Klinefelter syndrome: an argument for early aggressive hormonal and fertility management

Akanksha Mehta, M.D.

Fertility & Sterility, VOL. 98 NO. 2 / AUGUST 2012

Presentor: R3 孫怡虹 / Advisor: VS 鍾明廷

Klinefelter syndrome (KS)

- The most common **chromosomal disorder** in men
- Estimated prevalence:
  - General population: 0.2%
  - Infertile men: 3%
  - Men with non obstructive azoospermia: ~ 11%

- Frequently underdiagnosed:
  - **Wide phenotypic variation**
  - Lack of established screening programs
    - Categorized by X-chromosome polysomy
    - X disomy (47,XXY): Most common variant
  - < 10%: Diagnosed before puberty
  - 25%: Diagnosed during their lifetime

- Early diagnosis/management → Early interventions
  - Physical, occupational, speech therapy
    - Promoting normal physical development in adolescents with KS
      → may have positive psychological benefits
  - Combined with hormone manipulation therapy

⇒No RCT evaluating the efficacy or outcome

T replacement therapy (TRT)

- Goal:
  - Promote linear growth
  - Increase muscle mass
  - Preserve bone density
  - Development of 2nd sexual characteristics
- Impact on the fertility potential: unknown
  - Beneficial effect on semen volume
  - Exogenous T → suppressing testicular function
    → may conversely have a detrimental impact

Characteristic features

☆Small testes, Hypogonadism, Infertility

☆**Absolute or relative hypergonadotropic hypogonadism & impaired spermatogenesis**

- Higher grades of X chromosome polysomy ⇔ More severe clinical presentation
- Genetic mosaicism (46,XY/47,XXY) → usually a milder phenotype

Testosterone levels

- In infant: Usually normal
- During childhood: Appropriate response to gonadotropin stimulation
- To initiate puberty: Most have sufficient levels
- Progress maturity adequately: often fail

→Decelerated or poor progression of puberty *(Poor development of facial hair, masculinization, and emotional and social development delay)*

Testosterone levels

- During early puberty: ↑
- During mid-puberty:
  - Testosterone **Stabilize** (low ~ normal range)
  - LH & FSH: Progress ↑→ hypergonadotropic levels
  - Inhibin B: Concomitant ↓→ undetectable level

  ➔ *Progressive testicular steroidogenic dysfunction*

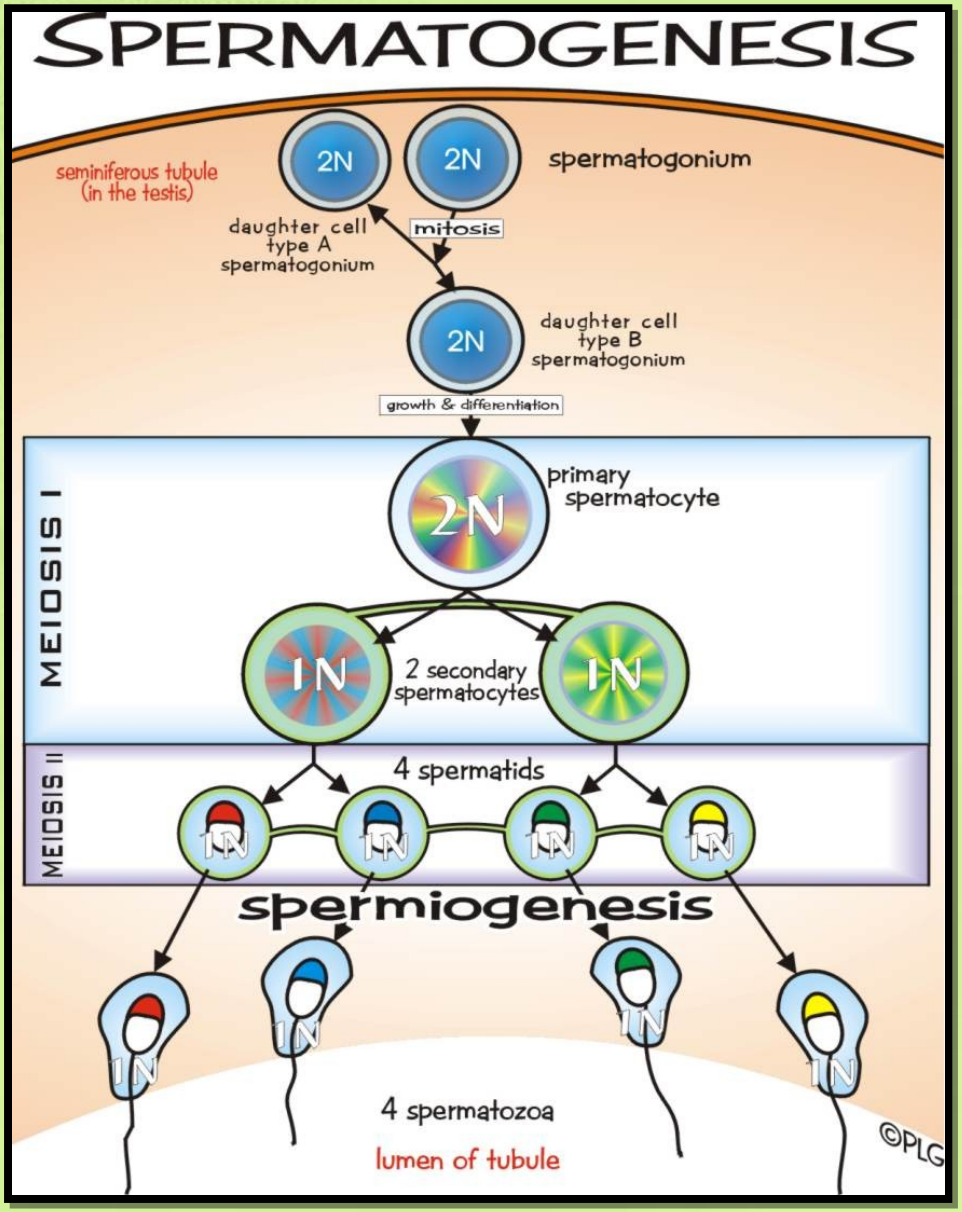
  - Mid-to-late puberty: Require exogenous T

