SEMEN EVALUATION AND SEMEN QUALITY IN ARTIFICIAL INSEMINATION

THERIOGENOLOGY LECTURES – A5

Prof. Sana HIRECHE

Created by Prof. Sana HIRECHE

OBJECT

- To understand the **importance of evaluating semen quality** in artificial insemination and to indicate certain **evaluation limitations**

INITIAL SCREENING

- Evaluations routinely conducted by the **AI laboratory** are used to determine whether the semen that is collected and processed is **satisfactory for use**

INITIAL SCREENING

- Semen is screened immediately following collection for quality and number of spermatozoa
  - **To eliminate any substandard ejaculates**

INITIAL SCREENING

- Avoids wasting expensive supplies, antibiotics, semen extender…
  - ***Substandard samples are not processed***

INITIAL SCREENING

- Semen that passes initial screening will be further :
  - **Extended**
  - **Cooled**
  - **Packaged into straws**
  - **Frozen**

POST-THAW EVALUATIONS

- **After freezing**, a representative **sample** is normally **thawed** and evaluated using various **laboratory tests :**
  - **Process quality control :** Reflect the ability of the semen to withstand the processing conditions
  - **Fertility prediction:** Give some indication of the potential fertility of the semen

FERTILITY HISTORY OF THE BULL

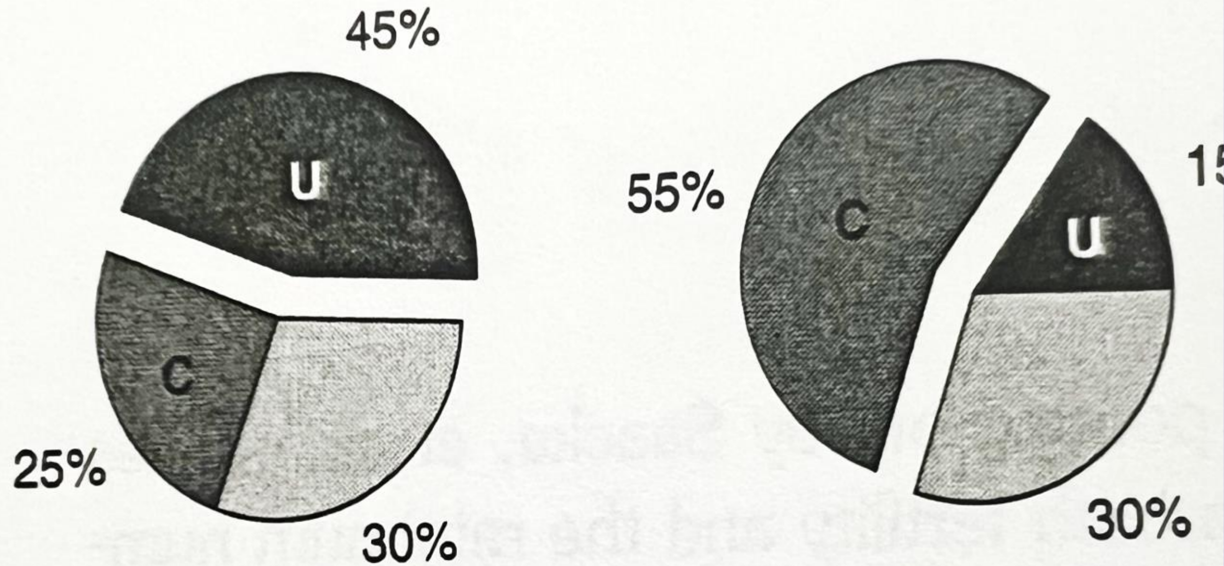
- The best indication of the fertility of the bull :
  - **His actual breeding record, estimated by non-return rate**
  - Interpretation of **semen quality test**

FERTILITY HISTORY OF THE BULL

- Males differ in both **fertility** and the **minimum number of sperm per inseminate** needed to reach the maximum fertility of which they are capable

ASPECTS OF SEMEN QUALITY IMPORTANT IN AI LABORATORY

- **Compensable** characteristics
- **Uncompensable** characteristics



Saake, 2000. *Fertility in the bovine male: current status and future prospects-An opinion*

Created by Prof. Sana HIRECHE

- **The Bull A** should be recognized and eliminated from AI service since the larger portion of this deficiency is **uncompensable** and ***cannot be oversome by increasing dosage in the inseminate***
- **Bull B** may require higher than normal dosages to achieve pregnancy rates comparable to his peers with minimal compensable deficiences

ASPECTS OF SEMEN QUALITY IMPORTANT IN AI LABORATORY

- **Increasing sperm dosage** to the **female** results in a response in fertility for **compensable** semen characteristics
- Differences in fertility among **bulls** cannot be removed by increasing sperm dosage for **uncompensable** semen characteristics

ASPECTS OF SEMEN QUALITY IMPORTANT IN AI LABORATORY

- **Immotile** or **obviously malformed sperm** would be **compensable** and have ***little effect on fertility*** if the **female is inseminated with sufficient numbers of competent sperm**

ASPECTS OF SEMEN QUALITY IMPORTANT IN AI LABORATORY

- **Viable abnormal ejaculates** are believed to be ***uncompensable*** because they may ***not be able*** to maintain or sustain the fertilization process
- Bulls with ***largely uncompensable semen characteristics*** would probably be **subfertile at any dosage**

Factors influencing the opportunity for a female to become pregnant

- The quality and quantity of frozen semen used to inseminate a cow or a heifer
- Heat detection accuracy
- Insemination skill
- Physiological stress from lactation
- Health and nutrition of the female

EVALUATION TESTS IN AI ORGANIZATIONS

- Many different tests are used by the AI organizations to monitor changes that occur in their programs
- These tests are not exact predictors of fertility
- These tests are effective quality control measures when used in an appropriate manner, by well trained personnel, using properly calibrated equipments

EVALUATION TESTS IN AI ORGANIZATIONS

- These tests can be influenced by :
  - **Individuals bulls**
  - **Extenders**
  - **Semen freezing and thawing rates …**

SEMEN EVALUATION

1. Once the semen sample has been collected the vial can be easily detached from the semen collection cone
2. Always keep the semen vial protected from the cold prior to evaluation
3. A simple and effective method is to hold the vial in a warm hand with the fingers completely encircling it

SEMEN EVALUATION

4. If evaluation is not going to occur right away the sample should be transferred to a 37 °C water bath

5. The chest pocket of a close-fitting pair of coveralls has also worked well for short-term storage, especially if the hands are needed for other tasks

SEMEN EVALUATION

- The skilled examiner should know how to assess :

- Color and consistency
- Volume
- Concentration
- Gross motility
- Individual motility
- Sperm morphology
- Percentage staining alive

SEMEN EVALUATION : VOLUME

- The **volume** is measured in prewarmed graduated glass tubes
- It's an indication that a representative sample was collected
- It's **influenced by the collection process** and **is not a relevant measure**

SEMEN EVALUATION : VOLUME

- The volume of an EEJ derived semen sample should not be compared with ejaculates from the same or different bulls

SEMEN EVALUATION : VOLUME

- In cases of **sperm accumulation**
  - The **“rusty load phenomenon”**
  - **25–40 ml** of concentrated semen may be collected easily during a single ejaculation
- **A typical EEJ-derived semen sample is 1–5 ml**

COLOR AND CONSISTENCY

- A good quality ejaculate should be **opaque, creamy-white in color**
- A yellow, buttery sperm is a variation of normal when it occurs in **concentrated samples** with no apparent aberrations

COLOR AND CONSISTENCY

- A pink or red sperm or even streaks of red in the semen sample are usually indicative of **blood contamination**

GROSS MOTILITY

- **Gross motility** or gross wave motion is influenced by the percentage of **progressively motile sperm**, the **sperm concentration**, and **the vigor or rate of speed of the motile sperm**

GROSS MOTILITY

- A drop of semen on a microscope slide is viewed using bright field microscopy at 40–125× magnification
- Vigorous swirls and eddies are indicative of a **concentrated semen with a very high proportion of vigorous motile sperm**

Evaluation microscopique de la semence

PINNACLE
SYSTEMS

Created by Prof. Sana HIRECHE

RATES OF PROGESSION (VIGOR)

- **5 :** Very rapid and vigorous forward motion. Swirls and eddies caused by movements of sperm are extremely rapid and changing constantly in neat semen preparations
- **4 :** Rapid progressive motion. Abruptly forming swirls and eddies are viewed in neat semen preparations

Created by Prof. Sana HIRECHE

RATES OF PROGESSION (VIGOR)

- **3 :** Steady progressive motion at a moderate speed. Swirls and eddies move more slowly across the field of view in neat semen preparations
- **2 :** Slow progression, including stop and start motion. No swirls or eddies are viewed in neat semen preparations

RATES OF PROGESSION (VIGOR)

- **1 :** Weak undulation or oscillatory motion
- **0 :** no discernable motility

Created by Prof. Sana HIRECHE

INDIVIDUAL MOTILITY

- Individual motility or more precisely percentage of progressively motile sperm is determined by placing a **2 to 4 mm drop of semen** on a **clean microscope slide** over which a **cover slip** is dropped in place

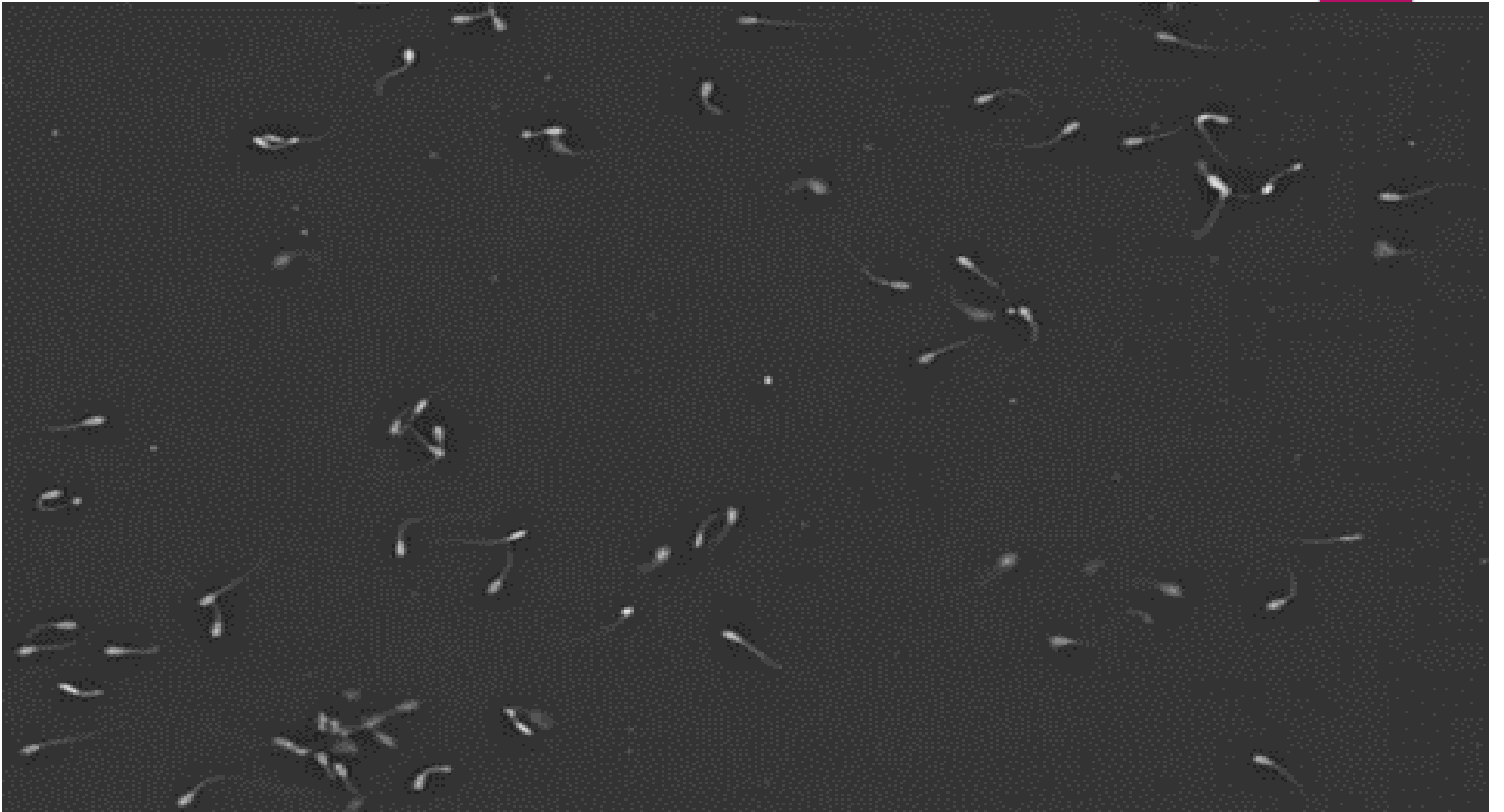
INDIVIDUAL MOTILITY

- The seminal fluid should spread just to the edge of the cover slip to form a thin layer in which the sperm can be viewed within the same focal plane

