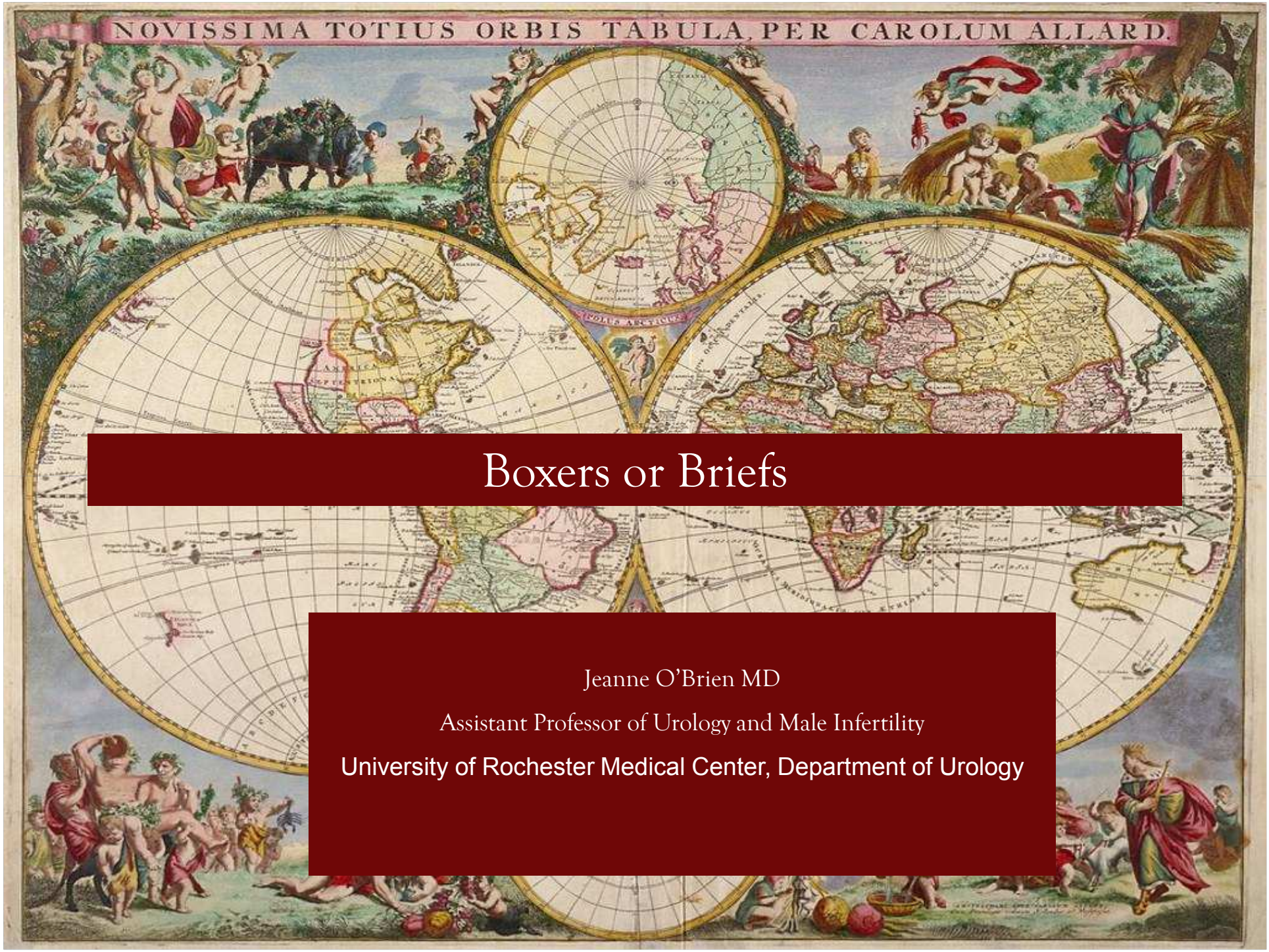

Boxers or Briefs

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Male Infertility: Definitions

- Primary infertility: inability to achieve pregnancy > 1yr
- Secondary infertility: previously fertile, now unable >1 yr
- Azoospermia: no sperm in semen
- Oligospermia: reduced sperm concentration <20 million/ml
- Asthenospermia: reduced percent motility <50%
- Teratospermia: reduced percent normal forms <30%
- IVF: in vitro fertilization
- ICSI: intra-cytoplasmic sperm injection

Etiology of Male Infertility

- Varicocele (35-40%)
- Idiopathic (25%)
- Infection (~10%)
- Genetic (~10%)
- Endocrine (<5%)
- Immunologic (<5%)
- Obstruction (<5%)
- Cryptorchidism (<5%)

Greenberg et al, J Urology 1978

Male Infertility: Evaluation

- **Basic Evaluation:**
  - History (Questionnaire)
  - Physical examination
  - Standard semen analysis
  - Hormonal evaluation
- **Optional Additional Evaluation:**
  - Genetic counseling and evaluation
  - Specialized sperm function tests
  - Imaging studies
  - Testis biopsy

Male Infertility: History

- **Duration of infertility**
  - Previous treatments
  - Female-factor (anovulation, tubal obstruction)

- Sexual history
  - timing and mechanics of intercourse
  - lubricants (peanut oil, olive oil, egg whites ok)

History

- Childhood & Development
  - cryptorchidism
  - pubertal development
- Medical History
  - systemic illness
- Surgical History
  - abdominal, pelvic or scrotal surgery

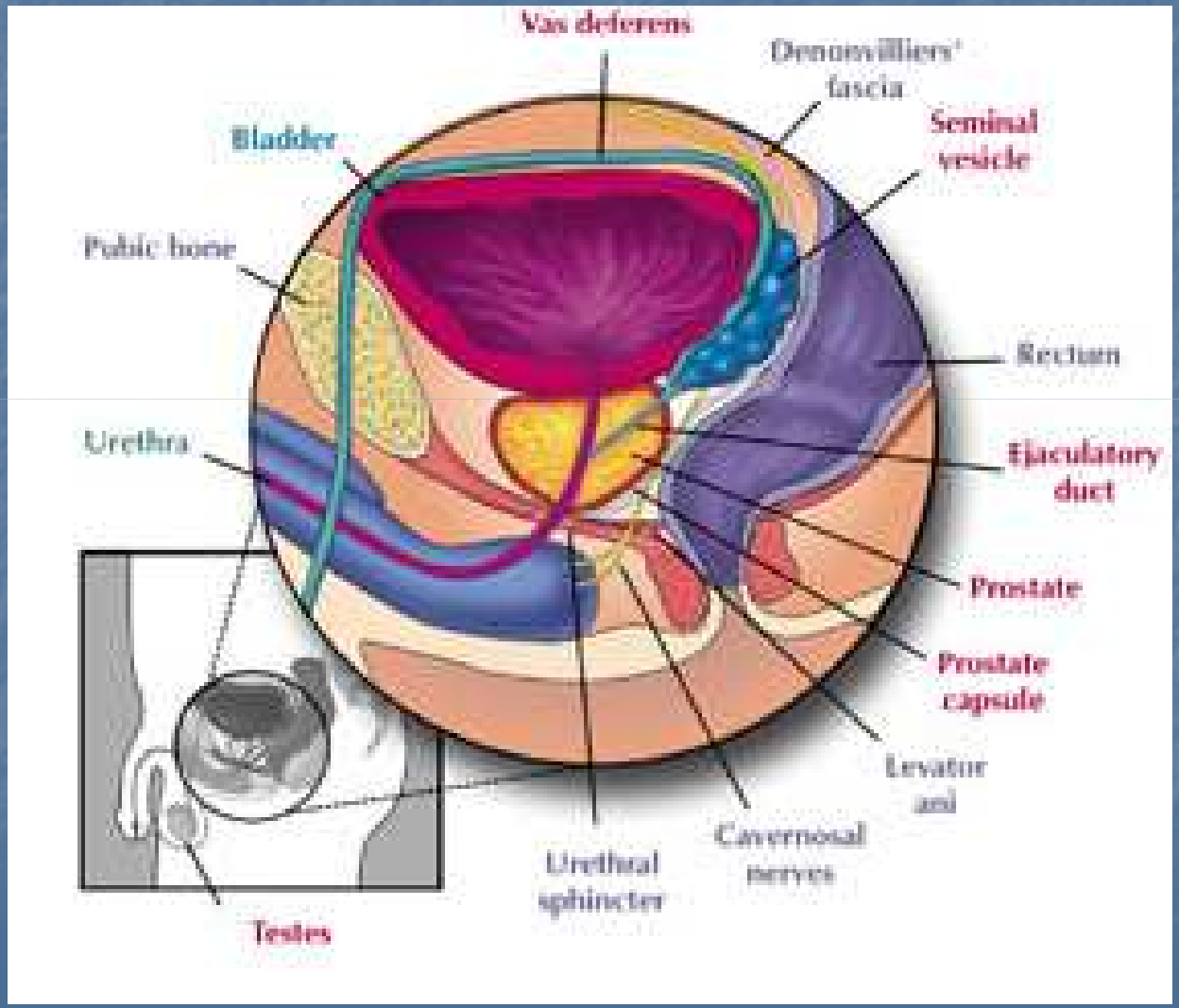
History

- Infections
  - STDs, prostatitis, orchitis (post-pubertal mumps)
- Environmental gonadotoxins
  - smoking
  - ETOH
  - radiation, chemicals, pesticides, chemotherapy
  - Heat exposure (short order cook, tanning booths, hot tub/bath)
- Medications (steroids, herbal supplements, hair growth products)

