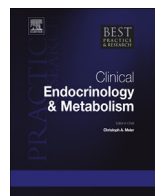




ELSEVIER

Contents lists available at ScienceDirect

Best Practice & Research Clinical
Endocrinology & Metabolismjournal homepage: www.elsevier.com/locate/beem

Male fertility preservation in DSD, XXY, pre-gonadotoxic treatments – Update, methods, ethical issues, current outcomes, future directions

Maria Grazia Giudice, MD, OBGYN Consultant, PhD Candidate in Andrology ^{a, b},

Federico Del Vento, MD, OBGYN Consultant, PhD Candidate in Andrology ^b,

Christine Wyns, MD, PhD, Head of Department of Gynecology-Andrology, Cliniques Universitaires Saint Luc ^{a, b, *}

^a Department of Gynecology-Andrology, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium

^b Institut de Recherche Expérimentale et Clinique (IREC), Université Catholique de Louvain, Avenue Mounier 52, 1200 Brussels, Belgium

ARTICLE INFO

Article history:

Available online xxx

Keywords:

fertility preservation

DSD

Klinefelter syndrome

This paper aims at reviewing the fertility preservation strategies that could be considered in several conditions at risk of spermatogonial depletion such as 46,XY disorders of sexual development, Klinefelter syndrome and after gonadotoxic treatment in males highlighting current knowledge on diseases and processes involved in infertility as well as future directions along with their specific ethical issues.

While sperm cryopreservation after puberty is the only validated technique for fertility preservation, for prepubertal boys facing gonadotoxic therapies or at risk of testicular tissue degeneration where testicular sperm is not present, cryopreservation of spermatogonial cells may be an option to ensure future parenthood. Promising results with transplantation and in vitro maturation of spermatogonial cells were achieved in animals but so far none of the techniques was applied in humans.

© 2019 Elsevier Ltd. All rights reserved.

* Corresponding author. Department of Gynecology-Andrology, Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, 1200 Brussels, Belgium.

E-mail address: christine.wyns@uclouvain.be (C. Wyns).

<https://doi.org/10.1016/j.beem.2019.01.002>

1521-690X/© 2019 Elsevier Ltd. All rights reserved.

Introduction

Several conditions can affect men's reproductive capacity during their lifespan such as gonadotoxic treatments for oncological diseases [1] or for benign hematological diseases requiring preconditioning chemotherapy before bone marrow transplantation, immunosuppressive therapies for autoimmune diseases [2], genetic disorders affecting fertility, and disorders of sex development (DSDs) [3].

Fertility preservation (FP) options may offer the possibility for future fatherhood and counseling about the different methods available should be provided to each patient (and/or his parents for minors) when facing such clinical situations.

While sperm cryopreservation is a validated technique in adult men and post-pubertal adolescents [4], for prepubertal boys or for young adult men presenting with azoospermia due to arrest of the spermatogonial maturation or a condition leading to progressive testicular degeneration, cryopreservation of spermatogonial stem cells (SSCs) might be the sole option, although this strategy is still experimental [5]. Cryopreserving the SSCs may be done by freezing whole testicular tissue or cell suspensions. If for cryopreservation of SSCs suspensions a mechanical or enzymatic tissue digestion method is required, immature testicular tissue (ITT) cryopreservation offers the advantage of maintaining cell connections required for SSCs maturation [6], albeit none of the two methods proved itself superior to the other so far [7]. The main goal of fertility preservation experimental programs is obtaining mature spermatozoa with reproductive potential. Active research is therefore ongoing to restore fertility with cryostored SSCs, both in vivo in animal models and in vitro using different culture systems [5].

This paper aims at reviewing fertility preservation strategies that could be considered in several conditions such as DSD, Klinefelter syndrome and after gonadotoxic treatment in males highlighting current knowledge on diseases and processes involved in infertility as well as future directions along with their specific ethical issues.

Methods

A systematic literature review was performed using PubMed electronic databases to search for the following query: (((Disorders Sex Development) OR (Klinefelter Syndrome) OR (Gonadotoxic treatment)) AND (Fertility Preservation) AND Human). Studies published in English language until the 31st July 2018 were included. Any additional references found in original articles or review papers that were found relevant and missing from the primary search were added. In Fig. 1 a flow chart describes the literature selection process.

Results

The literature review found studies on fertility for DSD, Klinefelter syndrome and following gonadotoxic therapies in the male. With regard to fertility preservation, studies were limited to Klinefelter patients and males facing gonadotoxic therapies but not available for DSD.

Disorders of sex development with 46 XY karyotype

DSD is a group of congenital conditions characterized by atypical development of chromosomal, gonadal or anatomic sex that leads to a discordance between the genital appearance and the chromosomal sex [8]. Infertility in DSD can be due to anatomical defects, abnormal gonad development, prophylactic gonadectomy and hormone replacement therapy to induce and sustain puberty [9].

Since the 2006 Consensus Conference, experts are trying to define and classify the different disorders, and to give directions for sex assignment based on diagnosis, surgical options, need for hormonal replacement therapy, future fertility options, family's opinion and cultural context [10].

DSD worldwide incidence is approximately 1:1000–1:4500 live births. Excluding simple cryptorchidism and hypospadias the incidence rate among subjects with a 46,XY karyotype to have a DSD has been estimated to be 1 in 20,000 births [11]. Most cases are recognized during the neonatal period [8] but the success in reaching a molecular diagnosis is relatively low although clinical and biochemical investigations may adequately orient the molecular diagnosis [12]. The EU COST Action BM 1330

