



**Male Infertility**

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**Section 1**

**Physiology and  
Anatomy of Male  
Infertility**

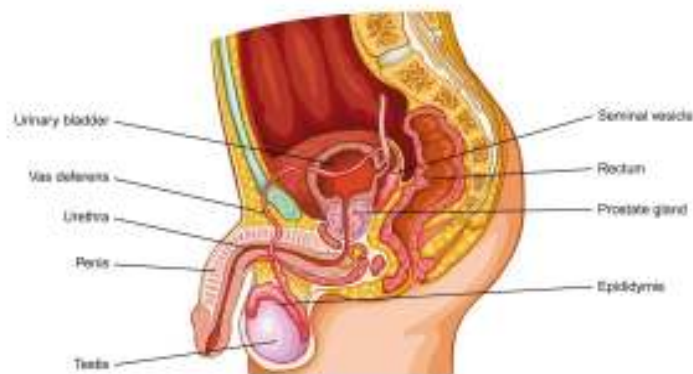


## Learning Objectives

After this section, participants should be better able to understand:

- The physiology and anatomy of male infertility

## Anatomy of the Male Reproductive System



## Development of Male Reproductive System

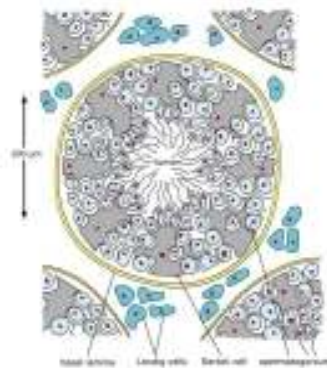
- Development of male reproductive system
  - Gonads are undifferentiated in the first weeks of fetal development
  - In the male fetus:
    - Sex determination is initiated by a sex-specific gene on the Y-chromosome
    - Influence of testosterone: the Wolffian ducts mature into the male genital system
    - Influence of Mullerian Inhibiting Substance (also known as anti-Müllerian hormone): suppresses development of the Mullerian ducts (which form the uterus and Fallopian tubes in the female)

Heffner LJ, Schust DJ. *The Reproductive System at a Glance*. Hoboken, NJ: John Wiley & Sons Ltd; 2010.; Robaire B. *Handbook of Andrology*. 2nd ed. Lawrence, KS: Allen Press, Inc.; 2010.; de Kretser DM, Temple-Smith PD., Kerr JB. et al. Anatomical and functional aspects of the male reproductive organs. In: Bandhauer J, Frick K, eds. *Disturbances in Male Fertility*. Berlin, Germany: Springer-Verlag; 1982: 1–131.; Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.; Glover TD, Barrat CLR, eds. *Male Fertility and Infertility*. Cambridge, United Kingdom: Cambridge University Press; 1999.



## Anatomy – Testes

- Sertoli cells
  - Nurse cells
  - Provides nutrition to developing sperm cells through spermatogenesis
  - Establish and maintain spermatogonial stem cell niches
- Leydig cells
  - Synthesise and release androgens in response to luteinising hormone



Robaire B. *Handbook of Andrology*. 2nd ed. Lawrence, KS: Allen Press, Inc.; 2010.; de Kretser DM, Temple-Smith PD., Kerr JB. et al. Anatomical and functional aspects of the male reproductive organs. In: Bandhauer J, Frick K, eds. *Disturbances in Male Fertility*. Berlin, Germany: Springer-Verlag; 1982: 1–131.; Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.; Glover TD, Barrat CLR, eds. *Male Fertility and Infertility*. Cambridge, United Kingdom: Cambridge University Press; 1999.



## Physiology – Spermatogenesis

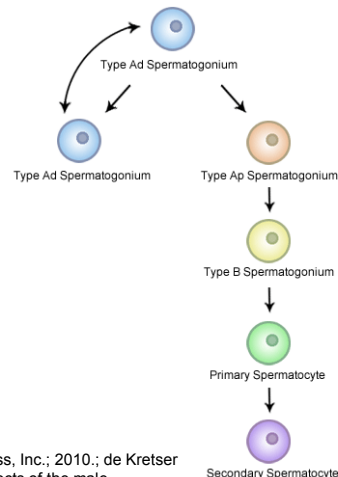
- Spermatogenesis is comprised of three stages:
  - Spermatocytogenesis: begun by primitive germ cells called spermatogonia and results in the formation of the so-called secondary spermatocytes
  - Spermatidogenesis: characterised by the formation of haploid spermatids from secondary spermatocytes due to second reduction division
  - Spermiogenesis: maturation of spermatids into motile spermatozoa

Robaire B. *Handbook of Andrology*. 2nd ed. Lawrence, KS: Allen Press, Inc.; 2010.; de Kretser DM, Temple-Smith PD., Kerr JB. et al. Anatomical and functional aspects of the male reproductive organs. In: Bandhauer J, Frick K, eds. *Disturbances in Male Fertility*. Berlin, Germany: Springer-Verlag; 1982: 1–131.; Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.; Glover TD, Barrat CLR, eds. *Male Fertility and Infertility*. Cambridge, United Kingdom: Cambridge University Press; 1999.



## Physiology – Spermatogenesis (con't.)

- Type A dark spermatogonia (Ad): can divide mitotically and reproduce by homonymous division
- Type A pale spermatogonia (Ap): heteronymous division
- Type B spermatogonia (B): divide mitotically to produce diploid intermediate cells called primary spermatocytes

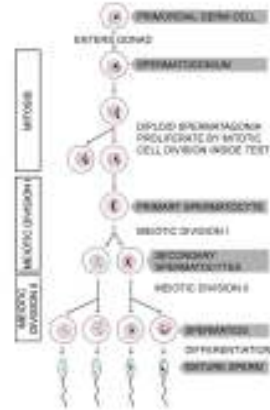


Robaire B. *Handbook of Andrology*. 2nd ed. Lawrence, KS: Allen Press, Inc.; 2010.; de Kretser DM, Temple-Smith PD., Kerr JB. et al. Anatomical and functional aspects of the male reproductive organs. In: Bandhauer J, Frick K, eds. *Disturbances in Male Fertility*. Berlin, Germany: Springer-Verlag; 1982: 1–131.; Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.; Glover TD, Barrat CLR, eds. *Male Fertility and Infertility*. Cambridge, United Kingdom: Cambridge University Press; 1999.



## Physiology – Spermatogenesis (con't.)

- Primary spermatocytes then enter the first meiotic prophase, in which their paired homologous chromosomes participate in crossing-over
- The cells then proceed with division I of meiosis to produce 2 secondary spermatocytes
- The 2 secondary spermatocytes derived from each primary spermatocyte proceed through meiotic division II to produce 4 spermatids
- Spermatids undergo morphological differentiation into sperm
- Cytological differentiation among spermatogonia, primary spermatocytes, and round spermatids is important in round spermatid injection (ROSI)

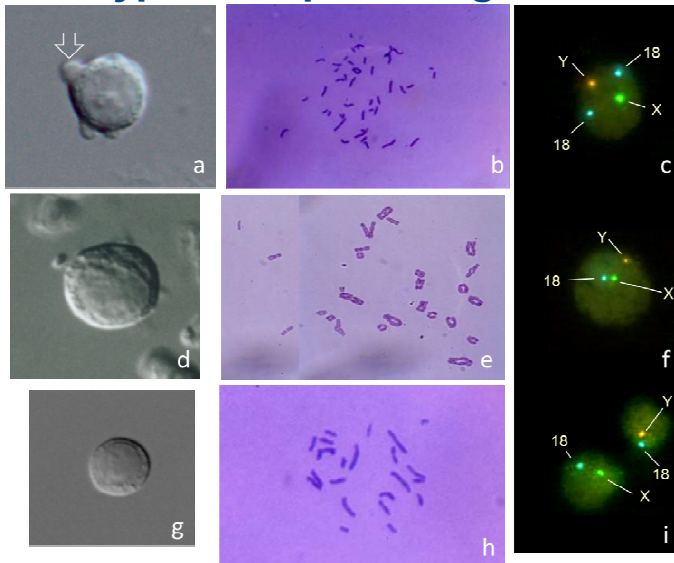


Spermiogenesis

Robaire B. *Handbook of Andrology*. 2nd ed. Lawrence, KS: Allen Press, Inc.; 2010.; de Kretser DM, Temple-Smith PD, Kerr JB, et al. Anatomical and functional aspects of the male reproductive organs. In: Bandhauer J, Frick K, eds. *Disturbances in Male Fertility*. Berlin, Germany: Springer-Verlag; 1982: 1–131.; Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.; Glover TD, Barrat CLR, eds. *Male Fertility and Infertility*. Cambridge, United Kingdom: Cambridge University Press; 1999.; Tanaka A, Nagayoshi M, Awata S, Mawatari Y, Tanaka I, Kusunoki H. Completion of meiosis in human primary spermatocytes through in vitro coculture with Vero cells. *Fertil Steril*. 2003;79 Suppl 1:795-801.



