Short Report

Evaluation of sexual function and micro-testicular sperm extraction in men with mosaic Turner syndrome

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ABSTRACT

Background. Men with mosaic Turner syndrome (TS) having karyotype 45,X/46,XY are infrequently seen. Their sexual function and fertility potential are not well documented. We describe the sexual function and outcomes of sperm extraction in 5 such men who were evaluated between 2008 and 2017.

Methods. Five phenotypic men diagnosed to have mosaic TS underwent detailed physical examination, semen analysis and evaluation of follicle-stimulating hormone, luteinizing hormone, prolactin and total testosterone. Blood, testis, oral mucosa and skin fluorescence *in situ* hybridization (FISH) analyses were done for evaluating the karyotype. Genomic DNA was extracted from peripheral blood for molecular analysis of azoospermic factor (AzF) deletions. Sexual function was assessed using the International Index of Erectile Function-5 (IIEF-5). One patient also underwent micro-testicular sperm extraction (micro-TESE) and intracytoplasmic sperm injection (ICSI).

Results. All 5 men had a mosaic 45,X/46,XY genotype and the sex-determining region (SRY) was positive in DYZ1-negative cells. None had a deletion in the AzF a, b or c regions. Sperm was detected in 3 patients through micro-TESE but ICSI could be done in only 1 patient. No embryo development was identified in time lapse (Embryoscope©) follow-up. It was observed that the rate of 46,XY was particularly high in gonadal tissues in the mosaic structure in patients detected to have sperms.

Conclusion. Patients with TS having 45,X/46,XY, SRY(+), with no AzF deletions, and a high percentage of 46,XY in the peripheral blood, especially in gonadal tissues, could have a healthy sexual life and possibly father a child through *in vitro* fertilization or ICSI upon detection of sperms with micro-TESE.

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INTRODUCTION

Turner syndrome (TS) usually occurs in 45,X monosomic women and 45,X/46,XY mosaicism is a rare, and probably under-diagnosed, condition. Its reported incidence is 1.5 per 10 000 newborns.[1] 45,X/46,XY mosaicisms are believed to occur due to the loss of chromosome Y after normal disomic fertilization. The ratio of the 2 cell lines varies in different organs and tissues and the phenotype of such patients is also variable. This could include male phenotype with normal male genitalia and bilateral testis but TS stigmata, such as bilateral streak gonads with persistent Müllerian structures and mixed gonadal dysgenesis. Phenotypically normal men may present with infertility and azoospermia.[2,3] Sex-determining region (SRY) may be positive in men with TS having 45,X genotype who may have a healthy Y chromosome with 45,X/46,XY mosaicism or a Y autosomal translocation.[4] It is known that the SRY on Yp induces testis development and is the transcription factor of the genes involved in the activation of the sex differentiation process.[4,5] Infertile Y mosaic men with karyotype 45,X/46,XY, SRY (+), who do not have Y chromosome deletions may be candidates for sperm retrieval for *in vitro* fertilization (IVF). We report the outcomes in such men who underwent micro-dissection testicular sperm extraction (micro-TESE) and intracytoplasmic sperm injection (ICSI) in our institution.

METHODS

Five men who presented with infertility between 2008 and 2017 underwent a detailed physical examination including cardiovascular examination. Their partners had a normal ovulatory cycle, 46,XX karyotype, and a mean age of 25.6 years. They reported sexual intercourse at least twice a week and their International Index of Erectile Function-5 (IIEF-5) and Female Sexual Function Indexes (FSFIs) were recorded. Following a semen analysis, assessment of follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, prolactin and oestradiol was done. Subsequently karyotype and Y chromosome microdeletion analysis was obtained. Three patients were diagnosed in 1996 in other clinics while in 2 patients the initial diagnosis was made in our clinic. The age of the subjects at the time of diagnosis ranged from 22 to 29 years. The karyotype analysis and fluorescence *in situ* hybridization (FISH) of all patients were done on cultured, phytohaemagglutinin-stimulated, peripheral blood lymphocytes, according to standard genetic procedures. FISH analysis was done by following the manufacturer's instructions with a SRY-specific probe (SRY, Yp11.3, DYZ1, DXZ1; Cytocell). A second FISH analysis was done on skin fibroblast culture cells, and the testicular tissue of patients were assessed with the same probe. Two hundred cells were analysed from 3 different tissues. Genomic DNA was extracted from peripheral blood of the patient using QIAamp DNA blood kits according to the manufacturer's instructions for molecular analysis of azoospermic factor (AzF) a, b, c regions. All patients also underwent scrotal ultrasonography and pelvic magnetic resonance imaging (MRI) to look for Müllerian structures. After discussion of the genetic diagnosis and obtaining consent, the men underwent micro-TESE with a 12.5×25 surgical microscope. Subsequently, 1 subject underwent ICSI with the retrieved sperms.

