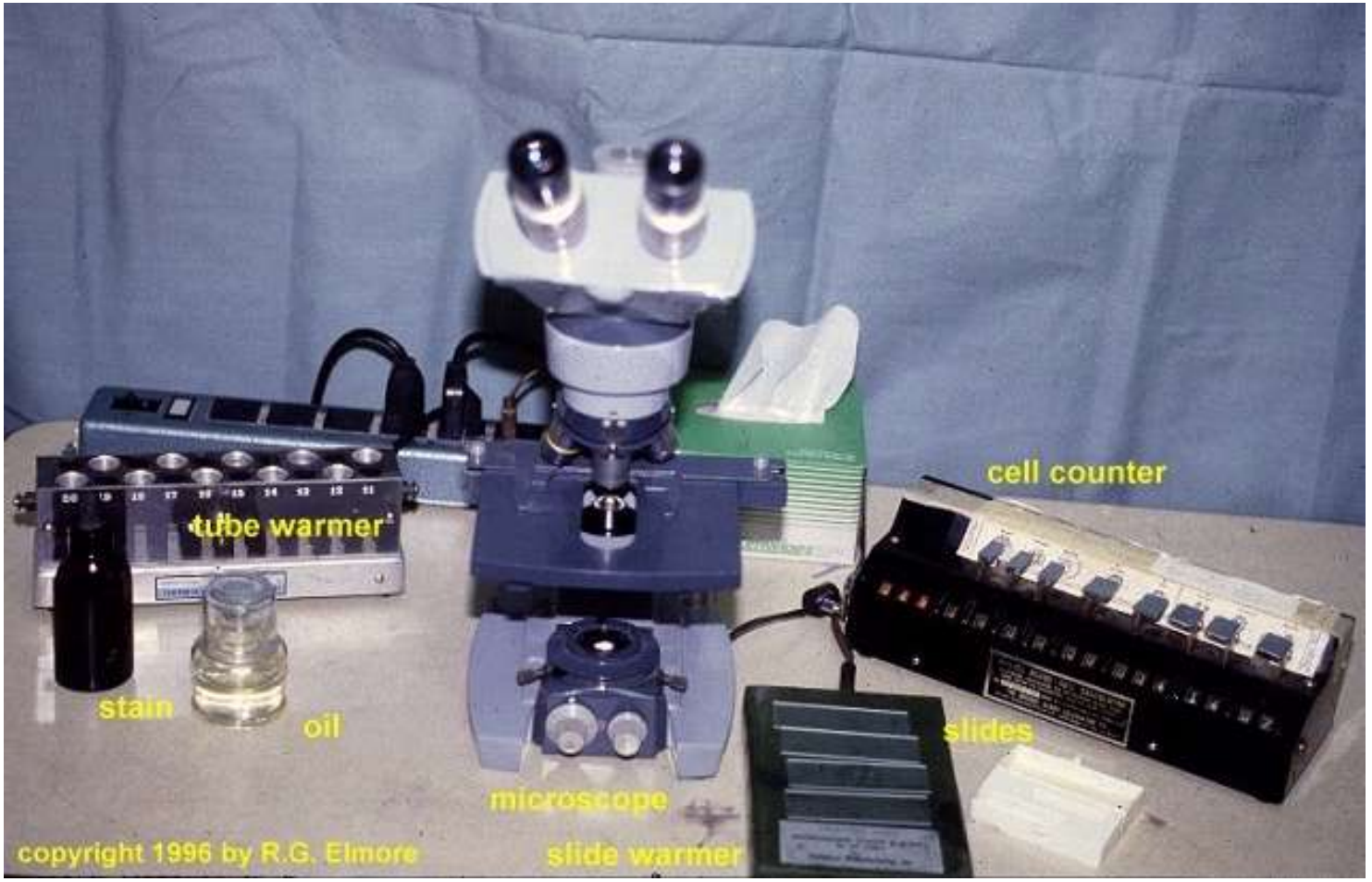

Microscopic evaluation

Dr Hany Lotfi

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1- Examination of non stained film

1- Evaluation of motility

2- Examination of stained film

1- Evaluation of live/dead percent

2- Evaluation of sperm abnormality

3- Evaluation of sperm ripeness

3- Estimation of sperm cell concentration

I- Examination of non stained film (sperm motility)

Factors adversely affecting sperm motility

1- Time elapsed between collection and examination

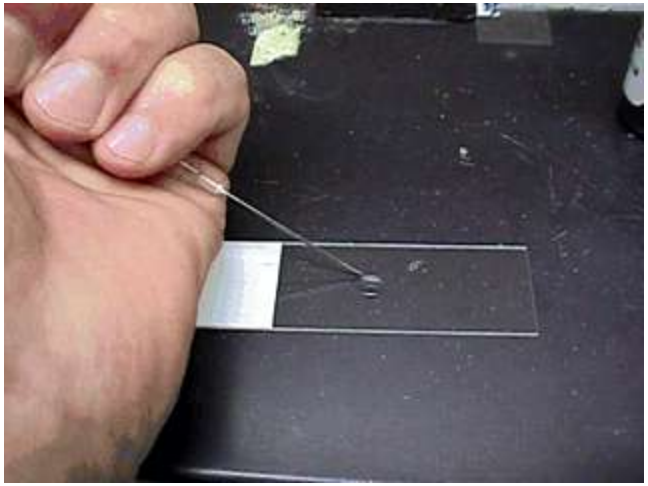
2- Exposure to unsuitable temperature

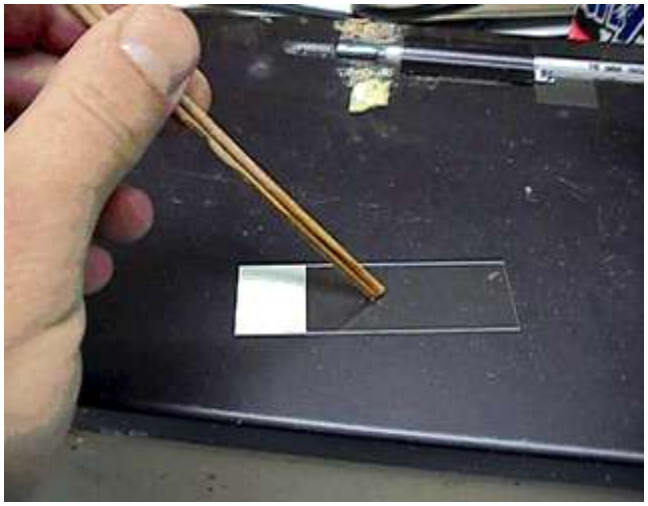
During collection and transportation

Period between collection and evaluation

During examination

A- Estimation of mass motility





Mass motility Mass motility 2

Descriptive and numerical scale for evaluation of microscopic wave pattern of semen from bull:

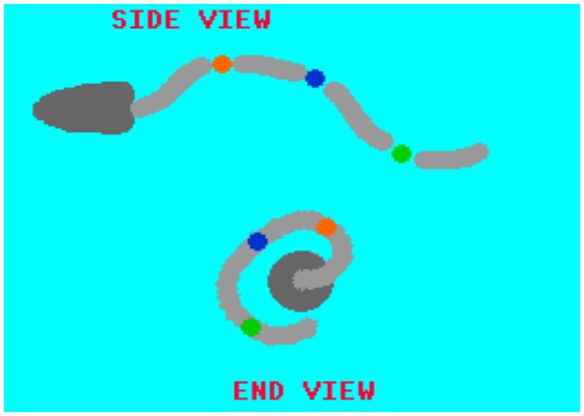
Descriptive scale	Numerical	Appearance of pattern
Excellent	**5**	**Extremely rapid and vigorous motion** (over than 90 % motile sperm)
Very poor	**4**	**Apparently rapid dark distinct wave** (~90 % motile sperm)
Good	**3**	**Slowly wavy motion** (50-80% motile sperm)
Fair	**2**	**Only strong rotatory movement** (less than 50% motile sperm)
Poor	**1**	**Week rotatory movement**
Very poor	**0**	**Waves not present, sperm cells are immotile**

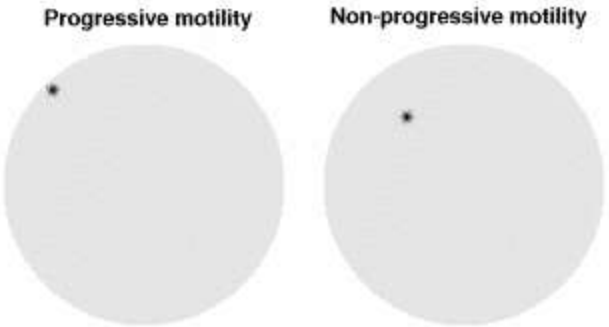
Mass motility grad

B- Estimation of individual motility

What is the normal motile sperm ?

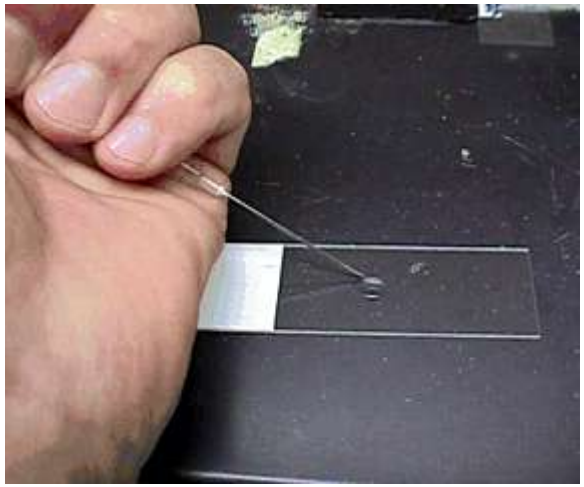
The normal motile sperm is the sperm that move from point to another in recty forward progressive motility by it own force

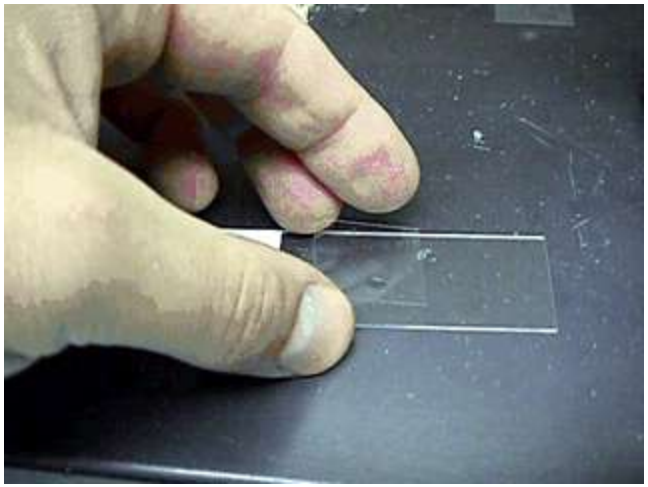


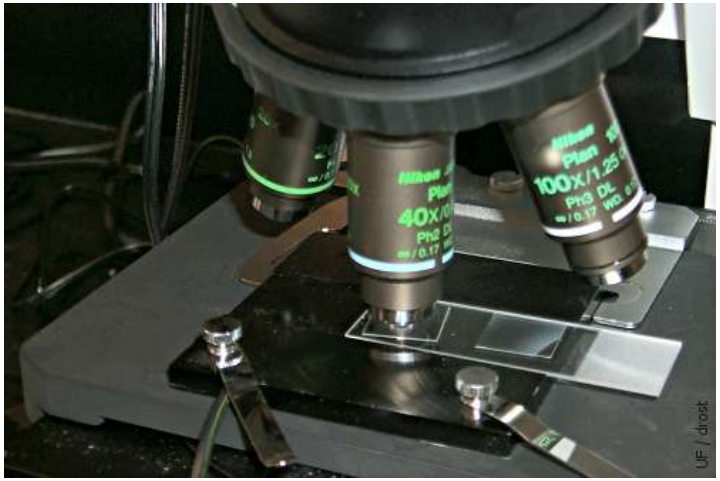


What are the abnormal sperm motility ?

Method







[Individual motility]

[Types of motility]

Descriptive and numerical scales for estimation of microscopic motility of sperm cells from the bull.

