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Gonadal tissue cryopreservation in transgender and gender diverse people

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In Brief Statement

Gender affirming treatments for gender dysphoria can impact fertility. This review describes the impact of gender affirming treatments on fertility and options to preserve fertility in transgender or gender diverse children, adolescents and young adults.

Abstract

Transgender individuals who pursue alignment with their gender identity, through medical treatments or surgery face challenges to family building because the medical community lacks the understanding or infrastructure to serve the reproductive needs of transgender or non-binary people. Fertility preservation (FP) offers a crucial opportunity for the transgender community, enabling individuals to exercise autonomy over their reproductive choices. While fertility preservation has been extensively studied in other populations such as cancer patients, the unique biology and clinical care of the transgender and gender diverse (TGD) individuals has challenged direct translation of what can be offered for cisgender individuals. Additionally, the FP services in transgender communities are reportedly under-utilized, despite the prevalent desire of TGD individuals to have children. This review aims to provide up-to-date information on the current standard of care and experimental FP options available to TGD individuals and their potential reproductive outcomes. We will also discuss the barriers to the success of FP utilization, from both the biology/medical aspect and the perspectives of TGD population. By recognizing the unique family building challenges faced by TGD people and potential areas of improvement, appropriate adjustments can be made to better support fertility preservation in the TGD community.

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Introduction

TGDFor transgender communities, understanding terminology is crucial for providing effective care. According to the World Professional Association for Transgender Health (WPATH) Standard of Care version 8 (SOC8) (Coleman et al., 2022), the term transgender or gender diverse (TGD) is used to describe individuals whose gender identities or expressions differ from the gender typically associated with the sex assigned to them at birth. Gender identity refers to an individual's internal sense of their gender, which is distinct from sexual orientation—defined as a person's patterns of emotional, romantic, and sexual attraction. Gender affirmation involves recognizing and validating TGDTGD individuals in their gender identity across social, medical, legal, and behavioral domains, or a combination of these (Poteat et al., 2023). Gender Affirming Medical and/or Surgical Therapy (GAMST) are the medical and surgical intervention to align person's body with their gender identity (Coleman et al., 2022). GAMST may include hormonal (Gender-Affirming Hormone Therapy: GAHT) and/or Gender-Affirming Surgery (GAS), the latter of which may include but is not limited to genital reconstruction, removal of gonads, and the surgery to enhance the secondary sex characteristics that affirm gender identity (Coleman et al., 2022). The evolution of terminology and diagnostic criteria show the efforts that have been made to remove stigma from transgender communities.

Transgender individuals represent a small yet growing segment of the global population, constituting approximately 0.6% of adults and 2.7% of children and adolescents (Scheim et al., 2024). The reported prevalence varies depending on regions, survey methodologies, and definitions used (Reisner et al., 2016). More inclusive definitions of transgender, counting non-binary, gender diverse and gender non-conforming persons, indicate that up to 4.5% of adults and 8.4% of children and adolescents fall within this category (Scheim et al., 2024). In the United States, according to The Williams Institute's 2022 report, 0.5% of adults (approximately 1.3 million individuals) and 1.4% of youth aged 13-17 (around 300,000 individuals) identify as transgender. Of the 1.3 million adults identifying as transgender, 38.5% (515,200) are transgender women, 35.9% (480,000) are transgender men, and 25.6% (341,800) are gender non-conforming (Herman et al., 2022). Notably, reported numbers are often higher among younger populations and may continue to rise (Zucker, 2017).

TGD people show improvement in quality of life, well-being, satisfaction in ones' body image, and sexual life after receiving gender affirming treatments (Coleman et al., 2022). The current recommendations for GAMST by the Endocrine Society and WPATH SOC8, can be categorized into the guidelines for TGD adults/adolescents with testes or ovaries (Coleman et al., 2022, Hembree et al., 2017). The Gender Affirming Hormonal Therapy (GAHT) for adult TGD people with testes requires both anti-androgen medications, such as Cyproterone or Spironolactone, and estrogen supplement, preferably estradiol. Whereas the protocol for adult TGD people with ovaries is testosterone monotherapy. The details of dosing and regimens varied among countries, possibly due

to availability, cost and familiarity of clinicians with drug choice (Tangpricha and den Heijer, 2017). In adolescents, the treatment usually begins by delaying puberty with GnRH agonists (GnRHa) to allow more time for the youth to explore their gender identity and ease the distress of entering puberty before GAHT is initiated. GAHT can also later encompass puberty-blocking treatment. The recommended age to initiate the GAHT using age of majority as previously mentioned in SOC7 – at least 16 years for GAHT and 18 years for surgery, has been updated. In SOC8, to initiate GnRHa or GAHT in the youth, they must exhibit an early sign of entering puberty (Tanner stage 2). Another important consideration is that the TGD individuals must be on stable GAHT treatment for at least 6 months before GAS in adults, 12 months in adolescents unless the GAHT is not desired or contraindicated. Nahata et al reported median age at which puberty blockers and cross-sex hormone therapy were prescribed was 15.0 (range 9–18 years) and 16.0 (range 14–18 years), respectively. Median age at first Endocrinology visit was 15.2 years (range 9–18 years) (Nahata et al., 2017).

The common indications to initiate treatment across all groups (transgender adults and adolescents of both genders) include 1) having marked and sustained gender incongruence, 2) having the ability to consent, 3) that the other possible causes of gender incongruence have been ruled out, and 4) that TGD individuals fully understand the effect and consequences of treatment and thus, the benefits and risks of GAHT should be discussed, including the risk if infertility.

This review is a narrative review intended to provide up-to-date and comprehensive information regarding fertility preservation (FP) options for TGD people. We will review standard of care and experimental options for FP; implications of gender affirming treatments for FP as well as future reproductive options. A literature search was conducted separately for each topic using Pubmed/MEDLINE combined database and hand search from the review references.

Effects of GAHT on fertility

GAHT showed unpredictable and negative effect to fertility. Therefore, the Endocrine Society, WPATH, American Society for Reproductive Medicine (ASRM), and European Society of Human Reproduction and Embryology (ESHRE) recommended counseling on the impact of GAMST on fertility and options for fertility preservation prior to and periodically during GAMST (Anderson et al., 2020, Ethics Committee of the American Society for Reproductive Medicine, 2021, Hembree et al., 2017, Coleman et al., 2022). The GAHT-prior counseling should include informing and discussing positive and negative effects of GAHT in every aspect, not limited to reproductive health. In this section, we will discuss the effect GAHT has directly on gametogenesis and fertility.