INDIVIDUAL MOTILITY

- Too much semen and the cover slip will float, making visualization of individual sperm more difficult, and very concentrated samples may be impossible to evaluate without dilution

PINNACLE
SYSTEMS


Created by Prof. Sana HIRECHE

Created by Prof. Sana HIRECHE

INDIVIDUAL MOTILITY – DILUTION

- Warmed sodium citrate solution
- Commercial semen extender
- Fresh sperm-free seminal fluid

INDIVIDUAL MOTILITY

- Overly-thick semen smears contribute to inaccurate estimates of motility
- Very thin semen smears can dry out too rapidly
- Use of a standard evaluation volume of semen delivered by microliter pipet and uniformly sized cover glasses greatly improve accuracy and repeatability

INDIVIDUAL MOTILITY

- Evaluation of multiple smears from the same semen sample results in a more representative estimation of the motility
  - Individual smears can vary

INDIVIDUAL MOTILITY- PROCEDURE

1. **5 µL** of the ejaculate (from artificial vagina or electroejaculation) or sperm suspension (from postmortem) is diluted in **495 µL of D-PBS** at **37 °C**
2. **10 µL** of the sperm suspension is deposited between a glass slide and coverslip at **37 ° C**
3. Individual motility is assessed subjectively **under a phase-contrast microscope at 400 × with a warmed stage at 37 ° C** by determining the percentage of motile sperm in **5 fields**

Created by Prof. Sana HIRECHE

INDIVIDUAL MOTILITY

- Individual motility is recorded as a percentage
- Minimum acceptable proportions of **30 % and 60 % being used by the respective SFT and Western Canadian Association of Bovine Practitioners' guidelines**

INDIVIDUAL MOTILITY

- Good quality bull semen typically will range **from 50 to over 90 % initial (pre-freezing)** progressive motility based on visual estimate

PHOTOGRAPHIC MOTILITY

- Track motility evaluations are more repeatable and objective than visual estimations
- Method developed by Van Dellen and Elliott (1978) employs time-exposure darkfield photomicrography of thawed semen samples using a specially modified counting chamber

PHOTOGRAPHIC MOTILITY

- Several exposures are made for each semen sample
- The film is developed and the motility % is obtained by counting the number of motility tracks in comparison to the number of single sperm cell images

CONCENTRATION

- A subjective evaluation of concentration should be made by examining the tube containing the semen to ensure that a representative sample has been obtained
- Semen samples must usually be diluted prior to sperm concentration evaluation

CONCENTRATION

- Accurate dilution is crucial to obtain reliable results
- Because diluent and semen sample volumes are usually small and dilution ratios are relatively large, even minor sampling errors can significantly affect the results

CONCENTRATION

- Semen must be thoroughly mixed and homogenized prior to sampling and representative aliquots of at least 25 µL should be used for dilution.
- Proper user, maintenance, and calibration of instruments used to prepare dilutions is very important.

CONCENTRATION

- The basic property required of diluents is the ability to disperse sperm and not interfere with the counting method
- Therefore, diluents are usually translucent solutions that prevent sperm from agglutinating

CONCENTRATION

- Simple salt solutions (e.g., **physiological saline**), buffered solutions (e.g., **phosphate-buffered saline**), and even **distilled water** can be used as diluents depending on the counting method

CONCENTRATION

- **Sperm immobilization** and **disruption** of the plasma membrane is essential when performing manual counts
- Sperm can be immobilized by adding **formalin** to the diluent or using diluents with low (**distilled water**) or high osmolarity (**10% saline solution**)

Autodiluters can be used for preparing samples for sperm concentration evaluation with great precision and accuracy

(Hamilton™ Microlab™ 600; www. fishersci.com/shop/products/microlab-600-diluter-dispenser-system/p-40 88795)

Phosphate-Buffered Saline

1. Add 800 mL of distilled water to a volumetric flask

2. Add sodium chloride (9 g/L), sodium phosphate dibasic (1.42 g/L), potassium. phosphate monobasic (0.24 g/L), and potassium chloride (0.2 g/L) to the flask

3. Stir for 15 min

Phosphate-Buffered Saline

4. Add distilled water to bring the volume up to 1000 mL

5. Measure the pH and adjust to 7.4 with hydrochloric acid or sodium hydroxide as needed

6. Store at 4 °C for 30 days

Buffered Formalin

1. Add 10 mL of formaldehyde to 990 mL of phosphate-buffered saline
2. Stir for 15 min
3. Store at 4 °C for 30 days

CONCENTRATION

- Poorly concentrated sample will be **watery and translucent containing <250 million sperm per milliliter**
- Concentrated sample, typical of a mature bull, will appear **creamy and grainy** when examined against a dark background **containing at least one billion sperm per milliliter**

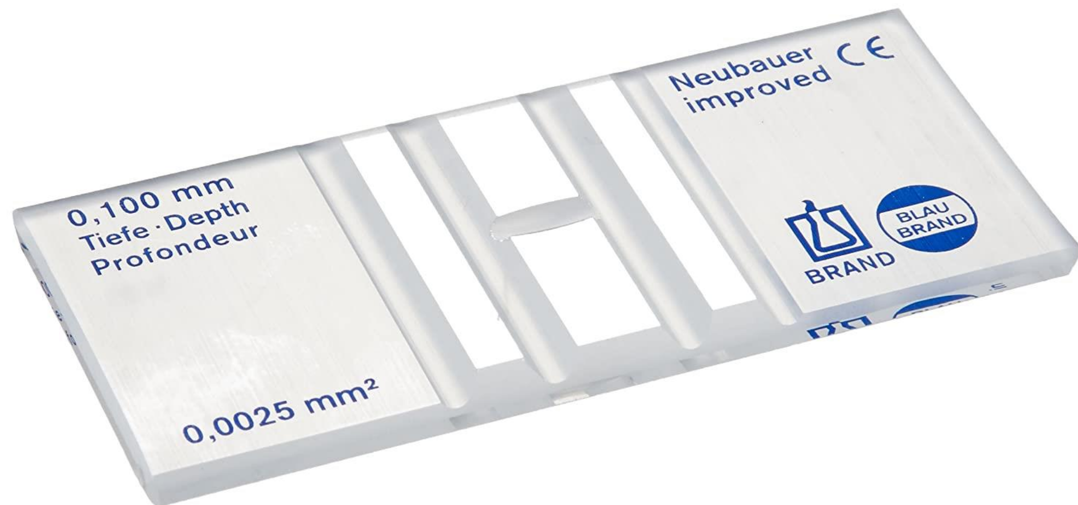
CONCENTRATION

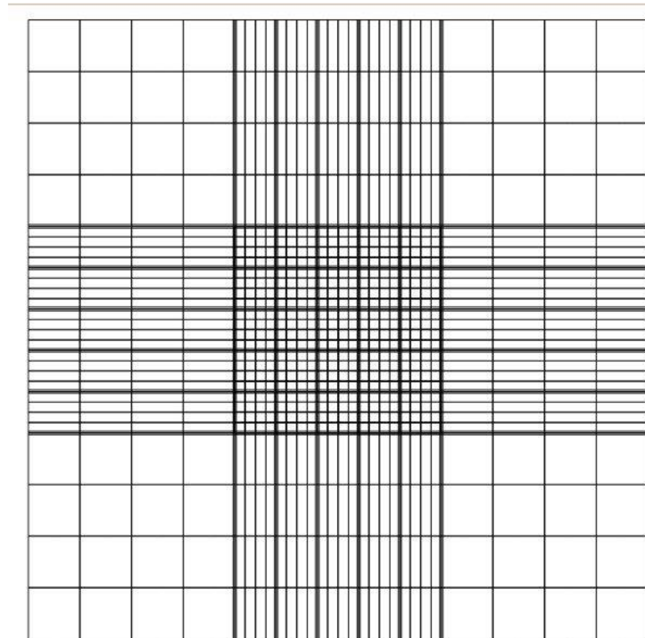
- Very concentrated, high-volume ejaculates occur in cases of sperm accumulation
- A watery, poorly concentrated sample from a mature bull is typical in cases of testicular degeneration

CONCENTRATION

- Closely linked to volume
- The concentration of a semen sample varies with the collection technique
- EEJ is not an appropriate method of determining the sperm-producing capability of a bull

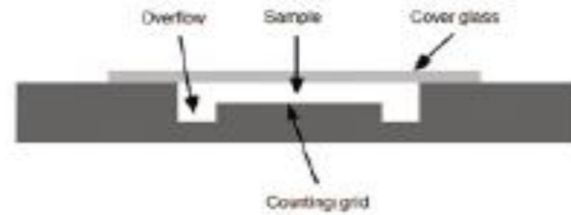
Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer





Created by Prof. Sana HIRECHE

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer



Created by Prof. Sana HIRECHE

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

- Improved Neubauer hemocytometer contains two separate counting chambers that are covered with a glass coverslip held at a specific height
- The chambers are filled by capillary action by loading the sample through a notch at the end of each chamber

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

- Counts are usually performed in the in the central 1 mm2 grid, or on the smaller 0.4 mm2 areas defined within the central grid (yellow)

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

- When performing the count, sperm heads that touch the left or lower grid boundary lines are counted (black ovals), whereas heads that touch the right or upper boundary lines (open ovals) are not counted

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

1. Ensure that the hemocytometer and coverslip are clean and dry

2. Secure the coverslip on the counting chambers by pressing it firmly onto the chamber pillars. Iridescence (Newton's rings) between the two glass surfaces indicates appropriate contact

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

3. Select desired dilution rate (DR):

(a) For neat semen: DR = 1:200 to 1:50

(b) For neat or extended semen: DR = 1:50 to 1:10

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

4. Dispense the appropriate volume of buffered formalin accord-ing to dilution rate using a pipette

5. Swirl or invert the semen container for at least 10 seconds

6. Aspirate the appropriate volume of semen according to dilution used (see table below) using a pipette

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

7. Wipe off semen from the outside wall of the pipette tip using a disposable wipe

Take care to ensure the entire circumference of the tip is wiped but avoid touching the tip opening and absorbing semen from inside the tip

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

8. Insert the pipette tip 3 mm below the meniscus of the aliquoted diluent and dispense the semen

Rinse the tip twice by pipetting "up and down" before removing the tip from the diluent

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

9. Swirl or invert the semen container for at least 10 s again and prepare the duplicate dilution following the same steps described above

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

10. Cap the tube with the diluted sperm suspension and vortex for at least 10 s

11. Using a pipette, aspirate 10µL of the diluted sperm suspension immediately after mixing to avoid settling

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

12. Touch the pipette tip carefully against the V-shaped groove of one of the chambers of the hemocytometer and depress the plunger slowly, allowing the chamber to fill by capillary action

- Take care not to overfill (coverslip movement) or underfill (no air) the chamber

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

13. Vortex the second dilution for at least 10 s and load the second chamber following the same steps

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

14. Store the hemocytometer horizontally for 2 min at room temperature to allow sperm to settle onto the chamber grid

- Use a humid chamber (e.g., water-saturated filter paper in a covered Petri dish) to prevent drying if necessary

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

15. Examine the hemocytometer with phase-contrast optics between 100–400× magnification

16. Tally the number of sperm on the upper left small square in the central grid with the aid of a laboratory counter

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

17. If there are more than 40 sperm in the first counted small square, then count all corner and the center square (5 squares total)

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

18. If there are less than 40 sperm in the first counted small square, then count all 25 squares

19. Switch to the second chamber of the hemocytometer and perform the replicate count on the same number of squares as the first replicate

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

20. Calculate the mean and the difference between the two counts

- If the difference is greater than 10% of the mean, discard the results and repeat the counts using the same diluted sperm suspensions
- If the difference is still greater than 10% after a second time, prepare new sperm suspensions and repeat the entire process

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

21. If the difference between the two counts is less than 10%, use the mean to calculate sperm concentration

- The variables used for the calculation include: (1) number of sperm counted in 1 mm^2 , (2) chamber depth, and (3) dilution factor .

