Should non-mosaic Klinefelter syndrome men be labelled as infertile in 2009?

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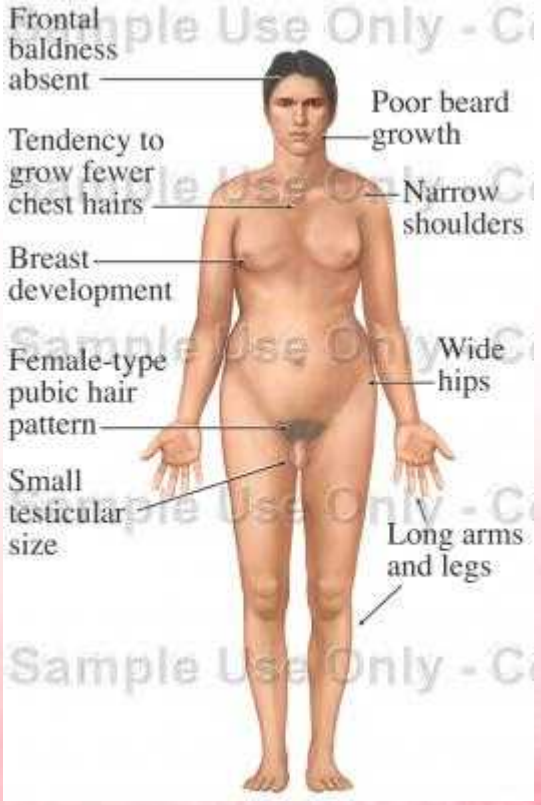
Klinefelter syndrome

- **First described in 1942**
- **Common genetic condition** - *1 in 500-1000 newborn males*
- **Various genotypes associated -** *extra chromosome inherited either from the mother or father at an approximately equal ratio*
  - **47 XXY** - most common, ~ 80% of non-mosaic Klinefelter
  - 48 XXXY
  - 49 XXXXY
  - 48 XXYY

- **Phenotypic appearances varies widely**
  - Enlarged breast
  - Sparse facial and body hair
  - Small, firm testes
  - Increased height
  - Decreased muscle mass

