

# 4. Assessment of Microbiological Examination

## Microbiological Assessment in Clinical Andrology

### 1. Purpose

- To detect **pathogenic bacteria, fungi, or viruses** in semen, preputial secretions, and the genital tract.
- To rule out **subclinical infections** that may affect fertility.
- To ensure **biosecurity** in semen used for **artificial insemination (AI)** or natural breeding.

### 2. Sample Collection

- **Semen sample:** collected via artificial vagina, manual collection, or electroejaculation (aseptic technique).
- **Preputial swab:** sterile swab inserted into preputial cavity.
- **Urethral swab** or **urine sample** (especially post-prostatic massage in dogs).
- **Testicular biopsy or aspirate** (rare, only if deep infection is suspected).

## 4. Assessment of Microbiological Examination

### 3. Laboratory Techniques

#### •Direct Microscopy:

- Wet mount (motility, leukocytes).
- Gram stain (morphology, inflammation).
- Diff-Quik or Giemsa (cell types, sperm morphology).

#### •Culture and Sensitivity:

- Aerobic and anaerobic culture.
- Fungal culture (Sabouraud agar).
- Incubation 24–72h.
- Antibigram for treatment planning.

#### •PCR / RT-PCR / qPCR:

- Detection of specific pathogens (e.g. *Brucella* spp., *Campylobacter fetus*, *Tritrichomonas foetus*).

#### •Special Stains:

- Ziehl-Neelsen for Mycobacteria.
- PAS or silver stain for fungi.

## 4. Assessment of Microbiological Examination

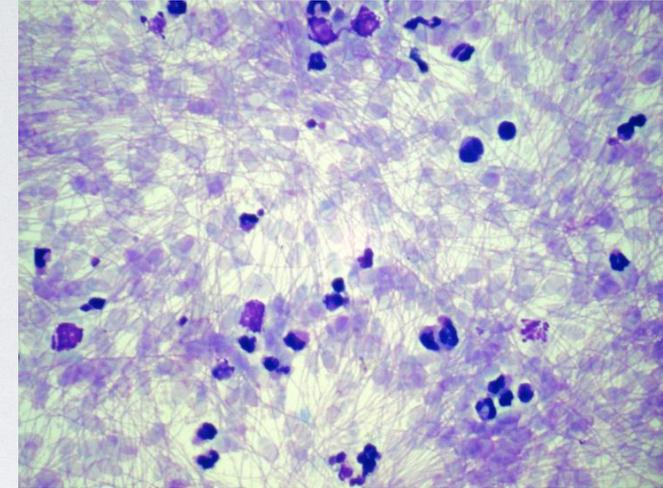
### 4. Common Pathogens

Species	Common Pathogens
Bull	Brucella abortus, Campylobacter fetus, Trichomonas foetus, Mycoplasma, Trueperella
Stallion	Pseudomonas, Klebsiella, Taylorella equigenitalis, Streptococcus zooepidemicus
Dog	E. coli, Brucella canis, Proteus, Staphylococcus intermedius, Mycoplasma canis
Ram/Buck	Brucella ovis, Actinobacillus, Histophilus somni, Mycoplasma, Pasteurella

## 4. Assessment of Microbiological Examination

### 5. Interpretation

- Normal flora may be present in low numbers (especially in preputial swabs).
- Presence of leukocytes + bacterial growth = suggestive of infection.
- Sperm motility decline in infected samples.
- PCR positive with no symptoms → subclinical carrier.



Semen smear from a ram infected with *Brucella ovis* containing several inflammatory cells (predominantly neutrophils).



Inflammation of the tail of the epididymis in a ram (a) and a buck (b). It was enlarged, felt hard, and appeared more echogenic than the testes (c, arrow)

### 6. Clinical Relevance

- Infection can lead to:
  - Reduced sperm motility and morphology.
  - Orchitis, epididymitis, prostatitis.
  - Transmission via natural breeding or AI.
- **Treatment:** systemic antibiotics based on antibiogram, local antiseptics, rest from breeding.

