Systematic review of fertility preservation options in transgender patients: a guide for plastic surgeons

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Abstract: Transgender patients often desire to have biological children. However, their reproductive potential is often negatively impacted by gender affirming surgery (GAS) such as gender confirmation surgery (bottom surgery) and medical hormone therapy. Therefore, counselling patients on fertility preservation options before initiating gender-affirming treatments is prudent to avoid reducing their reproductive potential. A systematic review of English, Spanish, Chinese, French and Turkish languages from 2000 to December 23rd, 2019, using the preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) guidelines, was conducted. The search strategy was designed and conducted by an experienced librarian with input from the study's principle investigator. Fifteen articles that report outcomes of fertility preservation options in transgenders were included. Eight articles described options for transgender women, six reported options for transgender men and one included both transgender women and transgender men. Semen cryopreservation and oocyte cryopreservation are the most common and available methods for fertility preservation in transgenders. Physician awareness of fertility preservation options in transgender patients is crucial to ensure informed discussions regarding reproductive options in the early phase of transition.

Keywords: Fertility preservation; transgender persons; plastic surgery; reproductive techniques; fertilization

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Introduction

Transgender patients are those whose gender identity differs from the gender assigned at birth (1). Around 1.4 million people in the United States are estimated to identify as transgender according to the William Institute (2). Many transgender individuals are misgendered and their physical appearance adds to their gender dysphoria leading them to pursue exogenous hormonal therapy and subsequent gender affirming surgery (GAS) (3,4). While these therapies have been shown to increase self-esteem, alleviate depressive symptoms and decrease symptoms of gender dysphoria (5), it can affect their reproductive potential. Hormonal therapy can cause oligo/azoospermia and erectile problems in transgender women and low ovarian reserve in transgender men. These effects can be reversible; however, orchiectomy and oophorectomy causes a permanent loss of fertility (6).

In order to pursue hormonal therapy, transgender individuals are required to present with persistent gender nonconformity or gender dysphoria which has emerged or has aggravated with puberty. In addition, any medical, social, and psychosocial conditions that can interfere with the hormones should be addressed and an informed consent should be obtained. Transgenders pursuing gender affirming genital surgery are required to live in their desire sex for a minimum of one year before the surgery (7). Plastic surgeons are involved in the care of transgender patients during their gender affirmation procedures and therefore, they should be aware of the fertility preservation options for this population. According to the World Professional Association for Transgender Health (WPATH) guidelines, healthcare professionals should discuss fertility preservation options with transgender patients prior to initiating gender affirming therapies (8,9).

In the last decade, the incidence of transgender women seeking sperm cryopreservation has increased compared with cisgender men (10). In a survey of 121 transgender women performed by De Sutter *et al.* (11), 77% reported that they would very interested in sperm banking if it was offered, and agreed that fertility preservation should be discussed with their healthcare provider. However, more than 90% of the responders reported that the loss of fertility secondary to gender confirmation therapies would not hinder their desire for pursuing such procedures. Another survey conducted by Wierckx *et al.* (12) showed that 62% of transsexual men wanted to have children. Moreover, transgender men with children scored better on vitality and self-perceived mental health status. In fact, parenting has been shown as a protective factor for suicide in transgender individuals (13).

The age in which transgender patients present to the physician has been trending down (11). In addition, there have been reports of low fertility preservation methods used among the transgender youth (14). This imposes a problem, since many of them have not yet defined their fertility wish or desire to have biological children; therefore, the importance of addressing fertility preservation options becomes crucial. While fertility preservation options have been used for oncologic patients, there is not many data on the fertility preservation options available for transgender patients. These options include sperm cryopreservation, surgical sperm extraction and testicular tissue cryopreservation for transgender women, and oocyte cryopreservation, embryo cryopreservation, *in vitro* maturation and ovarian tissue cryopreservation for transgender men (15). This systematic review aims to examine the outcomes of fertility preservation techniques for transgender patients and offer a guide for plastic surgeons during counselling of these patients.

We present the following article in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) reporting checklist (available at http://dx.doi.org/10.21037/atm-20-4523).

Methods

This study is a systematic review of aggregated published data so no institutional review board approval was required.

Population and outcomes

A systematic review of studies evaluating fertility preservation options among transgender patients was conducted. The population were transgender men and women.

Search strategy

A comprehensive review of the literature was performed in accordance to the PRISMA guidelines (16). A comprehensive search of several databases from January 1st, 2000 to December 23rd, 2019 was conducted. The databases included Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, and Daily, Ovid EMBASE, Ovid Cochrane Central Register of

Controlled Trials, Ovid Cochrane Database of Systematic Reviews, Ovid PsycINFO, and Scopus.

The search strategy was designed and conducted by an experienced librarian with input from the study's principle investigator. Controlled vocabulary supplemented with keywords was used to search for studies describing fertility preservation options in transgenders and was limited to English, Spanish, Chinese, French, Turkish, Portuguese, Arabic, and German languages. The actual strategy listing all search terms used and how they are combined is available in the Appendix 1.

Inclusion and exclusion criteria

Inclusion criteria were articles on medical fertility preservation options for transgender patients that reported outcomes of these procedures. All time peer-reviewed original articles, written in English, Spanish, Chinese, French and Turkish; and studies involving human subjects were included. Primary outcomes were semen and oocyte parameters. The secondary outcomes were pregnancy and live birth rates after using fertility preservation methods. Exclusion criteria were articles in the form of abstracts, clinical overviews, clinical reviews, systematic reviews, opinion pieces, editorial letters, media reports and theses/dissertations, and any studies with animal models.

Study screening

Two independent investigators (MY, SSB), screened the titles, abstracts, and full texts of the articles identified. The investigators were blinded to each other and disagreement between the reviewers was resolved by discussion and consensus. The authors extracted data regarding study type, sample size, population type, mean age, study period, fertility preservation method, use of gender affirming hormonal therapy, treatment length, ovarian stimulation medication, evaluation parameters and study results.

Quality assessment

The Newcastle-Ottawa Quality Assessment Tool was used to identify the risk of bias of all studies included in this systematic review. A score of 0 to 3 was considered to be as low quality, 4 to 6 was considered as intermediate quality; and 7 or more was considered as high quality.

Data extraction

Two data extraction spreadsheets were developed and agreed upon between the co-authors. The selected studies were comprehensively assessed; all relevant data were extracted and entered into the spreadsheets by MY and SSB. Information selected included author details, year of publication, country of study, study design, study period, sample size, population, fertility preservation method, gender affirming hormone therapy, sperm or ovarian stimulation treatment, outcome of interest and results. Disagreements were resolved by discussion and if needed by the senior author.

