Klinefelter's syndrome

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Klinefelter's syndrome is the most common genetic cause of human male infertility, but many cases remain undiagnosed because of substantial variation in clinical presentation and insufficient professional awareness of the syndrome itself. Early recognition and hormonal treatment of the disorder can substantially improve quality of life and prevent serious consequences. Testosterone replacement corrects symptoms of androgen deficiency but has no positive effect on infertility. However, nowadays patients with Klinefelter's syndrome, including the non-mosaic type, need no longer be considered irrevocably infertile, because intracytoplasmic sperm injection offers an opportunity for procreation even when there are no spermatozoa in the ejaculate. In a substantial number of azoospermic patients, spermatozoa can be extracted from testicular biopsy samples, and pregnancies and livebirths have been achieved. The frequency of sex chromosomal hyperploidy and autosomal aneuploidies is higher in spermatozoa from patients with Klinefelter's syndrome than in those from normal men. Thus, chromosomal errors might in some cases be transmitted to the offspring of men with this syndrome. The genetic implications of the fertilisation procedures, including pretransfer or prenatal genetic assessment, must be explained to patients and their partners.

Klinefelter's syndrome was first described in 1942 as an endocrine disorder characterised by small firm testes, gynaecomastia, hypogonadism, and higher than normal concentrations of follicle-stimulating hormone (FSH).[1] With a reported prevalence of 0·1–0·2% in the general population and of up to 3·1% in the infertile male population,[2–6] the syndrome is the most common form of male hypogonadism and of chromosome aneuploidy in human beings. Among 15 600 patients referred to our andrology clinic during the past 25 years, there have been 278 affected by Klinefelter's syndrome (1·8%).

About 80% of cases are due to the congenital numerical chromosome aberration 47,XXY; the remaining 20% have higher-grade chromosome aneuploidies (48,XXXY; 48,XXYY; 49,XXXXY), 46,XY/47,XXY mosaicism, or structurally abnormal X chromosomes.[7–9] The true prevalence of mosaic forms might be underestimated because chromosomal mosaicism can be present only in the testes, and the karyotype of peripheral leucocytes is normal.[10]

Many patients with Klinefelter's syndrome remain undiagnosed. Abramsky and Chapple[11] calculated that 10% of expected cases were identified prenatally and 26% were diagnosed in childhood or adult life because of hypogonadism, gynaecomastia, or infertility, leaving 64% undiagnosed. A large Danish national registry study confirms that Klinefelter's syndrome is widely underdiagnosed, with less than 10% of the expected diagnoses made before the age of puberty.[6]

Pathogenesis

The numerical chromosome aberrations in Klinefelter's syndrome arise by non-disjunction either during meiotic divisions occurring in germ-cell development or in early embryonic mitotic cell divisions. Incorrect meiotic divisions are predominant. In autosomal trisomies, paternal non-disjunctions account for only about 10% of all cases; however, paternal errors contribute more frequently to sex-chromosome aneuploidy. The 47,XXY condition has been extensively studied, and about half of all cases are paternally derived.[12] In theory, the errors by which maternal and paternal XXYs can arise differ substantially in nature. Maternal XXY can be caused by an error during meiosis I (MI) or meiosis II (MII), or through an error at an early mitotic division in the developing zygote. Errors at MI seem to be the most common source of maternal non-disjunction. By contrast, XXY of paternal origin can arise only by an MI error, since errors at MII or during an early cleavage division result in 47,XXX or 47,XYY conceptuses, not in 47,XXY (figure 1).[12] The relation between parental age and these different cytogenetic origins of 47, XXY is complex. Eskenazi and colleagues[13] found that men who have recently fathered a child with trisomy produce higher frequencies of aneuploid sperm, with an increase in XY sperm per year of age. Among maternally derived cases, there is a reported association

Search strategy and selection criteria

A computer-aided search of PubMed was done with the terms "Klinefelter('s) syndrome", "hypogonadism", "XXY", "male infertility", "reproduction", "ICSI", "TESE", and "fertility". For the sections on fertility and genetic counselling, publications from the past 8 years were selected, whereas frequently referenced landmark and highly regarded older publications were included for the earlier sections. References cited in retrieved articles and reviewed articles collected over 35 years were searched. Book chapters and systematic reviews were also included because they provide a more extensive overview. New data presented at international scientific meetings and clinical findings during the past 25 years in our andrology clinic were summarised. Our original data derive from the initial visits of a large group of patients with Klinefelter's syndrome. Only men not receiving testosterone replacement therapy at that time were included.

