Abnormalities of Spermatogenesis

Male Factor

- 40% of the cause for infertility
- Sperm is constantly produced by the germinal epithelium of the testicle
  - Sperm generation time 73 days
  - Sperm production is thermoregulated
    - 1° F less than body temperature
- Both men and women can produce anti-sperm antibodies which interfere with the penetration of the cervical mucus

Semen Analysis (SA)

- Obtained by masturbation
- Provides immediate information
  - Quantity
  - Quality
  - Density of the sperm
- Abstain from coitus 2 to 3 days
- Collect all the ejaculate
- Analyze within 1 hour
- A normal semen analysis excludes male factor 90% of the time
  - Morphology
  - Motility

Normal Values for SA

Volume	2.0 ml or more
Sperm Concentration	20 million/ml or more
Motility	50% forward progression
	25% rapid progression
Viscosity	Liquification in 30-60 min
Morphology	30% or more normal forms
pH	7.2-7.8
WBC	Fewer than 1 million/ml

Causes for male infertility

- 42% varicocele
- repair if there is a low count or decreased motility
- 22% idiopathic
- 14% obstruction
- 20% other (genetic abnormalities)

Abnormal Semen Analysis

- Azospermia
  - Klinefelter's (1 in 500)
  - Hypogonadotropic-hypogonadism
  - Ductal obstruction (absence of the Vas deferens)
- Oligospermia
  - Anatomic defects
  - Endocrinopathies
  - Genetic factors
  - Exogenous (e.g. heat)
- Abnormal volume
  - Retrograde ejaculation
  - Infection
  - Ejaculatory failure

Evaluation of Abnormal SA

- Repeat semen analysis in 30 days
- Physical examination
- Testicular size
- Varicocele
- Laboratory tests
- Testosterone level
- FSH (spermatogenesis- Sertoli cells)
- LH (testosterone- Leydig cells)
- Referral to urology

SEMEN ANALYSIS

Dr. Amal Baalash

Indications

- Assessment of fertility
- Forensic purposes
- Effectiveness of vasectomy - 2 samples 1 month apart negative
- Suitability for artificial insemination

Semen Analysis Include

- Macroscopic
  - viscosity
  - coagulation + liquifaction
  - volume
  - pH
- Microscopic
  - concentration/count
  - motility
  - morphology
  - viability

Motility &Viability must be performed within 1½ - 2 hrs of collection

REMEMBER

SEMEN IS A BODY FLUID

BIOHAZARDOUS

Semen Collection

- Name
- Period of abstinence - 2-7days
- Time of collection
- Entire ejaculate and not coitus interruptus in a wide mouth container
- Delivered within 1 hour of collection
- Avoid temperature extremes

Reference Ranges

- Volume 2.0-6.0 ml
- pH 7.2-8.0
- Count >20 million/ml
- Total count > 40 million
- Morphology > 30% normal form
- Viability > 75%(50% in other)
- WBC< 1million/ml
- RBC none

Macroscopic Examination

- Semen is viscous, yellow grayish.
- Forms gel-like clot immediately.
- Liquefies completely in 5-60 minutes; this must be complete before further testing (mix before further testing).
- Appearance: homogenous white-gray opalescence.
  - Brown/red in hematospermia
  - Dense white turbid if inflammation and high WBC