Effect of GAHT on spermatogenesis:

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GAHT effects on TGD individuals with testes are pervasive (Andrews et al., 2021). The severity of spermatogenesis defect can be represented using testis histopathology classification (McLachlan et al., 2007) and semen analysis. Histopathology findings of GAHT-exposed testicular tissues with regards to degree of spermatogenesis were summarized in Table 1. It is worth noting that androgen cessation is usually recommended before GAS-orchiectomy with 2-6 weeks duration depending on centers. These periods of androgen cessation may or may not have positive impact on spermatogenesis in the testicular tissue. However, the data are inconclusive, and the duration of hormonal cessation is unknown.

Testicular histology findings in TGD people with testes receiving GAHT showed evidence of complete spermatogenesis (normal/hypospermatogenesis) in 0-37% of the specimen, with 21-100% presence of germ cells. Studies found no correlations between evidence of spermatogenesis and the hormonal regimen, dosage, duration on GAHT, or time off GAHT before GAS, which may be attributed to small sample sizes. Nevertheless, these findings indicate the possibility of utilizing discarded testes at the time of GAS for fertility preservation. Utilization of tissues may include but is not limited to large-volume testicular sperm extraction (TESE) on discarded testes (Niederberger, 2020), and testicular tissue cryopreservation (TTC) for utilization of experimental approaches when technologies mature (please see section: Fertility preservation options –TGD people with testes -Experimental).

Effect of GAHT on oogenesis:

Regarding the ovarian histologic findings in testosterone-exposed TGD people with ovaries, there were studies that reported histological finding resembling those of polycystic ovarian syndrome (PCOS) (Spinder et al., 1989, Grynberg et al., 2010, Pache et al., 1991), the disease which also involves high testosterone exposure, and the studies that found no differences in number of primordial, early, antral follicles compared to control (Ikeda et al., 2013, De Roo et al., 2017, Bailie et al., 2023). Table 2 summarizes the important study designs from each report.

Fertility preservation options

There are still no standard guidelines regarding FP choices for TGD individuals. This may be due to limited evidence to make the recommendations. We will review standard of care fertility preservation options that have been offered to TGD individuals and experimental options that are offered at a very few centers with IRB approval. It is very important to note that, unlike in cancer patients, FP interventions are not usually offered until Tanner stage 2 (approximately 11 years old in female and 11.5 years old in male) is reached as this stage of development is required for GnRHa/GAHT initiation. Therefore, we will focus our review on findings from the peripubertal period and older.

Fertility preservation options for TGD people with testes

Two fertility preservation options are possible for TGD people with testes. The established and standard of care option is to cryopreserve a semen sample with sperm. Cryopreserved sperm can be thawed in the future to fertilize partner or donor eggs and establish a pregnancy. This method has extensive evidence supporting its use in adult cisgender males and is the only recommended standard protocol for adults facing gonadal threats, such as chemotherapy or total body radiation (Gassei et al., 2017, Practice Committee of the American Society for Reproductive Medicine, 2019, Oktay et al., 2018, Martinez, 2017). The second option is testicular tissue cryopreservation (TTC), which is typically reserved for prepubertal patients who are not producing sperm. TTC is experimental both for cisgender patients with a cancer diagnosis or TGD patients with a gender dysphoria diagnosis because there is no evidence yet that those tissues can be matured in the future to produce sperm. While many centers around the world provide TTC to cancer or bone marrow transplantation patients who are at risk if infertility, very few provide this service to TGD individuals who cannot or will not interrupt GAHT to collect and freeze a semen sample with sperm. TESE can be offered to TGD people with testes who are going to gender-affirming surgery, as the testes are typically removed during the GAS process and would otherwise be discarded. However, the long-term impact of GAHT prior to GAS is not known. Table 3 summarizes fertility preservation outcomes by semen collection and TESE based on age groups and history of GnRHa/GAHT exposure.

Standard of care FP options for TGD people with testes

Adult

Before the initiation of GnRHa or GAHT

Although sperm cryopreservation is recommended in adults who can produce sperm, collection of semen via masturbation may cause psychological distress and exacerbate gender dysphoria in some cases (Reckhow et al., 2023). Also, there is a high prevalence (47%) of orgasmic dysfunction in TGD people with testes, even before GAHT (Kerckhof et al., 2019). In such cases, alternative ways to obtained sperm such as Electro- or vibratory stimulation, TESE, Testicular Sperm Aspiration (TESE, TESA), or Epididymal Sperm Aspiration (PESA) among others, may be offered (Esteves et al., 2011). Adult TGDTGD people with testes also had poorer semen parameters (sperm concentration, total motile sperm count and/or morphology) compared to the WHO-referenced male, or healthy cisgender male control group even before GAHT (Li et al., 2018, de Nie et al., 2020, Rodriguez-Wallberg et al., 2021a) (Table 3). Although not directly evaluated in these reports, poor sperm parameters before GAHT were thought to be attributed to lifestyle or environmental factors such as the tucking of the testicles (Trussler and Carrasquillo, 2020). Additionally, cryopreserved semen from TGD individuals before GAHT showed that only 26% of the post-thawed samples were of adequate quality for intrauterine insemination (IUI), the cheapest and simplest assisted reproductive technology (ART) (de Nie et al., 2020).

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Hamada et al., 2015). Therefore, even when pursuing FP before GAHT, TGD patients with testes may need to plan for more expensive ARTs in the future, such as in vitro fertilization (IVF) with intracytoplasmic sperm injection (ICSI). However, Hamada and colleagues did report a case of fertilization and pregnancy using a single transwoman's cryopreserved sperm for IUI in a surrogate mother (Hamada et al., 2015).