Statistical analysis

The majority of studies were single-center experiences; therefore, a quantitative analysis was performed. Due to the high heterogeneity the studies, a meta-analysis was not conducted.

Results

Sample description

A total of 844 articles were identified, of which 396 were duplicated and were removed. A total of 448 abstracts were screened and 409 were excluded based on title and abstract screening. In total, 39 full articles were analyzed for eligibility, of which 15 met inclusion criteria. Reasons for exclusion were not articles unrelated to transgender patients (n=361), articles not reporting outcomes of fertility preservation methods (n=17), reviews (n=5) and commentaries (n=2) (*Figure 1*).

Fertility preservation options for transgender women (Tables 1,2)

Semen cryopreservation

Li *et al.* (10) performed a retrospective review of 78 transgender sperm bankers and compared them with 141 cisgender sperm bankers from the same private cryobank. Transgender specimens showed lower sperm concentration, total motile sperm count and post-thaw sperm parameters, as well as higher incidence of oligozoospermia. Only healthy cisgender men and transgender women with no reported previous hormonal therapy were included in this study.

Marsh *et al.* (17) conducted a prospective case-control

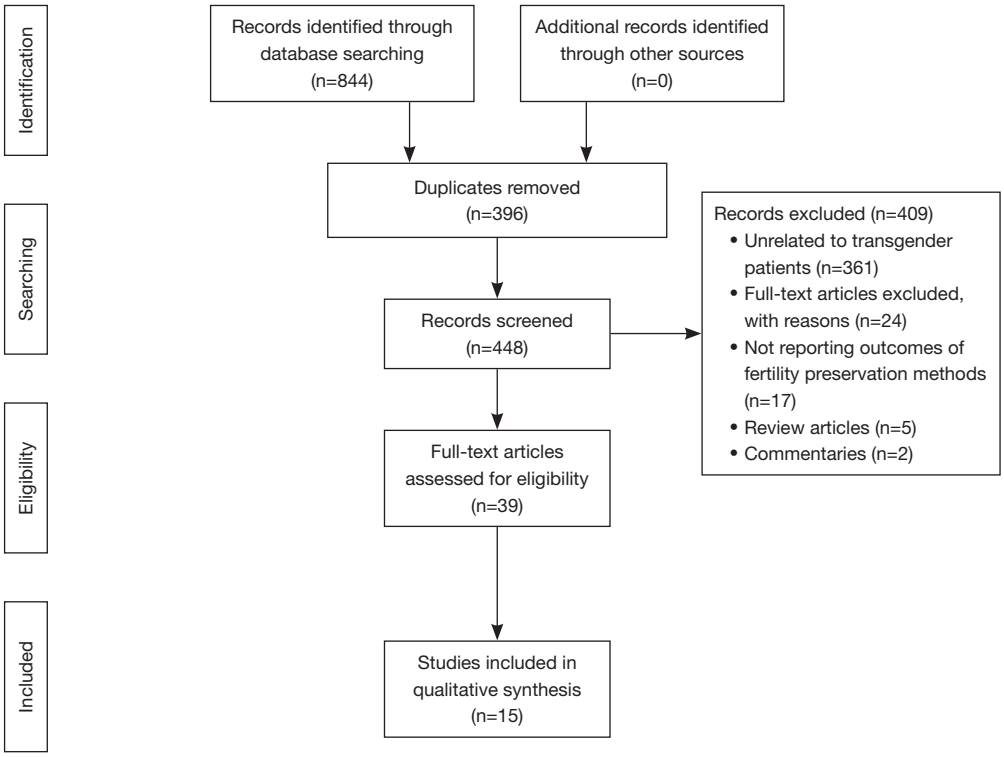

Figure 1 PRISMA flowchart.

study with 22 transgender women without current hormonal therapy treatment, and 17 cisgender men who had fathered a child in the previous 24 months. Semen collection occurred after at least seven days of sexual abstinence. The results showed lower semen parameters including sperm concentration, total motile sperm, volume, total sperm per ejaculate and normal sperm morphology in the transgender group. In addition, they reported a negative correlation between anxiety and stress and sperm concentration according to the Depression, Anxiety and Stress Scale (DASS-21).

Adeleye *et al.* (3) reported lower sperm concentration, percent of sperm motility and total motile count in patients on hormone treatment versus nontreatment. A total of 28 patients were included, 18 patients did not have any hormonal treatment, seven were on previous hormonal treatment and three remained on their hormones prior to semen collection. The median time of discontinuation ranged from 3 to 6.5 months. Additionally, lower semen parameters were found in patients on current gender affirming hormone therapy (GAHT) treatment versus those who previously used GAHT.

Jones *et al.* (18) described a case series of nine transgender women who underwent sperm cryopreservation. Of them, six patients had been on hormonotherapy for a mean duration of 0.33 months. A median of two sperm samples were cryopreserved. One patient obtained a successful intrauterine pregnancy following transfer of a cryopreserved embryo after intracytoplasmic sperm injection (ICSI) cycle.

Alford *et al.* (19) described a case report of a transgender woman who underwent a successful sperm cryopreservation after discontinuing estrogen and spironolactone therapy 2 months prior to attempting fertility preservation. At 6 months after stopping hormonotherapy, the patient had normalized her testosterone levels and semen parameters.

A study by Brik *et al.* (20) reported nine successful sperm cryopreservation among 13 patients. Nine patients successfully cryopreserved their semen to be used for intrauterine insemination or intracytoplasmic sperm injection. Of the remaining patients, one could not ejaculate, one had azoospermia and another had severe oligozoospermia with low post-thaw sperm parameters and no sperm with testicular sperm extraction. Of note, one patient had an inguinal testis, which could have influenced the outcomes. The authors did not record the hormonal therapy use.

