Genetic causes of male infertility and their impact on future generations

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Disclosures

Drs. Sadri and Howards have *no financial disclosures or conflicts of interest* to report relevant to this presentation.

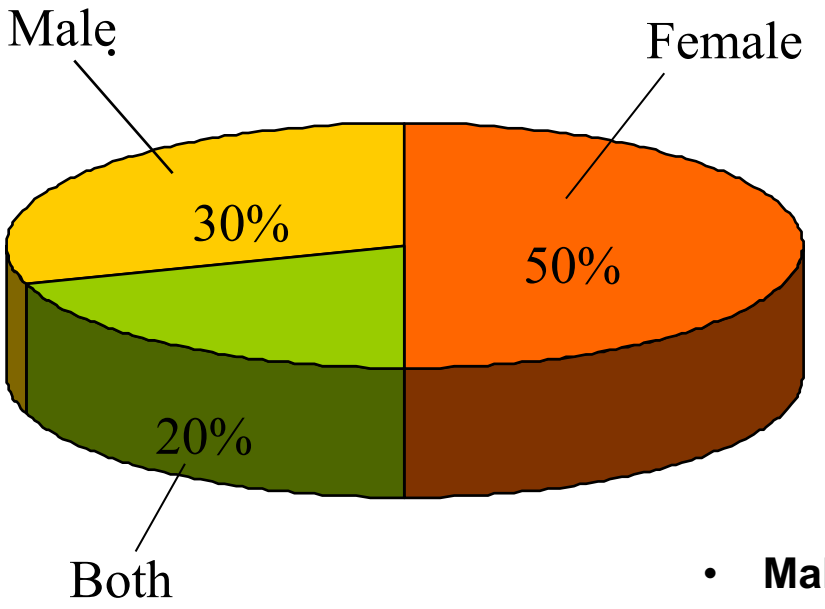
Learning objectives

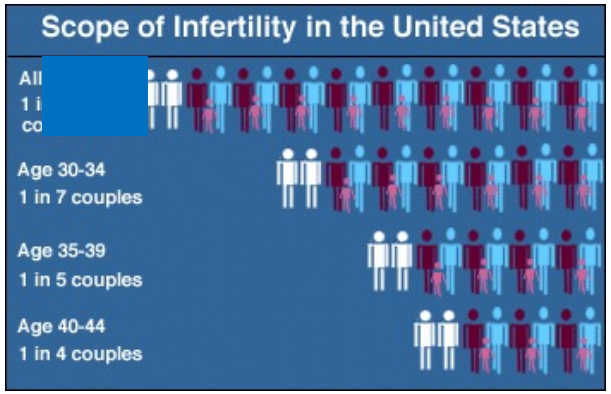
After this presentation, the learner should be able to:

- Explain the currently known Genetics etiologies of impaired sperm production
- Delineate indications for complex laboratory and diagnostic Genetics testing in infertile men
- Identify genetic abnormalities that may affect the health of offspring if assisted reproductive techniques are to be employed

Male and Female Infertility

- No conception after 1 yr of unprotected sexual intercourse is defined as possible infertility
- 85% of couples conceive with in 1 yr
- 50% of infertility involves male factor.





http://library.med.utah.edu/kw/human_reprod/seminars/seminar2A.html

- **Male infertility is a problem in 7% of all men**

Etiology of Male Infertility

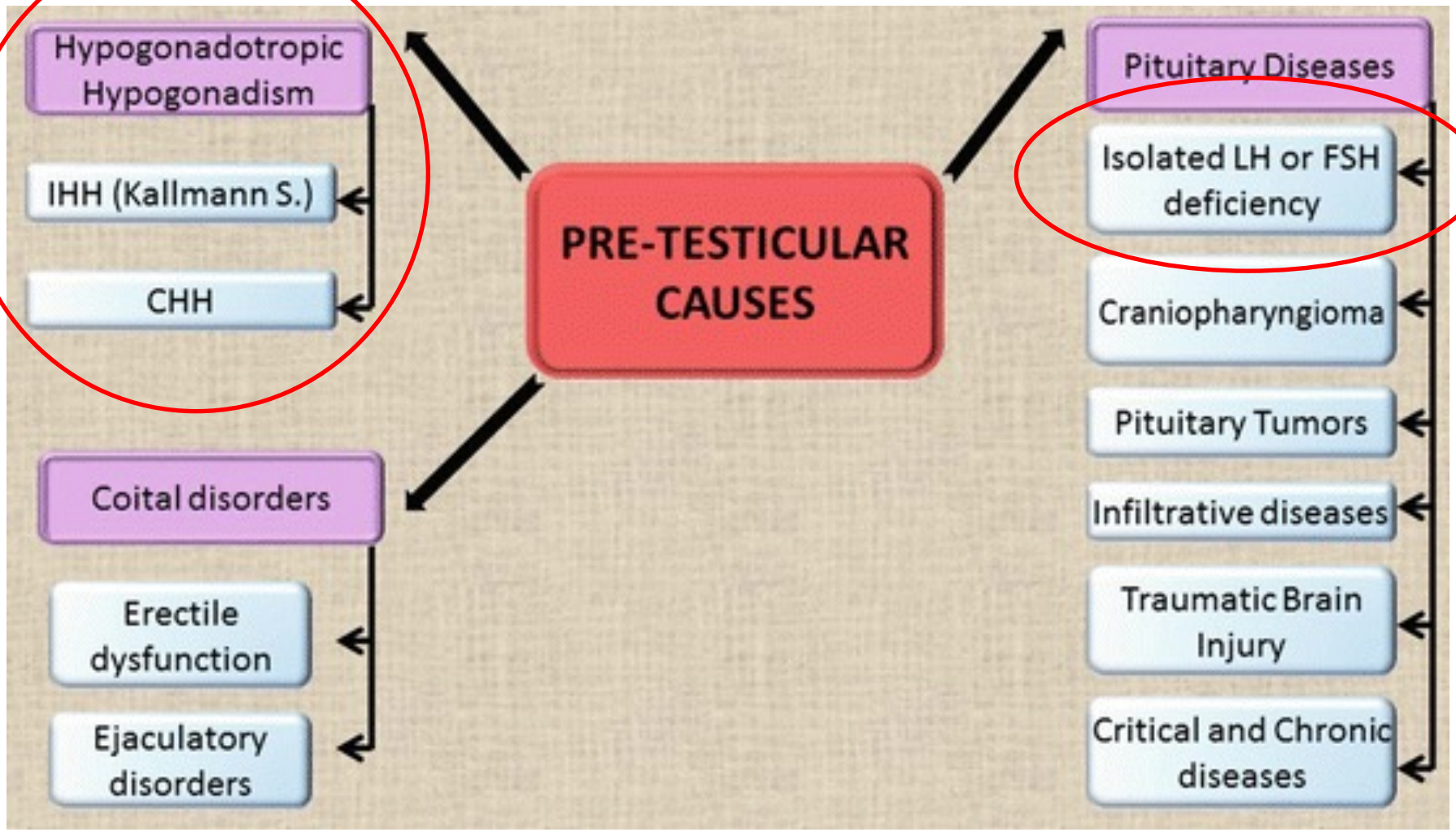
Category	*N*	*Percentage (%)*
Varicocele	629	26.4
Infectious	72	3.0
Hormonal	54	2.3
Ejaculatory dysfunction	28	1.2
Systemic diseases	11	0.4
Idiopathic	289	12.1
Immunologic	54	2.3
Obstruction	359	15.1
Cancer	11	0.5
Cryptorchidism	342	14.3
Genetic	189	7.9
Testicular failure	345	14.5
TOTAL	2,383	100.0

Source: Androfert, Center for Male Reproduction, Campinas, Brazil

Etiology of impaired sperm production

- Pre-testicular
- Testicular level
- Post-testicular

Genetic factors can be identified in each etiologic category



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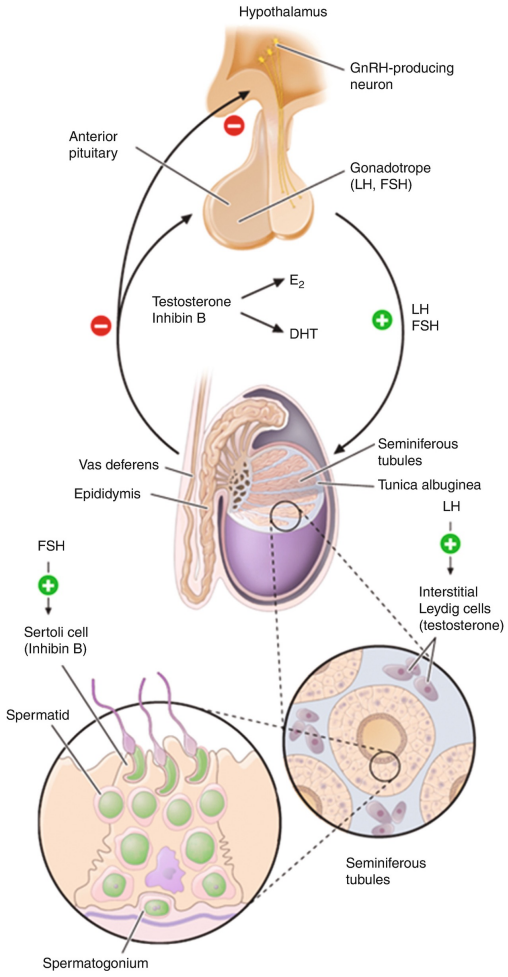
Hypogonadotropic Hypogonadism

A fully efficient hypothalamic–pituitary–gonadal axis is required for both endocrine and reproductive functions of the testis

Genetic factors causing deficit of gonadotropins **(LH, FSH)** may act at the hypothalamic or pituitary level and are responsible for the congenital forms of hypogonadotropic hypogonadism



Hypogonadotropic Hypogonadism

The diagnosis of congenital hypogonadotropic hypogonadism is normally made before adulthood because in the majority of cases it is associated with delayed puberty.