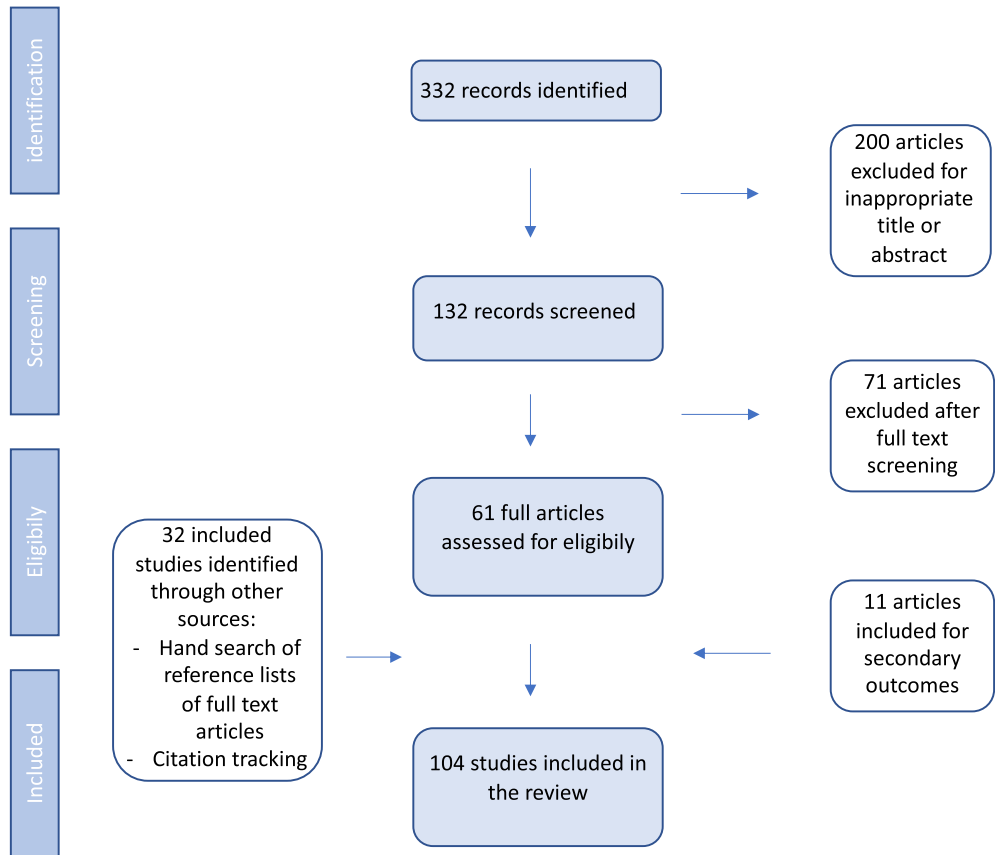


Fig. 1. Flow chart describing the literature selection process.

recommends a multidisciplinary approach in which the information on clinical phenotyping is considered in parallel with the biochemical data and genetic results in an integrative manner [13]. The possibility for fertility differs between the diagnostic groups but is significantly reduced in all types of DSD. In a multicenter study including 1040 patients with different DSD diagnoses only 14% of DSD patients reported at least one child and 7% of DSD patients were helped by ART [14].

DSDs are associated with an increased risk of developing a malignant germ cell tumor especially in case of abdominal testes [15]. The choice between a prophylactic orchidectomy or biopsy with fixation of gonads in a palpable position depends on many factors such as the macroscopic aspect of the gonads, the ability to reach a palpable position, the preference of parents and the predictability of future gender identity. Gonadectomy can be postponed until adolescence because the risk of germ cell cancer is low and gonadal preservation allows spontaneous puberty [16].

The decision of prophylactic gonadectomy in some DSD (see Table 1) and the abnormal gonad development in others impose a discussion on fertility and fertility preservation options if available [9].

The most common 46,XY DSD is the mixed gonadal dysgenesis, characterized by small sized gonads, hypotrophic Wolffian derivatives and ambiguous external genitalia. Infertility can be caused by the primary hypogonadism or by the genitals' anatomic defects [17]. In patients with testicular dysgenesis, seminiferous tubules lose their normal architecture over time and become hyalinized and atrophic [18]. Most of the children will be infertile in adulthood, with few reports where spermatogonia were identified and even a case of successful testicular sperm extraction (TESE) [19].

Other forms of DSDs are associated with enzymatic dysfunctions. The term congenital adrenal hyperplasia (CAH) refers to a group of diseases characterized by the defect of one of the five enzymes

involved in the conversion of cholesterol to testosterone. The most frequent condition is due to a defect of 21-hydroxylase where patients present normal testosterone levels and reduced cortisol and aldosterone production. Boys have a high risk to develop testicular adrenal rest tumors (TART) [20] and to present reduced fertility with abnormal semen parameters as a consequence of a compression effect of the tumor on the seminiferous tubules and suppression of the hypothalamo-pituitary-gonadal axis [21,22]. Depending on the semen quality, in vitro fertilization (IVF) or intra-uterine insemination (IUI) may be the solution in most of these cases [23].

Another enzymatic defect is the 5 alpha-reductase deficiency, an autosomal recessive disorder, where the conversion of testosterone to dihydrotestosterone is impaired. Infertility is the result of genitalia abnormalities (hypospadias, microphallus) and an abnormal secretory function that leads to a highly viscous semen and very small volumes of ejaculate [24], although sperm counts may be normal with possible paternity using IUI or IVF [25].

Similar genital anomalies due to lack of testosterone synthesis (and subsequently dihydrotestosterone) is found for deficiencies in 17-β-hydroxysteroid dehydrogenase-3 [26]. Usually men are infertile and need testosterone replacement therapy [27].

Infertility in androgen insensitivity syndrome (AIS) is due to androgen receptor mutations. Most of the cases present partial AIS with variable phenotypes and typical cryptorchidism histology, although fertility is possible with high dose testosterone (resulting in sperm parameters improvement) in combination with ICSI [28].

Another DSD called “non-hormonal” DSD presents with severe genital anomalies (micropenis, penile agenesis, cloacal exstrophy) [29,30]. Depending on the degree of the anomaly and the possible surgical results to guarantee a normal sexual life, assisted reproductive techniques can maximize the chances of fatherhood [18,31].

The rarest DSD is the Ovotesticular DSD, previously called hermaphroditism, characterized by the presence of ovarian and testicular tissue in the same individual [10]. The case of two children born after testicular sperm extraction and ICSI in the same patient with ovotesticular DSD has been reported [32].

To summarize, the reproductive potential of DSD patients should be assessed individually and fertility care individualized.

Although not always detected, most of the cases are due to mutations in the androgen receptor (AR) gene, of which over 1000 have been reported (Gottlieb et al., 2012). These disorders follow an X-linked pattern of inheritance being transmitted solely through healthy female carriers, as infertility is a hallmark of the condition in the affected male subjects.

Klinefelter Syndrome

The Klinefelter syndrome (KS), first described in 1942, is the most common sex chromosomal disorder in males with an estimated frequency of 1/450–1/600 newborns [37] and is characterized by the presence of one Y chromosome and two or more X chromosomes. The 47,XXY karyotype is the most common karyotype found in almost 80% of cases, while the other 20% present high-grade chromosome aneuploidies, 46,XY/47,XXY mosaicism or structurally abnormal X chromosomes [38].

Beyond being the most frequent genetic cause of azoospermia [39] and of different degrees of infertility, KS contemplates a wide variety of phenotypic and clinical characteristics, such as gynecomastia, small testes, hypergonadotropic hypogonadism, learning difficulties, and an increased risk of cardiovascular diseases [40,41].

Over the last decades, a rise in KS prevalence has been reported. Indeed, an increased age at the time of conception for both parents (4-fold increase in more than 40 year-old mothers) [37,42] and environmental factors interfering with paternal spermatogenesis (meiosis I) have been reported as being causative [43]. Furthermore, the diffusion of non-invasive prenatal testing technology is supposed to increase diagnosis of KS by 4–5-fold, raising the necessity for evidence-based care for these patients and their parents. Nowadays, 25% of KS patients get a post-natal diagnosis and less than 3% are diagnosed before puberty [37], while adult patients usually discover it because of symptoms linked to hypogonadism, sexual dysfunction and infertility [44].