Spermatogenic function of the testes

- During childhood: Lower testicular volumes
- Onset of puberty ~ mid puberty:
  - **Testicular growth** briefly ↑→ ↓thereafter
    - In unaffected boys: Result of germ cell proliferation (80% of the total testicular volume: seminiferous tubules)
    - In boys with KS: likely the result of interstitial cell proliferation & hyperplasia
  - Lower serum T & ↑gonadotropin levels

Spermatogenic function of the testes

- At the spermatogonium or early spermatocyte stage
  - Germ cell differentiation arrest
- Spermatogonia:
  - Difficulty entering meiosis
  - At the onset of puberty: Proceeding directly to apoptosis



Other characteristics

- **Seminiferous tubules:** Gradual deterioration
- **Tubular hyalinization**
- **Leydig cell hyperp**

Effect on Fertility

- *Historically, men with KS were considered infertile*
- Rare ejaculated sperm → Successful pregnancies have been previously reported
  - In the testes of patients with KS → Isolated foci of spermatogenesis can exist
  - With advances in ART during the past 2 decades → Made paternity possible
- **Fluorescence microscopy** for sperm sample:
  - Sample preparation is toxic to sperm → Impossible to appreciate *motility*

Effect on Fertility

- **Onset of puberty** ⇔ Progressive ↓in steroidogenic & spermatogenic functions of the testes