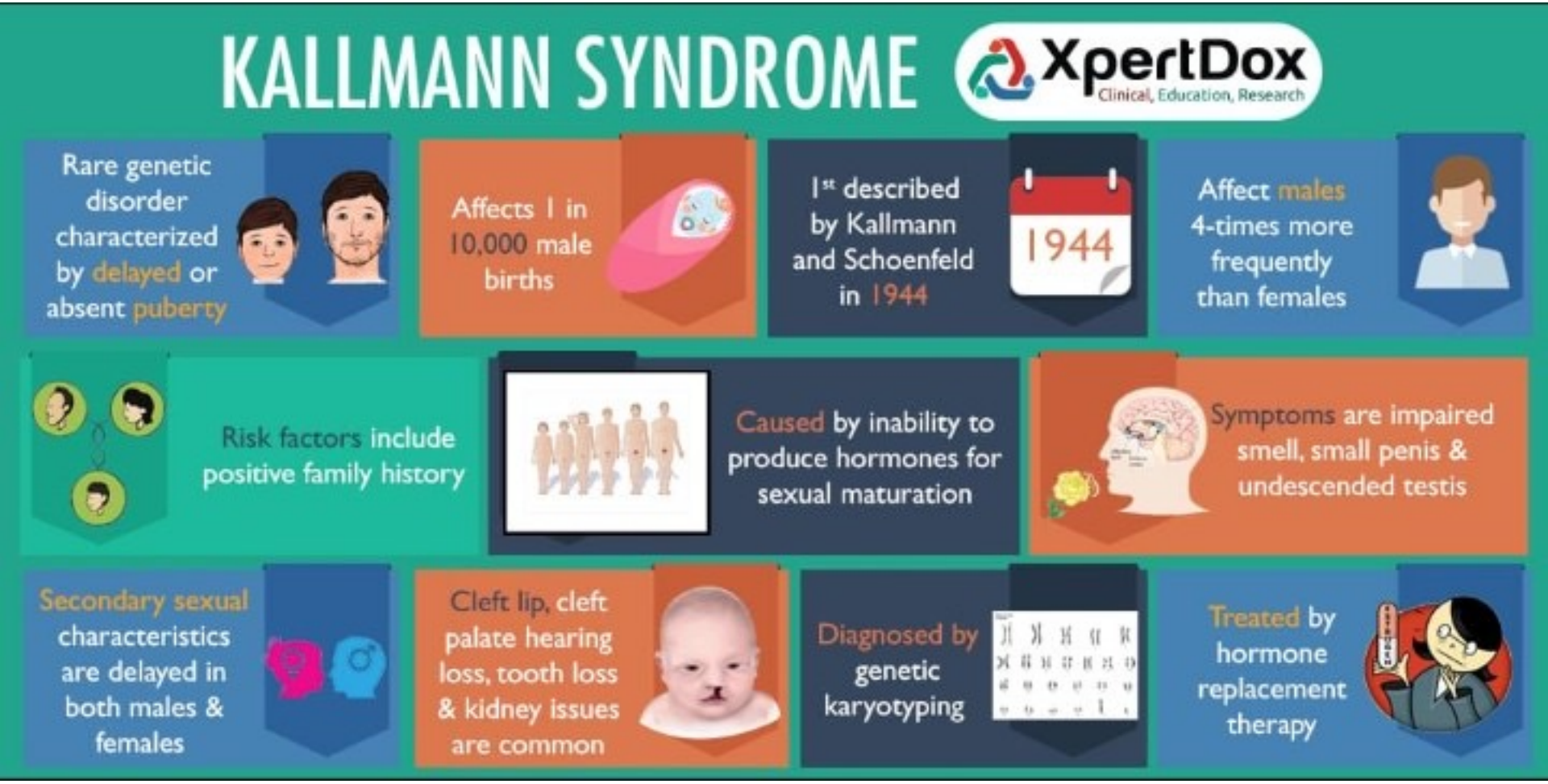
However, sometimes reduced spermatogenesis and mild hypoandrogenism may be the only symptoms and the diagnosis may be delayed till adulthood.

Genes can be screened for mutations in hypogonadotropic hypogonadism

Kallman Syndrome **Idiopathic hypogonadotropic hypogonadism (IHH)**

Table 1 Diagnostic Genetic Testing in Male Infertility

Gene or region	Indication for testing
Pretesticular	
KAL1	Kallmann syndrome
KAL2 (FGFR1)	Kallmann syndrome or normosmic IHH
PROK2/PROK2R	Kallmann syndrome or normosmic IHH
GnRHR	IHH (normosmic)
KiSS1/GPR54	IHH (normosmic)
FSH	Isolated FSH deficiency
LH	Isolated LH deficiency



https://steemit.com/steemstem/@shodiya/kallmann-syndrome-a-boy-in-a-man

Inheritance of Kallmann syndrome

- The inheritance of Kallmann syndrome can be X-linked (gene KAL1), therefore an affected father will transmit the mutation to his daughter who will have a 50% probability to generate a son with Kallmann syndrome.

- All other listed genes are autosomal, and the transmission of the disease maybe autosomal dominant (FGFR1) or recessive.

Kallmann versus IHH

- **Both can be managed by hCG or TRT**
- **Note that in some cases of IHH, long term testosterone treatment has lead to spontaneous reversibility of reproductive function**

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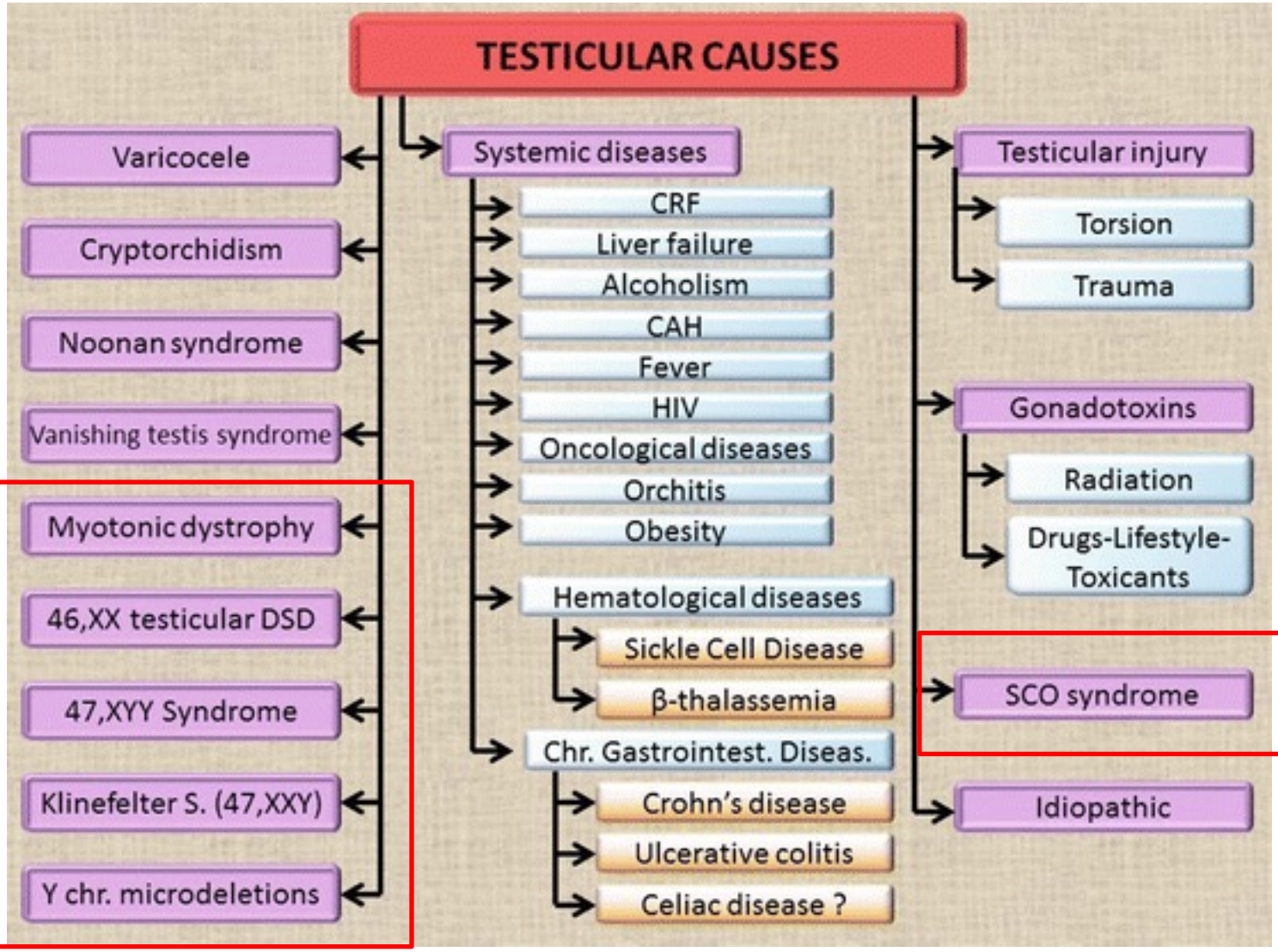
Home > Guidelines > Best Practices Statements > Male Infertility: Optimal Evaluation Best Practice Statement

Optimal Evaluation of the Infertile Male

- Patients with **acquired** hypogonadotropic hypogonadism should be evaluated for functioning and non-functioning pituitary tumors by measurement of serum prolactin and imaging of the pituitary gland.