After the initiation of GnRHa or GAHT

TGD people with testes whose GAHT treatment has been initiated without prior fertility preservation can collect sperm via the same means as the GAHT-naïve group, opening up more flexibility to those who were undecisive, prioritized initiation of GAHT or simply change their plan on family building. There is histologic evidence of complete spermatogenesis (Table 1) and the evidence to suggest that sperm can be recovered in the semen or by TESE after temporary cessation of gender affirming treatments in some cases (Table 3). Therefore, the state of GnRHa or GAHT should not preclude fertility preservation. TGD

Adolescent

Recommendations of the FP choice in adolescent TGD people with testes is still sperm cryopreservation. However, this may not be feasible in adolescents whose age is less than 15 years old because of high prevalence of azoospermia (no sperm in the ejaculate). A recent study in peripubertal cancer patients reported azoospermia in 66.7% of 12 year olds, 31.3% of 13 year olds and approximately 10% of 14-17 year olds, decreasing to 0% in 18-19 year olds (Halpern et al., 2019). Even if no sperm are found in the ejaculate, it is sometimes possible to retrieve sperm directly from the testis by TESE. Peri and colleagues reported that sperm recovery via TESE was successful in 68% of patients in the 13-17 year-old range with no prior gender affirming treatments (Peri et al., 2021) (Table 3). TGD

Experimental: Testicular tissue cryopreservation

Testicular tissue cryopreservation (TTC) has been offered and studied as an experimental FP approach in prepubertal cancer patients worldwide with the expectation that these tissues can be matured in the future to produce sperm from resident spermatogonial stem cells (SSCs) (Tran et al., 2022). Our center has extended this experimental FP option to young TGD patients (NCT05829928). This protocol is separate from our cancer patient TTC protocol because the risks and benefits for TGD patients are different than cancer patients. Our center is approved to cryopreserve testicular tissues for patients who have a diagnosis of gender dysphoria and are referred by their physician for fertility preservation. Patients must be ≥9 years old, getting ready to start or already on gender affirming treatments, and unwilling or unable to delay or interrupt GnRHa or GAHT to collect sperm. If patients are 12 years or older, we provide the option to search a portion of the tissue for sperm, similar to TESE. However, the majority of the tissue is cryopreserved with the expectation that SSCs in the tissue have the potential to produce sperm in the future. Peri and colleagues reported retrieval of sperm from the

testicular tissues of young TGD patients who were Tanner stage 3 or higher and when testis volumes were greater than 10-12 mL. Age, hormone levels and previous gender affirming treatments were not reliable determinants of whether sperm could be retrieved from testicular tissues (Peri et al., 2021). Therefore, Tanner staging and testis volume data may be useful in counseling young TGD patients about the potential future uses of their cryopreserved testicular tissue. Several studies showed the presence of undifferentiated germ cells (stem and progenitor spermatogonia) in TGD testicular tissue regardless of GAHT history, showing potential utility of cryopreserved testicular tissues in this group (Table 1). This may suggest that suspension of gender affirming treatments is not necessary prior to cryopreservation of testicular tissue with SSCs. TTC may also be possible when testes are being removed for GAS. However, there are no data on the function of germ cells that may remain in that tissue after long term GAHT treatment. Studies in animal models have shown different ways to utilize the cryopreserved testicular tissue in both tissue-based and cell-based approaches (reviewed in (Tran et al., 2022)). Future utilization of tissues requires different considerations than in cisgender cancer survivors because TGD people may not want the tissue or cells transplanted back into their body or go through puberty in the gender that would be required to mature their tissues/cells inside their bodies. Methods to mature testicular tissue or cells outside the body to produce sperm (see below) may be required but are in very early stages of development.

Potential uses of cryopreserved testicular tissues in reproduction: Considerations for TGD individuals

Testicular tissue or cell transplantation

Brinster and colleagues pioneered the method of spermatogonial stem cell transplantation more than three decades ago. Testicular cells (including SSCs) were injected into the seminiferous tubules of the testes where they regenerated spermatogenesis with sperm that were competent to fertilize and produce offspring (Brinster and Zimmermann, 1994, Brinster and Avarbock, 1994). Donor SSCs of any age are competent to regenerate spermatogenesis. In addition, cells that were thawed after 14 years of cryostorage could regenerate spermatogenesis (Wu et al., 2012), which is relevant in the context of fertility preservation in young patients. Testicular tissue grafting is an alternative approach that involves transplanting intact pieces of testicular tissue under the skin. Fresh or cryopreserved immature testicular tissue can be matured over several months in vivo and then recovered and dissected to release sperm that are competent to fertilize by IVF with ICSI and produce offspring (Honaramooz et al., 2002, Schlatt et al., 2003, Shinohara et al., 2002, Fayomi et al., 2019). Testicular tissue grafting is usually performed in castrated recipients, which may be germane to TGD patients after GAS. This approach works only with immature (prepubertal) testicular tissues and not adult tissues (Arregui and Dobrinski, 2014). It is not known whether testicular tissues from TGD patients where spermatogenesis is suppressed by gender affirming treatments would function more like adult tissues or immature prepubertal tissues in this context. However, it is noteworthy that when spermatogenesis was suppressed in mice with

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acyline (GnRH antagonist) prior to transplantation, grafts survived and produced spermatogenesis (Arregui et al., 2012).

Spermatogonial stem cell transplantation and testicular tissue grafting are mature technologies that have been replicated in numerous animal models including nonhuman primates (reviewed in (Tran et al., 2022)) and may be ready for translation to the human clinic. However, as indicated above, TGD patients may not want their testicular tissues or cells transplanted back into their body or to go through male puberty with testosterone production that is necessary for spermatogenesis to occur from transplanted testicular cells or tissues. Below we review ex vivo approaches to mature testicular tissues or cells and produce sperm. These methods are much earlier stage than the transplant approaches described above but may have valuable application for TGD patients who have cryopreserved their testicular tissues.

Xenotransplant into SCID/Nude mice or other animal hosts

An alternative to autologous transplantation is testicular tissue grafting into an animal host. Testicular tissue from several species (reviewed in (Tran et al., 2022)) can be transplanted under the dorsal skin or scrotal skin of immune-deficient SCID or nude mice and matured to produce sperm as well as offspring in rabbit (Shinohara et al., 2002), pig (Nakai et al., 2010) and monkey (Liu et al., 2016). In human, the most advanced germ cells produced by this technique were premeiotic spermatocytes, which have been reported for both immature and adult as well as fresh or frozen human testicular grafts (References can be reviewed in Table 4). It is unclear why prepubertal monkey testicular tissues can be matured to produce sperm in a mouse host, while human testicular tissues cannot. Perhaps other animal hosts, such as immune-deficient pigs (Boettcher et al., 2018) will support better development of human tissues. The risk of transmitting viruses or other xenobiotics from the animal host to the patient must be carefully considered (Kimsa et al., 2014, 2017). However, it is noteworthy pigs are actively being developed as organ donors for human patients (Kozlov, 2024).