RESULTS

The mean age of the subjects was 25.4 years (Table I) and the mean

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duration of marriage was 6.6 years. All had normal masculinization with a reduction in body hair and mean height of 164.4 cm (Fig. 1). All patients had palpable testes, vas deferens and epididymis. One patient had previously undergone laparoscopic gonadectomy for a streak gonad. No cardiovascular pathology was observed but 2 patients had a short metatarsus. The mean semen volume was 3.24 ml and all men had azoospermia. The mean volume of the testes was 2.3 ml on scrotal ultrasound (Fig. 1). No internal genitalia that could be associated with TS were detected on pelvic MRI. The mean hormone levels were 30.96 mIU/ml for FSH (normal 1.7–12 mIU/ml), 12.3 mIU/ml for LH (normal 1.1–7 mIU/ml), 3.67 pg/ml for total testosterone (normal 3.5–8.6 pg/ml), 50.44 pg/ml for oestradiol (normal <62 pg/ml) and 11.44 ng/ml for prolactin (normal 1.5–19 ng/ml). The average IIEF-5 score was 26.8; the mean FSFI of the partners was 27.4.

Genetic results

Two hundred metaphases were scored and the 45,X/46,XY ratios of patients obtained from 3 different tissues are summarized in Table II. Molecular analysis revealed that the *SRY* gene and the AzF a, b, c regions were present. It was observed that the *SRY* gene was translocated to the short arm of X in all the patients.

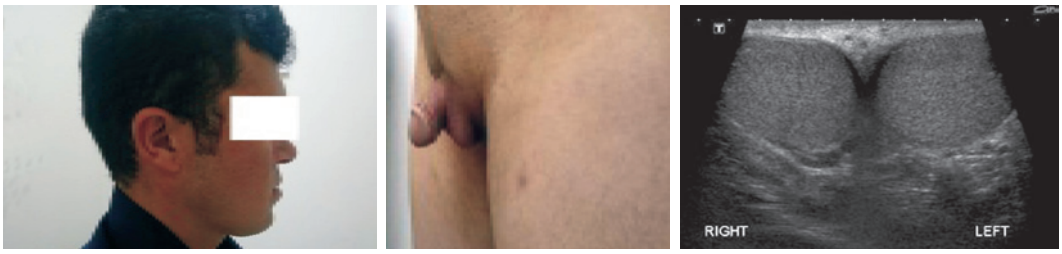
Micro-testicular sperm extraction, IVF/ICSI and pathology results

Distinct tubular structures were observed under the microscope during micro-TESE in only 1 patient (Fig. 2). Sperms were stained with Spermac stain to ensure a clearer tracking of existing sperms. Immotile sperms with head and tail abnormalities, with extremely rare morphology, were identified in 2 patients. One immotile sperm was detected on centrifugation following the collagenase procedure on testicular tissue after micro-TESE in 1