## 4. Assessment of Microbiological Examination

Disease	Stallion	Bull	Pig	Ram
Brucellosis	B (Blood)	B, S (Semen)	B	B, S
Leptospirosis	–	–	–	–
IBR/IPV	–	B, P (Preputial wash)	–	–
Leukosis	B, P, S	–	–	–
Haemophilus equigenitalis	B	B, P, S	–	–
Campylobacteriosis	–	S, P	–	–
Tuberculosis	–	A (Allergy test)	–	A
Paratuberculosis	A, B	A, B	A, B	A, B
Salmonellosis	S, F (Feces)	S, P, F	F	S, F
Internal parasites	F	F	F	F
External parasites	SC (Skin scraping)	SC	SK	SK
Contagious viral infections	S	P, S, Ö (Pre-ejaculate)	S	S, Ö

**(B):** Blood, **P:** Preputial wash, **S:** Semen, **P:** Pre-ejaculate, **(F):** Feces, **(SC):** Skin scraping, **A:** Allergy (test)

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IBR/IPV	–	B, P (Preputial wash)	–	–
Leukosis	B, P, S	–	–	–
Haemophilus equigenitalis	B	B, P, S	–	–
Campylobacteriosis	–	S, P	–	–
Tuberculosis	–	A (Allergy test)	–	A
Paratuberculosis	A, B	A, B	A, B	A, B
Salmonellosis	S, F (Feces)	S, P, F	F	S, F
Internal parasites	F	F	F	F
External parasites	SC (Skin scraping)	SC	SK	SK
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# 5. Assessment of sperm production and quality

How to collect semen ?

Teaser female

Dummy

Artificial Vagina

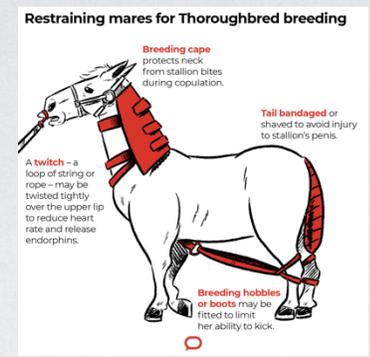
Post-mortem

Electro Ejaculation ?

Trans-rectal massage (Ampulla)

Digital Manipulation (Masturbation)

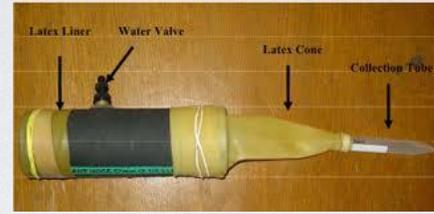
Urethral Catheterisation



Stallion



Bull



Ram & Buck



Bull

Ram & Buck



Dog



Cat



# Stallion

How to collect semen ?

## Artificial Vagina

Teasing  
Training



5-7 Fraction



Post-mortem

## Preferences



- Temperatures 42-45 C
- Pressure Must be recorded
- Lubrication Non spermacid

## Epididymal

Disposable sheeth ?

# Bull

How to collect semen ?

