Sperm Retrieval in Adolescents and Young Adults with Klinefelter Syndrome: A Prospective, Pilot Study

Leena Nahata, MD[1,*], Richard N. Yu, MD, PhD[2,*], Harriet J. Paltiel, MD[3], Jeanne S. Chow, MD[3], Tanya Logvinenko, PhD[2,4], Ilina Rosoklija, MPH[2], and Laurie E. Cohen, MD[5]

Objective To assess sperm retrieval rates in adolescents and young adults with Klinefelter syndrome, with the ultimate goal of improving fertility in this population. Secondary aims were to evaluate other clinical characteristics of the cohort and identify predictors of sperm retrieval.

Study design Patients 12-25 years of age with Klinefelter syndrome (47,XXY) were recruited at the Boston Children's Hospital. Physical examination, biochemical evaluation, scrotal ultrasonography, and semen analysis were performed. Neurocognitive data were collected. Microdissection sperm extraction (unilateral micro-testicular sperm extraction) was offered to individuals with no sperm in their ejaculates. Given the small sample size, analysis was primarily descriptive.

Results Fifteen patients were enrolled. None had sperm in their ejaculates. Ten patients underwent unilateral micro-testicular sperm extraction. Sperm retrieval rate was 50%. From a neurocognitive standpoint, subjects reported problems with peers, conduct, and overall difficulties. Incidentally, one-third of the patients were found to have testicular microlithiasis and 17% of subjects with renal ultrasound imaging had bilateral renal medullary nephrocalcinosis.

Conclusions This pilot study suggests that sperm retrieval rates in adolescents and young adults with Klinefelter syndrome are comparable with those reported in older men. However, larger studies are needed to confirm our findings. The clinical significance of the scrotal and renal ultrasound findings merits further investigation. *(J Pediatr 2016;170:260-5)*.

Trial registration ClinicalTrials.gov: NCT01817296.

Klinefelter syndrome affects 1 in 600 newborn males.[1,2] Most often caused by a meiotic disjunction event resulting in a 47, XXY karyotype, it is the most common chromosomal disorder in men and accounts for 11% of men with azoospermia.[3,4] Although the phenotype is heterogeneous,[5] there is a high incidence of neurocognitive and psychiatric comorbidities in this cohort, and >95% of individuals are unable to have biological children by natural means.[5,6]

Over the past 2 decades, techniques such as testicular sperm extraction (TESE) and microdissection TESE (unilateral micro-TESE) combined with intracytoplasmic sperm injection have revolutionized the management of infertile men.[7,8] These methods have resulted in similar paternity rates in patients with Klinefelter syndrome as in men with other causes of nonobstructive azoospermia, and the offspring appear to have normal karyotypes.[9] When surveyed, 90% of men with Klinefelter syndrome expressed a desire to have children and the majority were willing to pursue TESE in conjunction with intracytoplasmic sperm injection.[10] However, these methods have not been shown to be universally effective. Currently, the sperm retrieval rate in adults with Klinefelter syndrome appears to be approximately 50%, and one of the major predictors of successful retrieval in adults has been younger age.[8,11-13]

Based on these data, consideration of TESE or unilateral micro-TESE in adolescents with Klinefelter syndrome with sperm cryopreservation has been suggested.[8,14,15] However, because few studies have been done in this younger cohort, most clinical centers do not yet offer retrieval and cryopreservation in adolescence. Recently, a report of unilateral micro-TESE in adolescents and young adults with Klinefelter syndrome was published,[16] with retrospective data in 10 individuals with Klinefelter syndrome 14-22 years of age who were on testosterone and aromatase inhibitor therapy; sperm were retrieved in 7/10.[16] Subsequently, the first prospective study was published, in which sperm retrieval rates via bilateral TESE procedures were compared in adolescents and young adults vs older adults with Klinefelter syndrome who were not on hormone replacement therapy; sperm

CV	Coefficient of variation
ICC	Intraclass correlation coefficient
LH	Luteinizing hormone
SDQ	Strengths and Difficulties Questionnaire
TESE	Testicular sperm extraction

From the [1]Division of Endocrinology, Nationwide Children's Hospital, Columbus, OH; and Departments of [2]Urology and [3]Radiology, [4]Clinical Research Center, and [5]Division of Endocrinology, Boston Children's Hospital, Boston, MA

*Contributed equally.

Funded by Boston Children's Hospital (2012 House Officer Development Award). The authors declare no conflicts of interest.

http://dx.doi.org/10.1016/j.jpeds.2015.12.028

were retrieved from 52% of the younger cohort, with no significant difference between the 2 age groups.[17] Testicular volumes, age, and hormone levels were not predictive of sperm retrieval.[17] Neurocognitive/psychiatric data were not collected in either of these studies, so it is not clear that their cohorts were similar to other young Klinefelter cohorts described in the literature. Given these limited data, our primary goal was to prospectively assess sperm retrieval rates via unilateral micro-TESE in our cohort of adolescents and young adults with Klinefelter syndrome. Secondary aims were to collect neurocognitive data with a validated survey for a more comprehensive evaluation of the cohort, and to determine predictors of sperm retrieval.