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- Genetic anomalies related to **primitive testicular failure** can be detected in leukocytes or directly in spermatozoa

Table 1 Diagnostic Genetic Testing in Male Infertility

	Gene or region	Indication for testing
Leukocytes	*Testicular*	
	Chromosomal Anomalies (structural or numerical)	Azoospermia or sperm concentration <10 million/mL
	Y chromosome microdeletions	Azoospermia or sperm concentration <5 million/mL
	AR	Hypoandrogenized infertile male
	gr/gr[a]	Oligozoospermia
Spermatozoa	DNA integrity testing	
	sperm aneuploidy analysis fluorescence in situ hybridization (FISH)	

Chromosomal Abnormalities

- Karyotype abnormalities occur in about 0.4% of the **general population** and can affect the number or the structure of chromosomes

- The majority of chromosome abnormalities are generated during meiosis

- Patients with **spermatozoa <10 million/mL** show already a 10 times higher incidence (4%) of mainly autosomal structural abnormalities in respect to the general population

Chromosomal Abnormalities

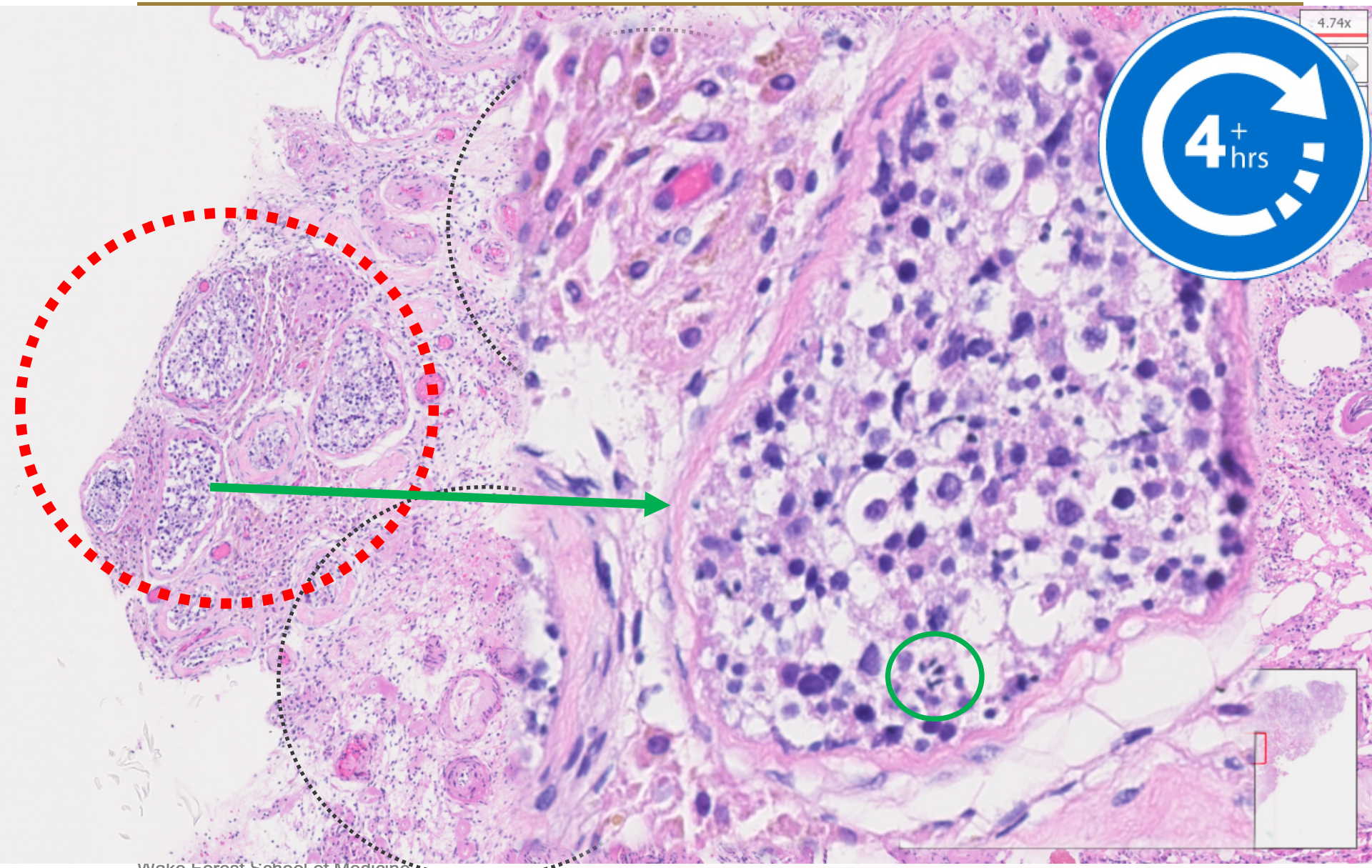
- Among **severe oligozoospermic** men (with spermatozoa <5 million/mL), the frequency increases to **7%**, whereas in **nonobstructive azoospermic men** it reaches the highest value,**15%.**

Klinefelter syndrome (47,XXY) represents the most common karyotype abnormality in severe male factor infertility, followed by
Ychromosomen terminal deletions (Yq-)
and
structural autosomal abnormalities

Klinefelter Syndrome

- Klinefelter syndrome is the most common sex chromosome abnormality in humans with an incidence of 1 in 600 live births and 1 in 300 spontaneous miscarriage
- It is also the most frequent chromosomal anomaly in azoospermic men (14%)
- About 80% of patients bear a 47,XXY karyotype, whereas the other 20% are represented either by 47,XXY/46,XY mosaics or higher grade sex chromosomal aneuploidy or structurally abnormal X chromosome

Microscopic Testicular Sperm Extraction



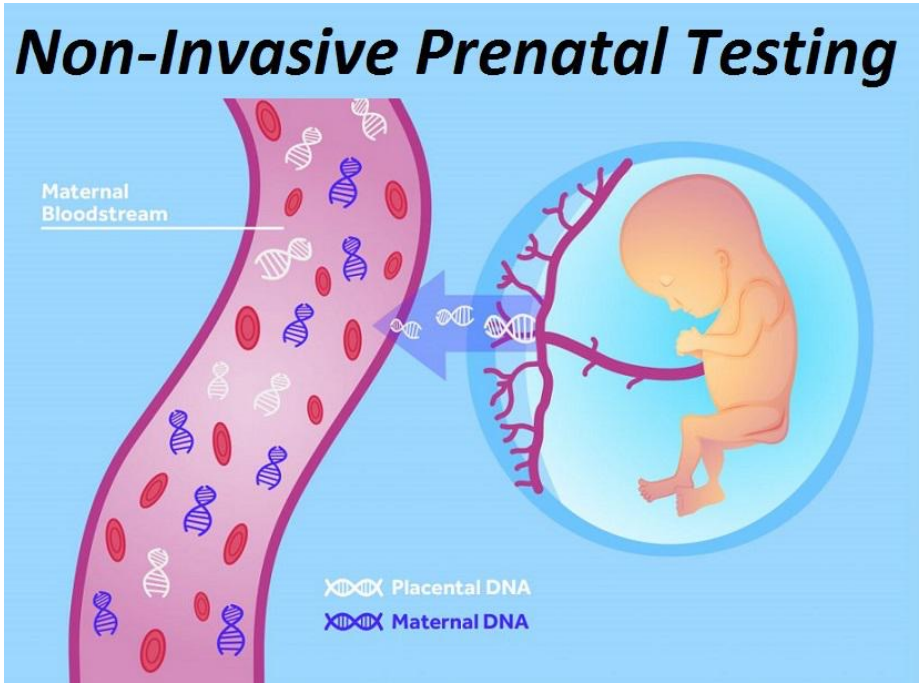
Inheritance of Klinefelter

- Although the large majority of subjects affected by Klinefelter syndrome are azoospermic, they may generate their own genetic children by undergoing TESE/ICSI, because they have an average of 30% to 50% of **testicular sperm recovery rate**.
- Based on sperm–FISH studies showing an **increased** frequency of sex chromosomal abnormalities and increased incidence of autosomal aneuploidies (disomy for chromosomes 13, 18, and 21), concerns have been raised about the chromosomal normality of the embryos generated through ICSI.

Inheritance of Klinefelter

- However, a study based on ICSI combined with PGD on 113 embryos shows that there is a significant fall in the rate of **normal embryos** for couples with Klinefelter syndrome, in respect to controls (54% vs. 77.2%). Due to the significant increase of sex chromosomal and autosomal abnormalities in the embryos of Klinefelter patients, ICSI along with PGD or NIPT should be strongly advised

NIPT (Non-Invasive Prenatal Testing)



- It's available any time after **9 weeks** of pregnancy (10-22 weeks)
- Routinely covered by <u>insurance</u> for women 35 years or older and women at high risk for genetic abnormalities

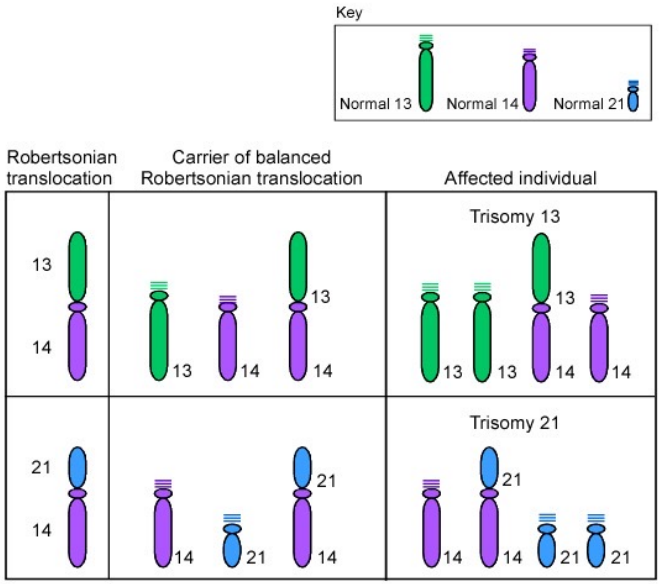
CHROMOSOME	N	SENSITIVITY	95% CI	SPECIFICITY	95% CI
Trisomy 21 (Down Syndrome)	951	99.99% (50/50)	92.89-100.0	99.89% (900/901)	99.38-100.0
Trisomy 18 (Edwards syndrome)	978	99.99% (32/32)	89.11-100.0	99.99% (946/946)	99.61-100.0
Trisomy 13 (Patau syndrome)	988	99.99% (12/12)	73.54-100.0	99.69% (973/976)	99.1-99.94
Monosomy X	904	99.99% (10/10)	69.15-100.0	99.89% (893/894)	99.38-100.0
XX	526	98.33% (59/60)	91.06-99.96	99.14% (462/466)	97.82-99.77
XY	560	99.99% (44/44)	91.96-100.0	99.99% (516/516)	99.29-100.0

Autosomal Abnormalities

- The most frequently found autosomal karyotype abnormalities are :
  - **Robertsonian translocations**
  - **Reciprocal translocations**
  - **Paracentric inversions, and marker chromosomes**

Robertsonian translocations

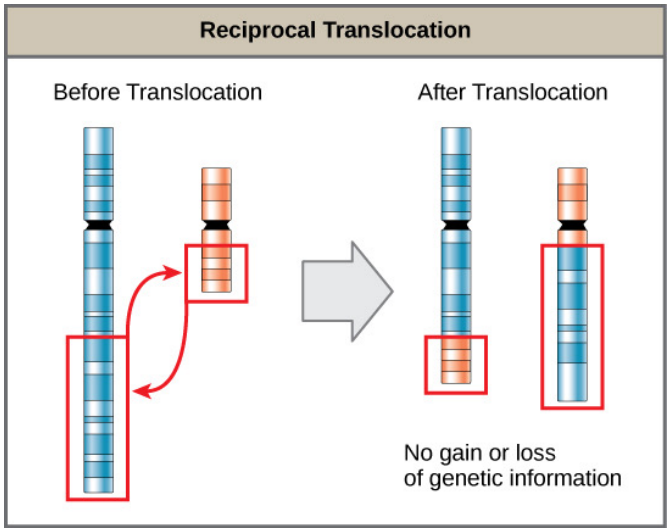
- Fusion of two acrocentric chromosomes



- This abnormality is rarely observed in azoospermicmen (0.2%) but is often found in oligozoospermic patients (about nine times higher in infertile men than in newborns).