TABLE I. Characteristics of male patients with Turner syndrome having 45,X/46,XY

Feature	Patient number					Total/Mean
	1	2	3	4	5	
Age at first evaluation (years)	22	9	5	7	23	13.2
Age of wife (years)	20	30	26	29	21	25.6
Duration of marriage (years)	5	7	8	10	3	6.6
Sex of rearing	Male	Male	Male	Male	Male	5
External genitalia	Male	Male	Male	Male	Male	5
Right and left gonads	+/+	+/+	+/+	+/+	+/+	5
Scrotal (volume/ml)	+	+	+	+	+	2.3
Streak gonadectomy	+	–	–	–	–	1
Orchiopexy (bilateral)	–	+	–	–	–	1
Semen volume (ml)	3.4	2.9	4.9	1.7	3.3	3.24
Regions AzF a, b, c	+	+	+	+	+	5
Sex-determining region (SRY)	+	+	+	+	+	5
Follicle-stimulating hormone (mIU/ml)	30.25	29.56	34.58	37.59	22.83	30.96
Luteinizing hormone (mIU/ml)	8.99	9.76	11.24	17.86	13.65	12.3
Total testosterone (pg/ml)	2.39	4.59	5.91	1.87	3.59	3.67
IIEF-5	28	27	26	24	29	26.8
FSFI	30	26	29	24	28	27.4
Length (cm)	164	170	162	167	159	164.4
Vas deferens	+/+	+/+	+/+	+/+	+/+	5
Micro-testicular sperm extraction	–	+	+	–	+	3
JHS score	1	6	6	2	7	–
Translocated SRY Xp	–	+	–	–	–	1
Karyotype	45X/46XY	45X/46XY	45X/46XY	45X/46XY	45X/46XY	45X/46XY
45,X cells (%)*	26.6	8.33	7.33	35.66	5.66	5.66/35.66
46,XY cells (%)*	73.3	91.7	92.3	64.3	94.3	64.3/94.3

* The average of the rate in different tissues was calculated to provide the percentages in 45,X/46XY IIEF-5 (International Index of Erectile Function-5) score of 20–25 indicates no erectile problem FSFI (Female Sexual Function Index): a total score below 26.55 indicates sexual dysfunction According to Johnsen histopathological score (JHS): 1=no cells in the seminiferous tubular lumen; 2=only Sertoli cells are present with no germ line cells; 6=no spermatozoa present and <10 spermatids present, 7=no spermatozoa, but many spermatids present AzF azoospermic factor



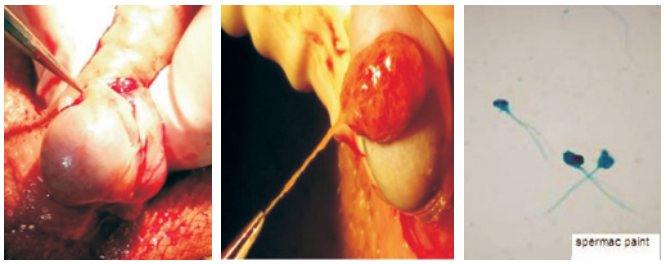
(a) (b) (c)

FIG 1. (**a**–**c**) Sample SRY (+) patients with 45,X/46,XY and no Y chromosome microdeletions and testes on scrotal ultrasound

TABLE II. Percentage of 45,X and 46,XY cell lines

Patient number	Karyotype cell line	Peripheral blood (%)	Right testis (%)	Left testis (%)	Total/Mean (%)
1	45X/46XY	14/86	25/75	41/59	26.6/73.3†
2*	46X/46XY	9/91	10/90	6/94	8.33/91.66†
3*	47X/46XY	3/97	8/92	11/89	7.33/92.33†
4	48X/46XY	30/70	37/63	40/60	35.66/64.33†
5*	49X/46XY	5/95	5/95	7/93	5.66/94.33†

* Patient with a high 46,XY rate in the mosaic Turner group with the male phenotype 45,X/46,XY † The average of the rate in different tissues was calculated to provide the percentages of 45,X/46,XY



(a) (b) (c)

FIG 2. (**a**–**c**) Man with Turner syndrome with 45,X/46,XY during microscopic-testicular sperm extraction: Testis/epididymis and tubular view under 12.5×25 surgical enlargement microscope. Large-headed and multiple-tailed sperms ×40, stained with Spermac of patient in whom sperm was detected

patient. No sperm was observed in 2 other patients. IVF/ICSI was done following the detection of a sufficient amount of sperms in only 1 patient among patients identified to have sperm; however, no embryo development was observed following time-lapse (Embryoscope©) follow-up.

