Testis Development and Reproductive Options in Males with Klinefelter Syndrome

Shanlee M. Davis, MD, Alan D. Rogol, MD, PhD, Judith L. Ross, MD

Author Contact Info

Shanlee M. Davis, MD, University of Colorado/Children's Hospital Colorado, 13123 East 16th Ave B264, Aurora, CO 80045, 720-777-6073, Shanlee.Davis@childrenscolorado.org

Alan D. Rogol, MD, PhD, University of Virginia, 685 Explorers Road, Charlottesville, VA 22911, 434-971-6687, adrogol@comcast.net
Consultant to: SOV Therapeutics, Trimel Pharmaceuticals, Novo Nordisk, Versartis, AbbVie

Judith L. Ross, MD, Department of Pediatric Endocrinology A.I. DuPont Hospital for Children/ Thomas Jefferson University, Department of Pediatrics, 833 Chestnut St., Philadelphia, Pennsylvania, 19107, jlross@nemours.org

Key Points

1. Klinefelter syndrome (KS) is common but underdiagnosed; non-invasive prenatal testing may increase the diagnosis rate by 4-5 fold, thereby increasing the demand for evidence-based research in the near future.
2. Testis development and function is abnormal from infancy and worsens with age, however the underlying molecular mechanisms for this have not been elucidated.
3. Due to lack of clinical trials, androgen supplementation practices vary between clinicians. Most often testosterone injections or gel are initiated in mid-puberty, as LH rises above the normal range, and continues lifelong.
4. With testicular sperm extraction (TESE), sperm can be obtained for fertilization in around half of men with KS. Small numbers of germ cells are present in around half of prepubertal and pubertal males with KS as well. Neither clinical or biochemical markers, nor age seem to accurately predict those who have focal areas of spermatogenesis.

Synopsis

Klinefelter syndrome (KS) is the leading genetic cause of primary hypogonadism and infertility in men.[1,2] The clinical phenotype has expanded beyond the original description of infertility, small testes and gynecomastia.[3]Animal models, epidemiological studies, and clinical research of males with KS throughout the lifespan have allowed us to better characterize the variable phenotype of this condition. This review will provide an overview on what is known of the epidemiology, clinical features, and pathophysiology of KS, followed by a more focused discussion of testicular development and the clinical management of hypogonadism and fertility in men with KS.

INTRODUCTION

Klinefelter syndrome (KS), defined by one or more extra X chromosomes in males, is the leading genetic cause of primary hypogonadism and infertility.[1,2]The clinical phenotype has expanded beyond the original description of infertility, small testes and gynecomastia.[3]Animal models, epidemiological studies, and clinical research of males of all ages with KS have allowed us to better characterize the variable phenotype of this condition. Scientific advances have led to fertility potential in about half of men with KS. Despite this, the molecular mechanisms underlying the nearly universal finding of primary gonadal failure remain elusive. If non-invasive prenatal testing becomes part of routine prenatal care as many suggest, the diagnosis of KS will increase by 4 to 5 fold, thereby raising the demand for high quality, evidence-based research to improve outcomes in boys and men with KS.[4]

EPIDEMIOLOGY

Population-based studies on newborns as well as adjusted prenatal screening rates yield an incidence of KS in ~1/650 males.[5-7] Approximately 3,075 infants with KS are born in the United States every year.[8]Based on historic data, it is reasonable to assume more than 2,000 of those infants will never be diagnosed. These statistics stem from a study in the United Kingdom in the 1990's which reported 10% of males with KS are diagnosed prenatally, 7% in childhood or adolescence, and another 17% in adulthood with the remaining 66% of males with KS never receiving a diagnosis.[9] As expected, the reasons for diagnosis depend on age, with developmental and behavioral concerns more common in younger children, pubertal delay in adolescence, and infertility in adulthood.[10]Diagnosis rates likely vary based on time period and geography and therefore may be different in the US in 2015 than it was in Europe20 years ago. It is also very probable that prenatal diagnoses will increase in the near future due to the increased utilization of

non-invasive prenatal testing that can screen for fetal aneuploidy with a simple maternal blood sample.[4] The actual incidence of KS may be increasing as well due to rising maternal age correlating with the risk for non-disjunction errors during meiosis resulting in fetal aneuploidy.[5,11,12]In fact, the most recent epidemiological study found the prevalence of KS to be 1/448 male births along with an overall higher rate of lifetime diagnosis of 50%.[13]

CLINICAL FEATURES

Hypergonadotropic hypogonadism and infertility are nearly universal in adult males with KS.[14] These features together with tall stature, eunuchoid body habitus, and gynecomastia define the cardinal findings described in the earliest literature on Klinefelter syndrome.[3]For the majority of affected males, manifestations are subtle and nonspecific, therefore falling below the threshold of clinical suspicion, particularly in childhood and early adolescence. Studies consistently report a higher prevalence of type 2 diabetes mellitus, dyslipidemia, fatty liver disease, hypercoagulability, and osteoporosis in adults, with evidence the metabolic dysfunction begins in childhood/adolescence.[10,15-19] Neurodevelopmental, behavioral, and psychosocial deficits are reported throughout the lifespan.[18-21]Toddlers with KS are at risk for motor and language developmental delays, while learning disabilities, internalizing and externalizing behaviors, and social difficulties may arise in school age and beyond.[21-25]Adolescents and adults can struggle with adaptive functioning skills including poor self-care.[26] Cognitive ability is usually in the normal range but lower than sibling controls, and verbal scores are about 10 points lower than performance domains.[27-30]Individuals ascertained by prenatal diagnosis may have fewer neurodevelopmental and psychosocial difficulties than those diagnosed postnatally, highlighting the importance of accounting for selection bias in research studies.[31]While 80-90% of males with KS have a non-mosaic 47,XXY karyotype, a smaller percentage will have mosaicism that is often associated

with a milder phenotype, or alternatively have more than one extra sex chromosome (48,XXYY, 48,XXXY, 49,XXXXY, 49,XXXYY), generally conferring a more severe phenotype.[32-35]

PATHOPHYSIOLOGY

The phenotypic heterogeneity in males with KS is likely influenced by genetic, epigenetic and environmental factors. Furthermore, given the universal testicular dysfunction in KS, it is difficult to determine what clinical features are due to hypogonadism and therefore modifiable by androgen supplementation, and what clinical features are manifestations of the aneuploidy itself. In adult men with KS, the presence of physical features such as higher body fat percentage, type 2 diabetes, decreased left temporal lobe gray matter, and autoimmune disease, correlate with degree of hypogonadism and/or lack of androgen supplementation, however these associations do not indicate causality.[18,36-39]Randomized controlled trials investigating androgen replacement in adults with KS have not been done, although several randomized controlled trials in children with KS are underway or recently completed.[40-42]In the KS mouse model, replacement of testosterone improves psychosocial dysfunction but not osteopenia or metabolic dysfunction.[43,44] More research is needed to understand the pathophysiology of the multiple phenotypic features of males with KS.

With the genetic basis for KS, based on an extra X chromosome, most of the focus is the more than 1,000 genes on the X chromosome that influence gonadal development, growth, and brain development. It is logical to assume the KS phenotype is secondary to a gene-dosage effect of extra genetic material on the X chromosome that escapes X-inactivation or polymorphisms of specific genes on the X chromosomes, such as the trinucleotide repeat length of the androgen receptor gene.[36,45-47] However, the complexity increases as gene expression on autosomes seem to be influenced by the presence of an extra X chromosome. In a microarray gene expression

analysis in the lymphocytes of 10 male subjects, half with 47,XXY, 480 autosomal genes were up-regulated in males with KS and over 200 were down-regulated.[48] Similar findings were found in testis transcriptome analysis with significant deregulation of gene expression in sertoli and leydig cells as well as germ cells.[49] Tissue-specific differences in autosomal DNA methylation and gene expression were identified in the post-mortem brain of a male with 47,XXY, including the gene SPAG1 (sperm associated antigen 1) on the long arm of chromosome 8 that codes for a protein thought to be essential for signal transduction pathways in spermatogenesis.[50,51] Differential gene expression of 35 genes correlated with clinical findings of insulin resistance, dyslipidemia, and coagulability.[52]Therefore, aneuploidy itself may result in epigenetic modulation of autosomal genes in a tissue-specific manner, contributing to the complexity in KS pathophysiology. As our knowledge of genetics and epigenetics advances, we will gain a better understanding of the underlying molecular mechanisms yielding gonadal failure as well as the other clinical features commonly found in men with this syndrome.

TESTICULAR DEVELOPMENT, FUNCTION AND PATHOLOGY

Case series and observational studies at various ages shed light on the natural history of testicular changes throughout the lifespan in males with KS. While it is clear the supernumerary X chromosome is the underlying etiology of testicular failure, the molecular mechanisms by which this occurs have not been fully elucidated. Although eventual germ cell failure is evident, it remains unknown whether the germ cells have a primary defector germ cell maturation is disrupted due to an abnormal gonadal milieu. Future investigation aimed at elucidating the underlying mechanisms will ultimately help develop measures to preserve testicular function.