History: Medications

- **Hormonal (pre-testicular)**
  - e.g. androgens, anti-androgens, estrogens
- **Gonadotoxic (testicular)**
  - e.g. chemotherapy/alkylating agents
- **Sperm-toxic (post-testicular)**
  - e.g. Ca-channel blockers

Anatomy of the male reproductive tract



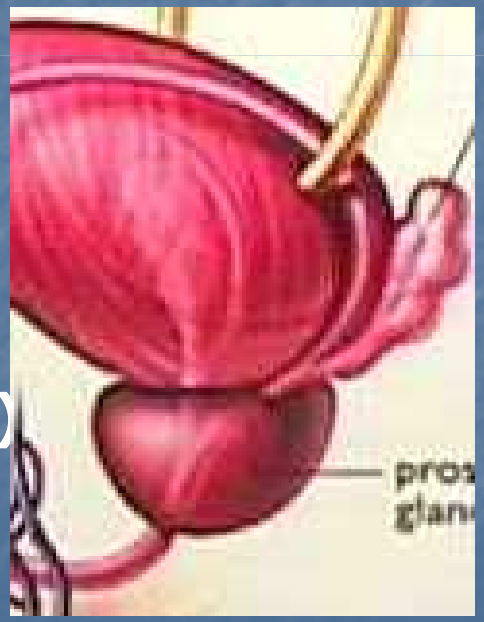
Physical Examination

- *General*
  - Body habitus (muscle mass), hair distribution
  - Evidence of normal virilization
- *CNS*
  - visual fields (r/o pituitary adenoma)
  - sense of smell (Kallmann's Syndrome - HypoHypo)
- *Abdomen/Pelvis*
  - Surgical scars

Physical Examination

Genital/Prostate

- **Penis:**
  - **length (normal development)**
  - **position of urethral meatus (deposition of semen)**
- **Prostate :**
  - **size**
  - **firmness**
  - **tenderness**
  - **presence of cysts (ejaculatory duct)**



Physical Examination

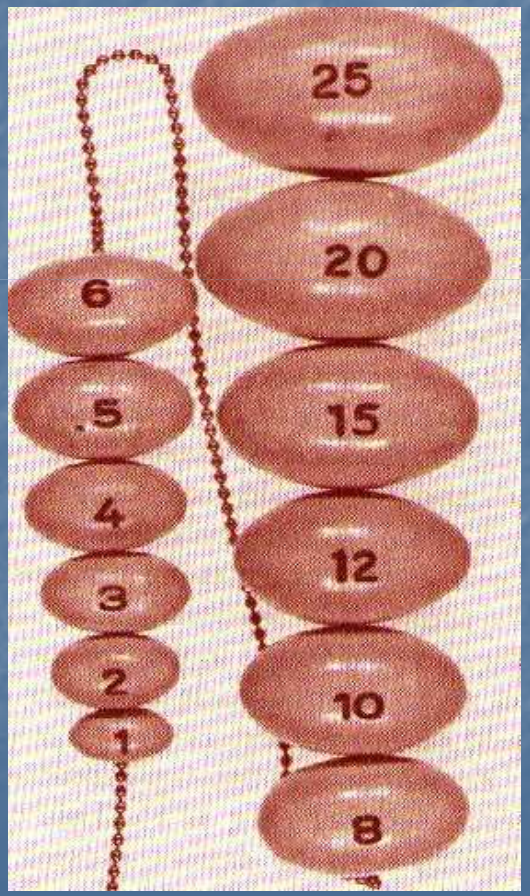
Testis:

-position (cryptorchid?)

-volume (normal ~15-25ml)*

-firmness (normal = firm)

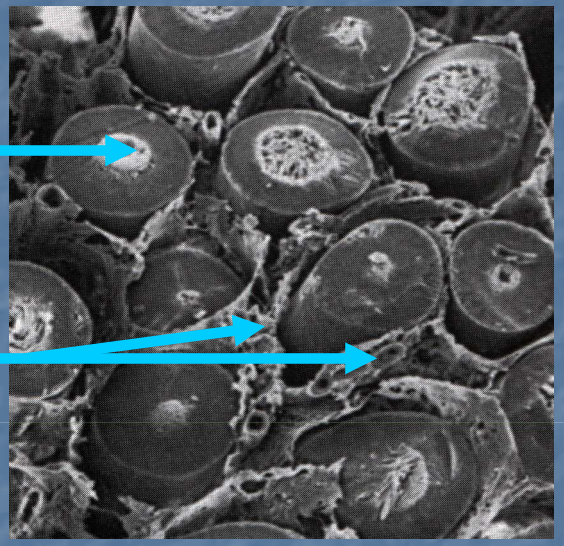
*Note: Normally, >70% of testis volume is from germ cells alone. Therefore, a soft and/or small testis is indicative of abnormal spermatogenesis.



Physical Examination

Testis:

- -Seminiferous tubules
  - Germ cells
  - Sertoli cells
- -Interstitium
  - Leydig cells
  - macrophages, endothelial cells



Spermatogenesis

- ~74 days in humans (epididymal transit ~15 days)
- Clinical correlate: Need to wait 3 months after any intervention (medical or surgical) to see a change in semen quality

Physical Examination

Epididymis:

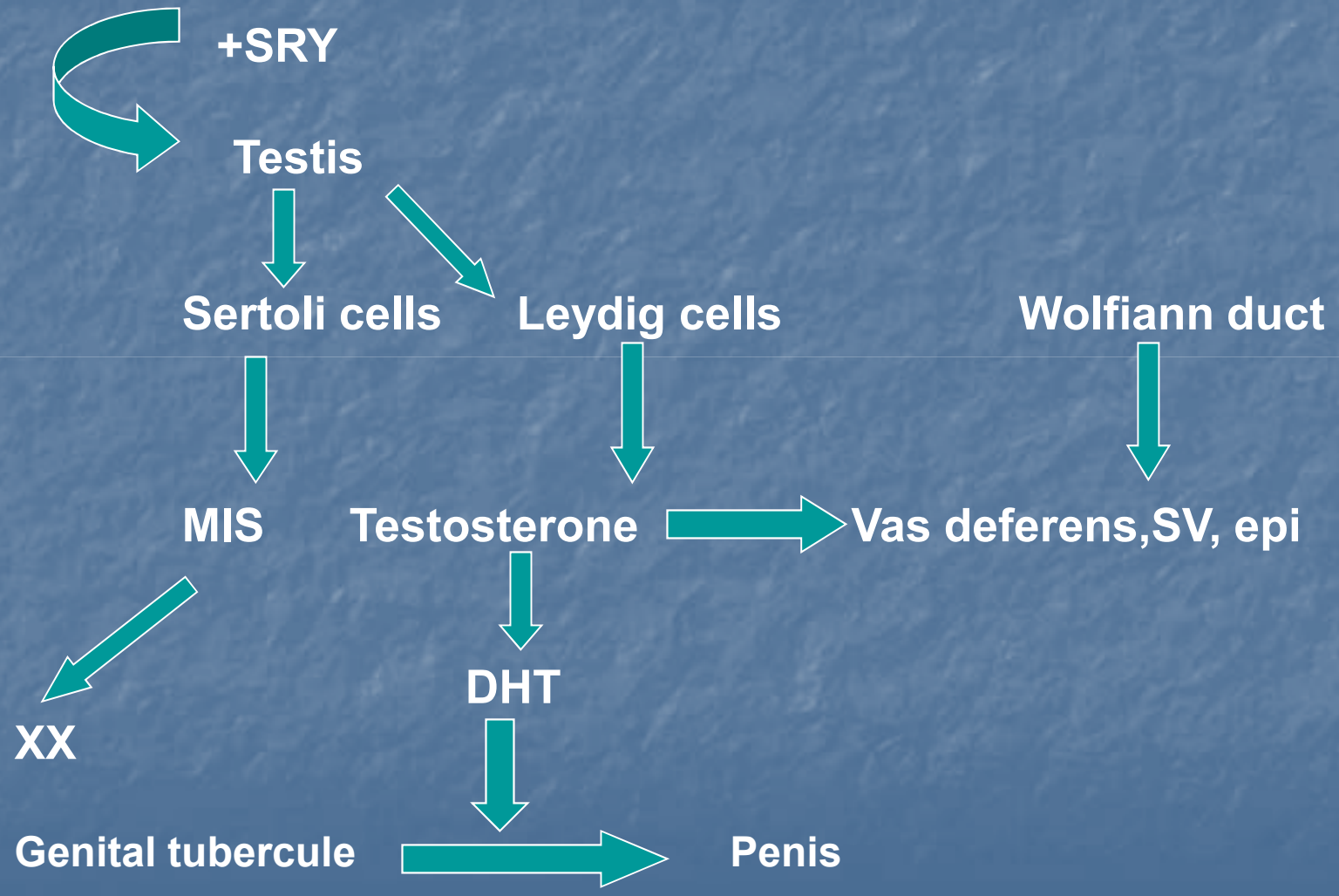
- -fullness
- -cystic changes

Vas deferens:

- -congenital absence of vas (CAVD)
  - Cystic fibrosis mutations
  - Woolfian duct anomalies

Overview of sexual differentiation in the male

(modified from Male Reproductive Biology, eds Lipshultz, Howards)



Varicocele: Diagnosis

- Definition: dilated testicular veins due to reflux of blood
- Established by physical examination (in a warm room)

  Grade 1: palpable with valsalva only

  Grade 2: palpable (> 1cm cord) without valsalva

  Grade 3: large, visible varicocele
- Other modalities used to diagnose a sub-clinical varicocele:ultrasound, venography, doppler stethoscope
- However, the subclinical varicocele does not require repair!

WHO *Fertil Steril* 1985

Howards *Fertil Steril* 1992

Varicocele

Etiology: probably multi-factorial

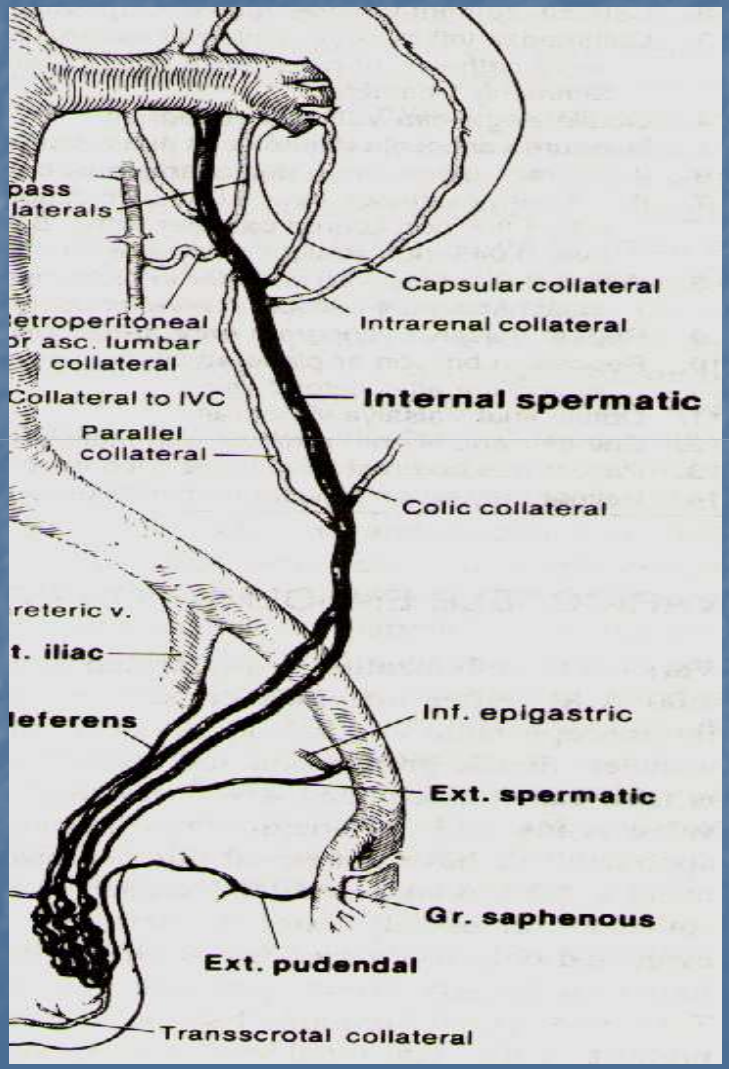
The absence or incompetence of venous valves resulting in reflux of venous blood

The anatomic differences (length, insertion) between the left and right internal spermatic vein.

Increased hydrostatic pressure

Buschi et al, *Am J Radiol* 1980

Braedel et al, *J Urol* 1994



Varicocele: Prevalence

- in the general male population ~ 15%
- in men with primary infertility ~ 35%
- in men with secondary infertility ~ 50-80%
- bilateral varicoceles ~ 15-50%
- isolated right sided varicocele rare

Clarke *JAMA* 1966

Greenberg et al, *J Urol* 1978

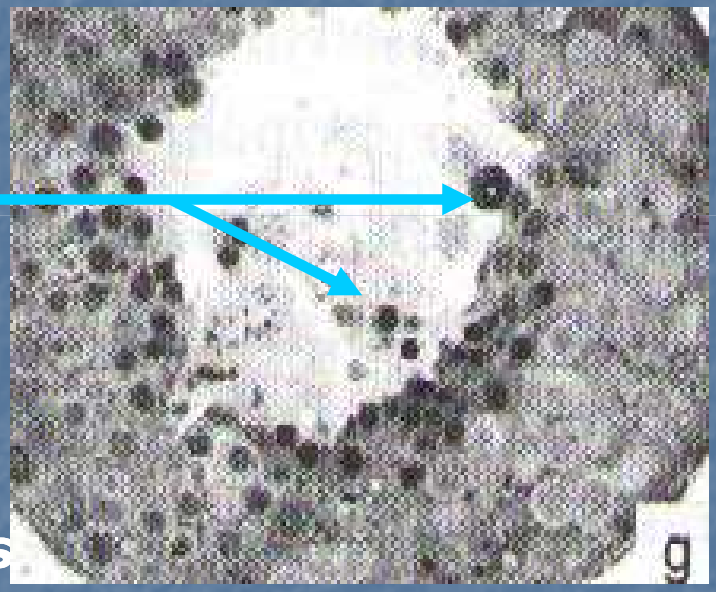
Varicocele-Induced Pathology

Testis atrophy

men with a left varicocele have a relative left testicular atrophy

Testis histology (non-specific)

- Hypospermatogenesis
- sloughing of germ cells
- Sertoli cell vacuoles
- Leydig cell hyperplasia



Leydig cell dysfunction

- Lower serum Testosterone (T) levels
- Blunted T rise in response to LH stimulation

Testicular Pain

- Mechanism unknown

Semen Analysis

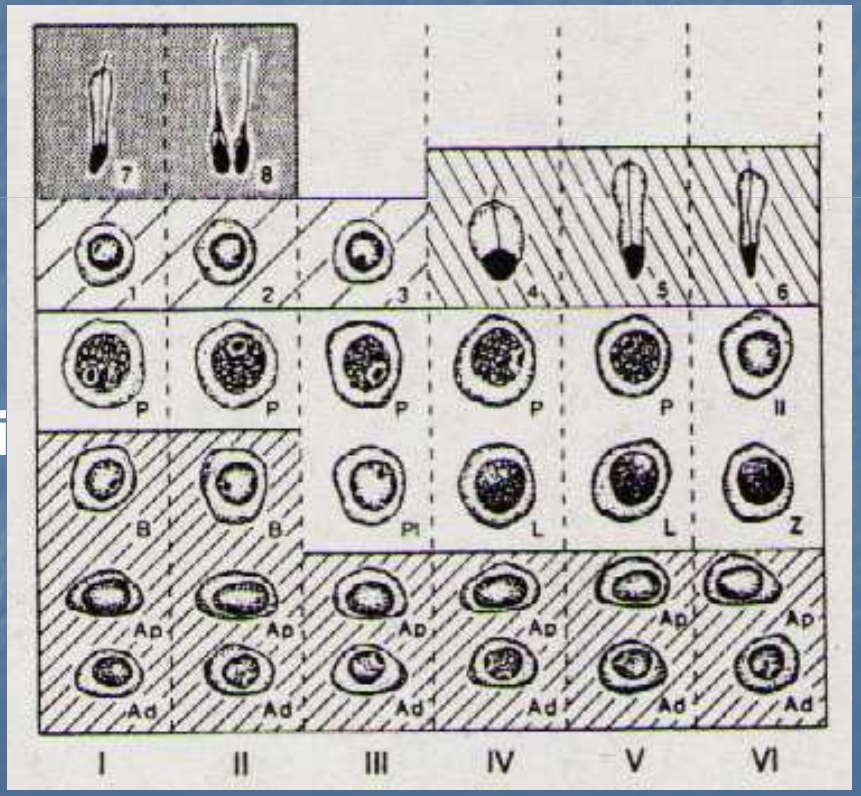
Semen Parameters	*Normal range (WHO)*
Volume	(1.5 - 5 mL)
Sperm density	(>20 million/mL)
Sperm motility	(>50%)
Sperm morphology	(>30% normal forms)
Leukocyte density	(<1 million/mL)