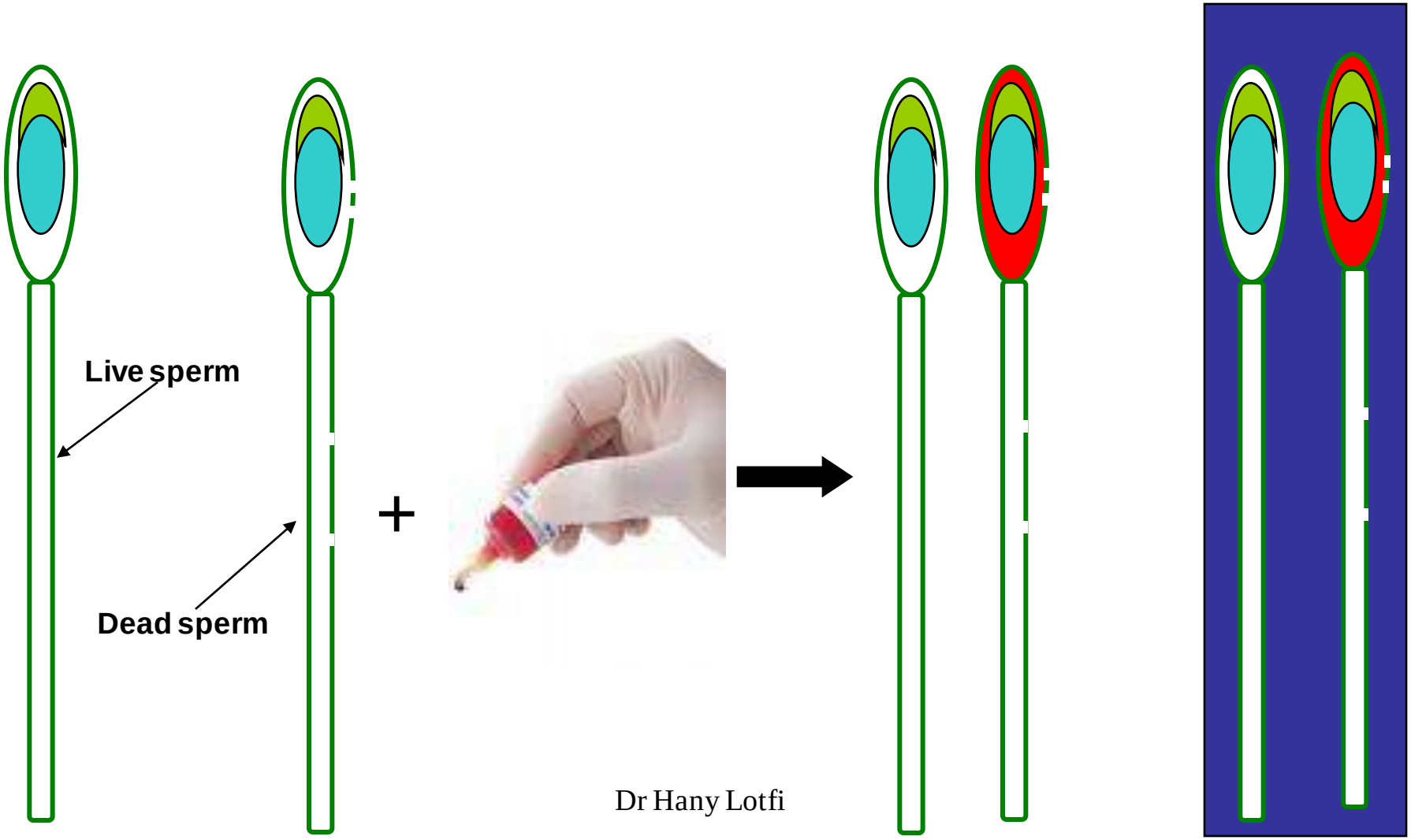
Motile cells %	Descriptive value	Numerical value
80 - 100	Very good	5
60 - 80	Good	4
40 - 60	Fair	3
20 - 40	Poor	2
0 - 20	Very poor	1

Grade individual motility

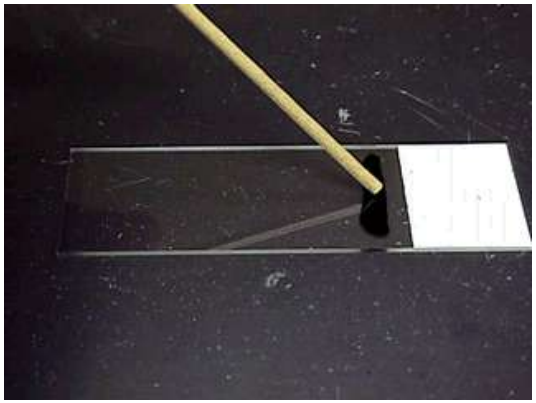
II- Examination of stained film

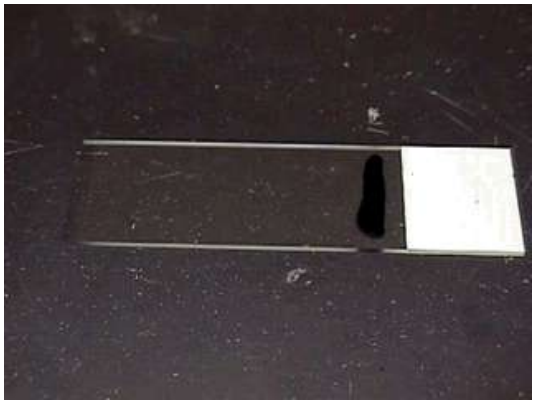
1- Examination of live dead percent (eosin nigrosin stain, vital stain)

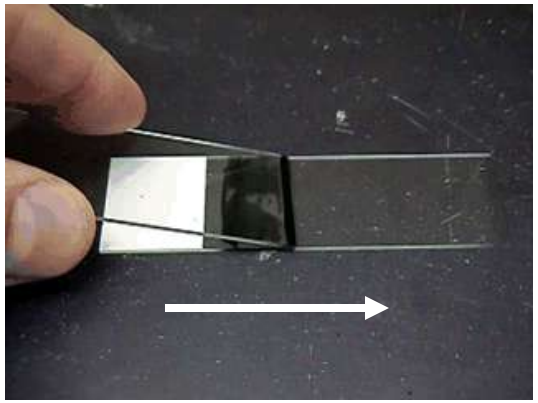
Idea



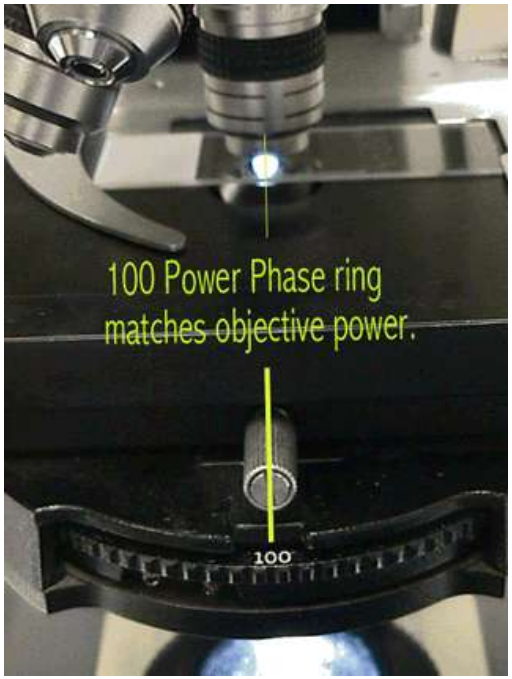
Method








OLYMPUS
JAPAN
100


100 Power Phase ring
matches objective power.
100

2- Examination of sperm morphology (abnormality)

Aim and indication

Type of stains

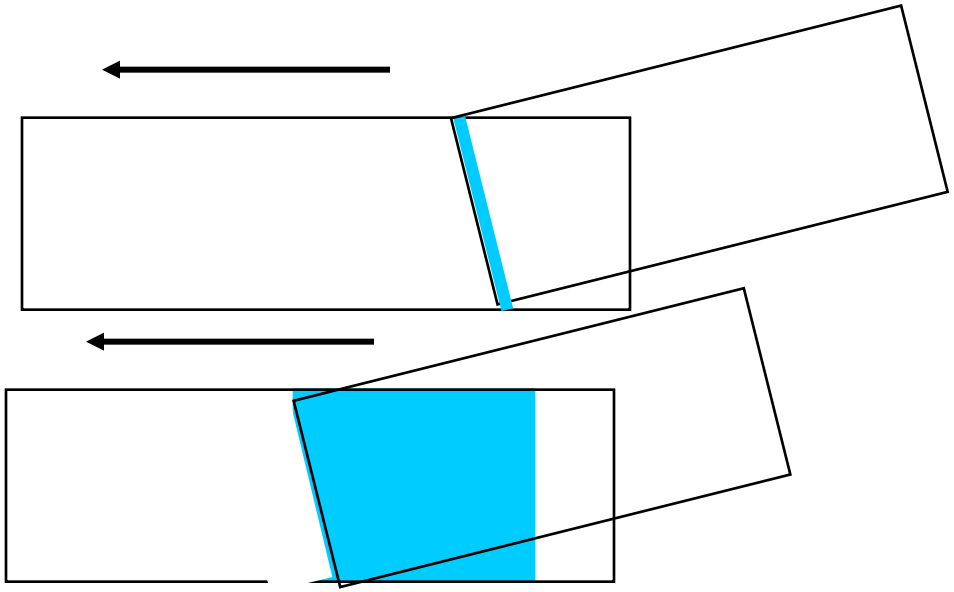
- Eosin nigrosin stain
- Nigrosin
- Indian ink
- Opal blue
- Toluidine blue
- Alkaline methyle violet