We have synthesized the currently available literature on testicular development in males with KS including testis size, histologic findings, and serologic gonadal function biomarkers in

Table 1. Much of our knowledge is based on evidence of marginal quality with small sample sizes, participant selection bias, and poor hormone assay quality. Many reports have been retrospective case series with significant inter- and even intra-study methodologic variability, limiting both the comparability and generalizability of the findings.

Fetal: The increased incidence of underdeveloped genitalia and cryptorchidism raise the concern for fetal androgen insufficiency, particularly during the second or third trimester.[14,53,54]Examinations of testes in second trimester fetuses with KS have had variable findings with approximately half reporting reduced germ cell numbers and half with normal histology.[55-61]Testosterone concentrations in amniotic fluid have been examined in six studies, with four of the six reporting no differences in total testosterone concentrations between male fetuses with 47,XXY (total n=33) and 46,XY.[60,62-66]In the largest of these studies, two of the 20 subjects with 47,XXY had testosterone levels in the female range, therefore there may be a minority of males with KS who have a defect in testosterone production inutero.[62]Testosterone levels in cord blood have been reported to be low (n=3), compared to controls (n=3), however this is far too small a sample size from which to draw conclusions.[67]None of these studies measured testosterone concentrations by liquid chromatography mass spectrometry, a method that has increased sensitivity and accuracy compared with older methods, particularly with testosterone concentrations <100 ng/dl (~3.5 mmol/L).[68]There have not been any studies examining other biomarkers of testicular function such as products of sertoli cells or insulin-like peptide 3 (INSL3), a hormone produced by leydig cells and critical for testicular descent.[69]At this time, there is insufficient evidence to determine if hypogonadism is present in the fetus with KS.

Infancy: Penile growth in the first months of life has been considered a biomarker for androgen exposure during the neonatal surge or "mini-puberty" of infancy.[70] Slow penile growth in the

first year of life in males with KS provides clinical evidence to support relative androgen deficiency in infancy.[70-72] Hypotonia, although certainly not specific for androgen deficiency, is frequently observed in infants with KS.[73]Testes are often small in infancy.[46,73,74]Testicular biopsies have shown lower number of spermatogonia in all case reports that included quantitative analysis, however the histological appearance of sertoli and leydig cells was typically normal. Three of these (total n=68) report lower median testosterone levels in KS, while the other two(total n=16)found normal or even high-normal testosterone levels. The single study that assessed testosterone concentrations with liquid chromatography/tandem mass spectrometry reported 87% of 38 infants with KS 16-120 days of life were below the median for controls and ~20% fell below the normal range.[80]Given the variability of the timing and peak of postnatal testosterone levels in normal infant males and the cross-sectional design of the majority of these studies in boys with KS, it is very difficult to determine if subtle deficits in the hypothalamic-pituitary-gonadal axis are present in some or all infant boys with KS.[82,83] The three studies that reported lower testosterone levels also reported normal LH levels, potentially raising the question of whether there is some degree of a central pituitary/hypothalamic defect as well as primary hypogonadism. The most recent of these studies reported INSL-3 levels within the normal range.[80]

Biomarkers of sertoli cell function including anti-mullerian hormone (AMH) and inhibin B (INHB) are broadly within normal ranges; however sertoli cell dysfunction may be present in some infants with KS.[80,81,84] In a study of 68 boys with KS under the age of 2 years, INHB was below the lower limit of normal in ~20%, while AMH was occasionally elevated in others.[80]FSH levels were elevated in 25%, although these were not the individuals that had low INHB levels. Overall, there is insufficient evidence to determine if hypogonadism occurs in infants with KS.

Prepubertal Childhood: Testicular volumes are small, often less than 1 mL, in pre-pubertal boys with KS.[46,71,73,85] Histologically, germ cell hypoplasia is appreciated while leydig and sertoli cells typically appear normal.[76,86,87]Childhood is typically considered the quiescent period of the hypothalamic-pituitary-gonadal axis development.[83]Baseline gonadotropin concentrations as well as stimulation testing with gonadotropin-releasing hormone are described as normal in the majority of studies of prepubertal boys with KS.[79,85,88]We have found a small but potentially significant number of boys with elevated gonadotropins for age (LH elevated in 7%, FSH elevated in 10%) in a large sample of 86 boys with KS, 4-11 years of age.[40] Serum testosterone concentrations in prepubertal boys with KS within the normal range for age, however the majority are in the bottom quartile.[72,85] It is also imperative to note that normal prepubertal hormone concentrations can be below the detection limit for many assays and testosterone radioimmunoassays in particular will overestimate the testosterone concentrations in children.[68]Sertoli cells make up the majority of the volume of the testes at this age, producing AMH and INHB even during this quiescent period.[89] In KS, small studies have found these biomarkers of sertoli cell function to be within the normal limits for age most often, however a few males with either low inhibin B or high AMH have been reported.[85,88,90] In a much larger sample of nearly 90 boys with KS, we have found a subset who have very low concentrations of AMH (13%) and/or low inhibin B (31%), while a quarter of subjects had rather elevated levels of AMH.[40]This raises the suspicion for sertoli cell dysfunction and in addition to germ cell depletion starting prior to external signs of puberty in boys with KS. However, it is difficult to conclude whether leydig cell dysfunction, in particular defective testosterone production, is present in childhood.

Puberty: Boys with KS in early puberty often have initial enlargement of testes to 6-8 mL, a rise in gonadotropins and testosterone to a pubertal range, and development of primary and second-

ary sex characteristics.[10,14,79,88,91,92] In mid-puberty, FSH rises and sertoli cell biomarkers decline – often to undetectable levels and testicular volumes decrease. In mid to late puberty, LH typically rises above the normal range and testosterone declines to low or low-normal for pubertal stage. In one study of six subjects followed longitudinally, INSL3 increased to low adult concentrations by a bone age of 12-13 years and then plateaued for the next two years, although the ratio of INSL3 to LH was much lower than healthy males.[93]Histologic evidence reveals near-absence of germ cells even in early puberty, and structurally abnormal support cells in half.[90]Clinical symptoms of hypogonadism at this age can include incomplete pubertal maturation, persistent pubertal (physiologic) gynecomastia, and relative tall stature.[14]

There is some evidence to suggest AMH declines more slowly during the peripubertal period in KS compared to XY males.[84] AMH is inversely related to intratesticular testosterone concentration as AMH gene transcription is down regulated in the presence of testosterone binding the androgen receptor on the sertoli cell.[94] An elevated AMH would therefore be consistent with lower intratesticular testosterone concentration, although intratesticular hormone concentrations in adolescents have not been reported. More studies on serum testicular function biomarkers in boys in early puberty may help to clarify this as it is possible these markers could predict timing of gonadal failure or future fertility potential. Overall, there is strong evidence to support hypogonadism with germ cells, sertoli cells, and leydig cells all being dysfunctional in the majority of boys with KS from mid-puberty on.

Adulthood: Unequivocal testicular dysfunction is observed in adults with KS. Testes are often even smaller than during puberty, and testicular histology typically reveals absence of germ cells (often a sertoli-cell only picture),fibrosis and hyalinization of the seminiferous tubules, and leydig cell hyperplasia.[14,71,95,96]FSH is universally elevated; LH is elevated in the great

majority.[10] Inhibin B is usually below the normal range, while AMH is often undetectable.[84,97] Testosterone concentration may be low or low-normal[10]. INSL3, another product of leydig cells critical for testicular descent and likely germ cell maturation and bone health, is also low.[98]

Intratesticular hormone concentrations have not been thoroughly investigated. Although low intratesticular testosterone would be suspected, a recent study found normal to elevated intratesticular testosterone in biopsies in men with KS.[99] These authors postulate an abnormal intratesticular vascular bed leading to inadequate secretion of testosterone systemically. Better understanding of the intratesticular hormonal milieu during the critical time of puberty may permit the development of targeted treatments to prevent the degeneration of germ cells, androgen deficiency, and infertility.

MEDICAL MANAGEMENT

Management of males with KS will involve routine physical examinations, ongoing evaluation for known clinical conditions associated with KS including developmental assessments, and potential androgen supplementation initiated in adolescence. If the diagnosis was made prenatally, a post-natal confirmation of the karyotype should be obtained. For this purpose and for any suspected KS diagnosis, routine chromosome analysis is sufficient, although high-resolution chromosome analysis and comparative genomic hybridization microarray would also reveal the diagnosis.

Infancy: Initial consultation with a pediatric endocrinologist is very important in this interval for reviewing testicular function and the role of androgen replacement with the family. Despite very little published data of prepubertal androgen treatment in infants with KS, we have found up to 1 in 5 boys with KS receive androgen treatment in infancy or early childhood.[40]Some of these infants will receive a short course of either intramuscular or topical testosterone for the indication

of micropenis or small phallus. Other clinicians have suggested testosterone treatment should be considered standard of care in infancy,[100] although no therapeutic benefits have been clearly delineated aside from penile growth. The only published data exploring benefits of testosterone in infancy was a recent retrospective study reporting higher scores on standardized developmental assessments in multiple cognitive domains at 3 and 6 years of age in boys who had received a short course of testosterone.[101]That retrospective study design which lacked blinding, randomization, or a delineated protocol significantly limits generalizability of these findings. A randomized trial of intramuscular testosterone during the mini puberty period has just started enrollment at Children's Hospital Colorado (NCT#02408445, SD, PI).

