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ABSTRACT

Background

Klinefelter syndrome (KS) is the most common cause of genetic male infertility, as most patients present azoospermia. In the testis, a massive decrease in the number of germinal cells is observed and this can begin early in childhood. Thus, it is possible to collect spermatozoa after sperm collection or thanks to testicular sperm extraction (TESE), but the chances finding spermatozoa are decreasing with the age. Sperm collection or TESE should be performed as early as possible. When KS is diagnosed during childhood or teens, fertility preservation could be beneficial. The minimal age for proposing fertility preservation remains controversial and there is no current recommendation about fertility preservation in young men with KS.

Design

In this context, we have conducted a systematic review of the results of fertility preservation in young patients with KS to discuss the optimal age range for offering fertility preservation, including or not a TESE.

Results

Six articles were included in the systematic review, with patients between 13 to 24 years-old. Except for one, all young men agreed for sperm collection following masturbation. Azoospermia was diagnosed in all patients presenting homogenous KS. One study reported the presence of spermatozoa in the ejaculate of a young man with mosaic KS. Fifty-eight young man for whom ejaculated sperm collection was unsuccessful have benefited from TESE. Testicular spermatozoa were found and frozen in 27 patients out of the 58 (46.5%). The chances of freezing viable testicular sperm between 14 and 23 years of age do not appear to depend on age.

Conclusion

Fertility preservation should be proposed in young men, but the optimal age for proposing the first sperm collection could be adapted according to the medical context and the psychological maturity of the young man.

Introduction

Klinefelter syndrome (KS) is a clinical syndrome associated with a sex chromosome aneuploidy characterized by the presence of cells with a 47,XXY karyotype, in male. Adult men usually present with gynecomastia and tall stature associated to low testosterone and elevated gonadotropin levels, as well as small and firm testes [1]. It is the most common cause of genetic male infertility, as most patients present azoospermia. However, in some rare cases, when aneuploidy is present in a mosaic state mixed with normal 46,XY cells, few spermatozoa can be found in the ejaculate. Most often, KS is diagnosed during adulthood, when investigating the cause of infertility. A testicular sperm extraction (TESE) is usually suggested in case of azoospermia, and testicular spermatozoa can be retrieved in approximately 50% of TESE [2], allowing *in vitro* fertilization (IVF) with intracytoplasmic sperm injection (ICSI). It has been suggested that the prognosis of TESE is correlated with age and that the chances of retrieving testicular spermatozoa are increased when TESE is performed as early as possible [3]. Indeed, spermatogenesis alterations likely worsen with age: it starts *in utero*, evolves slowly during childhood and accelerates during puberty. Histological study of a testicular tissue of an adult with KS usually shows fibrosis and hyalinization of seminiferous tubes, associated with hyperplasia of interstitial tissue [4]. In the testis, a massive decrease in the number of germinal cells is observed and this can begin early in childhood. In patients with KS, the peri-pubertal period is marked with progressive appearance of testicular fibrosis leading to progressive loss of spermatogonial stem cells (spermatogonia) and decreased testosterone secretion by Leydig cells leading to hypogonadism [5]. When KS is diagnosed during childhood or teens, fertility preservation of ejaculated sperm or TESE could be beneficial. However, masturbation for sperm recovery or testis surgery might be sensitive issues to be discussed with teenagers. Furthermore, the minimal age for proposing fertility preservation remains controversial as only

few data are available in the literature, and there is no current recommendation about the optimal age for proposing fertility preservation.

In this context, we have conducted a systematic review of the results of fertility preservation in young patients with KS to discuss the optimal age range for offering fertility preservation, including or not a TESE.

Material and methods

Documentary research and eligibility criteria

We searched for relevant reports published on PUBMED between January 2000 and April 2020, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations (Stewart et al, 2015).

The search strategy consisted of using the following combined search terms: "((Teenager [title/abstract]) or (Teenager [mesh terms]) or (prepubertal [title/abstract]) or (prepubertal [mesh terms]) or (post pubertal [title/abstract]) or (post pubertal [mesh terms]) or (child* [title/abstract]) or (child* [mesh terms]) or (young [title/abstract]) or (young [mesh terms]) or (adolescent [title/abstract]) or (adolescent [mesh terms])) and ((Klinefelter [title/abstract]) or (Klinefelter [mesh terms]) or (47,XXY [title/abstract]) or (47,XXY [mesh terms])) and ((Fertility preservation [title/abstract]) or ((Fertility preservation [mesh terms]) or (sperm freezing [title/abstract]) or (sperm freezing [mesh terms]) or (sperm banking [title/abstract]) or (sperm banking [mesh terms]) or (sperm cryopreservation [title/abstract]) or (Testicular sperm extraction [title/abstract]) or (Testicular sperm extraction [mesh terms]) or (Testicular sperm retrieval [title/abstract]) or (Testicular sperm retrieval [mesh terms]) or (Sperm extraction [title/abstract]) or (Sperm extraction [mesh terms]) or (Sperm retrieval [title/abstract]) or

(Sperm retrieval [mesh terms]) or (TESE [title/abstract]) or (TESE [mesh terms]) or (microTESE [title/abstract]) or (microTESE [mesh terms]))".