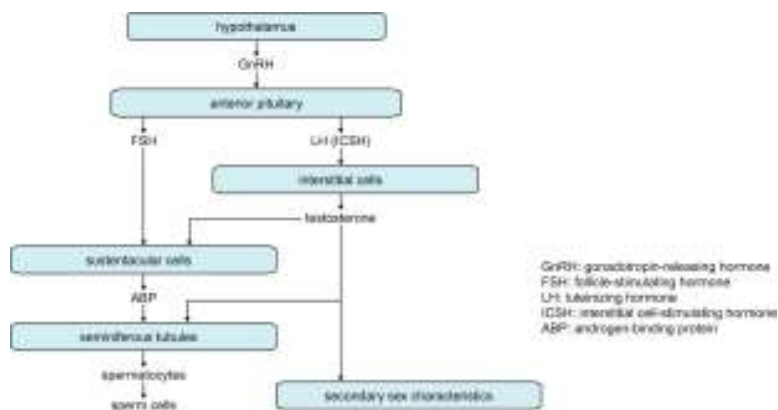
## Chromosomal Analysis and FISH Results for 3 Types of Spermatogenic Cells



## Physiology – The Temporal Course of Spermatogenesis

Spermatogonial mitosis	16 days
First meiosis	8 days
Second meiosis	16 days
Spermiogenesis	24 days
Total	~64 days

## Physiology – Hormonal Regulation of Spermatogenesis



Hormonal Regulation in the Male Reproductive System

Heffner LJ, Schust DJ. *The Reproductive System at a Glance*. Hoboken, NJ: John Wiley & Sons Ltd; 2010.; Robaire B. *Handbook of Andrology*. 2nd ed. Lawrence, KS: Allen Press, Inc.; 2010.; de Kretser DM, Temple-Smith PD., Kerr JB. et al. Anatomical and functional aspects of the male reproductive organs. In: Bandhauer J, Frick K, eds. *Disturbances in Male Fertility*. Berlin, Germany: Springer-Verlag; 1982: 1–131.; Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.; Glover TD, Barrat CLR, eds. *Male Fertility and Infertility*. Cambridge, United Kingdom: Cambridge University Press; 1999.

## Physiology – Sperm Pathway

- Duct system: epididymis
  - The epididymis is a narrow, tightly-coiled tube connecting the efferent ducts from the rear of each testicle to its vas deferens
  - The epididymis possesses numerous long atypical microvilli that are responsible for sperm transportation
  - During ejaculation, sperm flows from the lower portion of the epididymis (which functions as a storage reservoir)
  - During its transit in the epididymis, sperm undergo a maturation process
  - Final maturation is completed in the female reproductive tract

Manandhar G, Feng D, Yi YJ, et al. Centrosomal protein centrin is not detectable during early pre-implantation development but reappears during late blastocyst stage in porcine embryos. *Reproduction*. 2006;132:423-434.; Cooper TG, Yeung CH. Computer-aided evaluation of assessment of "grade a" spermatozoa by experienced technicians. *Fertil Steril*. 2006;85:220-224.



## Physiology – Sperm Pathway (con't.)

- Duct system: vas deferens
  - The vas deferens carries the spermatozoa<sup>1</sup>
  - Germ cells are mixed with ejaculatory secretions from the accessory sex glands and exit through the penile urethra<sup>2</sup>
  - Dependent upon neuroendocrine regulation<sup>3</sup>

1. Lipshultz LI, Howards SS. *Infertility in the Male*. 3rd ed. Maryland Heights, MO: Mosby; 1996.  
2. Nieschlag E, Behre HM, Nieschlag S, eds. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.  
3. Austin CR, Short RV, eds. *Germ cells and Fertilization*. Cambridge, United Kingdom: Cambridge University Press; 1972.



## Physiology – Sperm Pathway (con't.)

- Accessory glandular structure
  - Seminal vesicles: secretion is rich in fructose, which provides energy for the sperm passing through the tract
  - Prostate gland: secretes a slightly alkaline fluid into the urethra, which, similar to the alkaline fluid of the seminal vesicles, helps neutralise the acidic conditions in the vagina
  - Bulbourethral glands: secrete a viscous alkaline fluid into the vagina prior to ejaculation, which helps to neutralise the traces of acidic urine in urethra

Chughtai B, Sawas A, O'Malley RL, Naik RR, Ali Khan S, Pentyala S. A neglected gland: a review of Cowper's gland. *Int J Androl*. 2005;28:74-77.



## Physiology – Sperm Delivery

- Before fertilisation occurs, spermatozoa undergo certain modifications, which render them more susceptible to environmental conditions
  - Acrosome reaction: the enzymes located within the acrosome of spermatozoa cause modifications in the sperm, so that they can pass through zona pellucida of the oocyte into the perivitelline space
  - Hyperactivation is associated with changes in sperm motility, primarily beat frequency and flagellar curvature

Nieschlag E, Behre HM, Nieschlag S, eds. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.; Lipshultz LI, Howards SS. *Infertility in the Male*. 3rd ed. Maryland Heights, MO: Mosby; 1996.; Weinbauer GF, Luetjens CM, Simoni M, Nieschlag E. Physiology of testicular function. In: Nieschlag E, Behre HM, Nieschlag S. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.





## Physiology – Sperm Delivery (con't.)

- Capacitation
  - Involves a change in the components of the sperm plasma membrane, as well as in glycosylated proteins expressed at its surface, allowing the sperm to establish functional binding to receptors at the surface of the zona pellucida
  - Hyperactivation may be expressed before the acrosome reaction, which probably does not occur in most fertilising spermatozoa until they reach the zona surface

Nieschlag E, Behre HM, Nieschlag S, eds. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.; Lipshultz LI, Howards SS. *Infertility in the Male*. 3rd ed. Maryland Heights, MO: Mosby; 1996.; Weinbauer GF, Luetjens CM, Simoni M, Nieschlag E. Physiology of testicular function. In: Nieschlag E, Behre HM, Nieschlag S. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.



## Physiology – Oocyte Activation

### Sperm Factor, PLCzeta, and Egg Activation

- At fertilisation, mammalian eggs show repetitive transient rises in cytosolic free calcium ( $[Ca^{2+}]_i$ ), each of which is due to calcium ( $Ca^{2+}$ ) release from the endoplasmic reticulum through the inositol 1,4,5-trisphosphate (IP<sub>3</sub>) receptor
- During fertilisation, the sperm factor is released into the oocyte and induces a long-lasting series of  $Ca^{2+}$  spikes ( $Ca^{2+}$  oscillation) that is required for egg activation
- The  $Ca^{2+}$  spikes initiate the extrusion of cortical granules that block the entry of other sperm
- Maturation (M-phase) promoting factor (MPF) is inactivated by the  $Ca^{2+}$  oscillation, resulting in exit from metaphase II arrest
- Meiosis resumes with the formation of the second polar body and complete meiotic division, and 1-cell embryos with the male and female pronuclei attain the first cleavage division through nuclear envelope breakdown

Kuroda K, Ito M, Shikano T, et al. The role of X/Y linker region and N-terminal EF-hand domain in nuclear translocation and  $Ca^{2+}$  oscillation-inducing activities of phospholipase Czeta, a mammalian egg-activating factor. *J Biol Chem*. 2006;281:27794-27805.