Sperm Concentration Evaluation Using the Improved Neubauer Hemocytometer

(a) The central grid is 1 mm^2 ; therefore, if the entire grid was counted, no correction is necessary, whereas if only 5 small squares were counted, then the count must be multiplied by 5

(b) The depth of the hemocytometer chamber is 0.1 mm (100 µm); therefore, the volume over 1 mm^2 is 0.1 mm^3 or 0.0001 mL

Example

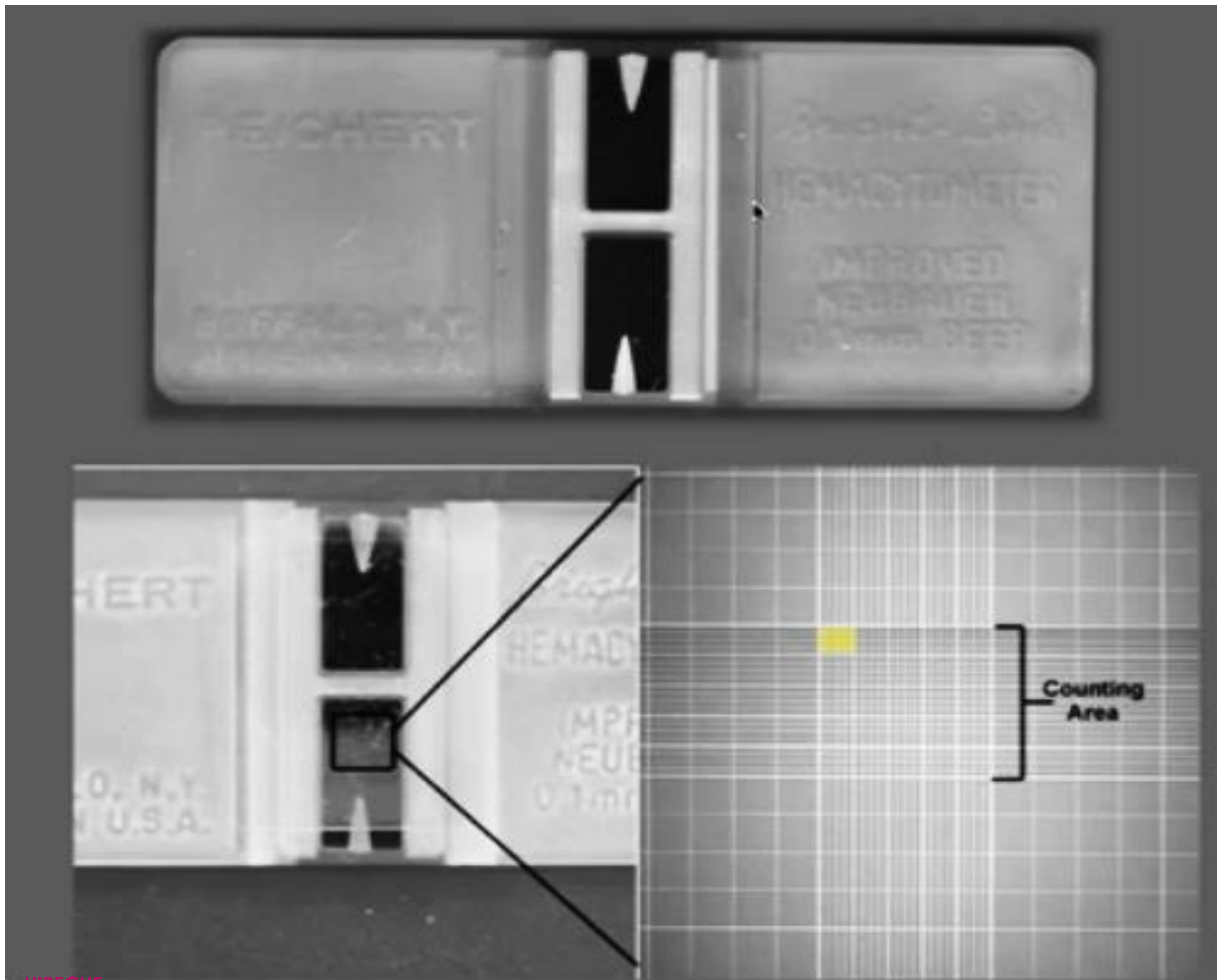
- Using a dilution of 1:100 and an Improved Neubauer hemocytometer, an average of 200 sperm are counted in five of the squares in the central grid
- In order to obtain the number of sperm in 1 mm^2 , the result is multiplied by 5, which total 1000

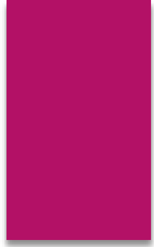
Example

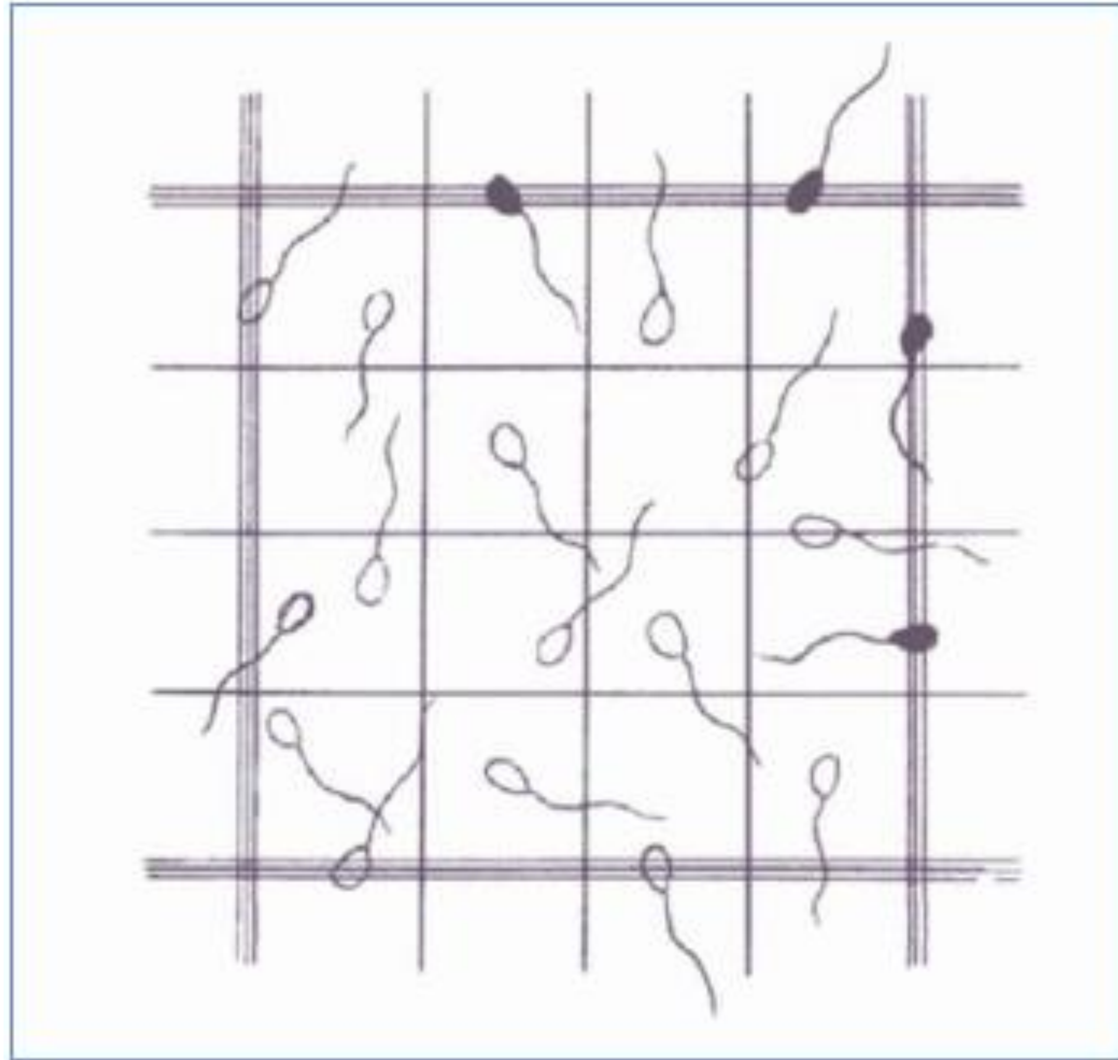
- When corrected for chamber depth, there are 1000 sperm in 0.1 mm3 or 0.0001 mL. Therefore, 1000 × 10,000 sperm are present in 1 mL
- Multiplying this number by the dilution factor (100), reveals that the concentration in the original sample is 1×10^9 sperm/mL

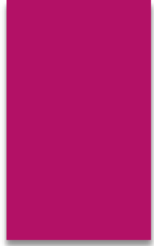
Suggested dilution rates for sperm concentration evaluation using the improved Neubauer hemocytometer

Dilution rate	Tube size	Diluent volume	Semen volume
1:200	5-mL culture	4.975 mL	25 µL
1:100	5-mL culture	2.475 mL	25 µL
1:50	5-mL culture	2.45 mL	50 µL
1:25	1.5-mL microcentrifuge	1.2 mL	50 µL
1:10	1.5-mL microcentrifuge	0.45 mL	50 µL