→As the critical time to address the **fertility potential**

- Early Sperm retrieval, Semen or testicular tissue cryopreservation

– Several invesigators recommend for patients with KS

– Timing & success may be influenced by HT (?)
→ A matter of debate

Aims

- Comprehensive search of the published literature

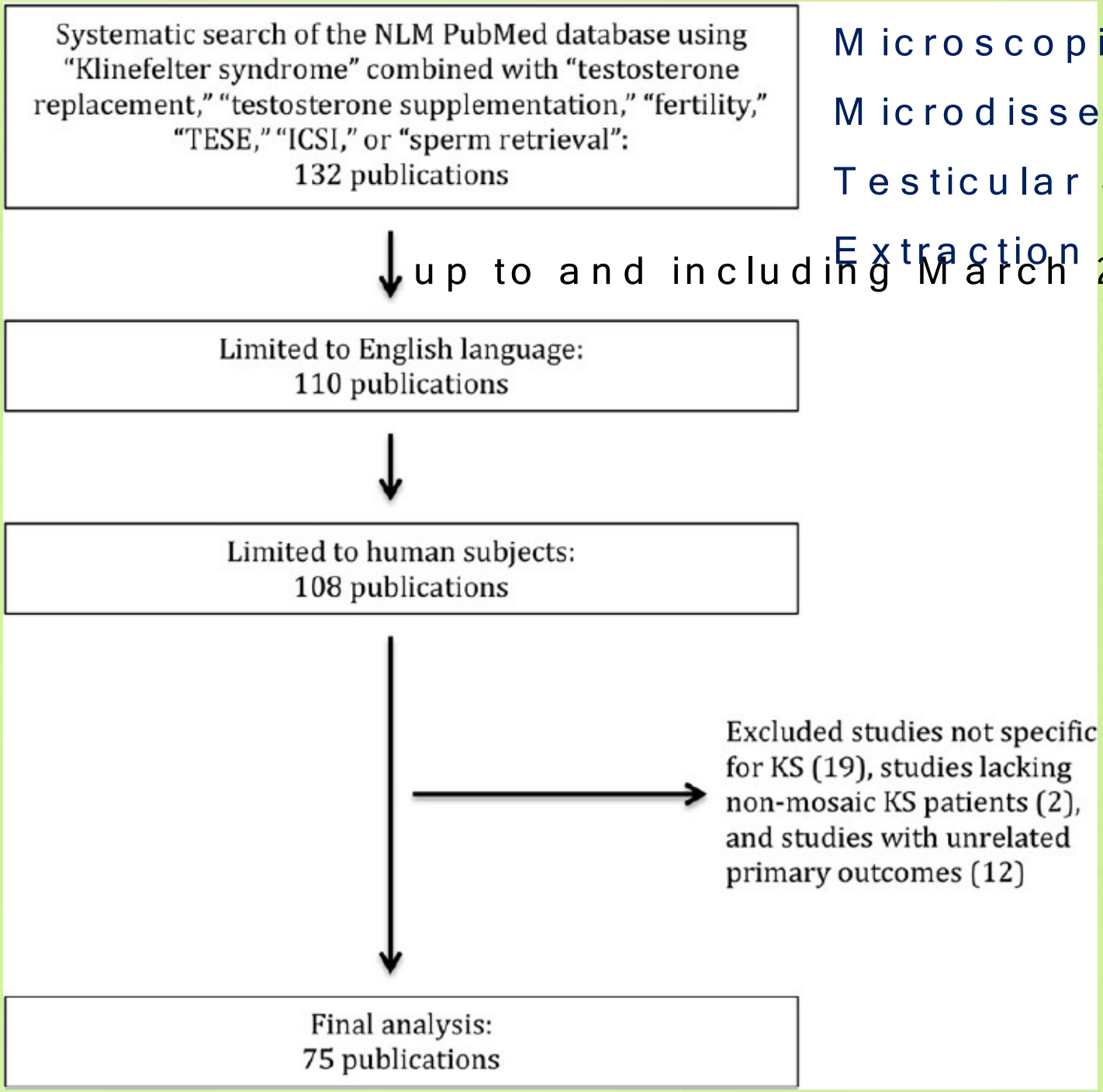
➔Investigate: The impact of **early HT** on **sperm retrieval rates** in patients with KS

➔Examine Predictors:

  - Sperm retrieval
  - Fertility outcomes are examined,

➔Discuss future directions: Fertility preservation in patients with KS

MATERIALS AND METHODS



Microscopic or Microdissection Testicular Sperm Extraction (TESE)

RESULTS

Studies evaluation

- Majority:
  - **Review articles**: Management of adolescents and adults with KS
  - **Case reports:** Achieved paternity in patients with KS, spontaneously or with ART
  - 16 studies (497 patients were analyzed):
  - **Evaluated success rates of sperm extraction**
  - *Optimal timing & modality of HRT: limited*
  - *Impact of HT on sperm retrieval or reproductive outcomes in men with KS: Lack*

Sperm retrieval rate

- Average: 51% (28%–69%)
  - Microdissection testicular sperm extraction (TESE): > non-microdissection TESE (61% vs. 47%)
  - *Previous TRT* (stopped at least 6 months) → microdissection TESE (20%–25%)
- Testicular histology: Sertoli cell-only pattern → 70%: had sperm found on micro-dissection TESE