## Conclusions

- The testes have 2 functions: spermatogenesis and steroidogenesis
- 4 mature sperm cells are produced through the first and second meiotic cell divisions, which start from spermatogonia Type B
- The spermatogenesis process is estimated to take approximately 64 days in humans
- Acrosome reaction, hyperactivation and capacitation are a series of modifications that are mandatory for fertilisation
- PLCzeta is a strong candidate for sperm factor, which is indispensable for oocyte activation



## Section 2

### Diagnostic Evaluation of the Infertile Male



## Learning Objectives

After this section, participants should be better able to perform a diagnostic evaluation of the infertile male, including:

- Clinical history
- Semen analysis
- Endocrine evaluation
  - Post-ejaculatory urinalysis
  - Ultrasonography
- Antisperm antibodies test (ASA)
- Sperm viability test
- DNA damage analysis



## Clinical History

### History of infertility

- Time trying to get pregnant
- Previous pregnancies
- Previous evaluation and treatments
- Contraception (methods and duration)

### Sexual history

- Potency/libido/orgasm
- Lubricants
- Frequency of sexual relations
- Marital and emotional status

### Infancy and development

- Puberty
- Gynecomastia
- Cryptorchidism
- Testicular torsion

### Medical history

- Systemic diseases (diabetes, multiple sclerosis, cancer, vasculitis)
- Respiratory infection
- Cystic fibrosis

### Surgical history

- Retroperitoneal surgery
- Pelvic surgeries (transurethral resection of the prostate, bladder neck plasty)
- Inguinal surgeries (herniorrhaphy, varicocelectomy, orchidopexy)
- Scrotal surgeries (vasectomy, hydrocelectomy)

Pasqualotto FF, Lucon AM, Sobreiro BP, Pasqualotto EB, Arap S. Effects of medical therapy, alcohol, smoking, and endocrine disruptors on male infertility. *Rev Hosp Clin Fac Med Sao Paulo*. 2004;59:375-382.; Pasqualotto FF, Pasqualotto EB, Sobreiro BP, Hallak J, Medeiros F, Lucon AM. Clinical diagnosis in men undergoing infertility investigation in a university hospital. *Urol Int*. 2006;76:122-125.



## Semen Analysis

Clinical significance of abnormalities observed in the main macroscopic parameters of seminal analysis

Parameters	Abnormalities	Clinical significance
pH (optimal value = 7.8)	Acidic: < 6.5–7	Low volume and non-coagulation indicate congenital absence of vas deferens, obstruction of ejaculatory duct, or partial retrograde ejaculation
Coagulation/liquefaction (normal: coagulates/liquifies within 20 min. at room 37°C)	Without coagulation Prolonged liquefaction	Congenital absence of the seminal vesicles Poor prostatic secretions
Color (normal: whitish-gray/pearl-white)	Yellowish color Reddish brown	Jaundice, carotenemia, drugs, and secondary hematospermia due to urethral bleeding or inflammation of the seminal vesicles. Other causes such as genital urinary tumors require work-up for exclusion.
Viscosity (normal: 4 mm threading)	> 6 mm No threading	Relevant when associated with low motility
Volume (normal volume: 2-4 mL)	0 (aspermia) < 2 mL (hypospermia)  > 6 mL	Retrograde ejaculation Loss of specimen during collection Partial retrograde ejaculation Short duration of sexual abstinence Prolonged sexual abstinence



Agarwal A, Bragais FM, Sabanegh E. Assessing sperm function. *Urol Clin N Am.* 2008;35:157–171.

## Normal Values of Macroscopic and Microscopic Semen Parameters

Semen parameters	Normal values	
	1999	2010
Semen volume (mL)	≥ 2.0	≥ 1.5
pH	≥ 7.2	≥ 7.2
Sperm concentration (10 <sup>6</sup> spermatozoa/mL)	≥ 20	≥ 15
Total sperm number (10 <sup>6</sup> spermatozoa/ejaculate)	≥ 40	≥ 39
Total motility (PR+NP)	≥ 50%	≥ 40%
Progressive motility (PR)	≥ 25%	≥ 32%
Sperm morphology (normal forms)	≥ 15%	≥ 4%
Vitality (live spermatozoa)	≥ 50%	≥ 58%
White blood cells (10 <sup>6</sup> mL)	< 1.0	< 1.0
Immunobead test (motile spermatozoa with bound beads)	< 50%	< 50%
MAR test (motile spermatozoa with bound particles)	< 50%	< 50%
Seminal zinc (μmol/ejaculate)	-	≥ 2.4
Seminal fructose (μmol/ejaculate)	-	≥ 13
Seminal neutral glucosidase (mU/ejaculate)	-	≥ 20

Cooper TG, Yeung CH. Computer-aided evaluation of assessment of "grade a" spermatozoa by experienced technicians. *Fertil Steril.* 2006;85:220-224.; World Health Organization. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction.* 4th ed. WHO Press; Geneva, Switzerland: 1999.; World Health Organization. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction.* 5th ed. WHO Press; Geneva, Switzerland: 2010.



## Nomenclature Related to Semen Quality

<b>aspermia</b>	no semen (no or retrograde ejaculation)
<b>asthenozoospermia</b>	percentage of progressively motile (PR) spermatozoa below the lower reference limit
<b>asthenoteratozoospermia</b>	percentages of both progressively motile (PR) and morphologically normal spermatozoa below the lower reference limits
<b>azoospermia</b>	no spermatozoa in the ejaculate (given as the limit of quantification for the assessment method employed)
<b>cryptozoospermia</b>	spermatozoa absent from fresh preparations but observed in a centrifuged pellet
<b>haemospermia (haematospermia)</b>	presence of erythrocytes in the ejaculate
<b>leukospermia (leukocytospermia, pyospermia)</b>	presence of leukocytes in the ejaculate above the threshold value
<b>necrozoospermia</b>	low percentage of live, and high percentage of immotile, spermatozoa in the ejaculate

World Health Organization. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction*. 5th ed. WHO Press; Geneva, Switzerland: 2010.; Grimes DA, Lopez LM. "Oligozoospermia," "azoospermia," and other semen-analysis terminology: the need for better science. *Fertil Steril*. 2007;88:1491-1494.; Eliasson R, Hellinga F, Lubcke F, et al. Empfehlungen zur Nomenklatur in der Andrologie. *Andrologia*. 1997;2:1257.



## Nomenclature Related to Semen Quality (con't.)

<b>normozoospermia</b>	total number (or concentration, depending on outcome reported) of spermatozoa, and percentages of progressively motile (PR) and morphologically normal spermatozoa, equal to or above the lower reference limits
<b>oligoasthenozoospermia</b>	total number (or concentration, depending on outcome reported) of spermatozoa, and percentage of progressively motile (PR) spermatozoa, below the lower reference limits
<b>oligoasthenoteratozoospermia</b>	total number (or concentration, depending on outcome reported) of spermatozoa, and percentages of both progressively motile (PR) and morphologically normal spermatozoa, below the lower reference limits
<b>oligoteratozoospermia</b>	total number (or concentration, depending on outcome reported) of spermatozoa, and percentage of morphologically normal spermatozoa, below the lower reference limits
<b>oligozoospermia</b>	total number (or concentration, depending on outcome reported) of spermatozoa below the lower reference limit
<b>teratozoospermia</b>	percentage of morphologically normal spermatozoa below the lower reference limit

World Health Organization. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction*. 5th ed. WHO Press; Geneva, Switzerland: 2010.; Grimes DA, Lopez LM. "Oligozoospermia," "azoospermia," and other semen-analysis terminology: the need for better science. *Fertil Steril*. 2007;88:1491-1494.; Eliasson R, Hellinga F, Lubcke F, et al. Empfehlungen zur Nomenklatur in der Andrologie. *Andrologia*. 1997;2:1257.