Hamanda *et al.* (21) performed a retrospective review of 29

Table 1 Study characteristics

Authors (year), Country	Fertility preservation method	Study design	Study period (years)	Sample size, population	Mean age ± SD/median age (range) (years)	GAHT (dose, administration technique)	GAHT median length (months)	Sperm/Ovarian stimulation medication (dose)
Li *et al.* (in 2018), USA	Semen cryopreservation	Retrospective case-control	2006–2016	78 MtF and 141 cisgender men	Cases: 24.1±7.6; Control: 36±9.4	None	N/A	Not specified
Marsh *et al.* (in 2019), USA	Semen cryopreservation	Prospective case-control	Not specified	22 MtF and 17 cisgender men	Cases: 26.70±1.85; Controls: 32.0±1.04	None	N/A	Not specified
Adeleye *et al.* (in 2019), USA	Semen cryopreservation	Retrospective cohort	2012–2018	28 MfF (18 with no prior GAHT use; 3 with prior use; 5 current users	18±39.9	Estrogen 2–6 mg oral/300 mcg transdermal/conjugated estrogen 0.625 mg/d plus; Spironolactone 50/100 mg oral BID/Finasteride 2.5–5 mg/micronized Progesterone 100 mg	Current GAHT users: 30 months; Prior users: 42 months (mean discontinuation 4.4 months)	Not specified
Barnard *et al.* (in 2019), USA	Semen cryopreservation	Retrospective cohort	2015–2018	10 MtF	19 (16 to 24)	Patient 1: leuprolide acetate (15 mg intramuscular injection every 28 days); Patient 2: spironolactone (100 mg daily) and estradiol (75 mg daily, transdermal patch)	Patient 1: 6 months; Patient 2: 26 months	Not specified
Jones *et al.* (in 2016), Canada	Semen cryopreservation	Retrospective cohort	2010–2014	9 MtF	28.5 (20 to 40)	6 patients on hormonal therapy (medications not specified)	0.33 months	Not specified
Alford *et al.* (in 2019)	Sperm cryopreservation	Case report	NA	1 MtF	26	Estrogen injection and spironolactone oral (doses not specified)	16 months prior to FP (discontinued 2 months prior to vaginoplasty)	FSH (75 units, 3 times/week) and clomiphene citrate (25 mg, daily)
Brik *et al.* (in 2018), Netherlands	Semen cryopreservation	Retrospective cohort	2011–2017	12 MtF	16.1±1.7	Not specified	Not specified	GnRH analog (dose not specified)
Hamada *et al.* (in 2014), USA	Sperm cryopreservation	Retrospective case series	2003–2011	29 MtF	Institution A: 17.5; Institution B: 28.4	Not specified	Not specified	Not specified

Table 1 (*continued*)

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Authors (year), Country	Fertility preservation method	Study design	Study period (years)	Sample size, population	Mean age ± SD/median age (range) (years)	GAHT (dose, administration technique)	GAHT median length (months)	Sperm/Ovarian stimulation medication (dose)
Broughton and Omurtag. (in 2017), USA	Semen cryopreservation; oocyte cryopreservation	Retrospective case series	2015–2016	3 couples with a transgender or gender-nonconforming member	31 (30 to 32)	Testosterone depot intramuscular in one case, none in remaining two cases (doses not specified)	26 months (discontinued for 3 months prior to FP)	GnRH antagonist/ analog (dose not specified)
Chen *et al.* (in 2018)	Oocyte cryopreservation	Retrospective case series	Not specified	5 FtM	16.4 (14 to 18)	None	N/A	FSH alone or a combination of FSH and hMG; 2,180 IU (range, 11–28)
Wallace *et al.* (in 2014), USA	Oocyte cryopreservation	Case report	NA	1 FtM	17	None	N/A	FSH, hMC, Ganirelix acetate and leuprolide acetate (dose not specified)
Maxwell *et al.* (in 2017), USA	Oocyte cryopreservation	Retrospective case series	Not specified	3 FtM	Range 17 to 32	None	N/A	Patient 1: GnRH antagonist (dose not specified) Patient 2 and 3: leuprolide acetate low-dose (dose not specified)
Adeleye *et al.* (in 2019), USA	Oocyte cryopreservation	Retrospective cohort	2015–2019	13 FtM and 13 cisgender women	22.4 (14.6 to 37.1)	7 patients discontinued testosterone prior to FP (doses not specified) 6 patients had not started hormonotherapy	Testosterone 46 months, discontinued 6 months (range 1–13) prior to FP	FSH, hMC (dose not specified)
Lierman *et al.* (in 2017), Belgium	Oocyte cryopreservation and *in vitro* maturation	Prospective cohort	Not specified	16 FtM	24.1±6.1	Testosterone (dose not specified)	13.4±5.3 months prior to FP	Not specified
De Roo *et al.* (in 2017), Belgium	Oocyte cryopreservation and *in vitro* maturation	Prospective cohort	2013–2015	40 FtM	24.3±6.15	Testosterone intramuscular, oral or transdermal (doses not specified).	14.5±6.6 months	Not specified

MtF, male-to-female; FtM, female-to-male; GAHT, Gender affirming hormonotherapy; FSH, follicle stimulating hormone; hMG, human menopausal gonadotropin; FP, fertility preservation.

Table 2 Evaluation parameters and results of fertility preservation methods; and quality assessment

Authors (year), Country	Evaluation parameters	Results of evaluation parameters	Newcastle-Ottawa
Li *et al.* (2018) USA	Semen parameters and cryosensitivity analysis	Lower semen parameters (median sperm concentration, count, TMSC, post-thaw sperm count, post-thaw TMSC) in MtF *vs.* cisgender men	Low
Marsh *et al.* (2019) USA	Semen parameters	Lower semen parameters (sperm concentration, total motile sperm, volume, total sperm per ejaculate) and normal sperm morphology in the transgender group *vs.* cisgender men.	Low
Adeleye *et al.* (2019) USA	Semen parameters and testicular pathology	Lower sperm concentration and percent of sperm motility, total motile count in patients on GAHT treatment *vs.* nontreatment	Low
		Lower semen parameters and specimen volume in current GAHT users *vs.* prior users	
Barnard *et al.* (2019) USA	Semen parameters	Patient 1: normal sperm parameters except low sperm morphology	Intermediate
		Patient 2: low sperm parameters, failed FP	
Jones *et al.* (2016) Canada	Semen parameters at two different visits	Semen parameters normal at the first visit, lower at second visit 1 intrauterine pregnancy after frozen embryo transfer of a cryopreserved embryo following ICSI	Intermediate
Alford *et al.* (2019) USA	Semen parameters	Recovery of testosterone levels and semen parameters 6 weeks after stopping hormonal therapy	Intermediate
Brik *et al.* (2018) Netherlands	Semen parameters	9 patients had good semen parameters for future intrauterus insemination or ICSI; 1 patient could not ejaculate; 2 patients had low semen parameters	Intermediate
Hamada *et al.* (2014) USA	Semen parameters	High rates of oligozoospermia, asthenozoospermia, teratozoospermia and low sperm motility	Intermediate
Broughton and Omurtag (2017) USA	Semen cryopreservation, oocyte cryopreservation and IVM outcomes	Couple 1: FtM patient failed pregnancy after 3 intrauterine inseminations and 1 IVF. His semen parameters were within normal limits	Intermediate
		Couple 2: FtM patient attempted *in vitro* fertilization using his oocytes followed by embryo transfer to his partner's uterus. 16 oocytes, of which 13 were matured oocytes and 7 were fertilized using intracytoplasmic sperm injection	
		Couple 3: MtF patient had a successful pregnancy after transferring two blastocysts to her wife's uterus; 16 oocyte retrieved, of which 9 were fertilized with sperm	
Chen *et al.* (2018) USA	Ovarian stimulation outcomes	18.2 oocytes (range, 11–28) retrieved; 14.2 oocyte (range, 8–25) mature and cryopreserved	Low
Wallace *et al.* (2014) USA	Oocyte cryopreservation results	39 COC identified, of which were 35 matured oocytes cryopreserved	Intermediate
Maxwell *et al.* (2017) USA	Oocyte cryopreservation cycle results; and pregnancy outcomes	Patient 1: 21 oocytes retrieved of them 17 mature oocytes were cryopreserved with vitrification	Low
		Patient 2: Twenty of the 45 oocytes were thawed, and 18 (90%) survived, with 17 (94%) fertilized after intracytoplasmic sperm injection. The embryos were cultured for 5–6 days, and 12 blastocysts underwent trophectoderm biopsy followed by vitrification. Twin pregnancy after embryo implantation.	
		Patient 3: 13 mature, 6 immature oocytes retrieved and cryopreserved. At the time of conception, 19 cryopreserved oocytes were thawed, of which 14 matured and 11 fertilized after intracytoplasmic sperm injection. Twin pregnancy after embyo transfer.	