Methods

This was an institutional review board-approved prospective clinical trial conducted at a single institution, Boston Children's Hospital (ClinicalTrials.gov: NCT01817296). Inclusion criteria were karyotype of 47,XXY and age of 12-25 years. Exclusion criteria were as follows: testosterone therapy within the past 6 months, history of epididymitis or orchitis within the past 6 months, history of testicular surgery or damage (eg, because of infection, gonadotoxic drugs, or trauma), or solitary testicle. The primary reason for excluding patients on testosterone therapy for the previous 6 months was to be able to compare our retrieval rates with results from adult studies in which patients were not on hormone therapy.[8]

A query of our institution's electronic medical record was performed to identify patients who met the inclusion criteria, and a recruitment letter was sent to the address on file for all of these patients along with an opt-out card. Anyone who returned the opt-out card was removed from the recruitment list. Two weeks later, the study team called the patients (if 18 years or older) or their families to explain the study via telephone. Those who agreed to participate and were not eliminated based on the exclusion criteria were scheduled for a study visit in the urology department, where informed consent and assent were obtained. The study schema is shown in **Figure 1** (available at www.jpeds.com).

Hormone Analysis and Ultrasound

At the first study visit, a brief history/physical examination was performed by a single examiner (a pediatric endocrinologist). Follicle-stimulating hormone and luteinizing hormone (LH) (electrochemiluminescence immunoassay, Roche Diagnostics, Indianapolis, Indiana, coefficient of variation (CV) 2.5%), testosterone (Liquid Chromatography Mass Spectrometry, Boston Children's Hospital, CV 4%-5.5%), and inhibin B (Enzyme-Linked Immunosorbent Assay, Beckman Coulter, Salt Lake City, Utah CV 9%) levels were drawn at 8 a.m. Scrotal ultrasound studies were performed for accurate measurement of testicular volumes, using ultrasound equipment with high frequency linear array transducers (Sequoia 512, 8-15 MHz transducer, Siemens Healthcare USA, Malvern, Pennsylvania for 8 subjects; iU22, 5-12 MHz transducer, Philips Healthcare, Bothell, Washington for 4 subjects; Logiq 9, 6-15 MHz transducer, GE Medical Systems, Milwaukee, Wisconsin for 3 subjects). Gray-scale images of the testes were obtained in transverse and longitudinal planes. Measurements of testicular length, width, and height were obtained using electronic calipers. Testicular volume was calculated using the formula for a prolate ellipsoid: $L \times W \times H \times 0.52$.[18] Any identified abnormalities were documented in transverse and sagittal planes. If a varicocele was noted, further evaluation with color and spectral Doppler ultrasound was performed with the patient at rest and following the Valsalva maneuver.

Ten subjects also underwent imaging of the kidneys and bladder with curvilinear and/or sector transducers (Sequoia 512, 2-6 and 1-4 MHz transducers, Siemens Healthcare USA, for 7 subjects; iU22, 1-5 MHz transducer, Philips Healthcare for 1 subject; Logiq 9, 1-5 MHz transducers, GE Medical Systems for 2 subjects), and 2 subjects had additional imaging of the kidneys (Sequoia 512, 2-6 MHz transducer, Siemens Healthcare USA for 1 subject; iU22, 2-5 MHz transducer, Philips Healthcare for 1 subject). Any focal or diffuse renal parenchymal abnormalities were documented in transverse and longitudinal planes. All ultrasound examinations were interpreted by the pediatric radiologist on service, as well as by 2 additional pediatric radiologists with particular expertise in pediatric genitourinary ultrasonography.

Surveys

Neurocognitive data were collected by 2 surveys. The first survey was the self-reported Strengths and Difficulties Questionnaire (SDQ), which is a validated behavioral screening tool used in adolescents to assess emotional symptoms, conduct problems, inattention, and peer relationship problems.[19,20] The other survey was an intake questionnaire developed by our institution's Developmental Medicine Program to collect information about various learning, developmental, and psychiatric issues.

Semen Analysis

After the initial study visit, subjects were encouraged to provide a semen sample by masturbation for semen analysis. Semen analyses were performed by the Reproductive Endocrinology Laboratory at Brigham and Women's Hospital in Boston, Massachusetts. Patients who had evidence of pubertal development (based on morning LH levels >0.7 IU/L) but had no sperm in their ejaculates, or who were unable or unwilling to produce an ejaculate, were offered unilateral micro-TESE to search for viable sperm.