In vitro maturation with testicular tissue organ culture

Sato and colleagues pioneered a method for culturing immature mouse testicular tissues at the air-liquid interface. Tissues matured over several weeks in culture and produced sperm that were competent to fertilize and produce offspring (Sato et al., 2011). Like the testicular tissue grafting, this approach only works with immature testicular tissues; and it is not yet known whether it would work with testicular tissues where spermatogenesis is suppressed by gender affirming treatments. Several groups have reported culturing human testicular tissues at the air-liquid interface. Tissues could be maintained for weeks to months with the maintenance of spermatogonia and occasional differentiation to produce spermatocytes or spermatids but not sperm (Medrano et al., 2018, Younis et al., 2023, Portela et al., 2019, de Michele et al., 2018). Komeya and colleagues reported that GAHT exposed testicular tissues could be maintained for two weeks in culture, but the

number of germ cells declined over that time (Komeya et al., 2021). Testing the fertilization potential of experimentally derived human sperm, using this approach or others, is necessary to demonstrate the safety and feasibility, but raises ethical concerns and is challenged by restrictive funding or laws in some states and countries.

De novo testicular morphogenesis in an animal host or organoid culture

Heterogeneous testis cell suspensions have the remarkable ability to reform seminiferous tubules, both in vivo and ex vivo. Testis cells from mice, sheep and pigs can be pelleted and transplanted under the skin of mouse recipients where they reform into seminiferous tubules, which sometimes contain spermatids and/or sperm (Honaramooz et al., 2007, Arregui et al., 2008, Kita et al., 2007). The fertilization potential of those sperm has not been tested and to our knowledge, in vivo de novo testicular morphogenesis has not been reported with human testis cells. Many groups have described methods for de novo testicular morphogenesis ex vivo, but none have yet produced sperm or offspring. Sakib and colleagues reported a microwell aggregation approach to produce 3D testicular organoids from neonatal or prepubertal testicular cell suspensions of mice, pigs, monkeys and humans. The tubules formed inside out and contained spermatogonia but did not support complete spermatogenesis (Sakib et al., 2019). Two studies reported human testicular organoids from adult (15+ years) formed in human testicular extracellular matrix (htECM). Baert and colleagues seeded heterogeneous prepubertal or adult human testis cell suspensions onto a 3-dimensional htECM scaffold that was shaped in the form of a tubule (Baert et al., 2017). Pendergraft and colleagues used a hanging-drop method to induce organoid formation from cultured adult human spermatogonia mixed with immortalized human Sertoli and Leydig cells suspended in a hydrogel of htECM (Pendergraft et al., 2017). Both approaches led to the production of organoids including germ cells and somatic cells but neither approach produced seminiferous tubule-like structures (Pendergraft et al., 2017, Baert et al., 2017). The Pendergraft study reported elongated spermatids, but since the starting point was adult tissues, it is impossible to determine whether those post-meiotic spermatids arose in culture or were already present in the original cell suspension (Pendergraft et al., 2017) (Table 4).

Standard of care FP options for TGD people with ovaries

Adult

Before the initiation of GAHT

Ovarian stimulation and oocyte cryopreservation can be done the same way as for cisgender females. Maxwell and colleagues reported successful four live births in two couples utilizing cryopreserved oocytes from GAHT-naïve adult TGD with ovaries followed by fertilization with donor sperm and embryo transfer into cisgender, sexually intimate, female partners (Maxwell et al., 2017, Adeleye et al., 2019a). TGD people with ovaries (with

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and without prior testosterone exposure) produced a similar number of oocytes, with a similar maturity rate as age/BMI matched cisgender women (Adeleye et al., 2019a, Leung et al., 2019) (Table 5).

After the initiation of GAHT

Oocyte cryopreservation and embryo cryopreservation can be offered even after the initiation of GAHT. However, Adeleye et al reported that the number of oocytes retrieved from GAHT naïve TGD with ovaries was higher than the group with prior GAHT who had suspended testosterone treatment for a median time of 6 months (Adeleye et al., 2019a). The main question in this scenario is whether or not to discontinue testosterone supplements before oocyte retrieval. Testosterone cessation has traditionally been encouraged to ensure good oocyte retrieval outcome, and the duration recommended is at least 3 months or until the return of menstruation (De Roo et al., 2016, Armuand et al., 2017). However, the necessity to suspend GAHT and resume menstruation requires further investigated because GAHT interruption can cause distress in TGD people (Armuand et al., 2017). (Greenwald et al., 2022)(Table 5).

Ovarian tissue cryopreservation (OTC) is no longer considered experimental by the ASRM (Practice Committee of the American Society for Reproductive Medicine, 2019), based in part on the evidence of more than 130 live births from transplanted ovarian tissues (Donnez and Dolmans, 2017). However, that guidance was based almost entirely on data from survivors of cancer or bone marrow transplantation who were adults at the time of OTC. Data on the transplantation potential of ovarian tissues that were cryopreserved during childhood or from TGD individuals on GAHT are limited or absent, respectively. Thus, it is reasonable to offer OTC as an experimental option until more transplantation and live birth data can be accumulated for those populations.

Adolescent

Before GAHT initiation

Oocyte cryopreservation is the standard fertility preservation option for hormone-naïve adolescent TGD people with ovaries. OTC could be offered at the time of GAS, but those patients have usually already initiated GAHT according to WPATH SOC 8 recommendations. According to WPATH SOC 8, GAS is usually recommended after 6 months of stable GAHT in adult and 12 months in youth unless the GAHT is not desired or contraindicated. This means that, in most cases, OTC with simultaneous GAS is generally not possible unless GAHT has begun. (Amir et al., 2020a)Embryo cryopreservation, which requires partner sperm, is not usually offered in adolescents. OTC for fertility preservation is not generally offered as a stand-alone option to adolescent TGD patients with ovaries, although it is offered at our center as an experimental protocol (NCT05863676).

After initiation of gender affirming treatments

Two studies have shown successful oocyte retrieval in adolescent TGD with ovaries who had GnRHa only, and who had prior testosterone use (Insogna et al., 2020, Barrett et al., 2022). Considerations for embryo freezing and OTC are the same as described above. Our center does not require cessation of GnRHa or GAHT prior to OTC. This may be a consideration for TGD people who do not want to interrupt their gender affirming treatments for fertility preservation.