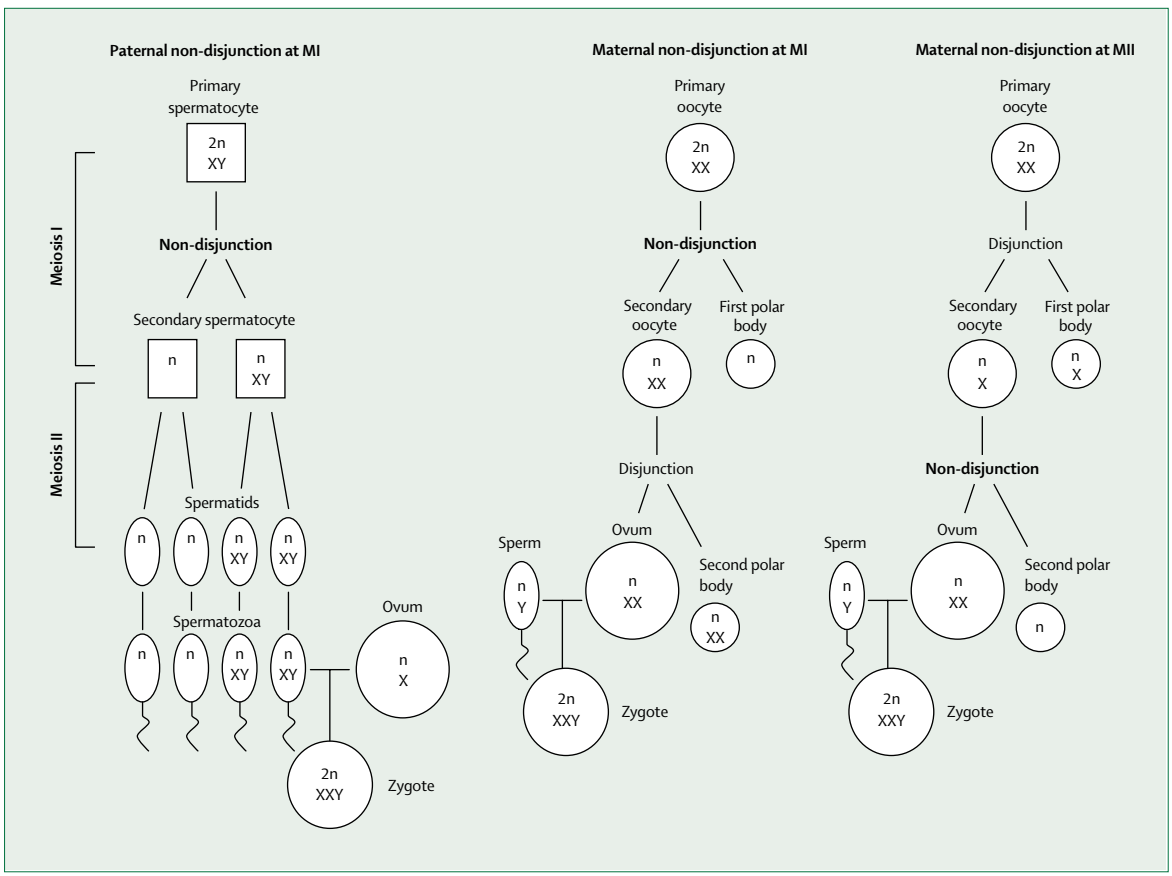

Figure 1: **Different forms of non-disjunction leading to the 47,XXY karyotype of Klinefelter's syndrome**

with increasing maternal age. This effect is limited to the subset of cases originating at MI; those originating at maternal MII appear to be independent of maternal age.[14] By contrast with that study and others,[15–17] we found no increased risk of conception of an affected child at advanced paternal or maternal age (figure 2).[9] Our findings accord with the evidence that sperm aneuploidies do not increase with age.[18,19]

Although Klinefelter's syndrome was described in 1942, and the XXY karyotype was demonstrated in 1959,[20] the underlying molecular mechanisms remain unknown. In animals, the presence of an extra X chromosome predestines the germ cells to a shortened lifespan. In various animal species and in human beings, a normal complement of primordial germ cells is present in the fetal testes of XXY males, but these cells degenerate at an accelerated rate during childhood.[21,22] Whether the defect in the XXY testis is intrinsic to germ cells or is due to the inability of the Sertoli cells to support normal germ-cell development is not known. Hunt and co-workers[23] showed that prenatal germ-cell proliferation is impaired in vivo, but not in vitro, which suggests a communication defect between Sertoli cells and germ cells in the differentiating XXY testis.

Because patients with Klinefelter's syndrome are not a homogeneous group, the karyotype of the lymphocyte lineage does not predict the chromosomal constitution of testis cells or the presence or absence of spermatogenesis.[24] The finding of testicular mosaicism in patients with the XXY karyotype in lymphocytes has high prognostic value for spermatogenesis.[25] Oligozoospermia has been reported in 46,XY/47,XXY mosaicism, and meiotic studies have shown various abnormalities: arrest of meiosis at primary spermatocyte or spermatid stages and foci of normal spermatogenesis in a few seminiferous tubules.[7,26–28] In patients with mosaicism, only 46,XY germ cells were assumed to be able to complete meiosis. However, since 1969, there have been suggestions that 47,XXY germ cells can go through meiosis and produce spermatozoa.[27,29,30] This hypothesis arises from results obtained by sperm karyotyping and, more recently, by DNA in-situ hybridisation, which allows rapid identification of spermatozoa with specific chromosomal aberrations.[30] These studies suggest that the frequency of 24,XY and 24,XX hyperhaploid spermatozoa is substantially higher than would be expected in patients with Klinefelter's syndrome. All of these studies were in patients with peripheral 46,XY/47,XXY mosaicism, and the low