General precautions

Alkaline methyle violet

Dilution

1:4 with % 1% NaCl



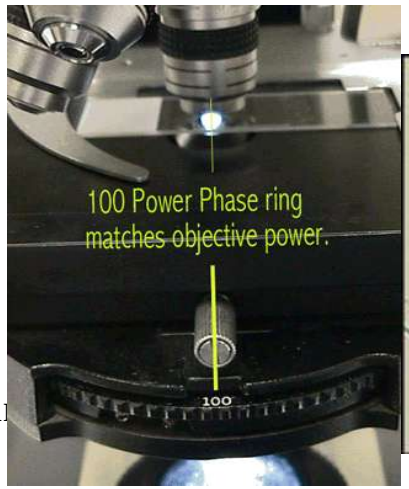
Preparation of the film

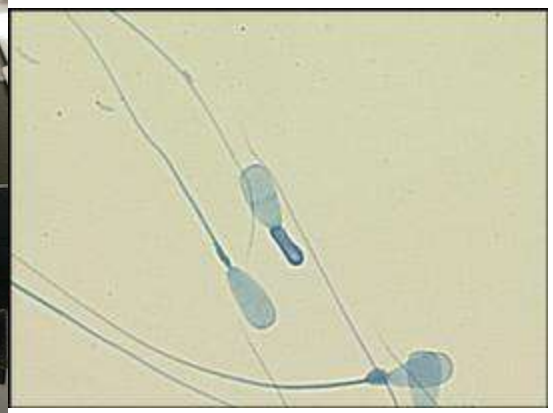
Staining

9 : 1

1% MV: 1% $NaHCo_3$

Observation



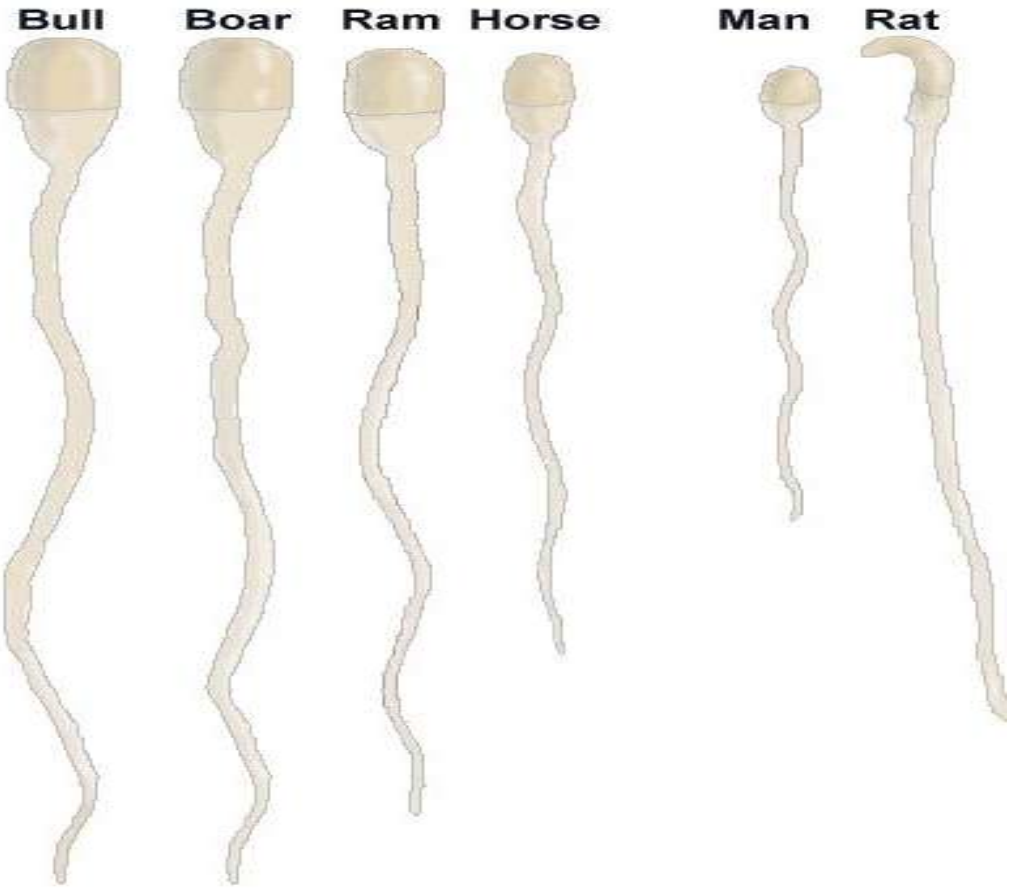


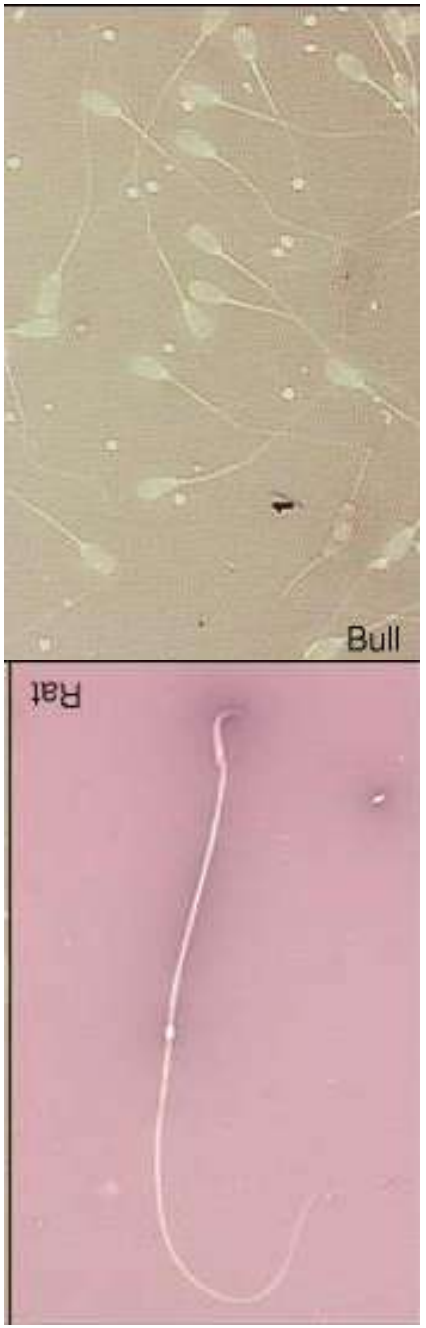
Opal blue

Indian ink

Nigrosin

Examination of the film



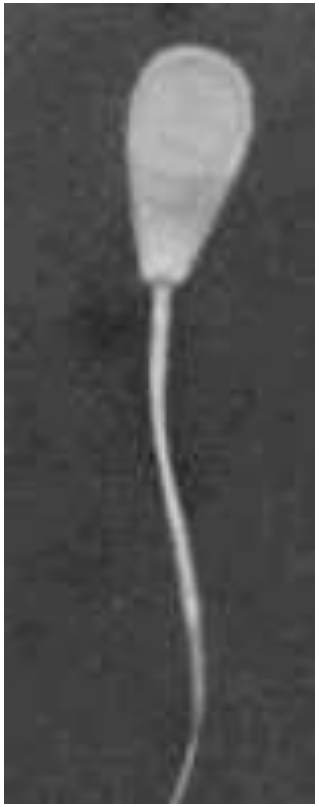


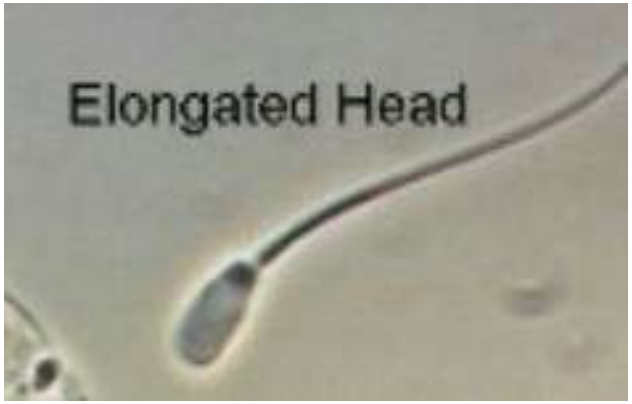
Type of sperm cell abnormality

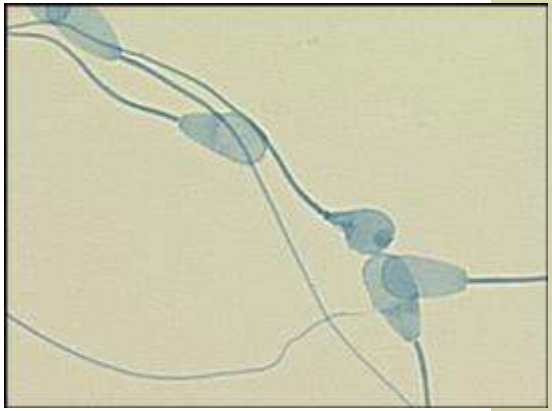
1- Primary sperm cell abnormalities

Abnormality include whole the sperm

Abnormality include the sperm head

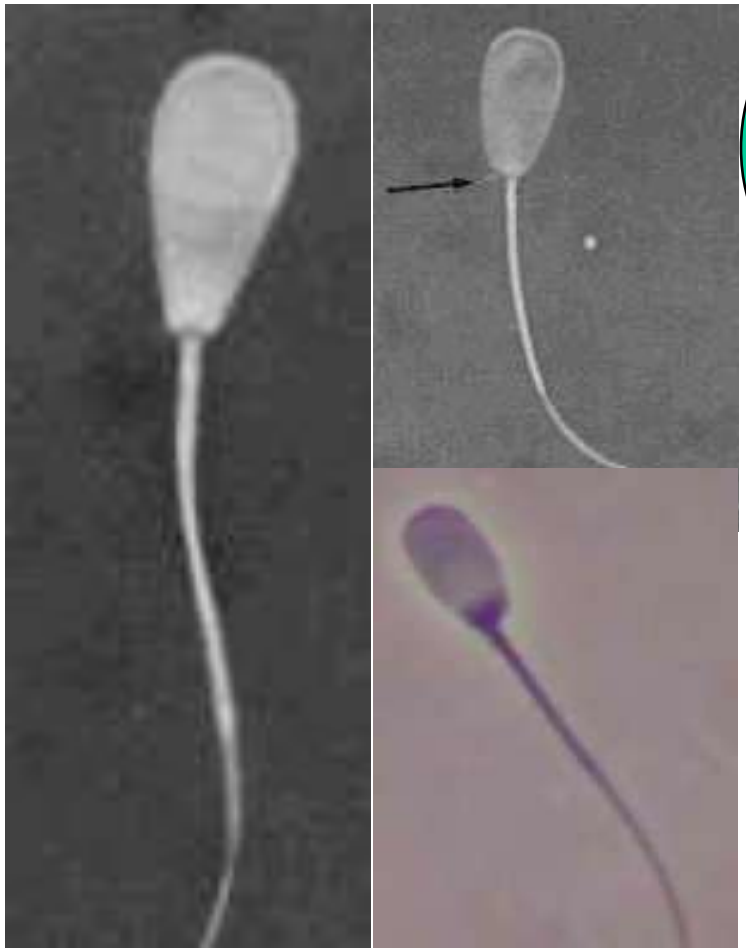


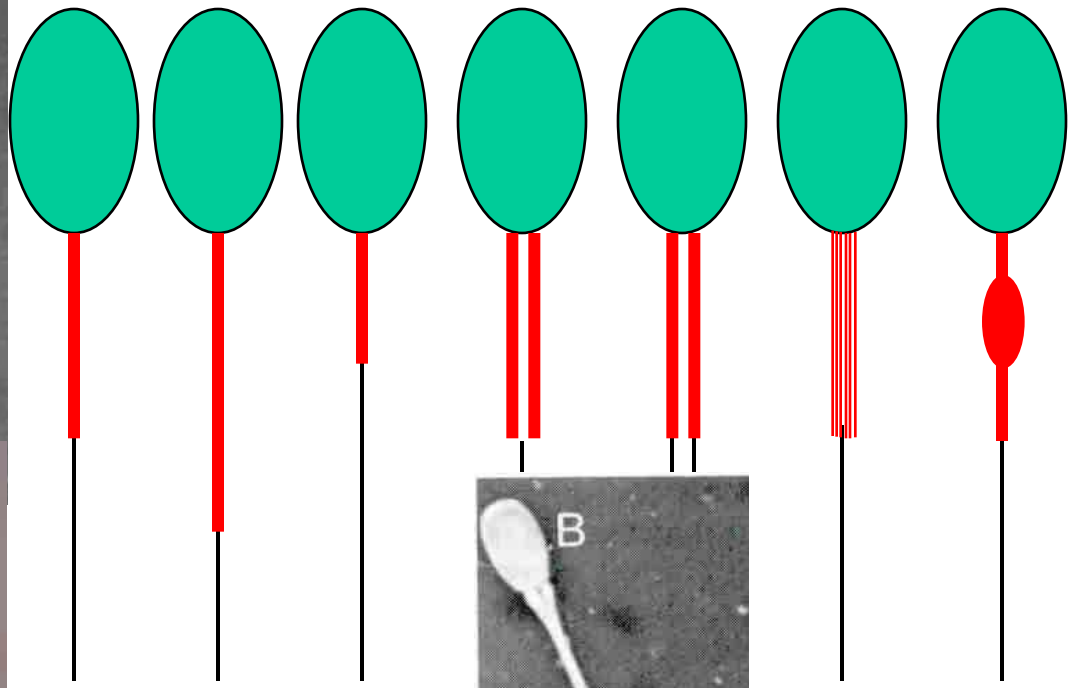


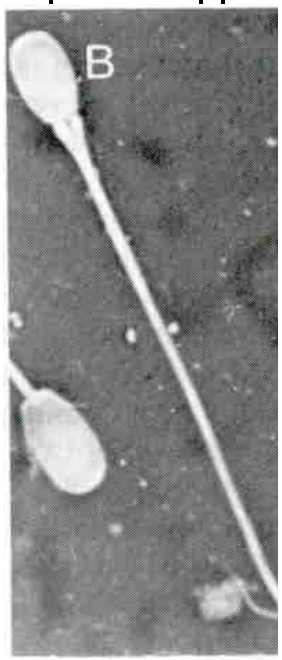


Lotfi

Abnormality include the middle piece







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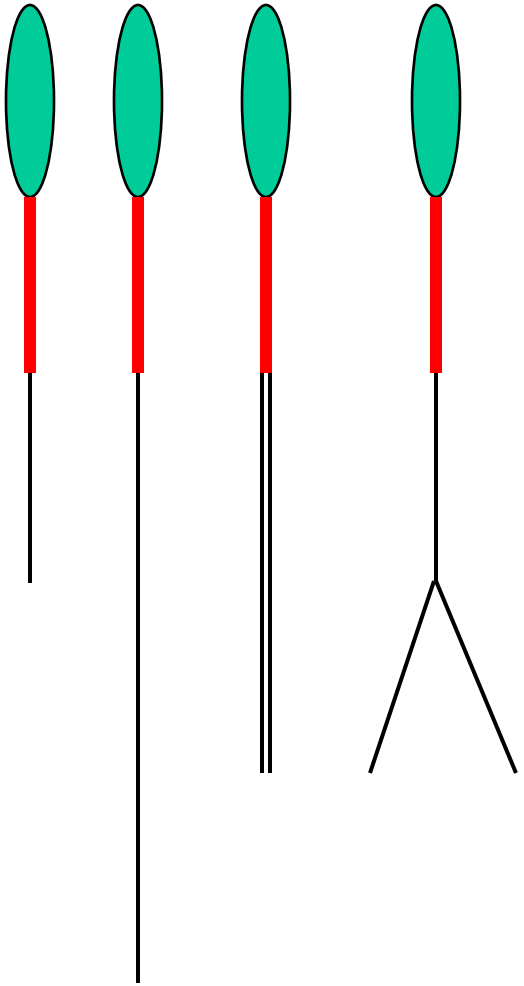
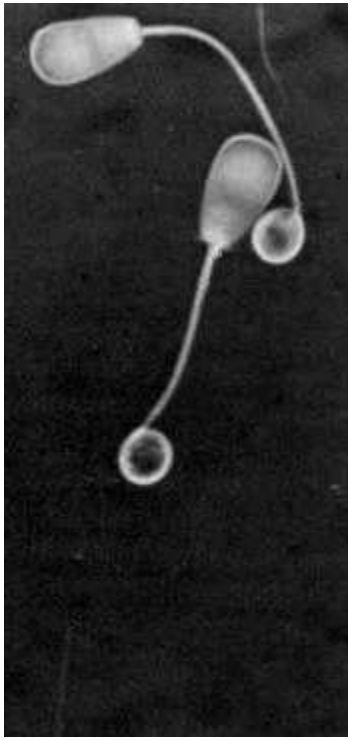
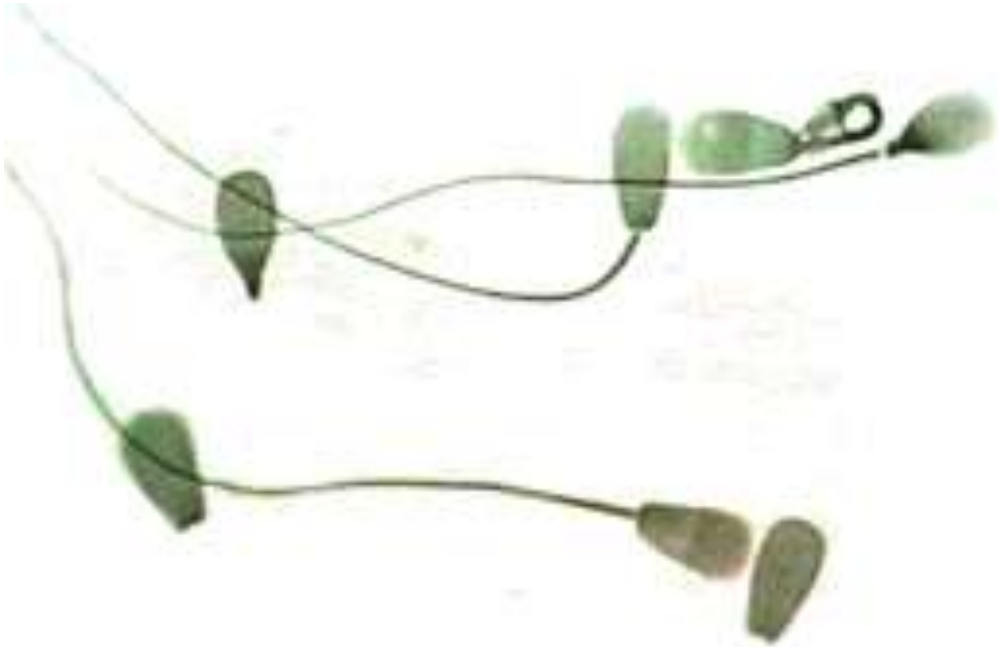
Abnormality include the main piece