Reciprocal translocations

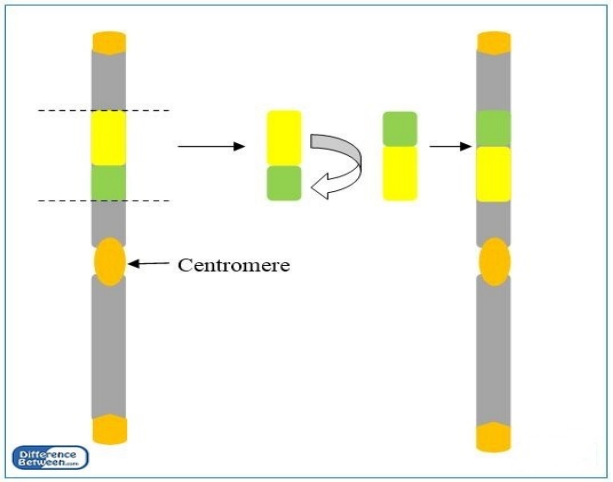
- Exchange of chromosome material between arms of two nonhomologous chromosomes; usually the exchange is conservative without loss of genetic material



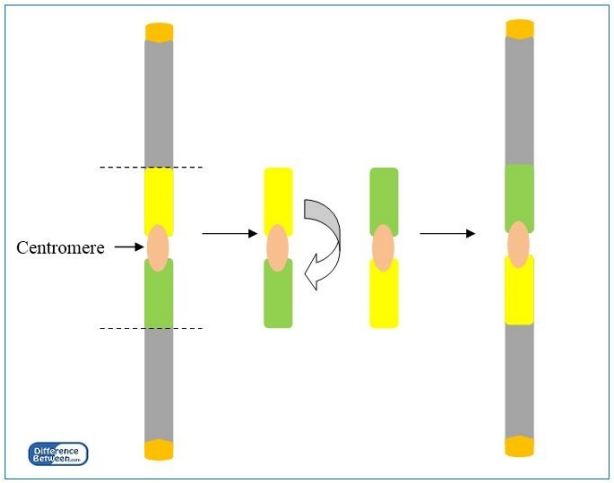
- The frequency of **balanced reciprocal translocations** is estimated to be 5 to10 times higher in infertile men than in the general population

Paracentric/Pericentric inversions

- These inversions result from two breaks within a single chromosome followed by a 180° rotation of the chromatin between these breaks



Paracentric inversion



Pericentric Inversion

- These rearrangements are 13 times higher in infertile men and probably interfere with meiosis, leading to a reduced rate of postmeiotic sperm development.

47,XYY Male

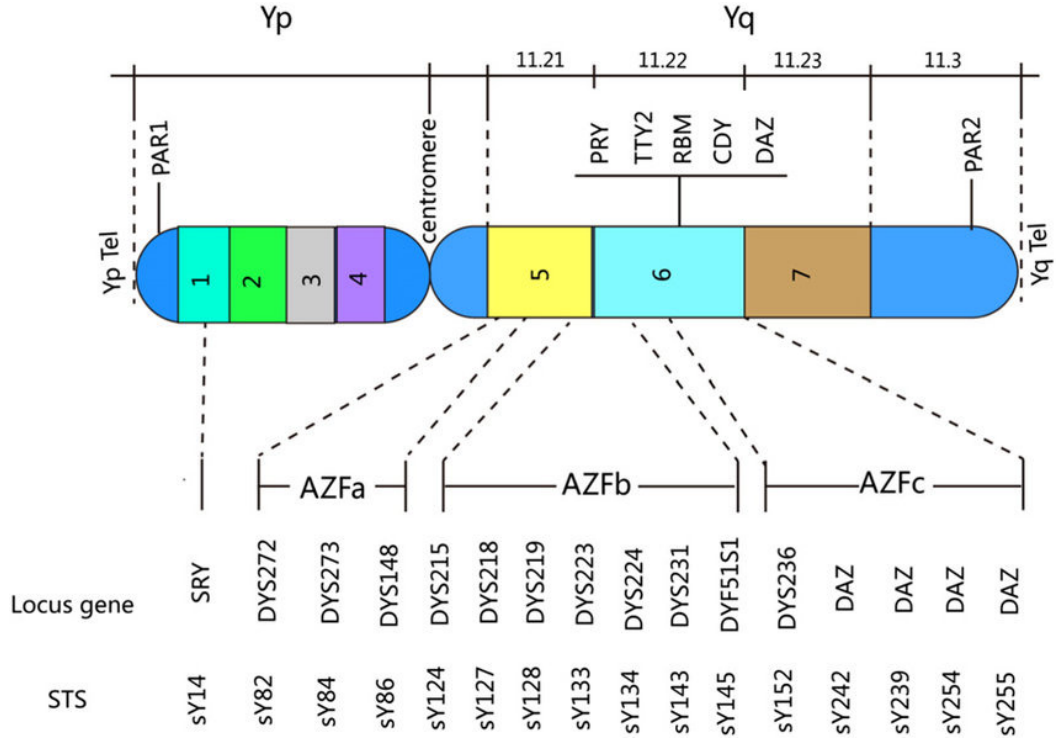
- The frequency of males with this karyotype is 1:750
- Carriers of this abnormality show a great diversity in the degree of spermatogenic impairment, ranging from severe oligozoospermia to apparent normality
- Distortion of sex vesicle formation is probably the major cause of disturbed spermatogenesis in these men

46 ,XX Male

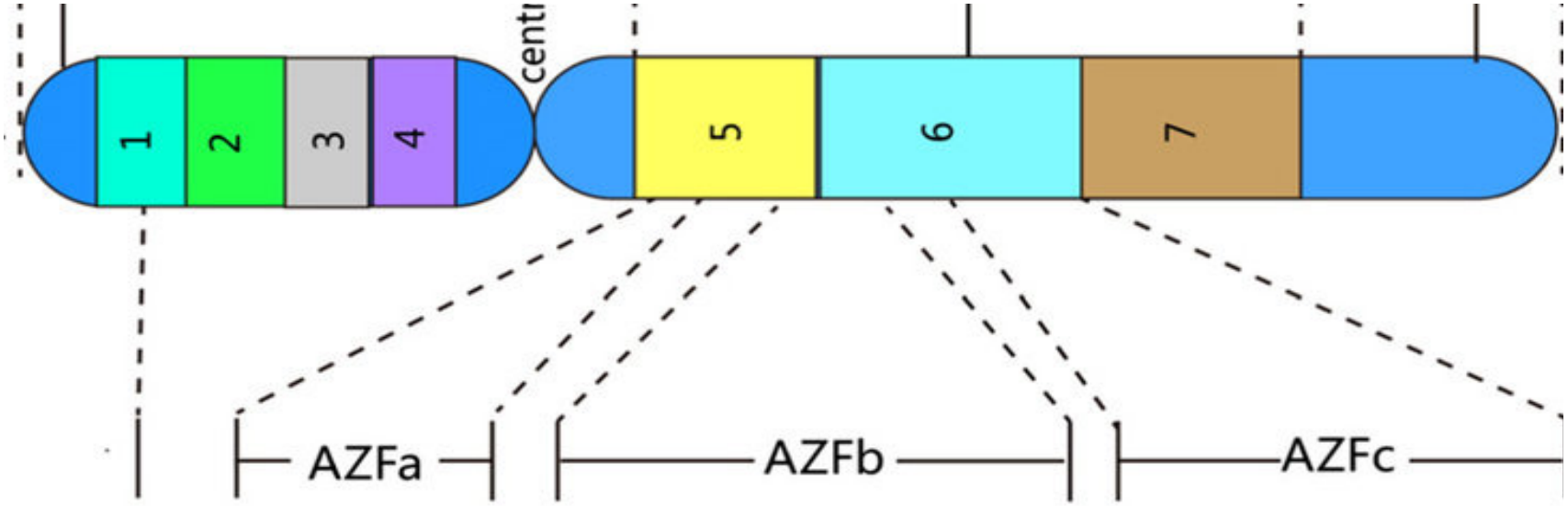
- This is a disorder of sex determination and occurs in about 1:20,000 newborns
- In about 80% of cases, XX maleness can be explained by the translocation of the SRY gene (encoding the testis-determining factor) to the X chromosome
- The phenotypic features of the syndrome are gynecomastia, female hair pattern, and small testes with azoospermia. Genital malformations such as hypospadias are rare

The Y Chromosome–Linked Infertility

- The long arm of the human Y chromosome (Yq) hosts a number of genes involved in spermatogenesis and several types of recurrent Yq deletions are firmly associated with spermatogenic failure



The clinical significance of Yq deletions



- The most frequently deleted region is AZFc (approximately 65–70%), followed by deletions of the AZFb and AZFb+c or AZFa+b+c regions (25–30%), whereas deletions of the AZFa region are extremely rare (5%)

- The complete removal of the AZFa and AZFb regions are associated with severe testicular phenotype Sertoli cell-only syndrome and spermatogenic arrest, respectively.

