology (germ-cell tumors, sex cord/stromal tumors and germ cell neoplasia in situ) and the date of testicular cancer diagnosis.[126] Data on testicular cancer diagnosis was further validated by comparing notes from the medical files with data obtained from PALGA.

Statistical analysis

Descriptive analyses were conducted to assess the characteristics of the cohort. Normally distributed data are presented as means with standard deviation and non-normally distributed data as medians with interquartile range (IQR). Mean estradiol and testosterone concentrations were calculated by averaging the results from measurements performed during GAHT. In people who had started GAHT prior to their first visit to our clinic, we used the first known start date of GAHT to calculate the most accurate treatment duration. Follow-up time was calculated as the number of years from the start of GAHT until, either the date of testicular cancer diagnosis, or the date of bilateral orchiectomy, or the date of death, or the date of the last visit to our gender identity clinic. To calculate the age-adjusted standardized incidence ratio (SIR), we used the observed cases and the expected cases of testicular cancer in our cohort. Only testicular cancer cases that occurred after the start of GAHT were included for analysis. For our primary research aim, we only included testicular cancer cases that were discovered due to symptoms (e.g. scrotal mass or infertility), since cis men are similarly diagnosed. Expected cases were calculated based on age-specific incidence rates obtained from the Netherlands Comprehensive Cancer Organization.[15] Since this organization also uses data from PALGA to generate the incidence rates of testicular cancer in the Dutch population, this allows for a reliable comparison. For the sake of clarity, we will refer to the reference population as cis men, although we were not able to verify if this was true for the whole population. The SIR with 95% confidence interval (95% CI) was calculated using a mid-exact P test. Since it remains largely unknown how GAHT during puberty affects testicular architecture in terms of testicular cancer risk, a subgroup analysis was performed for trans women who initiated GAHT when 18 years or older. Furthermore, in order to more accurately assess the effect of long-term hormone use, a subgroup analysis was performed for trans women with a follow-up time of 5 years or more. Lastly, to assess how often testicular cancer was discovered in orchiectomy specimens obtained during gender-affirming surgery, a subgroup analysis was performed for trans women whose testicular tissue was send for histopathological analysis at time of bilateral orchiectomy.

STATA Statistical Software, version 15.1 (Statacorp, College Station, TX, USA) and OpenEpi version 3.01 (www. OpenEpi.com) were used for statistical analyses.

RESULTS

A total of 8,015 people visited our gender identity clinic between 1972 and 2017 for either psychological, endocrine, or surgical treatment. After applying the in- and exclusion criteria, 3,026 trans women were included in the study cohort (Figure 1). The median follow-up time was 2.3 years (IQR 1.6-3.7) per person and the total follow-up time of the entire cohort was 11,223 years (Figure 2).

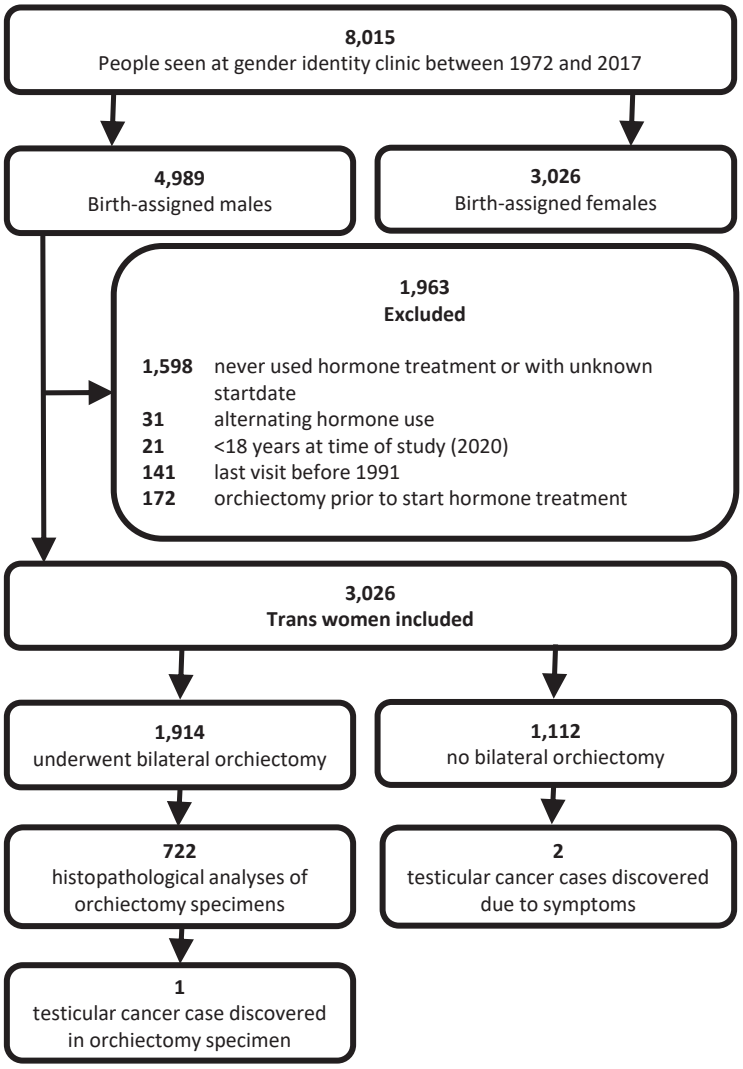


Figure 1. Study flowchart

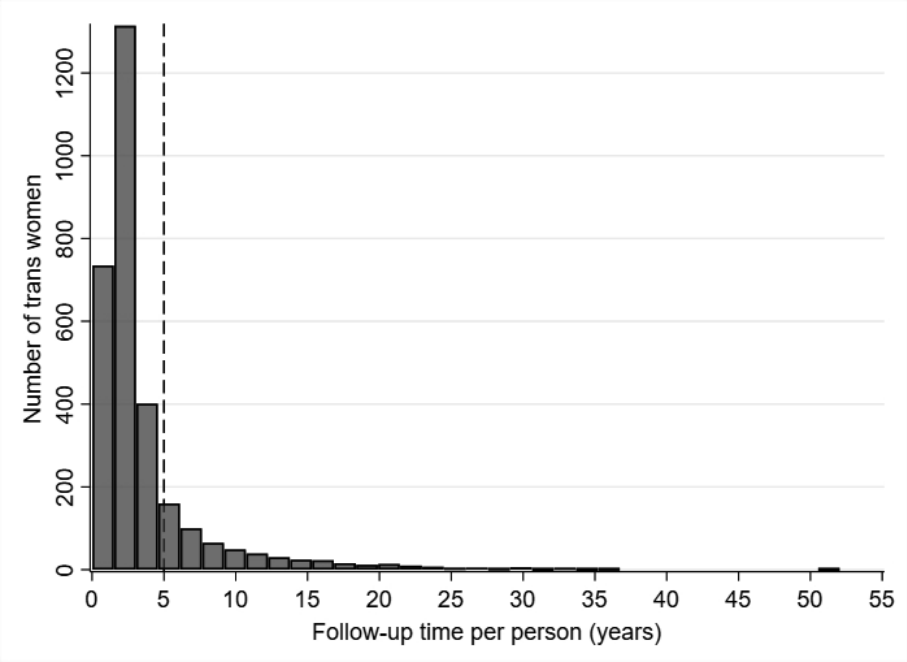


Figure 2. Follow-up time of study cohort. Dashed line indicates cohort for subgroup analysis performed for trans women with a follow-up time of five years or more.

The median age at start of GAHT was 29 years (IQR 22-41). At initiation of GAHT median serum testosterone and estradiol concentrations were in the normal male range. During GAHT median serum estradiol concentrations increased (181 pmol/L, IQR 110-296) and median serum testosterone concentrations decreased (1.0 nmol/L, IQR 0.6-1.3). In this cohort, 1,914 trans women (63%) underwent bilateral orchiectomy a median 2.3 years (IQR 1.7-3.4) after commencing hormonal treatment. Last known hormone measurements before bilateral orchiectomy did not differ from the averaged results during GAHT (data not shown). Table 1 shows the characteristics of the entire study cohort.

Table 1. Characteristics of study cohort (n=3,026)

Characteristics	n	Median (IQR) unless stated otherwise
Age at time of start HT, years	3,026	29 (22-41)
% history of cryptorchidism	35	1%
Serum testosterone concentration at initiation of GAHT, nmol/L	844	18.0 (12.0-23.0)
Serum estradiol concentration at initiation of GAHT, pmol/L	840	86 (68-109)
Serum testosterone concentration during GAHT, nmol/L	1,714	1.0 (0.6-1.3)
Serum estradiol concentration during GAHT, pmol/L	1,756	181 (110-296)
% bilateral orchiectomy	1,914	63%
Follow-up time, years	3,026	2.3 (1.6-3.7)
Total follow-up time, years	3,026	11,223

In total, three cases of testicular cancer were observed in the study cohort. Age at time of diagnosis of the three trans women with testicular cancer ranged from the second to the fourth decade of life. All three trans women were Caucasian, had no medical history for oncological diseases, and their family history was negative for testicular cancer. One person started treatment during adolescence, while the other two started GAHT in adulthood. Used anti-androgenic treatment involved tripterolin, cyproterone acetate, and spironolactone, resulting in serum testosterone levels below 2 nmol/L. Types of used estrogens included estradiol injections, estradiol patches, and estradiol valerate, resulting in mean serum estradiol concentrations between 150 and 300 pmol/L. Estrogens were used for a duration of 1 to 3 years prior to diagnosis. Histology showed non-seminoma (mature teratoma (95%), embryonal carcinoma (<5%) and yolk sac tumor (<5%)) in one case, pure seminoma grown into the rete testis in the second case, and a seminoma with syncytiotrophoblast cells in the third case. Tumor diameters ranged from 0.9 to 6.8 centimeters. None of the trans women had metastasis and all remained clinically stable after treatment.

Two of the previously mentioned testicular cancer cases were discovered due to symptoms of a painless scrotal mass. Based on age-specific incidence rates in cis men, we expected 2.4 cases of testicular cancer in our cohort. This resulted in a SIR of 0.8 with a 95% CI of 0.1 to 2.8. Subgroup analysis of 2,731 trans women who initiated GAHT when 18 years or older, showed two testicular cancer cases although 2.2 cases would have been expected, resulting in a SIR of 0.9 (95% CI 0.2-3.0). In the subgroup of 523 trans women with a follow-up time of at least 5 years (median 8.9 years, IQR 6.4-13.9, total 5,870 years), no testicular cancer cases were observed, although 1.2 cases would have been expected based on the age-specific incidence rates in cis men.

Of the 1,914 trans women who underwent bilateral orchiectomy, histopathological analysis of the resected specimens was performed in 722 trans women. Within this group, histopathological analysis showed testicular cancer in one case (0.1% of orchiectomy specimens obtained during GAS).

DISCUSSION

The aim of this study was to assess testicular cancer incidence in trans women using GAHT. A total of three testicular cancer cases were observed in our cohort, of which two were discovered due to symptoms and the third was encountered during routine histopathological analysis of the bilateral orchiectomy specimen. Our observations suggest that there is no difference in testicular cancer risk between trans women using GAHT and cis men. In addition, no testicular cancer cases were observed in trans women with a follow-up time of more than 5 years.

To the best of our knowledge, there is only one other epidemiological study that, in addition to other types of cancer, also assessed testicular cancer incidence in trans women, reporting an incidence ratio of 0.3 (95% CI 0.1-0.6) compared to cis men.[127] However, in contrast to our study this proportional incidence study lacked data on GAHT and GAS. Since trans women are no longer at risk for testicular cancer after bilateral orchiectomy, we feel that with our longitudinal data we were able to accurately calculate follow-up time, and hereby, adequately assess the effect of GAHT on testicular cancer risk.