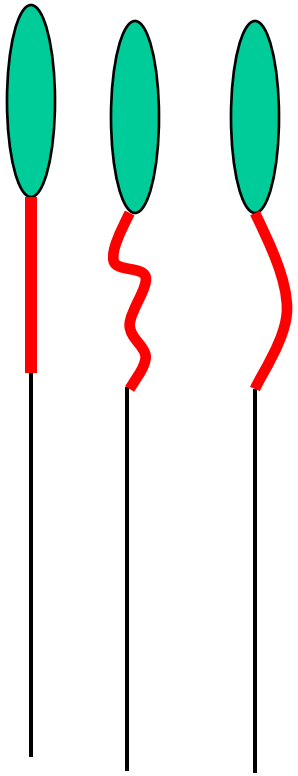


2- Secondary sperm cell abnormalities

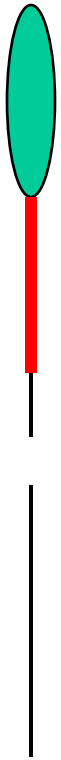
Abnormality include whole the sperm



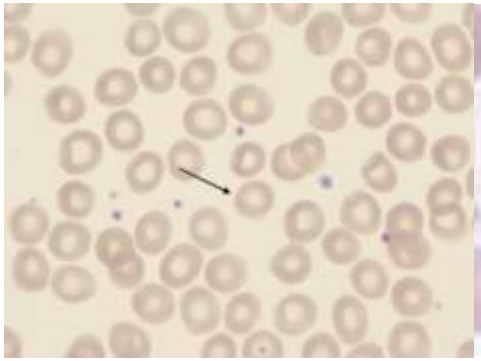
Abnormality include the middle piece

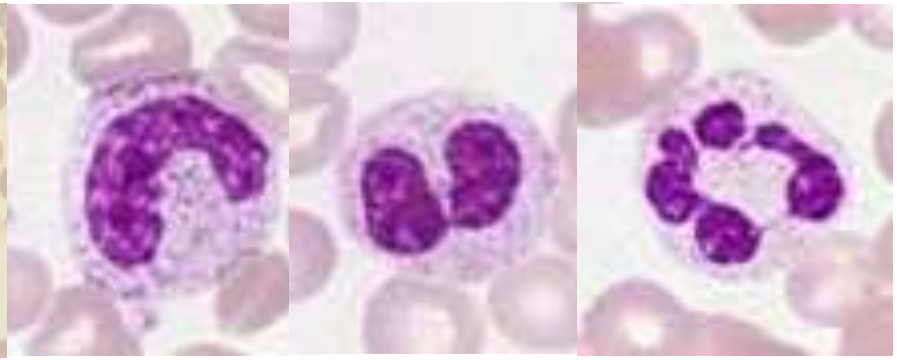


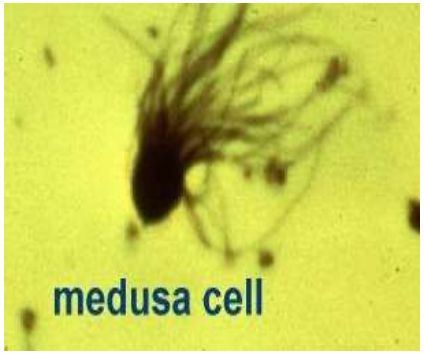
Abnormality include the main piece

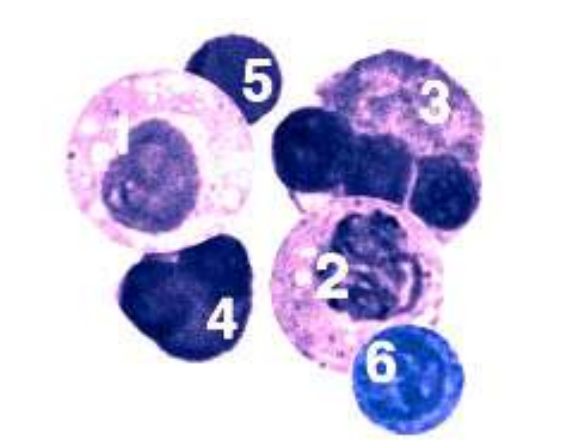


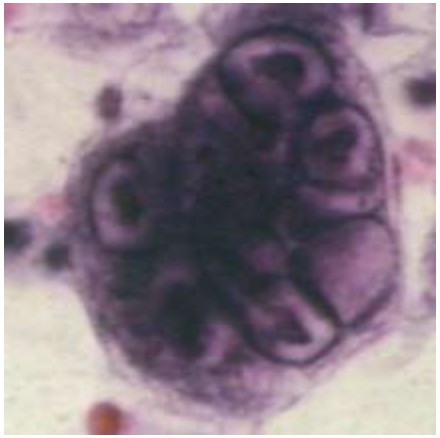
3- Miscellaneous abnormalities

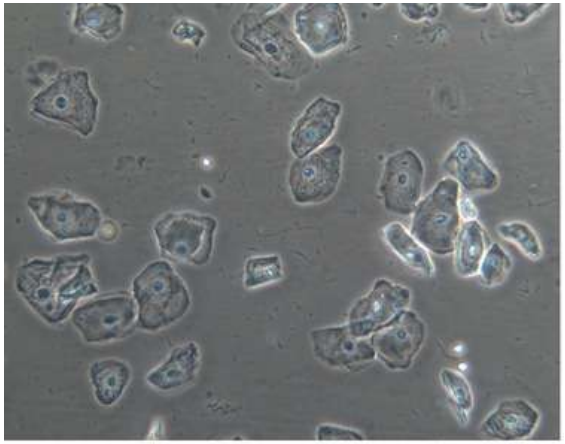


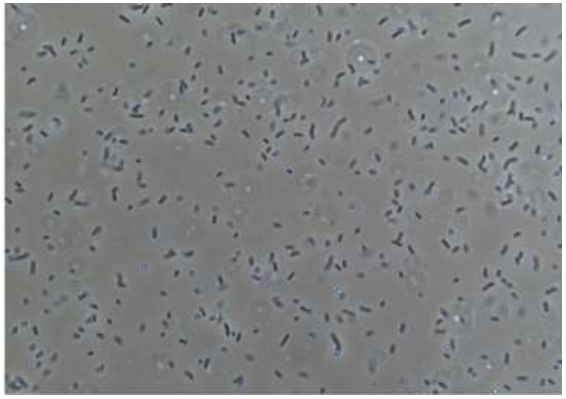


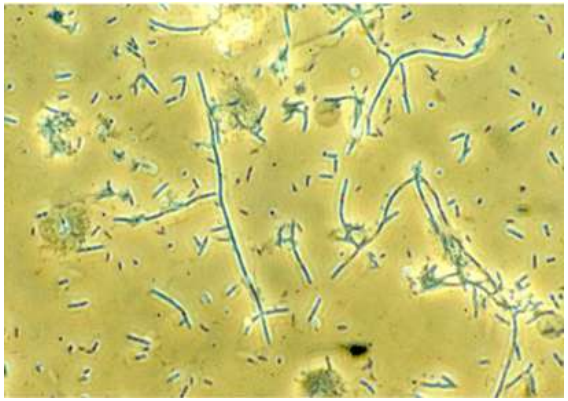








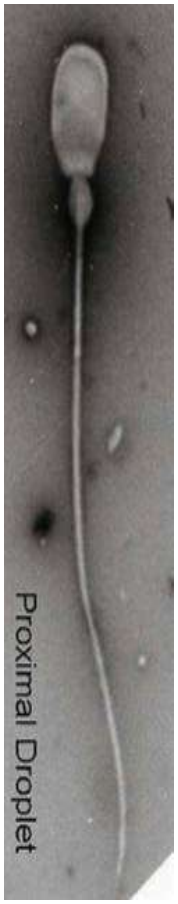


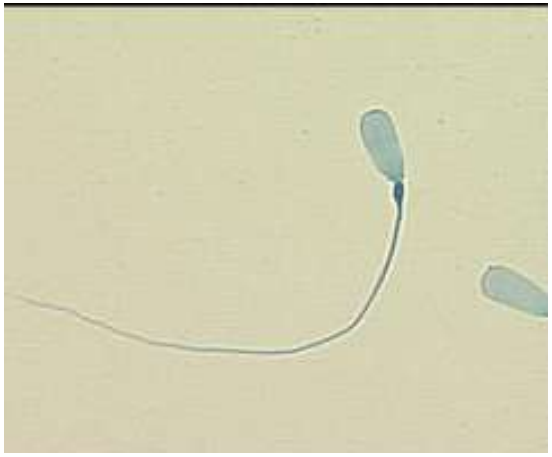


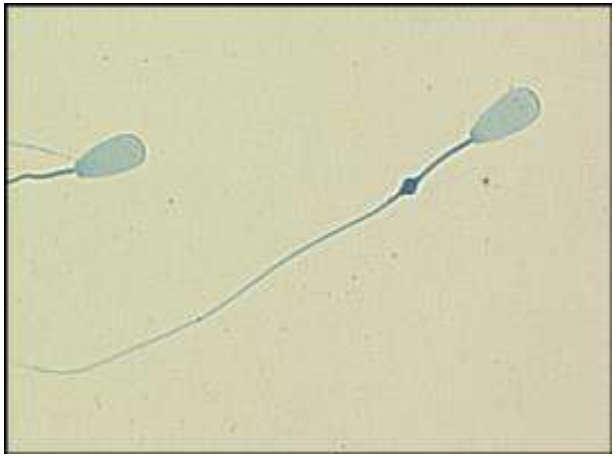
How to judge on the semen sample regarding the sperm cell abnormality

Dr Hany Lotfi

3- Examination of sperm ripeness







3- Estimation of sperm cell concentration (Sp. C. C.)

Definition **Number of the sperm / unit volume**

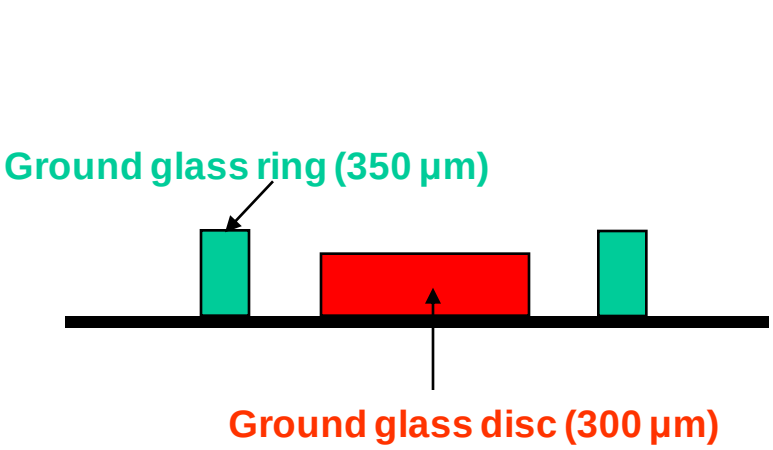
$$ml = cc^3 = 10^3\ mm^3$$

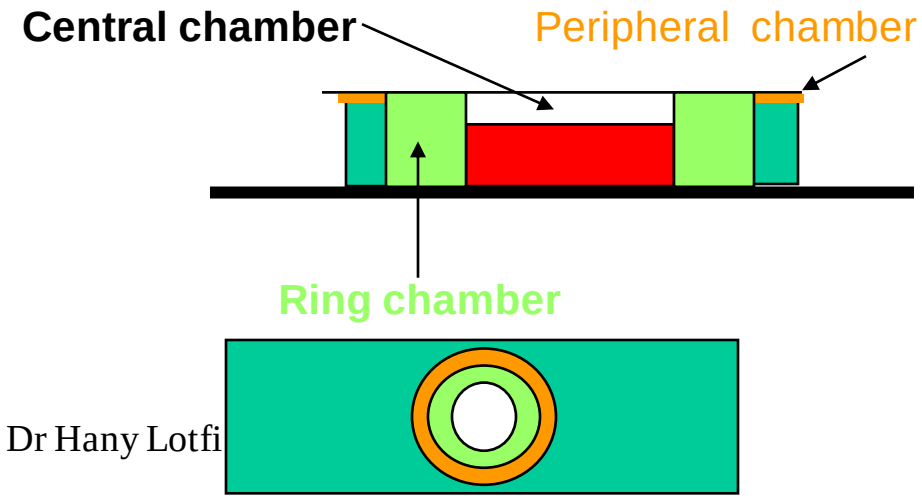
Importance

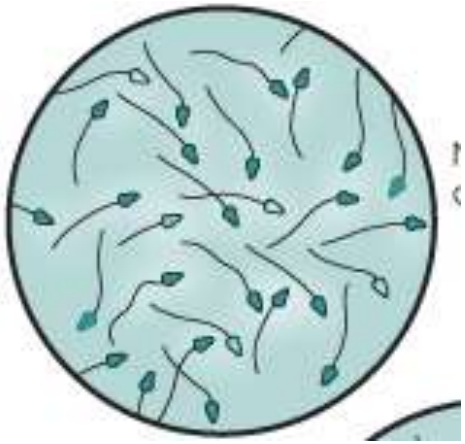
Method of detection of the sp.c.c.