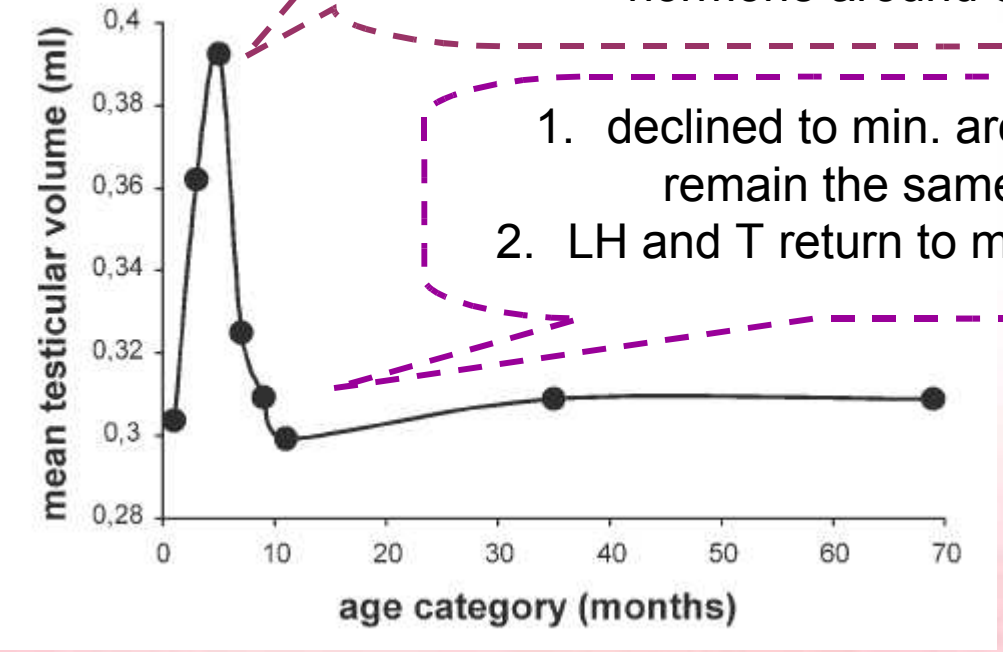


- **Only 25% of men with Klinefelter syndrome diagnosed**
  - ***Prenatally***
  - ***Around school age*** – learning and behavior difficulties, often related to poor language skills
  - ***Later in life with infertility***
    - make up 3% of infertile men and 11% of men with azoospermia
    - infertility caused by **degeneration of the germ cells** but exact mechanism not fully understood

Normal testicular development

1. from birth to 5 m/o, ↑to max. 0.44 cm^3
2. "mini-puberty": peak in gonadotropic hormone around 3-4 m/o

1. declined to min. around 9 m/o, remain the same till 6 y/o
2. LH and T return to min. at 6-9 m/o

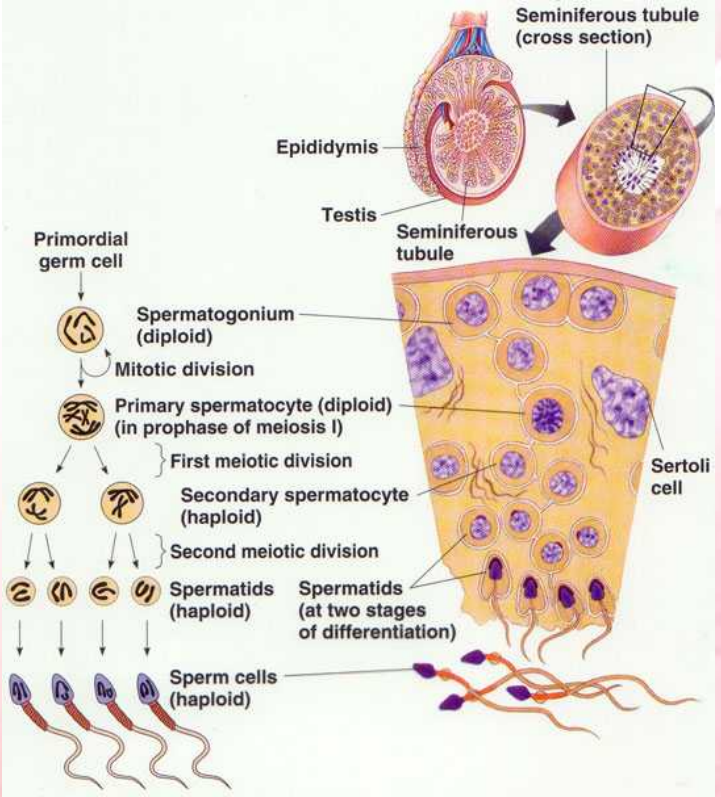


Kuijper et al., 2008

- ***Prepubertal*** boys have been shown to have **normal** testosterone, FSH, LH and inhibin B levels.

- ***At puberty***, impaired T and increasing LH
  - testicular size: initial increase to ~6 ml *(initial rise in **T** but level off into the low-normal range from 14 y/o)* but decrease to prepubertal size later *(∵ degeneration process : acceleration of germ cell depletion, hyalinization of tubules, Sertoli cell degeneration and hyperplasia of Leydig cells)*
  - Onset of puberty and secondary sexual characteristics: satisfactory

- testicular function:
  - germ cell differentiation at least partially **arrested** at the spermatogonium or early primary spermatocyte stage
  - Spermatogonia have difficulty entering meiosis → proceed to **apoptosis** at the onset of puberty



- ***In adulthood***:
  - hypergonadotrophic hypogonadism (low-normal T, high FSH and LH, undetectable inhibin B)
  - testicular histology: extensive fibrosis and hyalinization of seminiferous tubules and hyperplasia of interstitium
  - **extra X chromosome** thought to be responsible for infertility by causing degeneration of germ cells

Debate

- The degeneration of germ cells start *early in infancy*, leading to the absence or a significantly reduced # of germ cells even before puberty.
- The reduced # of germ cells seen in testicular biopsies on *fetuses* aborted at mid-trimester.
- normal testicular histology in 47XXY fetuses aborted at 17 and 20 weeks
- There may already be some impairment of Leydig cell function at birth but there are ***controversies*** regarding the presence of hypoandrogenism in infancy.