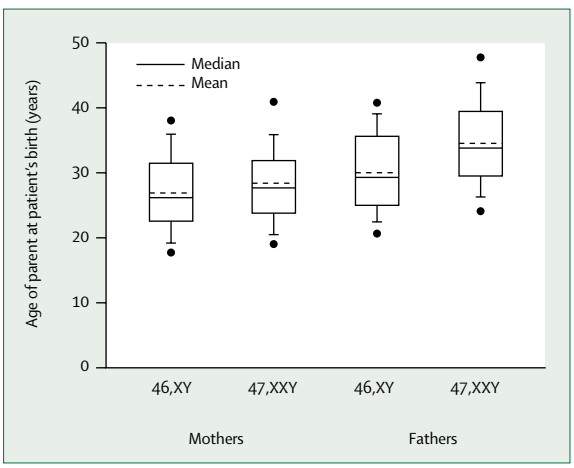

Figure 2: **Ages of parents at patient's birth for 228 patients with Klinefelter's syndrome (47,XXY) and 224 patients with normal karyotype (46,XY) referred to our andrology clinic for infertility or hypogonadism**
Points indicate data outside the 5th or 95th centile. Boxes indicate IQR, and error bars 5th to 95th centiles. Clinical features of the patients with normal karyotype were reported elsewhere.[9]

proportion of spermatozoa showing numerical sex-chromosomal abnormalities (about 3%) suggests that few 47,XXY germ cells are able to complete meiosis. Foresta and colleagues[7,31] have confirmed this hypothesis, demonstrating also that in patients with non-mosaic forms germ cells can undergo and complete the mitotic and meiotic processes.

The US National Institutes of Health recently sponsored a meeting on the topic of variations in phenotypes in Klinefelter's syndrome, which identified new research directions; in particular, the role of androgens and the X-linked androgen receptor should be elucidated.[32] The androgen receptor carries the CAG repeat (CAGn) polymorphism, the length of which is inversely associated with androgen action[33] and might thus contribute to the variation of phenotypes. In Klinefelter's syndrome the situation is complicated by the presence of at least two androgen-receptor alleles undergoing X-inactivation, whereby one of them becomes inactive in every cell. In women, a relation of CAGn length to polycystic ovary syndrome and a skewed inactivation of androgen-receptor genes with preferential expression of longer CAGn alleles have been suggested.[34]

We investigated the association of CAGn alleles with morphological and clinical traits in 77 patients with newly diagnosed and untreated 47,XXY Klinefelter's syndrome. Digestion of leucocyte DNA by methylation-sensitive *Hpa*II (after established procedures of X-chromosome inactivation analysis[34,35]) revealed significant skewing: the shorter CAGn allele was preferentially inactive. CAGn length was positively associated with height and the presence of gynaecomastia. A higher proportion of patients with short CAGn than of those with longer CAGn were in stable relationships. Thus, a significant modulation of androgen effects on the phenotype of Klinefelter's syndrome seems to be exerted via the CAGn polymorphism. This finding is aggravated by skewed inactivation of the more functional short CAGn allele.[36]

Clinical picture

The clinical picture of patients who come to medical attention varies according to age. Before puberty only discrete physical anomalies may be noticed—eg, slightly lower than normal testicular volume or long-leggedness. Sexual development may be normal before puberty and includes initiation of normal pubertal changes and normal pituitary gonadal function.[37] The physical appearance of a pathologically hypogonadal child may not differ clearly from that of a normal prepubertal boy. The evolution of physical and psychological changes in Klinefelter's syndrome from childhood until adulthood has been exhaustively investigated by Ratcliffe.[38]

In adolescence and after puberty the syndrome is characterised by small firm testes and varying symptoms of androgen deficiency. In our patients with Klinefelter's syndrome the mean bitesticular volume measured by ultrasonography is 5·5 mL (SE 0·3; table 1, figure 3). 118 (63%) of 186 patients had hypogonadism,