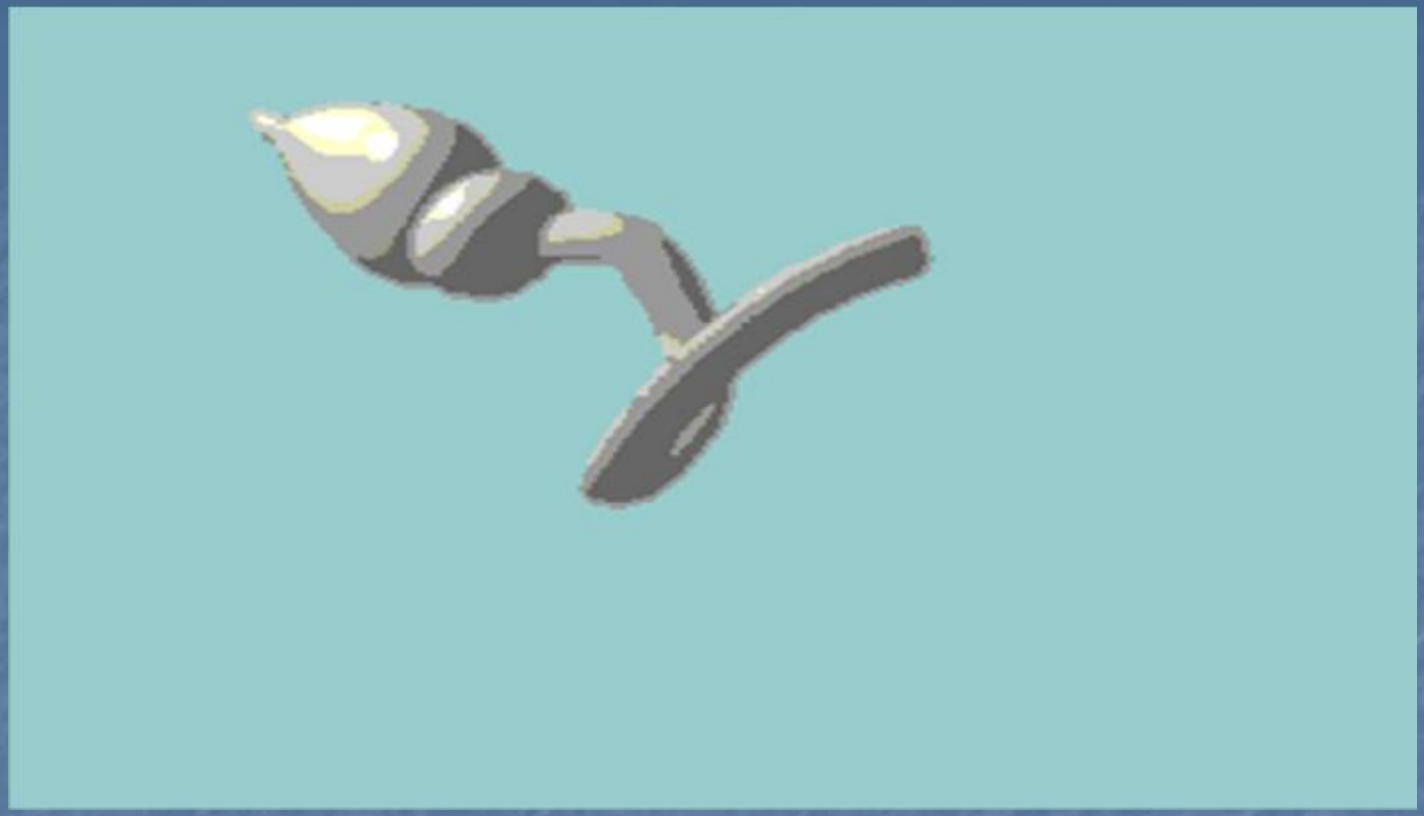
Macroscopic Examination

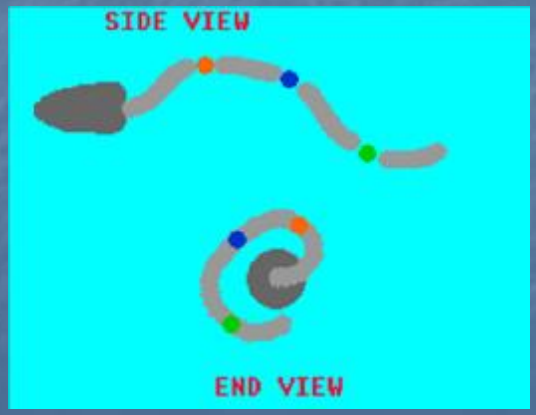
- Volume: in graduated cylinder to the nearest 0.1 ml or centrifuge tube free of contamination.
- Viscosity: 5ml pipette or plastic pipette
  - normal, more viscous, very viscous
- pH: important parameter of motility and viability 7.2-8.0; measured by pH paper.