Unilateral Micro-TESE

Those who consented for unilateral micro-TESE had a surgical visit scheduled. Unilateral micro-TESE was performed on 1 testicle by a single practitioner, a pediatric urologist with specialized training in performing this procedure. The procedure was limited to 1 testis based on feedback from the

institutional review board because of concern for damage or loss from the surgery. Under general anesthesia, 1 testicle was exposed through a scrotal incision. The tunica albuginea was incised transversely, and the seminiferous tubules were everted into the incision. The entire testis was explored using a high-powered surgical microscope, with which distinct areas of enlarged seminiferous tubules were sequentially identified and excised. Sperm extraction, processing, and storage were carried out at New England Cryogenic Center, with further microscopic analysis performed at the Massachusetts General Hospital Fertility Center Laboratory. A follow-up visit was scheduled for all patients in the urology clinic within 1 month of surgery to discuss the study results. For patients who underwent unilateral micro-TESE, surgical wound healing was also assessed at this visit.

Statistical Analyses

Given the small sample size, analysis was descriptive. Comparison of abnormality rates in the general population (assumed known) and the Klinefelter population was done based on our sample using a 1-sided binomial test (assuming that in the general population, the rate would be lower). Intraclass correlations for the ultrasound measurements done by the 3 radiologists were assessed. Average measurements obtained by the 3 radiologists for the same subject were correlated with the Prader orchidometer measurements performed in these patients at the time of the physical examination. Scores from Strengths and Difficulties Questionnaire in the Klinefelter cohort were compared with averages of the general population using a 1-sided Wilcoxon test (assuming that in the general populations, scores would be lower). Analyses were performed using R statistical software packages base, reshape2, and intraclass correlation coefficient (ICC).[21-23]

Results

A query of our institution's electronic medical record showed 95 individuals who were 12-25 years of age with a 47,XXY karyotype. Thirty-one could not be reached via the contact information on file, and 10 returned their opt-out cards. The study was discussed by telephone with the 54 remaining patients, of whom 26 declined to participate. The most common reasons for refusal were lack of psychological readiness to focus on fertility and inability to participate in the study because of distance constraints (primarily as a result of attending college out of state).

Twenty-eight patients expressed interest in participating in the study; 11 were ineligible because of testosterone therapy. The remaining 17 were scheduled for study visits; 3 of them did not attend and chose not to reschedule. One additional patient from out of state contacted the study team based on the Clinical Trials listing and travelled to Boston to participate in the study. Thus, from March 20, 2013, to August 12, 2013, a total of 15 patients were enrolled in the study, aged 15-23 years (Table I). None of the patients had ever received testosterone therapy. Five (33%) were diagnosed with Klinefelter syndrome prenatally, 3 (20%) were diagnosed prior to puberty in an evaluation for developmental delay/behavioral problems, and 7 (47%) were diagnosed during adolescence (primarily because of small testes).

Semen Analysis

One semen analysis was performed for each subject that was able to provide a specimen. Fourteen of the 15 subjects produced an ejaculate for semen analysis; 1 individual refused. All samples were centrifuged, and the pellet was analyzed by microscopy. None had sperm on semen analysis.

Sperm Retrieval

Ten of the 15 study patients (67%) chose to undergo unilateral micro-TESE. Detailed microscopic analysis was performed prior to cryopreservation and showed viable sperm in 4 patients. All 10 patients had their retrieved samples, tissue, and extracted fluid cryopreserved (3 vials per patient). To confirm our findings, repeat microscopic analysis was performed on 1 cryopreserved vial for each of

Table I. Sample characteristics

Subject	Age (y)	R testis (mL)	L testis (mL)	FSH	LH	Testosterone	Inhibin B	Unilateral micro-TESE/sperm retrieval
1	19	2.73	2.81	30.33	16.95	260	<10	Yes/yes
2	20	1.96	2.21	26.08	23.88	306	<10	
3	15	1.76	4.05	20.19	12.67	407	19	Yes/no
4	15	3.04	2.19	58.48	24.46	398	12	
5	16	1.96	1.77	51.56	29.39	425	<10	Yes/no
6	20	1.79	1.59	61.8	34.8	526	<10	Yes/no
7	23	2.09	1.76	42.97	30.86	507	<10	Yes/yes
8	18	1.77	1.85	53.7	25.43	258	<10	Yes/no
9	20	3.51	2.92	32.99	20.75	164	11	
10	16	1.59	1.20	37.95	18.47	563	17	
11	18	0.95	1.03	33.82	20.94	263	<10	
12	16	1.27	1.31	53.82	33.59	371	<10	Yes/yes
13	16	2.22	2.26	13.05	10.75	228	40	Yes/no
14	16	2.75	2.52	29.25	15.22	188	<10	Yes/yes
15	17	3.77	3.14	5.2	8.95	519	68	Yes/yes

FSH, follicle-stimulating hormone; *L*, left; *R*, right.

the 6 patients in whom sperm had not been identified initially. This showed viable sperm in 1 additional individual. Sperm retrieval rate in this cohort was 50%.