Selection of studies and data extraction

After removal of duplicates, the articles were first pre-selected by reading the title and abstracts by two independent readers (AL and CD). The pre-selected articles were then reclassified as "excluded", "doubtful" or "included". The "doubtful" articles were discussed between AL and CD to determine whether they can be retained or should be excluded. Any disagreement or uncertainty was resolved by a third reviewer (NS). The articles preselected as "retained" were then read in full text by two independent authors (AL and CD).

Synthesis of data

In order to characterize included studies, the following details were extracted: authors, year of publication, country, type of study, sample size, chromosomal aberration (homogeneous or mosaic), age group, sperm collection and results, TESE and results, testicular tissue histology.

Results

The database research allowed the selection of 80 articles, including five articles that were not written in English or French. After reviewing 75 titles and abstracts, 10 full-text articles were selected. Among them, six articles were included in the systematic review (Figure 1).

The number of patients in the included studies was small [4, 6-10], for a total number of 66 young men (Table 1). Included patients were 13 to 24 years-old. In one study, patients were treated with at least 1 year of topical testosterone and aromatase inhibitor [8] and a study

specified that any testosterone treatment was withdrawn 6 months before the first semen analysis and at least 9 months before testicular biopsy for patients [10]. Except for one, all young men agreed for sperm collection following masturbation. Azoospermia was diagnosed in all patients (n=63) presenting homogenous KS. One study reported the presence of spermatozoa in the ejaculate of a young man with mosaic KS [7]. Fifty-eight young man for whom ejaculated sperm collection was unsuccessful have benefited from TESE. Testicular spermatozoa were found and frozen in 27 patients out of the 58 (46.5%). Two teams reported freezing of testicular tissue or germ cells in patients with negative TESE [6, 9]. The chances of finding spermatozoa after TESE do not seem to depend on male age before 24 years-old (**Figure 2**). Regarding the procedure, microTESE is being increasingly used, especially among recent studies, and bilateral biopsy is usually preferred.

Discussion

Massive apoptosis of spermatogonia in young men with KS has led health care professionals to offer fertility preservation at young ages, as early as prepubescent stage in some cases [11]. Nevertheless, in the light of current evidence, the benefits of fertility preservation before puberty are highly questionable [12], since the chances of finding mature spermatozoa are null. A survey was recently submitted to various health professionals who are involved in the healthcare for KS patients (endocrinologists, pediatricians, urologists). This revealed that all practitioners promote fertility preservation during "late" puberty [13]. Similarily, in a recent review, Franik et al. underlined the low chances of obtaining mature spermatozoa for young patients under 16 years old as opposed to patients in the range of 16 to 30 years old [14]. Hence, the authors do not recommend fertility preservation before the age of 16. Although the number of patients who met the inclusion criteria of the present systematic review is limited, the results

show that the chances of finding spermatozoa in testicular tissue seem to be independent of age between 14 and 23 years old and do not seem to decrease with age between 14 and 18 years old in young men with KS. Consequently, we propose that fertility preservation could be offered to young patients from the age of 18.

While it remains difficult to determine an optimal age for offering fertility preservation in young men with KS [14], several parameters should however be considered. The initiation of androgen substitution therapy as early as possible following the diagnosis, including at puberty, has been advocated since a long while [15] in order to prevent the clinical consequences of hypogonadism. However, androgen therapy has a well-known detrimental effect on spermatogenesis [16]. Since hypergonadotropic hypogonadism may be compensated in 60% of KS adolescents between 15 and 23 years old resulting in a minimal endocrine testicular function that is sufficient to obtain a normal pubertal development [17], it may be preferable to defer fertility preservation to the age of 18 years old and start androgen therapy afterwards. If fertility preservation cannot be offered before the initiation of androgen treatments, optimization of intra-testicular testosterone levels by adjunction of anti-aromatases, anti-estrogens or hCG may be considered [8, 18]. As these treatments do not seem to compromise the chances of finding sperm in testicular tissue, they could constitute interesting alternatives to androgen disruption window, even if a potential impact of hormonal treatments on the quality of the spermatozoa, such as epigenetic marks, is not known [19]

Independently from age, the karyotype is predictive of the chances of retrieving spermatozoa. In non-mosaic situations, all the young KS men included in this study displayed azoospermia. Although spermatozoa may be found in the ejaculate of some individuals [17], this remains a rare situation that likely depends on the tissue-mosaicism, in particular in the testes. Hence, young men should be prepared to the risk of a negative outcome and the possible need of TESE.