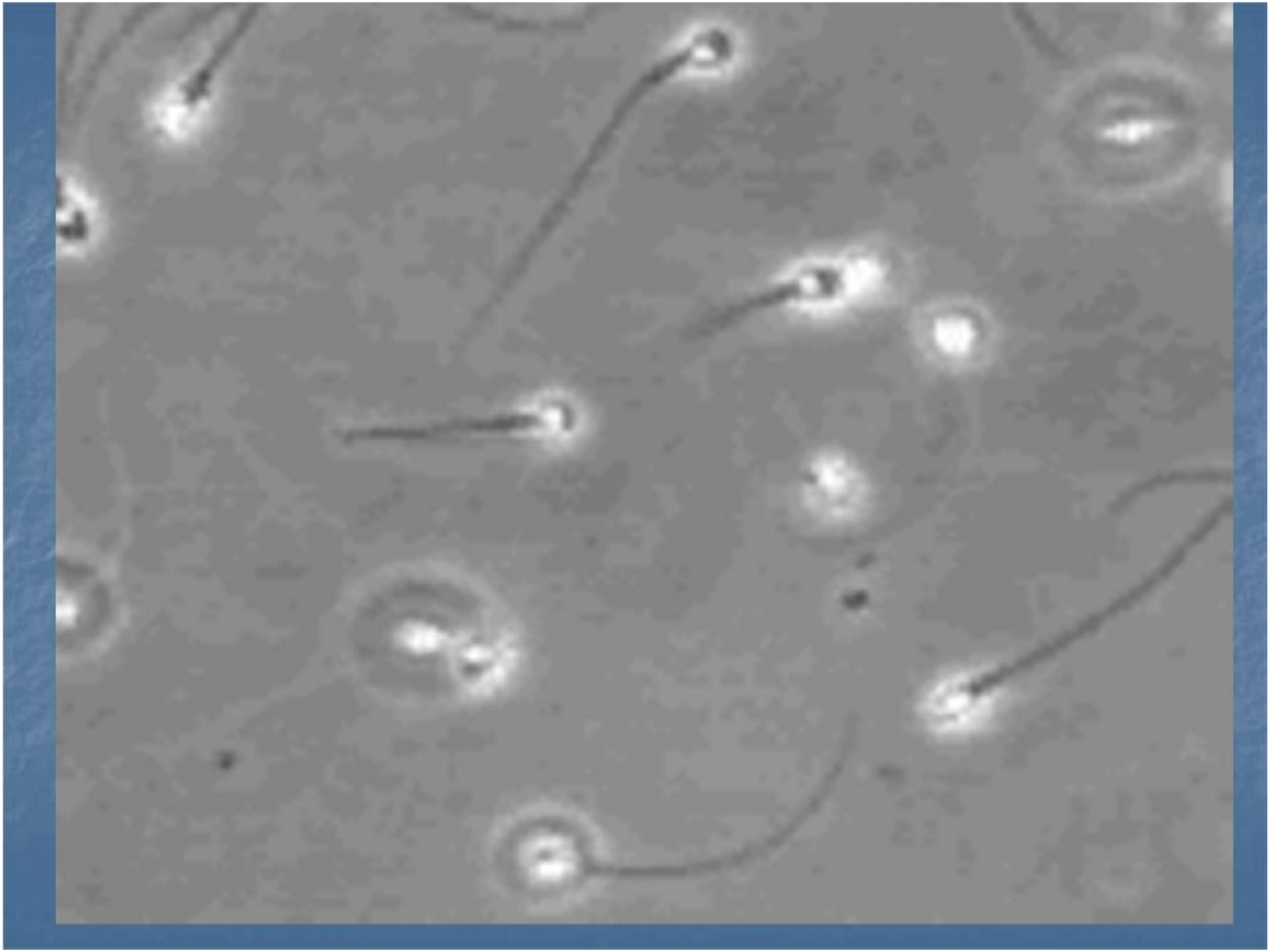
Motility

- While estimating count
- No stain
- Count 200 total sperm and then the motile
- Calculate the percentage of
  - Progressive motile
  - Sluggishly motile (<5 um/s)
  - nonmotile
- >50% motile