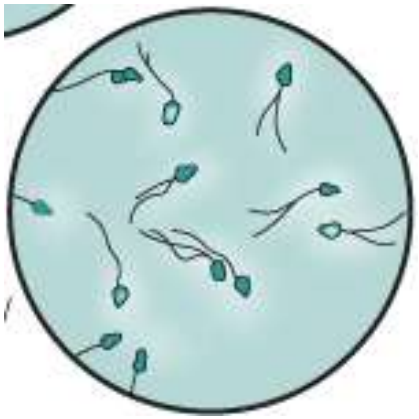
1- Density

2- Blom's comparing chamber (visual microscopical examination)



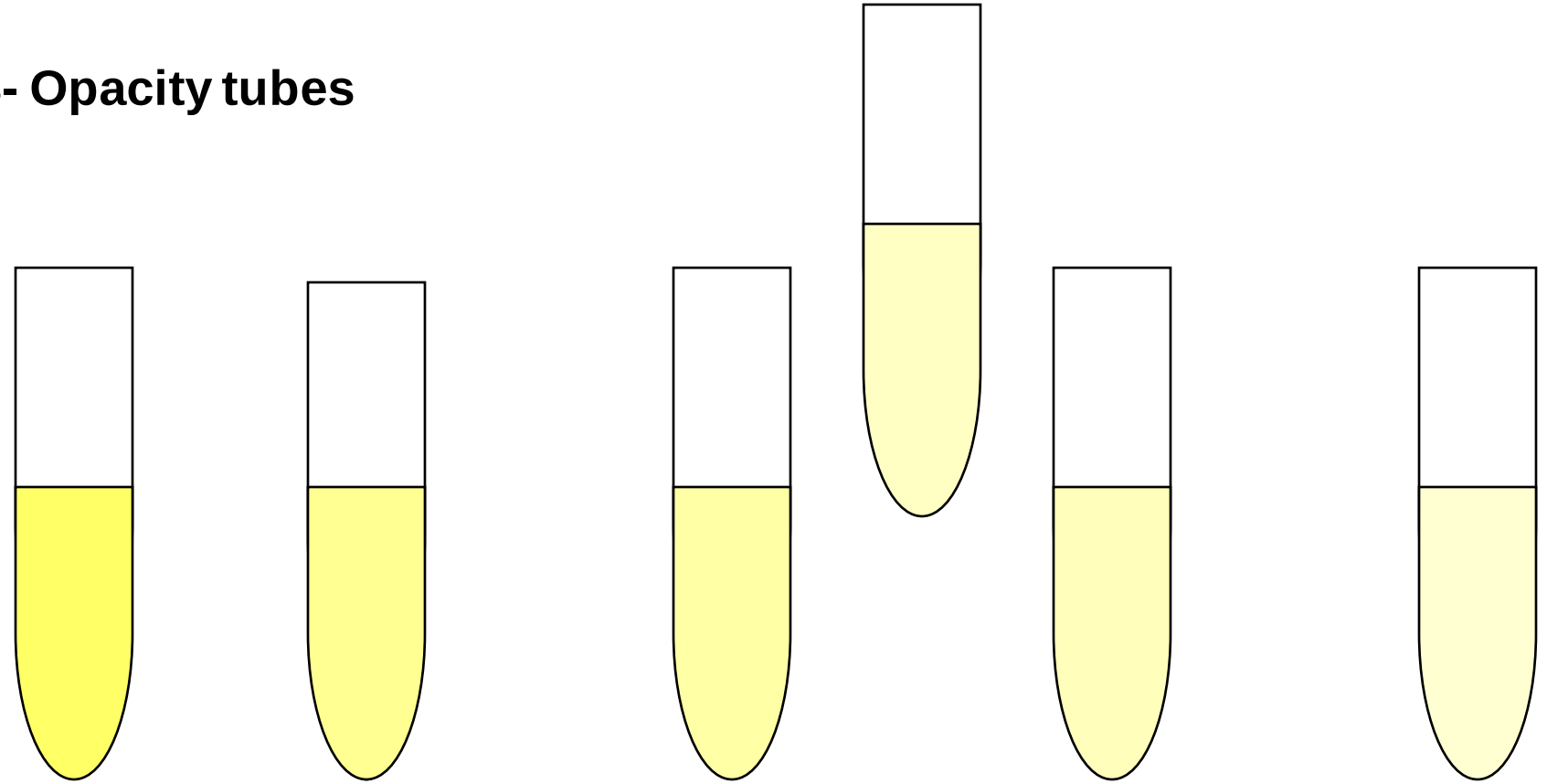






Density	Description	Approximate concentration/mm^3
D	Sperma densum	1.000.000 or more
SD	Sperma semidensum	500.000 - 1.000.000
R	Sperma rarum	200.000 - 500.000
OS	Oligospermia	Less than 200.000
A	Aspermia	Complete absence of sperm

3- Opacity tubes

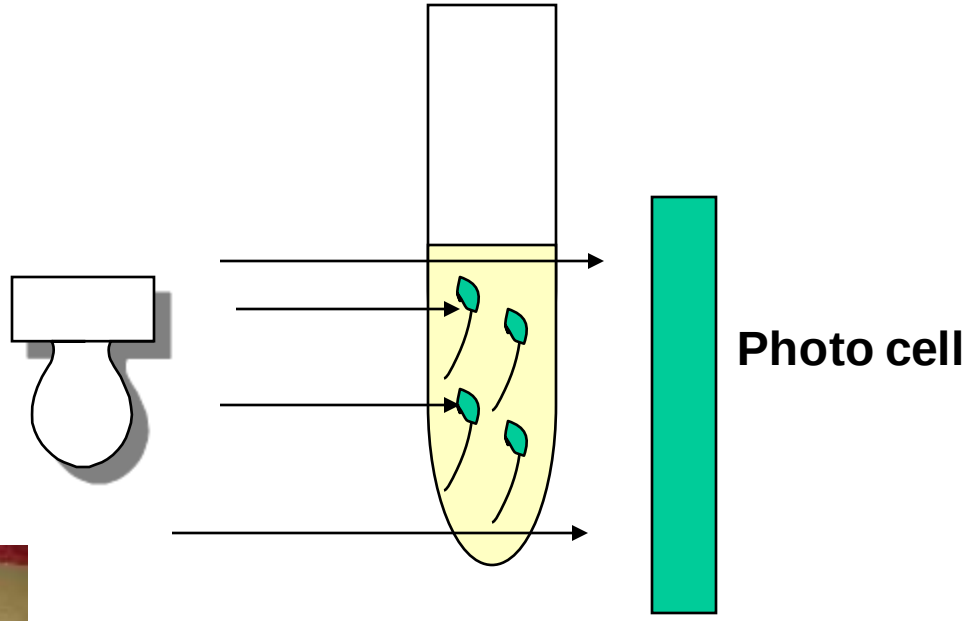


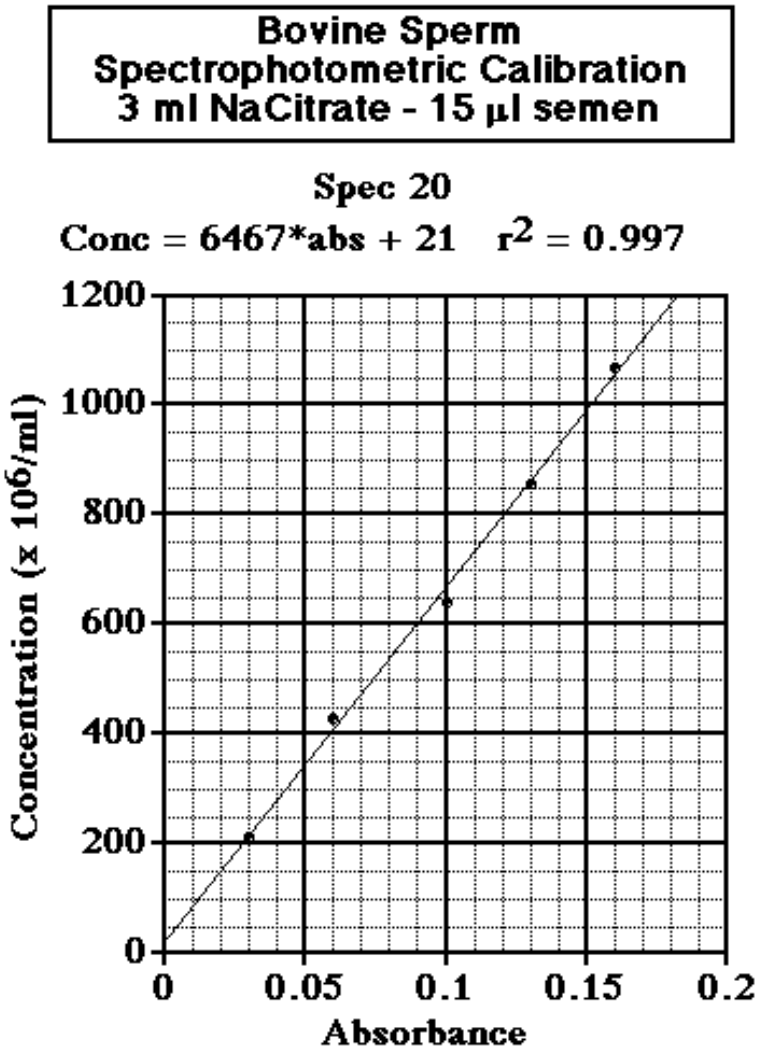
Sample of known Con.

$10^6/mm^3$ | 750×10^3 | 500×10^3 | 250×10^3 | $<250 \times 10^3$

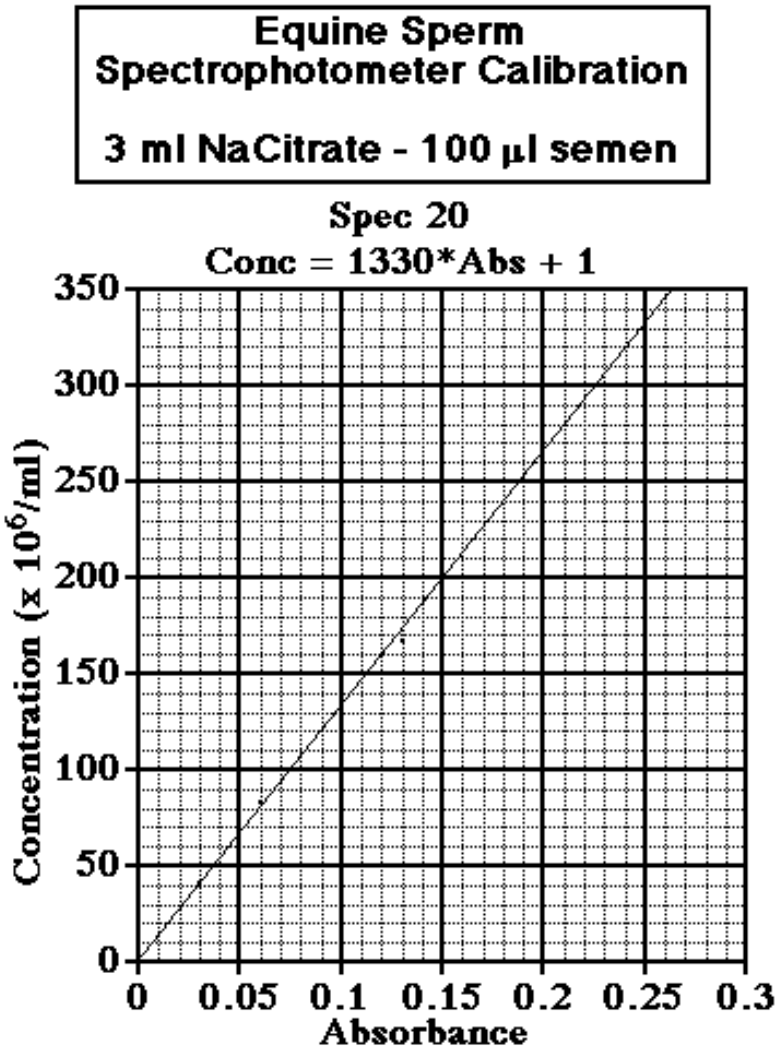
4- Calorimetric method (Absorptiometer, spectrophotometer)