	n	Mean (SE)	Median (IQR)	Range	Normal range
Age, years	189	29·9 (0·7)	28·6 (22·3–34·7)	18·2–74·5	..
Anthropometry					
Height, m	184	1·84 (0·01)	1·84 (1·78–1·90)	1·52–2·07	..
Bodyweight, kg	184	84·1 (1·3)	82·0 (72·0–93·3)	50–150	..
Body-mass index, kg/m^2	184	24·9 (0·4)	24·3 (21·7–27·5)	16·9–48·9	19–25
Arm span, m	138	1·84 (0·01)	1·84 (1·78–1·90)	1·52–2·08	..
Endocrinology					
LH, U/L	187	18·7 (0·5)	18·2 (13·7–21·5)	3·4–49·7	2–10
FSH, U/L	186	33·7 (1·1)	32·7 (23·8–41·3)	2·2–102·0	1–7
Testosterone, nmol/L	186	11·1 (0·4)	9·9 (7·0–16·2)	1·0–32·3	12–40
Free testosterone, pmol/L	126	220 (11·1)	205 (127·5–279·5)	10·8–692	> 250
SHBG, nmol/L	124	40·5 (1·8)	34·3 (26·0–53·0)	7·4–108·0	11–71
Oestradiol, pmol/L	162	82·4 (3·3)	81·1 (57·0–99·0)	8·4–262·0	<250
Bitesticular volume, mL, on ultrasonography	160	5·5 (0·3)	4·9 (3·0–7·0)	0·8–27·7	24–60

Table 1: **Clinical features of our adult patients with mosaic and non-mosaic Klinefelter's syndrome**

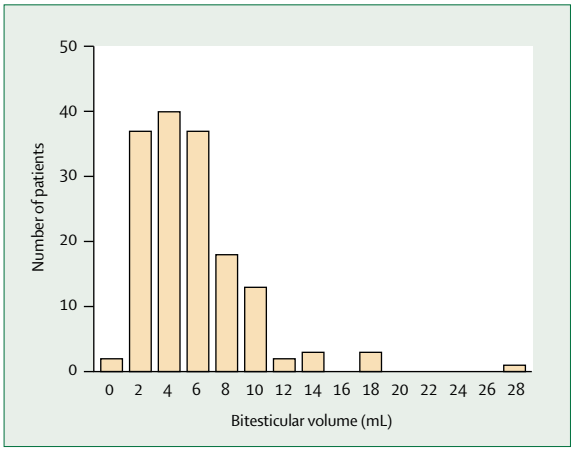

Figure 3: **Distribution of bitesticular volume measured by ultrasonography in 160 patients with Klinefelter's syndrome**

defined as testosterone concentration below 12 nmol/L. A history of maldescended testes was recorded in a higher proportion of our patients than in 10 469 consecutive patients attending our andrological clinic (27% *vs* 8%).[8]

At the time of puberty characteristic skeletal proportions begin to develop. These patients are generally of average height or taller. The increase in stature seems to be due to increased leg length; the presence of this feature before puberty suggests that it is not secondary to androgen deficiency but is probably related to the underlying chromosomal abnormality.[39] In contrast to typical eunuchoid tall stature, the arm span seldom exceeds the patient's height.[8,10] The low biacromial diameter seems to be caused by lowered plasma testosterone concentrations.[40] The anthropometric characteristics of our patients are reported in table 1.

After the age of 25 years about 70% of patients complain of decreasing libido and potency, and normal beard growth is present in only about a fifth of patients.[8] Osteoporosis and reduced muscle strength follow as a consequence of androgen deficiency.[41,42] Varicose veins, venous stasis ulcers, and thromboembolic disease are common, affecting up to a third of patients, with both non-mosaic and mosaic forms of the syndrome.[43–45] The increased thromboembolic risk in hypogonadal men has been explained by hypofibrinolysis due to androgen deficiency,[46] and testosterone substitution therapy has a favourable profibrinolytic effect.[47,48] Obesity, low glucose tolerance, and diabetes mellitus are also observed;[49,50] the risk of death from diabetes is greatly increased.[50]

During puberty nearly half of patients develop painless bilateral gynaecomastia of varying degrees (68 [38%] of 178 of our patients had past or present gynaecomastia).[38,51] The risk of developing mammary carcinoma, however, is no higher than in normal men,[52] as suggested in earlier studies based on small numbers of patients.[53]

Over 40 cases of extragonadal midline germ-cell tumours (mediastinal non-seminomatous germ-cell tumours) have been reported in patients with Klinefelter's syndrome,[52,54] most developing before the age of 30 years.[53] Increased frequencies of leukaemia and lymphoma have also been reported.[55] In our group of patients we have observed no obvious increase in haematological malignant disorders or midline germ-cell tumours.

The cognitive "phenotype" reflects not a general reduction of intellectual abilities but deficits in very specific domains of cognition, mainly language and executive functions (involved in concept formation, problem solving, task switching, inhibitory processes, speed of response, and planning), which seem similar to those observed in cytogenetically normal dyslexic children.[56,57] Early studies of Klinefelter's syndrome suggested an increased risk of psychiatric disturbances, criminal behaviour, and mental retardation,[58,59] but this notion could not be confirmed by later prospective studies based on chromosome surveys.[4,60]

The only prospective, longitudinal study with follow-up until adulthood of an entire cohort of individuals with Klinefelter's syndrome identified at birth shows that almost all affected boys have substantial medical, psychological, or social problems.[38] The relative risk of death in adult patients is significantly increased owing to diabetes and cardiovascular, respiratory, and gastrointestinal disorders.[50]

Nevertheless, such observations have to be regarded with caution, since two-thirds of cases of the syndrome are not detected and, hence, not characterised. The clinical picture we observe could be biased, showing only the extreme cases, whereas the men with less obtrusive phenotypes lead normal lives except for inadequate fertility due to meiotic malfunction.