Counting
Area



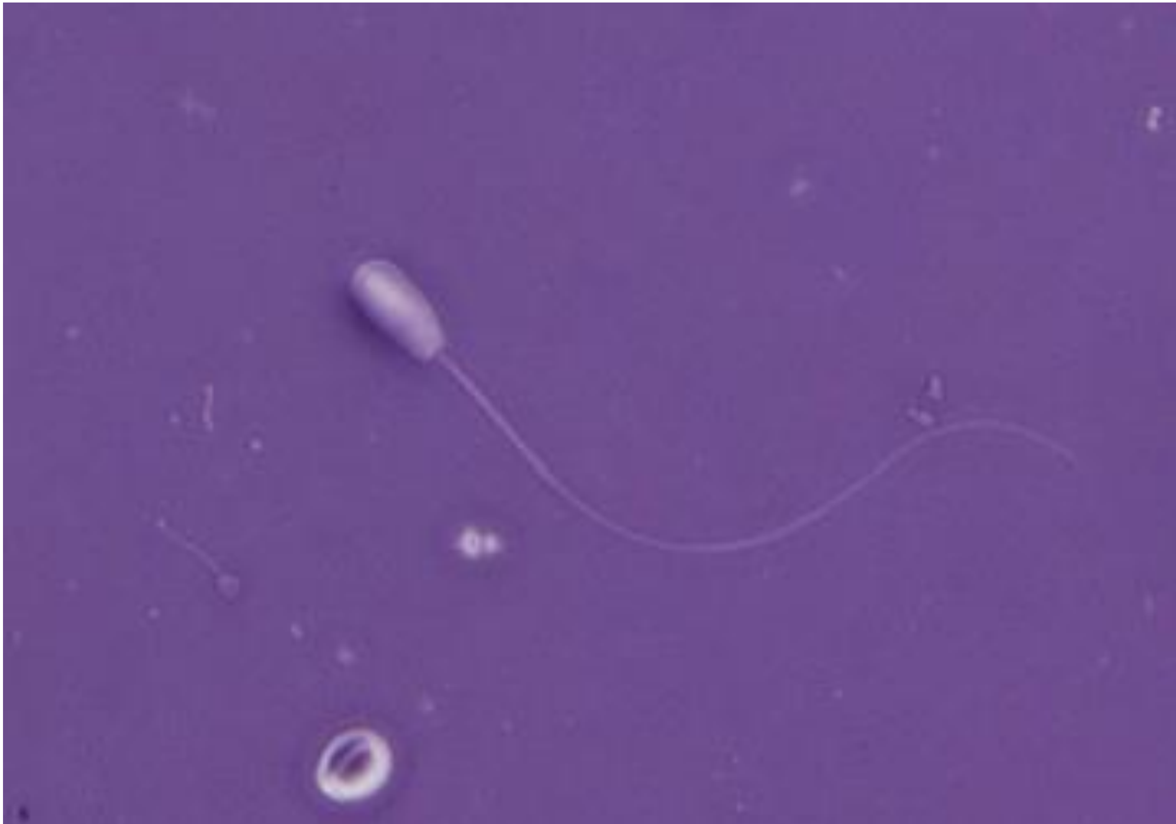


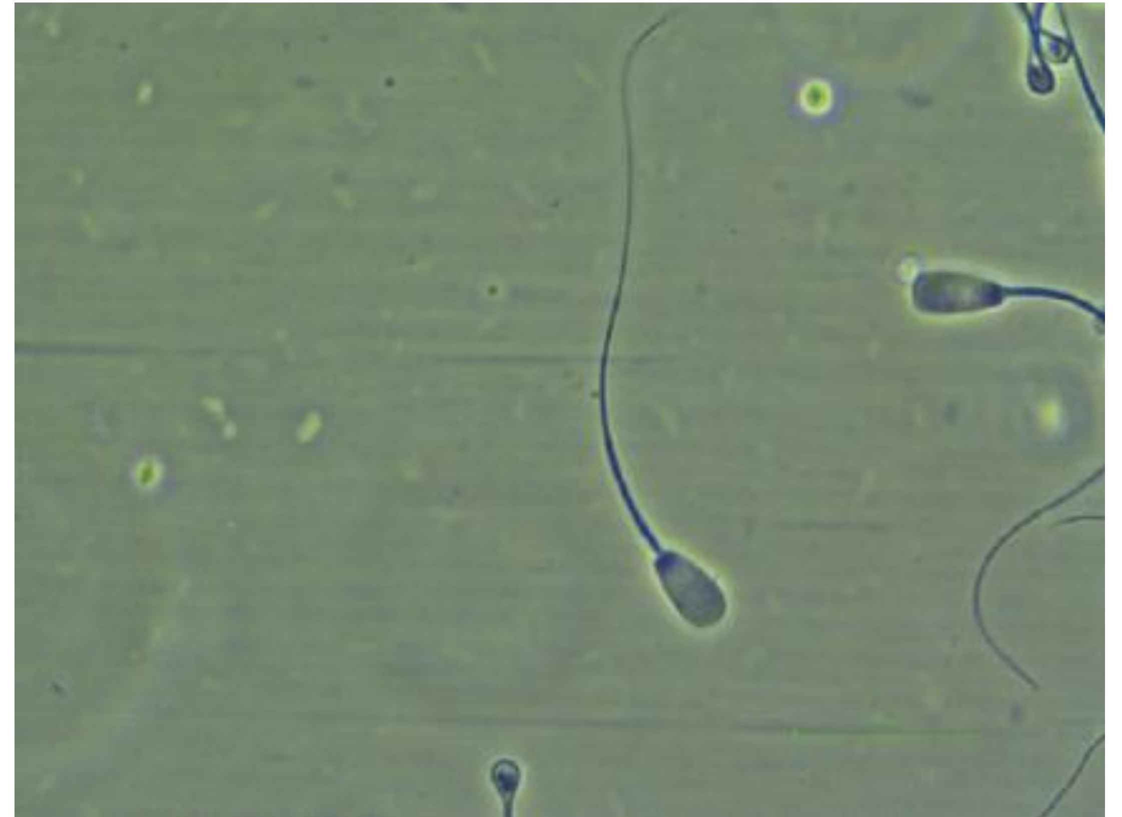


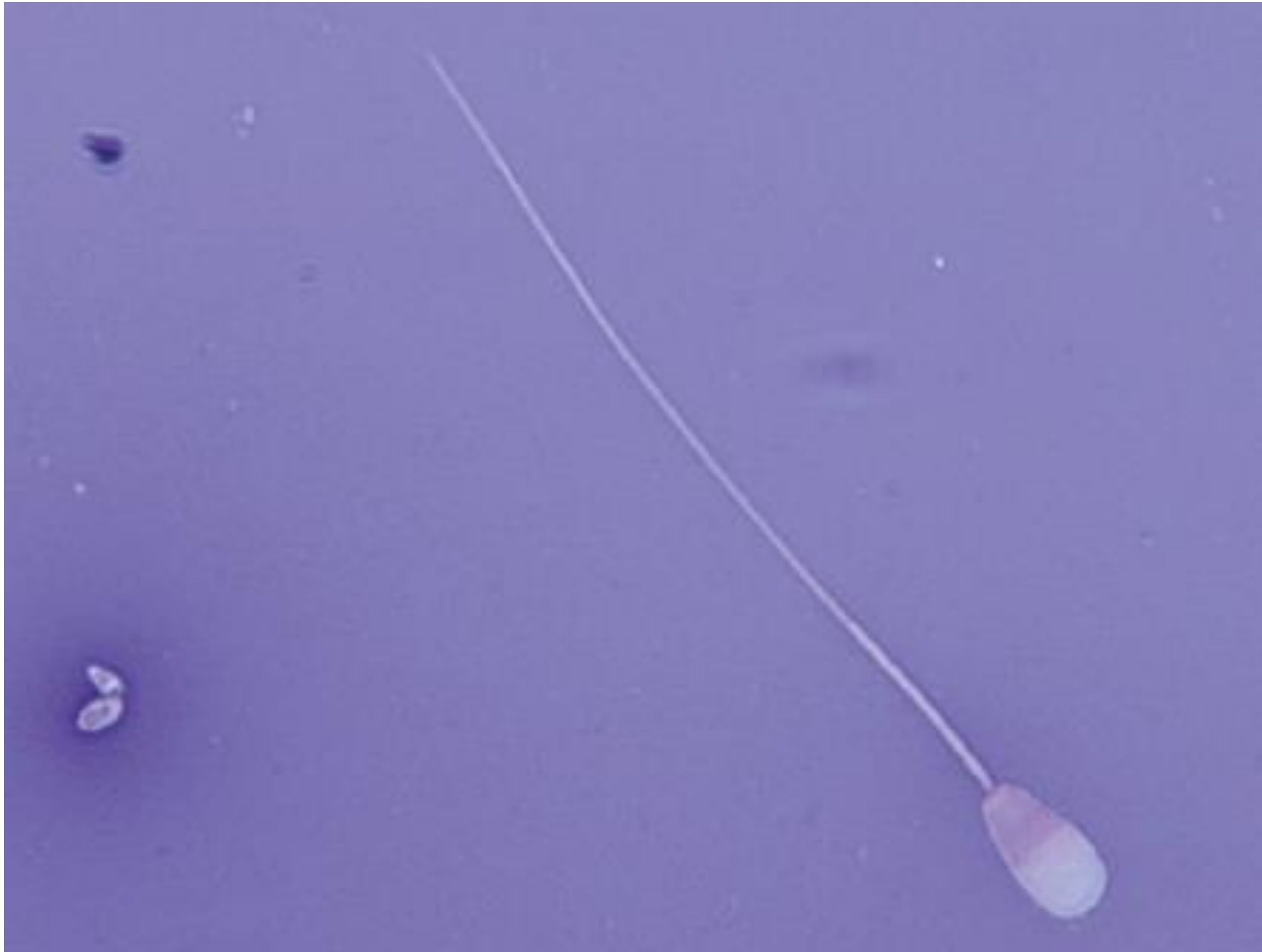
COMPUTER ASSISTED MOTILITY ANALYSIS

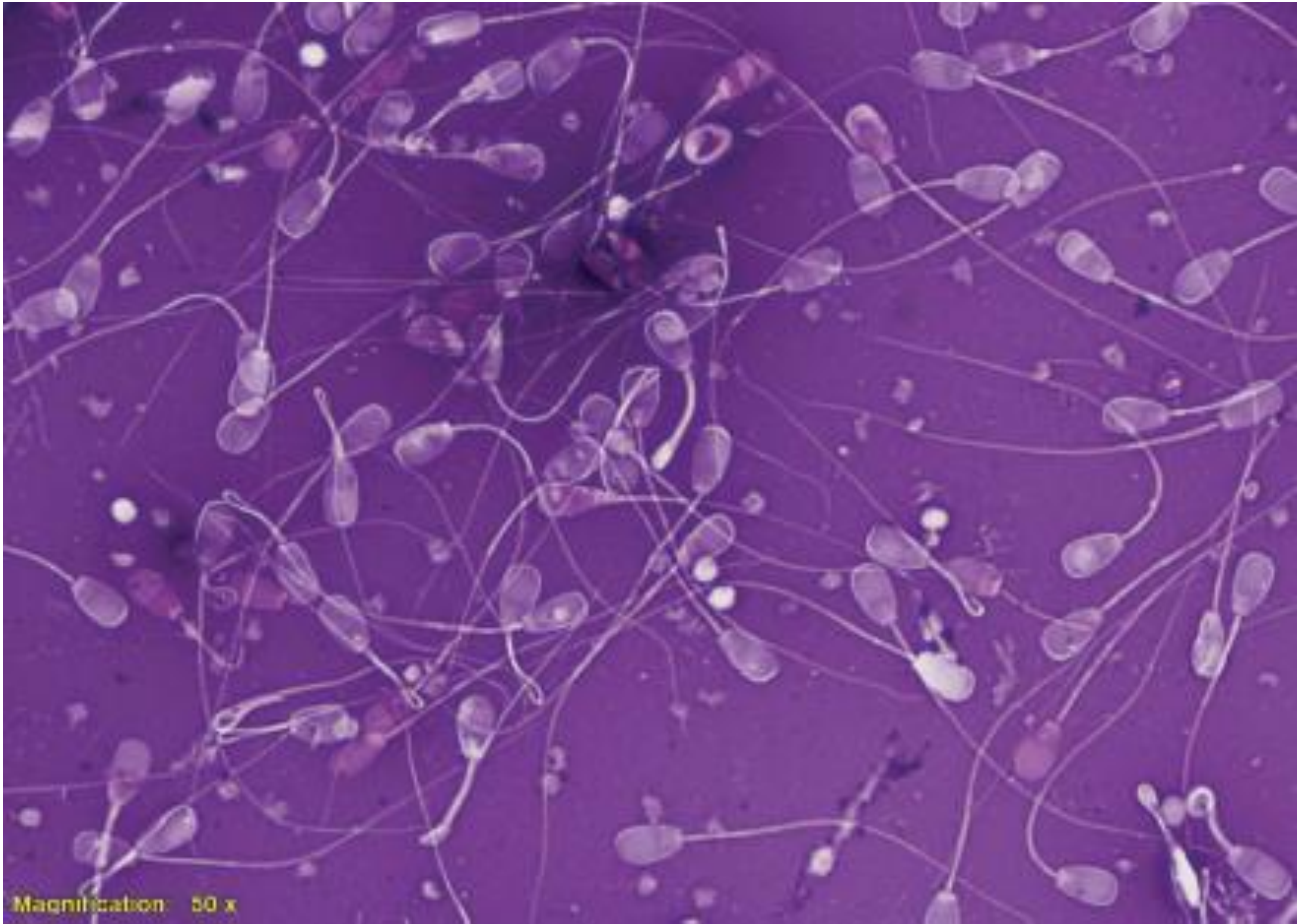
- Can generate detailed information on motion parameters of a semen sample including :
  - Curvilinear velocity
  - Average path velocity
  - Straight-line velocity
  - Linearity
  - Motile sperm
  - Progressively motile sperm
  - Lateral head displacement
  - Total count
  - Concentration