Infertility is a consequence of germ cell degeneration that commences already in utero during testis development, progresses slowly during infancy [45] and early childhood [46], and accelerates during

Table 1

Summarizes the different DSDs types with their related fertility outcomes, the need or not for orchidectomy and for FP measures.

Type of DSD	Fertility outcomes	Risk of Germ Cell Tumor	Prophylaxy	Fertility Preservation/ ART needed	Testicular function and histology	References
Gonadal Dysgenesis	Infertility One successful TESE	High	Orchidectomy/ orchidopexy and follow-up with biopsy	ITT cryopreservation	Tubules atrophy and hyalinization. Limited number of spermatogonia	[16,18,19]
CAH 21-hydroxylase deficiency	Subfertility especially in TART (<i>testicular adrenal rest tumors</i>)	Low High risk for TART	Not recommended	IUI or IVF/ICSI	Variable spermatogenesis and Leydig function	[21–23]
5-alpha-reductase deficiency type 2	Infertility or subfertility	Low	Not recommended	IUI or IVF/ICSI	Normal seminiferous tubules	[25,33,34]
17-beta-hydroxysteroid dehydrogenase deficiency	Azoospermia	Intermediate	Orchidopexy and follow-up with biopsy	ITT cryopreservation	Germ cell rare in adolescent tissue	[27,31,35]
Androgen action disorders	Subfertility	Intermediate	Orchidopexy and follow-up with biopsy	IUI or IVF/ICSI	Variable spermatogenesis	[16,28,36]
Severe genital anomaly	Infertility to normal fertility	Low	Not recommended	IUI or IVF/ICSI	Depending of the type of disorder	[18,31]
Ovotesticular DSD	Infertility One case report after TESE/ICSI	Intermediate- Low	Prophylactic gonadectomy or close surveillance	TESE – ICSI	Testicular and ovarian tissue in the same gonad	[9,32]

puberty and adolescence, eventually resulting in extensive fibrosis and hyalinization of the seminiferous tubules and hyperplasia of the interstitium in adult patients [47,48]. In addition, germ cell differentiation is arrested at the spermatogonia or early spermatocytes stages and it has been suggested that spermatogonia undergo apoptosis instead of entering meiosis at the onset of puberty [49]. Single residual foci with preserved spermatogenesis and spermatozoa able to generate offspring may be present in the testes of post-pubertal KS patients. Hence, spermatozoa can exceptionally be retrieved in the ejaculate of these patients [38], or in almost 40% of cases after testicular sperm extraction (TESE), and used as sperm source for ICSI [50,51] with a live birth rate (LBR) of 43% per ICSI cycle [52]. In a belgian cohort combining ICSI with preimplantation genetic testing (PGT), the rate of normal embryos was reduced, with increased risk of abnormalities for sex chromosome and autosomes, compared to control patients undergoing PGT for gender determination in couple with X-linked disease and using ejaculated sperm [53]. Genetic counseling should therefore be offered to couples with non-mosaic KS.

One of the prognostic factors for TESE success seems to be the age of patients with a higher percentage of positive TESE in younger men [54]. However, the testosterone level could also have an impact as reported in a small series of 68 men that underwent TESE where men with normal baseline testosterone had the best sperm retrieval rate (86%) [55]. Hence, KS patients presenting with low serum testosterone have been treated to increase their testosterone level with a variety of medications such as clomiphene citrate, human Chorionic Gonadotropin, recombinant FSH and aromatase inhibitors. Testosterone replacement therapy (TRT) has also been prescribed to support age-appropriate pubertal development and in the longer term to prevent osteoporosis, obesity, diabetes and metabolic syndrome [56]. However, TRT has also been considered having a negative influence on sperm retrieval rates found to be around 20–25% in men who previously received TRT [57,58]. Whether this is a permanent adverse effect of testosterone or, if and when TRT should be withdrawn before TESE is so far unknown. Table 2 summarizes sperm retrieval rates following hormone treatment before testicular sperm retrieval. Furthermore, in 18% of men where no spermatozoa were present during TESE and in half of adolescent boys where no meiotic germ cells were found, spermatogonia were observed [59]. Since testis degeneration accelerates with puberty, some researchers focused on the possibility to preserve fertility before complete tissue degeneration by cryopreserving SSCs for potential in vitro maturation in the future, although this subject is still controversial [60]. Indeed, on one hand, because of the lack of longitudinal studies, it is not known which adolescent patients will develop complete spermatogenesis after puberty [61]. On the other hand, the capacity of spermatogonia from KS patients to undergo meiosis is questionable [62].

It is still not clear if SSCs of KS patients present 47,XXY or 46,XY karyotypes but fluorescent in-situ hybridization experiments applied to ejaculated sperm showed 50–93% normal spermatozoa although with a higher proportion of hyperhaploid spermatozoa than in healthy men [63,64]. Consequently, two hypotheses on how spermatogenesis occurs in KS men have been raised: (1) 47,XXY spermatogonia have the potential to complete meiosis, explaining both the increase in sperm sex chromosomal aneuploidy and the presence of normal spermatozoa [65,66]; (2) spermatozoa arise from patches of 46,XY SSCs consecutive to “correcting mitotic errors” in the prenatal testis that might give rise to isolated testicular mosaicism, and the increased aneuploid sperm is due to meiotic errors caused by a compromised testicular environment [67,68]. Conclusions reached from studies analyzing directly the meiotic process in vivo or using an indirect deduction approach by analyzing spermatozoa are controversial. Further research is thus needed to unravel the mechanism of germ cells loss in 47,XXY patients and find fertility preservation methods. As autotransplantation of testicular tissue or cells is not an option due to the degenerative nature of the disease and the abnormal spermatogonial stem cell niche environment, in vitro maturation protocols for SSCs that might be retrieved in some of these patients should be developed.

Men facing gonadotoxic treatments

Due to great progress in oncological treatments with increasing survival rates estimated to be around 80% in children [71] and enhanced use of gonadotoxic therapies to treat some benign conditions [72], it is important to consider the long-term adverse effects of such therapies as a matter of quality of life on a steadily growing population. One of the major concerns with current treatment

Table 2

Summarizes sperm retrieval rates following hormone treatment before testicular sperm retrieval in KS patients.