Some clinicians recommend measuring testosterone, luteinizing hormone and follicle stimulating hormone during the neonatal surge, however the clinical utility of this information has not been established. Even among 46,XY males, the mini-puberty period is variable with regard to peak hormone concentrations and timing; therefore these data are not useful in providing evidence-based management decisions or prognostic assessments at this time.[82,102,103]It is quite possible a normal surge may have favorable prognostic implications, such as a milder phenotype, less hypogonadism, or improved fertility potential; however this has never been reported.

Childhood*:* The focus during the childhood years should be on educational and psychosocial development needs. There are no published randomized controlled trials of androgen supplementation in pre-pubertal boys with KS to date. A randomized controlled trial of oral oxandrolone administration in boys 4-12 years (NCT#00348946, JR, PI) was recently completed and published results are anticipated shortly. At this time there is no clinical indication for androgen treatment in pre-pubertal boys with KS.

Puberty: At the first sign of puberty or around the age of 10-12, boys warrant evaluation by a pediatric endocrinologist. Pubertal progression and growth should be monitored closely and gonadotropin and testosterone concentrations obtained at least annually during this time. Elevated gonadotropin concentrations or plateau of serum testosterone can be seen as puberty progresses and are important in determining when supplemental testosterone is warranted. Signs of relative hypogonadism such as poor muscle mass, persistent gynecomastia, and stalled virilization should be assessed. If the patient is obese or on antipsychotic medications, routine labs to screen for comorbidities should be performed every two years according to expert guidelines including cholesterol levels, hemoglobin A1C (or fasting glucose), and liver function tests.[104,105]We recommend these screening tests should also be performed in boys with KS and a normal BMI as well, since studies report greater visceral adiposity and a higher risk of these dysmetabolic conditions in all children and adolescents with KS.[15,16]Specifically,elevated LDL cholesterol was observed in 37% and insulin resistance in 24% of prepubertal boys with KS, despite BMI not differing from controls.[15]Although these abnormalities did not reach a threshold necessitating pharmacologic therapy, lifestyle modification, particularly with increased physical activity, would be beneficial and therefore screening around the time of puberty is reasonable and appropriate. There are no data to support the routine measurement of bone density in children or adolescents as bone mineral density has been described as normal.[16]

Due to a lack of definitive research, initiation of testosterone therapy in young adolescents with KS is predominantly clinician-preference. This decision is often based on progression of pubertal development, evolution of hypergonadotropic hypogonadism, development of physical symptoms of androgen deficiency such as persistent gynecomastia, and family preference. A randomized clinical trial of topical testosterone versus placebo in males with KS in early puberty

(NCT#01585831) examining psychosocial outcome measures is currently enrolling. When testosterone therapy is initiated, the favored options include intramuscular injections of a testosterone ester or transdermal testosterone gel.[106] Ongoing growth potential can be assessed with a bone age X-ray. A reasonable approach is to start at low doses (100 mg intramuscular injection every 4 weeks or 1 pump per day of 1% or 1.62% testosterone gel) and titrate up until clinical symptoms of hypogonadism improve and serum testosterone concentration is appropriate for stage of pubertal development. Testosterone formulations that have a prolonged duration of action or higher doses are not recommended in adolescents.

Adults: Recommendations for evaluation in adult men with KS include annual measurement of fasting glucose, lipids, hemoglobin A1c, thyroid function tests, and hematocrit as well as intermittent bone density measurement by dual-energy x-ray absorptiometry.[107,108] An interdisciplinary panel from France also recommended baseline and every two year chest x-rays, testes and breast ultrasonography, and echocardiography.[107] These recommendations are not necessarily all evidence-based for cost-effectiveness as research has been limited.

Untreated adults with KS often will meet criteria for male hypogonadism defined as a serum testosterone <300 ng/dl with clinical symptoms. The Endocrine Society Clinical Practice Guidelines advises on treatment of male hypogonadism, including KS.[109] Multiple formulations of testosterone are available and outlined in Table 2.

Exogenous testosterone can suppress LH, thereby reducing spermatogenesis and potentially decreasing fertility potential.[110] While high dose testosterone has the capability to be used as a male birth control method, the anti-spermatogenic effects are assumed to be temporary.[111,112] Some studies have found less successful sperm retrieval rates in men with KS who have previously been on testosterone treatment, while other, more recent studies have found no such asso-

ciation.[113-115] Although there is a lack of randomized controlled trials, the probable benefits of testosterone therapy include positive effects on body composition, bone health, and psychological wellbeing.[116-119] Overall these treatment advantages are more convincing than the theoretical risk of fertility decline, particularly with advances in reproductive endocrinology and assisted reproductive technology (ART).

FERTILITY & REPRODUCTION

The most common reproductive abnormality in KS is non-obstructive azoospermia (NOA), and approximately 11% of men with NOA will have KS.[10,120]In select populations, the ejaculate may contain motile sperm in up to 10% of men with KS; therefore birth control is advised if fertility is not desired.[35,90,121] However, spontaneous pregnancies are rare and, without ART, males with KS are nearly always infertile.[95]With recent advances of reproductive medicine, sperm can be retrieved via surgical testicular sperm extraction(TESE) in around 50% of men seeking biologic fertility. [113,114,122-125] These success rates are similar to males with NOA from other causes.[95,125]Retrieved sperm, either from ejaculate or TESE, are either used to fertilize an oocyte via intracytoplasmic sperm injection (ICSI) and/or cryopreserved for future ICSI.[124,126] This technology has significantly expanded the options for parenthood for men with KS beyond sperm donation and adoption; however it is often limited to those with access to large referral centers and monetary resources.

The sperm retrieval success may be increased with the use of micro-TESE, a technique that utilizes 20-25 times magnification to identify larger seminiferous tubules that are more likely to contain active spermatogenesis.[127,128] It is hypothesized these active spermatogenic foci represent germ cell mosaicism with 46,XY karyotype, potentially representing trisomy rescue dur-

ing meiosis.[96,123]Micro-TESE may have fewer complications than standard TESE including risk for hematoma, and post-surgical hypoandrogenism.[129]

Efforts to identify a consistent predictor for successful sperm extraction have not been fruitful. Testes size, serum hormone concentrations, physical signs of androgenization, age and history of exogenous testosterone treatment have all been proposed, but have largely failed to differentiate the ~50% of males who will have successful sperm retrieval with TESE.[130,131]Several studies have found greater success rates in sperm retrieval for younger men with KS, which conceptually makes sense, given the progressive decline in spermatogonia number described with age in men with KS.[114,132] Therefore, sperm cryopreservation as early as adolescence has been advocated, potentially even in early puberty prior to decline in inhibin B and rise in FSH.[122,133]However, spermatazoa were not found in the ejaculate of 13 adolescent boys with KS,[134]and testicular biopsies in adolescent males have found similar number of spermatogonia to those found in adults with even fewer spermatids.[90,133]Furthermore, several studies have not found age to be a factor in sperm retrieval from TESE, including a recent study where adult males age 25-36 had the same rates of success with TESE as males 15-24 years.[113]Younger males are also not seeking immediate fertility therefore requiring cryopreservation, which may yield lower fertilization and pregnancy rates compared to using fresh sperm.[135]Given these findings along with the high cost of sperm cryopreservation and ethical issues involved in using invasive means to obtain sperm in a minor, it seems most reasonable to wait until the male with KS is at an age where he can evaluate his options available for fertility, if desired, and provide his own consent to undergo ART.

While most specialists recommend discontinuation of exogenous testosterone, the use of other pharmacologic agents to enhance sperm retrieval success rates for men with KS is investi-

gational.[110,136-138]The three most commonly used medications all attempt to increase endogenous testosterone production, with the premise that higher intratesticular concentration will stimulate spermatogenesis.[137]The first, human chorionic gonadotropin, stimulates leydig cells by binding to the LH receptor, thereby increasing testosterone production if the leydig cell is at least partially functional.[137,139] This is currently the only FDA approved medication for male infertility, however no studies specifically in KS-related infertility have been done. Clomiphenecitrate is a selective estrogen receptor modulator that blocks the negative feedback at the level of the hypothalamus and pituitary thus increasing both LH and FSH secretion.[137,140]Reports of its use in KS date back to the 1970's, although the efficacy of clomiphene has not been proven in KS or men with sertoli-cell only morphology.[138,141]Finally,aromatase inhibitors increase the testosterone to estradiol(T/E2) ratio by inhibiting the conversion of testosterone to estrogen, thereby improving spermatogenesis by decreasing the negative inhibition of estrogen and stimulating FSH secretion as well as increasing testosterone levels.[137] Aromatase inhibitors increase sperm volume, sperm concentration and motility index in men with subfertility and a low T/E2 ratio (<10:1) in a non-randomized uncontrolled study, however results were less impressive in a KS subanalysis.[142-144]One study of males with KS and NOA who were treated with one of the above pharmacologic agents if baseline serum testosterone was<300 ng/dl found response to treatment (increase in serum testosterone) to be predictive of successful micro-TESE.[114]Others have shown comparable success rates without pre-treatment with these pharmacologic agents.[113]Algorithms have been proposed to help aid in determining which pharmacologic agents, if any, should be used prior to TESE, however the evidence base is largely limited to a single institution.[114,145]

The majority of offspring of men with KS are born with a normal karyotype[95], however research has demonstrated high rates of aneuploidy from 7-46% in spermatids of males with

KS.[123,146,147] One hypothesis for this increased risk of aneuploidy is 47,XXY spermatogonia progress through meiosis and yield hyperhaploid spermatozoa (24,XX and 24,XY).[123] Another potentially more probable hypothesis is that the germ cells that successfully progress through spermatogenesis are predominantly 46,XY however the surrounding testicular environment remains unfavorable and increases susceptibility of meiotic abnormalities.[96,148]This is consistent with findings of increased risk of autosomal aneuploidy (trisomy 21 and 18) as well as sex chromosome aneuploidy.[147]The routine use of pre-implantation genetic diagnosis of embryos fertilized by sperm from males with KS has been proposed, however, this remains an area of debate.[146,147]

In summary, males with KS seeking biological paternity today are no longer considered universally infertile. Various successful approaches for obtaining sperm have been described including first morning urine, (rarely successful)[79,149] ejaculation (up to 10%),[35,121] and TESE (around 50%).[95,114] Typically, the least invasive approaches are attempted first followed by surgical options. Mature sperm can either be used immediately for ICSI or alternatively cryopreserved for future use. Presently, cryopreservation of immature germ cells for the future hope of in vitro differentiation is experimental.