Potential uses of cryopreserved ovarian tissues in reproduction: Considerations for TGD individuals

Autologous transplantation

Cryopreserved ovarian tissue can be transplanted back to the donor on the ovary or pelvic site. Transplanted ovarian tissues can restore hormonal and reproductive function, including the possibility of in vivo conception and pregnancy. There are more than 180 live births after transplantation of cryopreserved ovarian tissues using in vivo conception or IVF (Khattak et al., 2022, Practice Committee of the American Society for Reproductive Medicine, 2019, Gellert et al., 2018, Donnez and Dolmans, 2017) Transplantation of ovarian tissues that were cryopreserved in prepuberty, adolescence or adulthood have resulted in live births (Table 6). To our knowledge, there are no reports of ovarian tissue transplantation in TGD individuals. While ovarian tissue transplantation is a robust technology, it probably requires GAHT cessation and production of estrogen from developing follicles. However, we note that ovulation appears to be possible while still on testosterone treatment (Asseler et al., 2024, Stark and Mok-Lin, 2022, Gale et al., 2021, White et al., 2024, Greenwald et al., 2022). Additional research may reveal protocols that enable follicle development in transplanted ovarian tissues without compromising gender affirming medical treatments.

Ovarian tissue oocyte followed by in vitro maturation (OTO/IVM)

OTC can be performed prior to the initiation of GAHT, During GAHT or concomitantly with ovariectomy as a part of the GAS. During ovarian tissue processing, the outer cortex of the ovary, which contains primordial follicles, is dissected away from the inner medulla and then cut into strips for cryopreservation. Small antral follicles that are present in the medulla are released into the dissection media and are usually discarded. Cumulus-oocyte complexes (COCs) retrieved from these medullary antral follicles can potentially be matured to produce MII oocytes or embryos that can be cryopreserved in parallel with the ovarian tissues (Cadenas et al., 2023). This approach does not require stimulation with exogenous hormones because the final steps of egg maturation occur in vitro. The birth of five healthy infants have been reported using this approach (Prasath et al., 2014, Uzelac et al., 2015, Segers et al., 2020)(Table 6). While data are limited in TGD with ovaries, studies showed normal oocyte distribution across all layers of ovarian tissue (de Michele et al., 2017, Bailie et al., 2023), though one study indicated higher yH2AX staining, marker for DNA breaks, in primordial germ cells compared to cisgender control (Bailie et al., 2023). The cumulus-oocyte complexes (COCs) that were extracted

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from the medulla resulted in MII oocytes after IVM with 87% normal spindle structure, also indicating the possibility of using ovarian tissue oocytes with IVM (OTO-IVM) in TGD people with ovaries and a history of GAHT. However, poor embryo development was noted in GAHT exposed in vitro-matured ovarian tissue oocytes recovered at the time of GAS during ovarian tissue processing(Lierman et al., 2021, Christodoulaki et al., 2023) and may be improved by spindle transfer (Christodoulaki et al., 2023). Thus, OTC earlier in transition before exposure to GAHT may be beneficial.

In vitro growth of primordial follicles followed by IVM in multistep culture

Cortical strips contain primordial follicles that can be extracted for in vitro development of primordial follicles (primordial follicle to antral follicle) and IVM (immature antral follicle to MII oocytes). The resulting MII can then be used for cryopreservation or fertilized for embryo transfer/cryopreservation. This approach has been studied as an alternative for cancer patients where chance of reintroducing of cancer is high. It shows possibility in TGD with ovaries whose primordial follicles are retained in the cortical strip, and the reversal of GAHT is not required at time of fertility restoration. While in vitro maturation from primordial follicles to mature MII oocytes and preimplantation embryos was described more than a decade ago in mice (Jin et al., 2010), IVM to mature MII oocytes has only been achieved when starting from growing primary and secondary follicles in primates and humans (Xu et al., 2013)(reviewed in (Hu et al., 2023)). An artificial ovary that reconstitutes the ovarian microenvironment ex vivo may provide a path forward (Amorim and Shikanov, 2016, Laronda et al., 2017).

Barriers to successful fertility restoration in TGD communities

Reproductive desire and/or interest in family building is high among transgender people, both adult and youth, but the FP services are reportedly under-utilized in many countries around the world. A meta-analysis using 76 studies showed 48.7-67.0% of transgender adolescents and 18.4-82.1% of transgender adults desired for children, but the FP utilization rates were 2-4% (Stolk et al., 2023). It is noteworthy that successful sperm/oocyte/gonadal tissue cryopreservation is only the beginning of the journey to successful family building. Multidisciplinary teams are required to ensure that TGD people have access to fertility preservation care and develop technologies that will enable them to use their cryopreserved cells or tissues for family building with minimal disruption to gender affirming care. Figure 1 shows the journey of a TGD person to have a biological child. For TGD people with testes, ejaculated sperm or sperm from testicular tissues can be used to fertilize partner or donor eggs using standard Assisted Reproductive Technologies (ARTs). If the partner has testes, egg donation for same-sex couple and surrogacy is often required. For TGD people with ovaries, fertility seems to be less affected by hormonal treatment compared to the TGD people with testes. Once oocytes are collected by hormonal stimulation or from ovarian tissues, partner or donor sperm and ART are required for fertilization and

conception. If the partner has ovaries, sperm donation for same-sex couples will be needed. Surrogacy is possible but may not be needed if their partner is a biological female who will carry the pregnancy.

Conclusions

The impacts of gender affirming treatments on fertility and family building should be discussed as before and throughout treatment. Explaining options for fertility preservation and restoration provides a sense of reproductive autonomy, even if the patient is unsure of their family building goals. Like FP for cancer patients, it is important to start these discussions early while the medical and research communities are still learning the impacts of gender affirming treatments on the ovaries, testes, eggs and sperm. Early intervention for FP may be important in some cases. For fertility preservation to accomplish its purpose (which is to allow TGD people to have biological children if they want to), it takes multidisciplinary teams, ranging from pediatric and adult endocrinologists, mental health professionals, reproductive medicine experts and scientists. Laws that support same sex parenting, egg/sperm donation for same sex couples and surrogacy will help ensure that TGD people have the same access to reproductive care as cisgender people. There is an unmet need for counseling and education to cisgender and TGD communities about the availability, accessibility and feasibility of fertility preservation and fertility restoration options for all people as well as the specific challenges and opportunities for TGD people.