Training

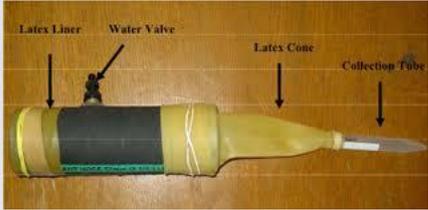


Phantom effect



Preferences

Artificial Vagina



- Temperatures 42-45 C
- Pressure Must be recorded
- Lubrication Non spermacid

Epididymal

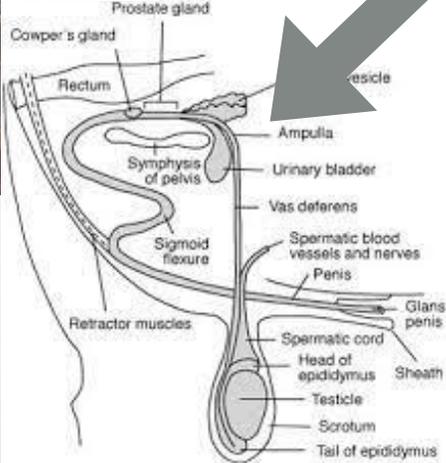
Ampulla Massage & Ej



Retrograd Flushing



Dissection



# Small Ruminant

Preferences

Training

Artificial Vagina

- Temperatures 42-45 C
- Pressure Must be recorded
- Lubrication Non spermacid



Epididymal

Ampulla Massage & Ej



Retrograd Flushing

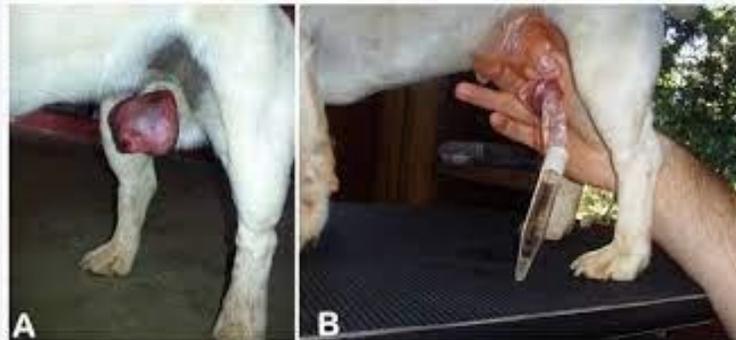


Dissection

## Digital Manipulation (Masturbation)

3 fractions

1. Prostate & urethral gland
- 2nd Sperm rich fraction
- 3rd. Prostate



## 5. Assessment of sperm production and quality

How to evaluate ?