In total, five case reports have been published on testicular cancer cases in trans women. Histopathological analyses showed seminoma in three cases, non-seminoma (mature teratoma) in one case, and mixed germ cell tumor (embryonal carcinoma (75%), immature teratoma (15%), seminoma (9%), and yolk sac tumor (<1%)) in the last case. Similar to the cases observed in our cohort, two trans women were referred to urologists because they felt a painless scrotal mass which was, in one case, already present since several months.[43,45] In the other three cases, testicular pathology was only discovered after extensive examination, initiated when antiandrogenic treatment failed to suppress serum testosterone concentrations.[41,42,44] This illustrates how diagnosis of testicular cancer in trans women may be delayed when people experience severe genital dysphoria, and may ignore or be unaware of abnormalities such as a testicular mass. Besides, physicians might not be aware of the presence of testicles during a consultation with a phenotypical woman, which can also lead to a delayed diagnosis. Improving awareness on this topic is important to provide proper care for the increasing number of trans women who may not undergo genital gender-affirming surgery. In addition, it is imperative that health care providers are counseled on working in a trans sensitive manner.[128,129] Furthermore, in line with recommendations for cis men, trans women with clinical risk factors such as a family history of testicular cancer, should be encouraged to regularly perform testicular self-examination.[16,130]

Several studies addressed the influence of GAHT on testicular tissue and mainly showed severe spermatogenic involution, reduced numbers of Leydig cells, seminiferous tubules with a decreased diameter or an absent lumen, heavy peritubular hyalinization, and fibrosis.[29] Because of depletion of germ cells, testicular volumes have shown to generally decrease by 25% within the first year of hormonal treatment.[131] However, in previous studies, no malignant changes were observed in the orchiectomy specimens of trans

women obtained during GAS.[104,119] To the best of our knowledge this is the first reported case of testicular cancer discovered during histopathological analysis of an orchiectomy specimen, even though our center implemented routine histopathological analysis of orchiectomy specimens obtained during GAS approximately ten years ago. For trans women who underwent bilateral orchiectomy outside the Netherlands or before routine histopathological analysis was implemented, no histology results were available. Since testicular cancer was discovered in only 0.1% of the 722 analyzed orchiectomy specimens, one can argue if this routine histopathological analysis is necessary when there is no suspicion of testicular pathology.

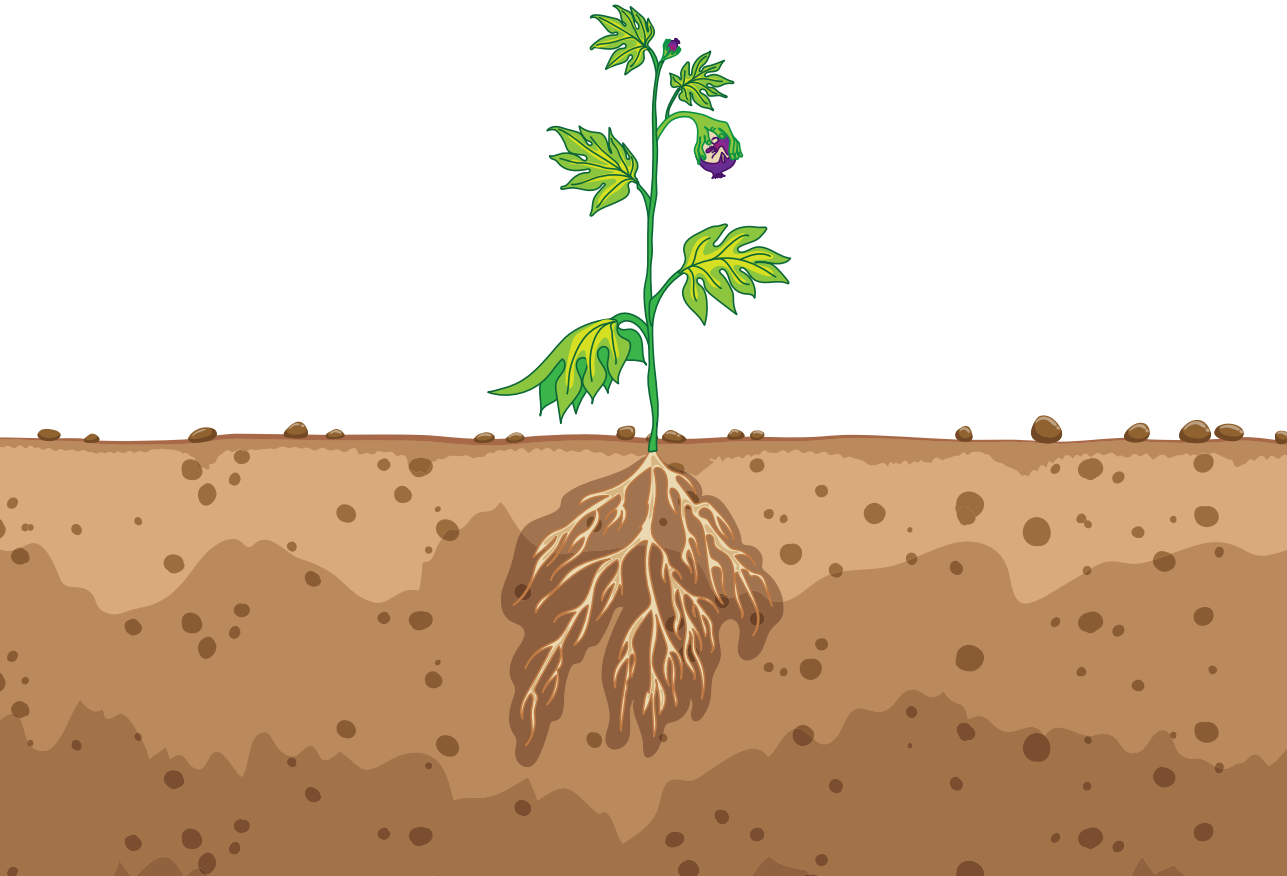
In literature, exposure to estrogens has been implicated as a risk factor for germ cell tumors. Studies in rodents have shown that exposure to high estrogen levels during either pre-natal or adult life induces testicular tumor formation, however it is unclear how such findings in animals apply to humans.[132] Several epidemiological studies have found a possible association between exposure to occupational and environmental estrogenic chemicals, such as pesticides and other endocrine disrupting agents, and increased testicular cancer risk but require further confirmation.[17-19,133] Also, studies have investigated the association between high maternal estrogen levels during the first trimester of pregnancy and the development of testicular cancer in offspring but failed to produce clear evidence for this estrogen excess hypothesis.[18,134] Limitations of these studies, however, include the indirect parameters that are used to assess the effect of estrogen exposure such as maternal age >30 years, being first-born, and twinning. We feel that, with our current study, we are able to more directly assess the influence of estrogens on testicular cancer risk, since the most profound difference between our cohort and cis men is the use of gender-affirming hormones. As we found that testicular cancer risk in trans women is not increased compared to cis men, our results do not support the hypothesis of a carcinogenic effect of post-natal exposure to exogenous estrogens on testicular tissue.

The major strengths of our study include the large cohort size consisting of young people with an age range in the peak incidence of testicular cancer. Also, follow-up time is adequately calculated by using the date that people were no longer at risk for testicular cancer or when they were last seen at our gender clinic. Furthermore, we were able to validate our data by linking our cohort to the Dutch national pathology database which has nationwide coverage.[126] Taken these factors into account, we feel that we are the first to report a reliable estimate of the testicular cancer risk in trans women.

A limitation of this study is that, despite the large cohort size, follow-up time is relatively short due to the fact that the majority of trans women decided to undergo bilateral orchiectomy directly after the required minimum of twelve months GAHT. Nonetheless, within the subgroup of 523 trans women using hormonal treatment for at least 5 years (median 8.9 years, range 5.0-52.1), no testicular cancer cases were observed, implying that a longer duration of hormonal treatment does not contribute to an increased testicular cancer risk. It might be worthwhile to repeat this study in ten years, to establish a larger

cohort size and a longer follow-up time, and hereby draw even more reliable conclusions on testicular cancer risk in trans women. Secondly, it was not possible to compare between GAHT protocols, since, on the one hand, many trans women change often between different types of prescribed estrogens over time and, on the other hand, they mostly use cyproterone acetate as antiandrogenic treatment. Furthermore, scrotal ultrasound to screen for the presence of testicular cancer was not routinely performed at initiation and during GAHT, but this was also not the case for the reference population, since guidelines advice against population-based screening.[16] Therefore, we do not expect that this affected our results.

In conclusion, this large nationwide cohort study in trans women using GAHT suggests that testicular cancer risk is comparable to the risk in cis men. Furthermore, results from our subgroup analysis in trans women with a long follow-up period, suggest that longer exogenous estrogen exposure does not increase the risk for the development of testicular cancer. This is reassuring for trans women who do not wish, or not have the option, to undergo genital gender-affirming surgery. However, awareness of the presence of the gonads remains important and regular testicular self-examination is recommended.



CHAPTER 8

PROSTATE CANCER INCIDENCE UNDER ANDROGEN DEPRIVATION: NATIONWIDE COHORT STUDY IN TRANS WOMEN RECEIVING HORMONE TREATMENT

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Published in Journal of Endocrinology and Metabolism; September 2020, 105(9):1–7

ABSTRACT

Context: Trans women (male sex assigned at birth, female gender identity) mostly use anti-androgens combined with estrogens and can subsequently undergo vaginoplasty including orchiectomy. Because the prostate remains in situ after this procedure, trans women are still at risk for prostate cancer.

Objective: The incidence of prostate cancer in trans women using hormone treatment.

Design: In this nationwide retrospective cohort study, data of participants were linked to the Dutch national pathology database (PALGA) and to Statistics Netherlands, to obtain data on prostate cancer diagnosis and mortality.

Setting: Gender identity clinic.

Participants: Trans women who visited our clinic between 1972 and 2016 and received hormone treatment were included.

Main Outcome Measures: Standardized incidence ratios (SIRs) were calculated using the number of observed prostate cancer cases in our cohort and the number of expected cases based on age-specific incidence numbers from the Netherlands Comprehensive Cancer Organization.

Results: The study population consisted of 2,281 trans women with a median follow-up time of 14 years (interquartile range 7-24), and a total follow-up time of 37,117 years. Six prostate cancer cases were identified after median 17 years of hormone treatment. This resulted in a lower prostate cancer risk in trans women compared to Dutch reference males (SIR 0.20, 95CI 0.08 to 0.42).

Conclusions: Trans women receiving androgen deprivation therapy and estrogens have a substantially lower risk for prostate cancer compared to the general male population. Our results support the hypothesis that androgen deprivation has a preventive effect on the initiation and development of prostate cancer.

INTRODUCTION

Transgender people experience an incongruence between the sex assigned at birth and their experienced or expressed gender.[70] People assigned male at birth who identify as male are defined as cis men, and those who identify as female are defined as trans women. In the Netherlands, the prevalence of gender dysphoria in birth-assigned males is approximately 1 in 2,800.[71] Transgender people may choose medical treatment to align their physical characteristics with their experienced gender, including gender-affirming hormone treatment and gender-affirming surgery. Hormone treatment for trans women consists of anti-androgens combined with estrogens.[2] Although gender-affirming hormone treatment is generally considered safe, there may be risks and side effects, such as thromboembolic events or the development of sex hormone-related cancers (e.g. breast cancer).[4,135,136]

Gender-affirming surgery involves bilateral orchiectomy, often combined with vaginoplasty and sometimes with breastaugmentation.[1] Although the prostate is biologically a male organ, prostatectomy is not performed during gender-affirming surgery because of the potential significant complications, such as incontinence. Therefore, trans women remain at risk for prostatic diseases after this procedure. It has been assumed that sex hormones, and androgens in particular, are involved in the pathogenesis of prostate cancer, because of the physiological dependency of prostate cells on androgens for functioning and proliferation.[22,23] In metastasized or advanced prostate cancer, androgen deprivation therapy is used to slow the progression of the disease.[24] However, a large meta-analysis showed no association between endogenous serum testosterone levels and prostate cancer incidence nor did it show an increased prostate cancer risk in hypogonadal men using testosterone replacement therapy.[25]

Androgen deprivation therapy in cis men is primarily used in patients diagnosed with advanced prostate cancer and only sporadically for other indications, such as to control sex impulses in patients with severe paraphilias.[46] Therefore, there is currently very limited data available about a potential preventive effect of long-term androgen deprivation on the occurrence of prostate cancer.