Options for fertility

- About 10% of patients with Klinefelter syndrome have a **mosaic form (46XY/47XXY).**
  - **presence of sperm in the ejaculate and subsequent paternities reported** *(Emre Bakiricioglu et al., 2006)*
- Men with non-mosaic Klinefelter syndrome have **azoospermia**, and are labeled as ***infertile.***
  - **donor insemination or adoption**

- Case report: spontaneous conception of a child from a non-mosaic Klinefelter father *(Laron et al., 1982)*
- **sperm** have been found in 7.7 and 8.4% of ejaculated semen samples of non-mosaic Klinefelter patients *(Kitamura et al., 2000; LanFranco et al., 2004)*

- with the ability to *retrieve testicular sperm* through testicular sperm extraction (TESE) and the expansion of *assisted reproduction* → possibility of having their own genetic child
- first child born in 1997 using **ICSI** for a non-mosaic Klinefelter man (Bourne *et al*., 1997)

- Foresta et al. (1999) performed FISH on testicular tissue of 10 non-mosaic Klinefelter men
  - residual spermatogenesis in 2 patients
  - Sertoli cells identified cytologically in all 10

- It is not common practice to offer surgical sperm retrieval to these men, at least in the UK.
- by systemic review to explore …
  - the **success of surgical sperm retrieval** in men with non-mosaic Klinefelter syndrome
  - the **outcome of pregnancies** in these couples

Materials and Methods

- Medline (1982–2009) and EMBASE (1982–2009) were searched by two authors (A.M. & G.F.) independently
  - Klinefelter
  - infertility
  - assisted conception
  - IVF
  - sperm injection
- All types of studies (RCTs and observational studies) selected
- no language restrictions

Inclusion criteria

- all articles that described surgical sperm retrieval in non-mosaic Klinefelter syndrome patients
- details of the method of sperm retrieval, # of patients, # of attempts and the success rate
- **Case reports**
  - ***excluded*** *to evaluate the success of sperm retrieval* to avoid bias
  - ***included*** *to explore pregnancy outcomes* -ART used irrespective of the method of sperm collection (from ejaculate or surgical sperm retrieval)

Exclusion criteria

- Studies that did not specify the genotype were excluded to avoid any bias *(∵ mosaic Klinefelter syndrome men can father spontaneous pregnancy)*
- In this review, we have concentrated on **non-mosaic Klinefelter syndrome men only**.

Outcome measures

- primary outcome : success rate of surgical sperm retrieval
- secondary outcome : live birth rate

Results

- 13 articles involving 373 men with non-Klinefelter syndrome
- The overall success rate of extracting sperm from patients with Klinefelter syndrome is 44% (16–60%)
  - **TESE** : 42% (95/228)
  - **micro-dissection TESE** : 55% (61/110), higher (P=0.010)