Masson trichromatic stain was applied in addition to the haematoxylin and eosin-stained sections of the testicular tissue, and basal membrane thickening was observed in 2 patients in whom no sperm was identified. The seminiferous tubules were generally surrounded by Sertoli cells in 3 patients in whom sperm was detected; few spermatocytes were observed in the germ line at the spermatogonium phase, while the Johnsen histopathological score was identified as 6, 6 and 7, respectively (a score of 6=absence of spermatozoa and <10 spermatids; a score of 7=a high number of spermatids and no spermatozoa). In patients in whom no sperm was detected, basal membrane thickening, absence of germ cells, predominance of Sertoli cells, Leydig cell proliferation and a Johnsen score of 1 and 2 was observed (a score of 1=no cells in the seminiferous tubular lumen; a score of 2=presence of only Sertoli cells and absence of germ cells).

DISCUSSION

Sexual differentiation is a complex process. Until the 6th week of gestation, the gonads of embryos of both sexes are indistinguishable. Testes develop in individuals with a 46,XY chromosome constitution, through the effect of the *SRY* gene. These are morphologically identifiable from week 7 to week 8 of gestation. In this process, the Sertoli cells are the first to become recognizable. These are organized as cells surrounding tubules and produce Müllerian-inhibiting factor, a hormone that, through local diffusion, leads to regression of the Müllerian duct derivatives (which give rise to the uterus, uterine tubes and proximal vagina). Leydig cells produce an androgen, testosterone, which induces Wolffian ducts to differentiate into epididymis, vas deferens, seminal vesicles and ejaculatory duct.[2,3,6] The loss of the Y chromosome following normal disomic fertilization through non-disjunction leads to the development of the 45,X cell line. Ovarian development is observed in such individuals with TS in the absence of chromosome Y and the external genitalia becomes female.[7] If the loss of Y occurs during the early post-zygotic cell division period, greater number of cell groups and tissues are affected. This impacts the distribution of cells with 45,X karyotype, leading to phenotypic differences which are attributed to the non-disjunction in the Y chromosome. TS with 45,X/46,XY mosaic male phenotype has been rarely reported. Generally, men present with infertility and azoospermia similar to our series.[6,8]

In patients with 45,X/46,XY complement, streak gonads and/or abnormal testes may also be seen along with Müllerian components. While bilateral testis and reduced virilization are present along with Müllerian components in some 45,X/46,XY, some patients may have bilateral scrotal testis and normal male genitalia as in our patients.[6,8] This phenotype is probably due to the high rate of 46,XY karyotype in the cells forming the gonads.[6] No internal organ association with TS was observed in our patients in the MRI assessment. The reason was thought to be the high rate of the 46,XY and SRY (+) in gonadal tissues. Clinical variations supporting this claim have also been observed in monozygotic twins.[9] Patients with mosaic TS having 45,X/46,XY karyotype had a normal height, with enhanced breast development rates and reduced somatic abnormality rate, as with our patients, compared to those with a 45,X karyotype.[8] In men who have a 45,X/46,XY mosaic TS and have undergone gonadectomy or have gonadal dysgenesis, sex steroid hormone therapies may be required to prevent loss of sexual characteristics and development of osteoporosis. However, none of our patients received sex steroid therapy and hormone values were normal in 45,X/46,XY despite hypergonadotropism.[6]

In a series of 14 subjects with 45,X/46,XY karyotype, there were 5 male and 9 female phenotypes.[6] Four of 5 men in this series had ambiguous genitalia while normal male genitalia were reported in 1 man.[6] SRY (+) was detected in all our patients; no abnormalities such as ambiguous genitalia, scrotal fusion, micro-penis and perineal urethral meatus were observed in our patients. It was presumed that this was due to the presence of the SRY gene translocated to the X chromosome through a post-zygotic translocation in the 45,X cell line and the high rate of karyotype 46,XY in the cells forming the gonads. An interesting finding was a SRY (+), TS man with 45,X. No Y chromosome was detected, the SRY gene was translocated to X and no spermatogenetic activity was observed due to the absence of chromosome Y in the individual followed up until the age of 12 years.[6] Mosaic karyotype was observed in all patients in our series. Although there was a mosaic structure in only 1 of our patients, it was observed that SRY was translocated to Xp. Moreover, the karyotyping performed in the testicular tissue retrieved from our patients was also 45,X/46,XY. It was observed in patients no. 2, 3 and 5 that the rate of 45,X/46,XY was high in favour of 46,XY in the gonads of those where sperm was detected at micro-TESE (Tables I and II).