The primary aim of this study was to investigate the incidence of prostate cancer in trans women receiving androgen deprivation therapy and estrogens. Furthermore, this study gives a unique opportunity to study the potential preventive effect of androgen deprivation on the initiation and development of prostate cancer in general.

METHODS

Study design and data collection

For this retrospective cohort study, data on subjects were obtained from their medical files including medical history, age at the start of hormone treatment, documented hormone use, and data on gender-affirming surgery. This database was linked to Nationwide Network

and Registry of Histopathology and Cytopathology in the Netherlands (PALGA) to obtain data regarding prostate cancer histology and the date of prostate cancer diagnosis.[137] Data with regard to mortality were obtained from Statistics Netherlands (CBS) to calculate follow-up time.[138]

The study protocol was assessed by the Ethical Review Board of the VU University Medical Centre Amsterdam. It was concluded that the Medical Research Involving Human Subjects Act (WMO) did not apply to this study, and necessity for informed consent was waived because of the retrospective design and the large study population. Transgender people or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

Study population

All individuals who visited the gender identity clinic of the Amsterdam UMC between 1972 and 2016 were identified. This cohort has been previously described as the Amsterdam Cohort of Gender Dysphoria.[71] For this study, only trans women who received hormone treatment were included. People who never used hormone treatment, of whom start dates of hormone treatment were unknown, who were under 18 years of age at the time of the study, or who used female and male hormones alternatingly, were excluded. Since data on prostate cancer diagnosis were obtained from PALGA, which covers histopathologic diagnoses since 1991, people were also excluded when their last visit to our clinic was before 1991.[137]

The prescribed hormone treatment for trans women generally consisted of a combination of anti-androgens and estrogens. In our cohort, the most commonly prescribed medication to achieve androgen deprivation was cyproterone acetate, only sporadically spironolactone was used. People were advised to discontinue anti-androgenic treatment after bilateral orchiectomy. Types of prescribed estrogens included oestradiol valerate, oestradiol patches, oestradiol gel, ethinyl oestradiol, conjugated estrogens, oestradiol implants, and oestradiol injections. From 2001 onward, mainly oestradiol valerate, oestradiol patches, or oestradiol gel were used. People who were younger than 18 years when they started hormone treatment, had often only used a gonadotropin-releasing hormone agonist, i.e. triptorelin, prior to the start with estrogens.

Statistical analysis

Characteristics of the cohort are expressed as means with standard deviations (SD) when normally distributed, and as medians with interquartile ranges (IQR) when non-normally distributed. When the cohort consists of less than ten individuals, ranges are given instead of interquartile ranges. To calculate the incidence rate for prostate cancer in our cohort, follow-up time was defined as years from the start date of hormone treatment until either prostate cancer diagnosis, date of death, or the end of the study period (January 25, 2019). To calculate age-adjusted Standardized Incidence Ratios (SIRs), we used the observed cases of prostate cancer and the expected cases based on age-specific incidence

rates obtained from the Netherlands Comprehensive Cancer Organization (IKNL).[139] Since IKNL generates prostate cancer incidence rates using data from the same source (PALGA), this allows for a reliable comparison. SIRs with 95% confidence intervals (95%CI) were calculated using a Mid-exact P test. Sub-group analyses were performed for trans women who underwent orchiectomy, and for those who did not. For these analyses, follow-up time of people who underwent orchiectomy was calculated from the date of surgery until one of the previously mentioned terminating events. Lastly, SIRs (95%CI) were calculated for different age categories.

STATA Statistical Software, version 14.1 (Statacorp, College Station, Texas, USA) and OpenEpi version 3.01 (www.OpenEpi.com) were used for statistical analyses.

RESULTS

A total of 6,793 individuals were identified, of whom 4,432 were birth-assigned males and 2,361 were birth-assigned females. After applying the inclusion and exclusion criteria, 2,281 trans women were included in this study (Figure 1). The median age at start of hormone treatment was 31 years (IQR 23-41). The median follow-up time was 14 years (IQR 7-24) per person and the total follow-up time of the entire cohort was 37,117 years. Table 1 shows the characteristics of the entire study cohort.

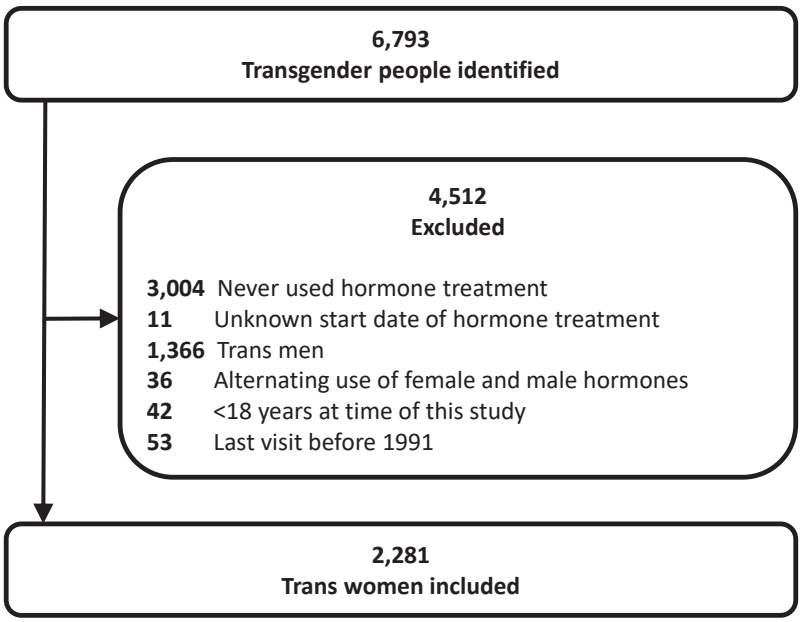


Figure 1. Study flowchart.

Table 1. Characteristics of study cohort.

	Total cohort (n=2,281)
Age at time of study (years)	50 (37-59)
Age at start of hormonal treatment (years)	31 (23-41)
Body Mass Index (kg/m²)	22.7 (20.5-25.6)^
% Caucasian ethnicity	96.7 (n=1579) ˜
% (former) smokers	39.0 (n=890)
% orchiectomy	68.9 (n=1572)
Follow-up time (years)	14 (7-24)
Total follow-up time (years)	37,117

Data available for: ^1,357 and ˜1,633 people of cohort.
Values are medians (interquartile ranges) unless stated otherwise.

Six people were diagnosed with prostate cancer, after median 17 years (range 10-24) of hormone treatment. The six trans women with prostate cancer had started hormone treatment at a median age of 47 years (range 38-58). Four of these six individuals had undergone orchiectomy, median 11 years (range 2-14) prior to the prostate cancer diagnosis. The median age at time of diagnosis was 64 years (range 53-77).

Histology reports of the six prostate cancer cases in our cohort showed adenocarcinoma in all cases. Gleason scores, the recommended prostate cancer grading system, were available for five trans women.[140] In all cases, there was at least one biopsy with a Gleason score of 7 or higher, suggesting a tumor with intermediate risk or higher. With a median of 18 ng/mL (range 5-1,722), serum levels of prostate specific antigen (PSA) were elevated at time of diagnosis in all cases.

Based on age-specific incidence rates, the number of expected prostate cancer cases in our cohort was 30. Since only six cases were observed in our cohort (16.2 cases per 100,000 years), the prostate cancer risk was considerably lower compared to Dutch cis males (SIR 0.20, 95%CI 0.08-0.42). This preventive effect holds in a subgroup analysis of trans women who underwent orchiectomy as part of their gender-affirming treatment (SIR 0.17, 95%CI 0.05-0.40, Table 2).

Table 2. Standardized Incidence Ratios for prostate cancer in trans women using hormone treatment

	Follow up time (years)	Observed cases	Dutch incidence rates (per 100,000 persons, per year)[139]	Expected cases	Standardized Incidence Ratio (95% confidence interval)
Age categories:					
<30 years	7,190	0	0	0	..
30-44 years	14,800	0	0.32	0	..
45-59 years	13,429	1	58.56	7	0.14 (0.01-0.70)
60-74 years	4,428	4	490.54	20	0.20 (0.06-0.48)
>75 years	535	1	567.68	3	0.33 (0.02-1.64)
Overall (n=2,281)	37,117	6		30	0.20 (0.08-0.42)
Subgroup analyses:					
Hormone treatment with orchiectomy (n=1572)	26,048	4	..	24	0.17 (0.05-0.40)
Hormone treatment without orchiectomy (n=709)	6,796	2	..	5	0.44 (0.07-1.47)

We were unable to perform analyses on different hormone treatment protocols, since on the one hand, many trans women change often between different types of prescribed estrogens over time and, on the other hand, they mostly use cyproterone acetate as anti-androgenic treatment.

DISCUSSION

Our study shows a five-fold decrease in prostate cancer risk in trans women using hormone treatment compared to the general male population of similar age. This observation provides new insight in the relationship between testosterone and prostate cancer risk. Where previously no association was found between serum testosterone concentrations and the incidence of prostate cancer, our results show that very low serum testosterone concentrations have a substantial preventive effect on the initiation and development of prostate cancer.

In this study, we linked the cohort of trans women with nationwide registries on prostate cancer and mortality. Therefore, we feel that the incidence we found of 16.2 prostate cancer cases per 100,000 years is a reliable estimate. In 2014, Gooren and Morgentaler reported an incidence of 2.0 prostate cancer cases per 100,000 person years in trans women.[141] This is lower than the reported incidence in our current study and is likely due to the absence of information on prostate cancer cases diagnosed in other centers and the lack of mortality data leading to an overestimation of follow-up time. Another cohort study showed a much higher incidence of 72 prostate cancer cases per 100,000 person years for trans women.[142] However, the higher incidence of prostate cancer in this American cohort may be explained by the fact that 38% of their study population consisted of trans women who had not undergone gender-affirming hormone treatment and were, therefore, not androgen deprived.[143]

Our study is not only relevant for health management of trans women. It also provides a unique insight into the relationship between serum testosterone levels and the occurrence of prostate cancer. A paradox in the current knowledge is that on the one hand, androgen deprivation slows the progression of metastasized or advanced prostate cancer, while on the other hand, higher endogenous serum testosterone or elevating testosterone concentrations in hypogonadal men do not increase prostate cancer risk.[24,25] A proposed theory suggests that the relationship between androgens and prostate cancer follows a saturation curve, as applies to the relation between PSA and testosterone levels.[144] This model states that if all androgen receptors are bound, a further increase of serum androgen concentrations produces no additional biological effects. Below this point of saturation, androgen concentration serves as the rate-limiting step in prostate tissue proliferation.[144] Hereby, the saturation model accounts for the slowed progression of prostate cancer by suppression of androgens below castration levels. As total testosterone concentrations in trans women receiving hormone treatment are generally below 1.7 nmol/L during hormone treatment, the observed low incidence implies that the saturation model might also apply to the initiation and development of prostate cancer.[2]

We also have to take the role of estrogen treatment into account which is used by these individuals. One might argue that the incidence of prostate cancer is not only influenced by androgen deprivation but also by the use of estrogens. Estrogens contribute to androgen deprivation through suppression of the hypothalamic-pituitary-gonadal axis and were initially used in prostate cancer treatment before the implementation of luteinizing hormone-releasing hormone agonists.[24] However, some theories also suggest a possible stimulating role of estrogens in the pathogenesis of prostate cancer.[145,146] Estrogen receptors are expressed in the human prostate. With respect to cell-autonomous actions of estrogens in prostate tissue, both pro- and anti-proliferative effects have been reported in the literature.[147] The estrogen receptor alpha has been shown to stimulate prostate cancer growth in preclinical models. Conversely, loss of expression of the estrogen receptor beta has been reported in prostate cancer tissues, implying a role as a tumor suppressor.[146] The exact role of estrogens in the pathogenesis of prostate cancer and its effect on the occurrence of the disease remains unclear and might be an interesting topic for future research.