Inheritance of Y deletions

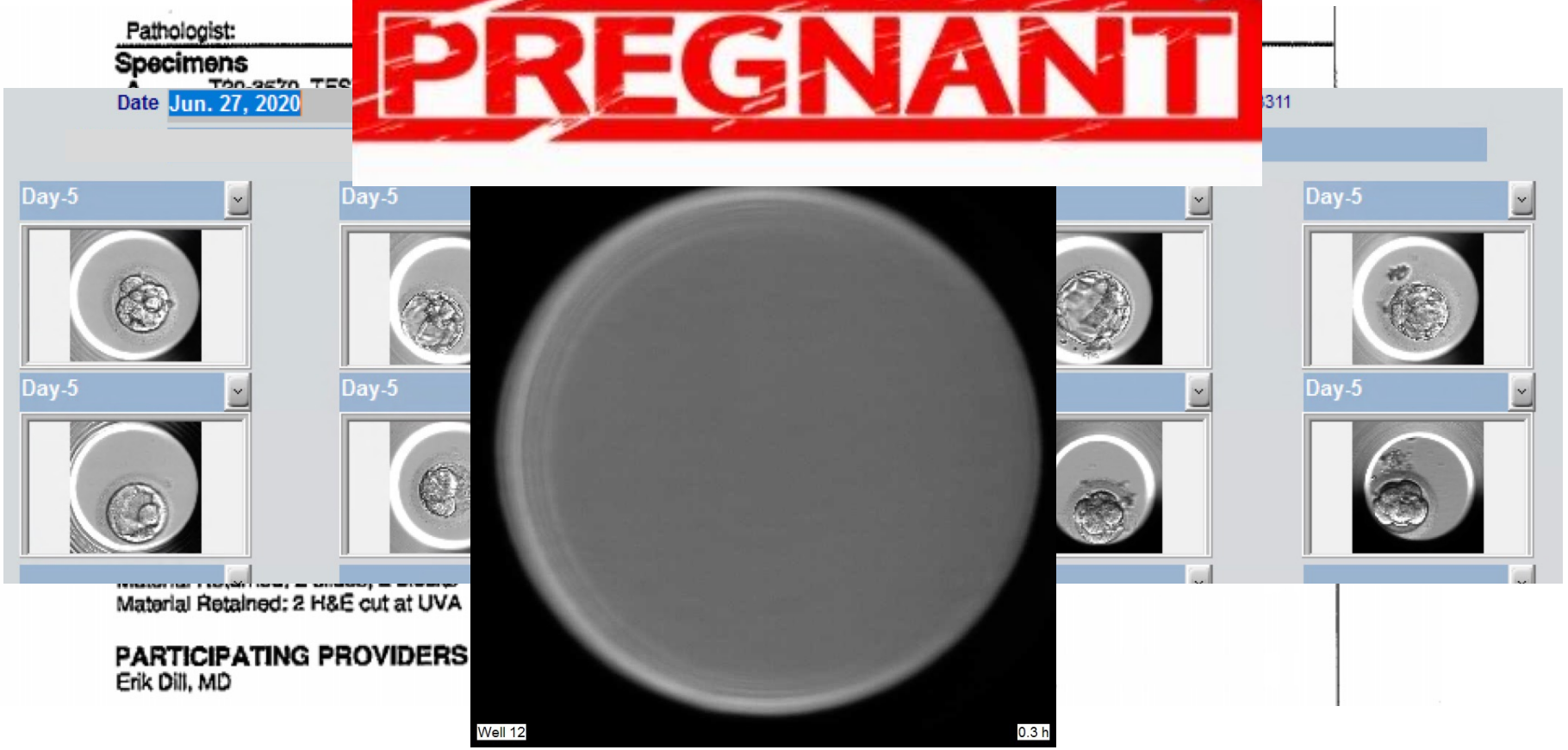
- Those Y deletions that are compatible with the presence of spermatozoa in the testis or in the ejaculate, are obligatory transmitted to the male offspring, therefore **genetic counseling** is mandatory

- It has been reported that a significant proportion of spermatozoa from men with Y microdeletion are nullisomic for sex chromosomes. This result indicates a potential risk for the offspring to develop 45,X0 Turner's syndrome and other phenotypic anomalies associated with sex chromosome mosaicism, including ambiguous genitalia.
- PGD can be offered to the couple both for sex selection and for avoiding the transfer of 45,X0 embryos

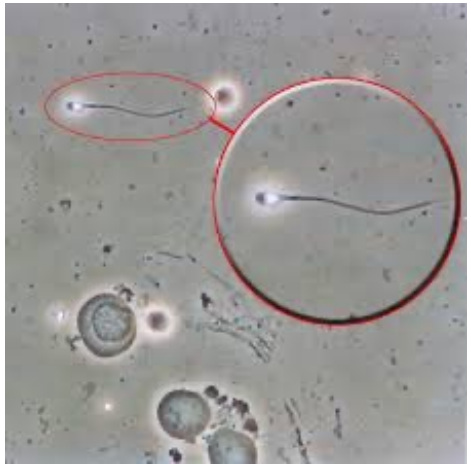
Microscopic Testicular Sperm Extraction (Micro-TESE)

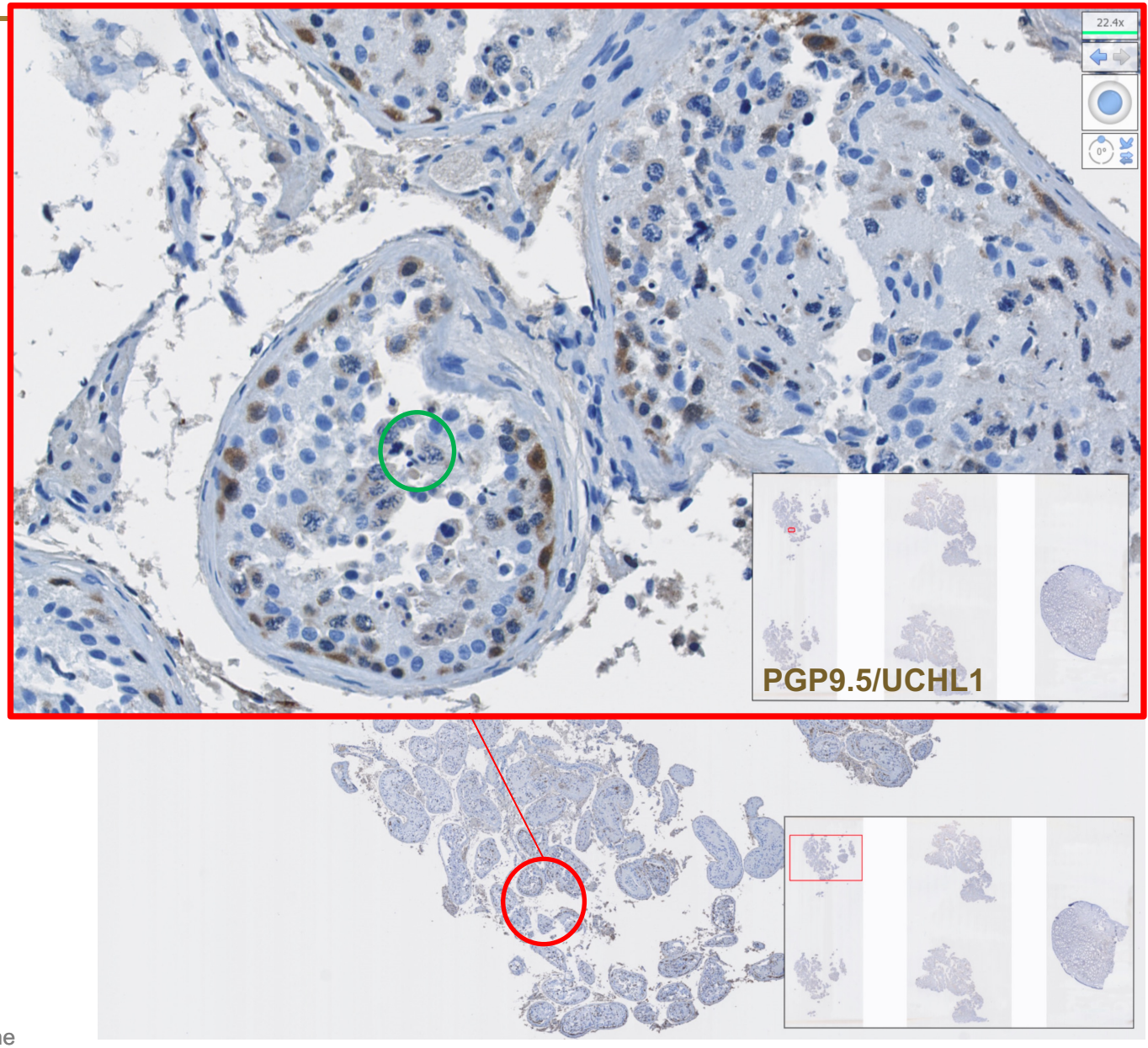
26 y old. AZFc deletion (DAZ & SPGY)
T 270 ng/dl (T 809 on Clomid + Anastrozole)
FSH 3.6 (FSH 12.4 on Clomid + Anastrozole)
LH 3.4 (LH 26.1 on Clomid + Anastrozole)
Testes sizes :