Future Considerations/Summary

We have learned a great deal in the past 70 years since the initial recognition of Klinefelter syndrome; however our understanding of the underlying pathophysiology as well as prevention or treatment of manifestations associated with the XXY karyotype is still remarkably limited. The greatest advances for men with the Klinefelter syndrome have arguably been in the field of reproductive endocrinology and ART. Less than two decades ago, males with KS were nearly invariably infertile, and now assisted fertility may be successful in half of them seeking to have a biological child. This technology will likely continue to advance rapidly, and it is difficult

to predict the possibilities that will exist when the infants born today seek assisted fertility 25 years from now. If future advances continue to require germ cells for fertilization, it will be prudent to understand the molecular mechanisms involved in germ cell apoptosis in general and in specific for men with KS permitting the exploration and implementation of preventative interventions. Research advances may make it possible to derive sperm from somatic cells, therefore preservation of germ cells may be unnecessary.

A less distant future consideration is the increased diagnosis rate of KS. There is currently active discussion to make non-invasive prenatal testing (NIPT) part of routine prenatal care independent of maternal age or risk factors.[4] Presuming positive screens for sex chromosome aneuploidy will be followed up with a diagnostic test via amniocentesis and/or post-natal karyotype, this change in practice would likely increase the number of infants diagnosed with KS by 10-fold. Thousands of parents and health care providers alike will be seeking evidence-based information on sex chromosome aneuploidies, both natural history and intervention to prevent common manifestations. As a research community, we need to focus efforts on patient-centered research outcomes; including predicting phenotypic variation and developing interventions to prevent the unwanted manifestations of KS, with the ultimate goal of helping millions of males with KS worldwide live healthy, normal lives.

Table 1: Summary and synthesis of primary literature on testicular development in KS

Testicular Volume (TV)	Histology	Leydig Cell Biomarkers	Sertoli Cell Biomarkers
Fetal			
No studies	**Quality:** Poor, ~6 case reports only. **Summary:** Approximately half of the case reports conclude reduced germ cell number.[55-61] **Conclusion:** Reduced germ cell numbers may be present in some boys with KS prior to birth.	**Quality:** Marginal. 6 studies, total 33 subjects. **Summary:** Mean total testosterone (TT) was normal; however TT was in the female range for 4/33 (12%). **Conclusion:** Amniotic TT is normal for majority, but a deficit in T production may be present in a subset (10-20%.)	No studies
Infancy			
Quality: Marginal, TV mentioned, but usually not compared to controls. **Summary**: Older studies generally report normal testes size at birth with lack of enlargement.[79] Two studies found lower testicular volumes than expected in infants(SDS –1.1).[46,75] **Conclusion:** Testicular volume may be normal at birth with less growth over the first year.	**Quality:** Poor, <10 case reports/series in infants <12 months. **Summary**: Most with normal appearance but quantitatively fewer germ cells. Germ cells inversely correlate with age. **Conclusion**: While support cells appear normal, germ cell depletion is already present in infancy and is possibly progressive.	**Quality:** Adequate, 5 total studies with 83 subjects. **Summary:** TT lower than expected in 3 of 5 studies (n=67),[73,80,81] normal in one (n=6)[79], and high-normal in another (n=10).[75] LH normal in all. INSL3 normal in one. **Conclusion:** Most likely subnormal serum TT during mini-puberty in majority of infants with KS.	**Quality**: Marginal, 3 studies with N~90 **Summary**: FSH, AMH and INHB usually within the normal ranges.[81,84] INHB low in ~20% in one study, few boys with high AMH.[80] **Conclusion**: Potentially sertoli cell dysfunction in a subset (<20%).
Childhood			
Quality: Adequate, reported in many studies. **Summary**: Multiple studies report small testes in the majority of boys; often <1mL.[10,71,92,150] mean -1.2 SDS.[46,85] **Conclusion**: Testes are smaller prepubertally.	**Quality:** Marginal, case reports or series, N~20, +selection bias. **Summary:** Fewer germ cells in all[76,87,90] number inversely correlates with age;[86]no germ cells were found in a case series including cryptorchidism.[76] Seminiferous tubules smaller,[87] leydig and sertoli cells normal but interstitial fibrosis and	**Quality:** Marginal, many studies report but assays poor. N~200. **Summary**: Most studies report LH and TT in normal prepubertal range.[10,91] With improved assays, TT is reported in the bottom quartile in majority;[85] LH to TT ratio is elevated;[14] possibly a low TT peak following stimulation.[92] INSL3 is	**Quality**: Marginal, few studies, N~125 **Summary**: Small studies report INHB and AMH as normal.[10,88,90] Larger study found low INHB in ~1/3 and abnormal AMH (high in ~25%, low in 13%).[40] **Conclusion**: Sertoli cell dysfunction may be present in a subset of boys.

	hylinization occurs in boys nearing puberty.[90] **Conclusion**: Depletion of germ cells occurs throughout childhood; degenerative changes in support cells may be beginning.	reported as normal (n=9).[93] **Conclusion**: Mild defects in leydig cells may be present but difficult to assess prepubertally.	
Puberty			
Quality: Adequate to excellent, many studies with various comparisons. **Summary**: Enlargement in early puberty to max range 3-10 mL.[92] Size plateaus midpuberty then decreases to ~3mL in T4-5 PH.[92] Even in early puberty, testicular size smaller than expected for degree of virilization.[150] **Conclusion**: Testes enlarge to pubertal size in most. Peak testicular size is variable but typically no more than ~8mL before decreasing to 3-5 mL in most by late puberty.	**Quality:** Adequate, case reports and cross sectional studies. **Summary**: Two studies only 6/15 boys in puberty had germ cells in biopsy; none with spermatatids.[90,133] Leydig cell hyperplasia in 9/15, fibrosis of the tubules in 15/15. Sertoli cell degeneration in 6/8.[90] **Conclusion**: Spermatogenesis is altered in all boys with KS; testicular support cells seem to become abnormal as puberty is initiated and fibrosis likely progresses with puberty.	**Quality:** Adequate, many cross-sectional and several longitudinal studies. Variability in TT assays. **Summary**: Median LH elevates by 13-14 years[10,92] and/or T3 PH.[88] TT rises possibly even faster/higher than controls and then plateaus[92] and can decline. ~25% have low TT.[10] LH to TT ratio nearly always high.[88] INSL3 is similar to controls until age 13 then plateaus rather than rising (n=14).[93] **Conclusion**: The majority of boys with KS will have evidence of leydig cell insufficiency by mid- to late puberty.	**Quality**: Marginal, several cross-sectional but rare longitudinal studies. Total N~100. Assay variation. **Summary**: Median FSH elevates by 12-13 years[10,93] and/or T2-3 PH.[88,92] FSH correlates with age.[10] INHB does not increase as expected in puberty,[97] then falls below normal range within a year of pubertal onset.[97] Delayed decline of AMH in early puberty.[84,151] **Conclusion**: The majority of boys with KS will have abnormal sertoli cell biomarkers by early to mid-puberty.
Adulthood			
Quality: Excellent, N>1,000, consistent. **Summary**: Smaller than controls in all.[10] Mean volume 3-3.5mL, range 1-8mL.[10,14] **Conclusions**: Adult men with 47,XXY universally have small testes.	**Quality:** Excellent, however ascertainment bias may be present. **Summary**: Sertoli cell only (SCO) picture most common, scarce patchy areas of germ cells with active spermatogenesis in some (around 50%).[123] Immature and degenerative sertoli cells, hyalinization of the tubules and leydig cell hyperplasia.[86] **Conclusions**: Germ cells are absent or rare; sertoli and leydig cells are abnormal, although normal patches may be present.	**Quality**: Adequate to excellent. **Summary:** LH elevated in 83-96%. [10,14] TT below normal in ~50%, lower half of normal in the rest. TT declines with age.[10] INSL3 is often low. **Conclusion**: Majority will have leydig cell dysfunction, however may be mild in a subset.	**Quality:** Adequate. **Summary:** FSH elevated in all,[10,84,88] however degree of elevation does not predict success or failure of TESE. AMH < -2 SD in 85%.[84] INHB below the lower limit of normal or undetectable in all.[97] **Conclusion:** Biomarkers of sertoli cell function (and germ cells) are nearly universally low in men with KS.