Idea



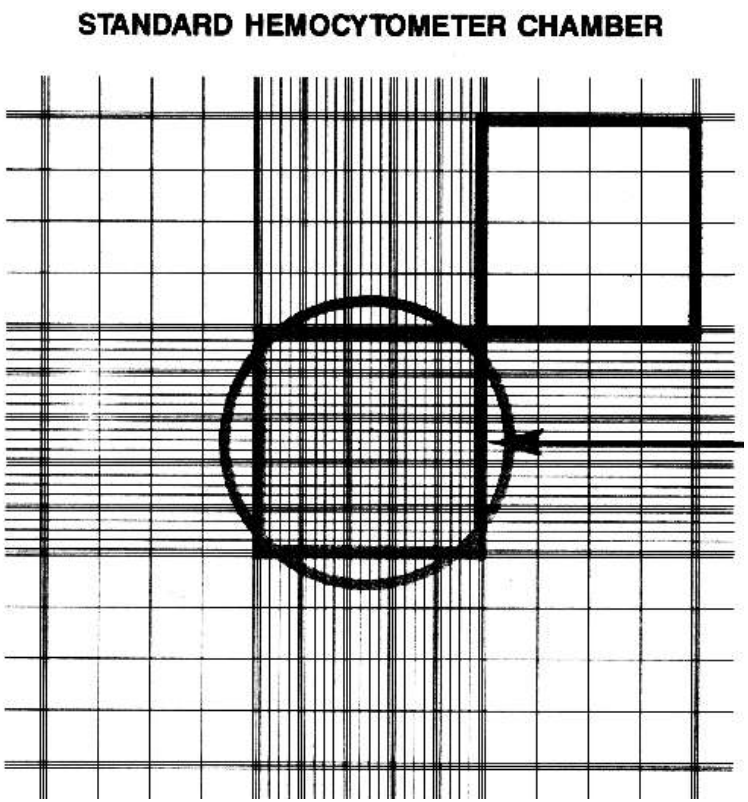


Calibration Curve for Bovine Semen



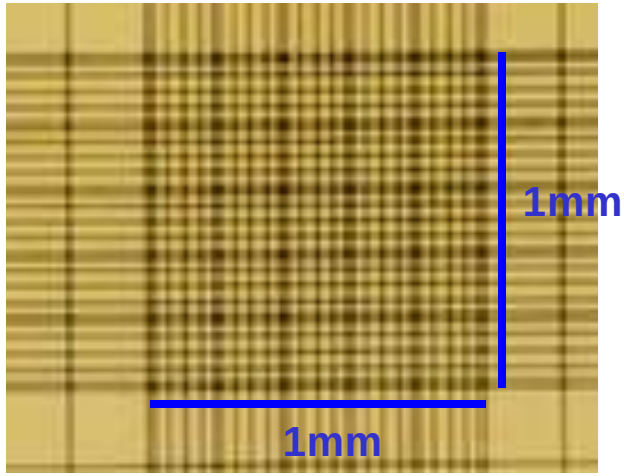
Calibration Curve for Equine Semen

5- Direct cell counting using the haemocytometer



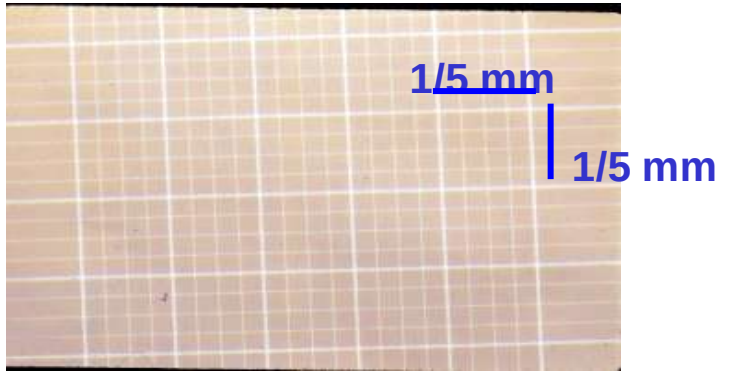
Size of the primary square = 1 mm^2

Volume of the primary square = 1 mm^3



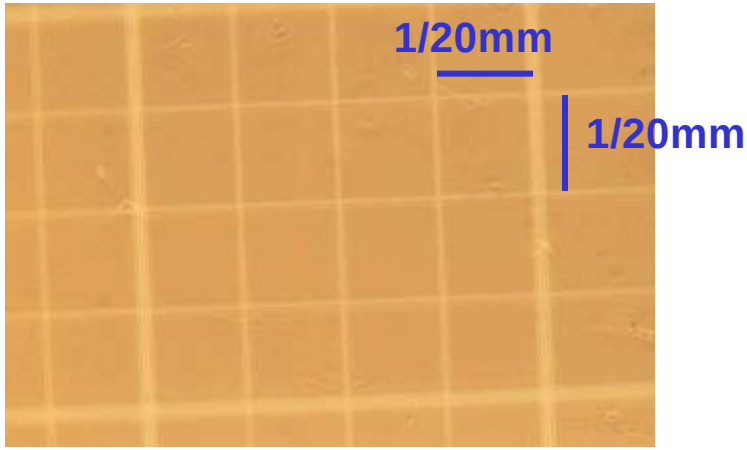
Size of the secondary square = 1/25 mm^2

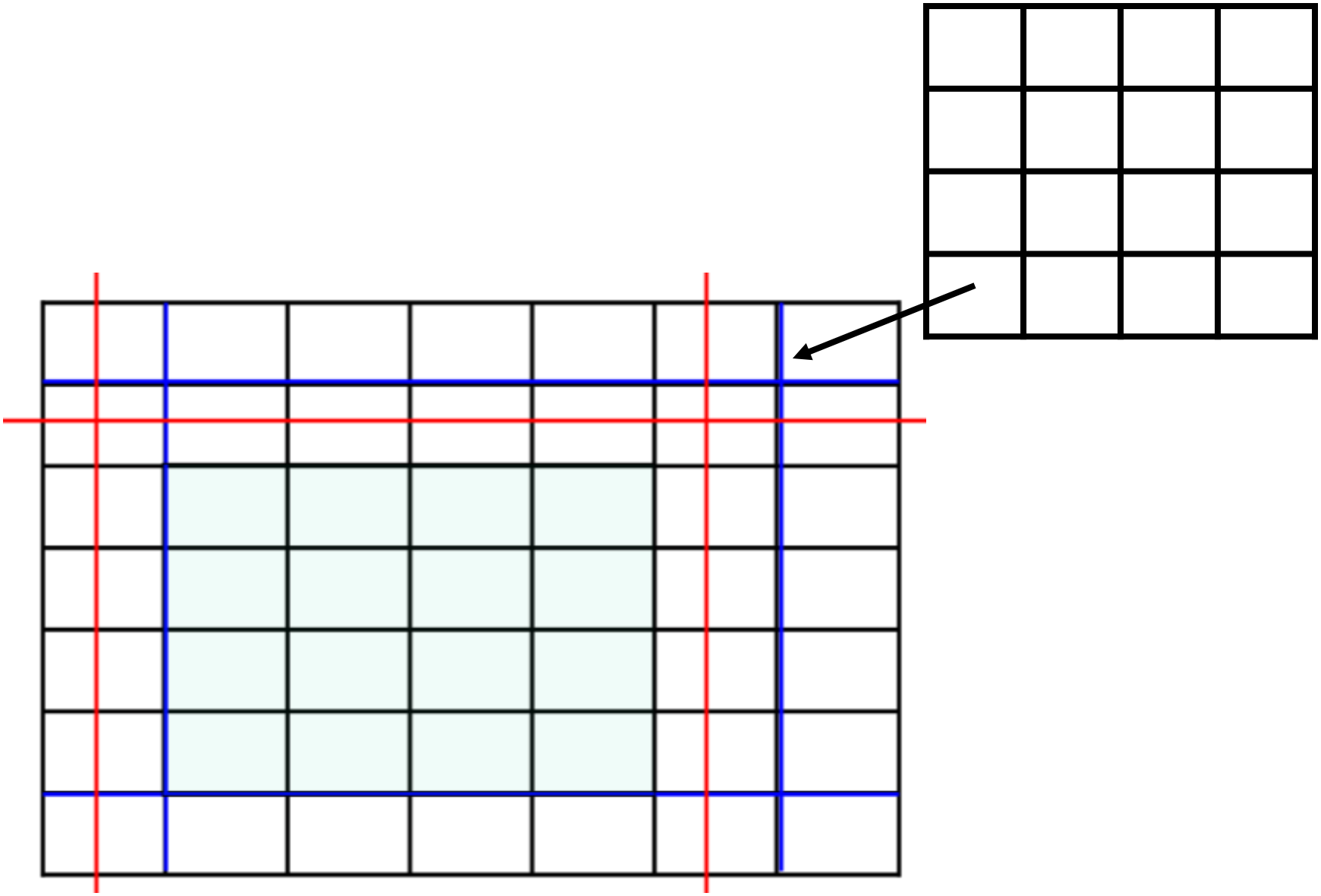
Volume of the secondary square = 1/250 mm^3



Size of the tertiary square = 1/400 mm^2

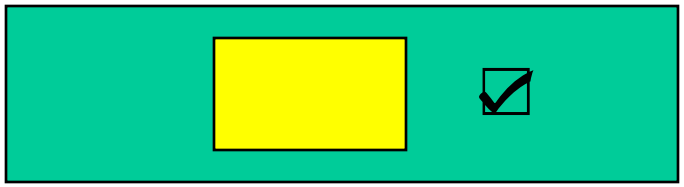
Volume of the tertiary square = 1/4000 mm^3