Macroscopically

Volume, pH, color, and viscosity,

Microscobically

Mass motility, Concentration, Motility, Viability (Dead/alive), Morphology



# 5. Assessment of sperm production and quality

Macroscopically

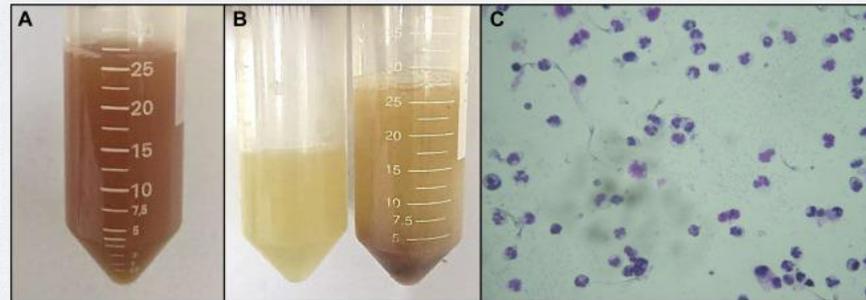


Clear & Contaminated

color



Hemospermia



Pyospermia

Volume



pH



# 5. Assessment of sperm production and quality

Microscopically

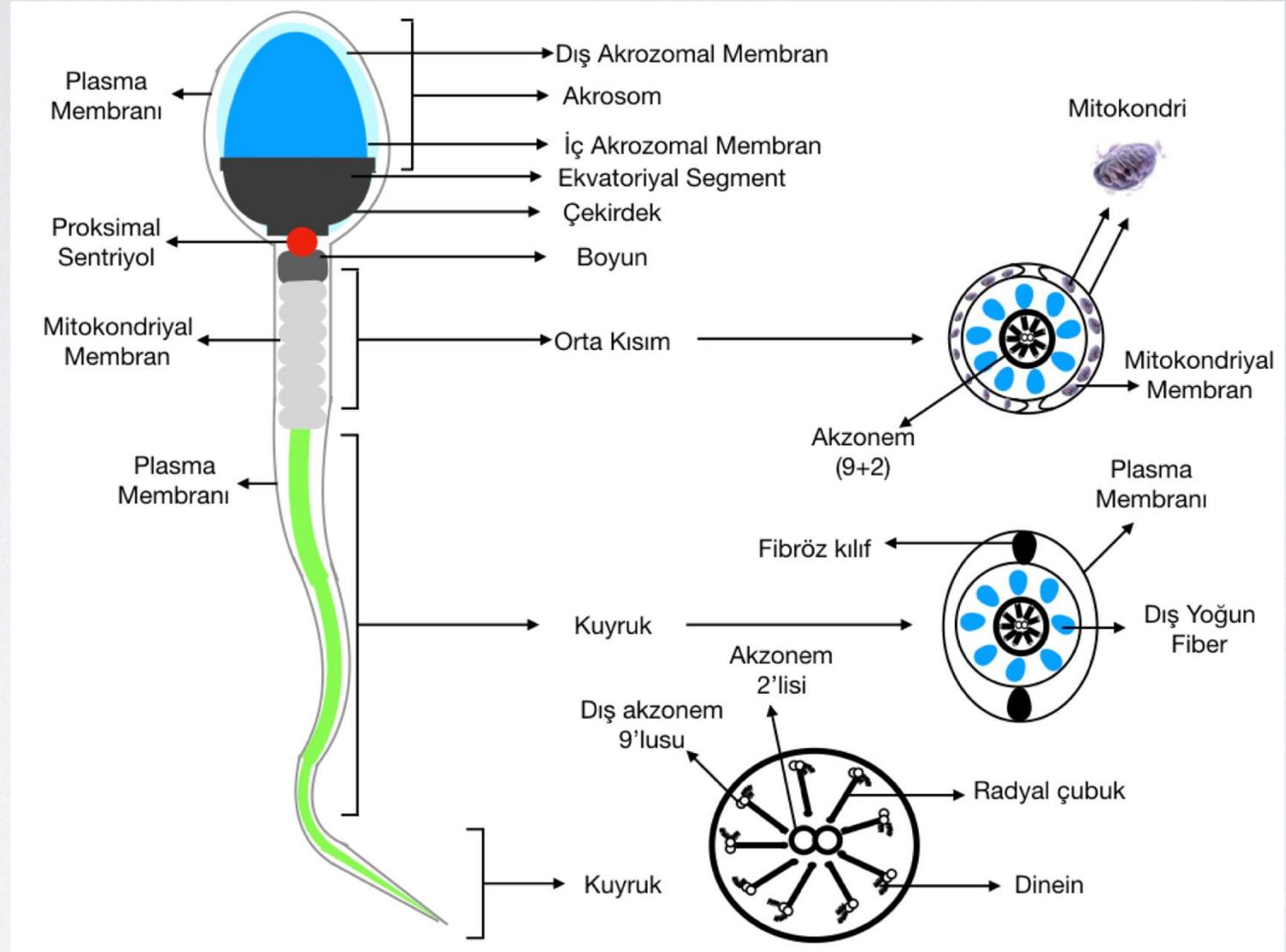
Mass Motility

Motility

Concentration

Viability (Live/Dead)

Abnormality



## 5. Assessment of sperm production and quality

Microscopically

Mass Motility

10 ×

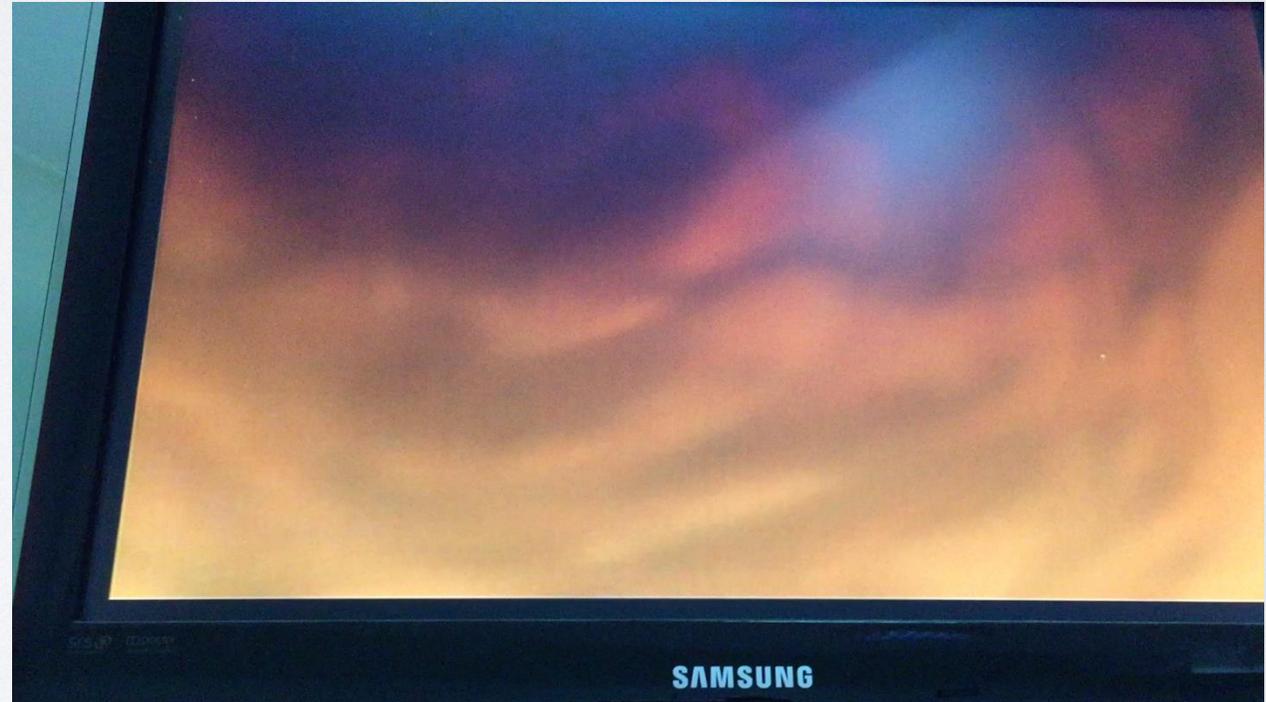
The consistency of the relationship for all breeds indicated that mass sperm motility is **predictive of the fertility resulting when sperm are used from a specific ejaculate.**