PH = pubic hair, T1-5 = Tanner stage 1-5, N=total number of subjects in the combined studies.

Table 2. Testosterone Formulations[109]

Formulation	Adult Regimen	Pharmacokinetic profile	Advantages	Disadvantages	Adolescent use
T cypionate or enanthate 200 mg/mL	150-200 mg IM every 2 wk or 75-100 mg/wk	Serum T peaks after the injection then gradually declines by the end of the dosing interval	Inexpensive, flexibility in dosing	Requires IM injection; peaks and valleys in serum T	Yes, preferred method when small doses are desired
T gel (1%, 1.62%, 2%)	5-10g daily	Stable levels of serum T can be attained in the range desired. Transdermal absorption may vary	Ease of application, minimizes variability in serum T	Potential skin-to-skin transfer; skin irritation; daily application	Yes, typically start at 1 pump/day and titrate
Transdermal T patch	5-10 mg daily (1-2 patches)	Stable levels of serum T can be attained in the range desired. Transdermal absorption may vary	Ease of application	Skin irritation (more frequent), daily application	Possibly. Lowest dose may be too high for many. Not well tolerated
Buccal bioadhesive T tablets	30mg controlled release, twice daily	Stable levels of serum T can be attained in the range desired. Absorbed from the buccal mucosa	More rapid metabolism, no transfer	Twice daily administration; buccal irritation	No
T pellets	3-6 subcutaneous implanted pellets	Serum T peaks at 1 month then sustained for 3-6 months	Eliminates daily administration, stable levels	Requires surgical incision; pellets may extrude; dose cannot be titrated	No
T nasal gel	11 mg nasally three times daily	Very quick peak and then trough	Ease of application and no transfer to others	Three times daily administration	No
T undecanoate	750 mg IM every 10 weeks	Very stable levels after loading doses	Stable long term levels avoiding peaks and troughs	Large (3mL) volume injection; fat pulmonary emboli	No

Modified from the Endocrine Society Clinical Care Guidelines of Testosterone Therapy in Androgen Deficiency Syndromes[109]

References

1. Gudeman SR, Townsend B, Fischer K, Walters RC, Crain D. Etiology of Azoospermia in a Military Population. *The Journal of urology.* Oct 16 2014.
2. Nakamura Y, Kitamura M, Nishimura K, et al. Chromosomal variants among 1790 infertile men. *International journal of urology : official journal of the Japanese Urological Association.* Feb 2001;8(2):49-52.
3. Klinefelter HF, EC R, Albright F. Syndrome Characterized by Gynecomastia, Aspermatogenesis without Aleydigism, and Increased Excretion of Follicle-Stimulating Hormone. *J Clin Endocrinol.* 1942;2:615-627.
4. Lo JO, Cori DF, Norton ME, Caughey AB. Noninvasive prenatal testing. *Obstetrical & gynecological survey.* Feb 2014;69(2):89-99.
5. Bojesen A, Juul S, Gravholt CH. Prenatal and postnatal prevalence of Klinefelter syndrome: a national registry study. *J Clin Endocrinol Metab.* Feb 2003;88(2):622-626.
6. Coffee B, Keith K, Albizua I, et al. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. *American journal of human genetics.* Oct 2009;85(4):503-514.
7. 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 original article series.* 1990;26(4):209-223.
8. Martin J, Hamilton B, Osterman M, Sally C, Mathews M. Births: Final data for 2013. *National Vital Statistics Reports.* 2/6/2015 2015;64(1).
9. Abramsky L, Chapple J. 47,XXY (Klinefelter syndrome) and 47,XYY: estimated rates of and indication for postnatal diagnosis with implications for prenatal counselling. *Prenatal diagnosis.* Apr 1997;17(4):363-368.
10. Pacenza N, Pasqualini T, Gottlieb S, et al. Clinical Presentation of Klinefelter's Syndrome: Differences According to Age. *International journal of endocrinology.* 2012;2012:324835.
11. Ferguson-Smith MA, Yates JR. Maternal age specific rates for chromosome aberrations and factors influencing them: report of a collaborative european study on 52 965 amniocenteses. *Prenatal diagnosis.* Spring 1984;4 Spec No:5-44.
12. Carothers AD, Filippi G. Klinefelter's syndrome in Sardinia and Scotland. Comparative studies of parental age and other aetiological factors in 47,XXY. *Human genetics.* Dec 1988;81(1):71-75.
13. Herlihy AS, Halliday JL, Cock ML, McLachlan RI. The prevalence and diagnosis rates of Klinefelter syndrome: an Australian comparison. *The Medical journal of Australia.* Jan 3 2011;194(1):24-28.
14. Aksglaede L, Skakkebaek NE, Almstrup K, Juul A. Clinical and biological parameters in 166 boys, adolescents and adults with nonmosaic Klinefelter syndrome: a Copenhagen experience. *Acta paediatrica.* Jun 2011;100(6):793-806.
15. Bardsley MZ, Falkner B, Kowal K, Ross JL. Insulin resistance and metabolic syndrome in prepubertal boys with Klinefelter syndrome. *Acta paediatrica.* Jun 2011;100(6):866-870.
16. Aksglaede L, Molgaard C, Skakkebaek NE, Juul A. Normal bone mineral content but unfavourable muscle/fat ratio in Klinefelter syndrome. *Archives of disease in childhood.* Jan 2008;93(1):30-34.
17. Jiang-Feng M, Hong-Li X, Xue-Yan W, et al. Prevalence and risk factors of diabetes in patients with Klinefelter syndrome: a longitudinal observational study. *Fertility and sterility.* Nov 2012;98(5):1331-1335.
18. Gravholt CH, Jensen AS, Host C, Bojesen A. Body composition, metabolic syndrome and type 2 diabetes in Klinefelter syndrome. *Acta paediatrica.* Jun 2011;100(6):871-877.

19. Bojesen A, Host C, Gravholt CH. Klinefelter's syndrome, type 2 diabetes and the metabolic syndrome: the impact of body composition. *Molecular human reproduction.* Jun 2010;16(6):396-401.
20. Swerdlow AJ, Higgins CD, Schoemaker MJ, Wright AF, Jacobs PA, United Kingdom Clinical Cytogenetics G. Mortality in patients with Klinefelter syndrome in Britain: a cohort study. *J Clin Endocrinol Metab.* Dec 2005;90(12):6516-6522.
21. Ross JL, Roeltgen DP, Stefanatos G, et al. Cognitive and motor development during childhood in boys with Klinefelter syndrome. *American journal of medical genetics. Part A.* Mar 15 2008;146A(6):708-719.
22. Salbenblatt JA, Meyers DC, Bender BG, Linden MG, Robinson A. Gross and fine motor development in 47,XXY and 47,XYY males. *Pediatrics.* Aug 1987;80(2):240-244.
23. Netley C, Rovet J. Verbal deficits in children with 47,XXY and 47,XXX karyotypes: a descriptive and experimental study. *Brain and language.* Sep 1982;17(1):58-72.
24. Walzer S, Bashir AS, Silbert AR. Cognitive and behavioral factors in the learning disabilities of 47,XXY and 47,XYY boys. *Birth defects original article series.* 1990;26(4):45-58.
25. Tartaglia N, Cordeiro L, Howell S, Wilson R, Janusz J. The spectrum of the behavioral phenotype in boys and adolescents 47,XXY (Klinefelter syndrome). *Pediatric endocrinology reviews : PER.* Dec 2010;8 Suppl 1:151-159.
26. Boone KB, Swerdloff RS, Miller BL, et al. Neuropsychological profiles of adults with Klinefelter syndrome. *Journal of the International Neuropsychological Society : JINS.* May 2001;7(4):446-456.
27. Ratcliffe SG, Masera N, Pan H, McKie M. Head circumference and IQ of children with sex chromosome abnormalities. *Developmental medicine and child neurology.* Jun 1994;36(6):533-544.
28. Rovet J, Netley C, Keenan M, Bailey J, Stewart D. The psychoeducational profile of boys with Klinefelter syndrome. *Journal of learning disabilities.* Mar 1996;29(2):180-196.
29. Bender BG, Linden MG, Robinson A. Verbal and spatial processing efficiency in 32 children with sex chromosome abnormalities. *Pediatric research.* Jun 1989;25(6):577-579.
30. Ross JL, Zeger MP, Kushner H, Zinn AR, Roeltgen DP. An extra X or Y chromosome: contrasting the cognitive and motor phenotypes in childhood in boys with 47,XYY syndrome or 47,XXY Klinefelter syndrome. *Developmental disabilities research reviews.* 2009;15(4):309-317.
31. Boada R, Janusz J, Hutaff-Lee C, Tartaglia N. The cognitive phenotype in Klinefelter syndrome: a review of the literature including genetic and hormonal factors. *Developmental disabilities research reviews.* 2009;15(4):284-294.
32. Fruhmesser A, Kotzot D. Chromosomal variants in klinefelter syndrome. *Sexual development : genetics, molecular biology, evolution, endocrinology, embryology, and pathology of sex determination and differentiation.* 2011;5(3):109-123.
33. Tartaglia N, Ayari N, Howell S, D'Epagnier C, Zeitler P. 48,XXYY, 48,XXXY and 49,XXXXY syndromes: not just variants of Klinefelter syndrome. *Acta paediatrica.* Jun 2011;100(6):851-860.
34. Tartaglia N, Davis S, Hench A, et al. A new look at XXYY syndrome: medical and psychological features. *American journal of medical genetics. Part A.* Jun 15 2008;146A(12):1509-1522.
35. Samplaski MK, Lo KC, Grober ED, Millar A, Dimitromanolakis A, Jarvi KA. Phenotypic differences in mosaic Klinefelter patients as compared with non-mosaic Klinefelter patients. *Fertility and sterility.* Apr 2014;101(4):950-955.