Declaration of Interest

The authors declare no conflicts of interest with the work presented in this manuscript.

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Author Contributions

CA wrote the initial draft of the manuscript and produced the figure and tables. KEO edited and revised the manuscript, figure and tables. Both authors approved the manuscript for submission.

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Table 1 Effect of GAHT on spermatogenesis in TGD adults with testes prior to gender affirming surgery

Study	Adults/ testes examined, *n*	Age *(years)	Normal *n* (%)	Hypo *n* (%)	Maturation arrest *n* (%)	Presence of germ cells *n* (%)	GAHT regimen	Duration on GAHT* (months)	Duration of cessation before GAS
Dabel *et al*. (2023)	25	28.1 (16 – 40)	0	0	SG:17 (68.0); SC: 5 (20.0); RS: 3 (12.0)	25 (100)§	Cyproterone acetate + estrogens	27.6 (11–66)	0-6 weeks
de Nie *et al*. (2021)	19	19.0 ± 1.5 (TS:2–3)	0	0	19 (100)	19 (100)	triptorelin or cyproterone acetate + estrogens (May include GnRHa in adolescent group, detail not specified)	5.9 ± 1.4	4 weeks
	10	19.6 ± 1.9 (TS:2–3)	0	0	10 (100)	10 (100)		6.8 ± 1.3	0
	35	19.7 ± 1.2 (TS:4–5)	0	2	33 (94.3)	35 (100)		4.1 ± 1.8	4 weeks
	14	19.3 ± 0.7 (TS:4–5)	0	3	11 (78.6)	14 (100)		2.8 ± 0.6	0
	62	34.5 ± 12.3	0	5	52 (83.9)	57 (91.9)		2.8 ± 1.9	4 weeks
	74	36.2 ± 12.2	0	1	63 (85.1)	64 (86.5)		2.3 ± 1.2	0
Sinha *et al*. (2021)	85	39 ± 16	7 (8.2)	17 (20.0)	24 (28.2)	24 (28.2)	Mixed regimen	48 (24-60)†	NS, likely continuous
Vereecke *et al*. (2020)	97	31.19 (23.25–45.78)†	0 (acrosin-negative)	0 (acrosin-negative)	SG: 85 (87.6)‡	85 (87.6)	Cyproterone acetate + estrogen	21.7 (15.2-28.4)†	2 weeks
Jiang *et al*. (2019)	141 testes	39 (30-53)†	0	57 (40.4)	unspecified spermatid present	114 (81)	Spironolactone, estrogen, progesterone	39 (24-65) †	2 weeks cessation of estrogen in vaginoplasty cases; the rest with continuous spironolactone or progesterone,
Jindarak *et al*. (2018)	173	26.09 ± 5.37	19 (11)	45 (26.0)	63 (36.4)	127 (73.4)	Mixed regimen	102.2 ± 55.2	4 weeks
Kent *et al*. (2018)	135	30 (18-76)†	6 (4)	0	17 (5.2)	28 (21%)	Spironolactone + estradiol and/or finasteride, progesterone	60 (12-684)†	NS
Matoso *et al*. (2018)	99 testes	33 (21-63)	0	0	SG:79 (80); SC:20 (20)	99 (100%)	Estradiol and/or spironolactone, finasteride, progesterone	6-240	NS
Schneider *et al*. (2015)	108	42 ± 12.1	26 (24.1)□		SG: 38 (35.19); SC:26 (24.07)	90 (83.3)	Mixed regimen	NS	Combined cohorts
	22		10 (45.5)						6 weeks
	51		22 (43.1)						2 weeks
	35		14 (40.0)						0 week

TGD = Transgender and gender diverse, GAHT = Gender-Affirming Hormone Therapy, MAGE-A4, marker for spermatogonia and early spermatocytes), boule homologue, RNA-binding protein (BOLL, marker for secondary spermatocytes and round spermatids), cAMP-responsive element modulator (CREM, marker for round spermatids) and acrosin (marker for acrosome visualization); SG, spermatogonia; SC, spermatocytes; RS, round spermatids; NS, not specified; TS, Tanner stage.

*Mean unless stated otherwise; †values are median (IQR); □complete spermatogenesis; ‡ Spermatogonia (MAGEA4+) positive (among these: 22 contained spermatocytes (BOLL+) and 14 contained spermatids (CREM+); § lower SG count/mm^2 seminiferous tubule compared to cisgender age-matched control;

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Table 2 Effects of GAHT on the ovarian tissues of TGD with ovaries

Effect/Study	**Donors, *n***	**Age (years)**	**Testosterone. exposure duration**	**Control**	**Summary of findings**
Consistent with ovarian syndrome-like change					
Pache *et al*. (1991)	17	25 (18-35)	21 months average	13 (Age: 29 (27-39))	– Cortex and stromal thickening compared to control – More antral follicles compared to control – Multiple cystic atretic follicles
Grynberg *et al*. (2010)	112	28.9 ± 0.9	2–9 years (3.7 ± 0.6)	None	– Cortex and stromal thickening – More than 12 antral follicles/ovaries (PCOS features) in 89 (79.5%)
Spinder *et al.* (1989)	26	26 ± 6	9–36 months	9 age-matched patients	– PCOS features in 18 (69.2%). (3/4 of stromal hyperplasia, multiple cystic follicles, collagenization of the tunica albuginea in 25 subjects (96.2%), and luteinization of stromal cells)
Comparable oocyte distribution to control, no PCOS features					–
Ikeda *et al*. (2013)	11	27-38	17 months – 14 years (median: 38 months)	10 age and BMI-matched oncology patients	– No differences in oocyte distribution numbers compared to control – Cortical and medullary hyperplasia noted
De Roo *et al.* (2017)	40	24.30±6.15	14.5±6.6 months	– Compare with previously published normal values – No internal control group	– No differences in oocyte distribution numbers compared to control – Cortical and medullary hyperplasia noted
Bailie *et al*. (2023)	8	27.6 ±1.7	18 months–10 years	31.8 ± 1.5 healthy donors	– Higher proportion of non-growing ovarian follicles, higher levels of DNA damage. – More growing follicles in transgender ovaries compared to control, but follicle health further deteriorated