(+) Predictors for sperm retrieval

- Young age
- Pre-op T levels: close to or within the normal range, either at baseline or with HT (aromatase inhibitors, clomiphene citrate [CC], or hCG)
- Larger testicular volume *(Madgar et al.)*
- Rare tubules with germ cells ⇔ spermatozoa (+)

Non specific predictor

- Serum [LH] or [FSH]
  ⇔ testicular spermatogenic function

- Testicular Doppler results, >10% mosaicism in peripheral lymphocytes or buccal tissue
  ⇔ successful sperm recovery

- Pretreatment testicular histology

DISCUSSION

Ejaculated sperm
from Adult men with KS

- ~8% have sperm present
  - Cryptozoospermia or severe oligospermia
  - Sperm concentrations < 1 x 10^6/mL
  - Impairment in sperm motility and morphology

- Aided with ART → Successful pregnancies

⇒Surgical sperm retrieval & ICSI

→ dramatically improved the fertility potential

Sperm retrieval

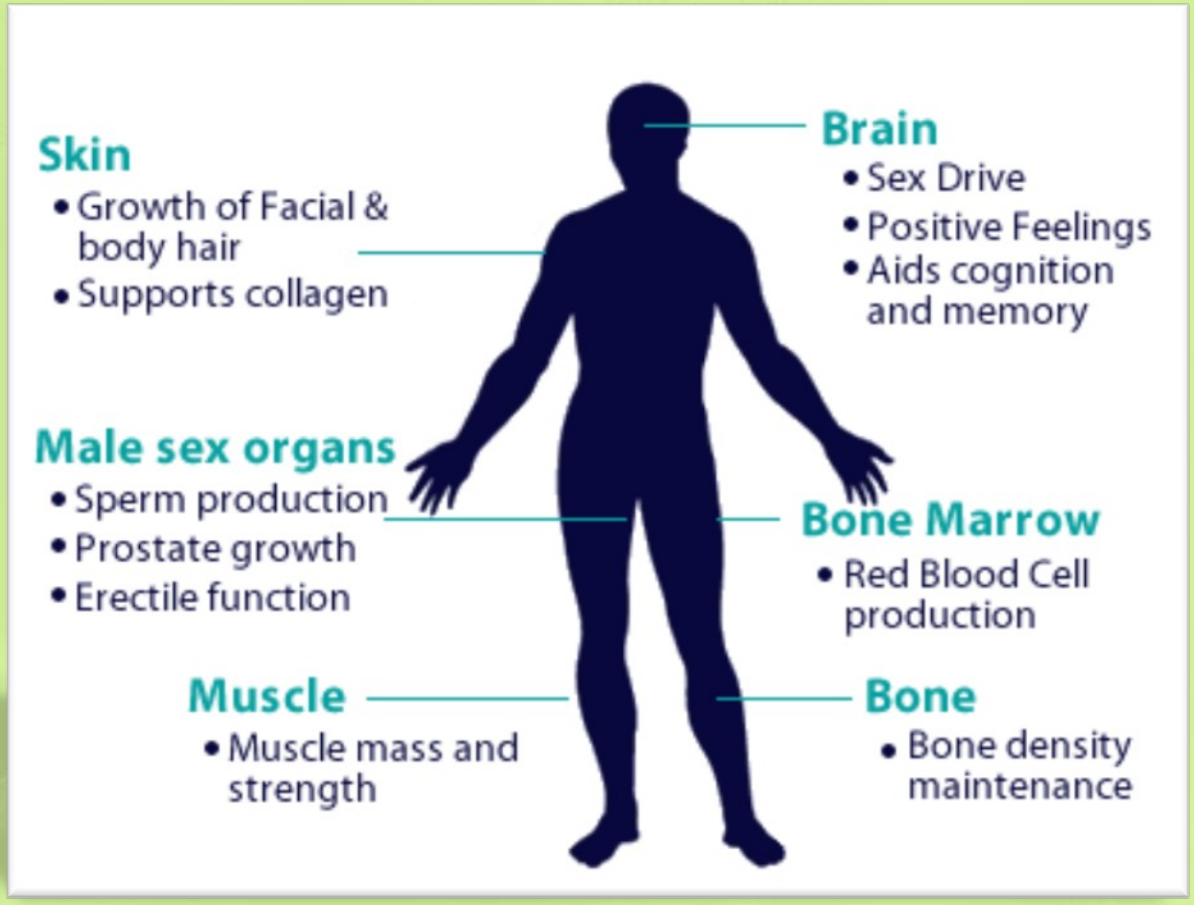
- 1st reported successful sperm retrieval: in 1996 (Tournaye et al.)
  - → 1st pregnancies achieved using ICSI of ejaculated and testicular sperm: 2 years later
  - → 101 children born to fathers with nonmosaic KS (underestimation)
- Use microdissection TESE
  → **Sperm retrieval rates ↑** in patients with KS (Considered equivalent to those in men with non obstructive azoospermia)