Hormone Analysis and Ultrasound

With the exception of 1 patient, all of the subjects had elevated levels of follicle-stimulating hormone and LH. Testosterone levels varied from slightly low for age to normal. Inhibin B levels were low for age in all subjects, and undetectable in many (Table I). Intraclass correlation for the ultrasound measurements provided by the 3 radiologists was high with left side ICC = 0.96 (95% CI 0.92-0.99) and right side ICC = 0.96 (95% CI 0.91-0.99) and allowed us to average measurements obtained by all 3 radiologists on the same subject (Table I). Testicular volumes detected by ultrasound did not correlate with orchidometry (Figure 2; available at www.jpeds.com).

There were several different testicular and renal lesions incidentally identified in the cohort. Notably, one-third of the sample was found to have testicular microlithiasis and 17% of subjects with renal imaging (13% of entire cohort) had bilateral renal medullary nephrocalcinosis. A comparison of reported prevalence rates in the general population vs this sample was done using binomial distribution (Table II).

Neurocognitive and Psychiatric Data

Nine subjects (60%) had been on an individualized educational program, 6 (40%) had received mental health/counseling services, and 4 (27%) had been diagnosed with attention deficit disorder. All 15 patients were given the SDQ (1 individual omitted some of the items as noted in Table III). As expected, scores (19.2 ± 7.6) showed significantly more problems with peers, conduct, and overall difficulties than in the general US population (averaging at 6.5 points; $P = .001$).

Discussion

Infertility may have a significant and devastating impact on young people. Given the high incidence of Klinefelter syndrome, investigating ways to improve fertility counseling and outcomes in these patients is of the utmost importance. Previous studies have shown successful sperm retrieval in approximately one-half of affected adults, and young age has been one of the primary established predictors of success.[8,11-13] This age effect is further supported by histologic studies that demonstrate normal spermatogonia at birth[24] followed by fibrosis and hyalinization of the seminiferous tubules and degeneration of germ cells.[4] Furthermore, testosterone, inhibin B, and gonadotropin levels are typically normal until early puberty,[25] after which gonadotropins quickly rise and testosterone levels plateau.[26] Based on all of this information, there has been increasing discussion about considering cryopreservation in adolescence, presumably before the decline of spermatogenesis.[14,15] However, there are minimal data available to compel a change in practice, and as a result, general pediatricians and pediatric subspecialists have been limited in the advice they can offer to these patients and families with regards to fertility preservation.

Until 2013, there were no promising results of testicular biopsies in young males with Klinefelter syndrome. However, studies had only been done in small groups of boys, many of whom may have been too young for spermatogenesis. Wikstrom et al[27] performed testicular biopsies in 14 boys with Klinefelter syndrome (median age 10.5 years) and were able to cryopreserve spermatogonia but no sperm in 7. Van Saen et al[28] found no sperm after unilateral biopsies in seven patients 13-16 years of age. Rives et al[29] performed bilateral testicular biopsies and found sperm in 1 of 5 patients 15-17 years of age.

Recently, a retrospective report of Klinefelter syndrome showed similar results of bilateral unilateral micro-TESE compared with what has been reported in adults,[16] followed by the first prospective study comparing sperm retrieval in adolescents vs adults.[17] Our sperm retrieval rate of 50% was similar to results of these studies, also suggesting that there is no clear benefit to performing TESE/unilateral micro-TESE in adolescence,[17] though our sample size was small and our subjects had never been on testosterone therapy (suggesting less severe gonadal dysfunction[12]). Aside from age, another potential proposed benefit of early cryopreservation has been to retrieve sperm prior to starting testosterone therapy. Though the effect of exogenous

Table II. Incidental scrotal and renal parenchymal findings

Scrotal abnormalities (total studies = 15)	Rate in general population	Number (%)	P*
Hydrocele	6.0	3 (20.0)	.0571
Varicocele	15.0	3 (20.0)	.3958
Microlithiasis	5.6	5 (33.3)	.0010
Spermatocele/cysts	30.0	6 (40.0)	.2784
Renal abnormalities (total studies = 12)			
Bilateral renal medullary nephrocalcinosis	N/A†	2 (16.7)	N/A†

N/A, not available.
**P* values are based on comparison to incidences reported in the general population.
†No information available about incidence in general population.

Table III. SDQ results

SDQ domains	Mean score US males (15-17 y) N = 1170	Klinefelter cohort score N = 15	*P* value (comparison of US males scores and Klinefelter cohort scores)
Total difficulties	6.5	19.2*	.0005
Emotional symptoms	1.3	2.7	.0080
Conduct problems	1.2	2.5	.0521
Hyperactivity-inattention	2.6	3.7	.1210
Peer problems	1.4	2.6	.0190
Prosocial behavior	8.5	8.2*	.6720

*Based on 14 subjects.

testosterone on sperm retrieval has not been assessed in any randomized trials in this population, treatment with testosterone >6 months prior to biopsy was not shown to affect retrieval in the recent study by Plotton et al.[17] Ongoing treatment with testosterone and an aromatase inhibitor did not appear to impair sperm retrieval rates in the retrospective study by Mehta et al.[16] We could not assess for differences because none of our subjects had ever been on testosterone therapy.