PREGNANT



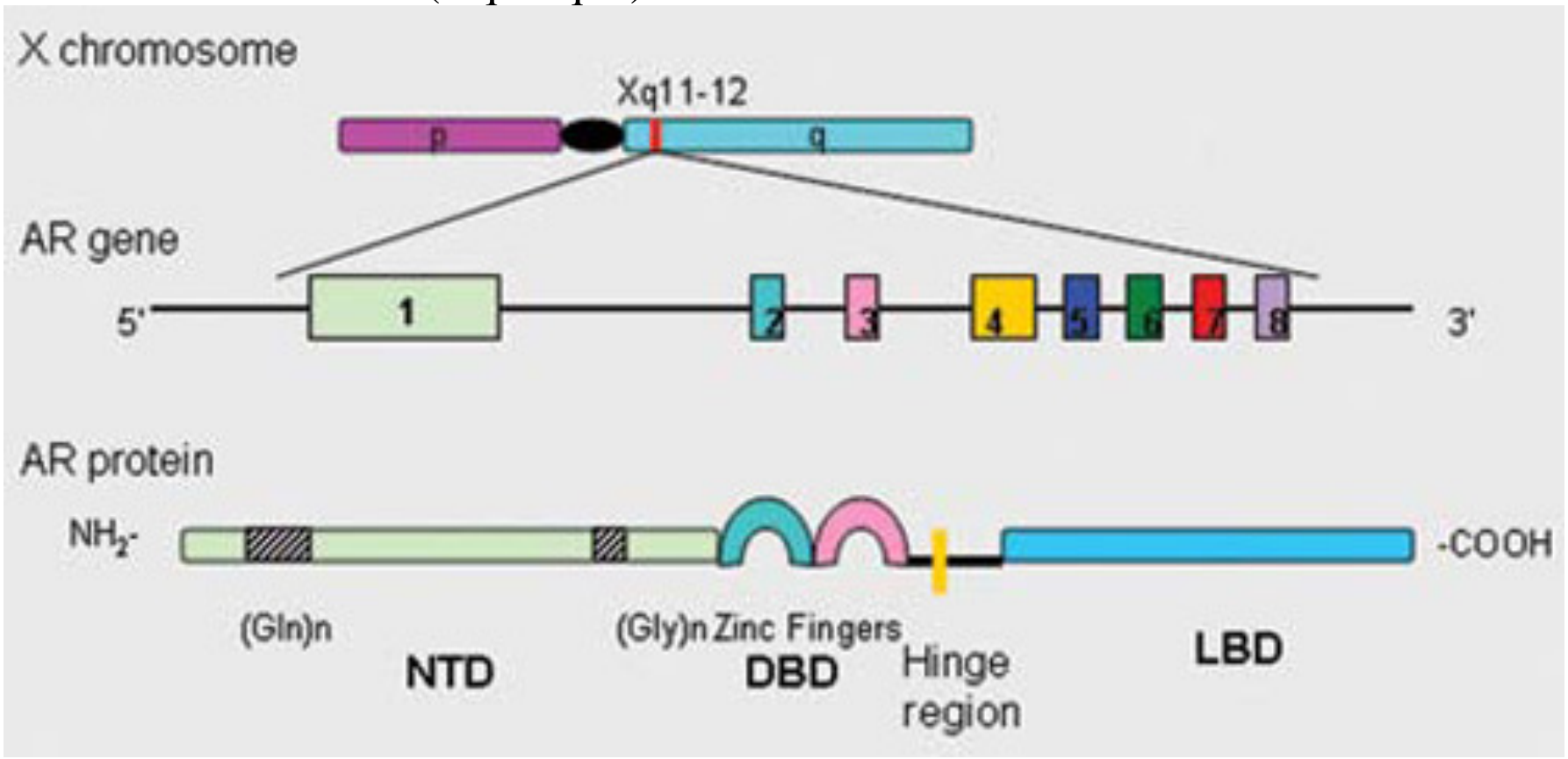
Pathology versus Embryology





Mutations and Polymorphisms in the Androgen Receptor

- The androgen receptor (AR) gene is located on the long arm of the X chromosome (Xq11-q12)



Sperm Chromosome Abnormalities

- Sperm DNA FISH analyses showed that even subjects with normal chromosomal constitution in their lymphocytes, but affected by infertility, have an increased risk for spermautosomal and sex chromosomal abnormalities (varying from 2–10 times higher than controls).

- Specific infertile phenotypes are at even higher risk than others such as round head only syndrome, macrocephaly, or a high percentage of multiflagellated sperm

Role for FISH analysis?

- Chr FISH (13,
- 1. I rs suf
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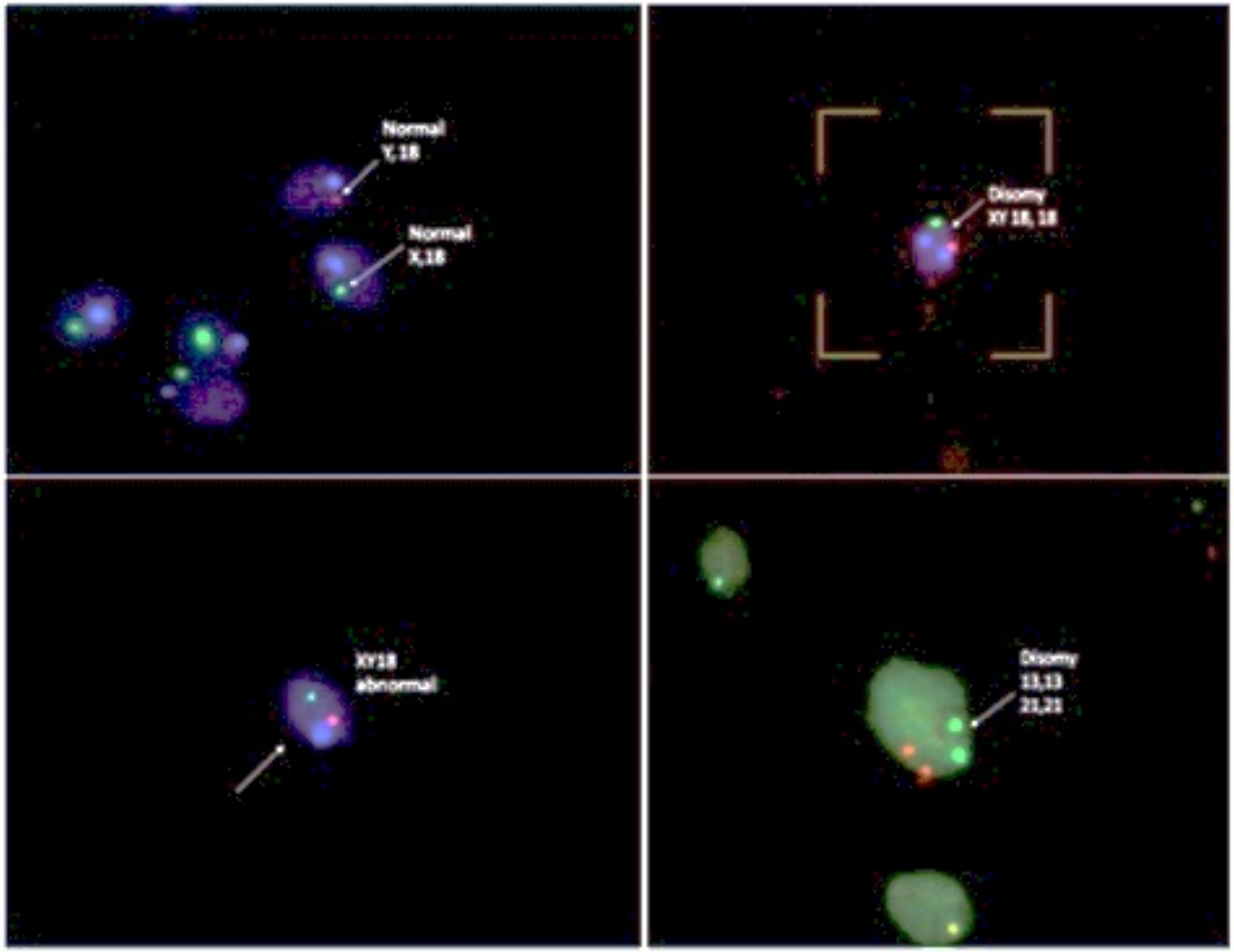
- 3. A and ger ose who RT

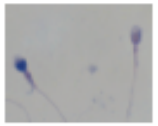
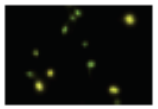
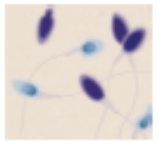
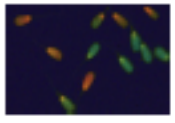
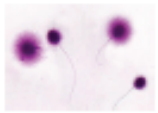
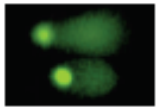


Sperm DNA Fragmentation (SDF)