Patients with chromosome mosaics commonly show very few clinical symptoms, and the testes may be normal in size. Endocrine abnormalities are also less severe, and gynaecomastia and azoospermia are less common.[39] In poly-X Klinefelter's syndrome, the phenotype progressively deviates from normal as the number of X chromosomes increases.[61] In XXXY and XXXXY, the frequency of almost any somatic anomaly is increased compared with XXY. Height is inversely related to the number of X chromosomes.[61]

Diagnosis

A suspected diagnosis can be based on the combination of typical clinical findings. The most important of these are very low testicular volume and firm consistency of the testes. However, even this very characteristic symptom may not be present in all cases (figure 3). Testicular size can be assessed by palpation and comparison with testis-shaped models of defined sizes (Prader orchidometer) or more precisely by ultrasonography.[62] A healthy European man has, on

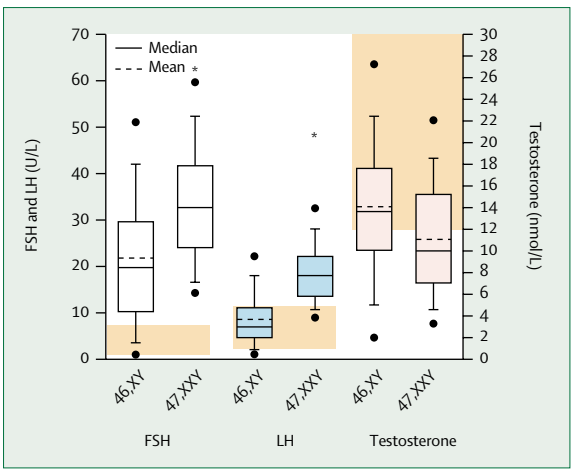

Figure 4: **Concentrations of FSH, LH and testosterone in 228 patients with Klinefelter's syndrome and in 224 patients with normal karyotype who were referred to our clinic for infertility or hypogonadism**
Points reflect data outside the 5th and 95th centiles. Boxes indicate IQR, and error bars 5th to 95th centiles. Shaded areas represent normal ranges. *Significant difference between 46,XY and 47,XXY groups (p<0·0001). Clinical features of the patients with normal karyotype were reported elsewhere.[9]

average, a testicular volume of 18 mL per testis; the normal range is 12–30 mL.

Some cases are detected among patients with azoospermia presenting to infertility clinics. Klinefelter's syndrome remains largely undiagnosed both because patients do not seek medical advice and because there is low awareness of the disease in health professionals. 60% of our patients with Klinefelter's syndrome were not suspected of having the disorder in the referring primary or secondary centre, despite previous external clinical investigations. However, once suspected by the experienced clinician, the diagnosis is confirmed in a high proportion; in a cohort of 309 suspected cases, 28% were confirmed by chromosome analysis.[9] Of the general and clinical features, testicular volume is the most sensitive, showing the smallest overlap between patients with and without the syndrome who are referred for medical assessment.

Barr-body analysis provides a quick and reliable screening test, with high sensitivity (82%) and specificity (95%).[9,63] Chromosome analysis in lymphocytes confirms the diagnosis of Klinefelter's syndrome.[9,64] This analysis occasionally shows a normal male karyotype. In these cases, karyotyping from skin fibroblasts or testicular biopsy samples can be used to confirm chromosome mosaicism, although karyotype analysis may also overlook tissue-specific mosaic Klinefelter's syndrome.

Serum testosterone concentrations increase during early adolescence in some patients, then begin to decrease by 15 years of age, being lower than normal in about 80% of adult patients with 47,XXY karyotype.[37] The exact mechanism of the androgen deficiency is unknown, and the degree of Leydig-cell dysfunction is variable. On average, the oestradiol concentration is higher than in normal men. Serum concentrations of sex-hormone-binding globulin (SHBG) are high, causing a further decrease in biologically active free testosterone. Concentrations of luteinising hormone (LH) and FSH are high in most cases (table 1, figure 4). FSH shows the best discrimination, and little overlap occurs with normal individuals, a consequence of the consistent damage of seminiferous tubules.[39] The androgen sensitivity index (LH concentration multiplied by testosterone concentration)[65] is high because the serum LH concentration is very high.[9] Concentrations of inhibin B are generally within the normal range in prepubertal boys with Klinefelter's syndrome but decrease significantly during late puberty,[66] because virtually all germ cells and the majority of Sertoli cells disappear.[67,68] Before puberty, plasma gonadotropin concentrations and the response to gonadotropin-releasing hormone do not differ from those in unaffected boys, but by the time of expected puberty plasma gonadotropin concentrations and the response to gonadotropin-releasing hormone are higher than normal.[37]