## The Evaluation and Discussion for WHO5 by ESHRE: Basic Semen Analysis (BSA)

- Improved accuracy and precision and reduced uncertainty of measurement in diagnostic laboratory andrology is welcome
- WHO has significantly contributed to these goals
- ESHRE's Basic Semen Analysis maintains standards of procedures that are not fully concordant with those recommended in the most recent WHO laboratory manual
- Recommendations provide results with the same or better quality than those recommended in WHO5



## Endocrine Evaluation

- Abnormal semen parameters, particularly when the sperm concentration is  $<10$  million/mL
- Impaired sexual function
- Other clinical findings that suggest a specific endocrinopathy
- The minimum initial hormonal measurement
  - Follicle-stimulating hormone (FSH)
  - Total testosterone
- Free testosterone, luteinising hormone (LH), prolactin, and second total testosterone when the total testosterone level is low ( $<300$  ng/mL)
- High serum estradiol level is observed in men with gynecomastia
- Inhibin B

Lipshultz LI, Howards SS. *Infertility in the Male*. 3rd ed. Maryland Heights, MO: Mosby; 1996.; Kumanov P, Nandipati K, Tomova A, Agarwal A. Inhibin B is a better marker of spermatogenesis than other hormones in the evaluation of male factor infertility. *Fertil Steril*. 2006;86:332-338.



## Basal Hormone Levels in Various Clinical States

Clinical condition	FSH	LH	Testosterone	Prolactin
Normal spermatogenesis	Normal	Normal	Normal	Normal
Hypogonadotropic hypogonadism	Low	Low	Low	Normal
Abnormal spermatogenesis <sup>a</sup>	High/normal	Normal	Normal	Normal
Complete testicular failure/ hypergonadotropic hypogonadism	High	High	Normal/low	Normal
Prolactin-secreting pituitary tumor	Normal/low	Normal/low	Low	High

<sup>a</sup> Many men with abnormal spermatogenesis have a normal serum FSH, but a marked elevation of serum FSH is clearly indicative of an abnormality in spermatogenesis.



## Post-ejaculatory Urinalysis

- Incomplete semen collection
- Retrograde ejaculation
- Ejaculatory duct obstruction
- Hypogonadism
- Congenital bilateral aplasia of the vas deferens (CBAVD)



## Ultrasonography

- Transrectal ultrasound
  - Examination of seminal vesicles and ejaculatory ducts
  - Low semen volume (exclude ejaculatory duct obstruction)
  - Ejaculatory abnormalities (hematospermia, anejaculation, painful ejaculation)
  - Abnormalities detected on digital rectal examination
- Scrotal ultrasound
  - Evaluation of testicular abnormalities
  - Should not be performed to detect subclinical varicocele



## Antisperm Antibodies (ASA) Test

- The antigenic properties of human sperm were reported as early as the end of 19th century and since then, antisperm antibodies have been considered by several authors as a possible causative factor in infertility, with significant levels
- Detected in the semen of 5-15% of infertile men, but in only 1-2% of fertile men
- Develop as a result of an accidental or iatrogenic breach of the blood–testes barrier or from obstruction of the male reproductive tract
- Adverse impact on male fertility by directly interfering with sperm motility and sperm-oocyte binding; indirectly by mediating the release of cytokines that can impair sperm function; and by impeding cervical mucus penetration
- Generally associated with poor sperm motility and reduced rate of natural pregnancy

Bohring C, Krause W. Immune infertility: towards a better understanding of sperm (auto)-immunity. The value of proteomic analysis. Hum Reprod. 2003;18:915-924.  
Chiu WW, Chamley LW. Clinical associations and mechanisms of action of antisperm antibodies. Fertil Steril. 2004;82:529-535.  
Mazumdar S, Levine AS. Antisperm antibodies: etiology, pathogenesis, diagnosis, and treatment. Fertil Steril. 1998;70:799-810.  
Sinisi AA, Di Finizio B, Pasquali D, Scurini C, D'Apuzzo A, Bellastella A. Prevalence of antisperm antibodies by SpermMARtest in subjects undergoing a routine sperm analysis for infertility. Int J Androl. 1993;16:311-314.





## Sperm Viability Tests

- Supravital dye
  - Eosin Y
  - Trypan blue
- Hypoosmotic swelling test (HOST)
- Pentoxifylline

World Health Organization. *WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction*. 5th ed. WHO Press; Geneva, Switzerland: 2010.; Liu J, Tsai YL, Katz E, Compton G, Garcia JE, Baramki TA. High fertilization rate obtained after intracytoplasmic sperm injection with 100% nonmotile spermatozoa selected by using a simple modified hypo-osmotic swelling test. *Fertil Steril*. 1997;68:373-375.; de Mendoza MV, González-Utor AL, Cruz N, Gutiérrez P, Cascales F, Sillero JM. *In situ* use of pentoxifylline to assess sperm vitality in intracytoplasmic sperm injection for treatment of patients with total lack of sperm movement. *Fertil Steril*. 2000;74:176-177.



## Hypoosmotic Swelling Test (HOST)

- Based on the principle that viable spermatozoa have intact membranes
- Characteristics
  - Swelling of the cytoplasmic space
  - Curling of the sperm tail
- Normal test results
  - >60% of spermatozoa with swelled and curling tail
- Test may be used to aid selection of viable spermatozoa for use in ICSI, especially in cases when only immotile spermatozoa are available

Jeyendran RS. *Sperm Collection and Processing Methods: a Practical Guide*. Cambridge, United Kingdom: Cambridge University Press; 2003.; Agarwal A, Bragais FM, Sabanegh E. Assessing sperm function. *Urol Clin N Am*. 2008;35:157-171.



## Mechanisms of DNA Damage in Male Germ Cells and Spermatozoa

- Apoptosis
- DNA fragmentation
- Reactive oxygen species (ROS) formation

Koppers AJ, De Iuliis GN, Finnie JM, McLaughlin EA, Aitken RJ. Significance of mitochondrial reactive oxygen species in the generation of oxidative stress in spermatozoa. *J Clin Endocrinol Metab.* 2008;93:3199-3207.



## Increase in Sperm DNA Damage

- Advanced age
- Cancer
- Cigarette smoking
- Post-testicular genital tract infection and inflammation
- Testicular hyperthermia
- Varicocele
- Finasteride

Saleh RA, Agarwal A, Nada EA, et al. Negative effects of increased sperm DNA damage in relation to seminal oxidative stress in men with idiopathic and male factor infertility. *Fertil Steril.* 2003;79 Suppl 3:1597-1605.