0	No movement
1	Movement, none forward
1+	Occasional movement of a few sperm
2	Slow, undirected
2+	Slow , directly forward movement
3-	Fast, but undirected movement
3	Fast, directed forward movement
3+	Very fast forward movement
4	Extremely fast forward movement



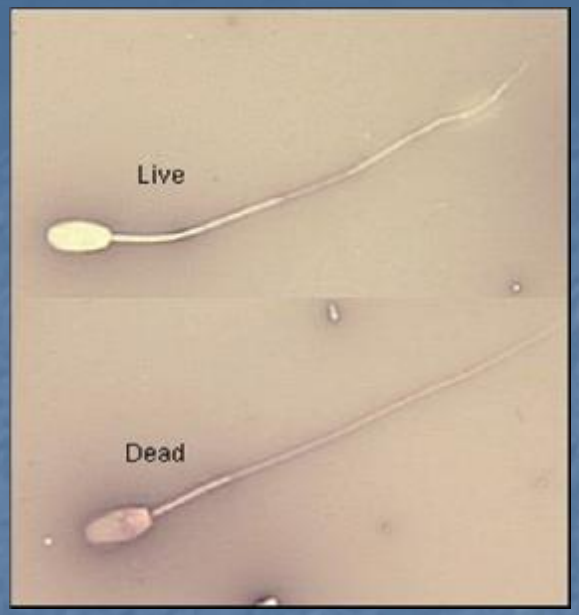

SIDE VIEW
END VIEW



Agglutination

- Reported when motile sperm stick to each other in a definite pattern.
  - Head-head
  - Tail-tail
  - Head-tail
- Immunological cause of infertility
