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Fertility preservation options in transgender people: A review

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Abstract
Gender affirming procedures adversely affect the reproductive potential of transgender people. Thus, fertility preservation options should be discussed with all transpeople before medical and surgical transition. In transwomen, semen cryopreservation is typically straightforward and widely available at fertility centers. The optimal number of vials frozen depends on their reproductive goals and treatment options, therefore a consultation with a fertility specialist is optimal. Experimental techniques including spermatogonium stem cells (SSC) and testicular tissue preservation are technologies currently under development in prepubertal individuals but are not yet clinically available. In transmen, embryo and/or oocyte cryopreservation is currently the best option for fertility preservation. Embryo cryopreservation requires fertilization of the transman's oocytes with a donor or partner's sperm prior to cryopreservation, but this limits his future options for fertilizing the eggs with another partner or donor. Oocyte cryopreservation offers transmen the opportunity to preserve their fertility without committing to a male partner or sperm donor at the time of cryopreservation. Both techniques however require at least a two-week treatment course, egg retrieval under sedation and considerable cost. Ovarian tissue cryopreservation is a promising experimental method that may be performed at the same time as gender affirming surgery but is offered in only a limited amount of centers worldwide. In select places, this method may be considered for prepubertal children, adolescents, and adults when ovarian stimulation is not possible. Novel methods such as *in-vitro* activation of primordial follicles, in vitro maturation of immature oocytes and artificial gametes are under development and may hold promise for the future.

Keywords Fertility preservation · Reproduction · Transgender health · Transgender

1 Introduction

Fertility preservation (FP) is the practice of improving the chances of biological reproduction in individuals when a significant risk of infertility is predicted [1]. Gender affirmation therapy, both hormonal and surgical, is one of the indications for FP [1] because gender affirming hormones adversely affect fertility and gender affirmation surgery may involve the removal of gonads. International guidelines recommend that healthcare personnel should discuss FP plans with transgender individuals before starting gender affirmation therapy in adults and adolescents, or puberty suppression treatment in children [1–3].

About 47% of transgender individuals wish to have a child to whom they are genetically related according to a study by Tornello and Bos [4]. A report using a self-questionnaire revealed that more than half of transmen desired to have a child and approximately one-third (37%) wished to have their gametes preserved before gender affirmation procedures [5]. While in transwomen the percentage seeking to have children varied from 15 to 51% depending on their sexual orientation [6, 7]. The common finding from these studies was the need of an FP plan provided by healthcare personnel before or during the transition process.

Efforts to demonstrate the need for transgender people to have an access to FP have been ongoing for more than a decade [3, 7]. The majority (76.2%) of the general non-

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transgender population agreed that transpeople should be able to conceive their own biological children [8]. However, transgender people still find difficulties in accessing FP services [9, 10]. Low access rates to FP service were reported in many centers [9, 11–14]. In adolescents undergoing gender affirmation therapy, the rate of formal fertility discussions with a specialist was 12.4%, and only 3–4.7% agreed to undergo FP procedures [13, 14]. A recent report by Wakefield et al., showed a considerably higher rate of FP discussions before starting treatment at 75% for adolescents and young adults, yet the actual referral rate for FP was only 13.5% [12]. There are many factors affecting the decision to undergo an FP process including laws, regulations, financial status, social climate and attitude of healthcare personnel [4, 9, 10, 13]. These factors are different in each country and tremendously affect the methods of treatment. For example, in Thailand assisted reproductive technologies are not allowed by the government for same-sex couples. In contrast, in Sweden, FP among same-sex couples is funded by the government [9]. Transgender people also report that the FP process potentially caused mental distress, increased gender dysphoria and adversely affected the view of their bodies [9]. A lack of competent and sensitive provider training can potentially prevent transgender patients from engaging in the FP process [9, 15].

Currently, there are multiple methods to conserve gametes in sex assigned at birth females. Embryo or oocyte cryopreservation is the treatment of choice for most adults. While still experimental and not offered widely yet, ovarian tissue cryopreservation (OTC) may be an option for adolescent and prepubertal children [2]. A distinct benefit of OTC is that it can be performed at the same time as gender affirming surgery without the need for ovarian stimulation or gynecologic examination. Long-term data however on live birth rates from OTC in transmen is not yet available.

For FP of post pubertal sex assigned at birth males, semen cryopreservation is widely available at most fertility centers and sperm banks. While testicular tissue cryopreservation, offered in prepubertal children, is still in an experimental phase [16].