## Evaluation of Sperm DNA Integrity

- TUNEL assay
- Sperm Chromatin Dispersion Assay (SCSA)
- Comet assay
- Impossible to choose sperm with intact DNA



## The Urgency for a Robust Clinical Test

- The study of DNA damage is highly relevant in the era of ART, particularly ICSI
  - These technologies bypass the barriers of natural selection
  - Subfertile men possess substantially more sperm DNA damage than do fertile men
  - Experimentally, sperm DNA damage has been shown to impact negatively on ICSI embryo development, pregnancy rates, and health of the offspring

Aitken RJ, De Lullis GN. On the possible origins of DNA damage in human spermatozoa. *Mol Hum Reprod.* 2010;16:3-13.; Carrell DT, Lui L. Altered protamine 2 expression is uncommon in donors of known fertility, but common among men with poor fertilizing capacity, and may reflect other abnormalities of spermiogenesis. *J Androl.* 2001;22:604-610.; de Yebra L, Ballescà JL, Vanrell JA, Bassas L, Oliva R. Complete selective absence of protamine P2 in humans. *J Biol Chem.* 1993;268:10553-10557.; Kodama H, Yamaguchi R, Fukuda J, Kasai H, Tanaka T. Increased oxidative deoxyribonucleic acid damage in the spermatozoa of infertile male patients. *Fertil Steril.* 1997;68:519-524.; Sakas D, Manicardi GC, Bizzaro D. Sperm nuclear DNA damage in the human. *Adv Exp Med Biol.* 2003;518:73-84.; Spanò M, Bonde JP, Hjøllund HI, Kolstad HA, Cordelli E, Leter G. Sperm chromatin damage impairs human fertility. The Danish First Pregnancy Planner Study Team. *Fertil Steril.* 2000;73:43-50.



## Sperm Epigenetics

- Epigenetics is the study of heritable changes in a chromosome without DNA alterations
- Epigenetic marks in sperm chromatin may play a role in regulating development of embryos, including:
  - Retained histones
  - MicroRNAs
  - Imprinted genes
  - DNA methylation
- The potential link between general sperm DNA damage and epigenetic alterations is not yet understood

Hammoud SS, Nix DA, Zhang H, Purwar J, Carrell DT, Cairns BR. Distinctive chromatin in human sperm packages genes for embryo development. *Nature*. 2009;460:473-478.



## Recommendation for Future Work

- Fundamental research is urgently required
- Standardisation of clinical assays
- Animal models
- High-quality clinical data is urgently required
- Long-term follow-up of ART children



## Conclusions

- Clinical history should be obtained very carefully
- The reference distributions in 2010 (WHO5) provide a description of the semen characteristics of recent fathers whose partner became pregnant within 12 months of stopping contraception use
- The ESHRE BSA will maintain standards of procedures that are not fully concordant (“WHO compliant”) with those recommended in WHO5



## Conclusions (con't.)

- An endocrine evaluation is indicated for men with the following
  - Abnormal semen parameters
  - Impaired sexual function
  - Other clinical findings that suggest a specific endocrinopathy
- The relationships among serum testosterone, LH, FSH, and prolactin concentrations help to provide an understanding of the source of abnormal total testosterone levels



## Conclusions (con't.)

- A low-volume or absent antegrade ejaculate suggests incomplete semen collection, retrograde ejaculation, lack of emission, ejaculatory duct obstruction, hypogonadism, or CBAVD
- Semen ASAs have generally been associated with poor sperm motility, and reduced natural pregnancy rates
- Sperm viability can be assessed by mixing fresh semen with a supravital dye such as eosin Y or trypan blue, or by the use of HOST and pentoxifilline



## Conclusions (con't.)

- The potential mechanisms often cited as a cause of DNA damage in the male germ line include abortive apoptosis, DNA fragmentation, and ROS formation
- Sperm DNA damage increases with advancing age, cancer, cigarette smoking, post-testicular genital tract infection and inflammation, testicular hyperthermia, varicocele, and use of finasteride
- The TUNEL, SCSA, and Comet assays are used to evaluate the integrity of sperm DNA, however, it is impossible to choose sperm for ICSI with intact DNA using these assays



## Conclusions (con't.)

- The term “DNA fragmentation” refers to denatured or damaged sperm DNA that cannot be repaired
- Sperm DNA damage is more common in infertile men and may contribute to poor reproductive performance. Sperm DNA damage is also associated with spontaneous recurrent miscarriage.
- Varicocele repair and antioxidant use may affect sperm DNA integrity
- Infertile men exhibit substantially more sperm DNA damage than fertile men although a small percentage of spermatozoa from fertile men also possess detectable levels of DNA damage



## Conclusions (con't.)

- An important issue with all these methods is whether the treatments used to prepare the sperm may themselves induce DNA damage
- It is essential that long-term comprehensive follow-up studies on ART children are performed to ascertain the safety of the procedures we currently use



## Section 3

### Genetic Evaluation of Male Infertility



### Learning Objectives

After this section, participants should be better able to:

- Perform a genetic evaluation of male infertility
  - Assess cystic fibrosis transmembrane regulator gene mutation (CFTR)
- Karyotypic Chromosomal Abnormalities
  - Klinefelter Syndrome
  - Translocations
  - Microdeletion of the Y Chromosome





## Genetic Evaluation of Male Infertility

Indication	Recommended tests
Sperm concentration (<math>< 5 \times 10^6</math> sperm/mL, unknown cause)	Y-chromosome microdeletion analysis Karyotype
Non-obstructive azoospermia	Y-chromosome microdeletion analysis Karyotype
Azoospermia or oligospermia and absence of 1 or 2 vas deferens	CFTR gene mutation
Obstructive azoospermia (unknown cause)	CFTR gene mutation
Repeated miscarriage	Karyotype
History of genetic syndromes in the family	Karyotype

Pinotti JA, da Fonseca AM, Bagnoli-Revinter VR. *Tratado de Ginecologia*. Revinter: Rio de Janeiro, Brazil; 2005.



## Genetic Screening

- Cystic fibrosis gene mutations
  - Almost all men with clinical cystic fibrosis exhibit CBAVD\*
  - 80% of men with CBAVD\* have documented mutations of the CFTR gene
- The prevalence of CFTR mutations also is increased among men with azoospermia related to congenital bilateral obstruction of the epididymides and those with unilateral vasa agenesis

\*CBAVD = Congenital bilateral absence of vas deferens

Anguiano A, Oates RD, Amos JA, et al. Congenital bilateral absence of the vas deferens. A primarily genital form of cystic fibrosis. *JAMA*. 1992;267:1794-1797.



## Karyotypic Chromosomal Abnormalities

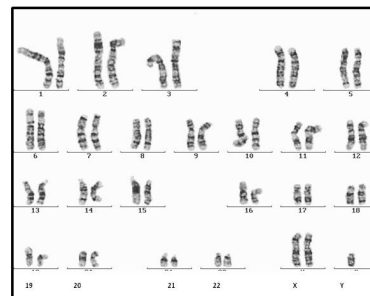
- 47,XXY
- Translocations: Robertsonian and reciprocal
- Sperm count
  - Azoospermia: 10-15%
  - Oligospermia (<5 million/mL): 5%
  - Normal: <1%
- Increased risk for miscarriages and for having children with chromosomal and congenital defects

Practice Committee of American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril*. 2012;98:294-301.; Van Assche E, Bonduelle M, Tournaye J, et al. Cyto genetics of infertile men. *Hum Reprod*. 1996;11(Suppl 4):1-25.; Ravel C, Berthaut I, Bresson JL, Siffroi JP; Genetics Commission of the French Federation of CECOS. Genetics Commission of the French Federation of C. Prevalence of chromosomal abnormalities in phenotypically normal and fertile adult males: large-scale survey of over 10,000 sperm donor karyotypes. *Hum Reprod*. 2006;21:1484-1489.; De Braekeleer M, Dao TN. Cyto genetic studies in male infertility: a review. *Hum Reprod*. 1991;6:245-150.



## Klinefelter Syndrome (47,XXY)

- Estimated frequency: 1/500-1/1000
  - 47,XXY: 80-90%
  - 46,XY or 47,XXY: 10-20%
  - 48,XXXY, 48,XXYY, 47,X, or X9,Y in remaining
- Origin of XXY aneuploidy is from the father in half of all cases
- No increased risk for aneuploidies in children born from Klinefelter syndrome father
- 4-fold increase with advanced maternal age (>40 years)
- No increased complete and incomplete deletions of AZFa, AZFb, or AZFc in men



Courtesy of Dr. Lucia Martelli, Cyto genetics Lab FMRP-University of Sao Paulo.