- Done on several HPF

Viability



- Supravital stain:
  - Eosin +/- Nigrosin
    - Viable do not take up the stain
- This distinguish live nonmotile from dead; it is important to compare viability and motility.

Morphology

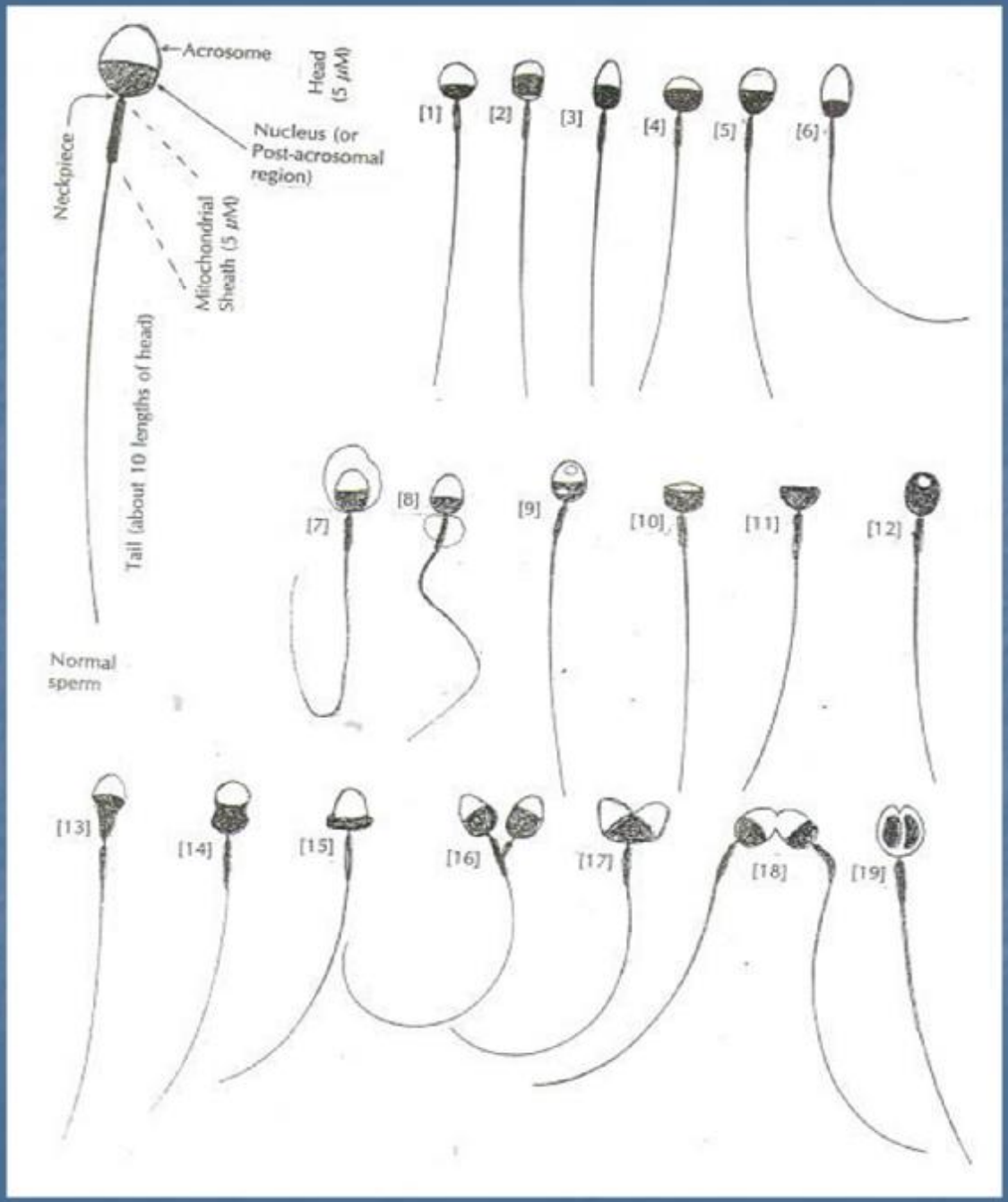
- *Smear:*
  - *H&E, Papanicolaou, Wright stains*
  - *Feathering like blood smear or 2 slides*
  - *Count and classify 100-200 spermatozoa*
  - *Examine the head, midpiece, tail*
- *Normal >30%*
- *Immature*
- *Abnormal*