This review primarily aims to explore both available and experimental options of FP for transgender people based on updated publications and guidelines to provide better comprehensive care for transgender people.

2 Fertility and fertility preservation options in transgender women

2.1 Effects of gender affirming hormones and medications on reproduction

Gender affirming hormone regimens for transgender women usually involve a combination of androgen lowering medications and estrogens. Commonly used medications to lower testosterone include: GnRH agonist, Cyproterone acetate (CPA) or spironolactone [1]. The most common form of estrogen given to transgender women is estradiol. These agents have different mechanisms of actions as well as different effects on fertility [17].

Cyproterone acetate (CPA), a synthetic progestin with anti-androgenic properties, is commonly prescribed in European countries and in Thailand. CPA competitively binds to testosterone receptors in brain and peripheral tissues, and as a result strongly inhibits spermatogenesis [17, 18]. CPA affects spermatogenesis in a dose-dependent fashion. Exposure to low dose CPA (5–10 mg) for 2–4 months can cause impairment of sperm quantity, motility and morphology [19–21]. Therefore, the standard dose recommended for transwomen of 25–50 mg/day [1] can potentially depress spermatogenesis. The effect of CPA on spermatogenesis seems to be reversible; however, the exact time course for restoration of sperm production after discontinuing CPA is yet to be defined. Meriggiola and colleagues reported a time to full restoration from complete suppression of sperm production (azoospermia) was 14–18.5 weeks depending on CPA dosage. However, a major limitation of this study was that these subjects were non-transgender men and they were exposed to both CPA and testosterone enanthate, which is also a potent inhibitor of spermatogenesis, for 16 weeks. Thus, these results may not be fully representative of what would be expected in transgender women [22, 23] and the reversibility after a prolong use is yet to define.

Spironolactone is a diuretic and anti-hypertensive medication with anti-androgenic properties [24]. Studies regarding spironolactone and spermatogenesis in humans are scarce. From animal models, spironolactone competitively binds both peripheral androgen receptors [25] and androgen receptors in the pituitary gland [26], and directly interferes with angiogenesis at the testicular level, which impairs spermatogenesis in rats [27, 28]. To the best of our knowledge, there is no study about the effect of long-term use of spironolactone on spermatogenesis in transwomen, thus the exact time course of spermatogenesis impairment and recovery after exposure is not known.

Another androgen lowering class of medications is GnRH agonist. This class of medications lowers serum testosterone to a castration level by competitively occupying and constantly stimulating GnRH receptors in the anterior pituitary gland, resulting in down-regulation of FSH and LH production by the pituitary. As a result, Leydig cells decrease testosterone production within 2–3 weeks after starting the treatment [29–31]. The effect of GnRH agonist therapy on spermatogenesis correlates to the dosage and duration of the exposure and is can be reversible unless there is testicular atrophy [29, 32, 33]. Linde et al., demonstrated a nadir point of sperm parameters at 7–18 weeks after 6–10 weeks of exposure to a GnRH agonist. Restoration of spermatogenesis occurred at 10–14 weeks after

cessation [32]. Depending on the dose, GnRH agonists may not entirely suppress spermatogenesis (due to incomplete FSH/LH suppression) [34, 35]. Due to a limited number of studies with long term exposure and follow up, the exact time frame of when and if spermatogenesis will be restored is still unclear. Therefore, the long-term effect of extended use of GnRH agonists on fertility is yet to be elucidated.

Apart from anti-androgens, estrogen also suppresses androgen production and spermatogenesis [17, 36, 37]. Long term exposure to estrogen causes a narrowing of the seminiferous tubule and total absence of Leydig cells [38]. Also, estrogen may interfere with the expression of androgen receptors and up regulate estrogen α receptors, which in combination could induce cell apoptosis in the pituitary and testes, leading to impairment of spermatogenesis [39, 40]. A study by Thiagaraj et al., demonstrated a varying degree of impairment of spermatogenesis after extended-use of estrogen (more than 6 years) [37]. Total absence of sperm or intact spermatogenesis activity can be observed in some study participants [37]. The recovery period for spermatogenesis after estrogen cessation is not known.