Table 2 (*continued*)

Table 2 (*continued*)

Authors (year), Country	Evaluation parameters	Results of evaluation parameters	Newcastle-Ottawa
Adeleye *et al.* (2019) USA	Ovarian stimulation outcomes with and without prior testosterone exposure	FtM patients without testosterone: 25.5 oocytes (range, 18–28); FtM patients with testosterone use: 12 oocytes (range, 4–26), P=0.038	Low
Lierman *et al.* (2017) Belgium	Ovarian histology, oocyte *in vitro* maturation and spindle analysis	Median 42.5 (5-174) COCs collected per transgender man Normal spindle structure and chromosomes alignment in both non-cryopreserved and the vitrified group (85.7% *vs.* 92.2%; P=0.27)	Low
De Roo *et al.* (2017) Belgium	Ovarian histology, oocyte *in vitro* maturation and spindle analysis	1,313 COC retrieved, of which 34.30% reached metaphase II with 87.10% of them showing normal spindle structure	Low

MtF, male-to-female; FtM, female-to-male; GAHT, Gender affirming hormonotherapy; COC, cumulus oocyte complexes; TMSC, total motile sperm count; ICSI, intracytoplasmic sperm injection; FSH, follicle stimulating hormone; IVM, in vitro maturation; hMG, human menopausal gonadotropin.

transgender women and reported altered semen parameters with high rates of oligozoospermia, asthenozoospermia, and teratozoospermia; in addition to low sperm motility in the majority of specimens. This study did not provide information on previous hormonal therapy use, and lacked a cisgender control group.

Barnard *et al.* (22) conducted a retrospective analysis of ten transgender women. Two of them had been on hormonal therapy before attempting semen cryopreservation and the remaining eight had not yet started hormonal therapy. Of the two patients who received hormonal therapy, one of them received treatment with leuprolide acetate (15 mg intramuscular injection every 28 days) for 6 months prior to specimen collection. The sperm parameters were assessed both at 3 and 6 months after stopping the hormonal therapy. At 3 months, the semen analysis showed only 12 total sperm with two of them being motile. At 6 months, only low morphology was reported with the remaining semen parameters within normal limits. The second patient who received hormonal therapy was treated with spironolactone (100 mg daily) and estradiol (75 mg daily, transdermal patch) for 26 months prior to sperm collection. The results showed persistent azoospermia at 2, 3, and 4 months after discontinuation of both medications, and the semen cryopreservation was unsuccessful.

Broughton and Omurtag (23) described the outcomes of three patients who underwent semen preservation. One of them was a transgender woman who cryopreserved four semen specimens with normal parameters and attempted three intrauterine inseminations (two natural cycles and one with clomiphene treatment); and one *in vitro* fertilization with one blastocyst; however, none of these attempts were were successful.

Other methods

A surgical sperm extraction consists of a percutaneous aspiration of sperm from the epididymis or testis. The specimen can be used for intracytoplasmic sperm injection or *in vitro* fertilization.

Another method is the immature testicular tissue cryopreservation that consists of a biopsy of testicular tissue from pre-pubertal patients. It remains an investigational method and it is the only option available for pre-pubertal males at the moment. There were no articles reporting outcomes of these techniques in our literature search.

Fertility preservation options for transgender men

Oocyte and embryo cryopreservation

Chen *et al.* (24) reported five cases of transgender men adolescents that successfully achieved oocyte cryopreservation before starting hormonal therapy. The mean number of oocytes retrieved were 18.22 (11 to 28), of which 14.2 (8 to 25) were mature and were cryopreserved.

Wallace *et al.* (25) described a case of a 17-year-old transgender man who successfully cryopreserved 35 mature oocytes prior to initiating hormonal therapy.

Maxwell *et al.* (4) described a case series of one adolescent and two adult transgender patients who underwent oocyte cryopreservation before initiating hormonal therapy. The number of cryopreserved mature oocytes collected ranged between 13 and 45. After 5–8 years, two patients used their

specimens and both achieved successful twin pregnancies.

Broughton and Omurtag (23) reported a case series with two transgender men successful pregnancies. One patient achieved a successful pregnancy after transferring two blastocysts to his wife's uterus. This couple attempted *in vitro* fertilization using the patient's oocytes followed by embryo transfer into his wife's uterus. A total of 16 oocytes were obtained, of which 13 were matured oocytes. At the end, seven were fertilized using ICSI. Prior to pursuing fertility preservation, he had been on intramuscular depot testosterone therapy for 26 months and discontinued it 3 months before attempting fertilization. At that time, his hormonal levels (antimullerian hormone, follicle-stimulating hormone, and estradiol) were equivalent to a premenopausal cisgender female. The second patient identified as non-binary, and both the patient and the cisgender partner underwent *in vitro* fertilization with donor sperm. After gonadotropin stimulation, the patient had 16 oocytes retrieved, of which nine were fertilized with sperm. The cisgender partner did not conceive after uterine implantation of her own blastocyst; however, she did achieve intrauterine twin pregnancy after implantation of two blastocysts from the patient.

Adeleye *et al.* (4) conducted a retrospective study of 13 transgender men and 13 cisgender women. Seven transgender patients discontinued testosterone prior to fertility preservation, and six patients had not yet started hormone therapy. Median testosterone use was 46 months and the median discontinuation was 6 months (1 to 13) prior to fertility preservation. The median number of oocytes collected in transgender men with testosterone use prior to oocyte collection [12 (4 to 26)] was significantly lower than that of transgender men without prior testosterone treatment [25.5 (18 to 28)], P=0.038).