In addition to debating the optimal time to pursue testicular biopsy, many patients and families inquire about the likelihood of successful sperm retrieval in the future. Unfortunately, there is currently no clinical method of identifying those in whom TESE/unilateral micro-TESE will be successful. No association has been found between sperm retrieval rates and hormonal markers[17,30] or testicular size, even when measured with ultrasound[16]; our study was underpowered to determine whether or not there were correlations. Ultrasound has been identified as the most accurate method for measuring testicular volume, particularly in patients with Klinefelter syndrome because of their small testicular size; our findings confirmed that orchidometry is inaccurate in this population.[31,32]

Theories about lower fertility potential in those who have more phenotypic severity have also not been confirmed. The reason for phenotypic variability in Klinefelter syndrome is unclear, but low androgen levels, androgen receptor polymorphisms, and/or imprinting may be partially responsible.[33-35] Zitzmann et al[35] showed that patients with Klinefelter syndrome and longer CAG repeat polymorphisms were significantly taller, had lower bone density, and were more likely to have gynecomastia; patients with shorter polymorphisms were more likely to live with a partner and work in professions requiring a higher education level. Other data have supported the influence of imprinting, with increased rates of speech and motor delays in patients with Klinefelter syndrome whose extra X chromosome was paternally, rather than maternally, derived.[33] Previous groups have indirectly assessed the potential correlation between neurocognitive and gonadal function by comparing sperm retrieval rates in those diagnosed in childhood because of neurocognitive problems vs those diagnosed prenatally or in adulthood because of infertility, with no significant differences found.[17] To our knowledge, ours is the first prospective study in young patients with Klinefelter syndrome to assess both sperm retrieval and neurocognitive status with a validated survey; our SDQ results suggest that our cohort was similar to those described in other pediatric studies.[5]

Notably, there were a significant number of incidental scrotal and renal abnormalities found in our study, some of which will require further evaluation and management. One-third of the patients had testicular microlithiasis, which is significantly higher than that reported in the general population (2%-8%) and somewhat higher than what was found in a previous study of Klinefelter syndrome (17.5%); the clinical implications remain unclear.[36-39] Another notable result of our study was that 17% of those who had renal imaging (13% of our entire cohort) were incidentally found to have bilateral renal medullary nephrocalcinosis. These patients have no known medical conditions to explain this, and to our knowledge, this abnormality has not been previously described in the Klinefelter syndrome population. Because this was not an expected finding, our study protocol did not include a renal ultrasound for all of the patients. However, at our institution, renal imaging is usually performed at the time of scrotal imaging; thus, 12 of the 15 patients happened to have these studies. Patients with renal medullary nephrocalcinosis should be evaluated to seek an underlying cause (such as renal tubular acidosis or a condition causing hypercalcemia) and may need long-term follow-up, as persistent/severe nephrocalcinosis can result in compromise of renal function.

The main limitation of this study is the small sample size; it should, therefore, be viewed as a pilot study. Studies of the population with Klinefelter syndrome typically have small sample sizes because only 25% of patients with Klinefelter syndrome are ever recognized, and <10% are diagnosed prior to puberty.[40] Further, discussing fertility preservation with adolescents is challenging, as many of them have not thought seriously about these issues, thereby limiting recruitment for these types of studies. In addition, given the experimental nature of testicular biopsy in adolescents with Klinefelter syndrome, we elected to perform the procedure on 1 testis only and not place the other testis at risk. We did not analyze the entire specimen after retrieval because most of the tissue was cryopreserved for potential extraction of sperm to be used in assisted reproduction. Also, the biopsies were not performed at a high-volume in vitro fertilization facility because of the age range of our cohort and the lack of a suitable facility nearby; thus, the tissue was not examined thoroughly while the patients were in the operating room. Retrieval rates may have been underestimated because of these factors. It is also notable that none of our subjects had ever been on testosterone therapy (suggesting milder gonadal dysfunction) because we opted not to discontinue testosterone therapy for the purposes of enrollment to allow for comparison with adult studies, thus limiting the generalizability of our findings.

Larger, randomized studies should be done in this population to further assess the impact of age and other factors, and to evaluate the impact of ongoing testosterone therapy on sperm retrieval. Results of such studies would help clinicians provide counseling to patients and families with Klinefelter syndrome about the impact of age on sperm retrieval. In addition, our incidental scrotal and renal findings will need to be investigated further to determine their clinical significance in this population. ■

Submitted for publication Sep 9, 2015; last revision received Oct 20, 2015; accepted Dec 7, 2015.