When estrogen is used in combination with an anti-androgen, severe impairment of spermatogenesis is expected along with a reduction in the number of gametes, alteration of Sertoli and Leydig cells, shrinkage of seminiferous tubule and vacuolization of surrounding connective tissue [17, 36, 41]. In addition, sperm parameters are impacted with the development of oligospermia, asthenospermia and teratospermia [42]. Different combinations of estrogen and antiandrogens can lead to different levels of fertility impairment [17, 36, 41]. The combination of estrogen with CPA or GnRH agonist appears to suppress spermatogenesis more than estrogen alone [36]. However, Leavy et al., found that a longer duration of combined anti-androgen and estrogen therapy might correlated with an increased likelihood of resumption of spermatogenesis. In this study, spermatids and spermatozoa were found in a transwoman exposed to estrogen and CPA for 6 years, while the other transwomen exposed to a shorter duration (18–36 months) of these medications presented no spermatid or spermatozoa at all [36]. Decreased sensitivity of androgen receptors and increased destruction of exogenous hormones were proposed to be the causes by the researchers [36]. Although it is believed that spermatogenesis impairment from these agents is reversible [41], some experts suggest that prolonged use of hormones might cause irreversible damage to testicular tissue [43]. In addition, the exact time frame of fertility restoration is not known.

2.2 Available options for fertility preservation in transgender women

Fertility preservation is best addressed before any hormonal treatment or genital-related surgery is undertaken [1, 3] because anti-androgen and feminization agents adversely affect spermatogenesis, while gender affirming surgery causes irreversible damage to the reproductive organs. FP therapies can also be offered anytime during transition before gender affirming surgeries. However a longer period of time on gender affirming hormones will progressively impact semen quality with increased frequency of oligospermia, asthenospermia, teratospermia and azoospermia [36, 41]. Although restoration of spermatogenesis can be possible after stopping gender affirming hormones, the time to restoration is uncertain, and discontinuing hormone therapy may lead to undesirable effects from increased endogenous testosterone production [41].

Semen cryopreservation using specimens obtained from masturbation is a standard FP protocol that has been in use for decades. For non-transgender patients, it is easy, reliable and relatively inexpensive. However, some transwomen relate the act of ejaculation to male gender and find this procedure psychologically distressing [42]. Additionally, testosterone depletion from gender affirming therapy may lead to difficulties in erection and ejaculation [1]. Electro-stimulation or penile vibratory stimulation can be used in difficult cases but availability might be limited [44]. Another option is surgical sperm retrieval (SSR), a surgical procedure that retrieves sperm directly from parts of the testis or epididymis. SSR is an established practice among worldwide fertility centers and can be performed at the time of gender affirming surgery. Data from post-chemotherapy patients with azoospermia demonstrate a 42–65% success rate of TESE (testicular sperm extraction) [45–47], yet the success rate in azoospermic transgender women is not known. The overview of FP choices is shown in Table 1. Testicular tissue cryopreservation in prepubertal patients where sperm production has not started is still under investigation [16].

Pregnancy outcomes associated with the use of cryopreserved sperm depend on sperm quality and the assisted reproductive technology (ART) method(s) used. Cryopreserved sperm of good quality and quantity can be used for an intrauterine insemination (IUI) procedure while poorer quality or limited quantities of sperm can only be used in *in-vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Also, because the likelihood of live birth following a cycle of IVF is substantially higher than IUI, if only a limited number of frozen sperm specimens are available, IVF is often recommended even if the quality and concentration of the frozen sperm are adequate. Hamada et al. found, even prior to the initiation of gender affirming hormone therapy, transgender women may have poorer sperm quality than those of non-transgender men [42]. This implies that the future use of IVF and ICSI might be employed more often by transgender women than expected. As gender identity and sexual orientation are two separate entities, the ART techniques should be individualized by considering the sex and fertility status of the partner along with local laws and regulations governing

Table 1 Overview of fertility preservation options

Method	Procedure	Status[a]	Application
Transfemale			
Semen cryopreservation	- Masturbation - Assisted ejaculation	Clinically available	- Use for IUI, IVF or ICSI (depending on quantity/quality of a sample and reproductive goals)
	- SSR	Clinically available	- Small amount of sperm for IVF with ICSI only
SSC cryopreservation		Experimental	- No established protocol (still in experimental phase)
Testicular Tissue cryopreservation		Experimental	- No established protocol (still in experimental phase)
Transmale			
Embryo cryopreservation	- COH followed by ultrasound guided egg retrieval under anesthesia - Fertilization: IVF/ICSI	Clinically available	- Typically requires repeat transvaginal ultrasounds although transabdominal monitoring may be an option - Requires partner or donor sperm for fertilization
Mature oocyte cryopreservation	- COH followed by ultrasound guided egg retrieval under anesthesia	Clinically available	- Typically requires repeat transvaginal ultrasounds although transabdominal monitoring may be an option - Male gamete is not required
Ovarian tissue cryopreservation (OTC)	- Strips of ovarian cortex are cryopreserved in liquid nitrogen - Can be performed at the same time as gender affirming surgery without ovarian hyperstimulation (not widely available)	Experimental	-Experimental basis when mature oocyte cryopreservation is not an option -Not certain to produce usable mature oocytes, pregnancy rates uncertain