In vitro maturation

Lierman *et al.* (26) conducted a prospective cohort study with 13 transgender men. All of them had received testosterone therapy at the time of fertility preservation for 53.6±21 weeks. The procedure consisted of collecting cumulus-oocyte-complexes (COC) at the time of GAS. A total of 680 COC were obtained which were then subjected to *in vitro* maturation, and the median number of COC collected per transgender men was 42.5 (5 to 174), which was higher in patients 20 years of age or younger. In total, 38.1% of COC reached metaphase II stage, of them, 133 oocytes underwent cryopreservation through vitrification and fixation for spindle staining; and 126 oocytes were immediately fixed for spindle staining. The results reported normal spindle structure and chromosomes alignment (92.2% *vs.* 85.7%; P=0.27) in both groups. The study included only patients 18 years or older. Since more COC were obtained in the younger group of patients, future studies that provide insight on the COC collected from young adolescents could provide more information about the fertility options in this population.

The prospective cohort study by De Roo *et al.* (27) included 40 transgender men and studied the hormone serum levels, ovarian histology and COC at the time of GAS. All patients had been previously treated with testosterone for 58.18±26.57 weeks. A total of 1,313 COC were retrieved, of which 34.30% reached metaphase II. Of them, 87.1% showed normal spindle structure.

Ovarian tissue cryopreservation

It consists of cryopreserving ovarian tissue during GAS. The ovarian tissue can later be transplanted onto the ovaries or grafted heterotopically for maturation of the oocytes. No studies were found in this review.

Discussion

Even though the number of transgender patients pursuing fertility preservation has been increasing, it remains an underutilized resource (16). This could be due to barriers to access to healthcare, such as high costs, low awareness, late referral when patients have advanced reproductive age, psychosocial reasons and family values (28,29). Plastic surgeons are involved in a critical part of the gender affirming journey. Gender affirming genital surgery may have an irreversible impact on fertility; thus, plastic surgeons should be aware of the options for fertility preservation in transgender patients, and offer counselling to these patients before undergoing any gender affirming procedures. If the patient has not undergone genital surgery, hormone therapy may be discontinued to allow for potential recovery of reproductive function. Many patients who present to the plastic surgeon have been utilizing hormones for a period of months, thus their reproductive potential may have been altered. A multidisciplinary approach with other specialists such as psychologists and endocrinologists is essential to ensure proper counselling about their fertility preservation options.

Current fertility preservation options for transgender women include sperm cryopreservation and testicular sperm extraction. Semen cryopreservation is the current most

simple and reliable technique (30). It can be achieved with masturbation or vibratory stimulation without the need for invasive procedures, and is available at many fertility clinics. If the sperm quality is low, multiple samples can be collected and additional methods such as intrauterine insemination (IUI) and in vitro fertilization (IVF) can be used to increase the chances of pregnancy. A testicular sperm extraction consists of a percutaneous aspiration of sperm from the epididymis or testis. It is an option for patients who are unable to ejaculate or for those with severe oligospermia or obstructive azoospermia. It requires only local anesthesia and can be used for IVF or ICSI (31).

For patients on hormonal therapy, spermatogenesis may be altered and the results are often unsuccessful. A study assessing spermatogenesis in fifty transgender women on hormonal therapy showed spermatogenesis in 20% of specimens and maturation arrest at the level of spermatogonia in 80% of the samples (32). For these patients, testicular sperm harvesting at the time of GAS can be contemplated. Immature testicular tissue cryopreservation is another experimental method for fertility preservation used in transgender patients. It involves biopsy of testicular tissue from pre-pubertal and adolescent patients, which then undergoes vitrification and is subsequently xenografted to induce spermatogenesis (33). It remains an investigational method that has shown successful results in animal studies, and it is the only option available for pre-pubertal males at the moment.

Several studies have shown that semen parameters including sperm concentration, total motile sperm count and post-thaw motility are lower in transgender women when compared to cisgender men (3,10,17,18,22). The etiology of these findings is unknown; however, some of the reasons could be wearing tight underwear, self-induced high scrotal positioning of the testes, psychological stress, genetic factors and gender affirming hormone therapy (21,34). Other factors such as obesity (35), smoking (36) and psychosocial stress (37) could influence semen parameters. It is well known that hormonal therapy negatively affects sperm parameters (28). Ideally, the best time for attempting fertility preservation is before any hormonal therapy to avoid adverse effects on fertility including testicular atrophy or testicular function disorders (38). Studies analyzing semen parameters of transgender women before starting hormonal therapy have reported them to be within normal limits (10,17,23). A study of four patients with prostate cancer who used gonadotropin agonist for at least 1 year, showed an inhibition of spermatogenesis on testicular histology after orchiectomy (39). Payer *et al.* (40) described partially arrested spermatogenesis and changes on Leydig cell morphology on testicular biopsies of six transgender women who had been on hormone therapy with estrogen for one to 8.5 years. Discontinuing the hormonal therapy may restore reproductive function; however, it may take up 3 to 24 months after stopping the medication. During this time, previous gender characteristics that were suppressed may return along with feeling of gender dysphoria. Alford *et al.* (19) reported a case of a transgender woman who showed recovery of testosterone levels and semen parameters 6 weeks after stopping hormonal therapy.

For transgender women who are seeking fertility preservation, semen cryopreservation is usually the first option offered. If the patient is taking gender affirming hormonal therapy, it should be stopped before semen collection. If the sperm quality remains low, other methods such as testicular sperm extraction or testicular sperm harvesting at the time of GAS can be attempted. Due to the many factor influencing semen parameters, as well as differences of semen quality between samples, it is difficult to accurately predict which transgender patient will have active spermatogenesis. Therefore, it is crucial that fertility preservation options be discussed before initiating exogenous hormonal therapy.

Transgender men have more challenges when attempting fertility preservation. Age plays a bigger role in transgender men than transgender women, since the rates of successful oocyte collection declines with increased age (41). In addition, the process is more expensive and less effective than sperm banking. It requires invasive procedures such as transvaginal ultrasounds and transvaginal aspiration of oocytes, which can be perceived as a physically and psychologically challenging experience, and can worsen gender dysphoria (42). In 2014, Wallace *et al.* (25) described the first case of oocyte cryopreservation before initiating hormone therapy in a transgender man. Since then, many other studies have also reported successful oocyte or embryo cryopreservation in transgender men (4,24).