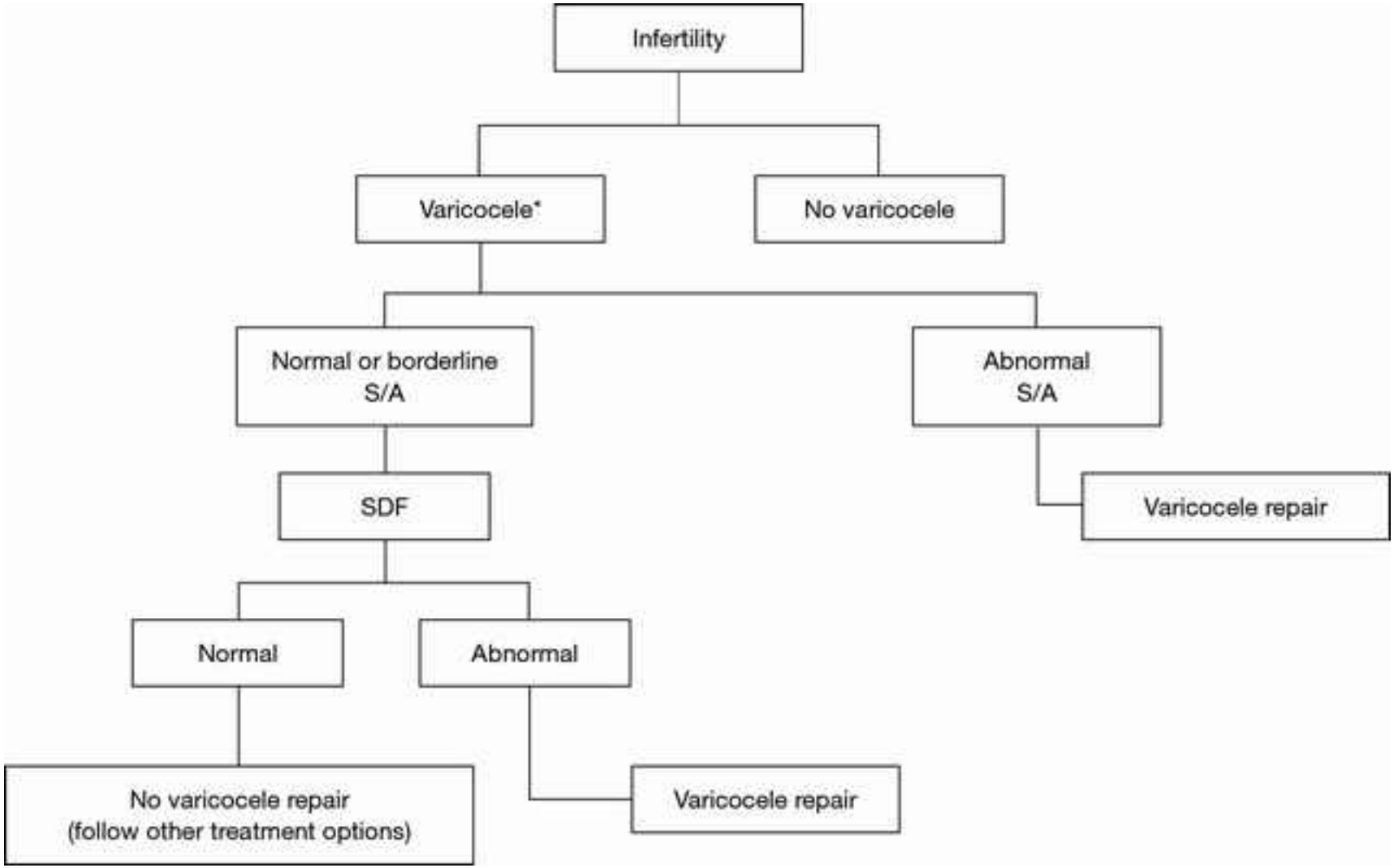
General Concepts

- Sperm of infertile men has ↑ level of DNA damage
- DNA damage is associated with impaired post fertilization embryo cleavage
- Seen as unexplained infertility or repeated early miscarriage

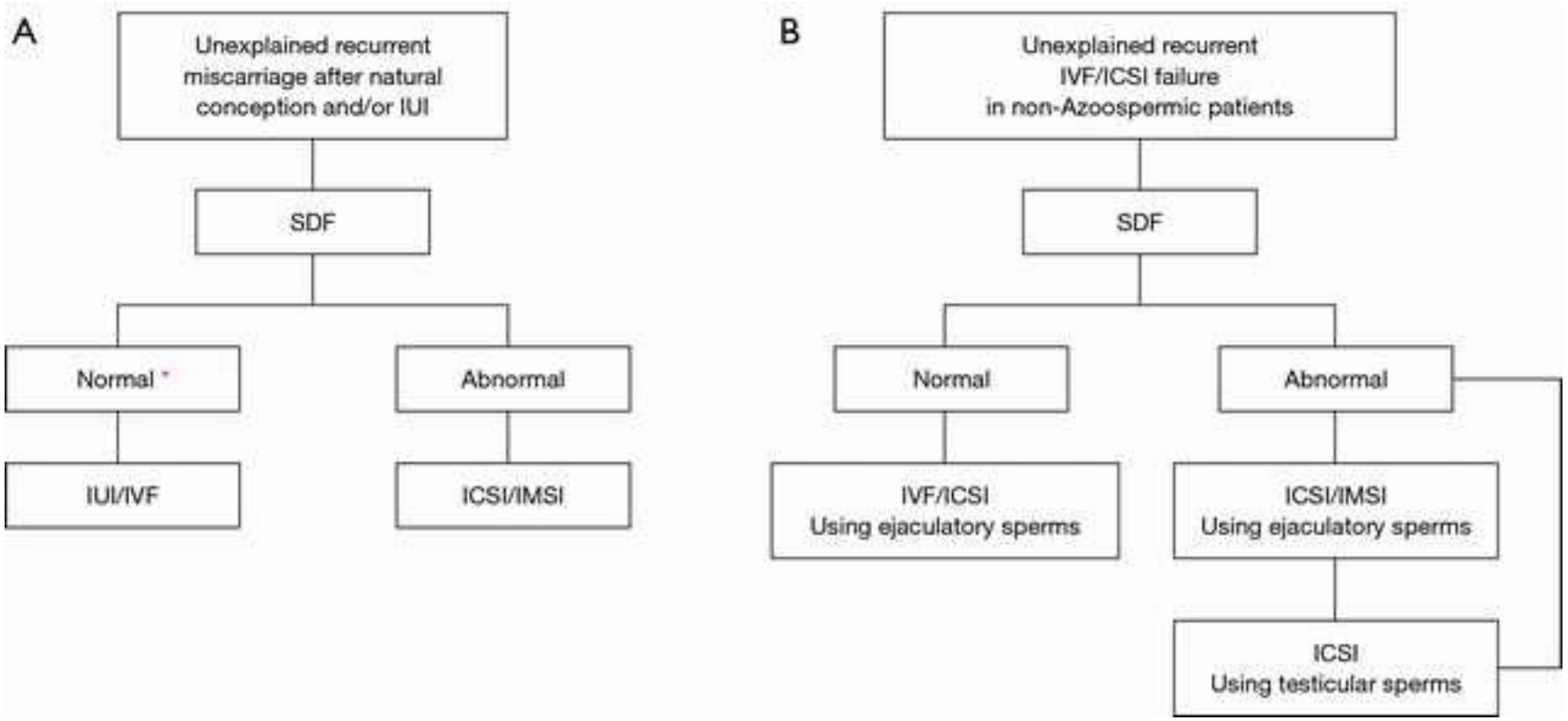
Sperm DNA fragmentation (SDF) testing methods

	Test	Principle	Advantage	Disadvantage
 [1]	AO test	Metachromatic shift in fluorescence of AO when bound to single strand (ss)DNA. Uses fluorescent microscopy	Rapid, simple and inexpensive	Inter-laboratory variations and lack of reproducibility
 [2]	AB staining	Increased affinity of AB dye to loose chromatin of sperm nucleus. Uses optical microscopy	Rapid, simple and inexpensive	Inter-laboratory variations and lack of reproducibility
 [3]	CMA3 staining	CMA3 competitively binds to DNA indirectly visualizing protamine deficient DNA. Uses fluorescent microscopy	Yields reliable results as it is strongly correlated with other assays	Inter-observer variability
 [4]	TB staining	Increased affinity of TB to sperm DNA phosphate residues. Uses optical microscopy	Rapid, simple and inexpensive	Inter-observer variability
 [5]	TUNEL	Quantifies the enzymatic incorporation of dUTP into DNA breaks. Can be done using both optical microscopy and fluorescent microscopy. Uses optical microscopy, fluorescent microscopy and flow cytometry	Sensitive, reliable with minimal inter-observer variability. Can be performed on few sperm	Requires standardization between laboratories
 [6]	SCSA	Measures the susceptibility of sperm DNA to denaturation. The cytometric version of AO test. Uses flow cytometry	Reliable estimate of the percentage of DNA-damaged sperm	Requires the presence of expensive instrumentation (flow cytometer) and highly skilled technicians
 [7]	SCD or Halo test	Assess dispersion of DNA fragments after denaturation. Uses optical or fluorescent microscopy	Simple test	Inter-observer variability
 [8]	SCGE or comet assay	Electrophoretic assessment of DNA fragments of lysed DNA. Uses fluorescent microscopy	Can be done in very low sperm count. It is sensitive and reproducible	Requires an experienced observer. Inter-observer variability

SDF and Varicocelectomy



SDF, Ejaculatory and Testicular Sperm



Majid Mirzazadeh, Hooman Sadri-Ardekani TAU 2017

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Home > Guidelines > Best Practices Statements > Male Infertility: Optimal Evaluation Best Practice Statement

Optimal Evaluation of the Infertile Male

RECOMMENDATION

- Karyotyping and genetic counseling should be offered to all patients with **nonobstructive azoospermia** and **severe oligospermia (<5 million sperm/ml).**
- There are insufficient data to recommend a minimal number of sequence tagged sites to test for in patients undergoing Y chromosome microdeletion analysis. Although the prognosis for sperm retrieval is poor in patients **having large deletions** involving **AZF region a or b**, the results of Y chromosome deletion analysis cannot absolutely predict the absence of sperm**.**