In our cohort, six trans women developed prostate cancer while receiving hormone treatment. It may have been possible that these six individuals harbored small foci of subclinical prostate cancer prior to the start of hormone treatment. Autopsy studies in healthy subjects who died of trauma, have shown microscopic foci of prostate cancer and high-grade prostatic intraepithelial neoplasia from the third decade of life onwards.[148-150] The lesions become more frequent and extensive as age increases. This complements our observation that trans women with prostate cancer had a median age of 47 years at start of hormone treatment. Trans women in previously reported cases of prostate cancer also started hormone treatment at older age.[151] Possibly, androgen deprivation slowed further progression of these carcinogenic foci for many years. The mechanisms of how these foci eventually might have evolved into prostate cancer, despite androgen deprivation, are perhaps similar to proposed mechanisms of the transition from hormone-sensitive to

castration-resistant prostate cancer. Different theories suggest that castration-resistant prostate cancers have developed mechanisms that enable them to use steroids from the circulation more efficiently through *de novo* androgen synthesis, changed function of the androgen receptor, or even through estrogen receptor signaling pathways.[145,146,152]

European guidelines advice against systematic population-based PSA screening for prostate cancer, since it does not increase survival and causes overtreatment.[153] Following these guidelines, routine population-based PSA screening, in both cis men and trans women, is not performed in the Netherlands. PSA testing is only recommended in people with an elevated risk of prostate cancer after counselling on the potential risks and benefits.[21] Given the low incidence of prostate cancer and lack of PSA reference values in this population, there is even less reason to perform routine screening in trans women. However, it remains important that trans women and their health care providers are aware of the presence of the prostate and the possibility of the development of prostate cancer despite low serum androgen levels.

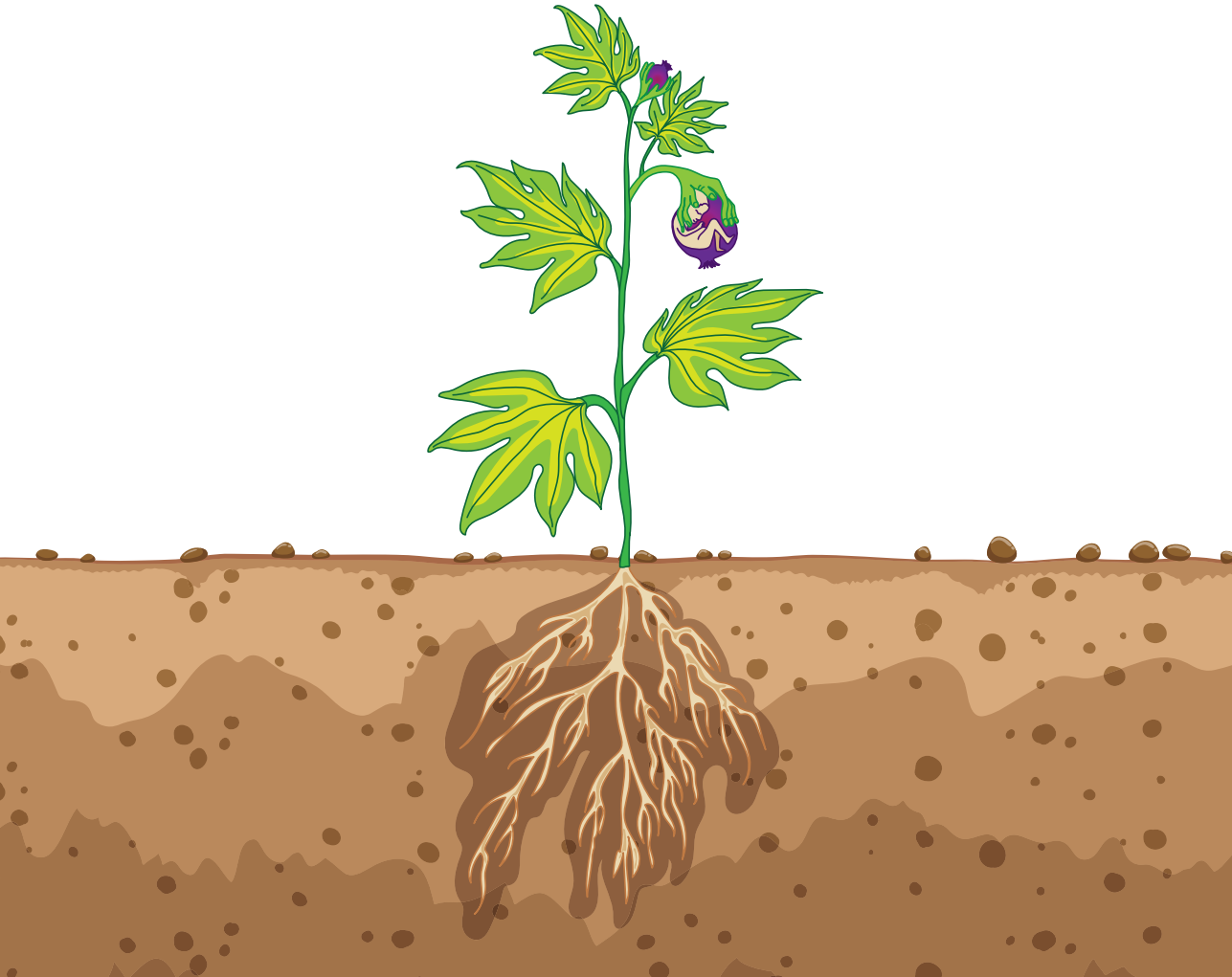
This study provides novel insights into prostate cancer risk in trans women receiving androgen deprivation and estrogen treatment. The major strengths of our study include the large cohort size consisting of people with a wide age range and the validation of our data by linking our cohort to the Dutch national pathology database. Also, our study has a long follow-up period which is adequately calculated using data on mortality from Statistics Netherlands. Furthermore, since the current practice for prostate cancer screening in the Netherlands is the same for cis men and trans women, i.e. no routine PSA testing, the role of detection bias in this study seems to be very limited.

A limitation of this study is the lack of information about the clinical symptoms of the six trans women with prostate cancer that led to the diagnosis, since the majority were diagnosed and treated in other hospitals than our clinic. Furthermore, information about family history, hormone use, and lifestyle, was missing or incomplete due to the retrospective design of our study. In particular, data on family history would have provided more insight into the genetic susceptibility for prostate cancer, which is known to be an important risk factor.[154] Although the influence of these risk factors on prostate cancer should not be underestimated, the most profound difference between our cohort and the reference population is gender-affirming treatment with anti-androgens and estrogens and eventually orchiectomy.

For future studies, it would be worthwhile to investigate the clinical symptoms in trans women leading to prostate cancer diagnosis. Further research is needed to understand the pathogenesis of prostate cancer in trans women receiving hormone treatment and how this influences treatment outcome compared to cis males.

CONCLUSIONS

In conclusion, this large nationwide cohort study in trans women receiving hormone treatment showed a five-fold decrease in prostate cancer risk compared to the general male population of similar age. This observation confirms our hypothesis that androgen deprivation has a preventive effect on the initiation and development of prostate cancer in general. Although the risk is much lower in trans women, prostate cancer in this population still occurs. Trans women, their general practitioners, and other health care providers should be aware of the possibility of the development of prostate cancer despite low serum androgen levels.



CHAPTER 9

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

This final chapter summarizes and discusses the findings of the work presented in this thesis. In addition, the strengths and limitations of this thesis will be discussed as well as the implications for clinical practice and future research. This thesis focused on andrological care in transgender women and aimed to obtain insight in topics such as the importance of fertility and family building, options for fertility preservation and the influence of GAHT on testicular and prostate tissue.

SUMMARY OF MAIN FINDINGS

In **chapter 2** we discussed the results of a survey study we conducted among transgender people of reproductive age (±30 year) who initiated treatment for gender dysphoria during adolescence, before 2014, and as a consequence were posed with the inevitable choice of giving up the possibility of genetic parenthood for legal gender recognition. Almost all participants of our study underwent gonadectomy before the abolition of this law, and as a result became permanently infertile. We found that, for various reasons, none of the participants pursued fertility preservation prior to initiation of treatment, but a substantial percentage indicated that, in retrospect, they would have wanted to do so. Moreover, 1 out of 6 would have wanted to keep their gonads. The majority of the study population reported to currently have a desire for children, to desire children in the future, or to have children. Furthermore, many participants advised adolescents who are currently in the process of starting treatment, to pursue fertility preservation and hereby keep all options for future family building open.

The preferred method for fertility preservation in trans women is cryopreservation of spermatozoa, obtained from a semen sample, prior to initiation of medical treatment. As described in **chapter 3**, we found that at time of semen cryopreservation, semen quality in trans women is significantly decreased compared to WHO data on semen quality in the general population. Furthermore, the vast majority of semen samples were only suitable for invasive and expensive reproductive techniques (IVF/ICSI) to establish a pregnancy in the future. In order to find an explanation for the impaired semen quality in our study population, we assessed the influence of factors known to have a negative impact on semen quality in the general population, such as demographic factors and lifestyle. Although smoking and a higher age at time of fertility preservation were found to correlate with an impaired progressive motility, it was insufficient to explain the overall decreased semen quality in this cohort.

Since it is often assumed that habitual behavior more typically observed in trans women (e.g. tucking, wearing tight undergarments, and a low ejaculation frequency) is explanatory for the impaired semen quality, we decided to conduct a consecutive study with prospectively obtained data on these lifestyle factors. In **chapter 4**, the results of this study are described. Semen quality was also impaired but no negative impact of age, BMI, smoking, alcohol consumption, cannabis use and medical history on the semen parameters was observed. However, when we assessed the influence of transgender specific lifestyle, it was found that approximately half of the study population reported to sometimes or always wear tight undergarments and that always wearing tight undergarments was associated with

having a total motile sperm count below 5 million. In addition, tucking was performed by 1 out of 4 trans women. In those with a tucking frequency of more than 8 times a month an association with low total motile sperm count was found, which was independent of demographic factors, lifestyle factors and medical history.

Although it is recommended to pursue fertility preservation before initiation of GAHT, not for everybody fertility preservation is available prior to the start of gender-affirming treatment. Besides, some trans women choose to keep the male gonads and discontinue gender-affirming medication when an active desire for children emerges. The use of GAHT negatively impacts spermatogenesis, resulting in a severely impaired semen quality or azoospermia.[72] The aim of the study described in **chapter 5,** was to determine whether loss of spermatogenesis can be reversed after GAHT is ceased. We included nine trans women, each of whom stopped GAHT for reproductive purposes, and we assessed their subsequent ability to produce sperm. Four participants stopped GAHT to conceive with their current partners; the remaining five wished to bank sperm to conceive in the future. It was found that after cessation of GAHT, serum testosterone levels returned to within the male reference range and in all nine individuals viable spermatozoa were found (3-27 months after cessation of GAHT). Three of the four trans women who stopped GAHT to naturally conceive with their current partners successfully did so after 4, 20 and 40 months. These results strongly suggest that the negative impact of GAHT on spermatogenesis can be reversed and may create an opportunity for those who develop a desire for children while already using GAHT. However, it may be difficult to predict how much time is needed for complete recovery of spermatogenesis since in some cases it took many months, during which time testosterone levels increased and are likely to have had negative physical and psychological consequences. Therefore, cessation of GAHT for reproductive purposes is not a feasible option for all trans women.