Mira1000 Semen Analyzer (CASA)

- *Aspermia:* No semen ejaculated
- *Hematospermia:* Blood present in semen
- *Leucocytospermia:* White blood cells present in semen
- *Azospermia:* No spermatozoa found in semen
- *Normospermia:* Normal semen parameters
- *Oligospermia:* Low sperm concentration
- *Asthenospermia:* Poor motility and/or forward progression
- *Teratospermia:* Reduced percentage of morphologicall normal sperm
- *Necrospermia:* No live sperm in semen

Other Sperm Abnormalities

- Head abnormalities:
  - absence
  - double head
  - micro/megalo
- Tail abnormalities:
  - coiled
  - kinked
  - lengthened


Acrosome
Head (5 μM)
Nucleus (or Post-acrosomal region)
Neckpiece
Mitochondrial Sheath (5 μM)
Tail (about 10 lengths of head)
Normal sperm
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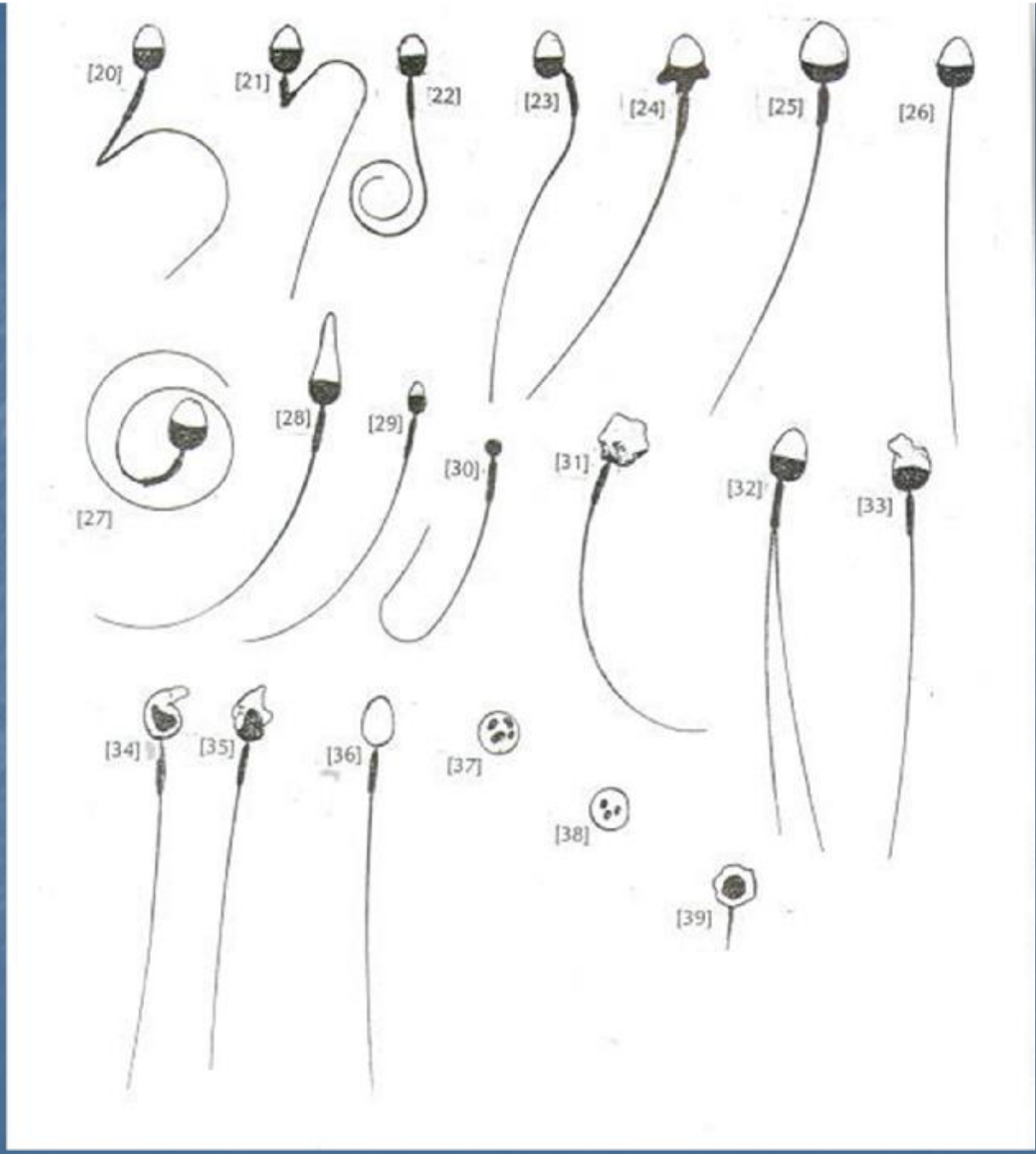
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Sperm Count

- Manual methods
  - Hemocytometer or counting chamber
- Computer assisted
- Oligospermia<20 million
- If azospermia: fructose level must be ordered to verify the integrity of the vas and seminal vesicles