Type of study	References	Nb of non-mosaic KS azoospermic patients (age range/median)	Type of study therapy	Duration of study therapy before TESE (Median/range)	Nb of patients taking T therapy	Breaking time of T therapy before TESE	Sperm Retrieval Rate
Retrospective	[57]	42 including 3 mosaic (24–52 y/32.8 y)	AI if T < 15.6 nmol/l or T/E2 < 100 Testolactone 2*50 –100 mg/d + hCG 2*1500 UI/w (n = 13) Testolactone 2*50 –100 mg/d (n = 19) Anastrozole 1 mg/d (n = 5) Anastrozole + hCG 2*1500 UI/w (n = 1) CC 25 mg/d (n = 3) rFSH (n = 1) none (n = 6) unknown (n = 6)	Median: 4 months	5	6–34 months before study treatment	Total 29/42 (69%) Previous T: 1/5 (20%)
Retrospective	[58]	68 (22–52 y/NA)	AI if T < 300 ng/dl (10.4 nmol/l) Testolactone 2*50–100 mg/d (n = 28) Anastrozole 1 mg/d (n = 9) Anastrozole + hCG (n = 1) If no response to AI: CC 25 mg/d (n = 3) or hCG alone (n = 4)	Median: 163 days (positive TESE) 164 days (negative TESE)	8	≥6 months before study treatment	Total 45/68 (66%) Previous T: 2/8 (25%)
Retrospective	[69]	10 (14–22 y/15.5)	Topical T therapy (2.5–10 g/d) to target T > 400 ng/dl and AI if T/E2 < 10 (1 mg anastrozole/day) max 24 months	NA/1–5 y	10	0	7/10 (70%)
Prospective comparative between age groups (15–23 y and >23 y)	[70]	41 (15–40 y/19.9)	Oral Testosterone undecanoate 2* 80 mg/d (n = 2) Testosterone enanthate i.m. 50–250 mg/3–4 w (n = 13) Testosterone undecanoate i.m. 1000 mg/3 months (n = 1) Unknown (n = 1)	1.5 y/NA * (positive TESE) 3.2 y/NA (negative TESE)	17	≥9 months	Previous T 9/17 (52.9%)* Without previous T 14/24 (59.1%)

TESE: testicular sperm extraction; y: years; AI: aromatase inhibitor; hCG: human Chorionic Gonadotropin; CC: Clomiphene Citrate; rFSH: recombinant Follicle Stimulating Hormone; T: testosterone; w: weeks; i.m.= intramuscular; NA= not available; *p=0.53 for difference between duration time of T treatment in group positive TESE et negative TESE; **p=0.98 for difference between groups with or without previous T treatment.

regimens is the gonadal dysfunction [73]. Indeed, testicular germ cells and especially type B spermatogonia have high mitotic and meiotic indices and are thus highly sensitive to the effect of chemo- and radiotherapy, although the most severe and definitive damage follows SSCs depletion (a subpopulation of undifferentiated type A spermatogonia) [74]. The testicular somatic cells are less affected but decreased testosterone levels due to Leydig cells impairment [75] as well as changes in cytokeratin expression and inhibin/follicle stimulating hormone (FSH) ratio as a reflect of Sertoli cells damage were reported [76].

The effects of these therapies are unpredictable although some poor prognostic factors for future fertility have been identified such as radiation doses ≥ 4 Gy to the testes, fractionation of testicular radiotherapy, alkylating agents based chemotherapy and high alkylating agent dose defined as cyclophosphamide equivalent dose above 5 g/m² [77,78].

The simplest strategy for FP of postpubertal patients able to provide an ejaculate is sperm cryopreservation before gonadotoxic treatment, a procedure that should be routinely proposed nowadays [79] and allows high success rates. Indeed, in a cohort of 272 male cancer survivors, the LBR after ICSI with cryopreserved sperm was 62.1% a result that was similar to couples treated for male infertility and even higher than the LBR in couple with healthy normozoospermic men undergoing the same treatment [80]. When ejaculation is not possible because of psychological factors or sexual immaturity, assisted ejaculation using penile vibration or electroejaculation under general anesthesia may be an alternative [81]. If assisted ejaculation fails after puberty or in peripuberty or when patients present with azoospermia, testicular sperm retrieval can be proposed.

When no mature spermatozoa can be found in the testis or in pre-pubertal boys, SSCs cryopreservation may be offered although the technique is still experimental. While no fertility restoration technique with cryostored human SSCs has been applied in humans, except for a clinical trial that was announced in 2003 [82] but without further report of follow-up data, encouraging results have been achieved in animals.

Briefly, research indicates that SSCs from prepubertal animals can be isolated, cultured in vitro and used for autologous transplantation resulting in spermatogenesis resumption with offspring in mice [83] and later in other species including non-human primates (for review see [84]). Autotransplantation of ITT also proved to be successful in several animal species including monkey, where complete spermatogenesis and fertile offspring were obtained (for review see Del Vento et al., 2018 [85]). Although autotransplantation of prepubertal testicular tissue was not performed in humans, xenotransplantation of human ITT to nude mice was used as a model to demonstrate that frozen-thawed and vitrified-warmed SSCs were still able to survive, proliferate and also differentiate up to the pachytene spermatocyte stage but development of mature spermatozoa was not achieved yet [86,87].

In oncological cured patients, an important concern is the risk to transplant cells or tissue containing cancer cells. Indeed, testicular infiltration was detected in 21% of young boys with leukemia [88] and xenotransplantation of leukemia rat testicular cells led to tumor transmission in the recipient nude mice [89]. In vitro maturation of SSCs to obtain mature spermatozoa may circumvent this risk. In mouse, spermatogenesis and healthy offspring were obtained after organotypic culture of fresh and frozen ITT [90,91].

With regards to human ITT, complete in vitro spermatogenesis has not been reproduced so far but a good functionality of Leydig cells, maturation of Sertoli cells with partial establishment of the blood-testis barrier and very recently development of haploid germ cells were achieved using a long term organotypic culture system [92–94]. However, the efficiency of the culture system has to be improved and the final maturation of the spermatids into spermatozoa still needs to be accomplished.

In another recent publication, Sun et al. using a culture system with Matrigel obtained haploid cells that could fertilize mouse oocytes from the testicular tissue of adult azoospermic patients [95].

These encouraging results motivate pursuance of both fertility preservation programs for prepubertal boys and research on fertility restoration methods using cryostored SSCs.

Ethical issues

A number of ethical issues regarding FP programs have been raised including the risk-benefit balance, the decision-making process, the patient's autonomy in the decisional process with for

minors the difficulty to take joint decisions with their parents, the patient/parental consent and child's ascent, and the possibility of transmitting a genetically determined condition [96,97]. Each health condition described in this paper has own peculiarities and without being exhaustive some of these specificities will be presented. In all these clinical situations, patients and their parents (for minors) face important decisions concerning future fertility at a moment when the patient's fertility is not always contemplated at the time of discussion.