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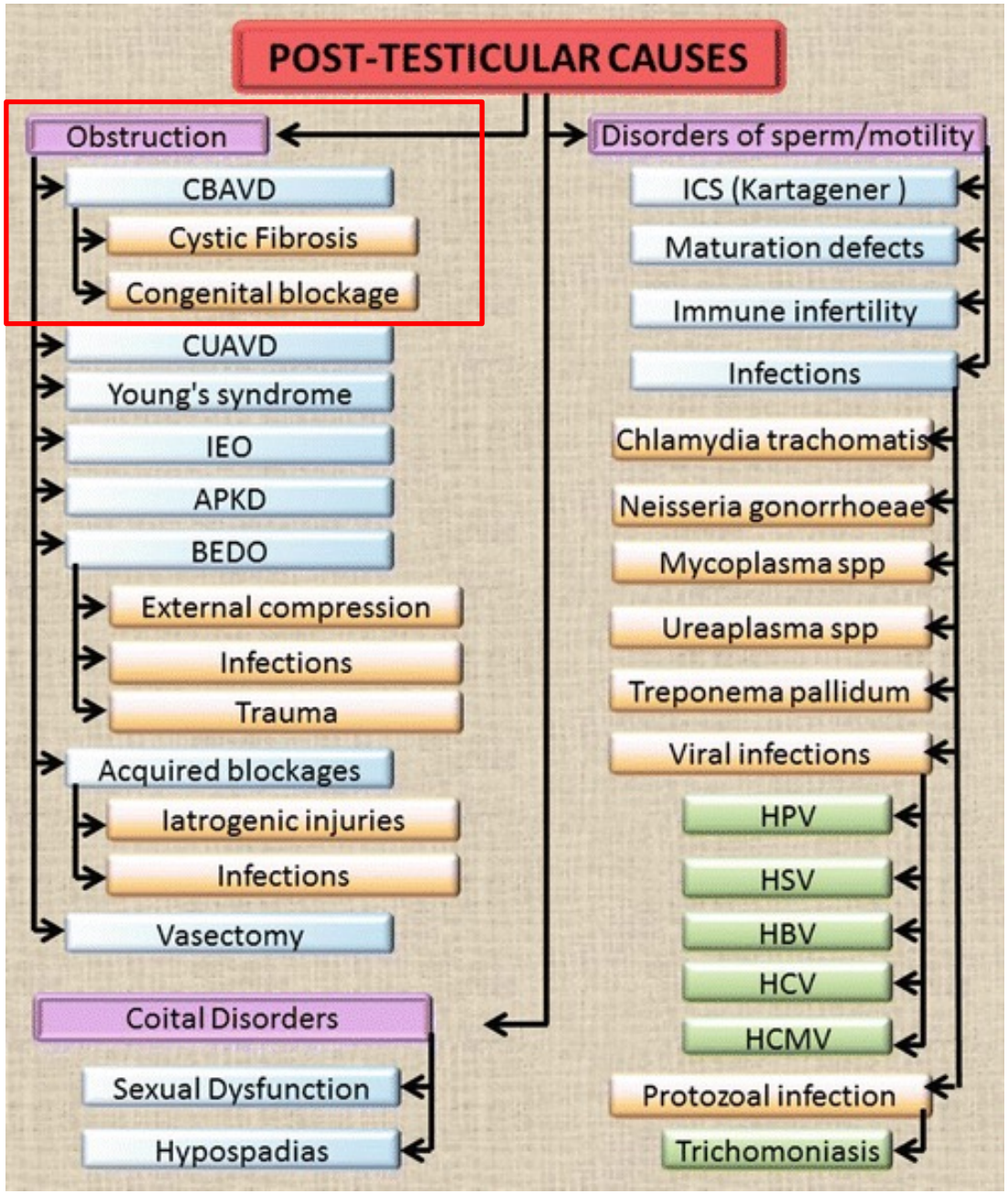
Home > Guidelines > Best Practices Statements > Male Infertility: Optimal Evaluation Best Practice Statement

Optimal Evaluation of the Infertile Male

RECOMMENDATION

- All patients with non-obstructive azoospermia due to **primary testicular failure** should be offered genetic testing



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CFTR (cystic fibrosis transmembrane conductance regulator) mutations

- The autosomal CFTR (cystic fibrosis transmembrane conductance regulator) gene (7q31.2)is highly mutated with **more than 1500 mutations** and variants described in the gene bank
- Depending on the severity of the reduction of functionally normal CFTR protein, the phenotype can be cystic fibrosis (CF) (generally due to the presence of two "**severe**" mutations) or "**mild** forms" of CF (combination of less severe mutations with a consequent reduction of functional CFTR protein below 50% but above 10%).
- Congenital agenesis of vas deferens (CAVD) is considered a "**mild form**" of CF.
- The most widely diffused mutation both in CF and CAVD is the **severe delta F508 mutation** (about70%of the total CF mutations in patients).

CFTR and Male Infertility

- Patients affected by CAVD may have sperm in their ejaculate (monolateral absence of vas deferens) or be azoospermic (congenital bilateral absence of vas deferens; CBAVD).

- All patients with CF have CBAVD.

Table 1 Diagnostic Genetic Testing in Male Infertility

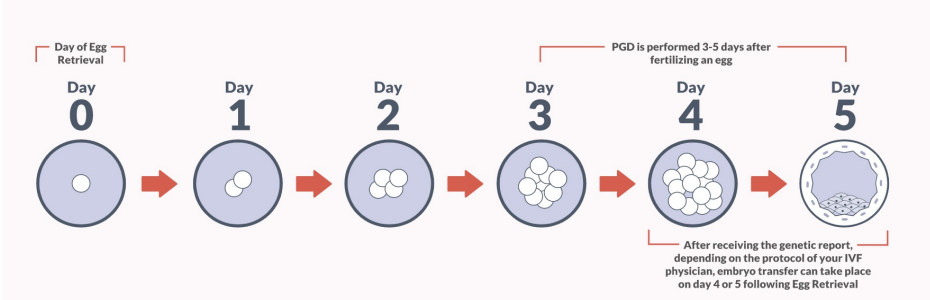
Gene or region	Indication for testing
Post-testicular	
CFTR	Congenital Absence of Vas Deferens (mono/bilateral) Idiopathic epididymal obstruction

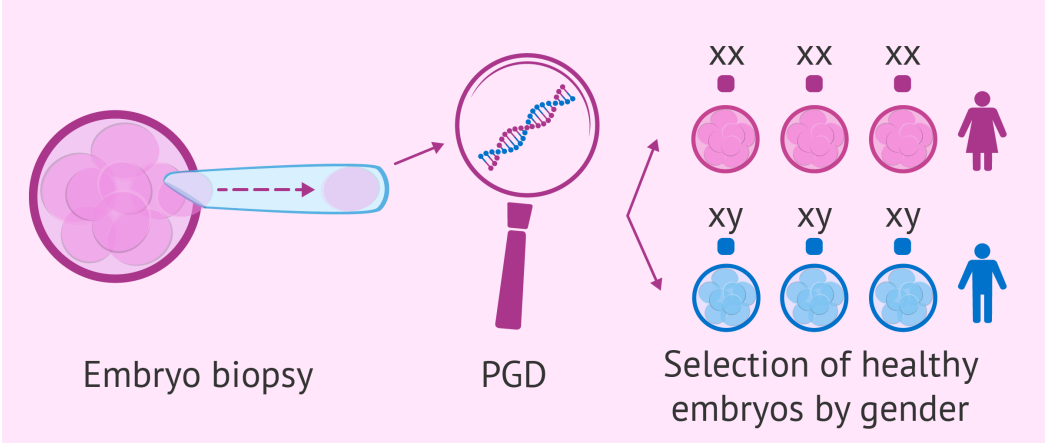
Inheritance of CFTR mutation

- The possibility to combine **testis biopsy** with intracytoplasmic sperm injection (ICSI), both CF or CBAVD patients may now generate their own biological children therefore **they can transmit** their CFTR mutations to their descendants
- Because the carrier frequency of CFTR mutations in persons with Northern European descent is **high (1:25),** the screening for CF gene mutations in the female partners of men with CAVD should be performed before assisted reproduction

CFTR mutation and PGD

- If mutations are detected in both partners (possibly performing a whole gene screening), the risk of an offspring with CF (or mild forms of CF such as CAVD, depending on the type and combination of mutations) is very high so preimplantation genetic diagnosis (PGD) should be advised to the couple





American Urological Association

Home > Guidelines > Best Practices Statements > Male Infertility: Optimal Evaluation Best Practice Statement

Optimal Evaluation of the Infertile Male

RECOMMENDATION

- Men with congenital bilateral absence of the vasa deferentia should be offered genetic counseling and testing **for cystic fibrosis transmembrane conductance regulator mutations**. The female partner should also be offered cystic fibrosis transmembrane conductance regulator mutations testing before proceeding with treatments that utilize the sperm of a man with congenital bilateral absence of the vasa deferentia.
- **Imaging for renal abnormalities** should be offered to men with unilateral vasal agenesis or congenital bilateral absence of the vasa deferentia and no evidence of cystic fibrosis transmembrane conductance regulator abnormalities.

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- Testing for cystic fibrosis transmembrane conductance regulator abnormalities should include at minimum a panel of common point mutations and the **5T allele**. There currently is **no consensus** on the minimum number of mutations that should be tested
- Gene sequencing may be considered in couples where the wife is a carrier and the husband with congenital bilateral absence of the vasa deferentia tests negative on a routine panel of cystic fibrosis transmembrane conductance regulator mutations.

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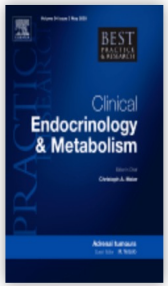
Optimal Evaluation of the Infertile Male

- Patients with unilateral absence of the vas and low volume azoospermia, may have a varient of CBAVD and should have CFTR and 5T testing and if positive do not need TRUS.

ELSEVIER

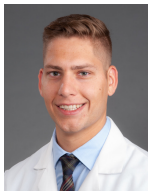
SPECIAL ISSUE



Best Practice & Research: Clinical Endocrinology & Metabolism

Editor-in-Chief: Christoph A. Meier

Clinical Endocrinology and Metabolism
Genetics of Male Infertility



1. **Infertility considerations in patients with Klinefelter Syndrome**
2. **Clinical implications of Y-chromosome microdeletions among infertile men**
3. **Genetic mutations contributing to non-obstructive azoospermia**
4. **Congenital Absence of the vas deferens Cystic Fibrosis Transmembrane Regulatory Gene Mutations**
5. **Genetic mutations of teratozoospermia**
6. **Kallman Syndrome and central non-obstructive azoospermia**
7. **Epigenetic implications in male infertility**
8. **Genetic underpinnings of asthenozoospermia**
9. **Genetic underpinnings of Teratozoospermia**
10. **Karyotypic Abnormalities resulting in Infertility Beyond Klinefelter Syndrome & Y-Chromosome Microdeletions**
11. **Sperm genetic abnormalities and contribution to embryo aneuploidy & miscarriage**
12. **Implications of small and long noncoding RNAs in male infertility**

Wake Forest School of Medicine

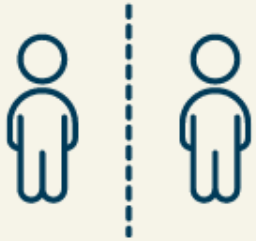
FUTURE SESSIONS

- Session One: Clinical investigation of the infertile male
- Session Two: Genetic causes of male infertility and their impact on future generations
- **Session Three: Medical Treatments for Male Infertility**
- **Session Four: Surgical Treatments and Assisted Reproductive Technology (ART) for Male Infertility**
- **Session Five: Ejaculatory disorders**
- **Session Six: Clinical investigation and laboratory analyses in male hypogonadism**
- **Session Seven: Testosterone deficiency syndrome, , Androgen replacement—indications and principles**
- **Session Eight: Female-to-Male Transsexualism**

THE THREE W'S

WASH YOUR HANDS

WATCH YOUR DISTANCE



WEAR A MASK