COH controlled ovarian hyperstimulation, *ICSI* Intracytoplasmic sperm injection, *IUI* intrauterine insemination, *IVF In-vitro* fertilization, *OTC* ovarian tissue cryopreservation, *SSC* spermatogonium stem cell, *SSR* surgical sperm retrieval

[a] Barcelona International Society for Fertility Preservation-ESHRE-ASRM 2015 expert meeting [2]

fertility preservation procedures [48]. For example, if the partner is a sex assigned at birth female with intact reproductive organs, intrauterine insemination (IUI) or IVF/ICSI can be considered depending on the quality of sperm and the number of frozen specimens available. In cases where a partner cannot provide sufficient oocytes of good quality, donor oocytes can be used. If the partner is a sex assigned at birth male, or a sex assigned at birth female but pregnancy is physically or psychologically impossible, surrogacy (with or without oocyte donation) is an option [1, 43, 48]. However, the usage rate of cryopreserved gametes, the live birth rates resulting from their use, and long-term health outcomes in this particular group are yet to be explored.

2.3 Available options for fertility preservation in transfeminine prepubertal and adolescent patients

Presently, there is no available means of preserving fertility in transgender girls who have not yet undergone spermarche. Testicular tissue and spermatogonium stem cell (SSC) cryopreservation may become viable options in the future, however both are still under investigation and successful fertility preservation has not yet been demonstrated in humans [16]. In adolescents mature enough to produce sperm, cryopreservation of sperm is a viable option, although not all adolescents will be able or willing to produce a specimen by masturbation. Spermarche occurs early in pubertal development with a median age of 12.7–13.4 years old [49–51]. Physical changes may not be a reliable predictor of an individual's spermarche, as testis may enlarge only slightly, pubic hair growth may not occur or may be in the early stages of development [51], and serum hormones often remain unchanged [52]. A large study that evaluated adolescents who underwent sperm banking (4345 adolescents, age 11–20 years) showed 93% success rate of collecting sperm and found a direct correlation with age [53]. The youngest age able to produce sperm in this study was 12.4 years old. The success rates were 81%, 91 and 95% in 11–14, 15–18 and 18–20 year old groups, respectively [53]. The youngest successful sperm collection was reported by Bahaur et al., in an 11 years old boy [54] and Nielsen et al, found that approximately 50% of tanner stage II and III boys had sperm in their urine [51]. Regarding sperm quality, while successful cryopreservation decreases at a concentration of less than 0.1×10^6 sperm/ml [16], there is no lower limit for

sperm concentration for cryopreservation since only a few sperm may be sufficient for ICSI. In addition, pregnancy rates from cryopreserved sperm are comparable to those with fresh sperm even in the setting of poor sperm quality [16, 55–57]. If masturbation is unsuccessful to collect sperm, assisted ejaculation or SSR under general anesthesia can be offered [16, 58].

In transgender adolescents using a GnRH agonist, testicular maturation is temporarily inhibited and will likely be restored after discontinued use [1]. A study of boys with precocious puberty who were treated with a GnRH agonist showed spermarche at 0.7 to 3 years after discontinuation [59], yet the data regarding time to spermarche in transgender patients treated with GnRH agonists is not well established. A prolonged period of stopping suppression in order to produce sperm for cryopreservation may lead to the development of male secondary sex characteristics. This may be unacceptable for some individuals and may lower the likelihood of them pursuing FP [14].

The psychological challenge of acquiring a specimen and the potential financial burden of semen processing and long-term cryopreservation is a concern. Adolescents may not fully realize the effects of gender affirming hormone therapy on their future reproduction and their desire to have children who are genetically related to them may change after they transition or when they reach adulthood [14]. Therefore, a child's parents or primary caregiver and a mental health professional should be included in the education and consent process [1].