The decisional process may be even more complicated when as in prepubertal boys an invasive technique has to be performed to collect SSCs while fertility restoration techniques still need to be developed in humans. Moreover, especially in the oncologic pediatric population, the moment of harvesting SSCs lies before the start of anti-cancer treatment in a phase of utter confusion where parents already have to deal with a serious diagnosis and its therapeutic consequences while the child, if mature enough to understand the procedure, has to project himself in the future at a moment when parenthood is not his concern. In a survey evaluating the FP program in this population, the decision on FP appeared to be positively influenced by the completeness of the information provided while pressure from doctors to start cancer therapy earlier had a negative impact on the decision [98]. It is therefore important to provide the patient and parents with adequate support within a multi-collaborative care pathway to help the decisional process and to allow a fast access to the FP procedure.

In KS prepubertal boys, unknowns on the reproductive potential of KS SSCs represent an even bigger ethical issue considering the invasiveness of the sampling procedure and the uncertainty regarding spermatogenic foci that may appear after puberty. Nevertheless, in a study questioning 49 pediatricians and 18 parents, FP measures were appreciated by most of the parents, as 83,3% agreed on performing a testicular biopsy [50].

In DSD patients, the major difficulty is the prediction of gender identity in children, a situation where parents and/or patients must take important decisions on surgical treatment including prophylactic gonadectomy or hormonal therapies. Sometimes gonadectomy is necessary due to the risk related of germ cell cancer and the possibility for FP should then be carefully balanced.

Because of the experimental character of some FP methods considering alongside the promotion of other ways of parenthood, such as adoption, is crucial and a multidisciplinary collaborative care pathway, including a team for substantial and detailed ethical analysis, is therefore essential to improve fertility counseling for individuals with DSD [99].

Future directions

Despite several limitations such as the lack of detailed knowledge of the spermatogenic process, the absence of study models for fertility restoration techniques with human SSCs and the scarcity of human ITT for research purposes, the field of FP is rapidly progressing.

So far autologous transplantation of ITT has been achieved in non-human primates with normal offspring [100] and no increased risk of developmental or epigenetic defects was observed following the procedure in rodents offspring [101]. Due to the risk of introducing malignant cells back to the patient, ITT transplantation should be limited to patients treated for benign conditions who became infertile. As ongoing preclinical studies on optimization of the transplantation procedure are progressing [102], it is likely that clinical trials could be considered in a near future. Regarding SSCs transplantation, although there is ongoing preclinical work, demonstration of the safety of cell propagation in culture before transplantation and of safe removal of cancer cells are the next challenges [103,104]. With regard to in vitro maturation of human prepubertal testicular tissue, although meiotic differentiation up to the spermatid stage was recently achieved [94] improvement of the procedure's efficiency and spermiogenesis to obtain mature spermatozoa must still be accomplished. To guarantee a safe clinical use of developed methods, future studies should focus on the genetic and epigenetic stability of the in vitro propagated and/or differentiated germ cells.

In addition, when it comes to KS patients, the reproductive potential of cryostored SSCs should be evaluated and the physiopathology of testicular tissue degeneration unraveled as first goals.

Genomic editing tools could also prove helpful in the future to obtain functional gametes where fertility restoration techniques fail due to genetic abnormalities. Correcting defects in spermatogenesis

caused by genomic mutations and preventing transmission of genomic diseases will be huge technical and ethical challenges of the future.

Summary

Fertility Preservation aimed at preserving the potential for genetic parenthood in adults of reproductive age or children who are at risk of sterility. In men FP can be realized by sperm cryopreservation; in prepubertal boys or men affected by testicular impairment only spermatogonial stem cells (SSCs) can be cryopreserved as experimental protocol. Researches are focusing on the model using to obtain mature spermatozoa. Several pathologies can affect testicular function as Disorders of Sex Development (DSDs) Klinefelter Syndrome (KS) or gonadotoxic therapies. DSD in 46,XY patients represent a large and heterogenous group of rare diseases with more or less severe infertility. Depending on the disorder, surgery, hormonal therapies and assisted reproductive technologies can help to father a child. DSD patients needed orchidectomy can only save SSCs. Klinefelter Syndrome is the most frequent genetic cause of azoospermia. Even if 43% of 47,XXY men will experience fatherhood by the help of in vitro fertilization, the disease is characterized by gonadal degeneration that is accelerated at the onset of puberty with the risk of permanent infertility in adulthood. Cryopreservation of SSCs before or after puberty could be the only option for future fatherhood. Men and boys needed a gonadotoxic treatment have high risk of infertility, therefore fertility preservation is recommended. While cryopreservation of mature spermatozoa remains a priority as the only validated technique, for prepubertal boys SSCs cryopreservation is so far the sole option to provide the patients with the hope of future parenthood.

Practice points

- Fertility preservation by sperm cryopreservation in post-pubertal boys and adult men is a routine practice.
- In Klinefelter Syndrome where mature sperm is not found or in prepubertal boys before gonadotoxic treatment or in prepubertal DSD before prophylactic gonadectomy only spermatogonial stem cells cryopreservation, as an experimental protocol, can be proposed.
- Research focused on transplantation or in vitro maturation of SSCs shows encouraging results in non-human primates.
- Fertility preservation in DSD remains a big challenge because of the difficulty of gender identity prediction in patients.

Research agenda

- To guarantee a safe clinical use of fertility restoration methods with cryostored SSCs, future studies should focus on the genetic and epigenetic stability of the in vitro propagated and/or differentiated germ cells.
- Genomic editing tools could prove helpful in the future to obtain functional gametes where fertility restoration techniques fail due to genetic abnormalities. Correcting defects in spermatogenesis caused by genomic mutations and preventing transmission of genomic diseases will be huge technical and ethical challenges of the future.
- To obtain mature spermatozoa by in vitro maturation of human prepubertal tissue improvement of the procedure's efficiency and spermiogenesis must still be accomplished.
- The reproductive potential of cryostored SSCs in KS patients should be evaluated and the physiopathology of testicular tissue degeneration unraveled as first goals.
- Need of prospective collection data in DSD to improve fertility care of DSD patients