Reprint requests: Leena Nahata, MD, Division of Endocrinology, Nationwide Children's Hospital, 700 Children's Dr, Columbus, OH 43205. E-mail: leena.nahata@nationwidechildrens.org

References

1. Coffee B, Keith K, Albizua I, Malone T, Mowrey J, Sherman SL, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. Am J Hum Genet 2009;85:503-14.
2. Nielsen J, Wohlert M. Sex chromosome abnormalities found among 34 910 newborn children: results from a 13-year incidence study in Arhus, Denmark. Birth Defects Orig Artic Ser 1990;26:209-23.
3. Foresta C, Galeazzi C, Bettella A, Marin P, Rossato M, Garolla A, et al. Analysis of meiosis in intratesticular germ cells from subjects affected by classic Klinefelter's syndrome. J Clin Endocrinol Metab 1999;84: 3807-10.
4. Klinefelter HF Jr, Reifenstein E Jr, Albright F. Syndrome characterized by gynecomastia, aspermatogensis, without a-Leydigism, and increased excretion of follicle stimulating hormone. J Clin Endocrinol Metab 1942;2:615-27.
5. Pacenza N, Pasqualini T, Gottlieb S, Knoblovits P, Costanzo PR, Stewart Usher J, et al. Clinical Presentation of Klinefelter's Syndrome: Differences According to Age. Int J Endocrinol 2012;2012:324835.
6. Nahata L, Rosoklija I, Yu RN, Cohen LE. Klinefelter syndrome: are we missing opportunities for early detection? Clin Pediatr 2013;52:936-41.
7. Palermo GD, Schlegel PN, Sills ES, Veeck LL, Zaninovic N, Menendez S, et al. Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with nonmosaic Klinefelter's syndrome. N Engl J Med 1998;338:588-90.
8. Fullerton G, Hamilton M, Maheshwari A. Should nonmosaic Klinefelter syndrome men be labelled as infertile in 2009? Hum Reprod 2010;25: 588-97.
9. Madureira C, Cunha M, Sousa M, Neto AP, Pinho MJ, Viana P, et al. Treatment by testicular sperm extraction and intracytoplasmic sperm injection of 65 azoospermic patients with nonmosaic Klinefelter syndrome with birth of 17 healthy children. Andrology 2014;2:623-31.
10. Maiburg MC, Hoppenbrouwers AC, van Stel HF, Giltay JC. Attitudes of Klinefelter men and their relatives towards TESE-ICSI. J Assist Reprod Genet 2011;28:809-14.
11. Okada H, Goda K, Yamamoto Y, Sofikitis N, Miyagawa I, Mio Y, et al. Age as a limiting factor for successful sperm retrieval in patients with nonmosaic Klinefelter's syndrome. Fertil Steril 2005;84:1662-4.
12. Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. J Urol 2009;182:1108-13.
13. Ferhi K, Avakian R, Griveau JF, Guille F. Age as only predictive factor for successful sperm recovery in patients with Klinefelter's syndrome. Andrologia 2009;41:84-7.
14. Mehta A, Paduch DA. Klinefelter syndrome: an argument for early aggressive hormonal and fertility management. Fertil Steril 2012;98: 274-83.
15. Plotton I, Brosse A, Lejeune H. Is it useful to modify the care of Klinefelter's syndrome to improve the chances of paternity?. Ann Endocrinol (Paris) 2010;71:494-504 [Article in French].
16. Mehta A, Bolyakov A, Roosma J, Schlegel PN, Paduch DA. Successful testicular sperm retrieval in adolescents with Klinefelter syndrome treated with at least 1 year of topical testosterone and aromatase inhibitor. Fertil Steril 2013;100:970-4.
17. Plotton I, Giscard d'Estaing S, Cuzin B, Brosse A, Benchaib M, Lornage J, 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;100:961-7.
18. Paltiel HJ, Diamond DA, Di Canzio J, Zurakowski D, Borer JG, Atala A. Testicular volume: comparison of orchidometer and US measurements in dogs. Radiology 2002;222:114-9.
19. Mathai J, Anderson P, Bourne A. Comparing psychiatric diagnoses generated by the Strengths and Difficulties Questionnaire with diagnoses made by clinicians. Aust N Z J Psychiatry 2004;38:639-43.