Structural alterations of the Y chromosome in the 46,XY cell line of the mosaic patients comprising AzF region have previously been described.[10] Interestingly, deletion of one or more loci in these regions can affect the development of the testes and cause impaired spermatogenesis.[6] We assessed the AzF a, b, c regions in our patients and observed no deletions. Although we could obtain

TABLE III. Stigmas identified in patients with 45,X/46,XY, sex-determining region (+) and no Y chromosome microdeletions

Feature	Patient number					Total
	1	2	3	4	5	
Short stature	+	–	–	–	+	2
Obesity	–	+	+	+	–	3
Low posterior implantation of hair	+	–	–	+	–	2
Epicanthic folds	+	–	–	+	+	2
Hypothyroidism	–	–	+	–	–	1
Posteriorly rotated ears	+	–	–	+	–	2
Short and webbed neck	+	–	–	+	–	2
Short and wide thorax	+	+	–	–	+	2
Cubitus valgus	–	–	+	–	–	1
Hypoplastic nails	+	+	+	–	–	3
Shortening of metatarsals	+	–	–	+	–	2
Karyotype	45X/46XY	45X/46XY	45X/46XY	45X/46XY	45X/46XY	45X/46XY
45,X cells (%)*	26.6	8.33	7.33	35.66	5.66	5.66/35.66
46,XY cells (%)*	73.3	91.66	92.33	64.33	94.33	64.33/94.33

* The average of the rate in different tissues was calculated to provide the percentages of 45,X/46,XY

an amount of sperm sufficient to perform IVF/ICSI in only 1 patient, we obtained sperm in 3 patients at micro-TESE. Although mature seminiferous tubules were detected in the pathological assessment of the testes, it was determined that they were disorganized, hyalinized and atrophic tubules.[11,12] We also observed basal membrane thickening and atrophic tubules in the trichromatic staining performed in patients in whom we could not detect any sperm. Contrary to the patients in whom we detected spermatogenetic activity or sperm (Table II; patients 1–4), the mosaic rate was in favour of 45,X, most notably in gonadal tissues.

It was reported that no pregnancy was achieved in the follow-up of 45,X/46,XY in a multicentre study.[13] It was also reported that a healthy, oligospermic adult male phenotype with karyotype 45,X/46,X, r(Y) could father a child with karyotype 47,XX, r(Y)/46,XX. Sperm was detected in this patient following m-TESE, but pregnancy could not be achieved in 2 ICSI cycles.[11,12]

Patients with male external genitalia can present with hypospadias and/or cryptorchidism.[14] Dysgenetic testes, gonadal streaks and contralateral dysgenetic testes are frequently observed.[13] One of our patients had previously undergone orchiopexy for