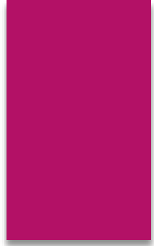
MORPHOLOGY

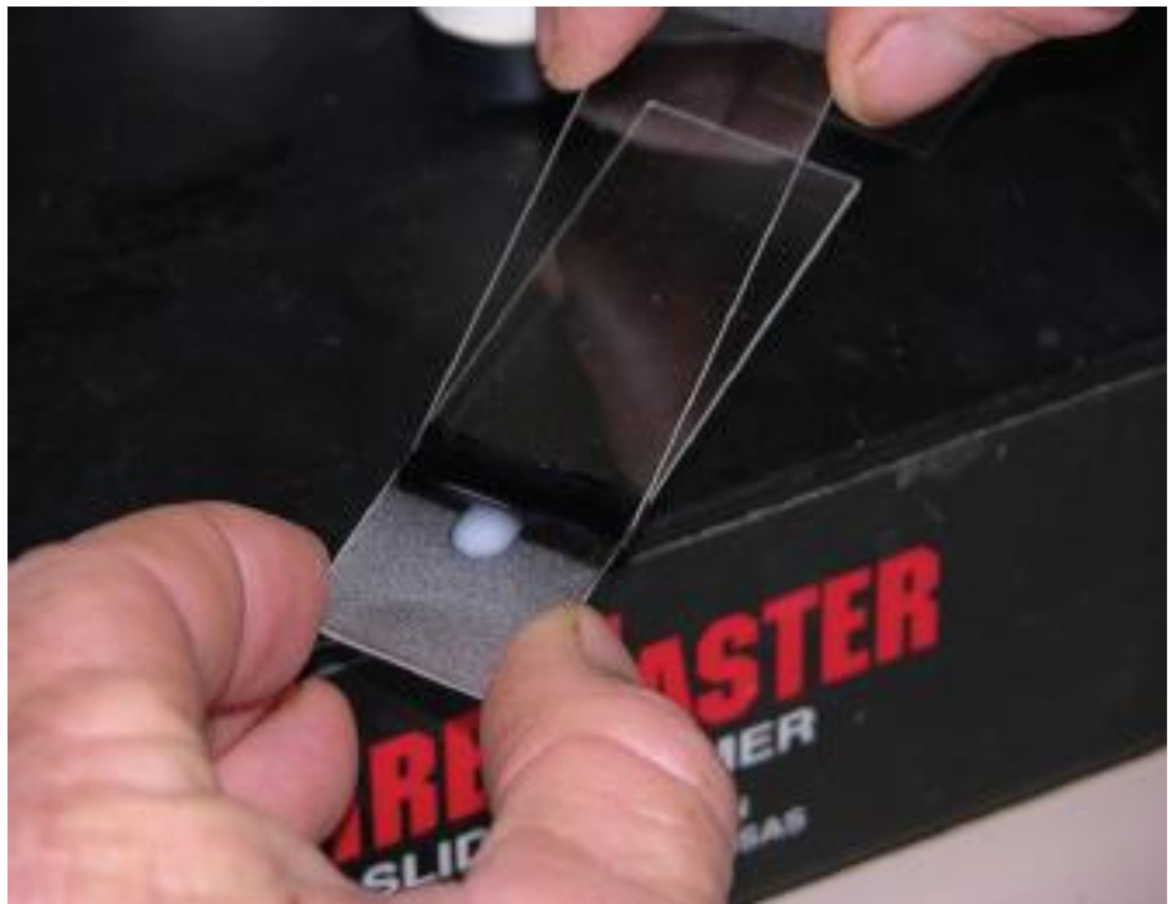


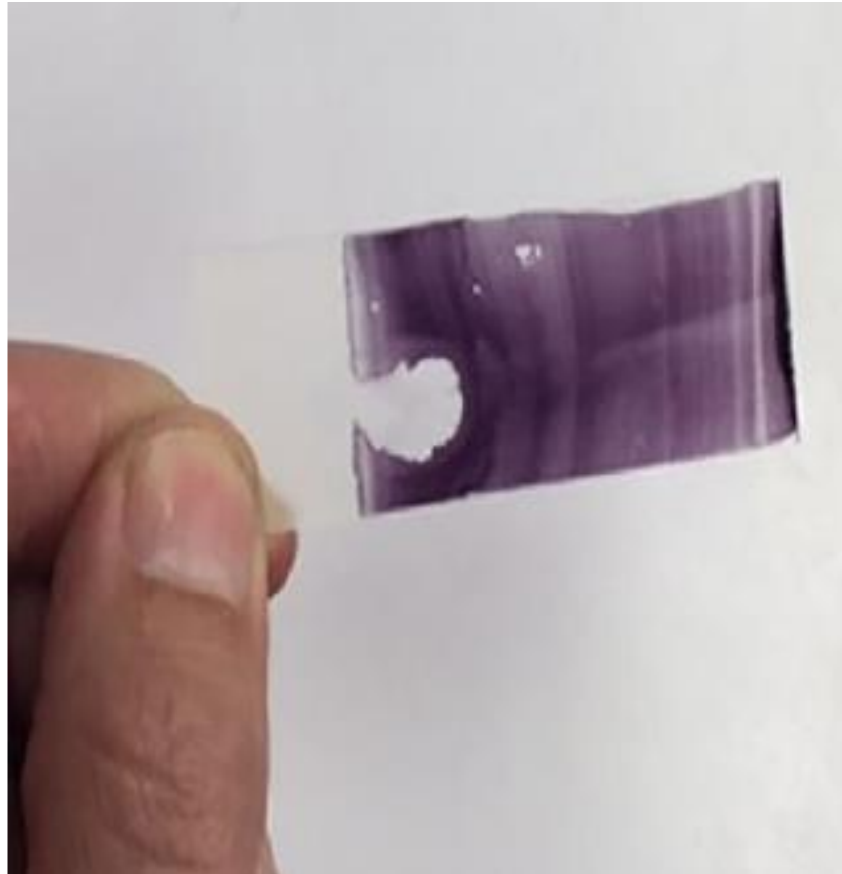





Magnification 50 x

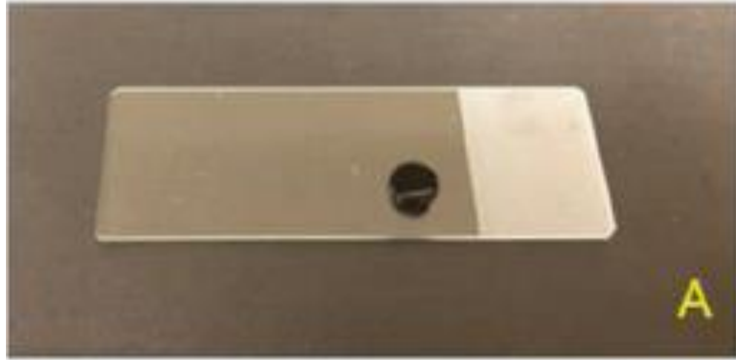
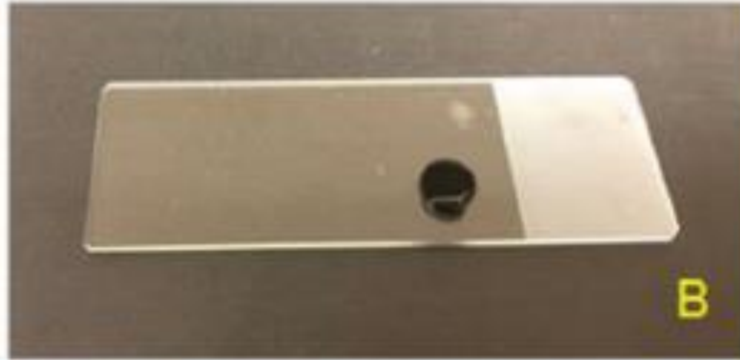
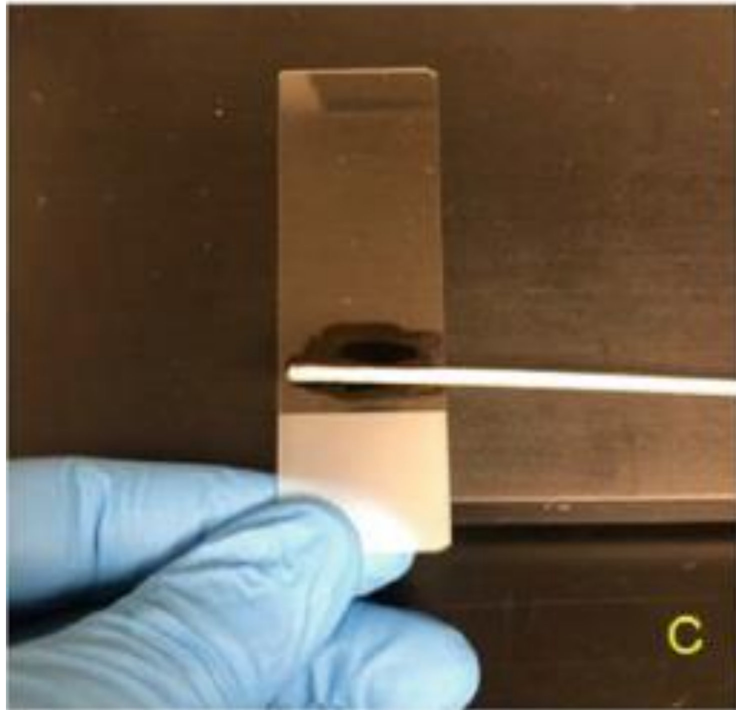
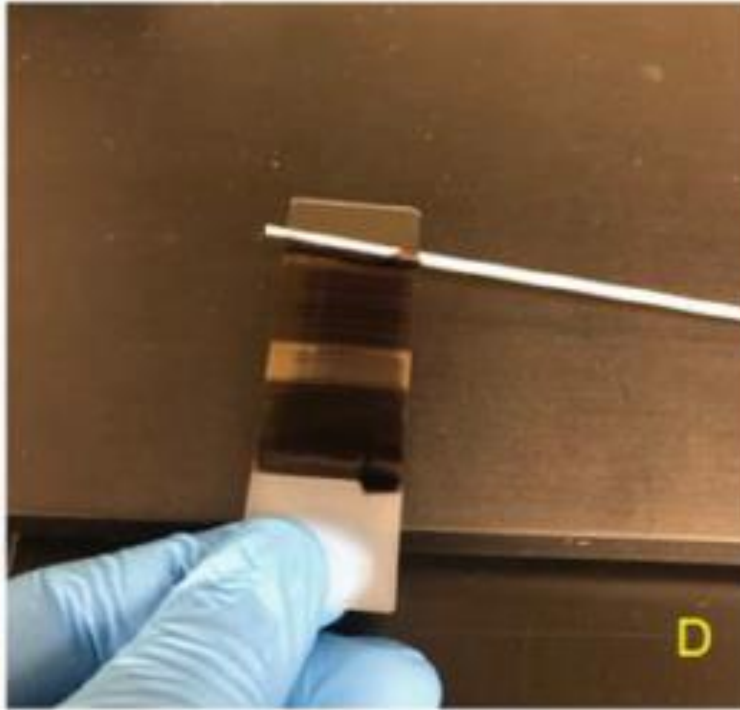






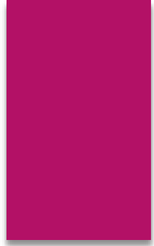
Slides should neither be too dark or too light, and when viewed under the microscope, the cells should be spaced in such a manner that they can each be individually examined