Mass motility rating system for ejaculated ram sperm

Rating	Microscopic appearance <sup>a</sup>
0	No swirl – nil or sporadic oscillation of individual sperm
1	No swirl – generalized oscillation of individual sperm only
2	Very slow distinct swirl
3	Slow distinct swirl
4	Moderately fast distinct swirl
5	Fast distinct swirl

<sup>a</sup> Drop of 5  $\mu$ L of raw semen deposited on a pre-warmed glass slide ( $\approx 37^\circ\text{C}$ ). Edge of the drop is observed at low magnification (10 $\times$  objective) on the thermally controlled stage of a phase contrast microscope.

Bull, Ram, Buck, Avian



## 5. Assessment of sperm production and quality

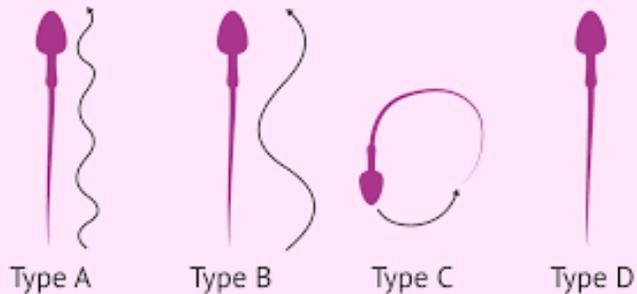
### Microscopically

Sperm motility is the ability of sperm to move efficiently.

### Motility



10 x



## 5. Assessment of sperm production and quality

### Microscopically

Asthenozoospermia

Asthenozoospermia (or asthenospermia) is the medical term for **reduced sperm motility**.

Oligozoospermia

Low sperm count

Azoospermia

No sperm in the ejaculate.

Aspermia

No fluid

Teratospermia

is a condition characterized by the presence of sperm with abnormal morphology.