36. Chang S, Skakkebaek A, Trolle C, et al. Anthropometry in Klinefelter Syndrome - Multifactorial Influences Due to CAG Length, Testosterone Treatment and Possibly Intrauterine Hypogonadism. *J Clin Endocrinol Metab.* Mar 2015;100(3):E508-517.
37. Bojesen A, Kristensen K, Birkebaek NH, et al. The metabolic syndrome is frequent in Klinefelter's syndrome and is associated with abdominal obesity and hypogonadism. *Diabetes care.* Jul 2006;29(7):1591-1598.
38. Patwardhan AJ, Eliez S, Bender B, Linden MG, Reiss AL. Brain morphology in Klinefelter syndrome: extra X chromosome and testosterone supplementation. *Neurology.* Jun 27 2000;54(12):2218-2223.
39. Bizzarro A, Valentini G, Di Martino G, DaPonte A, De Bellis A, Iacono G. Influence of testosterone therapy on clinical and immunological features of autoimmune diseases associated with Klinefelter's syndrome. *J Clin Endocrinol Metab.* Jan 1987;64(1):32-36.
40. Davis S, Lahlou N, Bardsley MZ, Temple M, Kowal K, Ross JL. Longitudinal Study of Boys with Klinefelter Syndrome Gives Evidence of Prepubertal Testis Defect. The Endocrine Society; 2015; San Diego, CA.
41. Davis S, Lahlou N, Bardsley MZ, Temple M, Kowal K, Ross JL. Longitudinal Gonadal Function Biomarkers of Puberty Onset in Klinefelter Syndrome. Pediatric Academic Society; 2015; San Diego, CA.
42. Bardsley MZ, Kowal K, Gamber R, Lahlou N, Ross JL. Androgen Replacement in Boys with 47,XXY Klinefelter syndrome: Influence on the Testicular Phenotype. Endocrine Society; 2014; Chicago, IL.
43. Chen X, Williams-Burris SM, McClusky R, et al. The Sex Chromosome Trisomy mouse model of XXY and XYY: metabolism and motor performance. *Biology of sex differences.* 2013;4(1):15.
44. Lue YH, Wang C, Liu PY, Erkilla K, Swerdloff RS. Insights into the pathogenesis of XXY phenotype from comparison of the clinical syndrome with an experimental XXY mouse model. *Pediatric endocrinology reviews : PER.* Dec 2010;8 Suppl 1:140-144.
45. Bojesen A, Hertz JM, Gravholt CH. Genotype and phenotype in Klinefelter syndrome - impact of androgen receptor polymorphism and skewed X inactivation. *International journal of andrology.* Dec 2011;34(6 Pt 2):e642-648.
46. 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. *The Journal of clinical endocrinology and metabolism.* Sep 2005;90(9):5041-5046.
47. 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.* Dec 2004;89(12):6208-6217.
48. Huang J, Zhang L, Deng H, Chang L, Liu Q, Liu P. Global transcriptome analysis of peripheral blood identifies the most significantly down-regulated genes associated with metabolism regulation in Klinefelter syndrome. *Molecular reproduction and development.* Jan 2015;82(1):17-25.
49. D'Aurora M, Ferlin A, Di Nicola M, et al. Deregulation of sertoli and leydig cells function in patients with klinefelter syndrome as evidenced by testis transcriptome analysis. *BMC genomics.* 2015;16:1356.
50. Liu N, Qiao Y, Cai C, et al. A sperm component, HSD-3.8 (SPAG1), interacts with G-protein beta 1 subunit and activates extracellular signal-regulated kinases (ERK). *Frontiers in bioscience : a journal and virtual library.* 2006;11:1679-1689.

51. Viana J, Pidsley R, Troakes C, et al. Epigenomic and transcriptomic signatures of a Klinefelter syndrome (47,XXY) karyotype in the brain. *Epigenetics : official journal of the DNA Methylation Society.* Apr 2014;9(4):587-599.
52. Zitzmann M, Bongers R, Werler S, et al. Gene expression patterns in relation to the clinical phenotype in klinefelter syndrome. *J Clin Endocrinol Metab.* Mar 2015;100(3):E518-523.
53. Fennoy I. Testosterone and the child (0-12 years) with Klinefelter syndrome (47XXY): a review. *Acta paediatrica.* Jun 2011;100(6):846-850.
54. Lahlou N, Fennoy I, Ross JL, Bouvattier C, Roger M. Clinical and hormonal status of infants with nonmosaic XXY karyotype. *Acta paediatrica.* Jun 2011;100(6):824-829.
55. Coerdt W, Rehder H, Gausmann I, Johannisson R, Gropp A. Quantitative histology of human fetal testes in chromosomal disease. *Pediatric pathology / affiliated with the International Paediatric Pathology Association.* 1985;3(2-4):245-259.
56. Murken JD, Stengel-Rutkowski S, Walther JU, Westenfelder SR, Remberger KH, Zimmer F. Letter: Klinefelter's syndrome in a fetus. *Lancet.* Jul 20 1974;2(7873):171.
57. Flannery DB, Brown JA, Redwine FO, Winter P, Nance WE. Antenatally detected Klinefelter's syndrome in twins. *Acta geneticae medicae et gemellologiae.* 1984;33(1):51-56.
58. Gustavson KH, Kjessler B, Thoren S. Prenatal diagnosis of an XXY foetal karyotype in a woman with a previous 21-trisomic child. *Clinical genetics.* Jun 1978;13(6):477-480.
59. Autio-Harmainen H, Rapola J, Aula P. Fetal gonadal histology in XXXXY, XYY and XXX syndromes. *Clinical genetics.* Jul 1980;18(1):1-5.
60. P C, J A. Histology of the testis in XXY fetuses. *Prenatal diagnosis.* 1979:336-337.
61. Jequier AM, Bullimore NJ. Testicular and epididymal histology in a fetus with Klinefelter's syndrome at 22 weeks' gestation. *British journal of urology.* Feb 1989;63(2):214-215.
62. Ratcliffe SG, Read G, Pan H, Fear C, Lindenbaum R, Crossley J. Prenatal testosterone levels in XXY and XYY males. *Hormone research.* 1994;42(3):106-109.
63. Kunzig HJ, Meyer U, Schmitz-Roeckerath B, Broer KH. Influence of fetal sex on the concentration of amniotic fluid testosterone: antenatal sex determination? *Archiv fur Gynakologie.* Sep 30 1977;223(2):75-84.
64. Carson DJ, Okuno A, Lee PA, Stetten G, Didolkar SM, Migeon CJ. Amniotic fluid steroid levels. Fetuses with adrenal hyperplasia, 46,XXY fetuses, and normal fetuses. *American journal of diseases of children.* Mar 1982;136(3):218-222.
65. Abeliovich D, Leiberman JR, Teuerstein I, Levy J. Prenatal sex diagnosis: testosterone and FSH levels in mid-trimester amniotic fluids. *Prenatal diagnosis.* Sep-Oct 1984;4(5):347-353.
66. Mean F, Pescia G, Vajda D, Felber JP, Magrini G. Amniotic fluid testosterone in prenatal sex determination. *Journal de genetique humaine.* Dec 1981;29(4):441-447.
67. Sorensen K, Nielsen J, Wohlert M, Bennett P, Johnsen SG. Serum testosterone of boys with karyotype 47,XXY (Klinefelter's syndrome) at birth. *Lancet.* Nov 14 1981;2(8255):1112-1113.
68. Moal V, Mathieu E, Reynier P, Malthiery Y, Gallois Y. Low serum testosterone assayed by liquid chromatography-tandem mass spectrometry. Comparison with five immunoassay techniques. *Clinica chimica acta; international journal of clinical chemistry.* Nov-Dec 2007;386(1-2):12-19.
69. Ivell R, Heng K, Anand-Ivell R. Insulin-Like Factor 3 and the HPG Axis in the Male. *Frontiers in endocrinology.* 2014;5:6.
70. Boas M, Boisen KA, Virtanen HE, et al. Postnatal penile length and growth rate correlate to serum testosterone levels: a longitudinal study of 1962 normal boys. *European journal of endocrinology / European Federation of Endocrine Societies.* Jan 2006;154(1):125-129.