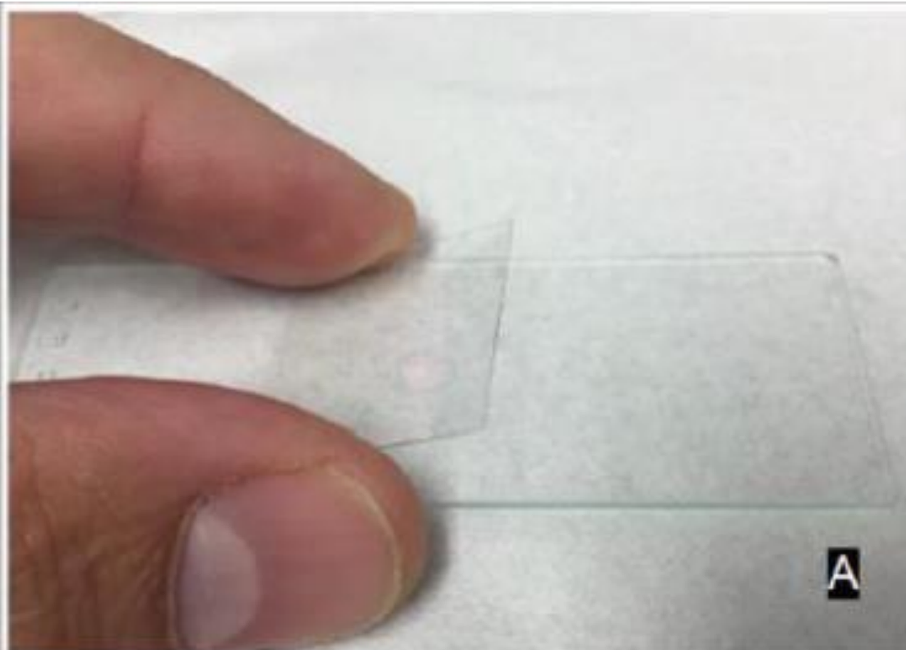
SPERMAC
STAIN
SPERMAC
STAIN
SPERMAC
STAIN
SPERMAC
STAIN

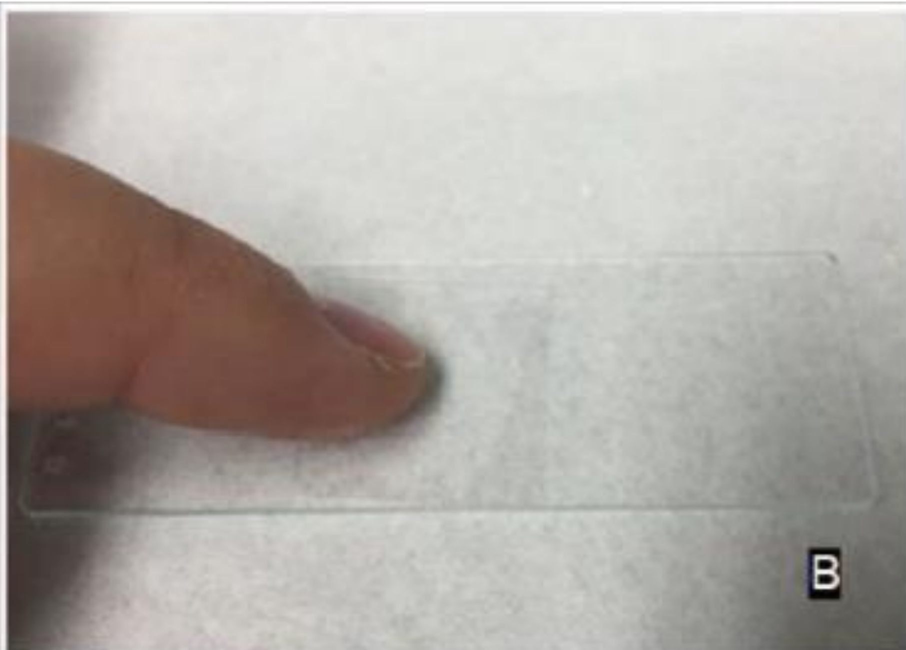

A

B

C

D

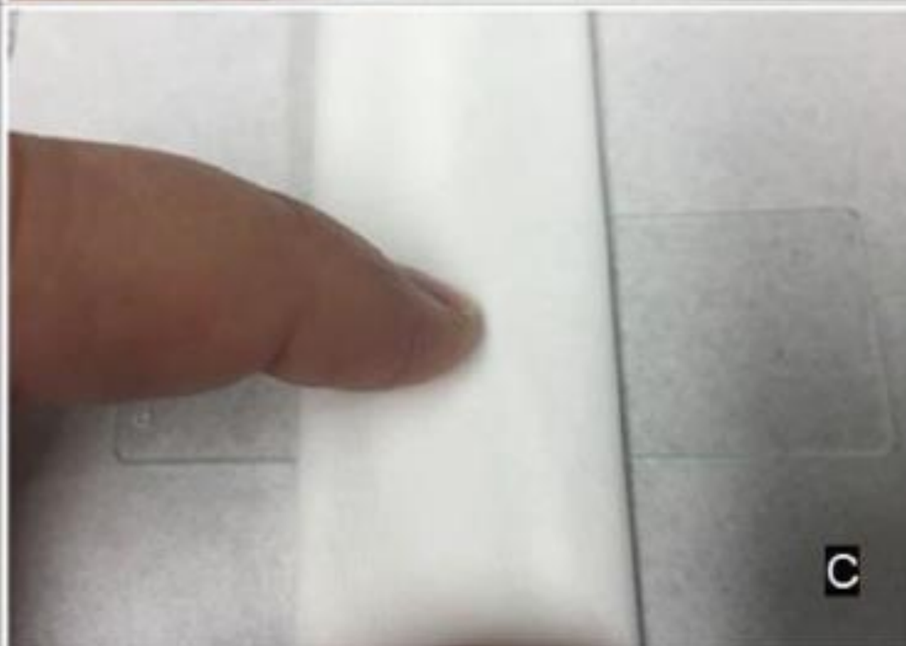
E

To prepare a stained slide, a drop of stain is placed on one side of a clean slide kept on a warming stage (A). Then, a slightly smaller drop of semen is placed close to the stain (B). The slide can be lifted from the warming stage with one hand and a wood applicator used to immediately mix the semen with stain (C). Using the same applicator, the mixture is spread down the length of the slide with a stopping and starting motion to create thick and thin areas (D). This will result in areas with more or less sperm and different stain intensity, giving the evaluator options to select areas with appropriate sperm density and stain contrast (E). Compared to other stains, eosin-nigrosin semen slides are easy and quick to prepare. Stained smears can be prepared for evaluation at a later time and can also be stored for reference