Advances in technology have increased the options and outcomes of fertility preservation in transgender men. In some cases, pregnancy rates fertility preservation methods have been reported to be similar to IVF cycles (43-46). The most common method for fertility preservation in transgender men is oocyte cryopreservation. It requires ovarian hyperstimulation with hormones, followed by transvaginal ultrasound—guided oocyte retrieval with oocyte cryopreservation. Embryo banking is another

option, however, it requires a sperm donor. Maxwell *et al*. reported successful pregnancies in transgender couples after oocyte cryopreservation (47). Immature oocyte aspiration at the time of GAS with *in vitro* maturation and oocyte cryopreservation is another method. There are two studies in transgender men who have attempted this technique and obtained normal spindle structure of the oocytes, which is necessary for subsequent fertilization (15,26). These studies were performed in patients 18 years old or older, and showed that most of the COC were obtained from the younger group of patients. Future studies on COC collected from young adolescents could provide more information about fertility options in this population.

Hormonal therapy has shown to have negative effects on ovarian tissue reserve and ovarian stimulation response to gonadotropin, with arrest of follicle maturation (6,8,9,27). Two studies (15,26) in this review reported successful results after stopping testosterone therapy; however the duration of testosterone therapy was relatively short, between 53 to 58 weeks; therefore, data on long-term testosterone use is still needed. Early oocyte retrieval before any gender affirming hormone treatment could prevent patients on having to temporarily stop their hormonal therapy if they decide to undergo fertility preservation in the future. Discontinuing testosterone may impose a regression in their gender affirming journey and could worsen gender dysphoria (42).

Limitations of this study are that several studies did not provide the dose of the hormone therapy or sperm/ovarian stimulation medication. In addition, only original peer reviewed articles were included, obviating other studies.

Conclusions

Improving physician and patient awareness on fertility preservation options in the transgender population is important. Several fertility preservation methods have shown to be effective in the transgender population. Our review shows that fertility preservation should be discussed early during gender transition and if possible, before any exogenous hormonal therapy is started, as the timeline for these interventions is important in order to preserve their reproductive potential as much as possible.

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References

1. Byne W, Bradley SJ, Coleman E, et al. Report of the American psychiatric association task force on treatment of gender identity disorder. Arch Sex Behav 2012;41:759-96.
2. Flores AR, Herman JL, Gates GJ, et al. How Many Adults Identify As Transgender in the United States? Los Angeles, CA: Williams Inst, 2016.
3. Adeleye AJ, Reid G, Kao CN, et al. Semen Parameters Among Transgender Women With a History of Hormonal Treatment. Urology 2019 Feb;124:136-41.
4. Adeleye AJ, Cedars MI, Smith J, et al. Ovarian stimulation for fertility preservation or family building in a cohort of transgender men. J Assist Reprod Genet 2019;36:2155-61.

5. Gorin-Lazard A, Baumstarck K, Boyer L, et al. Hormonal therapy is associated with better self-esteem, mood, and quality of life in transsexuals. J Nerv Ment Dis 2013;201:996-1000.
6. Hembree WC, Cohen-Kettenis P, Delemarre-Van De Waal HA, et al. Endocrine treatment of transsexual persons: An endocrine society clinical practice guideline. J Clin Endocrinol Metab 2009;94:3132-54.
7. Meyer W, Bockting WO, Cohen-Kettenis P, et al. The harry benjamin international gender dysphoria association's standards of care for gender identity disorders, sixth version. J Psychol Hum Sex 2002;13:1-30.
8. Coleman E, Bockting W, Botzer M, et al. Standards of Care for the Health of Transsexual, Transgender, and Gender-Nonconforming People, Version 7. Int J Transgend 2012;13:165-232.
9. Caanen MR, Soleman RS, Kuijper EAM, et al. Antimüllerian hormone levels decrease in female-to-male transsexuals using testosterone as cross-sex therapy. Fertil Steril 2015;103:1340-5.
10. Li K, Rodriguez D, Gabrielsen JS, et al. Sperm cryopreservation of transgender individuals: trends and findings in the past decade. Andrology 2018;6:860-864.
11. De Sutter P, Kira K, Verschoor A, et al. The desire to have children and the preservation of fertility in transsexual women: A survey. Int J Transgend 2003;6:3.
12. Wierckx K, Van Caenegem E, Pennings G, et al. Reproductive wish in transsexual men. Hum Reprod 2012;27:483-7.
13. Moody C, Smith NG. Suicide protective factors among trans adults. Arch Sex Behav 2013;42:739-52.
14. Nahata L, Tishelman AC, Caltabellotta NM, et al. Low Fertility Preservation Utilization Among Transgender Youth. J Adolesc Health 2017;61:40-4.
15. De Roo C, Tilleman K, Tsjoen G, et al. Fertility options in transgender people. Int Rev Psychiatry 2016;28:112-9.
16. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med 2009;6:e1000097.
17. Marsh C, McCracken M, Gray M, et al. Low total motile sperm in transgender women seeking hormone therapy. J Assist Reprod Genet 2019;36:1639-48.
18. Jones CA, Reiter L, Greenblatt E. Fertility preservation in transgender patients. Int J Transgend 2016;17:76-82.
19. Alford AV, Theisen KM, Kim N, et al. Successful Ejaculatory Sperm Cryopreservation After Cessation of Long-term Estrogen Therapy in a Transgender Female. Urology 2020;136:e48-50.
20. Brik T, Vrouenraets LJJJ, Schagen SEE, et al. Use of Fertility Preservation Among a Cohort of Transgirls in the Netherlands. J Adolesc Health 2019;64:589-93.
21. Hamada A, Kingsberg S, Wierckx K, et al. Semen characteristics of transwomen referred for sperm banking before sex transition: A case series. Andrologia 2015;47:832-8.
22. Barnard EP, Priya Dhar C, Rothenberg SS, et al. Fertility preservation outcomes in adolescent and young adult feminizing transgender patients. Pediatrics 2019;144:e20183943.
23. Broughton D, Omurtag K. Care of the transgender or gender-nonconforming patient undergoing in vitro fertilization. Int J Transgend 2017;18:372-5.
24. Chen D, Bernardi LA, Pavone ME, et al. Oocyte cryopreservation among transmasculine youth: a case series. J Assist Reprod Genet 2018;35:2057-61.
25. Wallace SA, Blough KL, Kondapalli LA. Fertility preservation in the transgender patient: Expanding oncofertility care beyond cancer. Gynecol Endocrinol 2014;30:868-71.
26. Lierman S, Tilleman K, Braeckmans K, et al. Fertility preservation for trans men: frozen-thawed in vitro matured oocytes collected at the time of ovarian tissue processing exhibit normal meiotic spindles. J Assist Reprod Genet 2017;34:1449-56.
27. De Roo C, Lierman S, Tilleman K, et al. Ovarian tissue cryopreservation in female-to-male transgender people: insights into ovarian histology and physiology after prolonged androgen treatment. Reprod Biomed Online 2017;34:557-66.
28. Ainsworth AJ, Allyse M, Khan Z. Fertility Preservation for Transgender Individuals: A Review. Mayo Clin Proc 2020;95:784-92.
29. Chen D, Kyweluk MA, Sajwani A, et al. Factors Affecting Fertility Decision-Making Among Transgender Adolescents and Young Adults. LGBT Health 2019;6:107-15.
30. Wallace WHB, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: Who is at risk and what can be offered? Lancet Oncol 2005;6:209-18.
31. Furuhashi K, Ishikawa T, Hashimoto H, et al. Onco-testicular sperm extraction: Testicular sperm extraction in azoospermic and very severely oligozoospermic cancer patients. Andrologia 2013;45:107-10.
32. Matoso A, Khandakar B, Yuan S, et al. Spectrum of findings in orchiectomy specimens of persons undergoing gender confirmation surgery. Hum Pathol 2018;76:91-9.