20. Bourdon KH, Goodman R, Rae DS, Simpson G, Koretz DS. The Strengths and Difficulties Questionnaire: U.S. normative data and psychometric properties. J Am Acad Child Adolesc Psychiatry 2005;44: 557-64.
21. Wickham H. Reshaping data with the reshape package. J Stat Softw 2007; 21:1-20.
22. Wolak ME, Fairbairn DJ, Paulsen YR. Guidelines for estimating repeatability. Methods Ecol Evol 2012;3:129-37.
23. Team RC. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2013.
24. Muller J, Skakkebaek NE, Ratcliffe SG. Quantified testicular histology in boys with sex chromosome abnormalities. Int J Androl 1995;18:57-62.
25. Wikstrom AM, Dunkel L, Wickman S, Norjavaara E, Ankarberg-Lindgren C, Raivio T. Are adolescent boys with Klinefelter syndrome androgen deficient? A longitudinal study of Finnish 47,XXY boys. Pediatr Res 2006;59:854-9.
26. Salbenblatt JA, Bender BG, Puck MH, Robinson A, Faiman C, Winter JS. Pituitary-gonadal function in Klinefelter syndrome before and during puberty. Pediatr Res 1985;19:82-6.
27. Wikstrom AM, Raivio T, Hadziselimovic F, Wikstrom S, Tuuri T, Dunkel L. Klinefelter syndrome in adolescence: onset of puberty is associated with accelerated germ cell depletion. J Clin Endocrinol Metab 2004;89:2263-70.
28. Van Saen D, Gies I, De Schepper J, Tournaye H, Goossens E. Can pubertal boys with Klinefelter syndrome benefit from spermatogonial stem cell banking? Hum Reprod 2012;27:323-30.
29. Rives N, Milazzo JP, Perdrix A, Castanet M, Joly-Helas G, Sibert L, et al. The feasibility of fertility preservation in adolescents with Klinefelter syndrome. Hum Reprod 2013;28:1468-79.
30. Koga M, Tsujimura A, Takeyama M, Kiuchi H, Takao T, Miyagawa Y, et al. Clinical comparison of successful and failed microdissection testicular sperm extraction in patients with nonmosaic Klinefelter syndrome. Urology 2007;70:341-5.
31. Shiraishi K, Takihara H, Kamiryo Y, Naito K. Usefulness and limitation of punched-out orchidometer in testicular volume measurement. Asian J Androl 2005;7:77-80.
32. Diamond DA, Paltiel HJ, DiCanzio J, Zurakowski D, Bauer SB, Atala A, et al. Comparative assessment of pediatric testicular volume: orchidometer versus ultrasound. J Urol 2000;164:1111-4.
33. Stemkens D, Roza T, Verrij L, Swaab H, van Werkhoven MK, Alizadeh BZ, et al. Is there an influence of X-chromosomal imprinting on the phenotype in Klinefelter syndrome? A clinical and molecular genetic study of 61 cases. Clin Genet 2006;70:43-8.
34. Zinn AR, Ramos P, Elder FF, Kowal K, Samango-Sprouse C, Ross JL. Androgen receptor CAGn repeat length influences phenotype of 47,XXY (Klinefelter) syndrome. J Clin Endocrinol Metab 2005;90: 5041-6.
35. Zitzmann M, Depenbusch M, Gromoll J, Nieschlag E. X-chromosome inactivation patterns and androgen receptor functionality influence phenotype and social characteristics as well as pharmacogenetics of testosterone therapy in Klinefelter patients. J Clin Endocrinol Metab 2004;89:6208-17.
36. Peterson AC, Bauman JM, Light DE, McMann LP, Costabile RA. The prevalence of testicular microlithiasis in an asymptomatic population of men 18 to 35 years old. J Urol 2001;166:2061-4.
37. Cooper ML, Kaefer M, Fan R, Rink RC, Jennings SG, Karmazyn B. Testicular microlithiasis in children and associated testicular cancer. Radiology 2014;270:857-63.
38. Heller HT, Oliff MC, Doubilet PM, O'Leary MP, Benson CB. Testicular microlithiasis: prevalence and association with primary testicular neoplasm. J Clin Ultrasound 2014;42:423-6.
39. Accardo G, Vallone G, Esposito D, Barbato F, Renzullo A, Conzo G, et al. Testicular parenchymal abnormalities in Klinefelter syndrome: a question of cancer? Examination of 40 consecutive patients. Asian J Androl 2015;17:154-8.
40. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. J Clin Endocrinol Metab 2003;88:622-6.