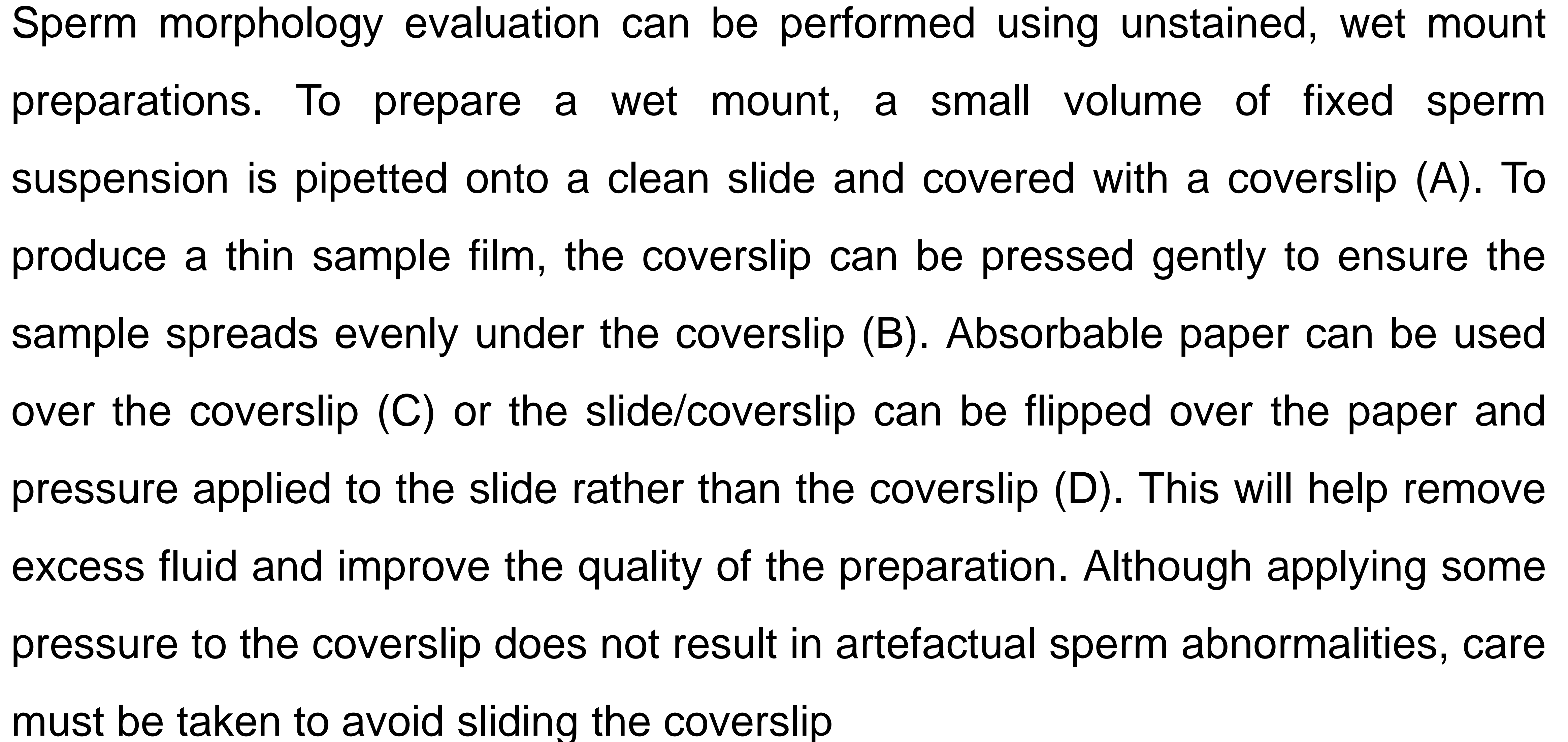



A

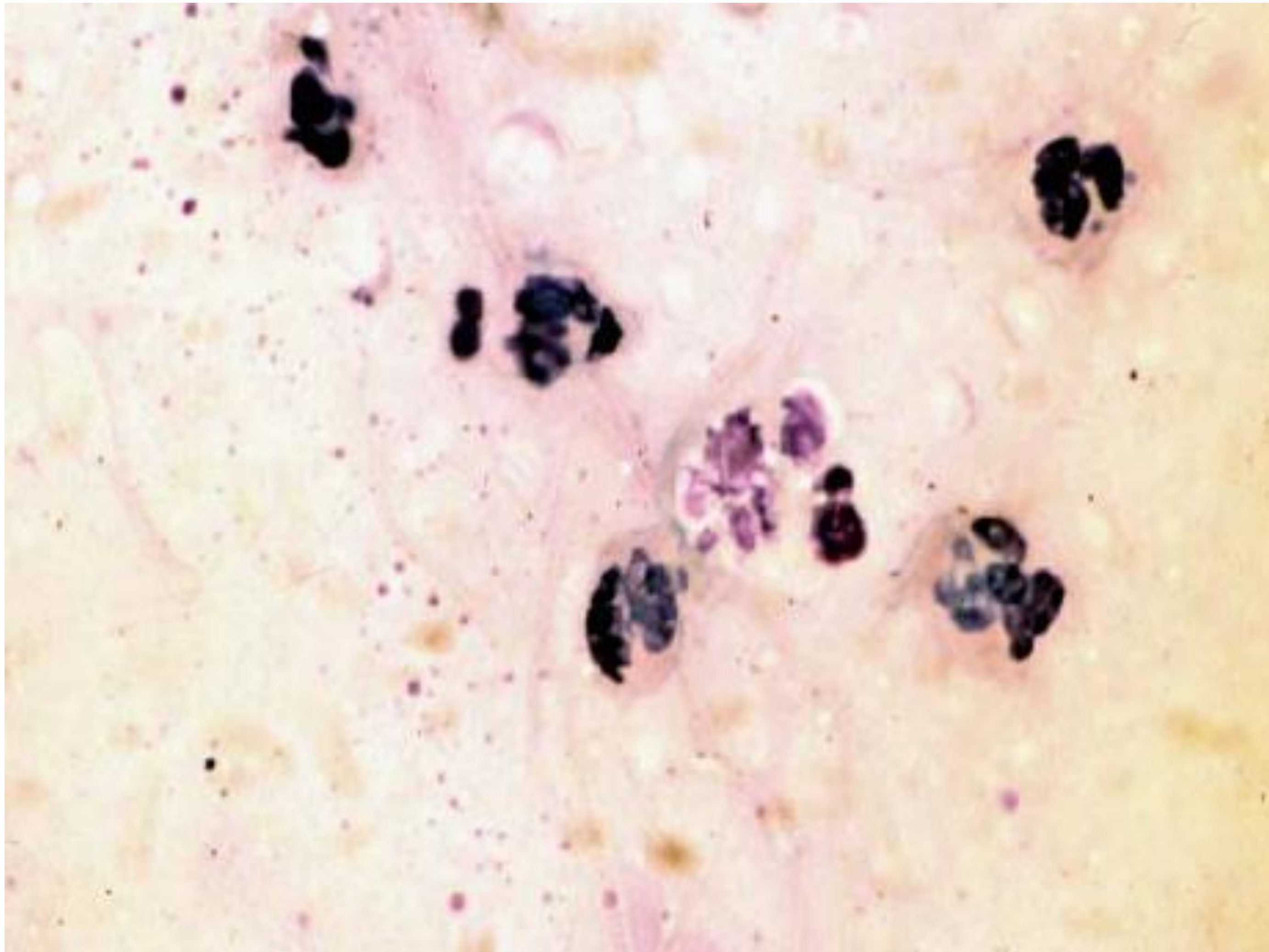

B


C

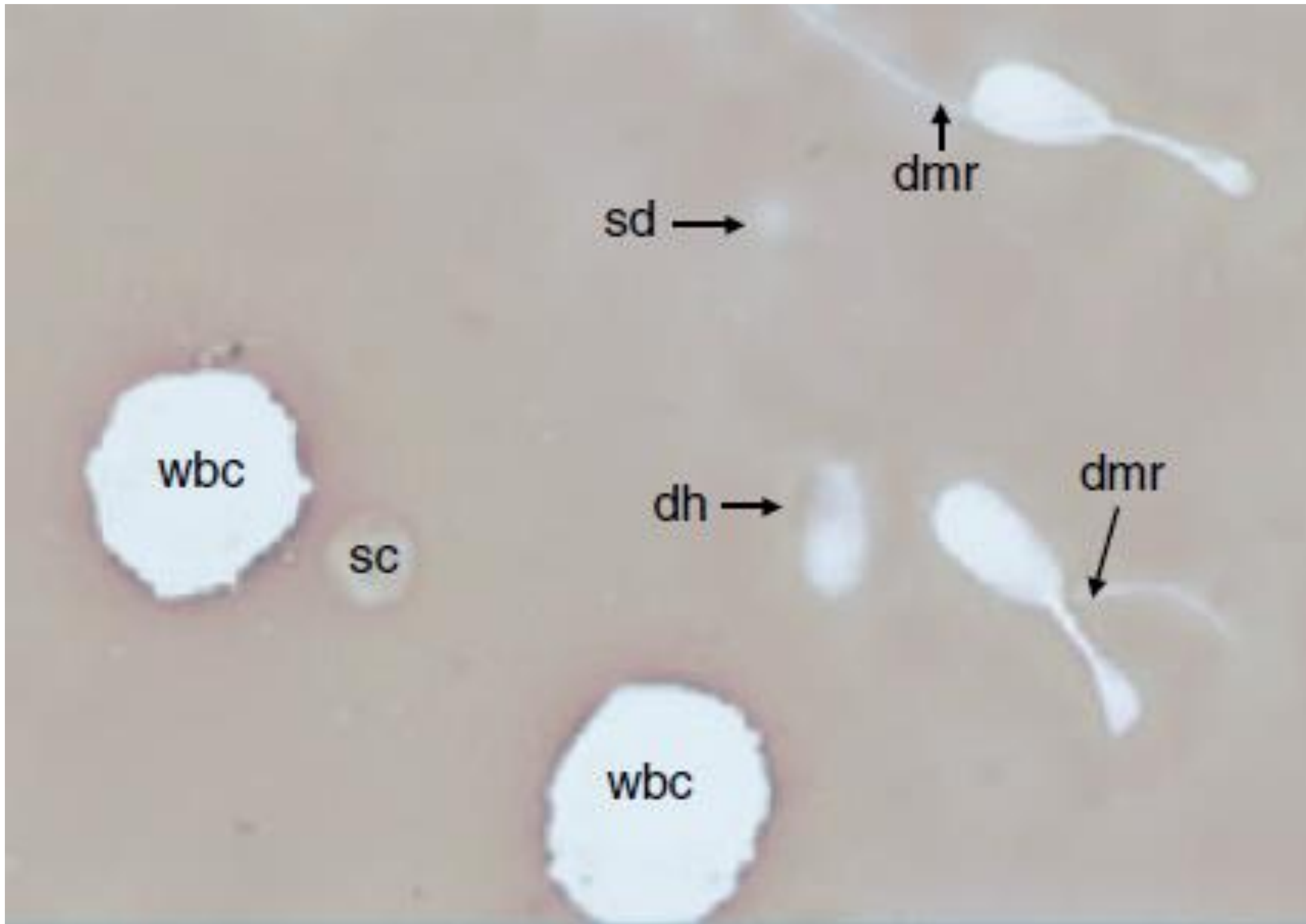
D



Sperm morphology evaluation can be performed using unstained, wet mount preparations. To prepare a wet mount, a small volume of fixed sperm suspension is pipetted onto a clean slide and covered with a coverslip (A). To produce a thin sample film, the coverslip can be pressed gently to ensure the sample spreads evenly under the coverslip (B). Absorbable paper can be used over the coverslip (C) or the slide/coverslip can be flipped over the paper and pressure applied to the slide rather than the coverslip (D). This will help remove excess fluid and improve the quality of the preparation. Although applying some pressure to the coverslip does not result in artefactual sperm abnormalities, care must be taken to avoid sliding the coverslip



White blood cells (neutrophils) on a Diff Quik stained smear. Note the faintly stained sperm.



White blood cells (wbc), a speroid cell (sc), detached head (dh), a shed droplet (sd), and distal midpiece reflexes (dmr) on an eosin-nigrosin stained smear

Cell counter with keys labeled for sperm cell morphology

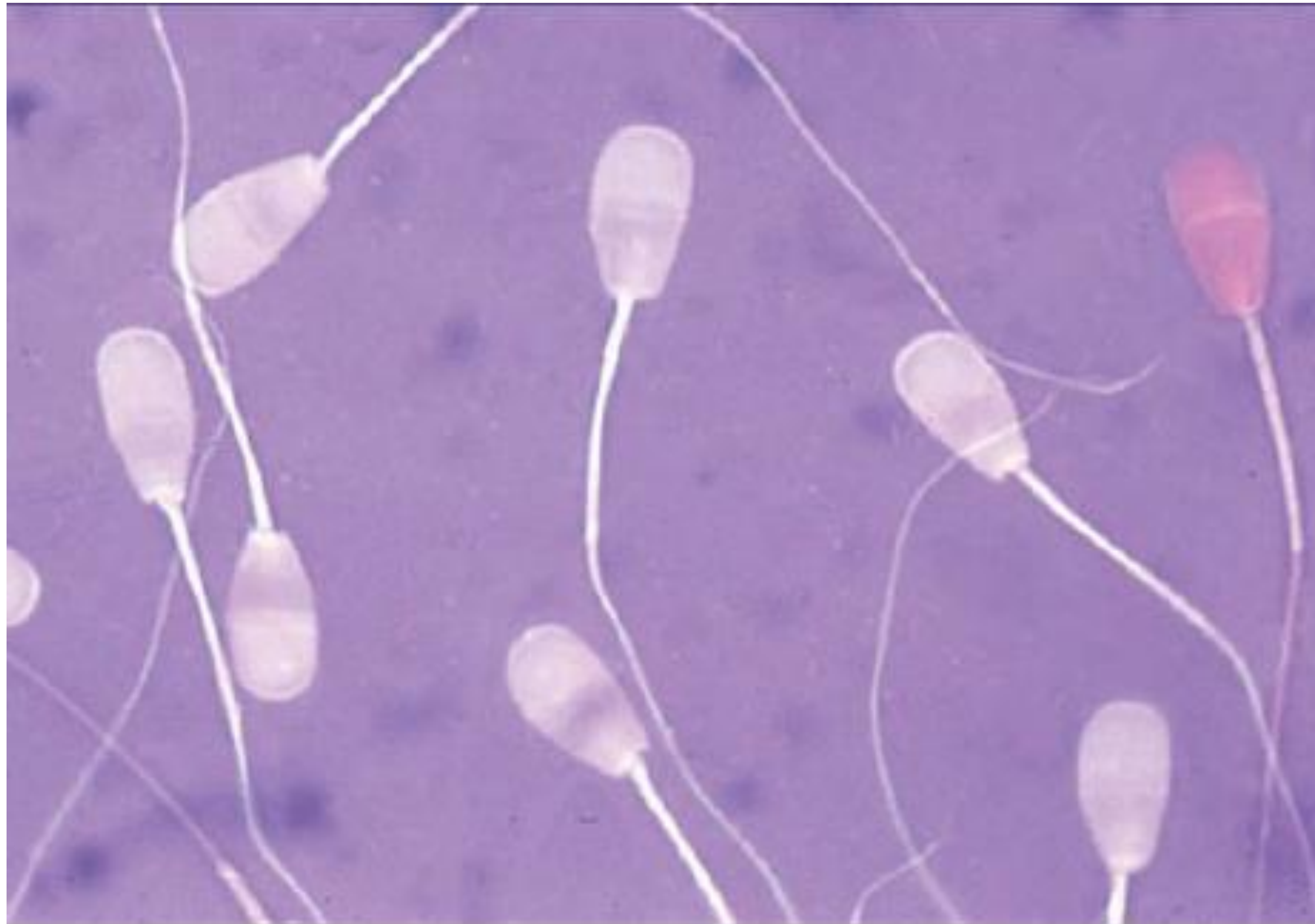
Sperm abnormality classification system

Head defects	Midpiece defects	Tail defects
Acrosome abnormality	Proximal cytoplasmic droplet	Tail stump defect
Pyriform head	Pseudodroplet	Coiled principal piece
Tapered head	Mitochondrial sheath defect	Double forms
Nuclear vacuolation	Dag defect	Accessory tail
Macrocephalic	Distal midpiece reflex	Bent principal piece
Microcephalic	Corkscrew sperm defect	Short tail defect
Detached head		

Koziol JH, Armstrong CL (2018) Manual for breeding soundness examination of bulls. Society for Theriogenology, Montgomery sperm concentration. Theriogenology 85: 1507–1527

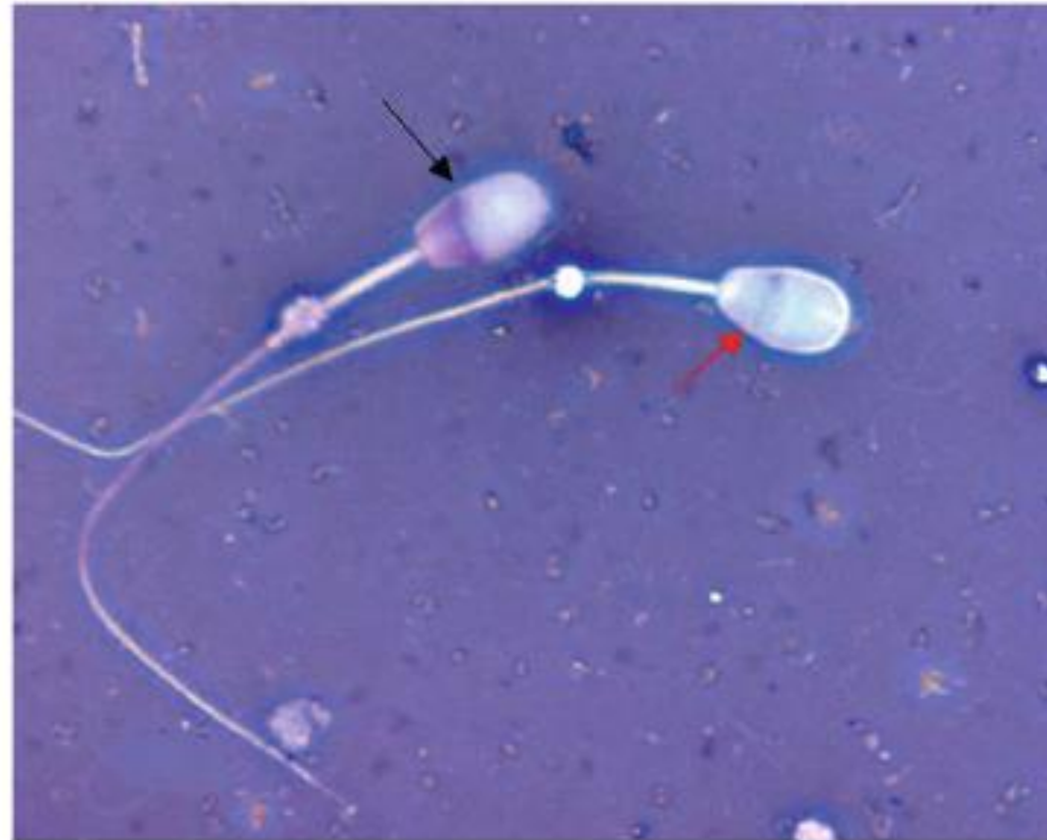
SPERM VIABILITY

- 5 µL of sperm diluted in D-PBS is mixed with 10 Ml of eosin-nigrosin stain in a slide at 37 °C, and after 20 s a smear is taken
- The smear is dried on a plate at 37 °C and the percentage of unstained spermatozoa is evaluated by counting 200 spermatozoa in a brightfield under a microscope at 400 ×