33. Kaneko H, Kikuchi K, Nakai M, et al. Generation of Live Piglets for the First Time Using Sperm Retrieved from Immature Testicular Tissue Cryopreserved and Grafted into Nude Mice. PLoS One 2013;8:e70989.
34. Eskiocak S, Gozen AS, Taskiran A, et al. Effect of psychological stress on the L-arginine-nitric oxide pathway and semen quality. Braz J Med Biol Res 2006;39:581-8.
35. Leisegang K, Henkel R, Agarwal A. Obesity and metabolic syndrome associated with systemic inflammation and the impact on the male reproductive system. Am J Reprod Immunol 2019;82:e13178.
36. Sharma R, Harlev A, Agarwal A, et al. Cigarette Smoking and Semen Quality: A New Meta-analysis Examining the Effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. Eur Urol 2016;70:635-45.
37. Shi X, Chan CPS, Waters T, et al. Lifestyle and demographic factors associated with human semen quality and sperm function. Syst Biol Reprod Med 2018;64:358-67.
38. Sapino A, Pagani A, Godano A, et al. Effects of estrogens on the testis of transsexuals: A pathological and immunocytochemical study. Virchows Arch A Pathol Anat Histopathol 1987;411:409-14.
39. Rajfer J, Swerdloff RS, Heber DM. Testicular Histology Following Chronic Gonadotropin-Releasing Hormone Agonist Treatment. Fertil Steril 1984;42:765-71.
40. Payer AF, Meyer WJ, Walker PA. The Ultrastructural Response of Human Leydig Cells to Exogenous Estrogens. Andrologia 1979;11:423-36.
41. Cil AP, Bang H, Oktay K. Age-specific probability of live birth with oocyte cryopreservation: An individual patient data meta-analysis. Fertil Steril 2013;100:492-9.e3.
42. Armuand G, Dhejne C, Olofsson JI, et al. Transgender men's experiences of fertility preservation: A qualitative study. Hum Reprod 2017;32:383-90.
43. Oktay K, Cil AP, Bang H. Efficiency of oocyte cryopreservation: a meta-analysis. Fertil Steril 2006;86:70-80.
44. Potdar N, Gelbaya TA, Nardo LG. Oocyte vitrification in the 21st century and post-warming fertility outcomes: A systematic review and meta-analysis. Reprod Biomed Online 2014;29:159-76.
45. Solé M, Santaló J, Boada M, et al. How does vitrification affect oocyte viability in oocyte donation cycles? A prospective study to compare outcomes achieved with fresh versus vitrified sibling oocytes. Hum Reprod 2013;28:2087-92.
46. Chang CC, Elliott TA, Wright G, et al. Prospective controlled study to evaluate laboratory and clinical outcomes of oocyte vitrification obtained in in vitro fertilization patients aged 30 to 39 years. Fertil Steril 2013;99:1891-7.
47. Maxwell S, Noyes N, Keefe D, et al. Pregnancy Outcomes after Fertility Preservation in Transgender Men. Obstet Gynecol 2017;129:1031-4.

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Appendix 1

Actual search strategies

Database(s): Ovid MEDLINE(R) 1946 to Present and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) Daily, EBM Reviews - Cochrane Central Register of Controlled Trials November 2019, EBM Reviews—Cochrane Database of Systematic Reviews 2005 to December 19, 2019, Embase 1974 to 2019 December 20

Search strategy

1	TITLE-ABS-KEY(((sex* or gender) W/1 (transition* or transform* or reassign* or chang*)) OR ((trans or intersex) W/1 (sexual* or gender* or male or men or women or female or people or person*)) OR (cross W/1 (sex* or gender*)) OR (trans W/1 gender*) OR "2 spirit person" OR "2-spirit person" OR crossgender* OR "gender dysphoria*" OR "gender identit*" OR "gender nonbinary" OR "gender nonconforming" OR "gender queer*" OR "Gender reassignment*" OR genderqueer* OR "gender-variant*" OR "male to female" OR "nonbinary gender*" OR "nonconforming gender*" OR "Sex reassignment*" OR transexual* OR transgender* OR transgenderism OR transpeople OR transperson* OR transsexual*)
2	TITLE-ABS-KEY ((fertility OR reproduction OR reproductive) W/2 (technol* OR preserv* OR cryopreserv* OR method*))
3	TITLE-ABS-KEY ((fertility OR reproduction OR reproductive OR "family planning"))
4	TITLE-ABS-KEY (((oocyte* OR embyo* OR ovarian OR ovaries OR ovary OR sperm* OR testicular OR testicle* OR gonad* OR gammete*) W/2 (preserv* OR cryopreserv* OR freez* OR frozen OR bank*)))
5	#2 or #3 or #4
6	#1 and #5
7	INDEX(embase) OR INDEX(medline) OR PMID(0* OR 1* OR 2* OR 3* OR 4* OR 5* OR 6* OR 7* OR 8* OR 9*)
8	#6 and not #7
9	DOCTYPE(ed) OR DOCTYPE(bk) OR DOCTYPE(er) OR DOCTYPE(no) OR DOCTYPE(sh) OR DOCTYPE(ch)
10	#8 and not #9
11	PUBYEAR AFT 1999 AND LANGUAGE(arabic or chinese or english or french or german or portuguese or spanish or turkish)
12	#10 and #11
13	TITLE-ABS-KEY((alpaca OR alpacas OR amphibian OR amphibians OR animal OR animals OR antelope OR armadillo OR armadillos OR avian OR baboon OR baboons OR beagle OR beagles OR bee OR bees OR bird OR birds OR bison OR bovine OR buffalo OR buffaloes OR buffalos OR "c elegans" OR "Caenorhabditis elegans" OR camel OR camels OR canine OR canines OR carp OR cats OR cattle OR chick OR chicken OR chickens OR chicks OR chimp OR chimpanze OR chimpanzees OR chimps OR cow OR cows OR "D melanogaster" OR "dairy calf" OR "dairy calves" OR deer OR dog OR dogs OR donkey OR donkeys OR drosophila OR "Drosophila melanogaster" OR duck OR duckling OR ducklings OR ducks OR equid OR equids OR equine OR equines OR feline OR felines OR ferret OR ferrets OR finch OR finches OR fish OR flatworm OR flatworms OR fox OR foxes OR frog OR frogs OR "fruit flies" OR "fruit fly" OR "G mellonella" OR "Galleria mellonella" OR geese OR gerbil OR gerbils OR goat OR goats OR goose OR gorilla OR gorillas OR hamster OR hamsters OR hare OR hares OR heifer OR heifers OR horse OR horses OR insect OR insects OR jellyfish OR kangaroo OR kangaroos OR kitten OR kittens OR lagomorph OR lagomorphs OR lamb OR lambs OR llama OR llamas OR macaque OR macaques OR macaw OR macaws OR marmoset OR marmosets OR mice OR minipig OR minipigs OR mink OR minks OR monkey OR monkeys OR mouse OR mule OR mules OR nematode OR nematodes OR octopus OR octopuses OR orangutan OR "orang-utan" OR orangutans OR "orang-utans" OR oxen OR parrot OR parrots OR pig OR pigeon OR pigeons OR piglet OR piglets OR pigs OR porcine OR primate OR primates OR quail OR rabbit OR rabbits OR rat OR rats OR reptile OR reptiles OR rodent OR rodents OR ruminant OR ruminants OR salmon OR sheep OR shrimp OR slug OR slugs OR swine OR tamarin OR tamarins OR toad OR toads OR trout OR urchin OR urchins OR vole OR voles OR waxworm OR waxworms OR worm OR worms OR xenopus OR "zebra fish" OR zebrafish) AND NOT (human OR humans or patient or patients))
14	#12 and #13