Practically all ejaculates from patients with 47,XXY karyotype show azoospermia. Sperm are observed only

	Karyotype	Age, years	BV, mL*	LH, U/L	FSH, U/L	Testosterone, nmol/L	Oestradiol, pmol/L	Sexual abstinence, days	Ejaculate volume, mL	Sperm		
										Motility†	Number ×10⁶/mL	Normal morphology (%)
1	47,XXY	18·6	2·8	19·2	37·5	9·9	94	4	1·1	40	0·6	10
2	47,XXY	30·9	3·0	14·7	21·0	8·6	82	..	..	37	7·6	18
3	47,XXY	23·8	2·8	15·4	21·1	11·1	88	4	1·7	0	<0·1	0
4	46,XY 47,XXY	31·9	27·7	4·0	8·8	27·5	101	8	3·1	10	3·1	4
5	47,XXY	19·7	5·1	8·9	16·5	22·6	69	4	2·7	40	0·1	25
6	47,XXY	19·3	..	40·0	28·0	10·0	..	..	..	2	0·1	0
7	47,XXY	20·2	5·1	28·7	41·2	19·0	34	4	3·0	0	<0·1	0
8	47,XXY	29·9	9·0	16·9	37·0	8·9	..	..	1·4	0	<0·1	0
9	47,XXY	34·8	7·2	13·5	20·9	13·5	82	2	2·5	15	<0·1	3
10	47,XXY	28·2	3·8	28·1	45·2	19·5	92	7	4·5	1	<0·1	0
11	47,XXY	20·6	..	85 ng/mL	8·7 ng/mL	14·0	104	5	5·0	54	4	7

*Bitesticular volume by ultrasonography. †% WHO grade a and b.[74]

Table 2: **Clinical features of patients with Klinefelter's syndrome and spermatozoa in the ejaculate**

Reference	n	Karyotype (peripheral leucocytes)	Testicular biopsy	Pregnancies	Liveborn children	Karyotype of child	Paternity testing
69	1	47,XXY	No	1	1	46,XY	HLA typing
70	1	47,XXY	No	1	1	46,XX	DNA fingerprinting

Table 3: **Reported pregnancies after natural intercourse in patients with Klinefelter's syndrome**

rarely, and exceptional cases of spontaneous paternity have been reported.[69,70] Histology of the testes generally reveals hyalinising fibrosis of the seminiferous tubules, absence of spermatogenesis, and relative hyperplasia of the Leydig cells.[21,71,72] However, the presence of tubules with residual foci of spermatogenesis has also been reported, with meiotic arrest at primary spermatocyte or spermatid stages and foci of normal spermatogenesis.[26,69,73] Of our 189 patients with Klinefelter's syndrome who were asked to provide a semen sample at the time of the first visit, 131 (69·3 %) were able to or agreed to provide an ejaculate.[74] Spermatozoa were observed in only 11 (8·4 %) of these men (table 2). The high proportion of patients able to ejaculate allows speculation on the patients' capacity to carry out reasonably successful sexual relations.

Management

When testosterone serum concentrations in patients with Klinefelter's syndrome are low, lifelong substitution therapy is indicated and should be started as early as possible to avoid symptoms and sequelae of androgen deficiency.[75]

Early recognition and treatment of Klinefelter's syndrome can significantly improve the patient's quality of life and prevent serious consequences. Early testosterone replacement results in increased masculinity, strength, libido, bone mineral density, and body hair.[75–77] It has a positive effect on mood and behaviour, improves goal-directed thinking and self-esteem, and reduces fatigue and irritability.[78,79] Gynaecomastia is not generally influenced by hormone therapy. If the patient wishes, surgical intervention can be sought from a surgeon experienced in mammary plastic surgery.[8] The slight anaemia characteristic of hypogonadal patients resolves under testosterone treatment.[80] Vascular endothelial functions, which are similar to those found in women, change to values observed in healthy eugonadal men with testosterone therapy.[81] Beneficial effects of testosterone on the cardiovascular system have been recently demonstrated in both eugonadal and hypogonadal men with chronic stable angina and in chronic heart failure.[82–85]

We emphasise that testosterone replacement therapy corrects symptoms of the androgen deficiency caused by Klinefelter's syndrome but has no positive effect on fertility.[86] In fact, testosterone blocks spermatogenesis at the stage of spermatogonial differentiation.[87] In patients with Klinefelter's syndrome who have sperm after testicular sperm extraction, administration of human chorionic gonadotropin (HCG) can increase serum testosterone concentrations, indicating that some functional testicular tissue is present,[88] Treatment with HCG before attempts at in-vitro fertilisation has thus