- Need at least 2 S/As (because parameters are highly variable)
- S/A is not a measure of fertility but fertility potential

In Vitro Maturation of Germ Cells

Spermatogenesis: orderly differentiation of immature germ cells to mature spermatozoa

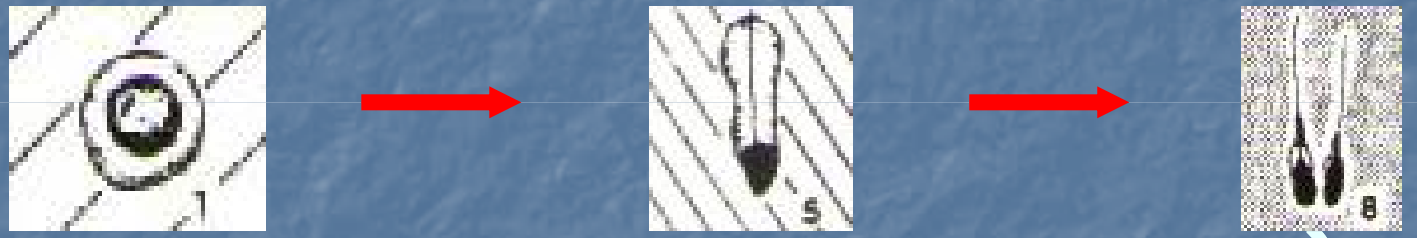
1. Mitotic phase
quantitative phase
2. Meiotic phase
generation of haploid spermati
3. Spermiogenesis
differentiation of spermatid



In Vitro Maturation of Germ Cells

Two separate events observed in vitro

1. Spermatid differentiation (round to elongated)



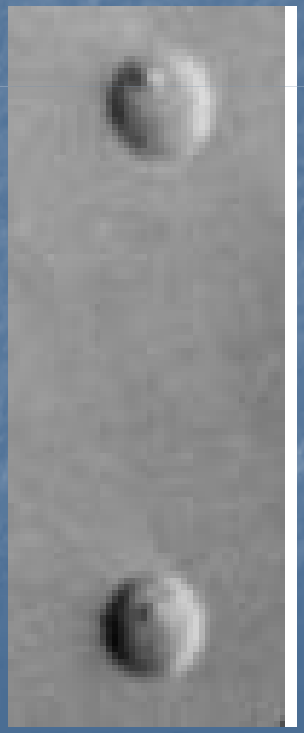
2. Meiotic progression (spermatocyte to spermatid)

Tesarik et al, *Hum Reprod* 1998, 2000, Tesarik et al, *Fertil Steril* 2002
Cremades et al, *Hum Reprod* 1999, Sousa et al, *Hum Reprod* 2002

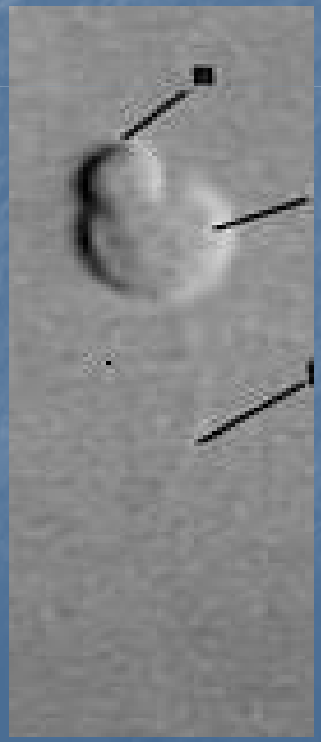
In Vitro Maturation of Germ Cells:

Assessment of in vitro maturation depends on serial sampling an
identification of most mature germ cell by light microscopy

Round spermatid



Elongating spermatid

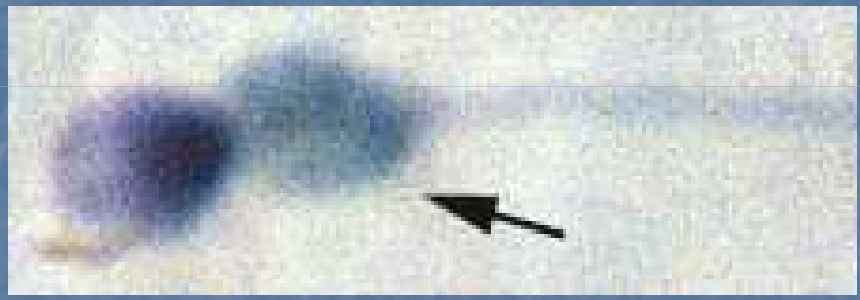


Elongated spermat

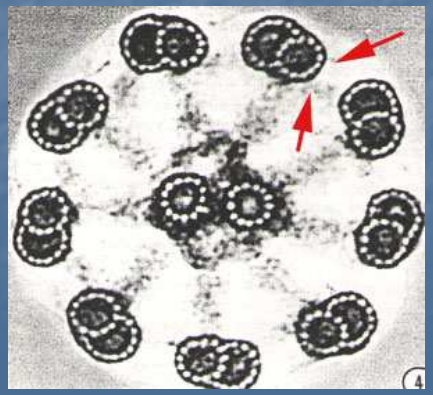
Human Ejaculate: Morphologic Abnormalities

Sperm head defects

Sperm mid-piece defects



Sperm tail defects



Semen Analysis: Critical Review

Guzick et al, *NEJM* 2001

Evaluated 765 infertile men and 696 fertile controls to determine semen parameter thresholds that best discriminate between fertile and infertile men.

Infertile couples

- part of a randomized Assisted Reproduction trial.
- female partners had a normal, complete evaluation (poorly controlled in prior studies).

Fertile controls

- recruited from prenatal classes.
- wives were pregnant or had delivered in the previous 2 yrs

Semen Analysis: Critical Review

Guzick et al, *NEJM* 2001

Methods:

2 semen samples were collected from each patient.

Technicians from the 9 centers were trained at a central site.

Stained sperm smears were sent to a central site for strict morphology assessment (by a single technician).

Statistical Analysis:

Classification-and-regression-tree (CART) analysis was used to define thresholds for classifying infertility

Receiver-operating-characteristic (ROC) curves were used to test the discriminatory power of each variable

Semen Analysis: Critical Review

Guzick et al, *NEJM* 2001

Results:

- Considerable overlap between sperm measurements from fertile and infertile men noted
- The odds of infertility increased with an increasing number of abnormal sperm measurements.
- % normal morphology has the greatest (albeit poor) discriminatory power

Area under ROC curve for normal morphology (0.66) is greater than for sperm concentration (0.60) & motility (0.59)

Semen Analysis: Critical Review

Guzick et al, *NEJM* 2001

Variable	Semen Measurement		
	Concentration	Motility	Morphology
	x10^6/ml	%	%normal
Fertile range	>48.0	>63	>12
Indeterminate range	13.5-48	32-63	9-12
Subfertile range	<13.5	<32	<9

Semen Analysis: Critical Review

Guzick et al, *NEJM* 2001

Conclusions:

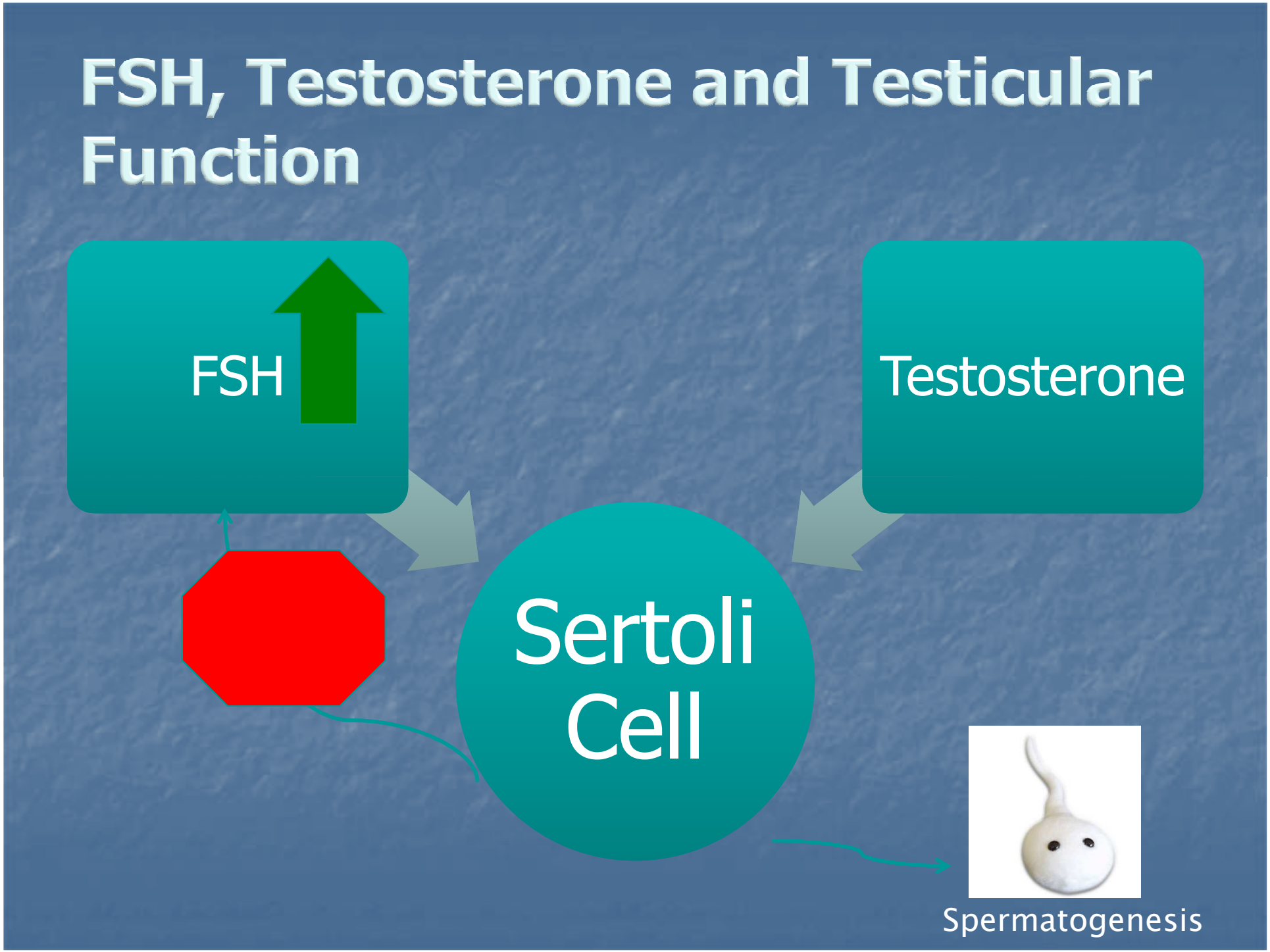
None of the semen parameters is a powerful discriminator

Using three categories is more clinically relevant

- Fertile --Fertile
- Indeterminate vs.
- Subfertile --Infertile

There is a need for identifying of new markers of male infertility

FSH, Testosterone and Testicular Function



What is "Normal FSH"

- Given the negative feedback on FSH, a "high" FSH can be indicative of testicular failure.
- What is "normal"?
- In our laboratory system, the normal range for FSH in the post pubescent male is defined as 1.4-18.1 mIU/ml.
- Nearly all male patients have "normal" FSH.

Mean Age	35
Mean Motility	33%
Mean Morphology	14%
Mean Volume	3 ml
Mean Sperm Conc.	41 mill/mil
Mean FSH	6.6 miU/ml
Mean Test/FSH	105
Normal Volume	412 (77%)
Abnormal Volume	126 (23%)
Normal Motility	120 (22%)
Abnormal Motility	418 (78%)
Normal Morphology	436 (81%)
Abnormal Morphology	102 (19%)
Azoospermia	63 (12%)
Oligospermia	192 (36%)
Normal Sperm Concentration	283 (53%)

Conclusion

- FSH level > 4.5 miU/ml showed statistically significant associations with abnormal sperm motility, concentration, and morphology ($p<0.0001$).

Semen parameters were more likely to be abnormal with decreasing testosterone/FSH ratios.

- Redefining normal FSH in infertile men would be valuable.

(O'Brien et al, AUA Chicago 2009)

Sperm DNA Integrity

Why examine sperm DNA integrity?

A need for better markers of male fertility potential

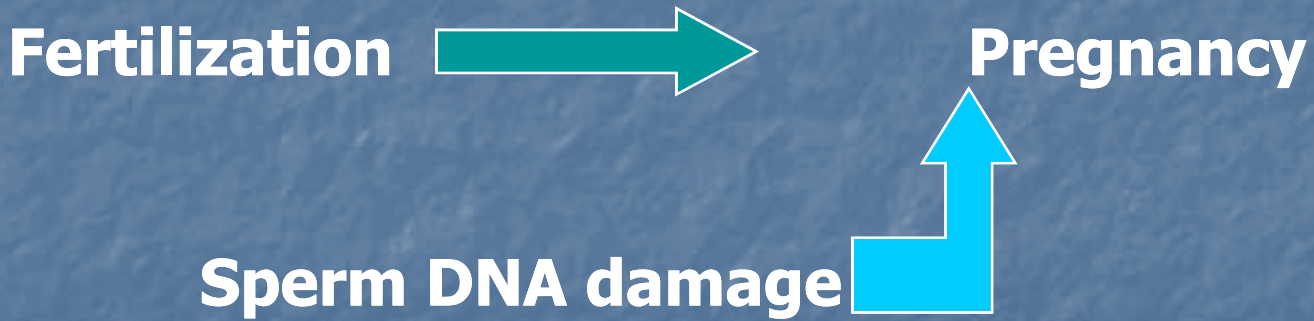
Advances in ART (IVF/ICSI) have led us to be more concerned about sperm DNA integrity

- because we have removed the natural barriers to fertilization
- Reports indicate increased birth defects with IVF/ICSI
- To understand the causes of IVF/ICSI failures

To better understand the basic biology of sperm function

DNA, fertilization, and pregnancy

- High levels of sperm DNA damage probably do not affect ***fertilization or early embryo development***
- May have an effect on ***pregnancy rates*** with advanced reproductive technologies (IVF and IVF/ICSI) and ***recurrent pregnancy loss*** with spontaneous conception



(Lopes, 1998; Host, 2000; Tomlinson, 2001, Tomsu, 2002; Benchaib, 2003; Carrell, 2003)

2 pronuclei, fertilization

Sperm DNA integrity important here – embryonic genome expressed

blastocyst

2 cell embryo

4 cell embryo

8 cell embryo

Human Sperm DNA: Characteristics

Highly compacted and packaged with protamines

Protamines held together by disulfide bonds

Exhibits high degree of integrity?

- **In fact, 10-15% of sperm have DNA damage (in fertile men)**

Biochemically inert?

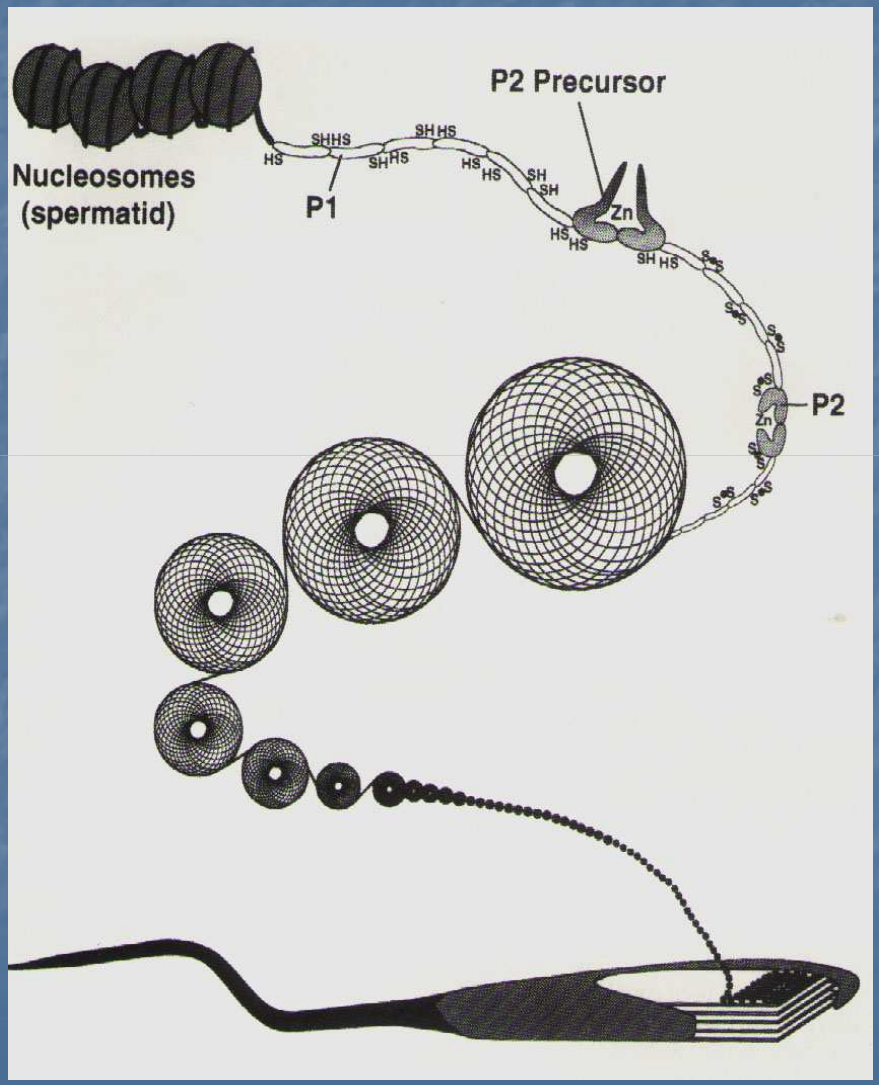
- **In fact, sperm DNA can be modified by endogenous endonucleases**