Sperm retrieval

- Freshly retrieved sperm → ICSI → ↑Pregnancy rate
- **Cryopreserved sperm**
  - Have been successfully used by several groups
  - ↓Repeat surgical procedures for sperm retrieval
  - A challenge when the sperm retrieved are limited in number or quality

Testosterone Replacement Therapy

- Impact: Difficult to ascertain
- Exogenous T
- Suppressive effect on testicular steroidogenic & spermatogenic function
  - Fully reversible ?
  - For what period of time ?
- Lack of data:
  - Unknown duration, route of therapy, rare case number
- Concomitant use at the time of surgery → Unknown impact on the number or quality of sperm retrieved



Testosterone Deficiency and HT

1. Testosterone Deficiency

- During normal male development: 3 physiological peaks of serum T
  - 1st: In the prenatal period
  - 2nd: Mini-puberty, during the 1st 2-4 months of life
  - 3rd: At adolescence
- *Whether **T deficiency** occurs during all 3 times in males with KS ?*
- *Whether **androgen therapy** should be considered when activation of the pituitary-gonadal axis first occurs ?*

Studies for serum T in infants with KS

- Several: A peak around 3 months of age
- 2 series: Lower mean levels versus controls
- Other: Normal or high normal levels
- Children with KS: normal levels of T, FSH, LH, inhibin B in the prepubertal period

  → Along with a normal serum T response to hCG stimulation

  → No indisputable evidence of **hypoandrogenism** in infants and prepubertal children with KS

2. Penile length

- A sensitive indicator of androgenization
- An inverse correlation to **CAG**n repeat length
  - Androgen receptor
  - Length polymorphisms may contribute to phenotypic variance of KS
- KS often < 46,XY *(not in the range of micropenis)*
- Treatment with **T** for decreased penile length:
  - Lack of alternative dosing regimens
  - Androgen therapy during infancy → Improved outcomes?
  - Considered on the basis on CAGn repeat length?

Current practice for patients with KS

- Initiation of therapy in early-to-mid puberty or at the onset of hypogonadism

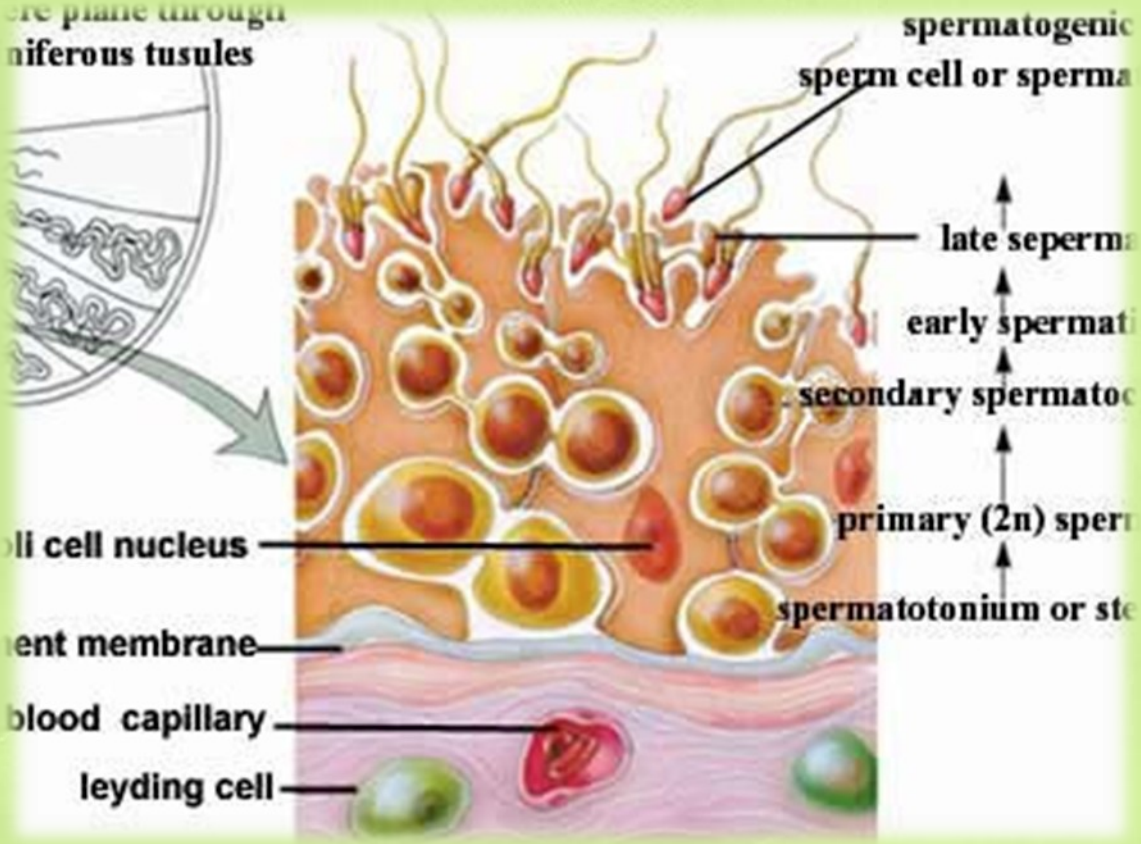
➔ Ensure the normal timing of completion of puberty