Lim AS, et al. *Hum Genet*. 1999;104:405-409.; Kruse R, et al. *Fertil Steril*. 1998;69:482-485.; Rives N, et al. *Mol Hum Reprod*. 2000;6:107-112.; Estop AM, et al. *Hum Reprod*. 1998;13:124-127.; Maiburg M, et al. *Fertil Steril*. 2012;98:253-260.; Fullerton G, et al. *Hum Reprod*. 2010;25:588-597.



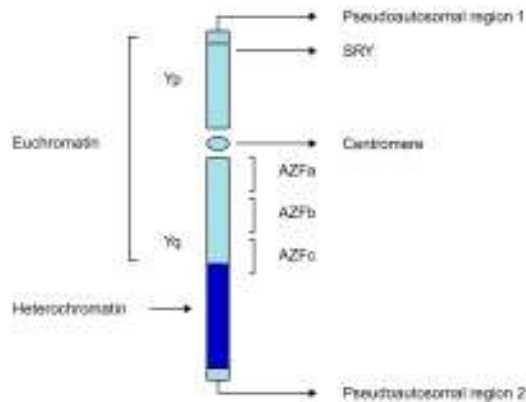
## Translocations

- Robertsonian
  - The risk of chromosomal imbalance at prenatal diagnosis is low, with approximately 1–2% of paternally-derived Robertsonian translocations being unbalanced<sup>1</sup>
- Reciprocal
  - Sperm karyotyping studies of 37 reciprocal translocated heterozygotes have shown that 19–77% of spermatozoa are unbalanced<sup>2-4</sup>
  - Frequency of paternally-derived translocation imbalances at prenatal diagnosis is about 12%, therefore, preimplantation genetic diagnosis is recommended<sup>1</sup>

1. Boué A, Gallano P. A collaborative study of the segregation of inherited chromosome structural rearrangements in 1356 prenatal diagnoses. *Prenat Diagn.* 1984;4:45-67.; 2. Estop A, Van Kirk V, Cieply K. Segregation analysis of four translocations, t(2;18), t(3;15), t(5;7), and t(10;12), by sperm chromosome studies and a review of the literature. *Cytogenet Cell Genet.* 1995;70:80-87.; 3. Martin R, Spriggs E. Sperm chromosome complements in a man heterozygous for a reciprocal translocation 46.XY,t(9;13)(q21.1;q21.2) and a review of the literature. *Clin Genet.* 1995;47:42-46.; 4. Cifuentes P, Navarro J, Blanco J, et al. Cytogenetic analysis of sperm chromosomes and sperm nuclei in a male heterozygous for a reciprocal translocation t(5;7)(q21;q32) by in situ hybridization. *Eur J Hum Genet.* 1999;7:231-238.



## Microdeletion of the Y Chromosome



Practice Committee of American Society for Reproductive Medicine. Diagnostic evaluation of the infertile male: a committee opinion. *Fertil Steril.* 2012;98:294-301.; Pryor JL, Kent-First M, Muallem A, et al. Microdeletions in the Y chromosome of infertile men. *N Engl J Med.* 1997;336:534-539.; Kent-First MG, Kol S, Muallem A, et al. The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Mol Hum Reprod.* 1996;2:943-950.; Jorgez CJ, Weedin JW, Sahin A, et al. Aberrations in pseudoautosomal regions (PARs) found in infertile men with Y-chromosome microdeletions. *J Clin Endocrinol Metab.* 2011;96:E674–E679.



## Microdeletion of the Y Chromosome Testicular Phenotypes

- Prevalence of Y microdeletions among subfertile severely oligozoospermic men stratified by sperm concentration

Sperm concentration (million/mL)	Total screened	AZFa (%)	AZFb (%)	AZFb+c (%)	AZFc (%)	Complete Yq (%)	Any Y microdeletion (%)
Azoospermic	1,153	4 (0.3%)	17 (1.4%)	31 (2.7%)	50 (4.3%)	18 (1.6%)	120 (10.4%)
> 0 to <1	257	0	0	1 (0.4%) <sup>a</sup>	25 (9.7%)	0	26 (0.1%)
1 - <5	181	0	0	0	3 (1.7%)	0	3 (1.7%)
Total	1,591	4 (0.3%)	17 (1.1%)	32 (2.8%)	78 (4.9%)	18 (1.1%)	149 (9.4%)

<sup>a</sup> Partial AZFb+c deletion that spared the centromeric portion of the AZFb region

Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. *Fertil Steril.* 2010;94:1753-1756.



## Microdeletion of the Y Chromosome Testicular Phenotypes (con't.)

- Outcomes of microdissection testicular sperm extraction (TESE) in azoospermic men stratified by Y microdeletion status

Etiology of azoospermia	Sperm retrieved	Sperm not retrieved	Total	Retrieval rate
AZFa	0	2	2	0%
AZFb	0	7	7	0%
AZFb+c	0	7	7	0%
AZFa+b+c	0	4	4	0%
AZFc	15	6	21	71.4% <sup>a</sup>
Nondeletedm idiopathic	188	197	385	48.8% <sup>a</sup>

Note: TESE = testicular sperm extraction; AZF = azoospermic factor.

<sup>a</sup>Comparison of retrieval rates in AZFc deleted men and idiopathically azoospermic nondeleted men,  $P < .05$  for Fisher's exact test).

Stahl PJ, Masson P, Mielnik A, Marean MB, Schlegel PN, Paduch DA. A decade of experience emphasizes that testing for Y microdeletions is essential in American men with azoospermia and severe oligozoospermia. *Fertil Steril.* 2010;94:1753-1756.



## Conclusions

- The prevalence of CFTR mutations is increased among azoospermic men with CBAVD
- Chromosomal analysis is indispensable for men with very severe oligozoospermia, azoospermia, small testicles, and repeated miscarriages
- Klinefelter Syndrome (47,XXY) has a frequency of 1/500 to 1/1000 men
- Origin of Klinefelter Syndrome remains unknown
- No significant increased risk for aneuploidies in children born from Klinefelter syndrome fathers



## Conclusions (con't.)

- The percentage of microdeletion of the Y chromosome increases according to the severity of spermatogenesis, but does not exceed 11%
- No sperm were found in men with microdeletion of AZFa, AZFb, AZFb+c, or AZFa+b+c, but some sperm were found in 71.4% of men with AZFc microdeletion
- There is no scientifically proven evidence that microdeletion of AZFa or AZFb causes male infertility, but the microdeletion is passed to the next generation



## Section 4

### Treatment of Male Infertility



### Learning Objectives

After this section, participants should be better able to discuss:

- ICSI
- Surgical treatment for azoospermia
- Microsurgical epididymal sperm aspiration (MESA)
- Microdissection (MD)-TESE
- Varicocele
- Treatment options for male infertility



## ICSI (Intracytoplasmic Sperm Injection)

- The first ICSI baby was born in 1992
- Consistent treatment to overcome severe male factor(s)
- ICSI results using epididymal, testicular, or ejaculated spermatozoa were similar to those obtained using conventional IVF
- A slight increase was observed in fetal chromosomal abnormalities and congenital malformation than after natural conception, but there was no difference between IVF and ICSI
- Clinical research in the long-term outcome of ICSI children is necessary

Van Steirteghem A. Celebrating ICSI's twentieth anniversary and the birth of more than 2.5 million children—the 'how, why, when and where'. *Hum Reprod.* 2012;27:1-2.