3 Fertility and fertility preservation options in transgender men

There are multiple forms of testosterone for inducing virilization including parenteral formulations (testosterone enanthate, cypionate or undecanoate) and transdermal preparations [1, 3]. Testosterone acts directly on end organs and induces male secondary sex characteristics. Although cessation of menstruation often occurs within 8–12 months [60], endometrial tissue in transgender males treated with testosterone often reveals varying morphology. Androgens can impair endometrial growth and function [61]. Accordingly, endometrial atrophy is the expected result of androgen exposure [62, 63], however recent observational studies found proliferative endometrial tissue in young transmen [60, 64]. In a study of 12 young transmen who underwent gender-affirming surgery, Loverro et al. found that all had active endometrium and 83.3% had proliferative endometrium [60]. In contrast, Grynberg et al. found that in 112 transmen exposed to androgens for at least 3 years, only 48% had proliferative endometrium while another 44.6% had atrophic change [64]. Importantly, eight of them had hyperplasia without cellular atypia and one case had focal adenocarcinoma. The mechanism for endometrial stimulation in transmen taking androgen therapy is yet to be determined, but unopposed estrogen from peripheral androgen conversion, in the absence of progesterone inhibition, and hyperinsulinemia might be the cause [60, 64]. Therefore, abnormal bleeding in transmen with a uterus requires an evaluation to rule out endometrial hyperplasia, malignancy, or other pathology.

The effect of testosterone on ovaries is inconclusive. Formerly, it was believed that long-term exposure to testosterone could induce PCOS (polycystic ovarian syndrome) [65, 66]. However, recent studies have not supported this hypothesis. Ikeda et al. found ovarian stromal and cortical hyperplasia during histological examination of resected ovaries obtained from transmen on testosterone therapy, but other morphologic features of the ovaries were not compatible with PCOS [67]. When evaluated by ovarian ultrasound, the prevalence of PCOS in long-term androgen exposed transmen was similar to those of non-transgender women [68]. In terms of reproduction, androgen therapy does not appear to disturb the ovarian follicular pool [69, 70]. Levels of AMH and Inhibin-B in serum appear normal [71] and the distribution of immature oocytes in ovarian cortex is comparable to those of non-transgender women [70]. Androgens inefficiently suppress ovulation, as ovulation and unplanned pregnancies during androgen exposure have been reported [60, 63, 72]. Loverro et al. found a quarter of young transboys taking testosterone continued to ovulate [60]. Therefore, if a transgender male is having unprotected intercourse with a male partner, contraception is recommended. If a desired pregnancy has occurred, cessation of testosterone is mandatory because of teratogenic effects.

In transgender men who are planning to conceive, the capacity to reproduce generally resumes soon after cessation of testosterone [72]. The effect of testosterone seems dose and duration related, yet confirming data is scarce. Studies in transmen show resumption of menstruation within 6 months after testosterone discontinuation [9, 72] and the majority of participants are able to conceive within 6 months [72]. The limitation of these studies was that information regarding conception was by self-report using a web-based tool with a limited number of participants. Although it is possible for transmen to reproduce after the initiation of hormone therapy, testosterone should be discontinued while attempting to conceive to lower the risk of teratogenicity [9, 48, 72]. The interruption of testosterone therapy and the ensuing pregnancy may lead to development of secondary female characteristics that might be unbearable for some individuals [9].

3.1 Available options for fertility preservation in transgender men

Available FP procedures in transmen are embryo cryopreservation, oocyte cryopreservation, and ovarian tissue cryopreservation (OTC) [73]. Standard methods to safeguard fertility are cryopreservation of embryos or oocytes obtained after

controlled ovarian hyperstimulation (COH) [2, 74]. Although embryo cryopreservation has been used for decades and yields reliable outcomes, sperm is needed. This may raise ethical and legal concerns for a future use [75, 76]. Oocyte cryopreservation involves no sperm and is a choice that offers transmen full autonomy over their gametes with fewer ethical concerns [77]. Vitrification is the preferred method of oocyte cryopreservation [78–80] as it offers comparable pregnancy rates to those of fresh oocytes [81, 82]. In prepubertal transboys, the only available option is OTC. OTC is still considered as an experimental procedure and is not widely available, yet live births have been reported [73]. It is noteworthy that as long as ovaries are not removed the options for spontaneous pregnancy, COH and mature oocyte cryopreservation or IVF in the future remain.