Table 1 Studies of success rates of sperm extraction

Author	No. of pts	Genotype	Mean/median age success failure	Drug therapy	No. of attempts	Procedure	Successful sperm retrieval (%)	Comments
Tournaye (1997)	15	Non-mosaic	Not available	nil	17	TESE	8/15(47)	
Friedler et al. (2001)	12	Non-mosaic	28 ± 3.2; 27.9 ± 4.5	nil	10	TESE	5/12 (42)	2 patients has sperm in ejaculate but subsequently underwent SSR (1x immotile sperm, 1x failed ICSI)
Levron et al. (2000)	20	Non-mosaic	Not available	nil	20	TESE	8/20 (40)	
Yamamoto et al. (2002)	24	Non-mosaic	Not available			TTB	12/24 (50)	Successful TTB was noted in men with 47XXY and 46XY spermatogonia in seminiferous tubules Unsuccessful TTB showed only 47XXY spermatogonia
Madgar et al. (2002)	20	Non-mosaic	32.5 32	nil	20	TESE	9/20 (45)	No significant difference in FSH, LH
Westlander et al. (2003)	18	Non-mosaic	30.8 ± 2.2; 34.5 ± 5.3		18 Repeat procedure done in the 5 successful patients in a second ICSI cycle	TESE	5/18 (28)	
Seo et al. (2004)	36	25 non-mosaic (11 mosaic)	31.2 ± 1.9; 32.3 ± 3.4	nil	36	TESE	4/25 non-mosaic (16)	No significant difference in success for age, FSH, testosterone, testicular volume
							6/11 mosaic (54)	Significant difference in success between non-mosaic and mosaic Klinefelter syndrome
Vernaeve et al. (2004)	50	Non-mosaic	29.5 + 1.3; 32.8 + 1.6	nil	24	TESE	24/50 (48)	No predictive power to age, testicular volume, FSH, FSH:LH ratio, testosterone, ASI
Okada et al. (2005a, b)	10	Non-mosaic	Not available	nil	10	mTESE	6/10 (60)	
Okada et al. (2005a, b)	51	Non-mosaic	31 (25–40); 38 (28–43)*	nil	51	TESE	26/51 (51)	
Emre Bakirdoglu et al. (2006)	74	Non-mosaic	31.6 ± 4.3; 35 ± 5.1	14 patients had testosterone replacement therapy, but stopped 6 months prior to mTESE	74	mTESE	42/74 (57)	No significance in success between FSH, LH, testosterone and testicular volume
Kyono et al. (2007)	17	Non-mosaic	30.2 ± 3.9; 37.6 ± 4.4	nil	17	TESE	6/17 (35)	Success was significantly improved in lower age group; no significant difference in LH, FSH, testosterone and testicular volume
Koga et al. (2007)	26	Non-mosaic	34.8 ± 3.3; 37.2 ± 5.8	nil	26	mTESE	13/26 (50)	No predictive power to age, testicular volume, LH, FSH, testosterone, estradiol, prolactin, inhibin B
					6 patients had unilateral mTESE 20 patients had bilateral mTESE (7/20 successful)			