GAHT = Gender-Affirming Hormone Therapy, TGD = Transgender and gender diverse, PCOS = Polycystic Ovarian Syndrome, BMI = body mass index, SD = standard deviation

Table 3 Fertility preservation (semen analysis or TESE outcomes) in TGD people with testes

Age group	Semen analysis or TESE outcome		
	No GnRHa and GAHT exposure	**With ongoing GnRHa/GAHT exposure**	**With previous GnRHa or GAHT exposure**
Adult	– Poor semen parameters compared to referenced samples in adult TGD with testes (Li et al., 2018, de Nie et al., 2020, Adeleye et al., 2019b, Rodriguez-Wallberg et al., 2021a, Hamada et al., 2015, Barda et al., 2023) – Poor semen parameter in post-thawed samples (de Nie et al., 2020, Hamada et al., 2015)	– Low semen parameters compared to previously-used GAHT and GAHT-naïve in adult TGD with testes (Adeleye et al., 2019b)	– Semen parameters poorer than GAHT-naïve TGD samples (Rodriguez-Wallberg et al., 2021a) – Semen parameters comparable with GAHT-naïve TGD samples. (Adeleye et al., 2019b, Barda et al., 2023) – Semen parameters higher than continuously-used GAHT (Adeleye et al., 2019b) – Normal semen parameters in 4 cases, lower in 4 cases with successful sperm recovery by masturbation, 1 case by TESE, and a natural conception was reported in 3 cases (de Nie et al., 2023)
Peripubertal	– Successful sperm retrieval rate with TESE in 68% (17/25), in 13-17 yo TGD with testes (Peri et al., 2021) – Normal semen parameters except for low percentage (3%) of normal morphology compared to normal reference per Modified Kruger criteria (>13%) in group with mean age 19.5 (16-24 yo, *n*=8) (Barnard et al., 2019)	No data	– 12 sperm (2 motile) found 3 months after suspending GnRHa; Normal semen sample 5 months after suspending GnRHa (age 17.5 at GnRHa initiation, 18 at retrieval, n=1); Azoospermic 4 months after suspending GAHT (age 18 at initiation, 19 at retrieval, *n*=1) (Barnard et al., 2019)

TESE = Testicular Sperm Extraction, TGD = Transgender and gender diverse, GnRHa = Gonadotropin Releasing Hormone Agonist, GAHT = Gender-Affirming Hormone Therapy, yo = years old

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Table 4 Developing technologies for maturing TGD patient testicular tissues/cells and producing sperm outside the patient's body. Evidence in human studies.

Method/Technique/Tissue/Cell types	**YO**	***n***	**Reference**
Xenotransplant into SCID or nude mice			
Tissue- based			
Prepubertal tissue			
Fresh tissue into dorsal skin			
Spermatogonia	10-11	4	Goossens et al. 2008
BOLL+spermatocytes			
Fresh tissue into scrotum			
Spermatogonia	5	1	Van Saen et al. 2010
Spermatocyte	12 – 13	2	Van Saen et al. 2010
	2 – 12	10	Poels et al. 2013
	3 - 9	3	Ntemou et al. 2019
Frozen prepubertal tissue into scrotum			
Spermatogonia	3 – 13	3	Van Saen et al. 2010
	2 – 12	11	Wyns et al. 2007
	2 – 15	6	Poels et al. 2014
Spermatocytes	7 – 14	5	Wyns et al. 2008
	2 – 12	10	Poels et al. 2013
Adult tissue			
Fresh tissue into dorsal skin			
Degenerated tissue			Schlatt et al., 2006
Spermatogonia			Geens et al., 2006
Fresh adult tissue into scrotum			
Spermatocyte			Van Saen et al. 2010
Frozen adult tissue into scrotum			
Spermatocyte			Van Saen et al. 2010
In vitro maturation with testicular tissue culture			
Tissue- based			
Immature	6-14		
Used fresh			
Spermatogonia			Portela et al., 2019
Used frozen			
Spermatogonia			Portela et al., 2019, de Michele et al., 2017
SYCP3+ primary spermatocytes			Medrano et al., 2018
SYCP3+ primary spermatocyte			Younis et al., 2023
Round spermatid			de Michele et al., 2018
Adult tissue			
Used fresh			
Spermatogonia			Jorgensen et al., 2014
Fresh and cryopreserved adult GAHT-exposed testicular tissue maintained for 2 weeks, no progression of spermatogenesis			Komeya et al., 2021
De novo testicular morphogenesis (organoid culture)			
Cell-based			
Fresh pubertal (age 15) and adult testicular cells			
Scaffold-based, or scaffold-free transwell			
Mitotically-active germ cells, normal somatic cells function and arrangement			Baert et al., 2017
Frozen prepubertal testicular cells			
Matrigel			
Inverted organization of spermatogonia and somatic cells			Sakib et al., 2019
Fresh and frozen adult testicular cells			
ECM			
Spermatogonia clusters, normal somatic cells function and arrangement			Baert et al., 2015
PRM2+ Elongated spermatids			Nikmahzar et al., 2023, Pendergraft et al., 2017

TGD = Transgender and gender diverse, GnRHa = Gonadotropin-releasing Hormone Agonist, GAHT = Gender-Affirming hormone Therapy, yo=years old, SYCP3 = Synaptonemal complex protein 3 (marker for primary spermatocytes), SCID = severe combined immunodeficiency, BOLL = boule homologue RNA-binding protein (marker for secondary spermatocytes and round spermatids)