71. Ratcliffe SG, Murray L, Teague P. Edinburgh study of growth and development of children with sex chromosome abnormalities. III. *Birth defects original article series.* 1986;22(3):73-118.
72. Stewart DA, Bailey JD, Netley CT, et al. Growth and development of children with X and Y chromosome aneuploidy from infancy to pubertal age: the Toronto study. *Birth defects original article series.* 1982;18(4):99-154.
73. Ross JL, Samango-Sprouse C, Lahlou N, Kowal K, Elder FF, Zinn A. Early androgen deficiency in infants and young boys with 47,XXY Klinefelter syndrome. *Hormone research.* 2005;64(1):39-45.
74. Ratcliffe SH. Development of children with sex chromosome abnormalities. *Proceedings of the Royal Society of Medicine.* Mar 1976;69(3):189-191.
75. Aksglaede L, Petersen JH, Main KM, Skakkebaek NE, Juul A. High normal testosterone levels in infants with non-mosaic Klinefelter's syndrome. *European journal of endocrinology / European Federation of Endocrine Societies.* Sep 2007;157(3):345-350.
76. Muller J, Skakkebaek NE, Ratcliffe SG. Quantified testicular histology in boys with sex chromosome abnormalities. *International journal of andrology.* Apr 1995;18(2):57-62.
77. Edlow JB, Shapiro LR, Hsu LY, Hirschhorn K. Neonatal Klinefelter's syndrome. *American journal of diseases of children.* Nov 1969;118(5):788-791.
78. Mikamo K, Aguercif M, Hazeghi P, Martin-Du Pan R. Chromatin-positive Klinefelter's syndrome. A quantitative analysis of spermatogonial deficiency at 3, 4, and 12 months of age. *Fertility and sterility.* Sep-Oct 1968;19(5):731-739.
79. Ratcliffe SG. The sexual development of boys with the chromosome constitution 47,XXY (Klinefelter's syndrome). *Clinics in endocrinology and metabolism.* Nov 1982;11(3):703-716.
80. Cabrol S, Ross JL, Fennoy I, Bouvattier C, Roger M, Lahlou N. Assessment of Leydig and Sertoli cell functions in infants with nonmosaic Klinefelter syndrome: insulin-like peptide 3 levels are normal and positively correlated with LH levels. *The Journal of clinical endocrinology and metabolism.* Apr 2011;96(4):E746-753.
81. Lahlou N, Fennoy I, Carel JC, Roger M. Inhibin B and anti-Mullerian hormone, but not testosterone levels, are normal in infants with nonmosaic Klinefelter syndrome. *J Clin Endocrinol Metab.* Apr 2004;89(4):1864-1868.
82. Forest MG, Sizonenko PC, Cathiard AM, Bertrand J. Hypophyso-gonadal function in humans during the first year of life. 1. Evidence for testicular activity in early infancy. *The Journal of clinical investigation.* Mar 1974;53(3):819-828.
83. Rey RA. Mini-puberty and true puberty: differences in testicular function. *Annales d'endocrinologie.* May 2014;75(2):58-63.
84. Aksglaede L, Christiansen P, Sorensen K, et al. Serum concentrations of Anti-Mullerian Hormone (AMH) in 95 patients with Klinefelter syndrome with or without cryptorchidism. *Acta paediatrica.* Jun 2011;100(6):839-845.
85. Zeger MP, Zinn AR, Lahlou N, et al. Effect of ascertainment and genetic features on the phenotype of Klinefelter syndrome. *J Pediatr.* May 2008;152(5):716-722.
86. Aksglaede L, Wikstrom AM, Rajpert-De Meyts E, Dunkel L, Skakkebaek NE, Juul A. Natural history of seminiferous tubule degeneration in Klinefelter syndrome. *Human reproduction update.* Jan-Feb 2006;12(1):39-48.
87. Ferguson-Smith MA. The prepubertal testicular lesion in chromatin-positive Klinefelter's syndrome (primary micro-orchidism) as seen in mentally handicapped children. *Lancet.* Jan 31 1959;1(7066):219-222.
88. Bastida MG, Rey RA, Bergada I, et al. Establishment of testicular endocrine function impairment during childhood and puberty in boys with Klinefelter syndrome. *Clinical endocrinology.* Dec 2007;67(6):863-870.

89. Grinspon RP, Loreti N, Braslavsky D, et al. Sertoli cell markers in the diagnosis of paediatric male hypogonadism. *Journal of pediatric endocrinology & metabolism : JPEM.* 2012;25(1-2):3-11.
90. 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.* May 2004;89(5):2263-2270.
91. Topper E, Dickerman Z, Prager-Lewin R, Kaufman H, Maimon Z, Laron Z. Puberty in 24 patients with Klinefelter syndrome. *Eur J Pediatr.* Sep 1982;139(1):8-12.
92. Salbenblatt JA, Bender BG, Puck MH, Robinson A, Faiman C, Winter JS. Pituitary-gonadal function in Klinefelter syndrome before and during puberty. *Pediatric research.* Jan 1985;19(1):82-86.
93. Wikstrom AM, Bay K, Hero M, Andersson AM, Dunkel L. Serum insulin-like factor 3 levels during puberty in healthy boys and boys with Klinefelter syndrome. *The Journal of clinical endocrinology and metabolism.* Nov 2006;91(11):4705-4708.
94. Aksglaede L, Sorensen K, Boas M, et al. Changes in anti-Mullerian hormone (AMH) throughout the life span: a population-based study of 1027 healthy males from birth (cord blood) to the age of 69 years. *J Clin Endocrinol Metab.* Dec 2010;95(12):5357-5364.
95. Fullerton G, Hamilton M, Maheshwari A. Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009? *Human reproduction (Oxford, England).* Mar 2010;25(3):588-597.
96. Sciurano RB, Luna Hisano CV, Rahn MI, et al. Focal spermatogenesis originates in euploid germ cells in classical Klinefelter patients. *Human reproduction (Oxford, England).* Sep 2009;24(9):2353-2360.
97. Christiansen P, Andersson AM, Skakkebaek NE. Longitudinal studies of inhibin B levels in boys and young adults with Klinefelter syndrome. *J Clin Endocrinol Metab.* Feb 2003;88(2):888-891.
98. Overvad S, Bay K, Bojesen A, Gravholt CH. Low INSL3 in Klinefelter syndrome is related to osteocalcin, testosterone treatment and body composition, as well as measures of the hypothalamic-pituitary-gonadal axis. *Andrology.* May 2014;2(3):421-427.
99. Tuttelmann F, Damm OS, Luetjens CM, et al. Intratesticular testosterone is increased in men with Klinefelter syndrome and may not be released into the bloodstream owing to altered testicular vascularization- a preliminary report. *Andrology.* Mar 2014;2(2):275-281.
100. Wosnitzer MS, Paduch DA. Endocrinological issues and hormonal manipulation in children and men with Klinefelter syndrome. *American journal of medical genetics. Part C, Seminars in medical genetics.* Feb 15 2013;163C(1):16-26.
101. Samango-Sprouse CA, Sadeghin T, Mitchell FL, et al. Positive effects of short course androgen therapy on the neurodevelopmental outcome in boys with 47,XXY syndrome at 36 and 72 months of age. *American journal of medical genetics. Part A.* Mar 2013;161A(3):501-508.
102. Winter JS, Faiman C, Hobson WC, Prasad AV, Reyes FI. Pituitary-gonadal relations in infancy. I. Patterns of serum gonadotropin concentrations from birth to four years of age in man and chimpanzee. *J Clin Endocrinol Metab.* Apr 1975;40(4):545-551.
103. Chada M, Prusa R, Bronsky J, et al. Inhibin B, follicle stimulating hormone, luteinizing hormone and testosterone during childhood and puberty in males: changes in serum concentrations in relation to age and stage of puberty. *Physiological research / Academia Scientiarum Bohemoslovaca.* 2003;52(1):45-51.
104. American Diabetes A, American Psychiatric A, American Association of Clinical E, North American Association for the Study of O. Consensus development conference on

antipsychotic drugs and obesity and diabetes. *The Journal of clinical psychiatry.* Feb 2004;65(2):267-272.