  Balhorn, *J Cell Biol* 1982
  Lavitrano et al, *Cell* 1989
  Perry et al, *Science* 1999
  Evenson et al, *Science* 1980
  Evenson et al, *Hum Reprod* 1999
  Zini et al, *Fertil Steril* 2001

Sperm DNA Packaging

- Binding of protamines (P1 and P2) to DNA replacing all other protein (histones)
- Formation of toroidal structures (each containing about 50 Kbp of DNA)
- Each sperm contains about 50,000 toroidal structures

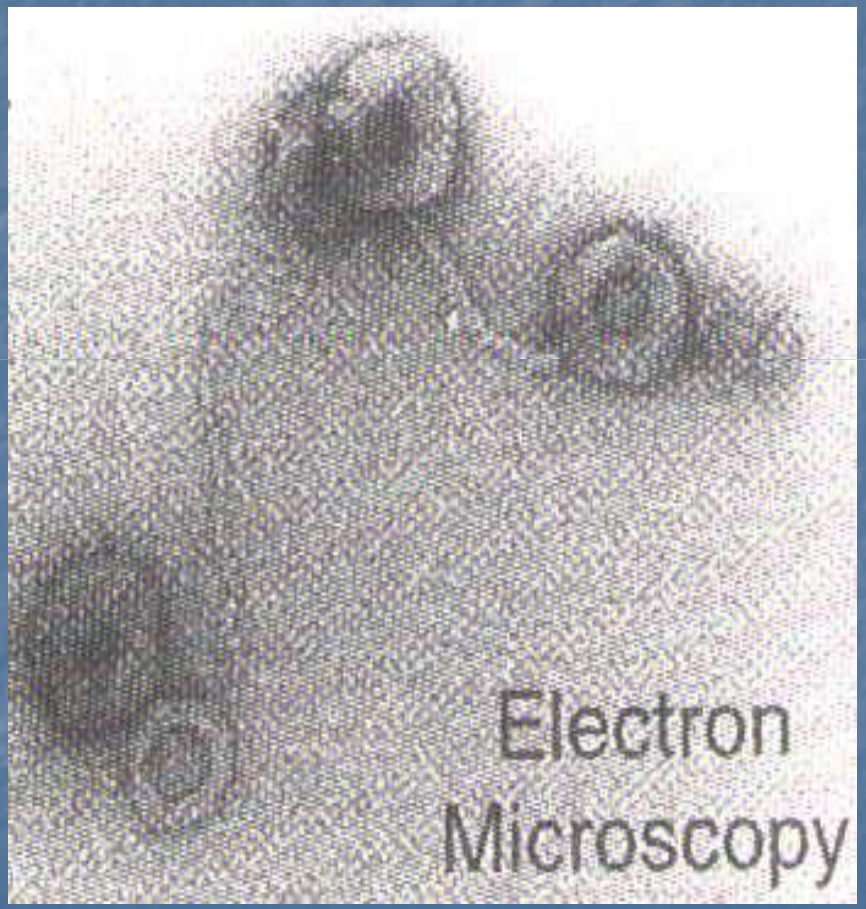
Balhorn, *J Cell Biol* 1982



Sperm DNA Packaging

Toroidal structures (each containing about 50 Kbp of DNA) seen by EM

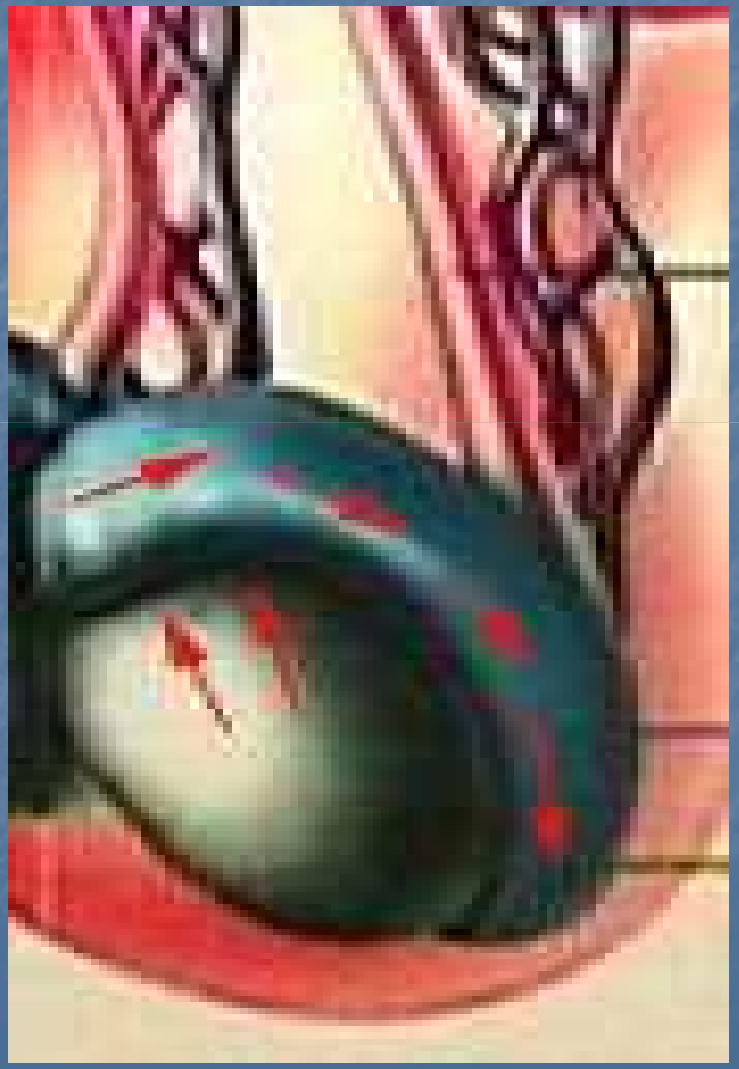
Balhorn, *J Cell Biol* 1982



Sperm DNA Packaging: Evolution During Epididymal Transit

Progressive DNA compaction through cross-linking of protamine –SH groups (both inter- and intra-molecular)

-SH + -SH ⟶ -S--S-



Kosower et al, *J Androl* 1992
Zini et al, *Urology* 2001

Human Sperm DNA Damage: Etiology

Intra-Testicular Causes

Protamine (P1,P2) deficiency is an important cause of sperm DNA damage (found in ~5% of infertile men) and mutations in the protamine gene cluster have been described.