References

- [1] Wallace WH, Anderson RA, Irvine DS. Fertility preservation for young patients with cancer: who is at risk and what can be offered? *Lancet Oncol* 2005;6(4):209–18.
- [2] Suehiro RM, Borba EF, Bonfa E, et al. Testicular Sertoli cell function in male systemic lupus erythematosus. *Rheumatology (Oxford)* 2008;47(11):1692–7.
- [3] Guercio G, Rey RA. Fertility issues in the management of patients with disorders of sex development. *Endocr Dev* 2014; 27:87–98.
- [4] Anger JT, Gilbert BR, Goldstein M. Cryopreservation of sperm: indications, methods and results. *J Urol* 2003;170(4 Pt 1): 1079–84.
- [5] de Michele F, Vermeulen M, Wyns C. Fertility restoration with spermatogonial stem cells. *Curr Opin Endocrinol Diabetes Obes* 2017 Dec;24(6):424–31.
- [6] Ogawa T, Ohmura M, Ohbo K. The niche for spermatogonial stem cells in the mammalian testis. *Int J Hematol* 2005; 82(5):381–8.
- [7] Pacchiarotti J, Ramos T, Howerton K, et al. Developing a clinical-grade cryopreservation protocol for human testicular tissue and cells. *Biomed Res Int* 2013;2013:930962.
- *[8] Lee PA, Nordenstrom A, Houk CP, et al. Global disorders of sex development update since 2006: perceptions, approach and care. *Horm Res Paediatr* 2016;85(3):158–80.
- [9] Van Batavia JP, Kolon TF. Fertility in disorders of sex development: a review. *J Pediatr Urol* 2016;12(6):418–25.
- [10] Lee PA, Houk CP, Ahmed SF, et al. International Consensus conference on intersex organized by the Lawson Wilkins pediatric endocrine S, the European society for paediatric E. Consensus statement on management of intersex disorders. International Consensus conference on intersex. *Pediatrics* 2006;118(2):e488–500.
- [11] Sax L. How common is intersex? a response to Anne Fausto-Sterling. *J Sex Res* 2002;39(3):174–8.
- [12] Alhomaidah D, McGowan R, Ahmed SF. The current state of diagnostic genetics for conditions affecting sex development. *Clin Genet* 2017;91(2):157–62.
- *[13] Audi L, Ahmed SF, Krone N, et al. GENETICS IN ENDOCRINOLOGY: approaches to molecular genetic diagnosis in the management of differences/disorders of sex development (DSD): position paper of EU COST Action BM 1303 'DSDnet'. *Eur J Endocrinol Eur Federat Endoc Soc* 2018;179(4):R197–206.
- [14] Slowikowska-Hilczner J, Hirschberg AL, Claahsen-van der Grinten H, et al. Fertility outcome and information on fertility issues in individuals with different forms of disorders of sex development: findings from the dsd-LIFE study. *Fertil Steril* 2017;108(5):822–31.
- [15] Oosterhuis JW, Looijenga LH. Testicular germ-cell tumours in a broader perspective. *Nat Rev Cancer* 2005;5(3):210–22.
- [16] van der Zwan YG, Biermann K, Wolffenbuttel KP, et al. Gonadal maldevelopment as risk factor for germ cell cancer: towards a clinical decision model. *Eur Urol* 2015;67(4):692–701.
- [17] Martinierie L, Morel Y, Gay CL, et al. Impaired puberty, fertility, and final stature in 45,X/46,XY mixed gonadal dysgenetic patients raised as boys. *Eur J Endocrinol Eur Federat Endoc Soc* 2012;166(4):687–94.
- [18] Hsieh MH, Hollander A, Lamb DJ, et al. The genetic and phenotypic basis of infertility in men with pediatric urologic disorders. *Urology* 2010;76(1):25–31.
- [19] Flannigan RK, Chow V, Ma S, et al. 45,X/46,XY mixed gonadal dysgenesis: a case of successful sperm extraction. *Can Urol Assoc J* 2014;8(1–2):E108–10.
- [20] Claahsen-van der Grinten HL, Otten BJ, Takahashi S, et al. Testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia: evaluation of pituitary-gonadal function before and after successful testis-sparing surgery in eight patients. *J Clin Endocrinol Metab* 2007;92(2):612–5.
- [21] Stikkelbroeck NM, Otten BJ, Pasic A, et al. High prevalence of testicular adrenal rest tumors, impaired spermatogenesis, and Leydig cell failure in adolescent and adult males with congenital adrenal hyperplasia. *J Clin Endocrinol Metab* 2001; 86(12):5721–8.
- [22] Claahsen-van der Grinten HL, Stikkelbroeck NM, Sweep CG, et al. Fertility in patients with congenital adrenal hyperplasia. *J Pediatr Endocrinol Metab* 2006;19(5):677–85.
- [23] Falhammar H, Nystrom HF, Ekstrom U, et al. Fertility, sexuality and testicular adrenal rest tumors in adult males with congenital adrenal hyperplasia. *Eur J Endocrinol Eur Federat Endoc Soc* 2012;166(3):441–9.
- [24] Cai LQ, Fratianni CM, Gautier T, et al. Dihydrotestosterone regulation of semen in male pseudohermaphrodites with 5 alpha-reductase-2 deficiency. *J Clin Endocrinol Metab* 1994;79(2):409–14.
- [25] Katz MD, Kligman I, Cai LQ, et al. Paternity by intrauterine insemination with sperm from a man with 5 alpha-reductase-2 deficiency. *N Engl J Med* 1997;336(14):994–7.
- [26] Cohen-Kettenis PT. Gender change in 46,XY persons with 5 alpha-reductase-2 deficiency and 17 beta-hydroxysteroid dehydrogenase-3 deficiency. *Arch Sex Behav* 2005;34(4):399–410.
- [27] Farkas A, Rosler A. Ten years experience with masculinizing genitoplasty in male pseudohermaphroditism due to 17 beta-hydroxysteroid dehydrogenase deficiency. *Eur J Pediatr* 1993;152(Suppl. 2):S88–90.
- [28] Tordjman KM, Yaron M, Berkovitz A, et al. Fertility after high-dose testosterone and intracytoplasmic sperm injection in a patient with androgen insensitivity syndrome with a previously unreported androgen receptor mutation. *Andrologia* 2014;46(6):703–6.
- [29] Reiner WG, Gearhart JP. Discordant sexual identity in some genetic males with cloacal exstrophy assigned to female sex at birth. *N Engl J Med* 2004;350(4):333–41.
- [30] Reiner WG, Kropp BP. A 7-year experience of genetic males with severe phallic inadequacy assigned female. *J Urol* 2004; 172(6 Pt 1):2395–8. Discussion 2398.
- [31] Higuchi T, Holmdahl G, Kaefer M, et al. International consultation on urological diseases: congenital anomalies of the genitalia in adolescence. *Urology* 2016;94:288–310.
- [32] Sugawara N, Kimura Y, Araki Y. Successful second delivery outcome using refrozen thawed testicular sperm from an infertile male true hermaphrodite with a 46, XX/46, XY karyotype: case report. *Hum Cell* 2012;25(4):96–9.