http://dx.doi.org/10.21037/atm-20-4523

SCOPUS

#	Searches
1	exp Transgender Persons/
2	exp Transsexualism/
3	exp Sex Reassignment Procedures/
4	exp Transgender/ or exp Gender Reassignment/ or exp Gender Dysphoria/
5	(((sex* or gender) adj (transition* or transform* or reassign* or chang*)) or ((trans or intersex) adj (sexual* or gender* or male or men or women or female or people or person*)) or (cross adj (sex* or gender*)) or (trans adj gender*) or "2 spirit person" or "2-spirit person" or crossgender* or "gender dysphoria*" or "gender identit*" or "gender nonbinary" or "gender nonconforming" or "gender queer*" or "Gender reassignment*" or genderqueer* or "gender-variant*" or "nonbinary gender*" or "nonconforming gender*" or "Sex reassignment*" or transexual* or transgender* or transgenderism or transpeople or transperson* or transsexual*).ti,ab,hw,kw.
6	1 or 2 or 3 or 4 or 5
7	((fertility or reproduction or reproductive) adj2 (technol* or preserv* or cryopreserv* or method*)).ti,ab,hw,kw.
8	(fertility or reproduction or reproductive).ti.
9	"family planning".ti.
10	((oocyte* or embyo* or ovarian or ovaries or ovary or sperm* or testicular or testicle* or gonad* or gammete*) adj2 (preserv* or cryopreserv* or freez* or frozen or bank*)).ti,ab,hw,kw.
11	fertility preservation/
12	reproductive techniques/
13	semen preservation/
14	cryopreservation/
15	or/7-14
16	6 and 15
17	16 not ((exp animals/ or exp nonhuman/) not exp humans/)
18	((alpaca or alpacas or amphibian or amphibians or animal or animals or antelope or armadillo or armadillos or avian or baboon or baboons or beagle or beagles or bee or bees or bird or birds or bison or bovine or buffalo or buffaloes or buffalos or "c elegans" or "Caenorhabditis elegans" or camel or camels or canine or canines or carp or cats or cattle or chameleon* or chick or chicken or chickens or chicks or chimp or chimpanze or chimpanzees or chimps or cow or cows or "D melanogaster" or "dairy calf" or "dairy calves" or deer or dog or dogs or donkey or donkeys or drosophila or "Drosophila melanogaster" or duck or duckling or ducklings or ducks or equid or equids or equine or equines or feline or felines or ferret or ferrets or finch or finches or fish or flatworm or flatworms or fox or foxes or frog or frogs or "fruit flies" or "fruit fly" or "G mellonella" or "Galleria mellonella" or geese or gerbil or gerbils or goat or goats or goose or gorilla or gorillas or hamster or hamsters or hare or hares or heifer or heifers or horse or horses or insect or insects or jellyfish or kangaroo or kangaroos or kitten or kittens or lagomorph or lagomorphs or lamb or lambs or llama or llamas or macaque or macaques or macaw or macaws or marmoset or marmosets or mice or minipig or minipigs or mink or minks or monkey or monkeys or mouse or mule or mules or nematode or nematodes or octopus or octopuses or orangutan or "orang-utan" or orangutans or "orang-utans" or oxen or parrot or parrots or pig or pigeon or pigeons or piglet or piglets or pigs or porcine or primate or primates or quail or rabbit or rabbits or rat or rats or reptile or reptiles or rodent or rodents or ruminant or ruminants or salmon or sheep or shrimp or slug or slugs or swine or tamarin or tamarins or toad or toads or trout or urchin or urchins or vole or voles or waxworm or waxworms or worm or worms or xenopus or "zebra fish" or zebrafish) not (human or humans or patient or patients)).ti,ab,hw,kw,mp.
19	17 not 18
20	19 not (mice or mouse or rat*).ti.
21	limit 20 to (english or spanish or chinese or french or turkish) [Limit not valid in CCTR,CDSR; records were retained]
22	limit 21 to (conference abstract or editorial or erratum or note or addresses or autobiography or bibliography or biography or blogs or comment or dictionary or directory or interactive tutorial or interview or lectures or legal cases or legislation or news or newspaper article or overall or patient education handout or periodical index or portraits or published erratum or video-audio media or webcasts) [Limit not valid in Ovid MEDLINE(R),Ovid MEDLINE(R) Daily Update,Ovid MEDLINE(R) In-Process,Ovid MEDLINE(R) Publisher,CCTR,CDSR,Embase; records were retained]
23	21 not 22
24	limit 23 to yr="2000 -Current"
25	remove duplicates from 24

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