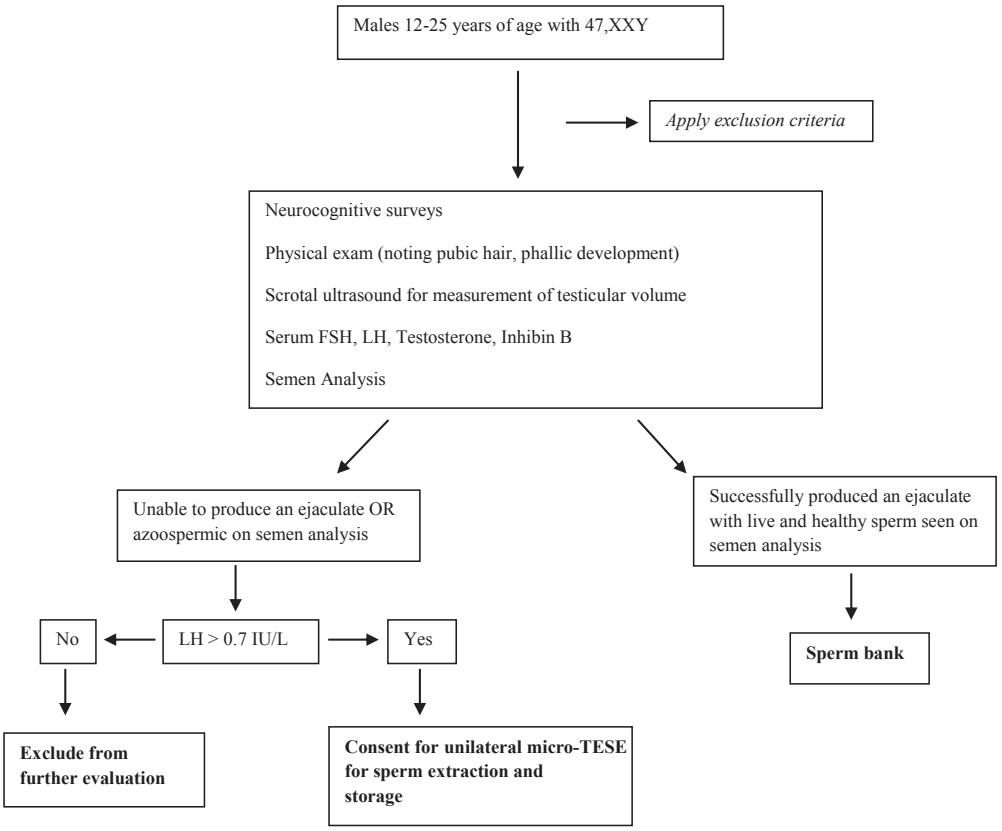

Figure 1. Study schema. *FSH*, follicle-stimulating hormone.

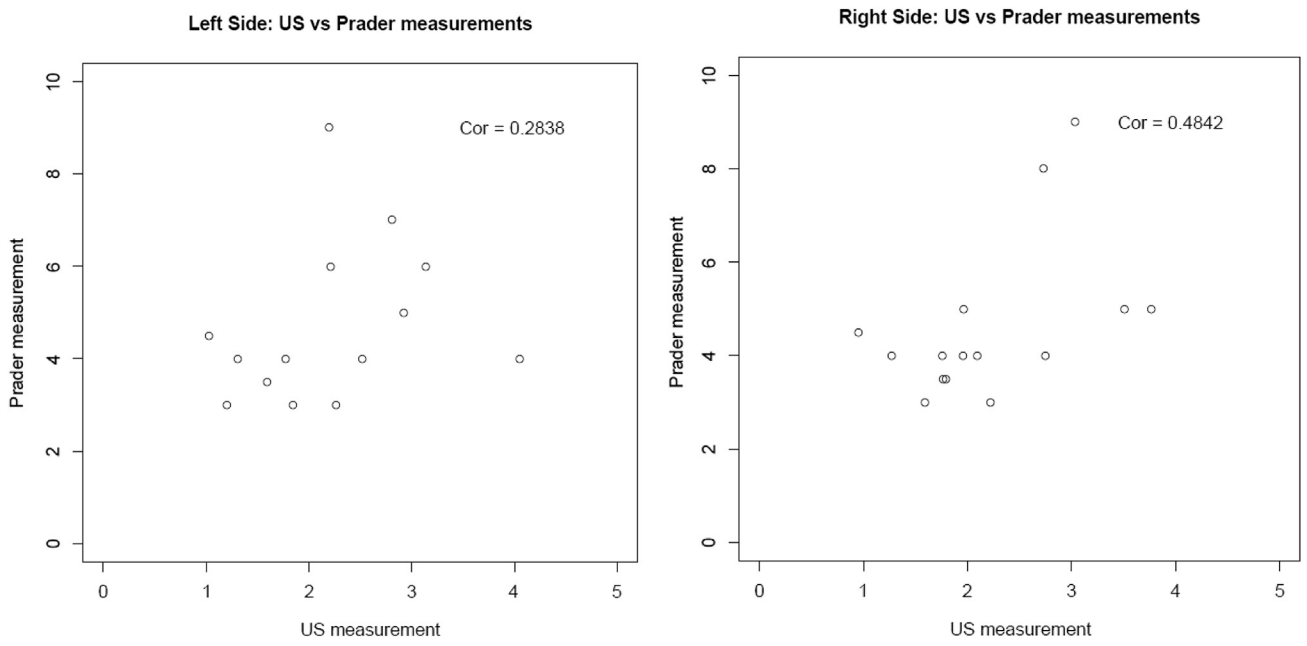

Figure 2. Correlation between Prader orchidometry and ultrasound measurements.