Hypospermia

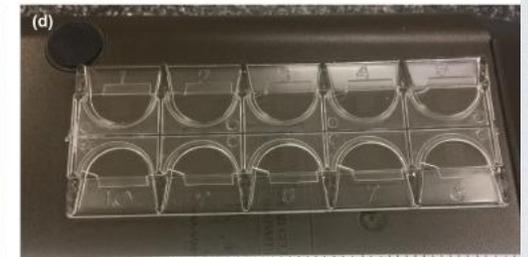
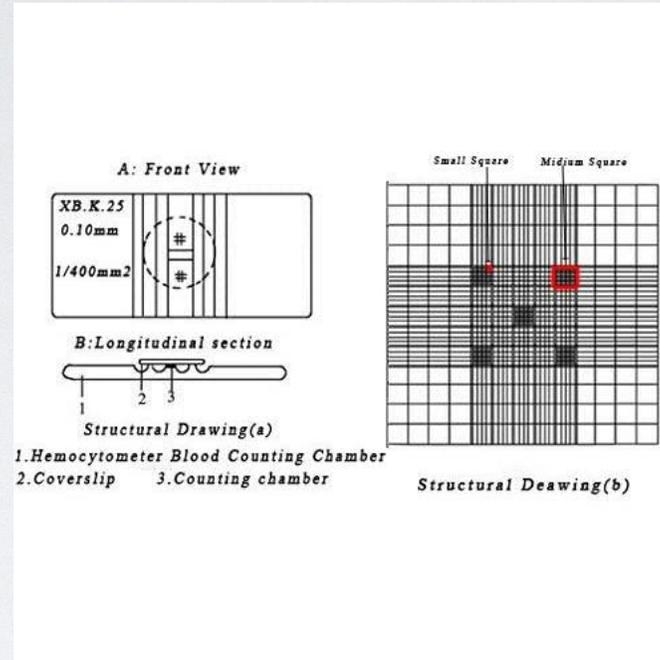
Low semen volume