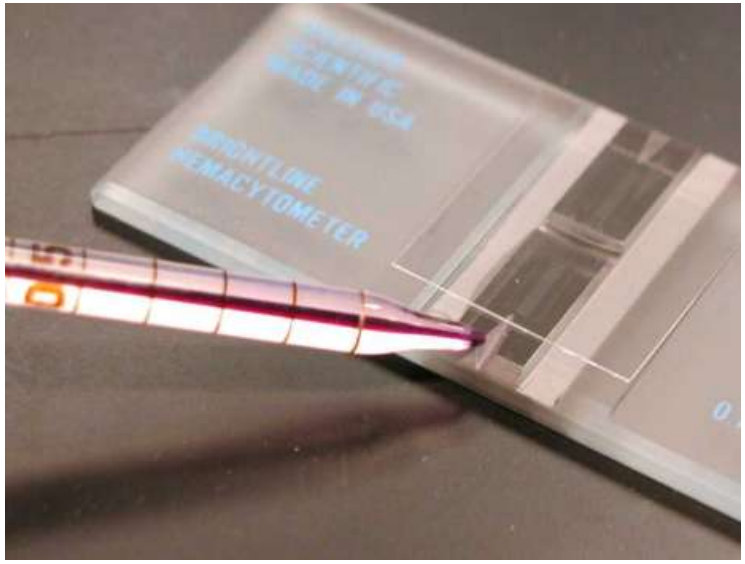


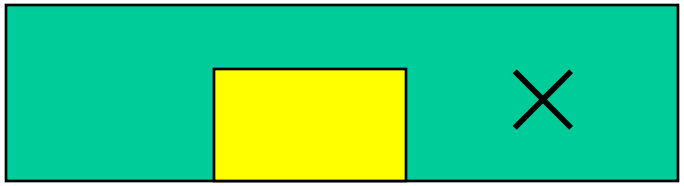


Methods

1- Preparation of haemocytometer







2- Preparation of the sample

Dilution

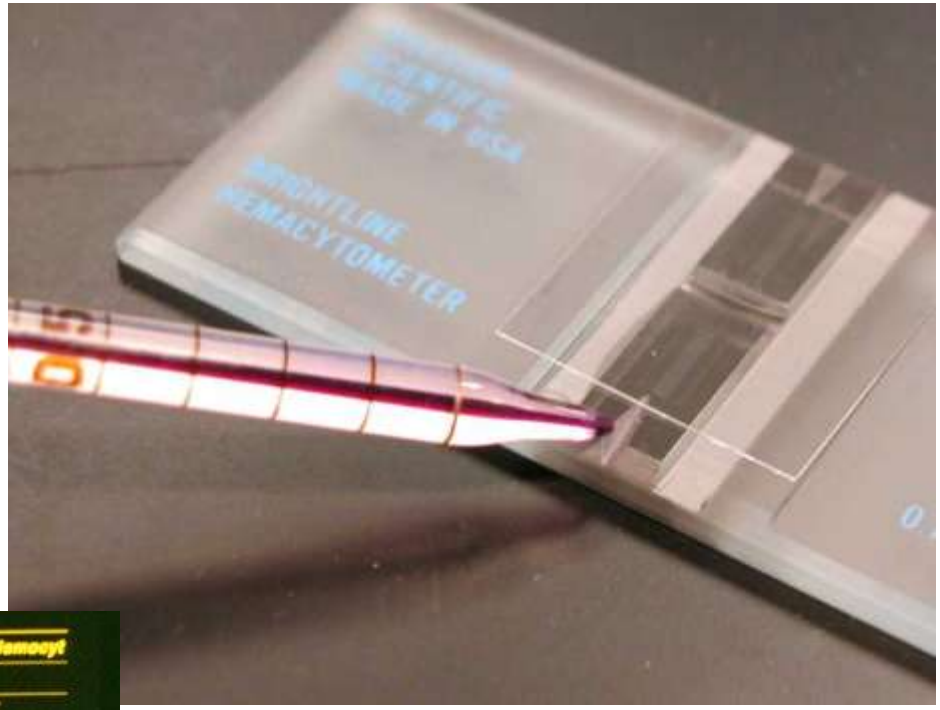
Killing of the sperm

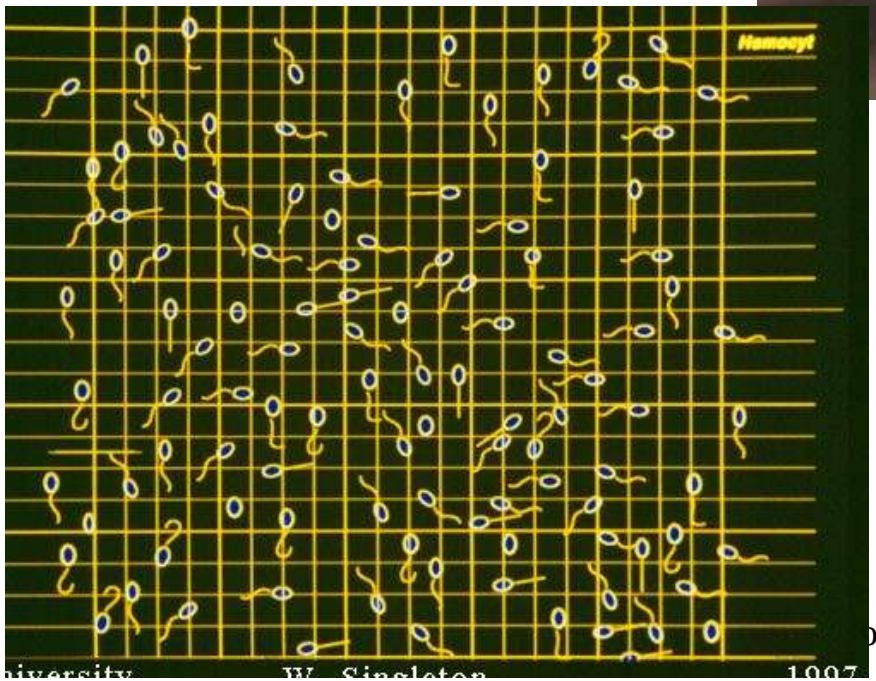
Staining of the sperm

0.01 % mercuric chloride + few drop of eosin in saline

2% sodium hydroxide + few drop of eosin in saline

3- Filling Haemocytometer





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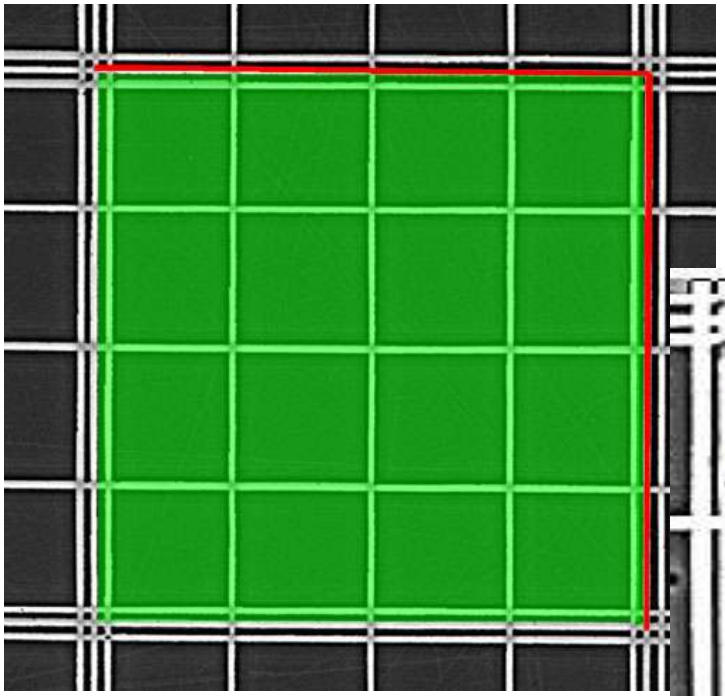
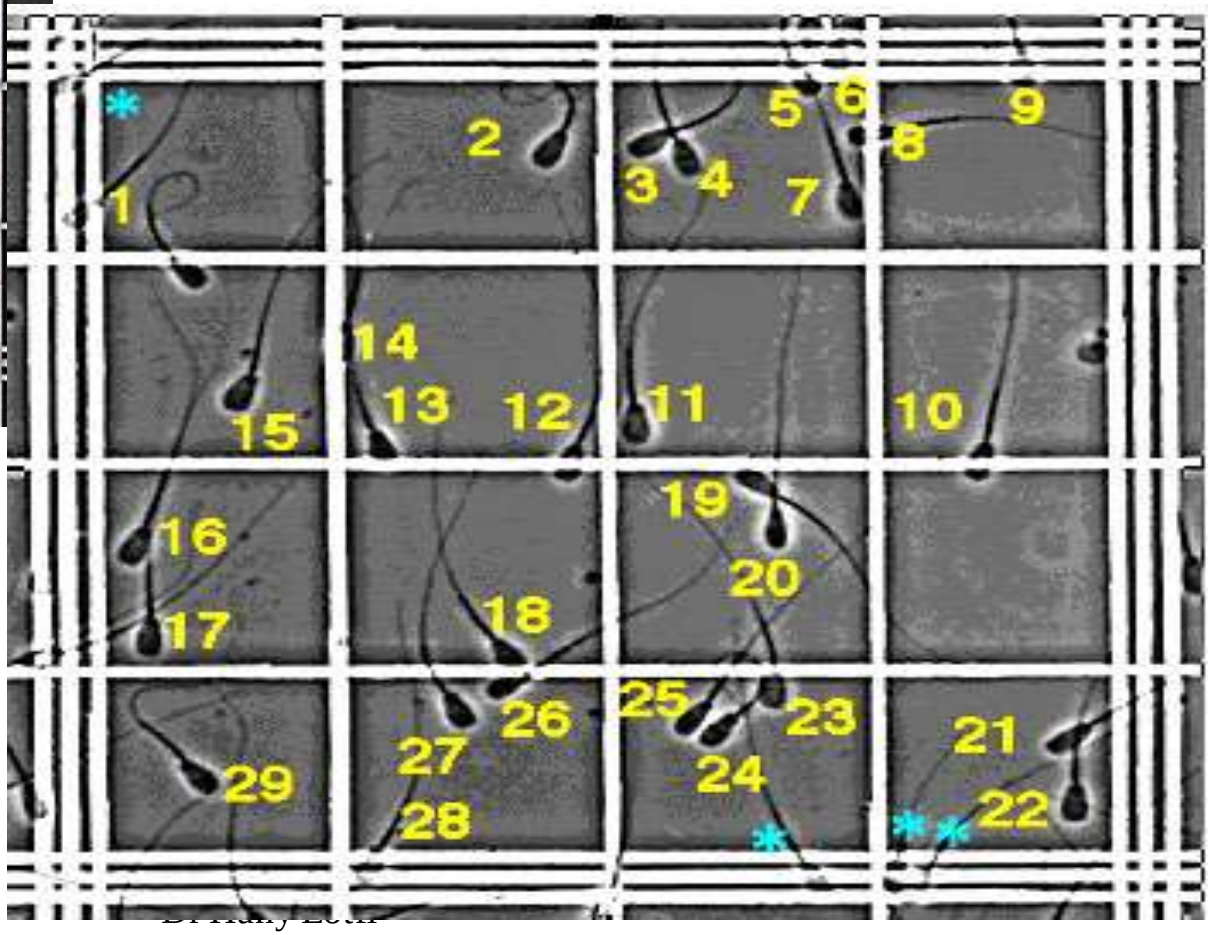
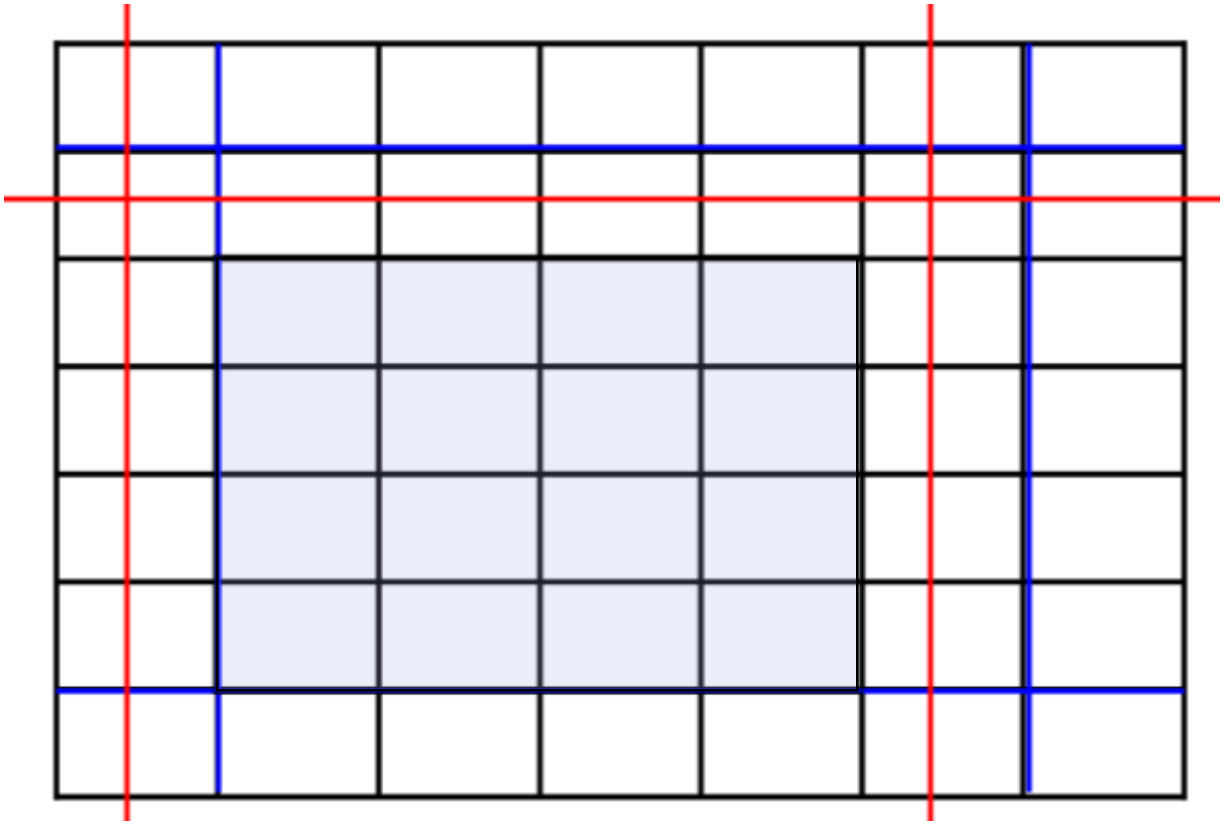
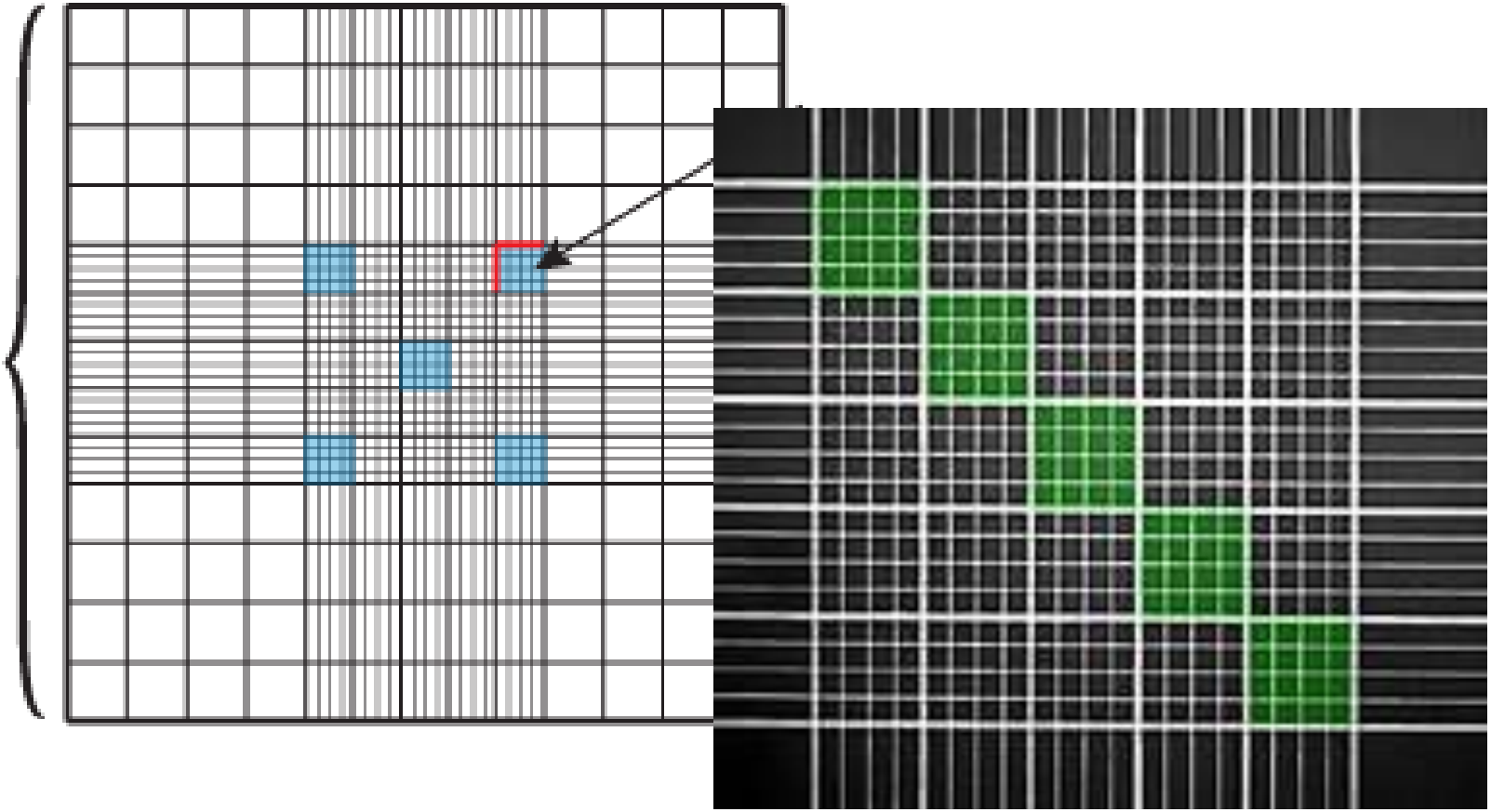
4- Counting







How many square you should count?