## Azoospermia

- Azoospermia is present in about 5% of infertile couples
- Non-obstructive azoospermia → testis
  - TESE
  - MD-TESE
- Obstructive azoospermia → epididymis
  - Percutaneous sperm aspiration (PESA)
  - MESA

Palermo G, et al. *Lancet.* 1992;340:17-18.; Nicopoulos JD, et al. *Fertil Steril.* 2004;82:691-700.; Pasqualotto FF, et al. *J Urol.* 1999;162:1626-1628.; Pasqualotto FF, et al. *Urol Int.* 2006;76:122-125.; Van Peperstraten A, et al. *Cochrane Database of Syst Rev.* 2008;(2):CD002807.; Belker AM, et al. *J Urol.* 1991;145:505-511.; Borges E Jr, et al. *Sao Paulo Med J.* 2002;120:122-126.; Borges E Jr, et al. *J Assist Reprod Genet.* 2003;20:33-37.; de Cássia Savio Figueira R, et al. *Fertil Steril.* 2008;91:727-732.; Elder K, Dale B. *In-vitro Fertilization.* Cambridge, United Kingdom: Cambridge University Press; 2010.; Lipshultz LI, Howards SS. *Infertility in the Male.* 3rd ed. Maryland Heights, MO: Mosby; 1996.; Nieschlag E, Behre HM, Nieschlag S, eds. *Andrology: Male Reproductive Health and Dysfunction.* 3rd ed. Berlin, Germany: Springer-Verlag; 2010.; Pasqualotto FF, et al. *J Urol.* 2002;167:1753-1756.; Pasqualotto FF, et al. *J Urol.* 2003;169:1779-1781.; Pasqualotto FF, et al. *Fertil Steril.* 2005;83:606-611.



## Microsurgical Epididymal Sperm Aspiration (MESA)

- Epididymal sperm retrieval was first described by Silber and colleagues in 1988 and subsequently named MESA by Patrizio and colleagues
- Advantages
  - High number and quality sperm
  - Easy technique for sperm collection
  - Easy cryopreservation
- Disadvantages
  - Invasive
  - Relatively expensive

Patrizio P, Silber S, Ord T, Balmaceda JP, Asch RH. First two births after microsurgical epididymal sperm aspiration. *Lancet*. 1988;2:1364.; Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred? *Hum Reprod*. 1999;14(Suppl 1):71-81.; Girardi SK, Schlegel P. MESA: review of techniques, preoperative considerations and results. *J Androl*. 1996;17:5-9.; Nicopoulos JD, Gilling-Smith C, Almeida PA, Norman-Taylor J, Grace I, Ramsay JW. Use of surgical sperm retrieval in azoospermic men: a meta-analysis. *Fertil Steril*. 2004;82:691-700.; Van Peperstraten A, Proctor ML, Johnson NP, Philipson G. Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. *Cochrane Database of Syst Rev*. 2008;(2):CD002807.; Silber SJ, Balmaceda J, Borrero C, Ord T, Asch R. Pregnancy with sperm aspiration from the proximal head of the epididymis: a new treatment for congenital absence of the vas deferens. *Fertil Steril*. 1988;50:525-528.



## Microsurgical Epididymal Sperm Aspiration (MESA) (con't.)

- Surgical technique
  - Scrototomy is usually performed under general (propofol + fentanyl citrate) or loco-regional (cord block) anesthesia (10 mL of 1% lidocaine + aneapine in equal quantities)
  - High quality sperm is retrieved in whitish regions and low quality sperm in yellowish regions with insertion of a glass pipette at a sharp angle
  - Easy to collect a large volume of sperm in the white color regions



Patrizio P, Silber S, Ord T, Balmaceda JP, Asch RH. First two births after microsurgical epididymal sperm aspiration. *Lancet*. 1988;2:1364.; Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred? *Hum Reprod*. 1999;14(Suppl 1):71-81.; Girardi SK, Schlegel P. MESA: review of techniques, preoperative considerations and results. *J Androl*. 1996;17:5-9.; Nicopoulos JD, Gilling-Smith C, Almeida PA, Norman-Taylor J, Grace I, Ramsay JW. Use of surgical sperm retrieval in azoospermic men: a meta-analysis. *Fertil Steril*. 2004;82:691-700.; Van Peperstraten A, Proctor ML, Johnson NP, Philipson G. Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. *Cochrane Database of Syst Rev*. 2008;(2):CD002807.





## Percutaneous Epididymal Sperm Aspiration (PESA)

- Advantages
  - Less invasive than MESA
  - Easily performed on an outpatient basis
  - Quick
  - More cost-effective than MESA
  - Fewer complications than after MESA
- Disadvantages
  - Difficult to collect sperm of the highest quality or large quantities due to the blind percutaneous puncture

Craft I, Bennet V, Nicholson N. Fertilising ability of testicular spermatozoa. *Lancet*. 1993;342:864.; Nieschlag E, Behre HM, Nieschlag S, eds. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.; Lipshultz LI, Howards SS. *Infertility in the Male*. 3rd ed. Maryland Heights, MO: Mosby; 1996.



## Percutaneous Epididymal Sperm Aspiration (PESA) (con't.)

- Surgical technique
  - PESA is performed on an outpatient basis with local anesthesia
  - Cord block anesthesia is administered by injecting 2 mL of 1% lidocaine without adrenaline in the pampiniform plexus
  - A 27-gauge needle is introduced into the proximal part of the epididymis, and a delicate suction is performed with a syringe



Craft I, Bennet V, Nicholson N. Fertilising ability of testicular spermatozoa. *Lancet*. 1993;342:864.; Nieschlag E, Behre HM, Nieschlag S, eds. *Andrology: Male Reproductive Health and Dysfunction*. 3rd ed. Berlin, Germany: Springer-Verlag; 2010.; Lipshultz LI, Howards SS. *Infertility in the Male*. 3rd ed. Maryland Heights, MO: Mosby; 1996.; Pasqualotto FF, Rossi-Ferragut LM, Rocha CC, Iaconelli A Jr, Ortiz V, Borges E Jr. The efficacy of repeat percutaneous epididymal sperm aspiration procedures. *J Urol*. 2003;169:1779-1781.



## Testicular Sperm Recovery by Excisional Biopsy (TESE)

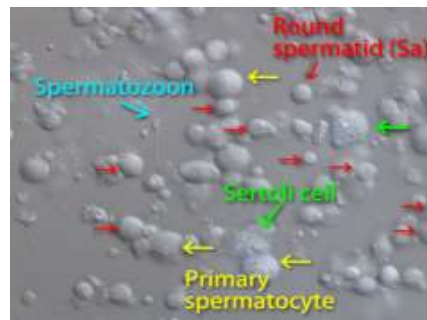
- Equivalent to a diagnostic testicular biopsy procedure
  - May be performed under local anesthesia
- Recovery rate:
  - Normal spermatogenesis → 100%
  - Maturation arrest/germ-cell aplasia → 50%
- Minimally invasive
- ICSI results with sperm obtained from patients with non-obstructive azoospermia remain poor

Girardi SK, Schlegel P. MESA: review of techniques, preoperative considerations and results. *J Androl.* 1996;17:5-9.; Nicopoulos JD, Gilling-Smith C, Almeida PA, Norman-Taylor J, Grace I, Ramsay JW. Use of surgical sperm retrieval in azoospermic men: a meta-analysis. *Fertil Steril.* 2004;82:691-700.; Sheynkin Y, Ye Z, Menendez S, Liotta D, Veeck LL, Schlegel P. Controlled comparison of percutaneous and microsurgical sperm retrieval in men with obstructive azoospermia. *Hum Reprod.* 1998;13:3086-3089.; Tournaye H. Surgical sperm recovery for intracytoplasmic sperm injection: which method is to be preferred? *Hum Reprod.* 1999;14(Suppl 1):71-81.; Schlegel P, Su L. Physiological consequences of testicular sperm extraction. *Hum Reprod.* 1997;12:1688-1692.; Van Peperstraten A, Proctor ML, Johnson NP, Philipson G. Techniques for surgical retrieval of sperm prior to intra-cytoplasmic sperm injection (ICSI) for azoospermia. *Cochrane Database of Syst Rev.* 2008;(2):CD002807.