Preparation

- Manual methods
  - Hemocytometer or counting chamber
- Computer assisted

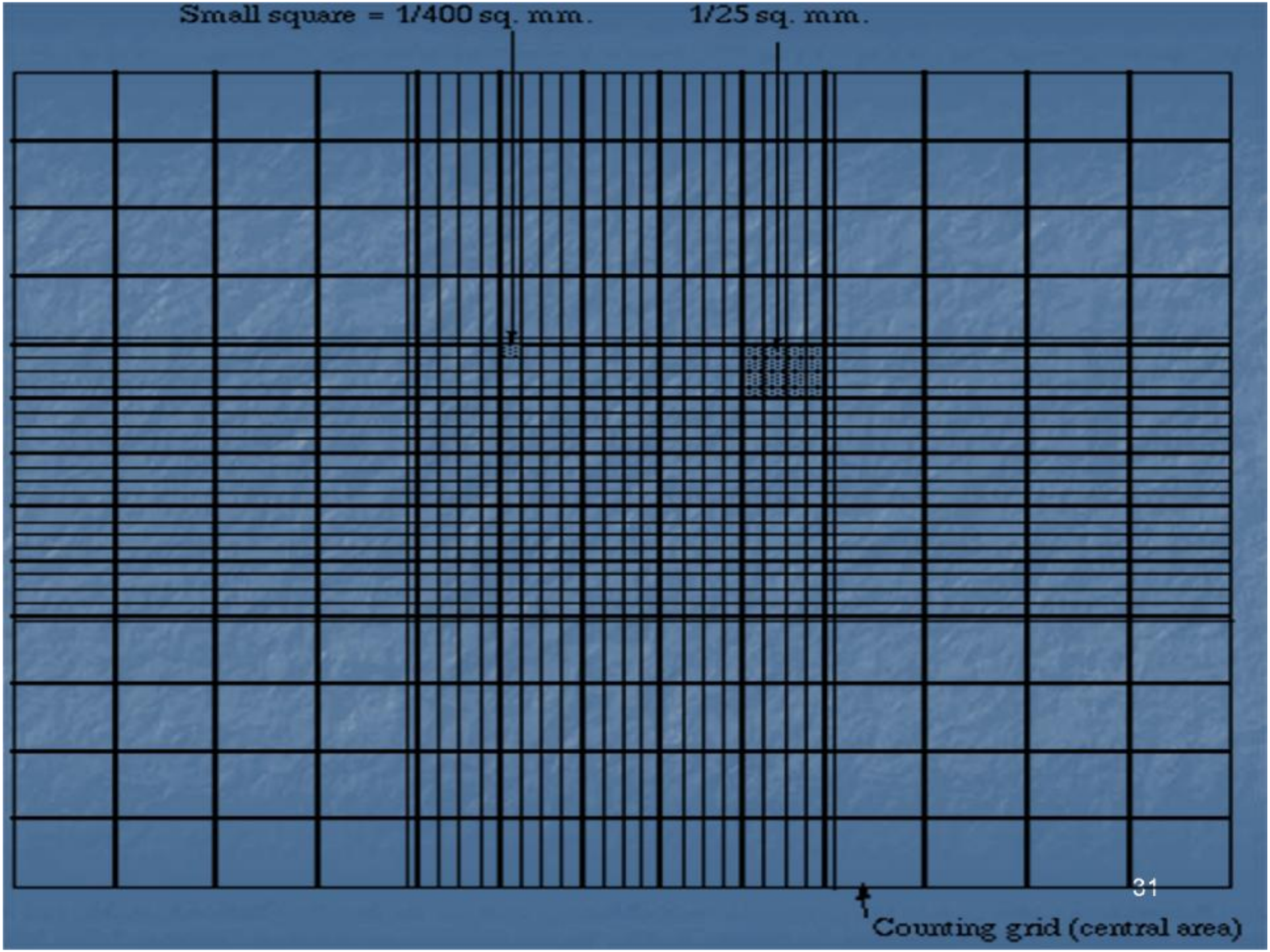
1. Thoroughly mix specimen and dilute 1:10 with diluent. (To obtain this dilution, dilute 100 uL of liquefied semen with 900 uL of diluent)
2. Thoroughly mix diluted specimen and allow a drop (10 - 20 uL) to into each side of the hemocytometer covered with a coverglass.
3. Allow chamber to stand for about 5 minutes in a humid container to prevent drying. During this period, the cells settle and can be more easily counted.

Preparation

4. After cells have settled, place chamber under a microscope
5. Count spermatozoa present in 5 1/25mm squares in center square millimeter X5 or sperms in one of the 9 large squares.

Only morphologically mature germinal cells with tails are counted.

No of sperms per large square X dilution factor (10) X Depth of chamber (10) X 1000 = count in million/ ml


Small square = 1/400 sq. mm.
1/25 sq. mm.
Counting grid (central area)

Sperm Count

- Decreased:
  - vasectomy (should be 0 after 3-6 months)
  - varicocele
  - primary testicular failure (Klinefelters)
  - secondary testicular failure
  - congenital vas obstruction
  - retrograde ejaculation
  - endocrine causes (prolactinemia, low testosterone)