Other clinical (testicular volume) or biological (hormonal status) markers were not sufficiently well documented in the selected studies to be used as prognostic markers in this review.

Importantly, a psychological support to the patients is recommended when proposing fertility preservation. Even if young men diagnosed with KS early in childhood are usually informed at a very early stage and may be comfortable with these questions, it is important to anticipate fertility issues beforehand. The possible absence of spermatozoa following semen collection and after TESE should be discussed with the young patient and his parents if he is minor. Sperm donation and adoption are also topics that can be addressed, even if some adolescents may not be mature enough or psychologically prepared to deal with these issues and anticipate the difficulties of an infertility journey [20]. Although it is usually admitted that maturity is acquired with age, each young man is different and evolves at his own pace. Consequently, the age of fertility preservation proposal can therefore be adapted to the emotional development of the young adult.

The surgical techniques of testicular biopsy were reported in the articles included in this review but available data is not sufficient to compare the efficiency of the different procedures. However, in young patient with testicular hypotrophy, microTESE may be more efficient and less invasive although at higher risk of postoperative hematoma [21].

Conclusion

Data published in young KS men raise the question of the minimal age to start fertility preservation procedures. The conventional approach is to look for spermatozoa in semen first, and, in case of azoospermia, suggest surgical extraction. The chances to obtain enough viable mature spermatozoa to be cryopreserved in the testicular biopsy seem to be independent of age between 14 and 23 years old. Although this result remains to be further confirmed in larger

series, it suggests that there would be no loss of chance to defer fertility preservation after TESE to the age of 18 years old for azoospermic KS young men. The optimal age for proposing the first sperm collection could hence be adapted according to the psychological maturity of the young man.

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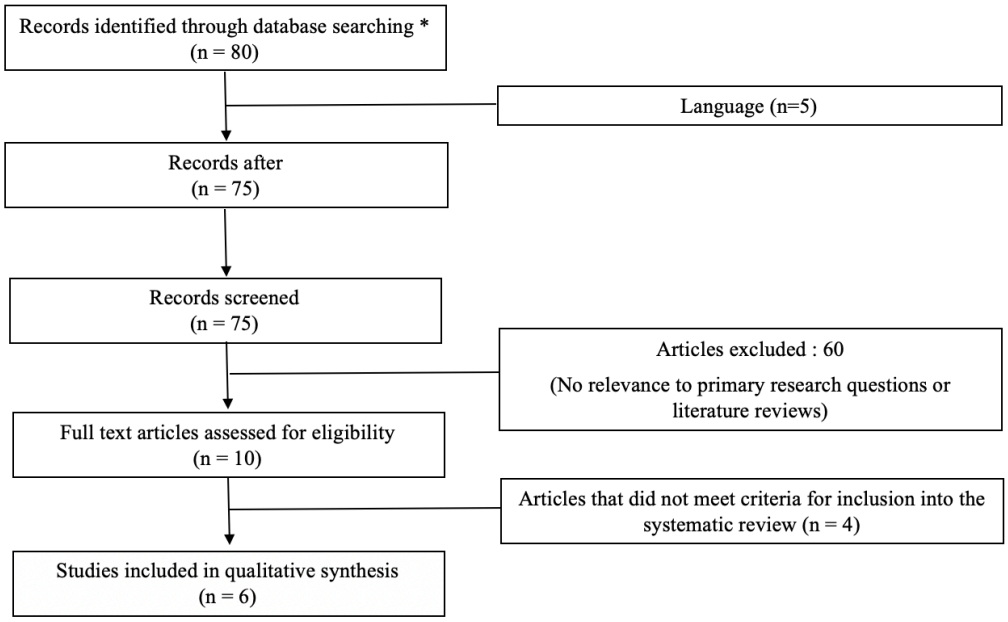