# 5. Assessment of sperm production and quality

Microscopically 10 ×

Concentration

1. Hemocytometer



2. Photometer



3. CASA

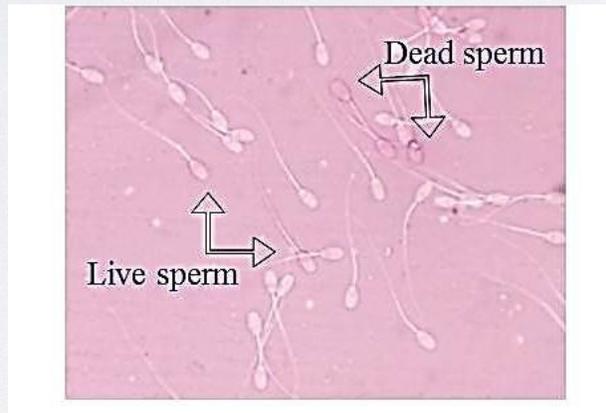


## 5. Assessment of sperm production and quality

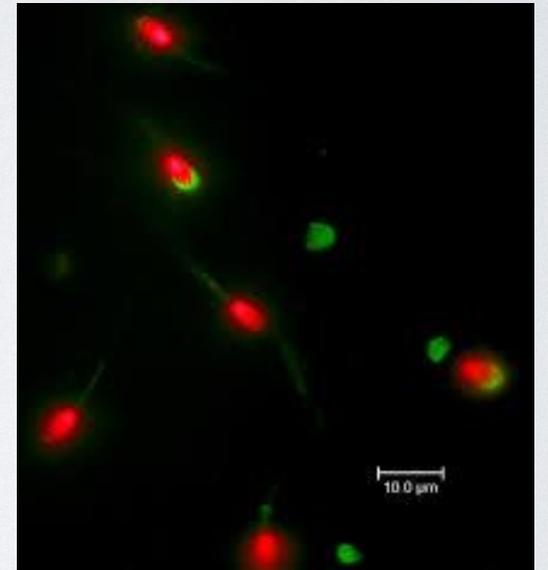
Microscopically 10, 20, 40 ×

Viability Sperm viability (SV), the proportion of live sperm in a sample

Eosin-Nigrosin



Fluorescein

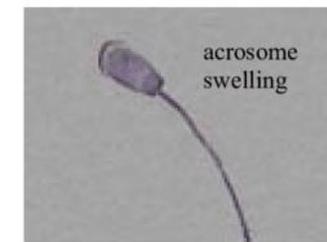
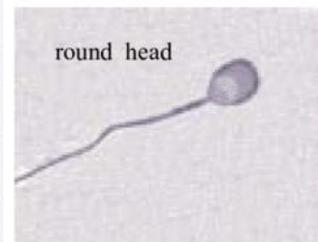
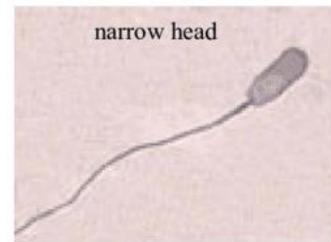
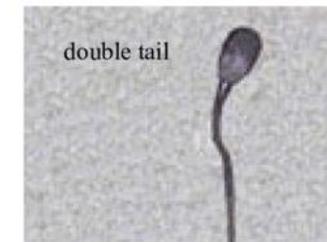
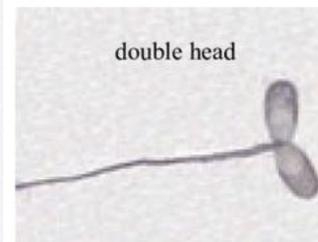
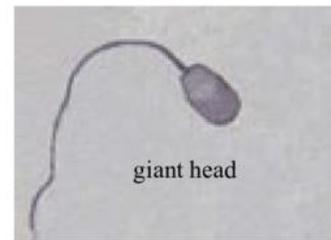
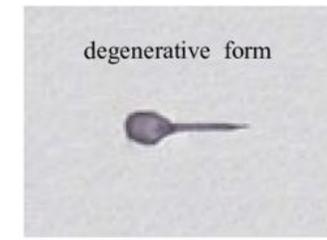
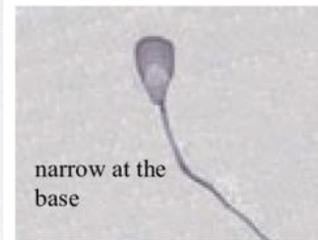
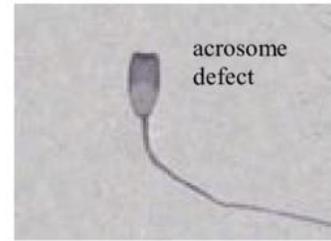
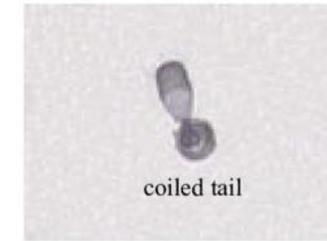
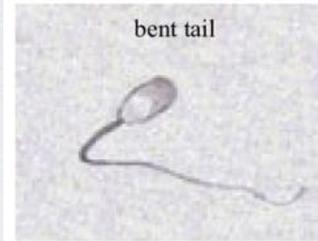
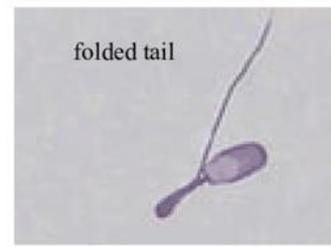
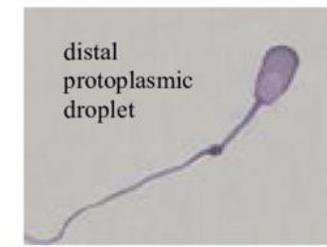
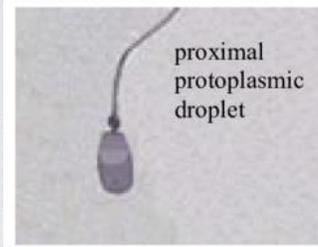