In embryo and mature oocyte cryopreservation, COH is essential to mature the oocytes in vivo. Gonadotropins are used to enhance oocyte maturation, and GnRH analogues or GnRH antagonists are used to prevent premature ovulation. During the process, patients undergo close monitoring with frequent measurement of serum estradiol and progesterone, as well as frequent transvaginal ultrasounds to monitor follicular growth. After approximately 10–14 days, oocytes are retrieved under sedation with ultrasound-guided transvaginal aspiration of the follicular fluid. Obtained oocytes are subsequently isolated, stripped of their cumulous, and only the mature oocytes (metaphase II with an extruded polar body) are cryopreserved. In cases of embryo cryopreservation, mature oocytes are either fertilized by conventional fertilization in which motile sperm are introduced into the culture environment and allowed to fertilize the oocytes naturally, or via intracytoplasmic injection (ICSI), in which sperm are injected into the oocyte to facilitate fertilization. Sperm from a known or an anonymous donor, or from an intimate partner can be used. Transmen who are considering embryo cryopreservation using sperm from an intimate partner must be carefully counseled, as the partner will need to consent to an embryo transfer or disposal of the embryos in the future.

Mature oocyte cryopreservation has, in recent years, transitioned from an experimental procedure with poor success rates to a robust means of preserving unfertilized eggs. [73]. It is now approved as a mean of FP by many organizations [2, 83]. Advancements in oocyte vitrification techniques have led to an improvement in live birth rates. In 2006, live birth rate per oocyte was less than 2% due to damages caused by ice crystal formation during the cryopreservation process [84]. Metaphase II stage oocytes are fragile and susceptible to ice formation because of their 1) high proportion of water, 2) large size, and 3) delicate chromosomal structure [83]. Advancements in vitrification techniques that avoid ice crystal formation have given rise to post-thaw survival rates of up to 85–97%, fertilization rates of 70–79%, and clinical pregnancy rates of 4.5–12% per thawed oocyte [79, 83]. For these reasons, oocyte cryopreservation has become the method of choice for FP in transmen who are not comfortable carrying their own pregnancy or plan to have their ovaries removed. It also alleviates social and ethical issues associated with the use of sperm from a donor or intimate partner, and gives full autonomy over the gametes to the owner [77].

A noteworthy point to consider in oocyte cryopreservation is the necessary number of oocytes to cryopreserve in order to provide an adequate means for successful family building in the future [85]. A study by Cobo et al., showed the correlation of cumulative live birth rates with the age at ovum pick up and the number of oocytes [85]. Cumulative live birth rates in women aged less than 35 was 15.4%, 40.8%, 60.5 and 85.2% in 5, 8, 10, and 15 cryopreserved oocytes, respectively. In patients over 35 years old, the cumulative pregnancy rate was significantly lower at 5.1%, 19.9%, and 29.7% when 5, 8 and 10 oocytes were retrieved [85]. Therefore, a target number of oocytes should be thoroughly discussed with patients. Depending on the age and ovarian reserve of the patient, the possibility of undergoing multiple cryopreservation cycles may be discussed. Although live birth rates from cryopreserved oocytes in transgender men is currently unavaliable, successful pregnancies from cryopreserved oocytes resulting in two twin pregnancies were reported [86]. More data is needed and each center should provide their own dataset for patient education and consent.

Transgender men using hormone therapy may find the oocyte cryopreservation process difficult due to the need to temporarily stop taking the hormone for varying lengths of time [9, 48, 72]. Cessation of testosterone could lead to unwanted physical changes and resumption of bleeding which may aggravate feelings of gender dysphoria in some individuals [9]. In addition, gynecologic procedures such as pelvic examination, transvaginal ultrasound and transvaginal oocyte retrieval [87, 88] could also cause emotional instability and psychological distress for some transmen [9]. In some cases, depending on body habitus and clinic, transabdominal monitoring may be possible. The addition of Letrozole in the stimulation protocol to avoid high-levels of estrogen, as is commonly done in estrogen receptor positive breast cancer patients, may temper some of these effects and help patients adhere to the treatment [9, 71]. Sensitivity training for medical personnel, who should be taught to use appropriate pronouns and avoid gender-related words, is important steps that may help patients feel more comfortable throughout the process [9].