Reference	n	Sperm retrieval, %	Type of spermatozoa used for successful fertilisation	Transferred embryos	Clinical pregnancies (as defined by fetal heartbeat)	Liveborn children	Karyotype of conceptus or neonate
91,92	20	50	Fresh	31	3 (singleton)	3	46,XY (2); 46,XX
93, 94			Frozen-thawed	8	1 (singleton)	*	
95	2	..	Fresh	9	2 (1 singleton, 1 twin)	3	46,XY (2); 46,XX
96	7	57	Fresh	4	1 (singleton)	1	46,XY
97	1	..	Fresh	3	1 (twin)	2	46,XY (2)
98	1	..	Fresh	3	1 (singleton)	1	46,XY
99	1	..	Frozen-thawed†	10	2 (twin; in 3 treatment cycles)	2	46,XY (2)
100	1	..	Fresh	3	1 (triplet)	2	46,XX; 46,XY, 1 47,XXY aborted
101	52	..	Fresh	..	1 (singleton)	1	46,XX
102	20	40	Fresh	..	4 (2 singleton, 1 twin, 1 triplet)	7	46,XY (4); 46,XX (3)
103	1	..	..	..	..	1	46,XX
104	12	42	Fresh	15	3 (2 singleton and 1 triplet)	4	4 healthy neonates; 1 47,XXY aborted
			Frozen-thawed	18	2 (1 twin, 1 abortion)	2	46,XY (2)
105	1	..	Fresh	3	1 (twin)	2	46,XY (2)
106	2	100	Fresh	6	2 (singleton)	2	46,XX; 46,XY
24	19	21	Fresh	..	4 (3 singleton, 1 miscarriage)	Not known	..
107	1	..	Frozen-thawed	4	1 (singleton)	1	46,XY
108	24	50	Fresh	..	4 (3 singleton, 1 twin)	5	46,XY (2); 46,XX (3)
109	12	55	Fresh	25	2 (singleton)	1	46,XX
110	8	..	..	..	4 (2 singleton, 2 twin)‡	3	..
Total	185	52	..	142	40	43	..

*Child stillborn at 23 weeks of gestation. †From same patient reported by Ron-El et al.[98] ‡Two pregnancies (one singleton, one 1 twin) were still under way when the paper was published.

Table 4: **Reported pregnancies after ICSI treatment with testicular spermatozoa of patients with non-mosaic Klinefelter's syndrome**

Reference	n	Number with spermatozoa in the ejaculate	Transferred embryos	Clinical pregnancies (as defined by fetal heartbeat)	Liveborn children	Karyotype of conceptus/neonate
111	1	1	4	1 (twin)	2	46,XY; 46,XX
112	1	1	3	1 (singleton; abortion 9th week)	..	46,XX
101	52	4	..	1 (abortion 8th week)	..	..
113	1	1	2	1 (singleton)	1	46,XX
94	20	1	2	1 (singleton)	1	46,XX
109	12	1	3	1 (triplet; abortion 18th week)	..	46,XY; 46,XX (2)
114	1	1	2	1 (twin)	2	46,XY; 46,XX
Total	88	10	16	7	6	..

Table 5: **Reported pregnancies after ICSI treatment with ejaculated spermatozoa of patients with non-mosaic Klinefelter's syndrome**

been suggested to improve the outlook for these patients,[88] but this approach has not been confirmed in controlled trials.

Current testosterone preparations include those for intramuscular administration (testosterone enanthate and cypionate), oral testosterone undecanoate, buccal testosterone, subdermal implants, and transdermal preparations.[75] The recently licensed long-acting testosterone undecanoate allows injection intervals of around 3 months and appears especially suited for substitution in younger patients with Klinefelter's syndrome.[75,89,90]

Patients should be advised about the existence of self-help groups, such as, for example, the UK Klinefelter Association (www.ksa.uk.co.uk), the US Klinefelter Organization (www.genetic.org/ks), and the Deutsche Klinefelter Syndrom Vereinigung (www.Klinefelter.org).

Fertility

Spermatogenesis is present at a very low rate in occasional patients with Klinefelter's syndrome, and very rare cases of spontaneous paternity have been reported (table 3). However, before the introduction of the ICSI technique, the fertility outlook for the vast majority of these patients was hopeless. ICSI offers the opportunity for reproduction even when spermatozoa are not present in the ejaculate, but only in the testis. The successful recovery of spermatozoa from azoospermic men with Klinefelter's syndrome by means of testicular sperm extraction was reported in 1996.[91] ICSI is now successful in patients with Klinefelter's syndrome, and pregnancies and livebirths have been reported. Most studies confirm ICSI of testicular spermatozoa (table 4),[24,91–110] and fewer reports describe delivery after ICSI with ejaculated spermatozoa (table 5).[94,101,109,111–114] Surgical sperm retrieval has revealed spermatozoa in up to half of patients with non-mosaic Klinefelter's syndrome selectively referred to centres specialising in assisted reproduction techniques.[93,104,115,116] This recovery rate is similar to that among all men with non-obstructive azoospermia.[116] A livebirth rate of 20% has been reported by Staessen and colleagues,[94] who investigated the outcome of ICSI in 20 couples in which the man had Klinefelter's syndrome. Moreover, testicular tissue can be successfully cryopreserved in patients with non-mosaic Klinefelter's syndrome without significant compromise of fertilisation and implantation rates.[93,94,104]