It may be especially difficult for trans women who initiated medical treatment in early puberty, as for them cessation of treatment is accompanied by irreversible and often unwanted physical changes such as a lowering of the voice and facial hair growth. Severe genital dysphoria may pose another barrier for fertility preservation, since semen cryopreservation requires masturbation which is non-negotiable for some young trans women.[54] In the study described in **chapter 6**, we assessed if there may still be options for fertility preservation in testicular tissue obtained during GAS, for those who are otherwise unable to have biological children. Outcome was compared between six subgroups, based on Tanner stage/age at start of medical treatment of cessation/continuation of GAHT prior to GAS. It was found that in a small percentage of trans women who initiated medical treatment in Tanner stage 4 or higher, spermatozoa could have been harvested from the orchiectomy specimen at time of GAS. In addition, the vast majority (> 85%) of trans women in our cohort could still opt for cryopreservation of testicular tissue harboring spermatogonial stem cells. We found that initiation of medical treatment in early-pubertal adolescents (Tanner stage 2-3) limits the ability to retrieve mature spermatozoa that can directly be used for assisted reproductive techniques, since in these orchiectomy specimens only immature germ cells were present. Lastly, we observed that testicular histology and spermatogenesis seemed more negatively

affected in those who initiated medical treatment as adults compared to those who initiated treatment during adolescence, despite the lower mean duration of medical treatment prior to GAS. A higher percentage of hyalinization of the seminiferous tubules was observed in the adult subgroup, as well as a complete absence of germ cells in 11% of orchiectomy specimens. The difference between the adult subgroup and the adolescent subgroups might be explained by age, lifestyle, higher dosages of estradiol, or the use of cyproterone acetate instead of GnRHa as testosterone suppressing therapy.

The use of GAHT may not only result in incomplete spermatogenesis and hyalinized seminiferous tubules, but might also affect testicular carcinogenesis. Specifically, a relative excess of exogenous estrogens during pre- or post-natal life (e.g. diethylstilbestrol, pesticides) is suggested to play a causal role in the development of testicular cancer.[17-19] To assess the safety of hormone treatment in terms of testicular cancer risk, we conducted a study to evaluate the incidence of testicular cancer in trans women using GAHT. As described in **chapter 7**, we observed a total of three testicular cancer cases in our cohort, of which two were discovered due to symptoms and the third was encountered during routine histopathological analysis of the bilateral orchiectomy specimen. Based on age-specific incidence rates in cis men, a similar amount of testicular cancer cases would have been expected, which suggest that testicular cancer risk in trans women is comparable to the risk in cis men. Furthermore, results from a subgroup analysis in trans women with a long follow-up period, suggest that longer exogenous estrogen exposure does not increase the risk for the development of testicular cancer.

The prostate is another part of the male genital tract that may be influenced by GAHT, since prostate cells are physiologically dependent on androgens for functioning and proliferation.[22,23] A paradox in the current knowledge is that, on the one hand, androgen deprivation slows the progression of metastasized or advanced prostate cancer, while, on the other hand, higher endogenous serum testosterone or elevating testosterone concentrations in hypogonadal men do not increase prostate cancer risk.[24,25] In trans women the prostate is not removed during gGAS because of the potential significant complications, such as incontinence. Therefore, they remain at risk for prostatic diseases after this procedure. In the study described in **chapter 8**, we assessed the prostate cancer incidence in trans women using GAHT and we hereby studied the potential preventive effect of androgen deprivation on the occurrence of prostate cancer. In line with our hypothesis, we found a 5-fold decrease in prostate cancer risk in trans women using GAHT compared with the general male population of similar age which confirms a preventive effect of androgen deprivation on the initiation and development of prostate cancer in general.

METHODOLOGICAL CONSIDERATIONS

The studies presented in this thesis have several strengths. In the majority of our studies we included large populations, especially in comparison with previous studies conducted on these topics. This allowed us to include multiple variables in the association models assessing the effect of certain factors on semen quality, and to perform subgroup analyses

in the cohort studies on the incidence of cancer in trans women. In addition, we were able to optimize our databases through collaborations with institutes such as the Nationwide Network and Registry of Histopathology and Cytopathology in the Netherlands (PALGA) and Statistics Netherlands (CBS) who provided us with reliable data on cancer diagnoses and mortality, and other departments such as the Reproductive Biology Laboratory who enabled the use of immunohistochemical markers to validate our findings regarding the most advanced germ cell types in testicular tissue.[137,138] Lastly, we experienced few missing data in the prospective cohort studies.

However, there are also some limitations. First, we did not include control groups in the studies on semen quality. Therefore, we could only assess the influence of demographics, lifestyle and medical history within the study population, but we were not able to assess if these factors were different from the general population. By comparing our data with data on lifestyle in the Dutch general population of similar age from CBS, and data on semen parameters in the general population of unscreened men from the World Health Organization (WHO), we aimed to overcome this limitation.[10,74] Another possible solution would have been to include a cis male control group who pursued fertility preservation for nonmedical reasons. Secondly, for both the survey study on the importance of fertility and family building, as well as for the case series on restoration of spermatogenesis following GAHT, it would have been valuable to also have a data on the study parameters before and (in the first years) during GAHT. This would allow for a more longitudinal observation of changes over time. Furthermore, for the study on the incidence of prostate cancer in trans women, we lacked information about the clinical symptoms that led to the diagnosis and the response to treatment, since the majority were diagnosed and treated in other hospitals than our clinic. This information would have been helpful to enhance early detection of prostate cancer in trans women and to provide recommendations regarding treatment in this population. Lastly, a limitation of the study on testicular cancer incidence in trans women is that, despite the large cohort size, follow-up time was relatively short due to the fact that the majority of trans women decided to undergo bilateral orchiectomy directly after the required minimum of twelve months GAHT.

FUTURE PERSPECTIVES

Implications for clinical practice

Andrological care is relatively new in the field of transgender medicine since, until 2014, strict transgender laws were in place which required a sterilization for legal gender recognition. Therefore, almost all trans women underwent gonadectomy, and reproductive wishes and the options for fertility preservation were hardly discussed. As a result, studies on family building, fertility (preservation) and the long term effects of GAHT on testicular tissue were scarce. Over the last couple of years the interest, and concomitant research output, in this field has increased substantially. Nowadays, there is a general consensus that it is essential to provide counseling about the effect of medical treatment on fertility and the currently available options for fertility preservation. Furthermore, the Ethics Committee

of the American Society for Reproductive Medicine states that fertility clinics should treat all requests for assisted reproduction without regard to gender identity status, since current data do not support concerns that children are harmed from being raised by transgender parents.[68] The results from our survey study, described in **chapter 2**, underline the importance of proper fertility counseling since the majority of transgender people developed a desire to have children and many would, in retrospect, have wanted to pursue fertility preservation or keep their biological gonads. Since several years, fertility counseling for adult transgender people is thoroughly embedded in the multidisciplinary approach of our gender identity clinic, and since last year the same has been implemented for people presenting during adolescence. As a result, the number of people pursuing fertility preservation has increased dramatically over the years.[110] However, since we found that semen quality is already decreased prior to the initiation of GAHT, the majority of cryopreserved samples can only be used for invasive and expensive assisted reproductive techniques to establish a future pregnancy (**chapter 3 and 4**). Therefore, it is very important to inform trans women about the influence of lifestyle on semen quality, specifically the negative impact of extensive tucking and wearing tight undergarments. Ideally, trans women should receive this information as early as possible, for example at time of referral to a gender identity clinic or during the diagnostic phase with the psychologist. In this way, trans women have enough time to adjust lifestyle in order to cryopreserve the best semen quality and hereby improve future reproductive options, without having to delay the start of GAHT. In addition, the option to keep the male gonads and, in a later stage, discontinue GAHT to naturally conceive with a female partner, should also be discussed with trans women. For those who can cope with a temporary cessation of GAHT, this is most likely the least invasive way to fulfill a desire for children and we observed positive results in trans women who have chosen this option in our study, described in **chapter 5**. However, health care providers should be aware of the physical and psychological consequences of increasing serum testosterone levels after cessation of GAHT, which may be very burdensome for trans women, and pay extra attention to their clients' wellbeing during this period.

The group of transgender people that is most challenging in terms of the options for fertility preservation, are trans female adolescents in early puberty. The combination of their physical immaturity (incomplete spermatogenesis) and psychosocial immaturity (a desire for children not being in their scope of vision) at start of puberty suppression poses serious challenges on providing the best possible fertility care. The equipoise of commencing medical treatment to avoid progression of puberty, and delaying treatment to enable semen cryopreservation as only option for biological children may be stressful, as puberty is accompanied by irreversible and often unwanted physical changes such as a lowering of the voice and facial hair growth. Our observation that up to 21% indicated to, in retrospect, have been too young at start of medical treatment to make proper decisions regarding fertility (**chapter 2**) raises complex questions on when and how to proceed with fertility impairing treatments. Internationally, there is increasing controversy over the provision of GAHT to adolescents, with the negative effect on fertility often cited as an argument for limiting adolescents' access to gender-affirming care.[108] However, since the majority felt that gender-affirming treatment was more important than staying fertile,

it seems even more harmful to deprive young transgender adolescents from treatment. Therefore, it is of utmost importance to find a way for fertility preservation for those who are unable to pursue semen cryopreservation prior to the initiation of treatment. The results of our study on testicular tissue obtained at time of GAS seem promising, since in a small percentage of trans women (who initiated treatment in Tanner stage 4 or higher) mature spermatozoa could have been harvested from the orchiectomy specimen (**chapter 6**). Furthermore, in all adolescents there were still germ cells present in the orchiectomy specimen. However, the downside of harvesting germ cells from testicular tissue is that such spermatozoa can only be used for invasive and expensive ICSI treatments, and the use of immature germ cells require techniques that are currently still experimental and far from the clinical realm. Therefore, cryopreservation of a semen sample prior to initiation of GAHT remains the preferred method of fertility preservation in transgender women, and harvesting germ cells from orchiectomy specimens might only be a considerable alternative in those for whom this is not an option.

Another interesting observation in the histological study on testicular tissue obtained at time of GAS was that seminiferous tubules and spermatogenesis seemed more negatively affected in trans women who initiated GAHT during adulthood (**chapter 6**). This may be related to the use of cyproterone acetate instead of GnRHa as testosterone suppressing therapy. Whereas GnRHa only leads to inhibition of gonadotropin secretion, cyproterone acetate also has progestative effects and acts as a direct antagonist of the androgen receptor. It hereby inhibits the influence of androgens on the androgen-dependent organs, among which the testes. The latter might have more profound and irreversible effects on testicular tissue. Because of unwanted side-effects of cyproterone acetate (e.g. increased risk for meningioma), transgender women commencing GAHT in our clinic above the age of 18 years now receive GnRHa as testosterone suppressing therapy instead of cyproterone acetate. The potential consequence of irreversible infertility might be an extra reason to not prescribe cyproterone acetate anymore.

Since several years, an increasing number of people with non-binary identities or less need to confirm to binary cis presentation, choose to keep their male gonads. Therefore, the results of our study assessing the influence of GAHT on testicular cancer risk become increasingly relevant (**chapter 7**). Our observation that testicular cancer risk in trans women is similar to the general population, and did not seem related to a longer period of exogenous estrogen exposure, is reassuring for trans women who do not wish to undergo genital GAS. Since guidelines advice against population-based screening for testicular cancer in cis males, and no increased risk exist in trans women, there seems to be no reason for extra precautions in the gender diverse population. However, awareness of the presence of the gonads remains important and regular testicular self-examination is recommended. Our findings also raise the question if it is still necessary to perform routine histopathological analysis of orchiectomy specimens when there is no suspicion of testicular pathology, since testicular cancer was discovered in only 0.1% of the 722 analyzed orchiectomy specimens. Especially, in cases where this tissue may be used for fertility preservation purposes instead.