In cases which ovarian simulation is unacceptable, ovarian tissue cryopreservation (OTC), a promising

experimental procedure, may be an option depending on whether there is a nearby institution that is able to offer this service. In institutions that have an appropriate research protocol and necessary facilities for doing so, ovarian tissue cryopreservation can be performed at the same time as gender affirming surgery without the need for controlled ovarian stimulation and multiple transvaginal ultrasounds [70]. The details of OTC are described in the prepubertal and adolescent section. However, there are important limitations to consider. Ovarian tissue that is cryopreserved is usually re-implanted to be used, and the transgender patient will need to stop using any hormones that may interfere with the function of the ovarian tissue. Additionally, he will be left with functional ovarian tissue that is difficult, if not impossible, to remove. Techniques to mature oocytes from cryopreserved tissue in order to be used with IVF are in development, but this remains highly experimental. Finally, ovarian tissue cryopreservation is considered experimental, and often requires access to a research institution with a protocol that specifically allows this technique to be offered to transgender patients. Also, this technique might not suit a person more than 35 years old, as there is a significant reduction of the number of cortical follicles in older patients [73, 89, 90].

3.2 Available options for fertility preservation in prepubertal tranmasculine children and adolescents

The only option for FP in prepubertal transmen is OTC. This experimental procedure is typically offered to prepubertal patients with cancer who require gonadotoxic treatment or oophorectomy [2, 73]. It is performed at institutions with a research protocol in place, which may specify precisely which population of patients it may be offered to (such as patients with cancer). In prepubertal patients with cancer, this method has been more widely used and outcomes are encouraging [73, 74, 91]. To date, more than 130 live-births have been reported from many research groups [73] and complications are uncommon [92].

The procedure for OTC includes ovarian cortex biopsy, cryopreservation, thawing, and utilization of gametes. A laparoscopic approach is most commonly used to obtain ovarian tissue [92–94]. Ovarian cortex, which contains large numbers of follicles, is removed for cryopreservation. Androgen exposure in transmen does not cause damage to oocytes in the tissue [70]. Partial removal of the ovarian cortex or removal of the entire ovary can be performed depending on institutional protocol and patient preference [70, 92]. Ovarian tissue can be stored for 4–5 h at 4 °C which facilitates transportation of the biopsied tissue and makes the procedure more accessible [95, 96]. Almost all live births reported to-date resulted from ovarian tissue that was cryopreserved using a slow freezing technique [73]. Vitrification, a newer method of cryopreserving tissue, has a limit number of pregnancy reports, but the procedure has become increasingly common and further reports are expected in the near future [97, 98]. In order to use cryopreserved ovarian tissue, the tissue must be reimplanted. There are 2 major sites to reimplant the tissue: orthotopic and heterotopic. Orthotopic reimplantation in the pelvis is more common [73, 92, 99] because the use of a heterotopic site (for example, a patient's arm or abdominal wall) yields inferior results [96]. Restoration of ovarian function usually occurs 4–6 month after reimplantation and may last up to 7 years with a mean duration of 4–5 years [96, 100]. Having up to 3 live births from one reimplantation has been reported [101]. Apart from producing oocytes, ovarian tissue reimplantation benefits women with ovarian failure in terms of endocrine function. However, in transgender males, the endocrine function of transplanted ovarian tissue may be detrimental. Therefore, where available, methods other than auto-reimplantation such *in-vitro* maturation (IVM) of immature oocytes may be considered but this is not widely available or efficient [70]. In conventional ovarian tissue preparation for cryopreservation, the ovarian medulla is removed and only the cortex is preserved. Using this technique, antral follicles located at the junction of medulla and cortex are discarded. Therefore, some institutions practice IVM (using antral follicles aspirated from an ovary or found in manipulation medium). [70, 102–104]. Chloë De Roo and colleagues [70] demonstrated the use of IVM in parallel with OTC in transgender patients. The study was carried out in 40 transmen with a mean androgen exposure of 58 weeks. The median number of cumulus oophorous complexes (COCs) obtained was 27 COCs per person, which was higher than 10–14 COCs per person reported in cancer patients [102, 104]. This may be explained by the accumulation of oocytes caused by FSH and LH down-regulation from androgen exposure [70]. Regarding oocyte maturation, COCs from transmen have a 34.3% maturation rate, which is comparable to 29–36% in those of cancer patients [102, 104]. In spindle analysis, a normal chromosome pattern was found in 87.1% of Metaphase II oocytes obtained via IVM. Based on these findings, oocytes that have been obtained using IVM from transmen may be suitable for future use. However, In studies examining the implantation rate of embryos produced from IVM oocytes, the implantation rate per embryo is significantly lower and the chance of early pregnancy loss is higher than embryos obtained from conventional means [105]. Therefore IVM of antral follicles should be considered at this stage as an experimental method in patients undergoing OTC, and should not be used as the single method of fertility preservation in these patients [102, 106].