Total number of the sperm in 5 secondary square = R

Total number of the sperm in 80 tertiary square = R

4- Calculation

Total number of the sperm in 80 tertiary square = R

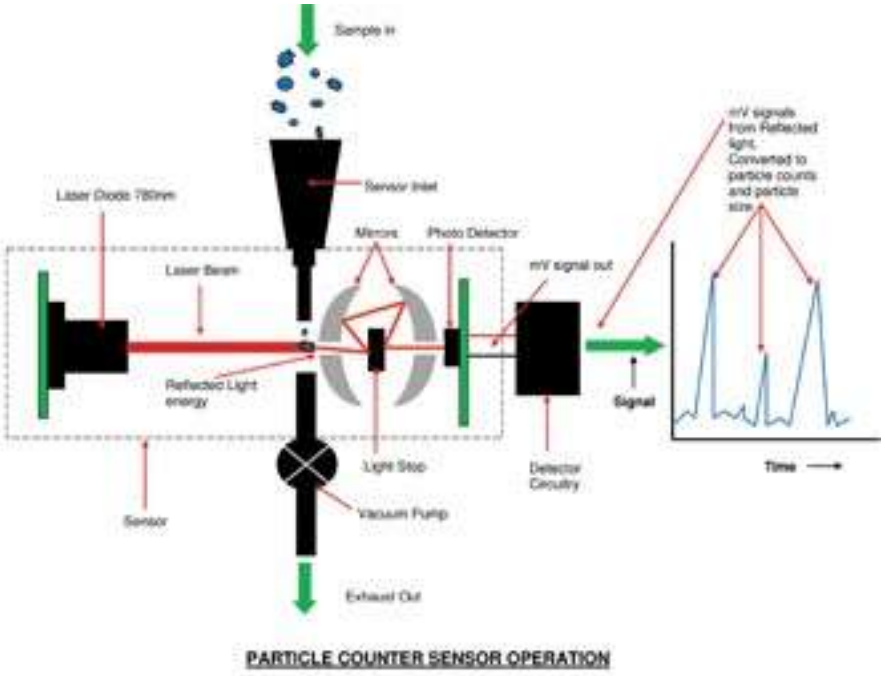
Total number of sperm in 80 x 1/4000 mm3 = R

Total number of sperm in 1/50 mm^3 = R

Total number of sperm in mm^3 = R x 50

Actual number of sperm in mm^3 = R x 50 x 200

6- Electronic sperm counter



PARTICLE COUNTER SENSOR OPERATION

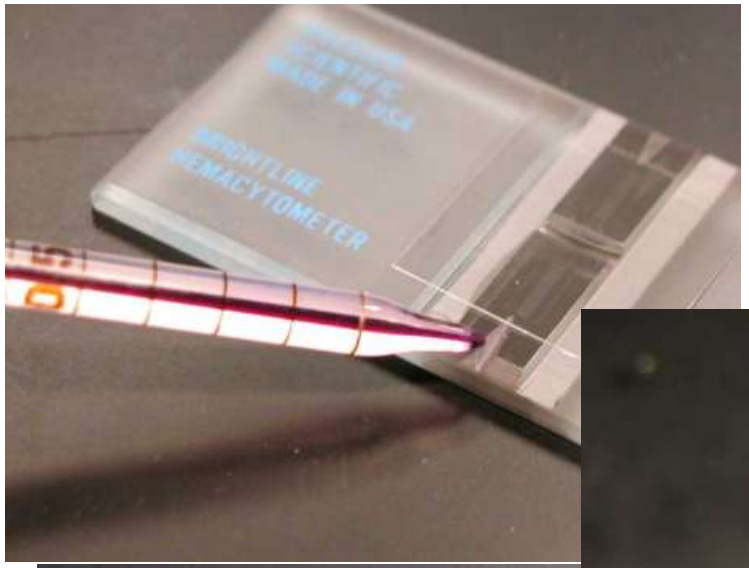
ny Lotfi

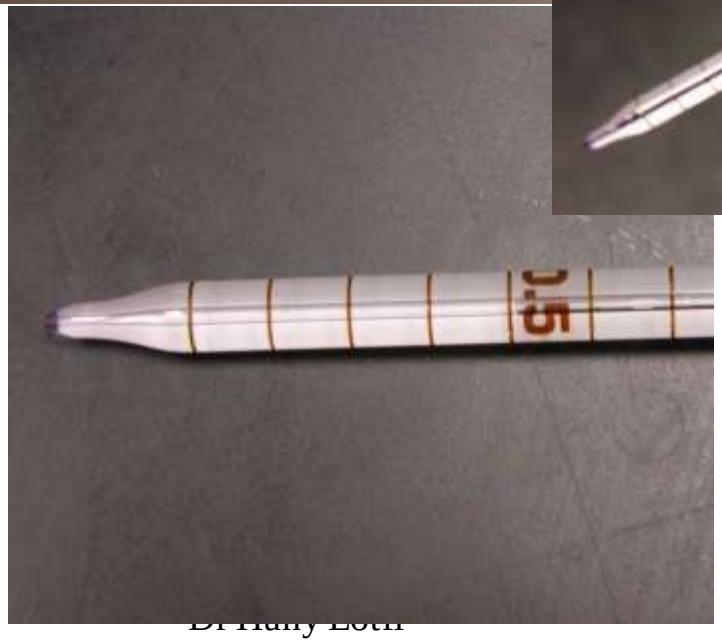
Sperm cell concentration in different species

Bull	**800,000 – 1000,000 / mm3**
Buffalo-bull	**600,000 – 1000,000 / mm3**
Ram	**2000,000 – 4000,000 / mm3**
Stallion	**50,000 – 200,000 / mm3**
Boar	**100,000 – 200,000 / mm3**
Dog	**50,000 – 100,000 / mm3**
Camel-bull	**500,000 – 600,000 / mm3**

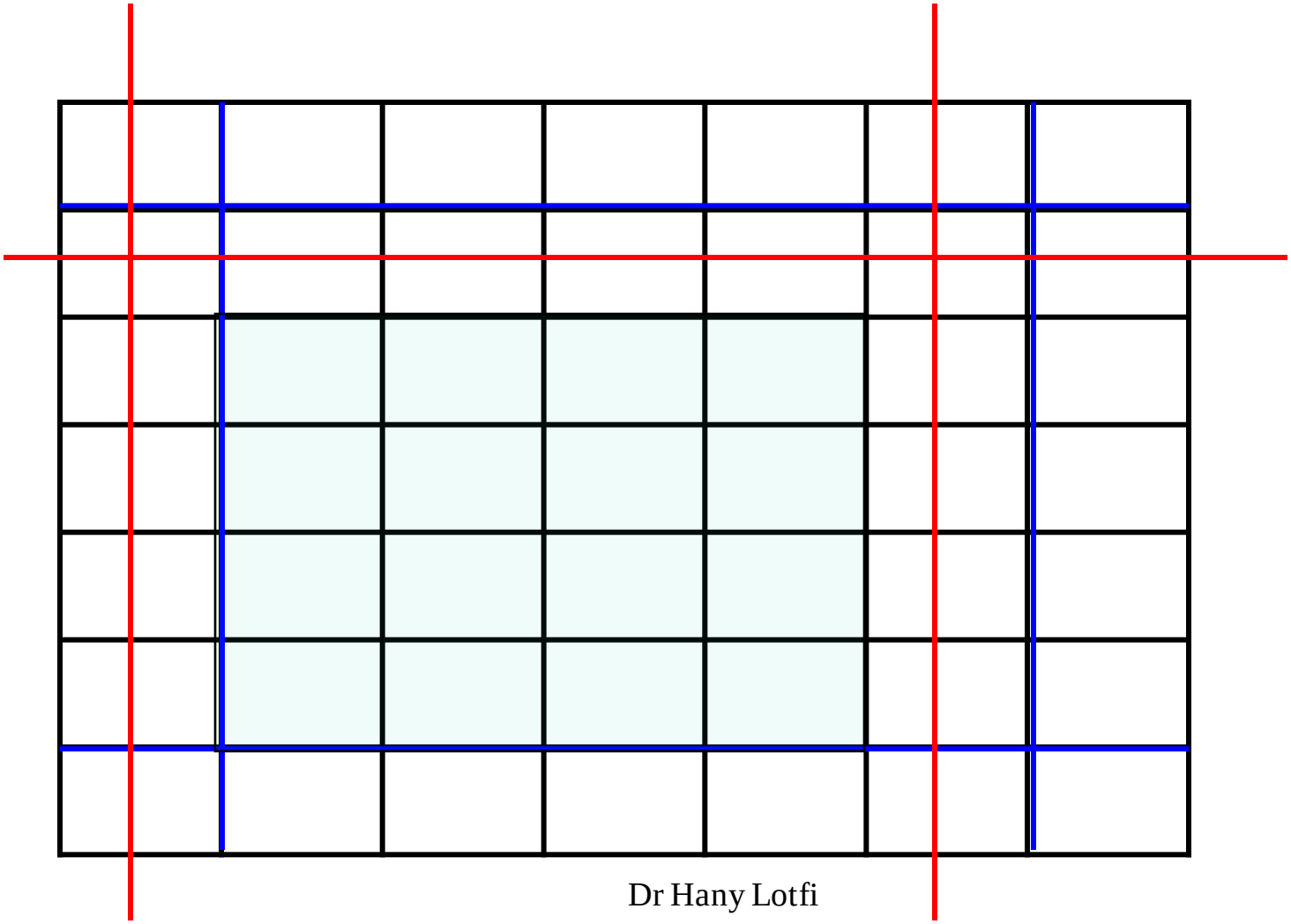
Computer assisted semen analyzer

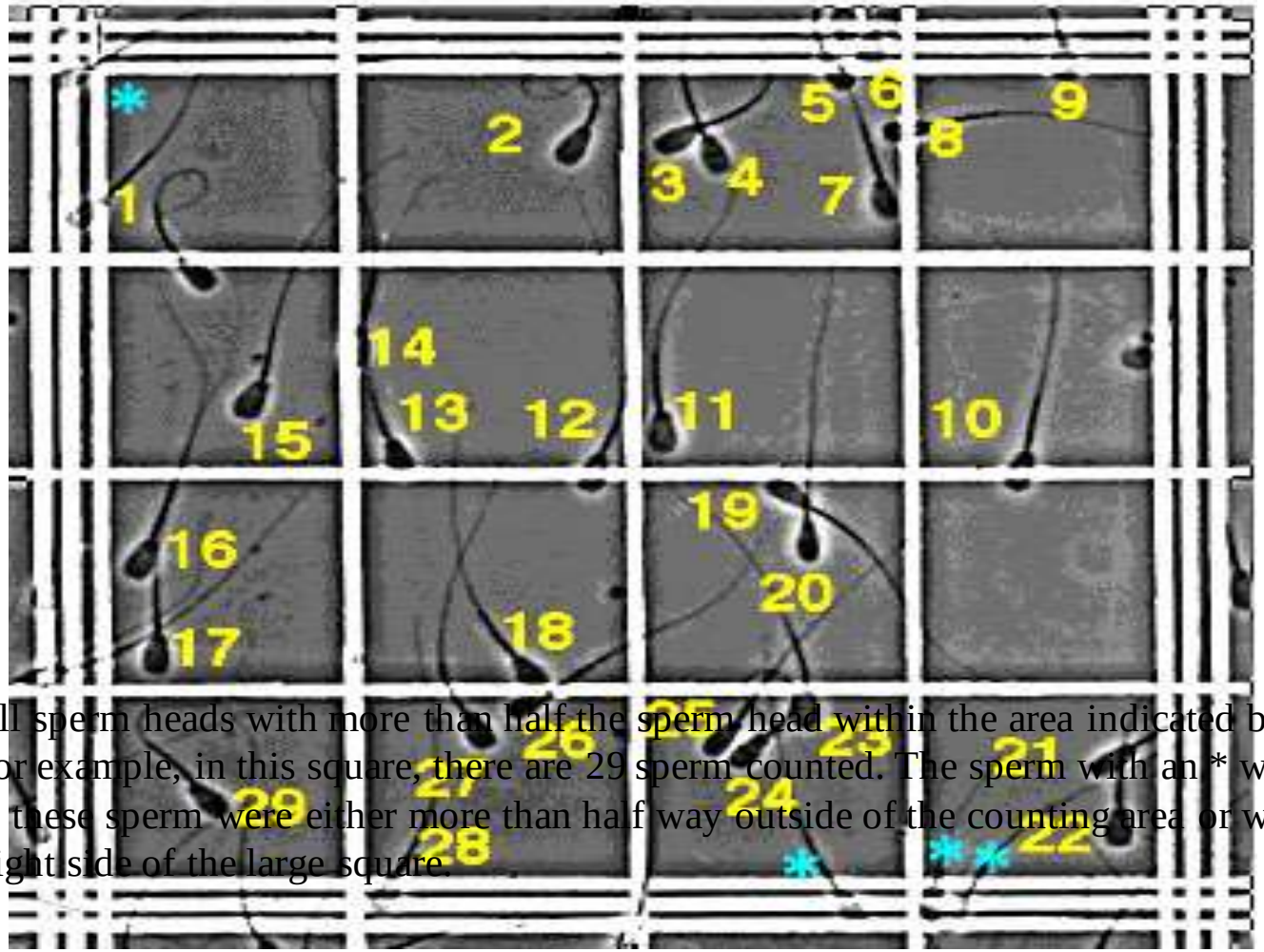

1.1
1.01
1
1
0.5
0.5


BRIGHTLINE
HEMACYTOMETER


0.5

The SQA-V Gold analyzer is a high-performance sperm analysis instrument used to test male fertility. It provides a precise and accurate 75-second automated semen analysis and delivers 16 clinical parameters, including count, motility (A+B+C), morphology, velocity, and functional sperm. Updated features include: full dynamic range; autotransfer to the included data manager; user-friendly interface; and an improved visualization system





Count all sperm heads with more than half the sperm head within the area indicated by a triple set of lines. For example, in this square, there are 29 sperm counted. The sperm with an * were not counted because these sperm were either more than half way outside of the counting area or were not on the top or right side of the large square.

In this square, there are 29 sperm counted. The pattern of counting begins at the top left and then proceeds through the 16 small squares. The sperm indicated with an * were not counted because these sperm were either more than half way outside of the counting area or were not on the top or right side of the large square. Note that sperm 6 was counted because although half way on the top edge of the counting area of the large square, this is one of the two sides (top and right) that will be counted in this type of a cituation. Sperm 5 and 9 were counted because they were more than half way below the top center line.

Dr Hany Lotfi