Figure 1 : Flow chart of study selection for systematic review

* PUBMED : between january 2000 and March 2020

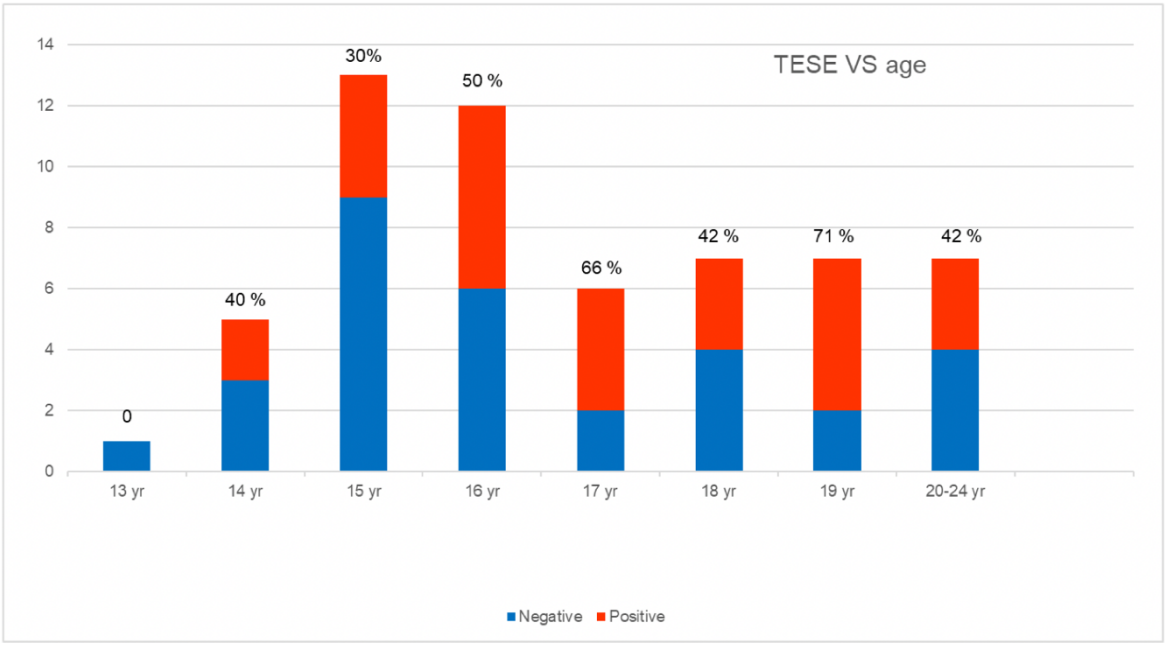

Figure 2 : TESE results according to patient's age

Authors, year, journal, country	Study	Enrollment (n=)	Age	Genotype	Sperm collection (SC)	SC Results	Testicular sperm extraction (TESE)	TESE Results	Cryopreservation	Testicular tissue histology	
										Spermatogenesis	Fibrosis
Damani MN *et al.*, 2001, Fertil Steril, USA	Case Report	1	15 years old	Non mosaic	n = 1	(+) = 0 (-) = 1	n=1 micro TESE unilateral	(+) = 1 (-) = 0	SPZ = 0 TESE = 1 TT = 0	-	NA
Van Saen D *et al.*, 2012, Hum Reprod, Belgium	Retrospective study	7	13-16 years old	Non mosaic	n = 7	(+) = 0 (-) = 7	n=7 TESE unilateral	(+) = 0 (-) = 7	SPZ = 0 TESE = 0 TT = 7	Incomplet (n=5)	No
Rives N *et al.*, 2013, Hum Reprod, France	Retrospective study	8	15-17 years old	Non mosaic (n=7) Mosaic (n=1)	n = 8	(+) = 1 (-) = 7	n=5 TESE bilateral	(+) = 1 (-) = 4	SPZ = 1 TESE = 1 TT = 0	incomplet (n=1)	Yes
Mehta A *et al.*, 2013, Fertilty and Sterility, USA	Case series	10 (*)	14-22 years old	Non mosaic	n = 10	(+) = 0 (-) = 10	n = 10 micro TESE 4 unilateral 6 bilataral	(+) = 7 (-) = 3	SPZ = 0 TESE = 7 TT = 0	NA	NA
Nahata L *et al.*, 2016, The Journal of Pediatrics, USA	Clinical trial	15	15-24 years old	Non mosaic	n=14	(+) = 0 (-) = 14	n=10 micro TESE unilatérale	(+) = 5 (-) = 5	SPZ = 0 TESE = 4 TT = 10	NA	NA
Plotton I *et al.*, 2016, The Journal of Pediatrics, France	Clinical trial	25 (**)	15-22 years old	Non mosaic	n = 25	(+) = 0 (-) = 25	n=25 TESE bilateral	(+) = 13 (-) = 12	SPZ = 0 TESE = 13 TT = 0	NA	NA

Table 1 : Characteristics of studies reporting fertility preservation in young men with Klinefelter syndrome

(*) Patients with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor.
(**) Testosterone treatment was withdrawn 6 months before the first semen analysis and at least 9 months before testicular biopsy.
(+) : positive, (-) : negative, NA : Not available : spermatogonia, SPZ : spermatozoa from semen, TESE : spermatozoa from biopsy testicular, TT : testicular tissue