- [33] Imperato-McGinley J, Peterson RE, Gautier T, et al. Androgens and the evolution of male-gender identity among male pseudohermaphrodites with 5 alpha-reductase deficiency. *N Engl J Med* 1979;300(22):1233–7.
- [34] Cools M, Nordenstrom A, Robeva R, et al. Caring for individuals with a difference of sex development (DSD): a Consensus Statement. *Nat Rev Endocrinol* 2018;14(7):415–29.
- [35] Chuang J, Vallerie A, Breech L, et al. Complexities of gender assignment in 17 beta-hydroxysteroid dehydrogenase type 3 deficiency: is there a role for early orchiectomy? *Int J Pediatr Endocrinol* 2013;2013(1):15.
- [36] Yong EL, Ng SC, Roy AC, et al. Pregnancy after hormonal correction of severe spermatogenic defect due to mutation in androgen receptor gene. *Lancet* 1994;344(8925):826–7.
- [37] Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab* 2003;88(2):622–6.
- *[38] Lanfranco F, Kamischke A, Zitzmann M, et al. Klinefelter's syndrome. *Lancet* 2004;364(9430):273–83.
- [39] Tuttelmann F, Werny F, Cooper TG, et al. Clinical experience with azoospermia: aetiology and chances for spermatozoa detection upon biopsy. *Int J Androl* 2011;34(4):291–8.
- [40] Groth KA, Skakkebaek A, Host C, et al. Clinical review: Klinefelter syndrome—a clinical update. *J Clin Endocrinol Metab* 2013;98(1):20–30.
- [41] Salzano A, Arcopinto M, Marra AM, et al. Klinefelter syndrome, cardiovascular system, and thromboembolic disease: review of literature and clinical perspectives. *Eur J Endocrinol Eur Federat Endoc Soc* 2016;175(1):R27–40.
- [42] Herlihy AS, Halliday J. Is paternal age playing a role in the changing prevalence of Klinefelter syndrome? *Eur J Hum Genet* 2008;16(10):1173–4. author reply 1174.
- [43] Morris JK, Alberman E, Scott C, et al. Is the prevalence of Klinefelter syndrome increasing? *Eur J Hum Genet* 2008;16(2):163–70.
- [44] Corona G, Petrone L, Paggi F, et al. Sexual dysfunction in subjects with Klinefelter's syndrome. *Int J Androl* 2010;33(4):574–80.
- [45] Mikamo K, Agueric M, Hazeghi P, et al. Chromatin-positive Klinefelter's syndrome. A quantitative analysis of spermatogonial deficiency at 3, 4, and 12 months of age. *Fertil Steril* 1968;19(5):731–9.
- [46] Muller J, Skakkebaek NE, Ratcliffe SG. Quantified testicular histology in boys with sex chromosome abnormalities. *Int J Androl* 1995;18(2):57–62.
- [47] Wikstrom AM, Raivio T, Hadziselimovic F, et al. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. *J Clin Endocrinol Metab* 2004;89(5):2263–70.
- *[48] Akglaede L, Wikstrom AM, Rajpert-De Meyts E, et al. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. *Hum Reprod Update* 2006;12(1):39–48.
- [49] Wikstrom AM, Dunkel L. Testicular function in Klinefelter syndrome. *Horm Res* 2008;69(6):317–26.
- [50] Ploton I, Brosse A, Groupe F, et al. [Infertility treatment in Klinefelter syndrome]. *Gynecol Obstet Fertil* 2011;39(9):529–32.
- [51] Gies I, Oates R, De Schepper J, et al. Testicular biopsy and cryopreservation for fertility preservation of prepubertal boys with Klinefelter syndrome: a pro/con debate. *Fertil Steril* 2016;105(2):249–55.
- *[52] Corona G, Pizzocaro A, Lanfranco F, et al. Sperm recovery and ICSI outcomes in Klinefelter syndrome: a systematic review and meta-analysis. *Hum Reprod Update* 2017;23(3):265–75.
- [53] Staessen C, Tournaye H, Van Assche E, et al. PGD in 47,XXY Klinefelter's syndrome patients. *Hum Reprod Update* 2003;9(4):319–30.
- [54] Vernaev V, Staessen C, Verheyen G, et al. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Hum Reprod* 2004;19(5):1135–9.
- [55] Akglaede L, Juul A. Testicular function and fertility in men with Klinefelter syndrome: a review. *Eur J Endocrinol Eur Federat Endoc Soc* 2013;168(4):R67–76.
- [56] Rogol AD, Tartaglia N. Considerations for androgen therapy in children and adolescents with Klinefelter syndrome (47, XXY). *Pediatr Endocrinol Rev* 2010;8(Suppl. 1):145–50.
- [57] Schiff JD, Palermo GD, Veeck LL, et al. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab* 2005;90(11):6263–7.
- [58] Ramasamy R, Ricci JA, Palermo GD, et al. Successful fertility treatment for Klinefelter's syndrome. *J Urol* 2009;182(3):1108–13.
- [59] Van Saen D, Tournaye H, Goossens E. Presence of spermatogonia in 47,XXY men with no spermatozoa recovered after testicular sperm extraction. *Fertil Steril* 2012;97(2):319–23.
- [60] Gies I, De Schepper J, Goossens E, et al. Spermatogonial stem cell preservation in boys with Klinefelter syndrome: to bank or not to bank, that's the question. *Fertil Steril* 2012;98(2):284–9.
- [61] Gies I, De Schepper J, Van Saen D, et al. Failure of a combined clinical- and hormonal-based strategy to detect early spermatogenesis and retrieve spermatogonial stem cells in 47,XXY boys by single testicular biopsy. *Hum Reprod* 2012;27(4):998–1004.
- [62] Maiburg M, Repping S, Giltay J. The genetic origin of Klinefelter syndrome and its effect on spermatogenesis. *Fertil Steril* 2012;98(2):253–60.
- [63] Foresta C, Galeazzi C, Bettella A, et al. High incidence of sperm sex chromosomes aneuploidies in two patients with Klinefelter's syndrome. *J Clin Endocrinol Metab* 1998;83(1):203–5.
- [64] Levron J, Aviram-Goldring A, Madgar I, et al. Sperm chromosome analysis and outcome of IVF in patients with non-mosaic Klinefelter's syndrome. *Fertil Steril* 2000;74(5):925–9.
- [65] Foresta C, Galeazzi C, Bettella A, et al. Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. *J Clin Endocrinol Metab* 1999;84(10):3807–10.
- [66] Yamamoto Y, Sofikitis N, Mio Y, et al. Morphometric and cytogenetic characteristics of testicular germ cells and Sertoli cell secretory function in men with non-mosaic Klinefelter's syndrome. *Hum Reprod* 2002;17(4):886–96.
- [67] Bergere M, Wainer R, Nataf V, et al. Biopsied testis cells of four 47,XXY patients: fluorescence in-situ hybridization and ICSI results. *Hum Reprod* 2002;17(1):32–7.