## Microdissection Testicular Sperm Extraction (MD-TESE)

- Small tissue samples may be taken using a microsurgical approach as proposed by Schlegel in 1999
- This technique may be very useful in patients with incomplete Sertoli cell-only syndrome, where there is a substantial difference in diameter for empty and filled tubules
- Thorough exploration with a microscope enables the surgeons to choose the largest tubules in the testis associated with more spermatozoa
- Better retrieval results with microsurgical sperm extraction compared to conventional open testicular retrieval



Schlegel P. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod.* 1999;14:131-135.; Schlegel P, Su L. Physiological consequences of testicular sperm extraction. *Hum Reprod.* 1997;12:1688-1692.; Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. *Fertil Steril.* 2002;77:873-878.; Sheynkin Y, Ye Z, Menendez S, Liotta D, Veeck LL, Schlegel P. Controlled comparison of percutaneous and microsurgical sperm retrieval in men with obstructive azoospermia. *Hum Reprod.* 1998;13:3086-3089.



## Varicoceles

- The incidence of varicocele is about 15% in the general population and roughly 40% in the infertile population
- Approximately 20% of males with varicocele have evidence of infertility, while the majority (80%) are fertile
- The exact mechanisms by which varicoceles affect fertility remain unclear

Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. *Fertil Steril*. 2002;77:873-878.; Pasqualotto FF, Lucon AM, de Góes PM, et al. Semen profile, testicular volume, and hormonal levels in infertile patients with varicoceles compared with fertile men with and without varicoceles. *Fertil Steril*. 2005;83:74-77.; Sigman M. There is more than meets the eye with varicoceles: current and emerging concepts in pathophysiology, management, and study design. *Fertil Steril*. 2011;96:1281-1282.



## Indications for Varicocele Treatment

- Palpable varicocele
- Documented infertility of the couple
- Female partner: normal fertility or a potentially correctable cause of infertility
- Male partner: abnormal semen analyses or abnormal results from sperm function tests
- Hypogonadism (low testosterone)
- Adult males who are not currently trying to conceive but have a palpable varicocele, abnormal semen parameters, and wish to leave their family planning options open for the future are also candidates for varicocele repair<sup>1</sup>

Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. *Fertil Steril*. 2002;77:873-878.



## Varicocele and Infertility

- The etiologic role of varicoceles in causing infertility remains controversial
- Effect of varicocele repair on pregnancy and live-birth rates
- There is no evidence to define the ideal treatment for men with subclinical varicocele
- Studies indicate an improvement in semen parameters after varicocele repair
- The mechanisms by which varicocele effect fertility are unclear

Sigman M. There is more than meets the eye with varicoceles: current and emerging concepts in pathophysiology, management, and study design. *Fertil Steril.* 2011;96:1281-1282.; Jarow JP, Sharlip ID, Belker AM, et al. Best practice policies for male infertility. *J Urol.* 2002;167:2138-2144.; Sharlip ID, Jarow JP, Belker AM, et al. Best practice policies for male infertility. *Fertil Steril.* 2002;77:873-878.; Evers JH, Collins JA. Surgery or embolisation for varicoceles in subfertile men. *Cochrane Database Syst Rev.* 2008;(3):CD000479.



## Primary Ciliary Dyskinesia (PCD) with Completely Immotile Sperm and Structurally Abnormal Sperm Tails

- Structurally abnormal, completely immotile sperm
- Kartagener syndrome (dextrocardia, bronchoectasia, chronic bronchitis) is a type of PCD
- Viable sperm found with HOST; healthy baby following ICSI or pentoxifylline possible

Eliasson R, Mossberg B, Camner P, Afzelius BA. The immotile-cilia syndrome. A congenital ciliary abnormality as an etiologic factor in chronic airway infections and male sterility. *N Engl J Med.* 1977;297:1-6.; Nagy ZP, Verheyen G, Tournaye H, Van Steirteghem AC. Special applications of intracytoplasmic sperm injection: the influence of sperm count, motility, morphology, source and sperm antibody on the outcome of ICSI. *Hum Reprod.* 1998;13(Suppl 1):143-154.; McLachlan RI, Ishikawa T, Osianlis T, et al. Normal live birth after testicular sperm extraction and intracytoplasmic sperm injection in variant primary ciliary dyskinesia with completely immotile sperm and structurally abnormal sperm tails. *Fertil Steril.* 2012;97:313-318.; Mangoli V, Mangoli R, Dandekar S, Suri K, Desai S. Selection of viable spermatozoa from testicular biopsies: a comparative study between pentoxifylline and hypoosmotic swelling test. *Fertil Steril.* 2011;95:631-634.



## Application of Artificial Oocyte Activation in Severe Male Factor Infertility Cases

- A deficiency in the mechanism of oocyte activation is the most common cause of fertilisation failure after ICSI
- Phospholipase C zeta (PLCzeta) plays an important role in fertilisation
- Calcium ionophore yields high fertilisation and pregnancy rates
  - 56.9% fertilisation rate
  - 39.7% clinical pregnancy
  - 11.8% spontaneous abortions

Sousa M, et al. *Hum Reprod.* 1994;9:2374-2380.; Nasr-Esfahani MH, et al. *Reprod Biomed Online.* 2007;14:422-429.; Yoon SY, et al. *J Clin Invest.* 2008;118:3671-3681.; Heytens E, et al. *Hum Reprod.* 2009;24:2417-2428.; Fujimoto S, et al. *Dev Biol.* 2004;274:370-383.; Nomikos M, et al. *Bioessays.* 2012;34:126-134.; Ebner T, et al. *Fertil Steril.* 2012;98:1432-1437.



## Aromatase Inhibitor

- Some men with severely defective sperm production have excess aromatase activity, reflected by low serum testosterone and relatively elevated estradiol levels
- Aromatase inhibitors can increase endogenous testosterone and serum testosterone levels
- Treatment of infertile males with the aromatase inhibitors, including testolactone, anastrozole, and letrozole, has been associated with increased sperm production and return of sperm in the ejaculate in men with non-obstructive azoospermia
- Use of the aromatase inhibitors represent off-label use of these agents

Schlegel PN. Aromatase inhibitors for male infertility. *Fertil Steril.* 2012;98:1359-1362.; Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. *J Urol.* 2001;165:837-841.  
Gregoriou O, Bakas P, Grigoriadis C, Creatsa M, Hassiakos D, Creatsas G. Changes in hormonal profile and seminal parameters with use of aromatase inhibitors in management of infertile men with low testosterone to estradiol ratios. *Fertil Steril.* 2012;98:48-51.



## Conclusions

- Since the introduction of ICSI, both epididymal and testicular spermatozoa have been used successfully to achieve pregnancies
- Many methods for epididymal or testicular sperm recovery have been proposed, and each has advantages and disadvantages
- Epididymal and testicular spermatozoa used successfully to achieve pregnancies
- MESA is the preferred method for patients with obstructive azoospermia



## Conclusions (con't.)

- PESA is easily performed on an outpatient basis; however the quality and quantity of aspirated sperm is inferior to that of MESA
- MD-TESE is more useful in non-obstructive azoospermia than conventional TESE
- Approximately 20% of males with varicocele have evidence of infertility, while most (80%) are fertile
- The exact mechanisms by which varicoceles affect fertility remain unclear
- The ideal treatment for men with subclinical varicocele remains unknown



## Conclusions (con't.)

- A positive (possibly non-specific) relationship exists between varicocele presence and sperm DNA fragmentation
- Repair for subclinical or clinical varicocele; post-operative improvement in sperm concentration but not in pregnancy rates
- PCD with completely immotile sperm can be rescued with HOST or pentxifiline
- Calcium ionophore and PLCzeta may be useful in cases of severe male factor infertility
- Aromatase inhibitor therapy is thought to be beneficial for men with a low T/E ratio



## References

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