Germ-cell depletion is evident even in the testes of 47,XXY infants and it progresses rapidly. Therefore, cryopreservation of semen samples might preserve future fertility in young men in whom Klinefelter's syndrome is identified before the time at which they present with infertility.[117] Cryopreservation of semen samples from boys in early puberty or containing very low numbers of spermatozoa is possible and should be offered to appropriate patients.[118,119]

Testicular biopsy has lately begun to be widely offered to patients with non-mosaic XXY karyotype. Histopathology is the best way to predict the likelihood of sperm recovery in patients with secretory azoospermia;[116,120,121] the predictive values of testicular volume, basal testosterone concentrations, and testosterone response to the HCG test have been shown by some investigators,[88] but not by others.[120,121]

Most infants born after ICSI with sperm from men with Klinefelter's syndrome have a normal karyotype (tables 4 and 5), as would be expected from the presence of high proportions of chromosomally normal spermatozoa in these men.[7,102,112] However, in studies of chromosomal abnormalities in ejaculated spermatozoa from patients with Klinefelter's syndrome, the incidence of sex-chromosomal hyperploidy varied from 0·9% to 2·5% in the mosaic form and from 2·5% to 21·6% in the non-mosaic form.[17] The proportion of hyperploid spermatozoa in men with Klinefelter's syndrome does not correlate with the proportion of 47,XXY cells in somatic tissues. Overall, a higher risk of fathering a 47,XXY or 47,XXX child after successful fertilisation treatment has to be taken into account.

Moreover, increased frequencies of autosomal aneuploidies in spermatozoa from men with non-mosaic Klinefelter's syndrome have recently been reported. Hennebicq and co-workers[122] found a higher frequency of disomy 21 in the spermatozoa of such patients, indicating an important risk of trisomy 21 in offspring if the patients were candidates for ICSI. Morel and colleagues[123] found significant differences from normal men in the frequency of autosomal disomy for chromosomes 13, 18, and 21 in patients with

Klinefelter's syndrome. Since several studies have also shown an increased frequency of autosomal aneuploidy in the spermatozoa of men with normal karyotype and oligozoospermia or oligoasthenoteratozoospermia,[124] the relation of the increased frequency of disomy for some autosomes to Klinefelter's syndrome or to the oligoasthenoteratozoospermia associated with it is still an unresolved issue.[123]

To date, two different hypotheses have been proposed to explain the high frequency of genetically imbalanced spermatozoa in patients with Klinefelter's syndrome. The first is that 47,XXY spermatogonia undergo meiosis to produce hyperploid spermatozoa; the second is that the rare breakthrough patches of spermatogenesis in XXY men are due to the presence of normal XY germ cells but, as a result of a compromised testicular environment, these cells are susceptible to meiotic abnormalities.[94,125,126]

Genetic counselling

As in all cases of severe male-factor infertility necessitating ICSI, the genetic risks resulting from this procedure should be discussed with each couple. Preimplantation genetic diagnosis by embryo biopsy offers an efficient tool for embryo selection.[104] To date, 49 healthy children have been born by use of ICSI, and the conception of one 47,XXY fetus has been reported (tables 4 and 5). Preimplantation genetic diagnosis in couples affected by Klinefelter's syndrome has allowed the identification of a lower rate of normal embryos compared with controls, in whom the technique is used to determine sex. It has also confirmed the increased risk of sex-chromosome and autosome abnormalities.[94,126] Without preimplantation genetic diagnosis, the chance of selection and transfer of abnormal embryos is high because even embryos with normal morphology have a distinct percentage of abnormalities.[127] With preimplantation diagnosis, the identification of abnormalities in a cohort of morphologically good-quality embryos prevents the transfer of those destined not to implant or to be spontaneously aborted.[94]

Preimplantation genetic diagnosis is not available in all centres for technical or legal reasons, so the options for prenatal genetic diagnosis should be discussed with each couple, with account taken of the possibility that the diagnosis of a fetus with a sex-chromosome abnormality could be more acceptable to subfertile patients,[128] especially those who carry a sex-chromosome anomaly, than to people of normal fertility.[99] Nevertheless, prenatal diagnosis has led to induced abortion in up to 70% of the cases, a high proportion for a syndrome of variable phenotype that can present with a very benign clinical picture.[6,11,129,130] A significantly lower rate of pregnancy termination was found by Meschede and colleagues,[131] who hypothesise that the difference from other studies depends on cultural differences in parental perception of sex-chromosomal polysomies as well as characteristics of genetic counselling at their institution. Parents' decisions to terminate the pregnancy are influenced by the health professional who provides the counselling; the affected pregnancy is more likely to continue if the counselling is given by a genetic specialist.[132,133] Thus, genetic counselling of all men with Klinefelter's syndrome is recommended, and close collaboration between genetic departments and fertility clinics may be of great value to the patients who can receive information about their genetic disorder and the risk to their offspring.

Conflict of interest statement
None declared.

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