➔ Prevent the symptoms and sequelae of long-term androgen deficiency

- No specific guidelines of TRT
  - IM, Transdermal applications
  - Implantable T pellets in noncompliant adolescents (one case report)

In this andrology setting practice

- Typically initiate TRT **after** the onset of puberty
- Topical T gel
  - Appropriately & adequately ↑serum T levels in the majority of patients
  - Avoids anxiety & needle phobia (IM)
  - Compliance can be challenging for some adolescents
- Aromatase inhibitors (KS can also have ↑E2 or ↑E2/T)
  - Adolescents with gynecomastia or central obesity
  - Particularly poor response to T gel



Impaired Spermatogenesis

Testicular degeneration in KS

- Etiology: Not well understood
  1. ↑Expression of **genes** on the supernumerary X chromosome
  2. Intratesticular **hormonal** imbalance
  3. Defects in spermatogonial **stem cells**
  4. Abnormal **apoptotic activity** of Sertoli & Leydig cells
  - 47,XXY testis → impaired **spermatogenesis**

  ? Intrinsic to germ cells

  ? Inability to support normal germ cell

Spermatogonia/Germ cell

- May begin during fetal or neonatal life
  - Progressive decline in the number of **spermatogonia**: during the first year of life in infants (Mikamo et al.)
  - Other quantitative studies: normal **germ cell** counts
  - In **prepubertal boys** with KS
  - Diminished number or complete absence of Spermatogonia
  - With normal-appearing Sertoli and Leydig cells

- In **early adolescence** with KS
  - Majority: have germ cells in their testes
  - Immunohistochemical studies: Number of Spermatogonia markedly ↓(especially adult dark spermatogonia)
- Onset of **puberty**
  - Accelerated & progressive depletion of germ cells → Elevation in serum gonadotropin levels

Sertoli cells

- Transforming → mature adult cells during puberty
- Express androgen receptors
  - Smaller proportion
  - In the cell cytoplasm (rather than the cell surface)
    - ↓[Inhibin B] during mid & late puberty → reflect the loss of Sertoli cell number & function
    - Secretory dysfunction