Since the prostate is not removed during GAS, it is reassuring that we observed a 5-fold decrease in prostate cancer risk in trans women using GAHT (**chapter 8**). Currently, European guideline advice against population-based PSA screening, since it does not increase survival and causes overtreatment, and to only perform PSA testing in people with an elevated risk of prostate cancer after counselling on the potential risks and benefits.[21,153] However, it still remains one of the most controversial topics in the urological literature.[155] Given the low incidence of prostate cancer and lack of PSA reference values in trans women, there seems even less reason to perform routine screening in this population. However, it remains important that trans women and their healthcare providers are aware that the prostate is not removed during GAS and there is still a possibility of development of prostate cancer despite low serum androgen levels. In our gender identity clinic, all trans women with an elevated risk for prostate cancer (e.g. positive family history) are referred to a urologist to discuss the benefits and harms of PSA-based screening, as well as the lack of PSA reference values under androgen deprivation therapy.

Implications for future research

As already mentioned in the paragraph on methodological considerations, we identified several limitations of the current studies. Longitudinal data about the perspectives of transgender adolescents on family building and the importance of genetic parenthood are needed. Since nowadays, all transgender adolescents in our gender identity clinic receive fertility counseling from a fertility specialist prior to the start with puberty suppression as well as before initiation of GAHT, they may have different experiences than the participants of our survey study. For future studies it would be worthwhile to evaluate how adolescents experience this fertility counseling and how it affects decisional conflict and regret regarding reproductive choices.

In addition, studies should be conducted on how trans women would feel about fertility preservation options in orchiectomy specimens obtained during GAS and to identify facilitators and barriers for implementation of such techniques in clinical practice. Furthermore, it is important to assess how GAHT influences the quality of germ cells and the safety of using cells harvested from orchiectomy specimens for reproductive techniques, and to continue research on the feasibility of i*n vitro* spermatogenesis so that transgender adolescents, who are otherwise unable to have genetically-related children, will be able to retain this possibility by cryopreserving testicular tissue containing spermatogonial stem cells. When such techniques are actually implemented in clinical practice, it is important to asses live birth-rate, as well as long-term follow up of children born from these gametes.

Besides, more research is needed on how prostate cancer develops in trans women using GAHT. Since these tumors developed under androgen deprived conditions, underlying pathological mechanism are perhaps similar to proposed mechanisms of the transition from hormone-sensitive to castration-resistant prostate cancer, which might have implications for the treatment options and outcomes in trans women. To gain more knowledge on this topic, it would be interesting to investigate the clinical symptoms in trans women leading to prostate cancer diagnosis, as well as the receptor status in prostate cancer tissue of trans women.

Lastly, it might be worthwhile to repeat the cohort studies on cancer incidence in ten years, to establish a larger cohort size and a longer follow-up time, and hereby draw even more reliable conclusions on testicular and prostate cancer risk in trans women.

CONCLUSIONS

Overall, the results of this thesis are reassuring for trans women. We identified life style factors that negatively influence semen quality in trans women, which enables optimization of fertility counseling on how to adjust this lifestyle prior to semen cryopreservation. Furthermore, we found that there may still be options for fertility preservation after initiation of GAHT, either by a temporary cessation of GAHT, or by harvesting germ cells from testicular tissue obtained during GAS. Lastly, we observed no increased risk for testicular and prostate cancer in trans women using GAHT. Taken all these results into account, the perspectives for (new) life after transition seem positive.

APPPENDICES

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LIST OF ABBREVIATIONS

AUTHOR AFFILIATIONS

SUMMARY IN DUTCH – NEDERLANDSE SAMENVATTING

PORTFOLIO

ABOUT THE AUTHOR

ACKNOWLEDGEMENTS – DANKWOORD

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LIST OF ABBREVIATIONS

GnRH	gonadotropin-releasing hormone
GnRHa	gonadotropin-releasing hormone agonists
GAHT	gender-affirming hormone treatment
GAS	gender-affirming surgery
gGAS	genital gender-affirming surgery
TESE	testicular sperm extraction
LH	luteinizing hormone
FSH	follicle stimulating hormone
TMSC	total motile sperm count
WHO	World Health Organization
IUI	intrauterine insemination
IVF	in vitro fertilization
ICSI	intracytoplasmic sperm injection
VUmc	VU University Medical Center
WMO	Medical Research Involving Human Subjects Act
OR	odds ratio
95% CI	95% confidence interval
SD	standard deviation
SIR	standardized incidence ratio
PALGA	Nationwide Network and Registry of Histopathology and Cytopathology in the Netherlands
CBS	Statistics Netherlands
IKNL	Netherlands Comprehensive Cancer Organization
PSA	prostate specific antigen

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SUMMARY IN DUTCH – NEDERLANDSE SAMENVATTING

Dit proefschrift gaat over de andrologische zorg voor transvrouwen. Andrologie is een medische discipline die zich bezighoudt met de anatomie, functies en aandoeningen van het mannelijk geslachtsorgaan. Transvrouwen zijn personen die het geslacht 'man' hebben toegewezen gekregen bij de geboorte, maar die zich identificeren als vrouw. Thema's die in dit proefschrift worden beschreven zijn kinderwens, vruchtbaarheid en het risico op het krijgen van kanker in het mannelijk geslachtsorgaan. Deze onderwerpen zijn relevant omdat transvrouwen ervoor kunnen kiezen om medische behandelingen te ondergaan om hun lichaam aan te passen aan hun gender identiteit. Ondanks dat deze behandelingen de kwaliteit van leven van transvrouwen verbeteren kunnen er ook negatieve kanten aan zitten, zoals het verlies van vruchtbaarheid en een verhoogd risico op sommige typen kanker (zoals borstkanker). Omdat er tot 2014 een wet van kracht was in Nederland die transgender personen verplichtte om onvruchtbaar te worden als zij wettelijk van geslacht wilden veranderen, is er tot die tijd weinig aandacht geweest voor deze onderwerpen. Met dit proefschrift willen we daar verandering in brengen en perspectieven bieden voor (nieuw) leven na transitie.

Medische behandelingen en hun invloed op het mannelijk geslachtsorgaan

De medische behandelingen voor transvrouwen bestaan uit hormoonbehandeling en operaties. Voor personen jonger dan 18 jaar worden deze behandelingen voorafgegaan door een periode van puberteitsremming, waarbij verdere vermannelijking door de puberteit wordt tegengegaan door de eigen hormoonproductie te blokkeren. Als adolescenten 16 jaar of ouder zijn en tenminste zes maanden puberteitsremmers hebben gebruikt kan de behandeling worden aangevuld met hormonen. De hormoonbehandeling voor transvrouwen bestaat uit een combinatie van anti-mannelijke hormonen (anti-androgenen) en vrouwelijke hormonen (oestrogenen). Effecten van de hormoonbehandeling zijn onder andere de ontwikkeling van borstgroei, het krijgen van een meer vrouwelijke vetverdeling en een afname van (gezichts-)beharing. Na tenminste één jaar hormoonbehandeling te hebben gehad, kunnen transvrouwen ervoor kiezen om operaties te ondergaan. Verschillende typen operaties voor transvrouwen zijn onder andere; het verwijderen van de zaadballen (orchidectomie), vaak in combinatie met het creëren van schaamlippen en een vaginaholte (vaginaplastiek), een vervrouwelijkende gezichtsoperatie en een borstvergroting.

Blokkade van de productie van mannelijke hormonen (androgenen) heeft een negatieve invloed op de aanmaak van zaadcellen, hierdoor is het grootste deel van de transvrouwen gedurende de hormoonbehandeling onvruchtbaar. Nadat zij een orchidectomie hebben ondergaan zijn transvrouwen permanent onvruchtbaar. Daarom worden transvrouwen met een kinderwens geadviseerd om hun zaadcellen in te vriezen voor eventueel toekomstig gebruik. Verder weten we dat hormoonbehandeling invloed heeft op zaadbalweefsel en de prostaat. Eerder onderzoek liet zien dat het volume van deze organen afneemt gedurende de hormoonbehandeling en bestaan er theorieën over een rol van de geslachtshormonen in het ontstaan van kanker in de zaadballen en de prostaat.

Belangrijkste bevindingen per hoofdstuk

In **hoofdstuk 2** worden de resultaten besproken van een enquêteonderzoek dat we uitvoerden onder transgender personen in de reproductieve leeftijd (±30 jaar) die medische behandelingen startten tijdens hun adolescentie (<21 jaar), vóór 2014, en als gevolg daarvan voor de onvermijdelijke keuze werden gesteld om de mogelijkheid van genetisch ouderschap op te geven voor wettelijke geslachtserkenning. Bijna alle deelnemers van onze studie lieten hun geslachtsorganen verwijderen vóór de afschaffing van deze wet, en werden als gevolg daarvan permanent onvruchtbaar. Wij stelden vast dat, om uiteenlopende redenen, geen van de deelnemers koos voor vruchtbaarheidsbehoud voor de start van medische behandelingen, maar een aanzienlijk percentage gaf aan dat, achteraf gezien, zij dit wel hadden willen doen. Bovendien zou 1 op de 6 de geslachtsorganen hebben willen behouden. De meerderheid van de onderzoekspopulatie gaf aan op dit moment een kinderwens te hebben, in de toekomst kinderen te willen, of kinderen te hebben. Bovendien adviseerden veel deelnemers aan adolescenten die zich momenteel in het behandelingsproces bevinden, om te kiezen voor vruchtbaarheidsbehoud en hierbij alle opties voor toekomstige gezinsvorming open te houden.

De voorkeursmethode voor vruchtbaarheidsbehoud bij transvrouwen is het invriezen van zaadcellen, verkregen uit een zaadlozing, vóór het begin van een medische behandeling. Zoals beschreven in **hoofdstuk 3**, vonden we dat op het moment van invriezen de zaadkwaliteit bij transvrouwen aanzienlijk verminderd was ten opzichte van de zaadkwaliteit in de algemene bevolking. Bovendien was de overgrote meerderheid van de spermamonsters alleen geschikt voor invasieve en dure voortplantingstechnieken om daarmee in de toekomst een zwangerschap tot stand te brengen. Om een verklaring te vinden voor de verminderde zaadkwaliteit in onze studiepopulatie, onderzochten we de invloed van factoren waarvan bekend is dat ze een negatieve invloed hebben op de zaadkwaliteit in de algemene bevolking, zoals demografische factoren en leefstijl. Hoewel roken en een hogere leeftijd op het moment van invriezen geassocieerd bleken met een verminderde progressieve beweeglijkheid, was dit onvoldoende om de algehele verminderde zaadkwaliteit in dit cohort te verklaren.

Omdat vaak wordt aangenomen dat bepaalde gewoonten die meer typisch zijn voor transvrouwen (bijv. tucken (het wegstoppen van de zaadballen in het lieskanaal), het dragen van strak ondergoed, en een lage ejaculatiefrequentie) verklarend zijn voor de verminderde zaadkwaliteit, besloten we een vervolg studie uit te voeren met prospectief verkregen gegevens over deze leefstijlfactoren. In **hoofdstuk 4** worden de resultaten van deze studie beschreven. De zaadkwaliteit was ook in deze studie verminderd, maar er werd geen negatieve invloed van leeftijd, BMI, roken, alcoholconsumptie, cannabisgebruik en medische voorgeschiedenis op de zaadkwaliteit waargenomen. Toen we echter de invloed van transgenderspecifieke leefstijl analyseerden, bleek dat ongeveer de helft van de studiepopulatie aangaf soms, of altijd, strak ondergoed te dragen en dat het altijd dragen van strak ondergoed geassocieerd was met het hebben van een VCM van minder dan 5 miljoen zaadcellen. Bovendien gaf 1 op de 4 transvrouwen aan te tucken. Bij degenen die aangaven meer dan 8 keer per maand te tucken werd een associatie met een lage VCM gevonden, die onafhankelijk was van demografische factoren, leefstijlfactoren en medische voorgeschiedenis.