Table II Summary of published pregnancies of Klinefelter syndrome patients

Author	No. of patients with ejaculated sperm	No. of patients with TESE sperm	Pregnancies	Miscarriages (biochemical pregnancy)	Live births	Comment
Laron et al. (1982)	1	0	1	0	1	Spontaneous conception
Terzoli et al. (1992)	1	0	1	0	1	Spontaneous conception
Honda et al. (2000)	1	0	1	0	1	2 embryos transferred
Staessen et al. (1996)	0	3	1	1 biochemical pregnancy	0	Preimplantation genetic diagnosis performed on all embryos
Bourne et al. (1997)	1	0	1	0	2	Frozen sperm, unsuccessful transfer at first cycle. Two embryos transferred at second cycle—twin birth
Hirney et al. (1997)	1	0	1	1	0	Miscarriage at 9 weeks gestation, normal karyotype
Tournaye et al. (1997)	0	5	3	1 biochemical pregnancy	2	
Palermo et al. (1997)	0	2	2	0	3	1 twin birth
Reubinoff et al. (1998)	0	2	1	0	1	Sperm retrieved by testicular fine needle aspiration; PGD of one embryo showed 47XXY
Nodar et al. (1999)	0	1	1	0	2	Pregnancy initially with 3 sacs (early fetal demise); twin birth
Ron-El et al. (1999)	0	1	1	0	1	
Kitamura et al. (2000)	3	0	3	2	1	
Levron et al. (2002)	0	8	4	0	7	One triplet and one twin pregnancy
Ron-El et al. (2000a, b)	0	1	1	0	2	Three fetal poles seen at 6 weeks gestation. Chorionic villus sampling at Week 10. Two normal fetuses, one with Klinefelter's karyotype (47XXY). Selectively reduced at Week 13
Ron-El et al. (2000a, b)	0	1	2	1	3	One live birth using fresh sperm; three cycles using frozen testicular sperm—miscarriage, failed implantation and successful twin pregnancy
Greco et al. (2001)	0	1	1	0	2	Twin birth
Kyono et al. (2001)	0	1	1			Pregnancy in 20th week at time of publication
Podalkis et al. (2001)	0	2	2	0	2	
Cruger et al. (2001)	1	0	1	0	1	
Friedler et al. (2001) (2 cycles described)	0	5	3	0	4	68% fertilization rate at ICSI—fresh sperm 3 embryos transferred per patient. Triplet pregnancy reduced to twin after one with 47XXY diagnosed prenatally
			2	1	2	58% fertilization rate at ICSI—cryopreserved thawed testicular sperm—1 healthy twin pregnancy
Rosenlund et al. (2002)	0	1	1	0	1	First attempt did not fertilize with fresh testicular sperm.
						Two attempts with frozen sperm—2 embryos transferred, no pregnancy; 2 embryos transferred, 1 pregnancy
Bergere et al. (2002)	0	3	2	1	1	
Yamamoto et al. (2002)	0	12	4	0	5	1 twin birth
Tachdjian et al. (2003)	1	0	1	0	2	Twin birth
Komori et al. (2004)	1	1	3	0	3	2 embryos transferred/cycle. 2 ICSI cycles/couple—1 fresh, 1 cryopreserved embryos
Seo et al. (2004)	0	4	2	1	1	
Okada et al. (2005a, b)	0	26	12	2	12	2 twin deliveries
Okada et al. (2005a, b)	0	6	4	1	3	Cryopreserved thawed sperm; transfer at 4–8 cell stage; 10 cycles
Schiff et al. (2005)	0	29	18	0	21	
Yarali and Bozdag (2006)	0	1	1	0	1	3 frozen embryos transferred; 7 cell stage. Previous failed transfer of 3 fresh embryos
Koga et al. (2007)	0	13	4	2	2	
Kyono et al. (2007)	0	6	5	0	8	5 x fresh sperm, 1 x frozen sperm; 3 couples had 1 cycle; 2 couples had 2 successful cycles (1 twin); 1 couple unsuccessful
Viodan et al. (2007)	0	2	2	0	3	1 twin pregnancy
Greco et al. (2008)	0	1	1	0	1	Frozen sperm and frozen oocyte. Single embryo transfer; 8 cell stage. Previous failed transfer of 3 embryos in separate cycle

- genetic children born to **non-mosaic** Klinefelter fathers : after ART (101) , spontaneous (2)
- 12 twin and 3 triplet conceptions
- Most did not have *Preimplantation Genetic Diagnosis* (PGD)
- 2 fetuses diagnosed prenatally as 47XXY genotype (both were part of triplet then reduced to twin)
- Postnatal genotyping – no published case

Discussion

- ***non-mosaic Klinefelter men can have potential for fertility by aid of ART***
- The presence of sperm in the ejaculate was an important finding but not all spermatozoa found are capable of fertilization.

- **Surgical sperm retrieval** -- ICSI
  - success rate with micro-TESE (55%) ***similar to*** pts with non-obstructive azoospermia with normal karyotype
  - first pregnancy using surgical sperm retrieval published in 1996 *(Staessen et al., 1996)*
  - first child born using ICSI in 1997 *(Bourne et al., 1997)*
- 101 children born to non-mosaic Klinefelter father – may be underestimated # (some not published)

- Why is this option not offered routinely to affected men in the fertility clinic ?

or

- Why is it not discussed with men who are diagnosed with this condition in childhood?

- **unanswered questions** :
  - the genetic risks to the offspring
  - the ability to predict success of sperm retrieval
  - the appropriate time for such a discussion

What are the genetic risks to offspring?