105. Barlow SE, Expert C. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. *Pediatrics.* Dec 2007;120 Suppl 4:S164-192.
106. Rogol AD, Tartaglia N. Considerations for androgen therapy in children and adolescents with Klinefelter syndrome (47, XXY). *Pediatric endocrinology reviews : PER.* Dec 2010;8 Suppl 1:145-150.
107. Radicioni AF, Ferlin A, Balercia G, et al. Consensus statement on diagnosis and clinical management of Klinefelter syndrome. *Journal of endocrinological investigation.* Dec 2010;33(11):839-850.
108. Groth KA, Skakkebaek A, Host C, Gravholt CH, Bojesen A. Clinical review: Klinefelter syndrome--a clinical update. *J Clin Endocrinol Metab.* Jan 2013;98(1):20-30.
109. Bhasin S, Cunningham GR, Hayes FJ, et al. Testosterone therapy in men with androgen deficiency syndromes: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* Jun 2010;95(6):2536-2559.
110. Samplaski MK, Loai Y, Wong K, Lo KC, Grober ED, Jarvi KA. Testosterone use in the male infertility population: prescribing patterns and effects on semen and hormonal parameters. *Fertility and sterility.* Jan 2014;101(1):64-69.
111. Forti G, Vannucchi PL, Borghi A, Giusti G, Fusi S, Serio M. Effects of pharmacological doses of testosterone and dihydrotestosterone on the hypothalamic-pituitary axis function of Klinefelter patients. *Journal of endocrinological investigation.* Aug 1983;6(4):297-300.
112. Liu PY, Swerdloff RS, Christenson PD, Handelsman DJ, Wang C, Hormonal Male Contraception Summit G. Rate, extent, and modifiers of spermatogenic recovery after hormonal male contraception: an integrated analysis. *Lancet.* Apr 29 2006;367(9520):1412-1420.
113. Plotton I, d'Estaing SG, 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.* Mar 2015;100(3):961-967.
114. Ramasamy R, Ricci JA, Palermo GD, Gosden LV, Rosenwaks Z, Schlegel PN. Successful fertility treatment for Klinefelter's syndrome. *The Journal of urology.* Sep 2009;182(3):1108-1113.
115. Schiff JD, Palermo GD, Veeck LL, Goldstein M, Rosenwaks Z, Schlegel PN. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab.* Nov 2005;90(11):6263-6267.
116. Wang C, Cunningham G, Dobs A, et al. Long-term testosterone gel (AndroGel) treatment maintains beneficial effects on sexual function and mood, lean and fat mass, and bone mineral density in hypogonadal men. *J Clin Endocrinol Metab.* May 2004;89(5):2085-2098.
117. Nielsen J, Pelsen B, Sorensen K. Follow-up of 30 Klinefelter males treated with testosterone. *Clinical genetics.* Apr 1988;33(4):262-269.
118. Wang C, Eyre DR, Clark R, et al. Sublingual testosterone replacement improves muscle mass and strength, decreases bone resorption, and increases bone formation markers in hypogonadal men--a clinical research center study. *J Clin Endocrinol Metab.* Oct 1996;81(10):3654-3662.
119. Wang C, Swerdloff RS, Iranmanesh A, et al. Transdermal testosterone gel improves sexual function, mood, muscle strength, and body composition parameters in hypogonadal men. *J Clin Endocrinol Metab.* Aug 2000;85(8):2839-2853.
120. Van Assche E, Bonduelle M, Tournaye H, et al. Cytogenetics of infertile men. *Human reproduction (Oxford, England).* Dec 1996;11 Suppl 4:1-24; discussion 25-26.

121. Lanfranco F, Kamischke A, Zitzmann M, Nieschlag E. Klinefelter's syndrome. *Lancet.* Jul 17-23 2004;364(9430):273-283.
122. 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. *Fertility and sterility.* Oct 2013;100(4):970-974.
123. Yamamoto Y, Sofikitis N, Mio Y, Loutradis D, Kaponis A, Miyagawa I. Morphometric and cytogenetic characteristics of testicular germ cells and Sertoli cell secretory function in men with non-mosaic Klinefelter's syndrome. *Human reproduction (Oxford, England).* Apr 2002;17(4):886-896.
124. Friedler S, Raziel A, Strassburger D, Schachter M, Bern O, Ron-El R. Outcome of ICSI using fresh and cryopreserved-thawed testicular spermatozoa in patients with non-mosaic Klinefelter's syndrome. *Human reproduction (Oxford, England).* Dec 2001;16(12):2616-2620.
125. Bakircioglu ME, Ulug U, Erden HF, et al. Klinefelter syndrome: does it confer a bad prognosis in treatment of nonobstructive azoospermia? *Fertility and sterility.* Apr 2011;95(5):1696-1699.
126. Plotton I, Brosse A, Cuzin B, Lejeune H. Klinefelter syndrome and TESE-ICSI. *Annales d'endocrinologie.* May 2014;75(2):118-125.
127. Deruyver Y, Vanderschueren D, Van der Aa F. Outcome of microdissection TESE compared with conventional TESE in non-obstructive azoospermia: a systematic review. *Andrology.* Jan 2014;2(1):20-24.
128. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Human reproduction (Oxford, England).* Jan 1999;14(1):131-135.
129. Okada H, Dobashi M, Yamazaki T, et al. Conventional versus microdissection testicular sperm extraction for nonobstructive azoospermia. *The Journal of urology.* Sep 2002;168(3):1063-1067.
130. Vernaeve V, Staessen C, Verheyen G, Van Steirteghem A, Devroey P, Tournaye H. Can biological or clinical parameters predict testicular sperm recovery in 47,XXY Klinefelter's syndrome patients? *Human reproduction (Oxford, England).* May 2004;19(5):1135-1139.
131. Gies I, De Schepper J, Van Saen D, Anckaert E, Goossens E, Tournaye H. 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. *Human reproduction (Oxford, England).* Apr 2012;27(4):998-1004.
132. Sabbaghian M, Modarresi T, Hosseinifar H, et al. Comparison of sperm retrieval and intracytoplasmic sperm injection outcome in patients with and without Klinefelter syndrome. *Urology.* Jan 2014;83(1):107-110.
133. Van Saen D, Gies I, De Schepper J, Tournaye H, Goossens E. Can pubertal boys with Klinefelter syndrome benefit from spermatogonial stem cell banking? *Human reproduction (Oxford, England).* Feb 2012;27(2):323-330.
134. Aksglaede L, Jorgensen N, Skakkebaek NE, Juul A. Low semen volume in 47 adolescents and adults with 47,XXY Klinefelter or 46,XX male syndrome. *International journal of andrology.* Aug 2009;32(4):376-384.
135. Madureira C, Cunha M, Sousa M, et al. Treatment by testicular sperm extraction and intracytoplasmic sperm injection of 65 azoospermic patients with non-mosaic Klinefelter syndrome with birth of 17 healthy children. *Andrology.* Jul 2014;2(4):623-631.
136. Ramasamy R, Ricci JA, Leung RA, Schlegel PN. Successful repeat microdissection testicular sperm extraction in men with nonobstructive azoospermia. *The Journal of urology.* Mar 2011;185(3):1027-1031.

137. Chehab M, Madala A, Trussell JC. On-label and off-label drugs used in the treatment of male infertility. *Fertility and sterility.* Mar 2015;103(3):595-604.
138. Hussein A, Ozgok Y, Ross L, Rao P, Niederberger C. Optimization of spermatogenesis-regulating hormones in patients with non-obstructive azoospermia and its impact on sperm retrieval: a multicentre study. *BJU international.* Mar 2013;111(3 Pt B):E110-114.
139. Shiraishi K, Ohmi C, Shimabukuro T, Matsuyama H. Human chorionic gonadotrophin treatment prior to microdissection testicular sperm extraction in non-obstructive azoospermia. *Human reproduction (Oxford, England).* Feb 2012;27(2):331-339.
140. Hussein A, Ozgok Y, Ross L, Niederberger C. Clomiphene administration for cases of nonobstructive azoospermia: a multicenter study. *Journal of andrology.* Nov-Dec 2005;26(6):787-791; discussion 792-783.
141. Adamopoulos DA, Loraine JA, Ismail AA, Foss GL. Endocrinological studies in patients with Klinefelter's syndrome treated with clomiphene. *Journal of reproduction and fertility.* Jun 1971;25(3):409-416.
142. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. *The Journal of urology.* Feb 2002;167(2 Pt 1):624-629.
143. Schlegel PN. Aromatase inhibitors for male infertility. *Fertility and sterility.* Dec 2012;98(6):1359-1362.
144. Patry G, Jarvi K, Grober ED, Lo KC. Use of the aromatase inhibitor letrozole to treat male infertility. *Fertility and sterility.* Aug 2009;92(2):829 e821-822.
145. Reifsnyder JE, Ramasamy R, Husseini J, Schlegel PN. Role of optimizing testosterone before microdissection testicular sperm extraction in men with nonobstructive azoospermia. *The Journal of urology.* Aug 2012;188(2):532-536.
146. Ferlin A, Garolla A, Foresta C. Chromosome abnormalities in sperm of individuals with constitutional sex chromosomal abnormalities. *Cytogenetic and genome research.* 2005;111(3-4):310-316.
147. Staessen C, Tournaye H, Van Assche E, et al. PGD in 47,XXY Klinefelter's syndrome patients. *Human reproduction update.* Jul-Aug 2003;9(4):319-330.
148. Mroz K, Hassold TJ, Hunt PA. Meiotic aneuploidy in the XXY mouse: evidence that a compromised testicular environment increases the incidence of meiotic errors. *Human reproduction (Oxford, England).* May 1999;14(5):1151-1156.
149. Gies I, De Schepper J, Goossens E, Van Saen D, Pennings G, Tournaye H. Spermatogonial stem cell preservation in boys with Klinefelter syndrome: to bank or not to bank, that's the question. *Fertility and sterility.* Aug 2012;98(2):284-289.
150. 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. *Pediatric research.* Jun 2006;59(6):854-859.
151. Wikstrom AM, Hoei-Hansen CE, Dunkel L, Rajpert-De Meyts E. Immunoexpression of androgen receptor and nine markers of maturation in the testes of adolescent boys with Klinefelter syndrome: evidence for degeneration of germ cells at the onset of meiosis. *J Clin Endocrinol Metab.* Feb 2007;92(2):714-719.