Hoewel het wordt aanbevolen aan transvrouwen om vruchtbaarheidsbehoud na te streven vóór het begin van de hormoonbehandeling, is dit op dat moment soms niet voor iedereen beschikbaar. Bovendien kiezen sommige transvrouwen ervoor om hun zaadballen te behouden en te stoppen met de hormoonbehandeling wanneer er een actieve kinderwens ontstaat. De hormoonbehandeling heeft een negatieve invloed op de zaadcelproductie, wat resulteert in een sterk verminderde zaadkwaliteit of een afwezigheid van zaadcellen in de zaadlozing. Het doel van de studie beschreven in **hoofdstuk 5** was om te bepalen of verlies van zaadcelproductie kan worden teruggedraaid na het stoppen van de hormoonbehandeling. We includeerden negen transvrouwen, die elk hun hormoonbehandeling stopten voor reproductieve doeleinden, en we beoordeelden hun daaropvolgende vermogen om zaadcellen te produceren. Vier deelnemers stopten met hun hormoonbehandeling om een spontane zwangerschap na te streven bij hun vrouwelijke partner; de overige vijf wilden zaadcellen invriezen voor de toekomst. Na het stoppen van de hormoonbehandeling bleken de testosteronwaarden in het bloed weer terug te keren naar binnen het mannelijke referentiebereik en bij alle negen deelnemers werden beweeglijke zaadcellen gevonden (3-27 maanden na het stoppen van de hormoonbehandeling). Drie van de vier transvrouwen die met hun hormonen stopten om hun partner op natuurlijke wijze zwanger te maken, slaagden daar na 4, 20 en 40 maanden in. Deze resultaten wijzen er sterk op dat de negatieve invloed van de hormoonbehandeling op de zaadcelproductie kan worden omgekeerd en een kans kan bieden aan degenen die een kinderwens ontwikkelen terwijl ze al hormonen gebruiken. Het is echter moeilijk te voorspellen hoeveel tijd nodig is voor een volledig herstel van de zaadcelproductie, aangezien het in sommige gevallen vele maanden duurde, gedurende welke tijd de testosteronspiegel steeg en waarschijnlijk negatieve lichamelijke en psychologische gevolgen heeft gehad. Daarom is het staken van de hormoonbehandeling voor reproductieve doeleinden niet voor alle transvrouwen een haalbare optie.

Het kan vooral moeilijk zijn voor transvrouwen die in de vroege puberteit met de medische behandeling zijn begonnen, omdat voor hen het staken van de behandeling gepaard gaat met onomkeerbare en vaak ongewenste lichamelijke veranderingen, zoals een verlaging van de stem en de ontwikkeling van gezichtsbeharing. Ernstige genitale dysforie kan een andere belemmering vormen voor vruchtbaarheidsbehoud, aangezien voor het opwekken van een zaadlozing masturbatie vereist is wat voor sommige jonge transvrouwen onbespreekbaar is. In de studie beschreven in **hoofdstuk 6**, onderzochten we of er nog steeds mogelijkheden zijn voor vruchtbaarheidsbehoud in zaadbalweefsel verkregen tijdens genderbevestigende genitale chirurgie, voor diegenen die anders niet in staat zijn om biologische kinderen te krijgen. De resultaten werden vergeleken tussen zes subgroepen, gebaseerd op het Tanner-stadium en leeftijd bij aanvang van de medische behandeling en het staken, danwel voortzetten van de hormoonbehandeling voorafgaand aan de genitale operatie. Er werd vastgesteld dat bij een klein percentage van de transvrouwen, die hun medische behandeling startten in Tanner stadium 4 of hoger, rijpe zaadcellen konden worden geoogst uit het zaadbalweefsel. Bovendien zou er voor de overgrote meerderheid (> 85%) van de transvrouwen in ons cohort nog de mogelijkheid bestaan voor het invriezen van zaadbalweefsel met daarin voorlopercellen (stamcellen)

van zaadcellen. Wij vonden dat het starten van medische behandeling bij vroeg-puberale adolescenten (Tanner stadium 2-3) de mogelijkheid beperkt om rijpe zaadcellen te verkrijgen die direct gebruikt kunnen worden voor geassisteerde voortplantingstechnieken, omdat in hun zaadbalweefsel alleen stamcellen aanwezig waren. Ten slotte zagen we dat het zaadbalweefsel en de zaadcelproductie negatiever leken te zijn beïnvloed bij degenen die als volwassene met medische behandeling waren begonnen dan bij degenen die tijdens de adolescentie met de behandeling waren begonnen, ondanks de gemiddeld kortere duur van de medische behandeling voorafgaand aan de genitale operatie. Het verschil tussen de volwassen subgroep en de adolescente subgroepen zou verklaard kunnen worden door leeftijd, leefstijl, hogere doseringen oestrogenen, of het gebruik van cyproteronacetaat in plaats van GnRHa als testosterononderdrukkende therapie.

Het gebruik van hormoonbehandeling kan, naast de hierboven beschreven effecten, ook van invloed zijn op de ontwikkeling van zaadbalkanker. Met name een relatieve overmaat aan oestrogenen zou een oorzakelijke rol kunnen spelen bij de ontwikkeling van zaadbalkanker. Om de veiligheid van hormoonbehandeling met betrekking tot het risico op zaadbalkanker te beoordelen, hebben wij een studie uitgevoerd om de incidentie van zaadbalkanker bij transvrouwen met hormoonbehandeling te evalueren. Zoals beschreven in **hoofdstuk 7**, hebben we in totaal drie gevallen van zaadbalkanker in ons cohort waargenomen, waarvan er twee werden ontdekt als gevolg van symptomen en de derde werd aangetroffen tijdens het gebruikelijke histopathologische weefselonderzoek na de geslachtsbevestigende genitale chirurgie. Op basis van leeftijdsspecifieke incidentiecijfers bij cismannen zou een vergelijkbaar aantal gevallen van zaadbalkanker zijn verwacht, wat suggereert dat het risico op zaadbalkanker bij transvrouwen vergelijkbaar is met het risico bij cismannen. Bovendien suggereren de resultaten van een subgroep analyse bij transvrouwen met een lange follow-up-periode dat een langere blootstelling aan oestrogenen het risico op de ontwikkeling van zaadbalkanker niet verhoogt.

De prostaat is een ander deel van het mannelijk geslachtsorgaan dat door hormoonbehandeling kan worden beïnvloed, aangezien prostaatcellen afhankelijk zijn van androgenen voor hun werking en groei. Een paradox in de huidige kennis is dat enerzijds androgeenonderdrukking de progressie van gemetastaseerde of gevorderde prostaatkanker vertraagt, terwijl anderzijds een verhoogde androgeenspiegel het risico op prostaatkanker niet doet toenemen. Bij transvrouwen wordt de prostaat niet verwijderd tijdens geslachtsbevestigende genitale chirurgie vanwege de mogelijke aanzienlijke complicaties, zoals ongewild urineverlies. Daarom blijven zij na deze procedure risico lopen op aandoeningen van de prostaat. In de studie beschreven in **hoofdstuk 8**, hebben we de incidentie van prostaatkanker bij transvrouwen met hormoonbehandeling onderzocht en we hebben hierbij het mogelijke preventieve effect van androgeenonderdrukking op het optreden van prostaatkanker bestudeerd. In overeenstemming met onze hypothese vonden we een 5-voudige daling van het prostaatkankerrisico bij transvrouwen die hormoonbehandeling gebruikten in vergelijking met de algemene mannelijke populatie van vergelijkbare leeftijd. Deze bevindingen bevestigen het preventieve effect van androgeenonderdrukking op de ontwikkeling van prostaatkanker in het algemeen.

Conclusies

Over het algemeen zijn de resultaten van dit proefschrift geruststellend voor transvrouwen. We identificeerden leefstijlfactoren die een negatieve invloed hebben op de zaadkwaliteit van transvrouwen, wat het mogelijk maakt om de counseling over vruchtbaarheid te optimaliseren met informatie over hoe zij hun leefstijl kunnen aanpassen voorafgaand aan het invriezen van zaadcellen. Bovendien stelden we vast dat er nog steeds opties zijn voor vruchtbaarheidsbehoud na het starten van de hormoonbehandeling, hetzij door het tijdelijk staken van de hormoonbehandeling, hetzij door het oogsten van (voorlopercellen van) zaadcellen uit zaadbalweefsel tijdens de geslachtsbevestigende genitale chirurgie. Ten slotte hebben we geen verhoogd risico op zaadbal- en prostaatkanker vastgesteld bij transvrouwen die hormoonbehandeling gebruiken. Al deze resultaten in aanmerking genomen, lijken de vooruitzichten voor (nieuw) leven na de transitie positief.

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PORTFOLIO

List of publications

I de Nie*, JD Asseler*, M Arnoldussen, S Baas, ALC de Vries, JAF Huirne, TD Steensma, M den Heijer, NM van Mello. *Reflecting on the importance of family building and fertility preservation: transgender people's experiences with starting gender-affirming treatment as adolescent.* Submitted.

I de Nie, NM van Mello, E Vlahakis, C Cooper, A Perie, M den Heijer, A Meißner, JAF Huirne, KC Pang. *Successful restoration of spermatogenesis following gender-affirming hormone therapy in transgender women*. Submitted.

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Presentations at (international) conferences and symposia

2021

Reflecting on the importance of family building and fertility preservation: transgender people's experiences with starting medical treatment as adolescent. Oral presentation as part of Symposium 'Evaluation of early medical treatment for gender dysphoria in adolescence: a long term follow-up study into adulthood'. European Professional Association for Transgender Health (EPATH), Götenborg, Sweden.

A prospective cohort study on factors impairing semen quality in transgender women. Oral presentation. European Professional Association for Transgender Health (EPATH), Götenborg, Sweden.

Tight undergarment and tucking impair semen quality: a prospective cohort study in trans women. Poster presentation and pitch. European Association of Urology (EAU), Virtual conference.

2020

Influence of transgender specific factors on semen quality. Oral presentation. World Professional Association for Transgender Health (WPATH), Virtual conference.

Prostate cancer incidence under androgen deprivation: a nationwide cohort study in trans women receiving hormone treatment. Poster presentation. European Association of Urology (EAU), Virtual conference.

Impaired semen quality in trans women: prevalence and determinants. Poster presentation. European Association of Urology (EAU), Virtual conference.

Prostate cancer incidence under androgen deprivation: a nationwide cohort study in trans women receiving hormone treatment. Oral presentation. Dutch Endocrine Meeting (DEM), Noordwijkerhout, The Netherlands.

2019

Impaired semen quality in trans women: prevalence and determinants. Poster presentation. International Society for Fertility Preservation (ISFP), New York, United States of America.

The incidence of prostate cancer in transwomen receiving hormonal treatment. Oral presentation. European Professional Association for Transgender Health (EPATH), Rome, Italy.

Prostate cancer risk in trans women receiving hormonal treatment. Oral presentation. Research Institute AG&M PhD retreat, Putten, The Netherlands.

2018

Do lifestyle factors influence semen quality in transwomen? Oral presentation. World Professional Association for Transgender Health (WPATH), Buenos Aires, Argentina.

Fertility preservation in transwomen, a retrospective database analysis. Research Institute AG&M PhD retreat, Putten, The Netherlands.

Courses and workshops

Scientific Integrity, 2020, Vrije Universiteit, Amsterdam, The Netherlands.
Regression techniques, 2020, EpidM, Amsterdam, The Netherlands.
Principles of epidemiological data analysis, 2019, EpidM, Amsterdam, The Netherlands.
Creative tools for scientific writing, 2019, Artesc, Delft, The Netherlands.
Writing a scientific article, 2018, Taalcentrum VU, Amsterdam, The Netherlands.
Workshop 'Male fertility preservation', 2018, European Society of Human Reproduction and Embryology, Amsterdam, The Netherlands.
BROK course, 2018, the Netherlands Federation of UMCs, Amsterdam, The Netherlands.