- increased numerical abnormalities in spermatozoa from XXY male
- various theories to explain the rate of **aneuploidy**:
  - XXY testis might be populated by XXY germ cells → formation of both normal and disomic spermatozoa
  - testis might be populated by XY germ cells but owing to deficiencies in XXY testicular environment, germ cells susceptible to various meiotic errors

abnormal germ cell line

- 47XXY germ lines are **unable** to undergo mitosis and meiosis, ***probably because of the presence of two functional X chromosomes***
  - any sperm found in such patients probably originates from normal germ line

→47XXY germ cells **able** to undergo meiosis and lead to abnormal gametes then abnormal offspring

Compromised environment

- Studies on XXY male mice showed few germ lines found in adult testis are exclusively of XY karyotype *(Mroz et al., 1999)*
  - meiotic aneuploidies found in the sperm probably relate to a compromised testicular environment
- non-mosaic Klinefelter pts → produce sperm **(germ cell mosaics)** → only 46XY cells complete meiosis
- abnormal testicular environment affects the spermatocytes and increases segregation errors

- A recent study demonstrated spermatogenic foci in 55% of non-mosaic Klinefelter patients (6/11).
  - subsequent FISH analysis showed all 92 spermatogonia to be **euploid 46XY** and can therefore form normal gametes.

Sciurano et al., 2009

- This provides a rationale for the high success rate in **TESE combined with ICSI** in this group of men.
- **genetic risk higher but the same as for azoospermia with normal karyotype**

Need for PGD ?

- Current aneuploidy rate in Klinefelter syndrome
- Potential risk to embryo of PGD
- More cost and delay in ET until blastocyst stage

- A small study of 20 Klinefelter syndrome patients, 46% had abnormal PGD.
  - sex chromosome abnormalities (3.1 vs. 13.2%)
  - autosomal abnormalities (5.2 vs. 15.6%)
  - ploidy abnormalities (4.3 vs. 10.6%)
  - abnormalities in chromosomes 18 and 21

Staessen et al., 2003

- an increase in chromosomal abnormalities in sperm retrieved surgically from non-obstructive azoospermic men (even when they have normal karyotype) compared with obstructive azoospermia and to ejaculated sperm (11.4 vs. 1.8 vs. 1.5%) especially for chromosomes XY, 18 and 21

Palermo et al., 2002

Can we predict the success of sperm retrieval?

- various factors explored to predict the success rate of surgical sperm retrieval :
  - Physical features (**age**…)
  - Biochemistry (serum testosterone, FSH, LH)
  - Testicular volume
  - FISH of lymphocytes
  - Testicular ultrasound

Small # of pts ➔ no known predictor

What does the future hold?

- As **age** increases, the potential for successful sperm retrieval decrease as hyalinization of tubules.
- for males diagnosed in childhood or teenager → advised to have families earlier *(option limited if diagnose made in adulthood)*
- Around the time of puberty seems to be the best time for sperm retrieval → initial increase in testicular size
  - case report of a 15 y/o boy

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- germ cell degeneration accelerates dramatically at the onset of puberty ➔ to retrieve germ cells at an early stage for cryopreservation and future utilization
- only 50% (7 out of 14) of Klinefelter boys (10.1–14 years) had germ cells in their testis ➔ severely impaired fertility even in the peripubertal period
- ejaculated sperm should be checked first for the presence of spermatozoa, prior to surgical attempt

- understanding of the procedure and its implications :
  - epididymitis, scrotal hematoma and testicular hydrocele
  - risk of not obtaining any sperm and hypogonadism
  - irreversibly reduced serum testosterone levels at 6 and 12 months following TESE and micro-TESE *(Okada et al., 2004)*
  - transient decline in serum testosterone levels in some patients following repetitive procedure *(Everaert et al., 2006)*
  - emotional impact

Conclusion

- It is possible for a man with non-mosaic Klinefelter to father a genetic child through the use of ART.
- Males diagnosed with Klinefelter syndrome need to be informed about and offered such choices.
- Ethical issues need to be clarified.
- counseling with geneticists with respect to the potential risks

Thank you !