→Unsuccessful attempts at testicular sperm

Leydig cells

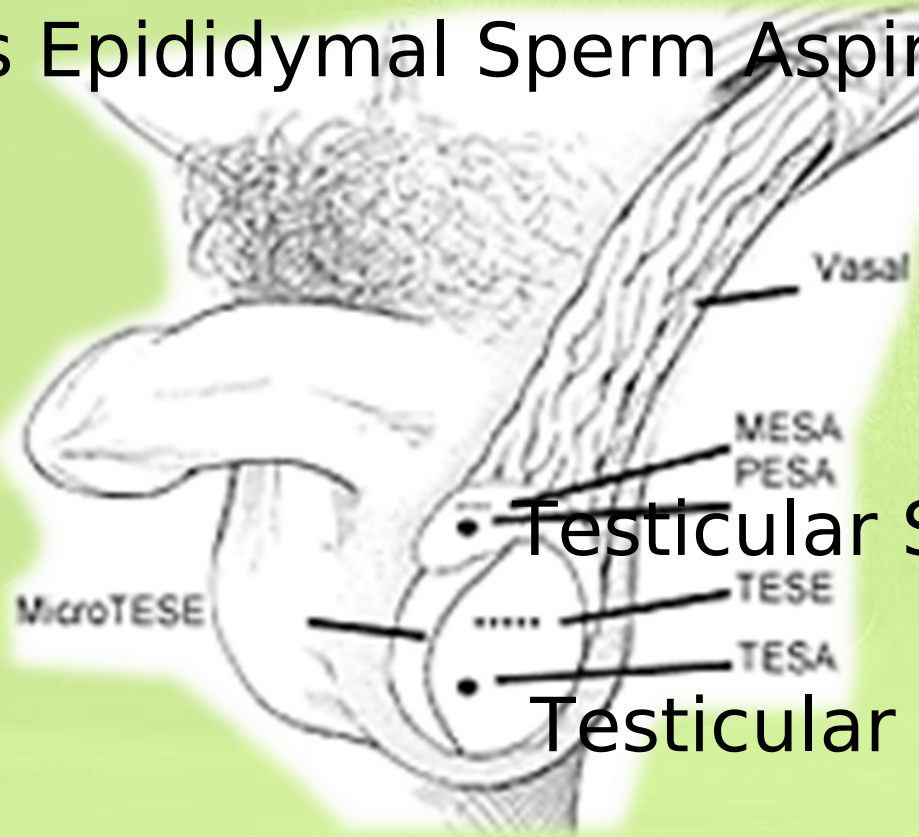
- Failure → impaired **steroidogenesis**
  - May be intrinsic to Leydig cells
  - Germ cell depletion
  - Sertoli cell dysfunction
  - Elevated intratesticular E2 levels

Germ cell

- Differentiation into mature spermatozoa → arrested in the testes of boys with KS
  - Early at the level of type A spermatogonia, **before meiotic division** *(Testicular biopsies, 14 adolescents)*
  - **Meiotic arrest later** *(Other investgators)*
    - At the 1° spermatocyte or spermatid stages
    - With rare foci of normal spermatogenesis

crosurgical Epididymal Sperm Aspiration

rcutaneous Epididymal Sperm Aspiration



Testicular Sperm Extraction

Testicular Sperm Aspiration

Early Sperm Retrieval

- In men with KS → Secondary decline in testicular function
  - Progressive, beginning in puberty & worsening during adulthood
  - Younger age: A positive predictor of sperm retrieval
  - Sperm retrieval during adolescence may have led to even better sperm retrieval rate
- *Case report (Damani et al.):*
  - Successful cryopreservation of sperm-containing testis tissue, a 15 y/o adolescent boy with KS

(Selective DNA fluorochrome & fluorescent microscopy, *Mehta et al., Poster 53, American Society of Andrology Meeting 2012*)

- Adolescents with KS aged 12–20 y/o → ejaculated semen samples → 70% present with Sperm

(Recommendation from this paper)

- Routinely semen cryopreservation in adolescents who have sperm present in the ejaculate
- Positive limited experience: Surgical sperm retrieval in 3 adolescents with KS

Testicular dissection for sperm extraction

- Negative effects on testicular function:
  - Temporary decline in serum T
    - → Recovers 12–18 months postoperatively
    - → One report: No improvement after 12 months post conventional or microdissection TESE
    - → Another report: Recovered to 50% of baseline after micro dissection TESE
  - Irreversible testicular atrophy & hypogonadism → much less common

- *The negative effects on testicular function*

- May reflect **preexisting testicular dysfunction** in the study population that worsened postoperatively
  - No account for the wide range of T levels among the subjects
  - No account for the possibility of natural decline in T over time in men with KS

Sperm retrieval and cryopreservation

- Recommendation -

- As early as possible
- Before the initiation of exogenous T therapy
- Early to mid puberty
  - Brief ↑in testicular size
  - Serum hormone concentrations are relatively within the normal range