De Yebra et al, *J Biol Chem* 1993
Bianchi et al, *Biol Reprod* 1993
Kramer et al, *Genet Test* 1997

Topoisomerase II and transition protein abnormalities may be a cause of sperm DNA damage (required for repair of induced DNA breaks during super-coiling)

Balhorn, *J Cell Biol* 1982
Kierzenbaum, *Mol Reprod Dev* 2001
Bissoneault, *FEBS Lett* 2002

Cellular apoptosis

Sakkas et al, *Exp Cell Res* 1999

Human Sperm DNA Damage: Etiology

Post-Testicular or External Causes

Febrile Illness may be a cause of sperm DNA damage

Evenson et al, *J Androl* 2000

Semen oxidants (or reactive oxygen species - ROS) can induce sperm DNA damage

- **-ROS cause sperm DNA oxidation and fragmentation in vitro**

  Aitken et al, *Biol Reprod* 1998
  Irvine et al, *J Androl* 2000

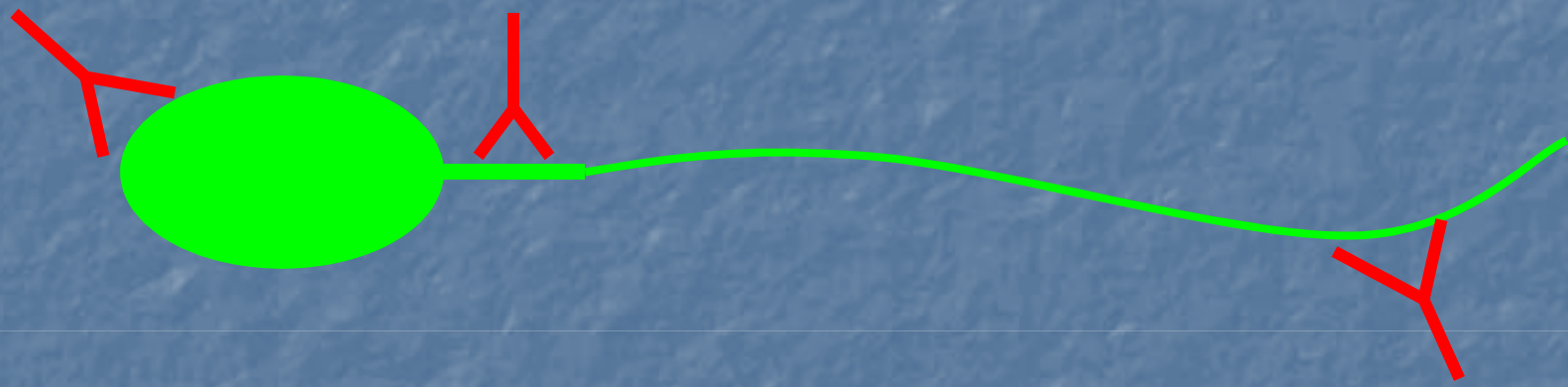
- **-Residual sperm cytoplasm (a cytologic feature associated with ROS production) correlates with sperm DNA damage**

  Fischer et al, *Urology* 2003

Potential causes of DNA fragmentation

- Varicoceles *(Saleh, 2003; Fischer, 2003; Zini, 2000)*
- Chemotherapy and radiation *(Chatterjee, 2000; Deane, 2004; Kobayashi, 2001)*
- Cigarette smoking *(Mak, 2000; Kunzle, 2003; Potts, 1999, Sun, 1993)*
- Reactive oxygen species and leukocytospermia *(de Laminrande, 1993; Zini, 1995, Twigg, 1998; Iwasaki, 1992; Zini, 1993)*
- Apoptosis *(Baccetti, 1996; Sakkas, 2003)*
- Protamine deficiency *(Cho, 2003)*

Antisperm Antibodies (ASAs)



5-10% of male infertility attributed to ASAs

? causative factor in male infertility

? association with ↓ motility

? association with ↓ sperm-egg binding

Antisperm Antibodies: Etiology & Incidence

In women:

Incidence: 9% of infertile vs 4% of fertile women

Etiology: chronic exposure to sperm antigens?

In men:

Incidence: 10% of infertile vs 3% of fertile men

Etiology: injury to blood-testis barrier?

- Obstruction (post-vasectomy reversal)
- Infection (orchitis)
- Trauma/Torsion
- Varicocele/cryptorchidism

Antisperm Antibodies: Testing

Indications: ↓ motility, sperm agglutination

Available tests:

- **Direct:** immunobead or MAR (mixed antiglobulin rxn)
- **Indirect:** SAT, ELISA, MAR
- Others (flow cytometry, radio-labeled)

Direct (on sperm) is more valid than indirect (serum, mucus, seminal plasma) ASA test. Therefore, must be cautious about studies reporting indirect ASA test results.

Hypo-Osmotic Swelling Test (HOST)

Jeyendran et al.1984,

- Curling of tail in 'viable' and straight tail in 'dead' sperm
- **Principle:** that 'viable' sperm have functionally intact plasma membrane
- **Poor viability predicts poor IVF success**
- **May use to select 'viable' testicular (often immotile) sperm**

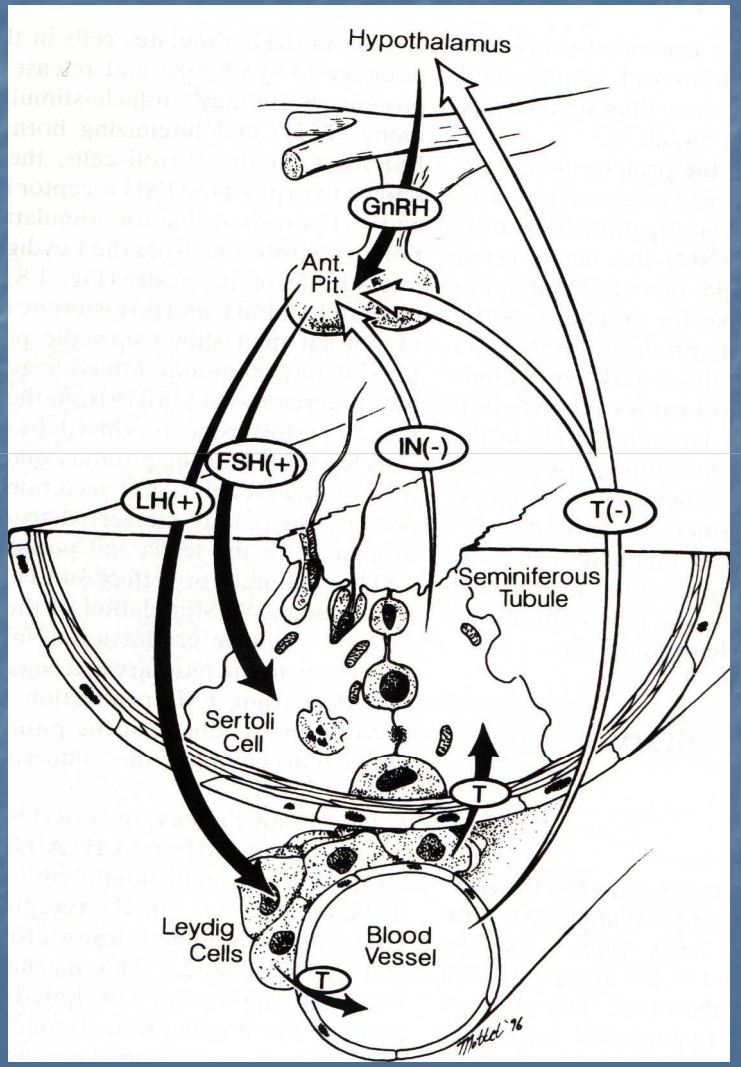
Hormonal Evaluation

Indication:

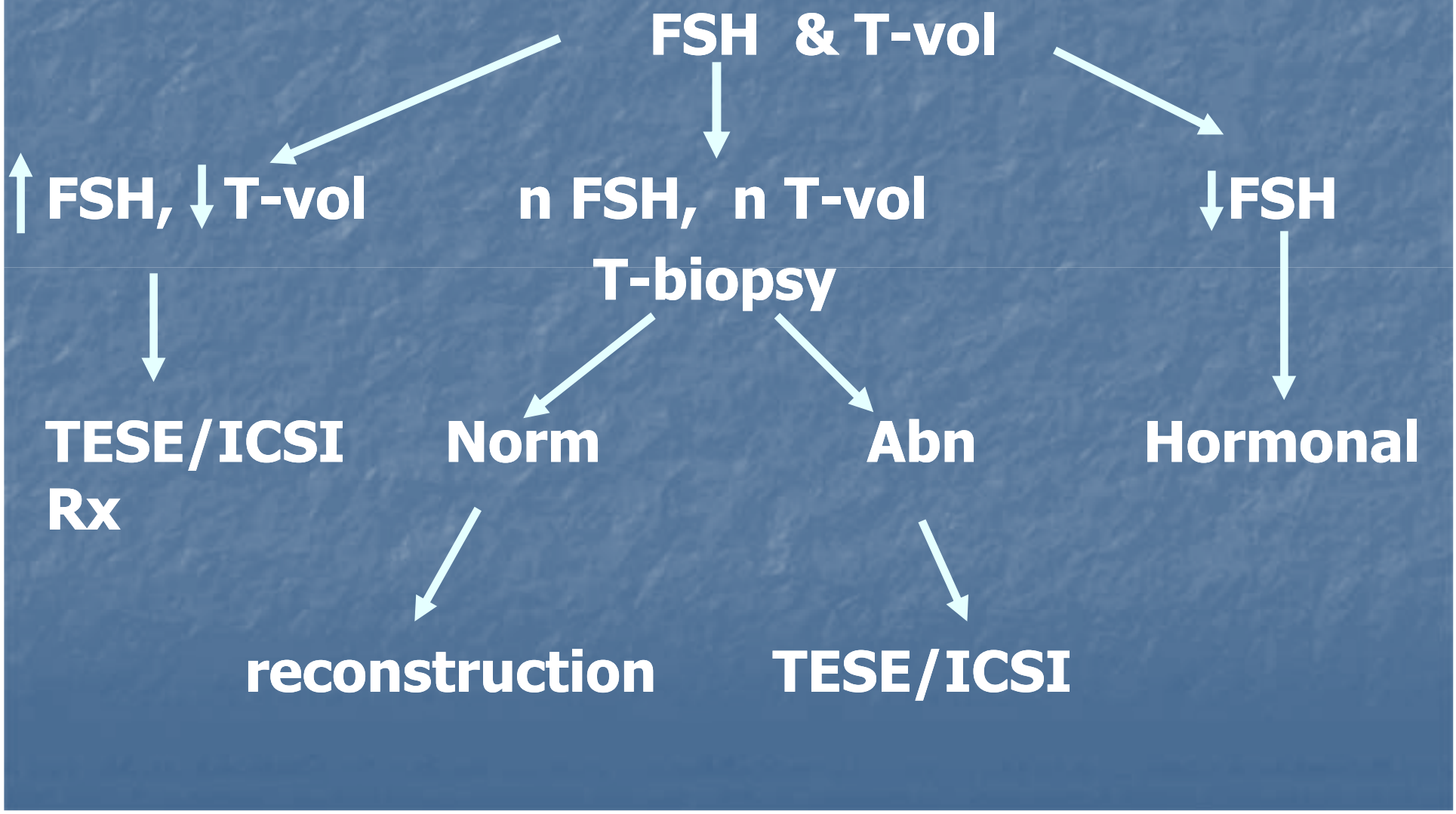
Abnormal semen parameters

Most useful in azoospermic men (to help differentiate between obstruction and primary T failure)

FSH
LH
Testosterone
Prolactin
(Estradiol)



Azoospermia: Normal semen volume



Genetic Evaluation

Karyotype analysis

Abnormal karyotype in ~3-5% of infertile men

Klinefelter's (47 XXY); 1-2% of infertile men

Y- chromosome micro-deletions

7-10% of infertile men vs. ~2% of fertile men

Cystic Fibrosis (CF) gene mutations

Carrier frequency;

~80% in CBAVD vs. ~30% of infertile vs. ~4% fertile men

Pryor et al, 1997, Oates et al, 1992, Mak & Jarvi, 1997

Genetic evaluation is recommended in all infertile men with severe semen parameters in order to assess and prevent possible iatrogenic transmission of genetic mutations

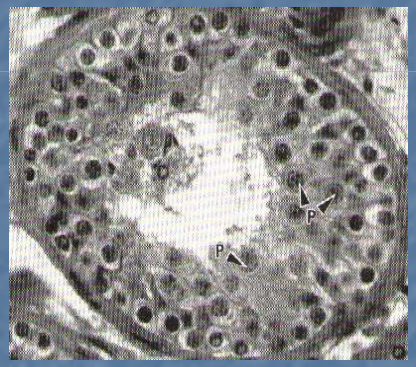
Non-Obstructive Azoospermia (NOA): Etiology

- Idiopathic
- Genetic (chromosomal abn., Y-microdeletion)
- Cryptorchidism
- Iatrogenic (devascularization injury)
- Infectious (post-pubertal mumps orchitis)
- Testicular torsion
- Chemotherapy/radiotherapy-induced
- Medication (hormonal)-induced
- Hormonal deficiency (Kallmann's syndrome or IHH)
- Aneiaculation

Non-Obstructive Azoospermia (NOA)

- **Hypospermatogenesis**
  (0-6 mature spermatids/tubule)

- **Maturation arrest**
  (absence of mature spermatids)



- **Sertoli-cell only**

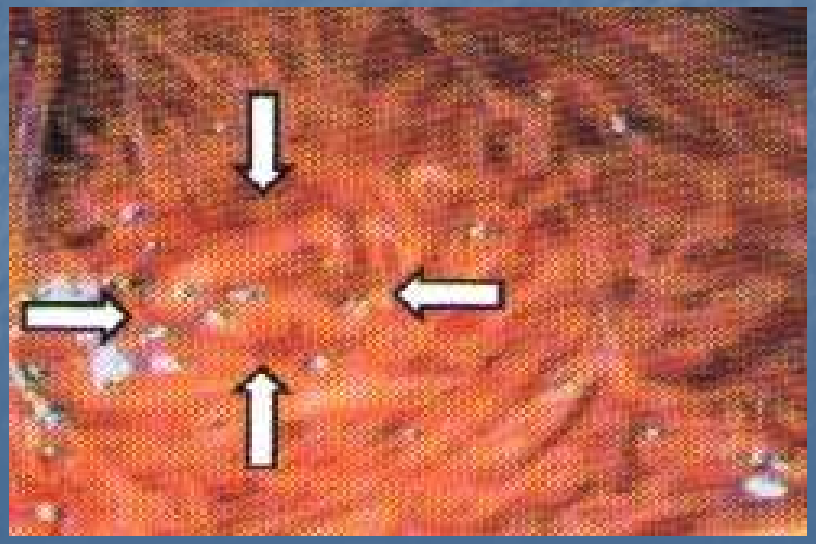
Non-Obstructive Azoospermia (NOA): Management Options

- Sperm retrieval from the testis (micro-TESE)
- Genetic counseling for risk to offspring
- Donor sperm, adoption
- TESE: testicular sperm extraction

Micro-Testicular Dissection

- Small areas of spermatogenesis may be distinguished from areas of Sertoli cell-only by microscopic examination
- Microdissection enhances sperm yield and reduces volume of tissue excised

  Schlegel et al, *Hum Reprod* 1999



Obstructive Azoospermia (OA): Clinical features

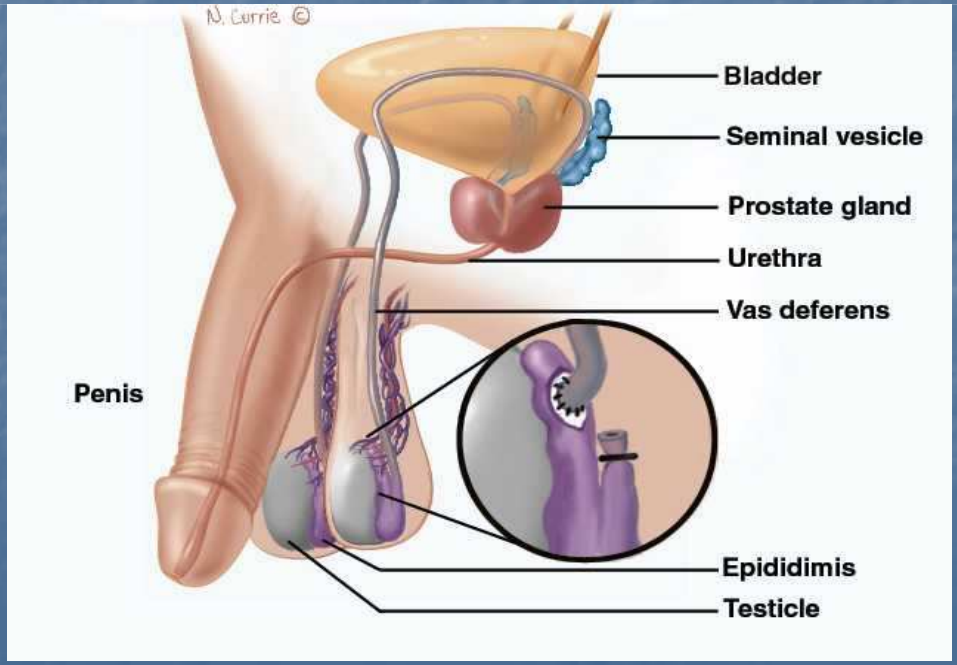
- Normal testicular volume (>15 cc)
- Normal serum levels of FSH, LH, testosterone
- Normal or reduced semen volume (obstructive)
- CBAVD - congenital bilateral absence of the vas
- Normal testicular biopsy (normal spermatogenesis)

Obstructive Azoospermia (OA): Etiology

- Idiopathic OA
- Iatrogenic OA (hernia repair, orchidopexy)
- Infectious (gonorrhea, chlamydia)
- Traumatic
- Genetic - CF with CBAVD or variant thereof

Obstructive Azoospermia (OA): Management Options

- Reconstructive surgery (vasal, epididymal)
- Resection of ejaculatory duct (cyst)
- Sperm retrieval from site proximal to obstruction
- Genetic counseling for CF patients



Conclusion

- Male infertility is multifactorial
- Hormones, physiology, environment, anatomy and DNA all play a role
- It is the delicate balance of all of these factors that must be weighed in order to optimize male fertility
- Every evaluation is different and every treatment strategy is geared toward the individual patient and circumstance and must always take into account the female partner

Conclusion

- So, boxers vs. briefs?
- Boxers every time