## 5. Assessment of sperm production and quality

Microscopically 60, 100 x

Abnormality

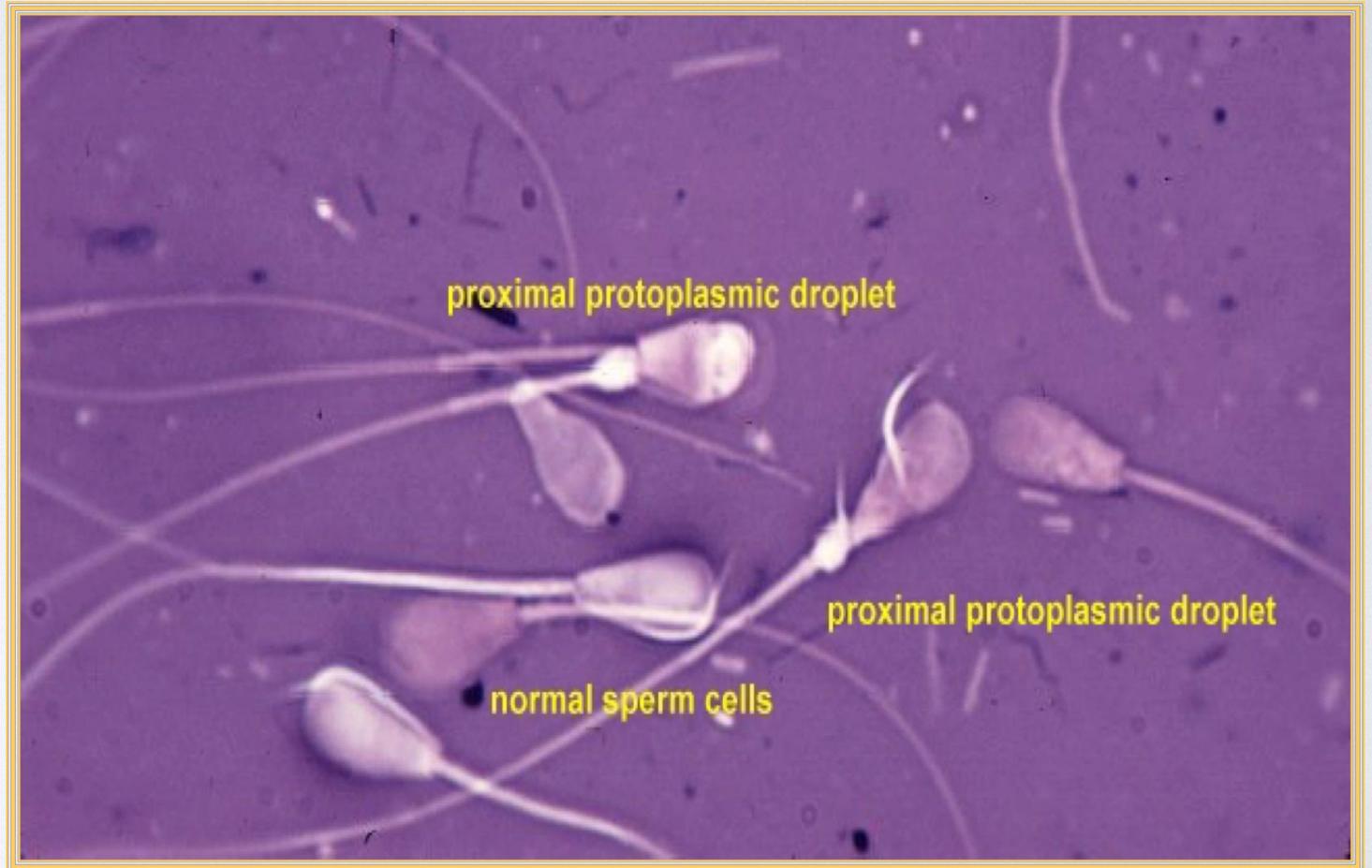
the proportion of abnormal sperm in a sample



## 5. Assessment of sperm production and quality

Microscopically

Common Abnormalities



## 5. Assessment of sperm production and quality

Parameter	Finding	Terminology
Semen Volume	No ejaculate/semen	Aspermia
	Less than normal	Hypospermia
	More than normal	Hyperspermia
Spermatozoa Concentration	No spermatozoa in ejaculate	Azoospermia
	Total sperm count in semen/ejaculate is below lower reference limit	Oligospermia
	Sperm concentration equal to or above lower reference limit	Normospermia
	Higher than normal	Polyspermia
	No spermatozoa seen in fresh/native semen, but visible after centrifugation (3000g, 15 min)	Cryptozoospermia
Motility	Percentage of progressively motile spermatozoa below reference value	Asthenozoospermia
Morphology	Morphologically abnormal spermatozoa percentage above upper reference limit	Teratozoospermia
Dead Spermatozoa	Very low percentage of live sperm; mostly dead sperm present (with or without sediment)	Necrozoospermia
	Some may recover motility	Akinozoospermia (Note: Rare term, typically refers to sperm that appear immotile but may regain motility)
Contamination	Red color (blood contamination)	Hematospermia
	Yellow color (urine contamination)	Urospermia
	Green color (pus contamination)	Pyospermia
Leukocytes	Leukocyte count above threshold in ejaculate	Leukospermia
Acrosome Absence	Acrosome not formed	Globozoospermia