  ➔May be the best time to consider sperm retrieval

Sperm retrieval and cryopreservation

- Recommendation -

- Non-T-based HTs (hCG, CC, aromatase inhibitors)
  - Theoretically stimulate **testicular steroidogenesis**
  - May be considered before planned surgical sperm extraction in hypogonadal patients

- The decision should be made on an individual basis

Genetics Risks to Offspring

Genetics Risks to Offspring

- Questionnaire-based survey of patients with KS
  → 90% expressed a desire to father children
  → 70% : TESE-ICSI
  → majority of offspring have been healthy, with a normal 46,XX or 46,XY chromosomal complement
  → ***47,XXY has certainly been reported → SA or elective termination***

- ↑Sex & autosomal chromosomal **aneuploidy** in sperm
  - Specific to KS offspring ?
  - Reflective of the rate seen in ICSI offspring in general?
- Theories:

1. 47,XXY germ cells → complete meiosis → produce hyperhaploid spermatozoa
2. Rare foci of 46,XY germ cells → susceptible to meiotic errors due to the abnormal testicular environment → Resulting in hyperhaploid sperm

Evidences from studies

1. Similar chromosomal patterns:

47,XXY (Sertoli cells, Spermatogonia, 1° spermatocytes) → Hyperhaploid (2° spermatocytes, spermatids, spermatozoa)

→ Suggest common 47,XXY origin

Evidences from studies

1. Sertoli cells: 47,XXY karyotype

   Germ cell lines in the KS testis: 46,XY

   → Euploid meiotic spermatocytes & Normal haploid gametes

(Mouse models…)

- Donor XY germ cells → haploid germ cells in the XXY environment

→ Testicular environment may be less important than the chromosomal complement of the germ cell line

Pre-implantation genetic diagnosis

- Recommend for Embryos obtained using TESE-ICSI
  - ∵↑Chromosomal abnormalities potential
  - *(Staessen et al.)* Rate of normal embryos for KS couples significantly < normal controls (54% vs. 77.2%)

Pre-implantation genetic diagnosis

- Not routinely used
  - Lack of availability in most center
  - Majority of offspring of KS couples are normal
  - KS fathers using ART have fewer than theoretically expected XY or XX disomic sperm & embryos
- Higher rate of chromosomal abnormalities detected in preimplantation embryos remains a concern

  ➔The use of preimplantation genetic diagnosis in KS couples undergoing ICSI should be

Future Directions

Cryopreservation of testicular tissue

(Containing mature spermatozoa)

- Used for evolving technologies (Spermatogonial maturation ex vivo) → Experimental at present

- Human testicular tissue
  - Can be cultured for several weeks without essential loss of spermatogonia
  - Spermatogenesis can take place under culture conditions → Normal spermatids with some fertilization potential

Cryopreservation of testicular tissue

(Containing mature spermatozoa)

- Consider in…
  - Non-azoospermia, Identified at puberty or before, ready for fatherhood
  - Severely oligospermic or cryptozoospermic patients
  - Younger adolescent, Spermatogonia in the seminiferous tubules, No more differentiated cell types

Conclusion

Early hormone substitution therapy

- Recommended in the patients with KS:
  - → Complete normal pubertal development
  - → Prevent adverse consequences of hypogonadism
- T supplementation
  - During the first 2–3 months of life: Benefit is unclear
  - Recommend initiation after the onset of puberty *(serum T levels being to decline)*

Assisted Reproductive Therapy

- Cryopreservation of semen samples
  - Possible from boys with KS in early puberty
  - Containing very low numbers of spermatozoa
  - Should before initiating T supplementation
- Surgical sperm retrieval
  - For fertility preservation in adolescents:
    - Unable to provide a semen sample
    - Azoospermic

Men with non-mosaic KS

- Limited testicular volume
- Extensive tubular sclerosis
- Markedly ↑[FSH]

☆Sperm retrieval + TESE & micro-dissection

TESE → Possible for 50%–70%

- Based on results from different centers
- Along with ICSI → ↑↑ability to father children

Boys with KS

- Option for fertility preservation in boys with KS
  - Immature germ cells or spermatogonia
  - → In vitro maturation, using ART
  - → Into mature spermatozoa

    at least, elongated spermatids capable of fertilizing ova

THANK YOU FOR LISTENING