4 Future methods

4.1 *In-vitro* activation (IVA) and *in-vitro* maturation (IVM) of immature oocytes

Currently, immature oocytes in the ovarian cortex can be activated and mature naturally after reimplantation resulting in both endocrine function and oocyte production. However, reimplantation of ovarian tissue in transmen leads to unfavorable female hormone production. An attempt to establish a novel technique to activate and mature these immature oocytes in cryopreserved tissue outside the body will benefit patients not only by avoiding the unwanted endocrine effects of tissue reimplantation but also by providing the means to create a large number of mature oocytes. Preantral follicles can be obtained via ovarian tissue harvesting and subsequently matured to the Metaphase II stage. These oocytes can then be cryopreserved in parallel with cortex cryopreservation to enhance the gamete pool [70, 102–104]. In primordial, primary, and secondary follicles, *in-vitro* activation (IVA) and maturation is far more complicated. In 2003, Kuwamara and colleagues became the first group to successfully activate immature oocytes and use them to produce a live birth [107]. There were two methods used in this study: 1) mechanical fragmentation of ovarian tissue that enhances actin polymerization and interrupts the ovarian Hippo signaling pathway, and 2) treatment with Akt stimulators. After this discovery, babies born to premature ovarian failure patients have been reported [108–110]. Yet, this IVA protocol is not compatible with transmen, as the procedure currently involves reimplantation of the ovarian tissue and restoration of endocrine function is unavoidable. However, the advancement demonstrates the possibility of using an *in-vitro* process to obtain mature oocytes for IVF without the necessity of tissue reimplantation in the future.

4.2 Spermatogonium stem cells

Spermatogonium stem cells (SSCs) or type A spermatogonia, a cell located at the basal layer of the seminiferous tubule, is a precursor of spermatogenesis throughout a man's life. SSC from testicular tissue biopsy presents a possible mean of fertility restoration. Possible methods for fertility restoration include direct injection of SSCs into the testes, testicular tissue reimplantation, and *in-vitro* maturation of SSCs. However all of these methods are currently theoretical [16]. Also, because endocrine function from reimplanted SSCs or testicular tissue is undesirable in transwomen, it is reasonable to conclude that *in-vitro* maturation of SSCs would be preferable for most transmen. This technique is more complicated than the others for fertility restoration. Special culture systems such as 3D culture or organ culture is required [111]. Sun and colleagues recently reported the successful creation of human spermatid from SSCs using a 3D culture system [112]. These spermatids showed normal gene profiles and DNA methylation and were able to create hybrid embryos [112]. However, creating human embryos via this method has never been reported and there are significant ethical and safety considerations associated with doing so.

4.3 Artificial gametes

The creation of artificial gametes from somatic cells is a theoretical mean of fertility treatment that would enormously benefit anybody who lacks mature gametes. This includes infertile patients with azoospermia or premature ovarian failure, or transgender people who have already undergone gender affirmation surgery. Primordial germ cells, pluripotent stem cells and induced pluripotent stem cells are theoretical sources of gamete production [113]. In 2016, Zhou et al. was the first group to report successful *in-vitro* creation of functional male gametes in mice. Fertile offspring were produced from spermatid-like haploid cell derived from embryonic stem cells [114]. Later, in November 2016, Hikabe and colleagues reported successful creation of oocytes from skin cells in a mouse model. These oocytes were able to be fertilized, resulting in live litters [115]. A more recent report from Cambridge University demonstrated that it is possible to create mouse embryos from two embryonic stem cell lines (requiring no gametes at all) in a 3D culture system [116]. Although this promising breakthrough has shed light on new theoretical means of treating infertility, it still is a long way from becoming a valid method for clinical use [2, 113].

5 Conclusion

An increasing number of transgender people are seeking fertility preservation therapy, yet access to treatment is still limited. Laws, regulations, financial status and social climate are currently major factors influencing the decision to undergo fertility preservation. There are many clinically approved methods available worldwide including sperm cryopreservation for transgender women and embryo and oocyte cryopreservation for transgender men. Experimental procedures involving the use of testicular tissue or spermatogonium stem cells and ovarian tissue cryopreservation holds promise as a technique for FP for prepubertal transgender children and transgender adults in whom the use of standard fertility preservation procedures are not possible. Given the advancement of technologies in FP, physicians should discuss the potential options of FP with transgender people before hormone therapy and gender affirmation surgery are undertaken.

Compliance with ethical standards

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

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