Other activities

Member of Clinical Network Gonadal Endocrinology, Dutch Society for Endocrinology, 2020-present
Amsterdam Urology Research Meetings (monthly), 2020-2021
Gender Endocrinology Research Meetings (GERO, monthly), 2018-2021
Research association Obstetrics and Gynecology Meetings (OVGO, monthly), 2018-2021
(Organization of) Genderteam Journal Club (quarterly), 2018-2021
Supervision of students writing a bachelor or master thesis, 2018-2021
Giving lectures for Bachelor Amsterdam University College and Gezondheid en Leven, and Master Biomedical Sciences, 2018-2021

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ABOUT THE AUTHOR

Iris de Nie was born in Naarden on the 14th of October 1990, and grew up in Weesp. After finishing her pre-university training at the Gemeentelijk Gymnasium in Hilversum, she took a gap year in which she travelled and worked as a volunteer in Argentina. In 2009, she started studying Medicine at the VU University in Amsterdam. During her studies she followed an internship in Paramaribo (Suriname) and did her scientific internship in Cape Town (South Africa). During her internships she became particularly enthusiastic about gynecology and urology and decided to conduct an eldest residency and an internship of choice in these departments. After her graduation she worked as a junior doctor (ANIOS) in the field of Andrology at the Center for Reproductive Medicine at the AMC. In this period, her mentor Andreas Meißner taught her the ins and outs of male fertility including procedures such as testicular sperm extraction (TESE) and microsurgical epididymal sperm aspiration (MESA). In 2017, she met Norah van Mello who was setting up fertility care for transgender people at the Center of Expertise on Gender Dysphoria at the VUMC, and who asked Iris to assist her in setting up andrological care for transgender women. She started her work at the Center of Expertise on Gender Dysphoria in January 2018, where she combined clinical work with a PhD project under the senior supervision of prof. dr. Martin den Heijer and prof. dr. Judith Huirne. During the first years, her clinical work focused mainly on counseling adult transgender women about fertility preservation options and on endocrinological care for transgender people. During the final year of her PhD, she dedicated her clinical work to fertility care for transgender youth and adolescents, combined with andrological care for cisgender men at the Urology department of the AMC. Iris lives in Amsterdam with her partner Mark, and currently works as a junior doctor at the Urology department of the OLVG.

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ACKNOWLEDGEMENTS – DANKWOORD

Omdat een echt proefschrift niet compleet is zonder een dankwoord, én omdat het nooit tot stand was gekomen zonder de hulp van anderen, wil ik bij deze een aantal mensen bedanken (hopelijk zonder in al te veel clichés te vervallen).

Allereerst wil ik de deelnemers van de verschillende studies, beschreven in dit proefschrift, bedanken voor hun tijd en het gebruik van hun gegevens. Door de grote hoeveelheid aan lopende onderzoeken binnen het KZcG hebben velen van jullie deelgenomen aan meerdere onderzoeken, ik waardeer het dan ook enorm dat jullie je ook voor mijn onderzoeken beschikbaar hebben gesteld. Zonder jullie belangeloze inzet waren deze resultaten en bevindingen nooit tot stand gekomen!

Verder ben ik mijn promotieteam enorm dankbaar voor hun begeleiding; hun geduld (als ik alles perfect wilde hebben voordat iets rond gestuurd kon worden), hun bevestiging (als ik weer eens twijfelde of mijn resultaten wel goed en interessant genoeg zouden zijn), hun sturing (als ik door de bomen het bos even niet meer zag), maar bovenal de vrijheid om mijn promotietraject vorm te geven zoals ik zelf wilde en te kunnen focussen op onderwerpen die mij het meeste aanspraken. Beste prof. dr. Huirne, beste Judith, bedankt voor jouw heldere inzichten, snelle en duidelijke feedback, en praktische oplossingen. Ik vind het inspirerend hoe jij in je dagelijkse werkzaamheden empathie en krachtdadigheid feilloos weet te combineren en ben heel blij dat ik als uro/endo buitenbeentje toch deel mocht uitmaken van jouw onderzoekersgroep. Beste prof. dr. den Heijer, beste Martin, ik wil je enorm bedanken voor de kans die je me hebt gegeven om me op dit onderwerp te mogen storten, zonder jou was dit promotietraject er niet geweest. Ondanks dat wellicht niet alle onderwerpen van dit proefschrift jou evenveel aanspraken heb je me altijd gesteund in mijn ideeën en heb je mij m'n gang laten gaan in mijn functie als 'bruggenbouwer'. Ik heb er veel respect voor hoe jij, als begeleider van hoofdzakelijk vrouwelijke promovendi, ook probeert op de hoogte te blijven van de persoonlijke ontwikkelingen en regelmatig polst 'of het allemaal nog een beetje gaat'. Dan mijn co-promotoren dr. Meißner en dr. van Mello. Lieve Andreas, jij hebt mij vanaf moment één aangestoken met jouw enorme passie voor de Andrologie en mij, als pas afgestudeerde basisarts, aan de hand genomen om mij de fijne kneepjes van het vak te leren. De mate van geduld, opleidingsgerichtheid en veiligheid die jij tentoonspreidt zijn ongekend, ik had me geen betere introductie tot het vak kunnen wensen. Ook de afgelopen jaren heb ik je altijd mogen storen voor een vraagje en schoof je al je andere bezigheden voor me aan de kant als ik iets van je nodig had, soms tot grote frustratie van anderen. Bedankt dat je er altijd voor me bent. En dan tot slot, lieve Norah, wat een eer dat ik jouw eerste gameetje mocht zijn en daarmee deel werd van jouw missie om de fertiliteitszorg voor transgender personen op de kaart te zetten. Jij hebt me wegwijs gemaakt binnen de genderzorg en (het politieke mijnenveld van) het genderteam, een eindeloze stroom van onderzoeksideeën op me afgevuurd, honderden keren naar me geluisterd als ik weer iets te klagen had, me peptalks gegeven als ik er even helemaal klaar mee was, en bovenal gesteund en gefaciliteerd in alles wat ik nodig had om dat

traject succesvol te kunnen volbrengen. Jouw creativiteit en ruimdenkendheid en mijn behoefte aan structuur en overzicht bleken een perfecte combinatie, ik ben er nog lang niet aan toe om dat te laten eindigen!

Ook mijn collega's wil ik bedanken voor de gezelligheid en prettig samenwerking de afgelopen jaren. Allereerst natuurlijk Ilona, jouw inzet voor de transvrouwen is enorm waardevol en daarnaast heb je me ontzettend geholpen met de inclusies en dataverzameling voor sommige van de, in dit boekje beschreven, studies. Dank daarvoor! Joyce en Asra, ik ben heel blij dat ook jullie nu deel zijn van de gameetjes en heb genoten van jullie vrolijkheid, zelfspot en soms ook wel naïviteit. Anna, Marijn en Maeghan, mijn Journal Club buddies, wat was het soms verfrissend om even buiten de endo-bubbel te kunnen stappen en met jullie te sparren over onderzoeksideeën, omgang met collega's of supervisie, of gewoon over het leven. Alle onderzoekers van ZH4A, de OVGO en de Urologie Amsterdam onderzoeksgroep, bedankt voor de gezelligheid en het delen van kennis en tips. Lieve endo-onderzoekers; Maartje, Nienke, Chantal, Christel, Mieke, Marieke, Dennis, Ceranza, Moya, Daan, Marianne, Milou, Margot, Benthe, Noor, Lidewij, Sophie en Sarah, wat is de groep gegroeid in de afgelopen jaren! Ik vind het waanzinnig om te zien hoe iedereen zich stort op z'n eigen onderwerp binnen de transgenderzorg en hoe daarmee de diversiteit steeds verder toeneemt. Bedankt voor alle gezellige uitjes, etentjes, borrels, racefiets tochtjes en bootcampsessies. In het bijzonder wil ik kamertje 56/#AMBERALERT bedanken voor het delen van lief en leed, de extreme lol en flauwe humor, de klaagzangen, de huilbuien, de eindeloze hoeveelheid kopjes thee en natte latte, de goede gesprekken, de hulp en de adviezen. Bedankt voor alle gezellige jaren! Ook de collega's aan de andere kant van de Amstel, Callista en Ans, wil ik graag bedanken voor de 'vruchtbare' samenwerking. Jullie kennis van de spermatogenese is ongekend, en ondanks jullie pogingen om mij bij te spijkeren voelt het naast jullie alsof ik de basis pas net een beetje onder de knie heb. Jullie vermogen om door te zeten tot iets perfect is, geduld en oprechte aandacht te hebben voor projecten, en overal kansen voor nieuw onderzoek in te zien, zijn enorm inspirerend. Bij deze wil ik natuurlijk de overige medewerkers en studenten van het Fertiliteitslaboratorium ook bedanken voor hun ondersteuning. Tot slot spreek ik graag mijn dank uit aan alle collega's van het IVF centrum voor het respectvol ontvangen van onze populatie, en het KZcG voor de saamhorigheid, diversiteit, gezelligheid en betrokkenheid die ik heb ervaren de afgelopen jaren!

Buiten werk zijn er ook een heleboel mensen belangrijk geweest voor dit promotietraject, voor de welkome afleiding, het aanhoren van mijn plannen/frustraties/bevindingen, maar bovenal het vieren van de publicaties en successen. Bij deze wil ik al mijn vrienden en familie bedanken voor hun luisterend oor en interesse. Ik wil een aantal mensen noemen in het bijzonder. Lieve Floor, vanaf de eerste seconde van het introductieweekend van Geneeskunde waren wij studievriendinnen en dat is nooit meer overgegaan. Samen bij Nereus, samen wonen, samen naar Suriname, en zo leuk dat we zelfs een jaartje samen onderzoeker zijn geweest! Ondanks dat je er niet live bij kunt zijn sta je in gedachten naast me op dat podium. Lieve Daphne, ook wij hebben (zo goed als) de hele studie samen doorlopen en heel wat uren samen op het water doorgebracht. Deze jaren vormen de basis

voor een extreem hechte vriendschap waar ik ontzettend blij mee ben. Ik ken niemand die zo attent is als jij en hoop dat je me vergeeft dat ik daar geenszins bij in de buurt kom. Jouw zorgzaamheid, betrokkenheid en extreem goede geheugen maken dat ik me geen betere paranimf kan wensen dan jij! Lieve Daan, toen jouw komst werd aangekondigd door Martin moet ik toegeven dat wij als onderzoeksgroep een beetje achterdochtig waren, ineens zou er een man in ons vrouwencluppie komen die in 2 jaar zou promoveren. Maar wat was jij een aanwinst voor het team; jouw relativerend vermogen en droge humor, gecombineerd met je beheersing van de klaagkunst en precies weten wanneer je een gespreksonderwerp gewoon even aan je voorbij moet laten gaan, maakten dat je perfect aansloot bij onze kamer. Door de jaren heen ben ik je als goede vriend gaan beschouwen, kon ik altijd bij je terecht met verhalen of voor advies en mocht ik je altijd bellen met STATA-paniek. Ik ben daarom super blij dat jij mijn paranimf wil zijn! Lieve Lin, lieve pap en mam, bedankt voor het warme nest waar ik vandaan kom en de basis vormt voor wie ik ben. Jullie onvoorwaardelijke liefde en steun betekenen de wereld voor me. Ik hou van jullie. Tot slot, lieve Mark, jij bent mijn allergrootste fan en staat altijd voor me klaar. Wat een feest om de afgelopen jaren samen in het onderzoeksschuitje te zitten en elkaar verder te kunnen helpen, ik jou met wat statistiek, jij mij met wat Engelse grammatica. Ik bewonder het enorm hoe jij duizend ballen tegelijk in de lucht houdt zonder jezelf (en mij) uit het oog te verliezen, en mij stimuleert om het beste uit mezelf te halen. Ik ben je oneindig dankbaar voor wie je bent en wat je voor mij betekent.

I hope you live a life you're proud of.
And if you find that you're not,
I hope you have the strength to start all over again.

F.S. Fitzgerald