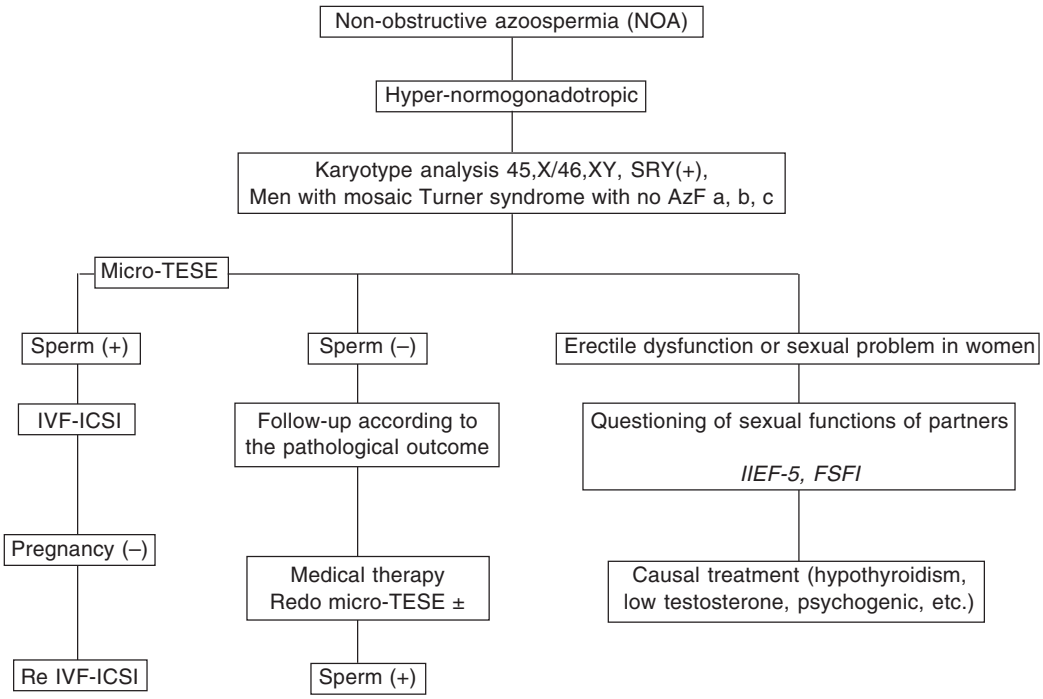


FIG 3. Treatment diagram proposed for male patients with mosaic Turner syndrome having 45,X/46,XY, sex-determining region (+) and no Y chromosome micro deletions

IIEF-5 International Index of Erectile Function-5 FSFI Female Sexual Function Index micro-TESE microscopic-testicular sperm extraction SRY sex-determining region AzF azoospermic factor IVF *in vitro* fertilization ICSI intracytoplasmic sperm injection

undescended testis along with removal of a streak gonad. No sperm was detected at micro-TESE in this patient.

Individuals with 45,X/46,XY mosaicism having male external genitalia may also have short stature.[15] The mean height of our patients was 164.4 cm. The slower growth in these patients appears to be related to deficiencies of both their hypothalamic–gonadal axis and primary gonadal function.[7]

Some major abnormalities, such as cardiovascular and renal abnormalities, and dysmorphia, such as low implantation of hair, shortening of metacarpal and metatarsal bones and a short and webbed neck, which are observed in individuals with TS, may also be observed among patients with 45,X/46,XY mosaicism.[16] Our patients had abnormalities in hair, weight, height and epicanthus folds. The rate of 45,X in the mosaic structure was observed to be higher in these patients compared with those devoid of these stigmata (Table III).

Mosaic patients with 45,X/46,XY are also at a higher risk for development of malignancies. Tumour frequency varies between 10% and 15%, especially for gonadoblastoma and dysgerminoma for mosaic patients. Tumours are observed mostly in the second decade of life. Therefore, it is necessary to monitor these patients.[17]

Another major issue in these patients is the gender of rearing the child. Although several factors are involved in this process, the degree of virilization of the external genitalia and the presence of gonads with testicular features in the labioscrotal folds are the most important.[18,19] In our patients, the external genitalia were male due to the presence of SRY and a high rate of 46,XY karyotype in the mosaic structure, especially in the gonads and they were all raised as male (Fig. 1). However, there is no consensus on what would be the best way of determining the gender of rearing.[20] Cools *et al*.[21] stated that the masculinization score for the external genitalia that was suggested by Ahmed *et al*.[22] could reflect both gonadal differentiation and the risk of tumours in patients with 45,X/46,XY mosaicism (malignancy risk seems to be inversely related with this score).[6] Our approach to managing male patients with mosaic TS having 45,X/46,XY, SRY(+) genotype with no AzF deletions of the Y chromosome is summarized in Fig. 3.

Conclusions

Patients with mosaic TS having 45,X/46,XY present with variable phenotypes, have a complex genetic structure and need a thorough multidisciplinary evaluation. Among these patients, those who are positive for SRY, have no AzF deletions and a higher percentage of 46,XY in the peripheral blood, especially in gonadal tissues may have a healthy sexual life and sperm detectable in micro-TESE.

Conflicts of interest. None declared

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