Table 5 Oocyte cryopreservation fertility outcomes in TGD with ovaries

Age group	Oocyte cryopreservation		
	No GAHT exposure	**With continued GAHT at collection**	**Stop GAHT at collection**
Adults	– 2 case reports of live births with cryopreserved oocytes and/or embryos (Maxwell et al., 2017). – 3 pregnancies conceived with oocytes from TGD people with ovaries (Adeleye et al., 2019a). – No differences in the number of retrieved oocytes, MII oocytes, and maturity rate between GAHT-naïve and GAHT-exposed adult TGD people with ovaries, and between all adult transmen and age/BMI-matched cisgender females (Adeleye et al., 2019a, Leung et al., 2019), or between cisgender female and GAHT-exposed TGD people with ovaries (Leung et al., 2019). – The amount of FSH used for ovulation induction was significantly lower than for cisgender women. Peak estradiol levels, number of oocytes retrieved, number of MII oocytes and oocyte maturity rate were comparable to cisgender women (Amir et al., 2020b).	– 1 case report LB Fresh transfer reciprocal IVF (Greenwald et al., 2022). – Case report 1 LB with fresh transfer (White et al., 2024). – successful oocyte retrieval (Cho et al., 2020, Gale et al., 2021, Stark and Mok-Lin, 2022). – Successful oocyte retrieval, fertilization and embryo development in two cases. One blastocysts from one case was transferred and resulted in a live birth with no pregnancy or neonatal complications (Moravek et al., 2023).	– Discontinue for 6 months – 2 pregnancies (fresh transfer and embryo cryopreservation)(Adeleye et al., 2019a). – 5 LBs -IUI with donor sperm, IVF and reciprocal IVF, no freezing (Ghofranian et al., 2023). – 7 LBs with fresh or frozen transfer (Leung et al., 2019). – Six cases. Time on testosterone ranged from 14-144 months. Time of cessation ranged from 5-22.5 months. Peak estradiol levels, number of oocytes retrieved, number of MII oocytes and oocyte maturity rate were comparable to cisgender women. Fertilization with donor sperm resulted in good quality embryos in five out of six cases. Embryos were cryopreserved for future embryo transfer in another country (Amir et al., 2020b).
Pubertal/adolescent	– Successful oocyte retrieval (Barrett et al., 2022, Chen et al., 2018, Insogna et al., 2020). – No difference in the number of retrieved oocytes, MII oocytes, and maturity rate between GAHT-naïve transmen oocyte and age-matched cisgender female cancer patients before chemotherapy (Amir et al., 2020a).	– No data.	– Successful oocyte retrieval in GnRHa-only, and who had history of prior testosterone use (Insogna et al., 2020).

TGD = Transgender and gender diverse, GAHT = Gender-Affirming Hormone Therapy, LB = live birth, IVF = in vitro fertilization, IUI = intrauterine insemination.

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Table 6 Developing technologies for maturing TGD patient ovarian tissues and producing eggs in vivo or in vitro

Methods	**Cell- or tissue- based technique**	**GAHT cessation at time of fertility restoration**	**Evidence**		
			Tissue/ Group	**Results**	**Reference**
Autologous transplantation	Tissue-based	Yes	Adult tissue	Live births	Reviewed in Donnez & Dolmans, 2017, Gellert et al. 2018, Khattak et al. 2022
			Prepubertal and Adolescent tissue	Live births	Demeestere et al. 2015, Matthews et al. 2018, Rodriguez-Wallberg et al. 2021b
			No evidence in TGD people with ovaries		
OTO/IVM	Cell-based	No	Cisgender	All age group (cancer) – 50-76.9% fertilization rate, pregnancy rate not reported due to no utilization	Reviewed in Mohd Faizal et al. 2022
				Pregnancy reported in benign pelvic arteriovenous malformation	Segers et al. 2015
				oocyte aspiration during cesarean section	Hwang et al. 1997
				Live births (LB rate after embryo transfer = 43%)	Segers et al. 2020
				cancer patients	Prasath et al. 2014, Uzelac et al. 2015, Kedem et al. 2018
			TGD adults with ovaries		
				Normal spindle after thawing	Lierman et al. 2017
				poor embryonic progression after fertilization	Lierman et al. 2021
				may be overcome by spindle transfer	Christodoulaki et al. 2023

TGD = Transgender and gender diverse, OTO/IVM = ovarian tissue oocyte/in vitro maturation, IVG/IVM = In vitro growth/in vitro maturation

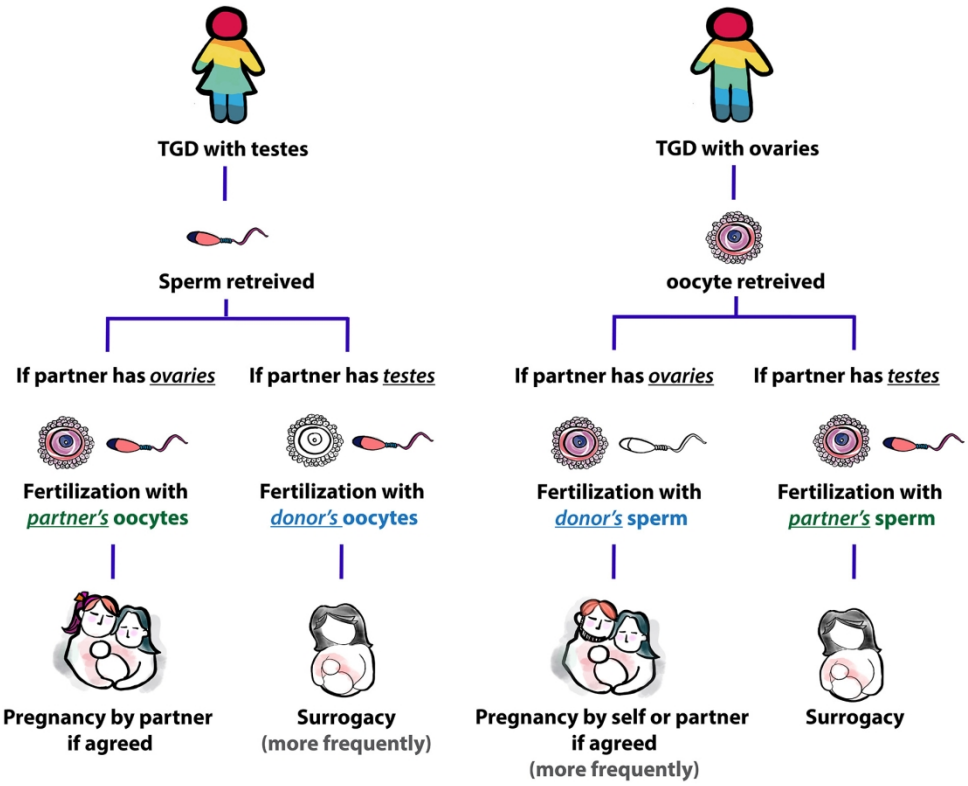

Figure 1. Journey of TGD people to have a biological child.

168x146mm (300 x 300 DPI)