- [68] Sciarano RB, Luna Hisano CV, Rahn MI, et al. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Hum Reprod* 2009;24(9):2353–60.
- [69] Mehta A, Bolyakov A, Roosma J, et al. Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor. *Fertil Steril* 2013;100(4):970–4.
- [70] Plotton I, Giscard d'Estaing S, Cuzin B, et al. Preliminary results of a prospective study of testicular sperm extraction in young versus adult patients with nonmosaic 47,XXY Klinefelter syndrome. *J Clin Endocrinol Metab* 2015;100(3):961–7.
- [71] Hudson MM. Reproductive outcomes for survivors of childhood cancer. *Obstet Gynecol* 2010;116(5):1171–83.
- [72] Choux C, Cavalieri M, Barberet J, et al. [Immunosuppressive therapy and fertility preservation: indications and methods]. *Rev Med Interne* 2018;39(7):557–65.
- [73] Howell S, Shalet S. Gonadal damage from chemotherapy and radiotherapy. *Endocrinol Metab Clin N Am* 1998;27(4):927–43.
- [74] Meistrich ML. Effects of chemotherapy and radiotherapy on spermatogenesis in humans. *Fertil Steril* 2013;100(5):1180–6.
- [75] Howell SJ, Radford JA, Ryder WD, et al. Testicular function after cytotoxic chemotherapy: evidence of Leydig cell insufficiency. *J Clin Oncol* 1999;17(5):1493–8.
- [76] Bar-Shira Maymon B, Yogev L, Marks A, et al. Sertoli cell inactivation by cytotoxic damage to the human testis after cancer chemotherapy. *Fertil Steril* 2004;81(5):1391–4.
- [77] Wyns C, Curaba M, Vanabelle B, et al. Options for fertility preservation in prepubertal boys. *Hum Reprod Update* 2010;16(3):312–28.
- [78] Green DM, Liu W, Kutteh WH, et al. Cumulative alkylating agent exposure and semen parameters in adult survivors of childhood cancer: a report from the St Jude Lifetime Cohort Study. *Lancet Oncol* 2014;15(11):1215–23.
- [79] ASCO recommendations on fertility preservation in cancer patients: guideline summary. *J Oncol Pract* 2006;2(3):143–6.
- [80] Garcia A, Herrero MB, Holzer H, et al. Assisted reproductive outcomes of male cancer survivors. *J Cancer Surviv* 2015;9(2):208–14.
- [81] Wyns C. Fertility preservation: current prospects and future challenges. *Gynecol Endocrinol* 2013;29(5):403–7.
- [82] Radford J. Restoration of fertility after treatment for cancer. *Horm Res* 2003;59(Suppl. 1):21–3.
- [83] Brinster RL, Avarbock MR. Germline transmission of donor haplotype following spermatogonial transplantation. *Proc Natl Acad Sci USA* 1994;91(24):11303–7.
- [84] Galdon G, Atala A, Sadri-Ardekani H. In vitro spermatogenesis: how far from clinical application? *Curr Urol Rep* 2016;17(7):49.
- [85] Del Vento F, Vermeulen M, de Michele F, et al. Tissue engineering to improve immature testicular tissue and cell transplantation outcomes: one step closer to fertility restoration for prepubertal boys exposed to gonadotoxic treatments. *Int J Mol Sci* 2018;19(1).
- *[86] Wyns C, Van Langendonck A, Wese FX, et al. Long-term spermatogonial survival in cryopreserved and xenografted immature human testicular tissue. *Hum Reprod* 2008;23(11):2402–14.
- [87] Poels J, Van Langendonck A, Many MC, et al. Vitricification preserves proliferation capacity in human spermatogonia. *Hum Reprod* 2013;28(3):578–89.
- [88] Kim TH, Hargreaves HK, Chan WC, et al. Sequential testicular biopsies in childhood acute lymphocytic leukemia. *Cancer* 1986;57(5):1038–41.
- [89] Hou M, Andersson M, Eksborg S, et al. Xenotransplantation of testicular tissue into nude mice can be used for detecting leukemic cell contamination. *Hum Reprod* 2007;22(7):1899–906.
- *[90] Sato T, Katagiri K, Yokonishi T, et al. In vitro production of fertile sperm from murine spermatogonial stem cell lines. *Nat Commun* 2011;2:472.
- *[91] Yokonishi T, Sato T, Komeya M, et al. Offspring production with sperm grown in vitro from cryopreserved testis tissues. *Nat Commun* 2014;5:4320.
- *[92] de Michele F, Poels J, Giudice MG, et al. In-vitro formation of the blood-testis barrier during long-term organotypic culture of human prepubertal tissue: comparison with a large cohort of pre/peripubertal boys. *Mol Hum Reprod* 2018 May 1;24(5):271–82.
- [93] de Michele F, Poels J, Weerens L, et al. Preserved seminiferous tubule integrity with spermatogonial survival and induction of Sertoli and Leydig cell maturation after long-term organotypic culture of prepubertal human testicular tissue. *Hum Reprod* 2017;32(1):32–45.
- *[94] de Michele F, Poels J, Vermeulen M, et al. Haploid germ cells generated in organotypic culture of testicular tissue from prepubertal boys. *Front Physiol* 2018;9(1413).
- [95] Sun M, Yuan Q, Niu M, et al. Efficient generation of functional haploid spermatids from human germline stem cells by three-dimensional-induced system. *Cell Death Differ* 2018 Mar;25(4):747–64.
- [96] Petropanagos A. Testicular tissue cryopreservation and ethical considerations: a scoping review. *J Bioeth Inq* 2017;14(2):217–28.
- [97] Patrizio P, Caplan AL. Ethical issues surrounding fertility preservation in cancer patients. *Clin Obstet Gynecol* 2010;53(4):717–26.
- [98] Wyns C, Collienne C, Shenfield F, et al. Fertility preservation in the male pediatric population: factors influencing the decision of parents and children. *Hum Reprod* 2015;30(9):2022–30.
- [99] Cutas D, Hens K. Preserving children's fertility: two tales about children's right to an open future and the margins of parental obligations. *Med Health Care Philos* 2015;18(2):253–60.
- [100] Liu Z, Nie YH, Zhang CC, et al. Generation of macaques with sperm derived from juvenile monkey testicular xenografts. *Cell Res* 2016;26(1):139–42.
- [101] Goossens E, De Rycke M, Haentjens P, et al. DNA methylation patterns of spermatozoa and two generations of offspring obtained after murine spermatogonial stem cell transplantation. *Hum Reprod* 2009;24(9):2255–63.
- [102] Poels J, Abou-Ghannam G, Decamps A, et al. Transplantation of testicular tissue in alginate hydrogel loaded with VEGF nanoparticles improves spermatogonial recovery. *J Contr Release* 2016;234:79–89.

- [103] Dovey SL, Valli H, Hermann BP, et al. Eliminating malignant contamination from therapeutic human spermatogonial stem cells. *J Clin Invest* 2013;123(4):1833–43.
- [104] Mulder CL, Catsburg LAE, Zheng Y, et al. Long-term health in recipients of transplanted in vitro propagated spermatogonial stem cells. *Hum Reprod* 2018;33(1):81–90.