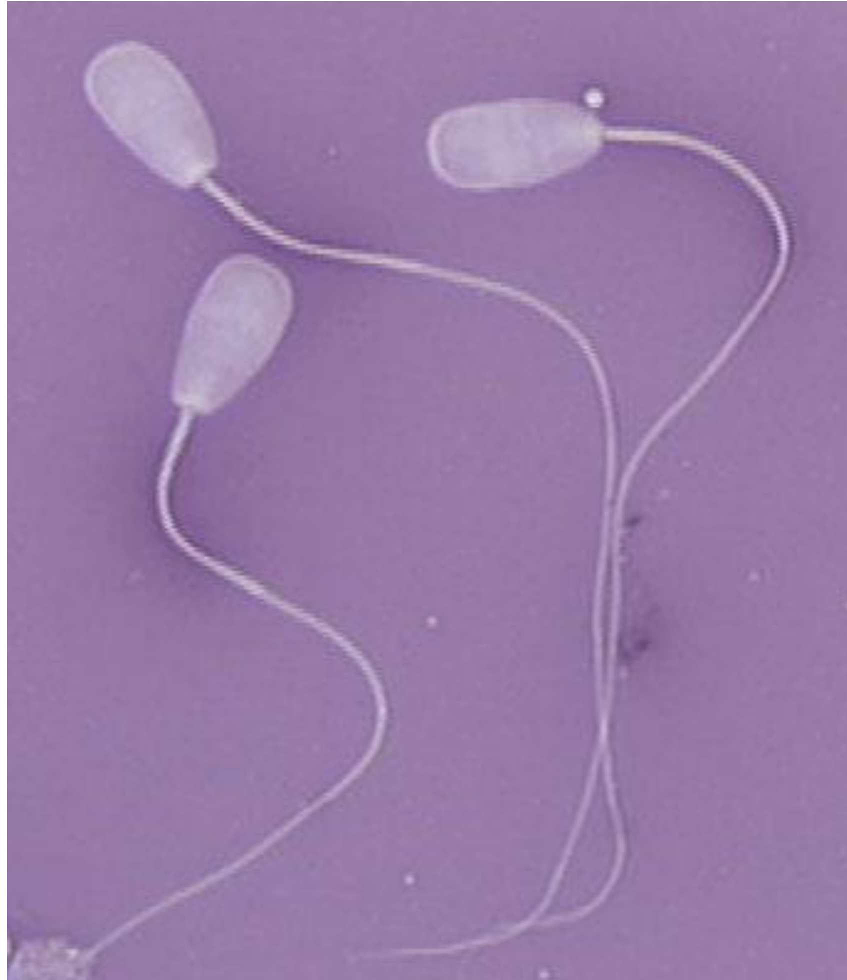
Normal sperm stained with eosin-nigrosin. The dark staining sperm is dead.

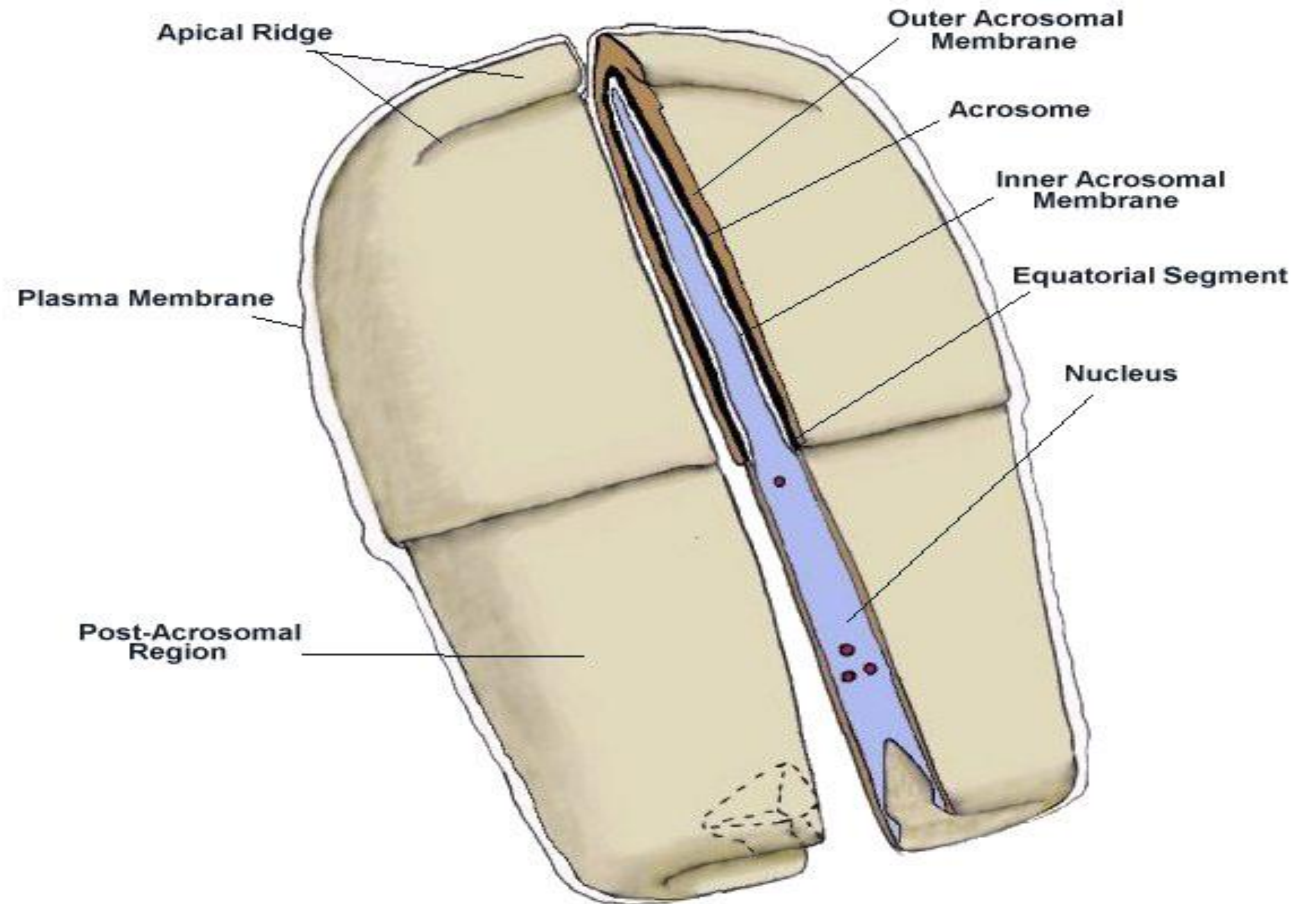
SPERM VIABILITY



Red arrow: live spermatozoa
Black arrow: dead spermatozoa

Spermatozoïde bovin





Listing of recognized sperm defects

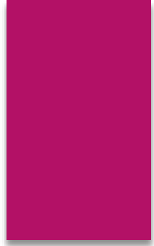
1) Pyriform heads
2) Tapered heads
3) Microcephalic/macrocephalic heads
4) Vacuoles – diadem, single vacuoles, confluent vacuoles
5) Clumped DNA
6) Detached (loose) heads – normal, abnormal
7) Decapitated defect
8) Rolled-crested-giant head syndrome
9) Teratoids
10) Knobbed acrosome – beaded, flattened
11) Ruffled and detached acrosomes
12) Distal midpiece reflex
13) Mitochondrial sheath defects
14) Dag defect
15) Stump tail defect
16) Corkscrew defect
17) Pseudodroplet defect
18) Coiled principal piece
19) Proximal cytoplasmic droplets

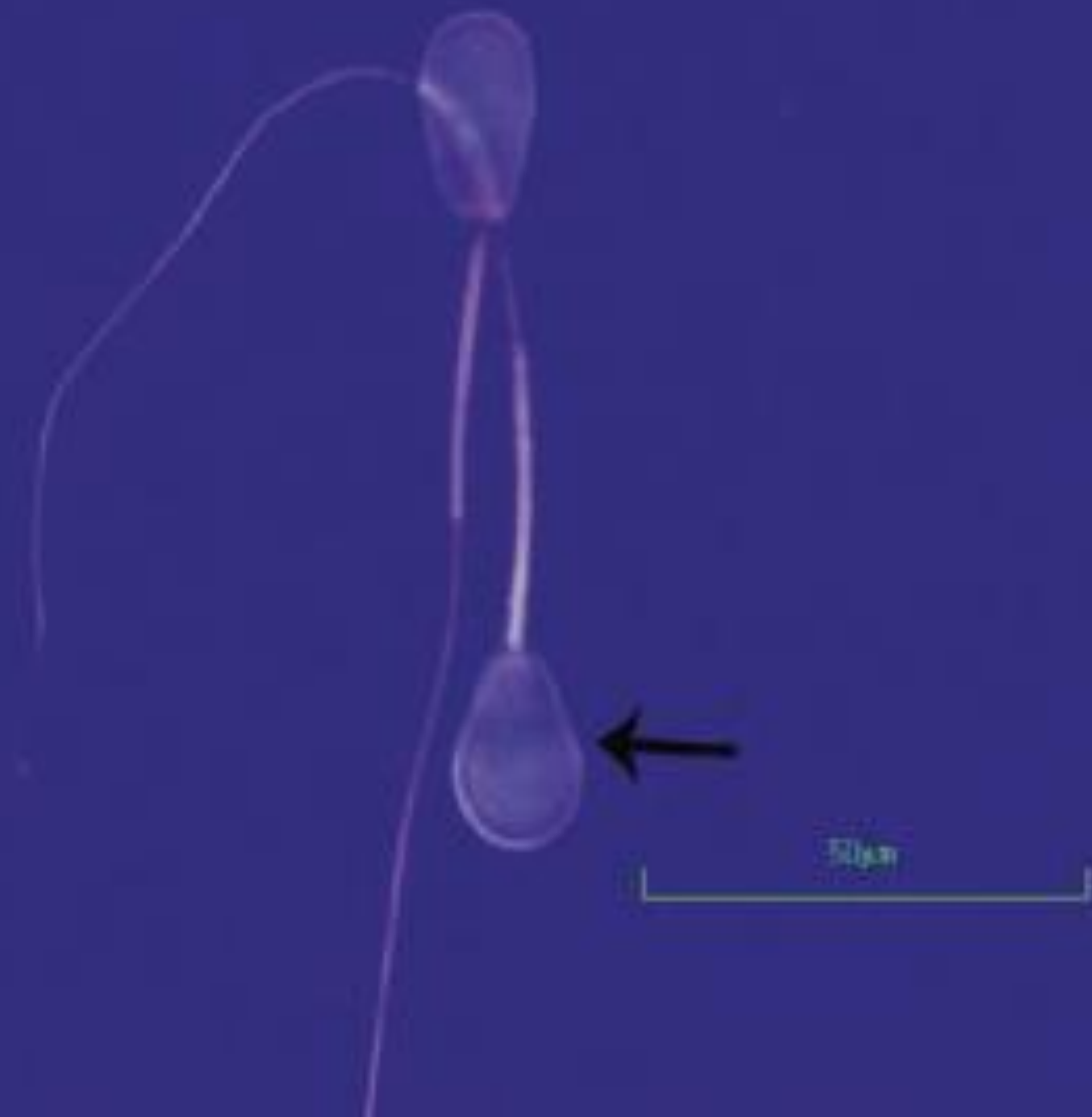
Head abnormalities

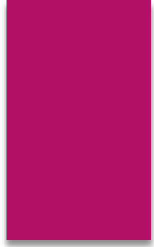
- The prevalence of **pyriform head defects** are relatively high and are either **the most or second most prevalent defect in an ejaculate**
- The classical pyriform sperm has **a pear- shaped head**, a **normal acrosome**, and a **narrow post- acrosomal region**

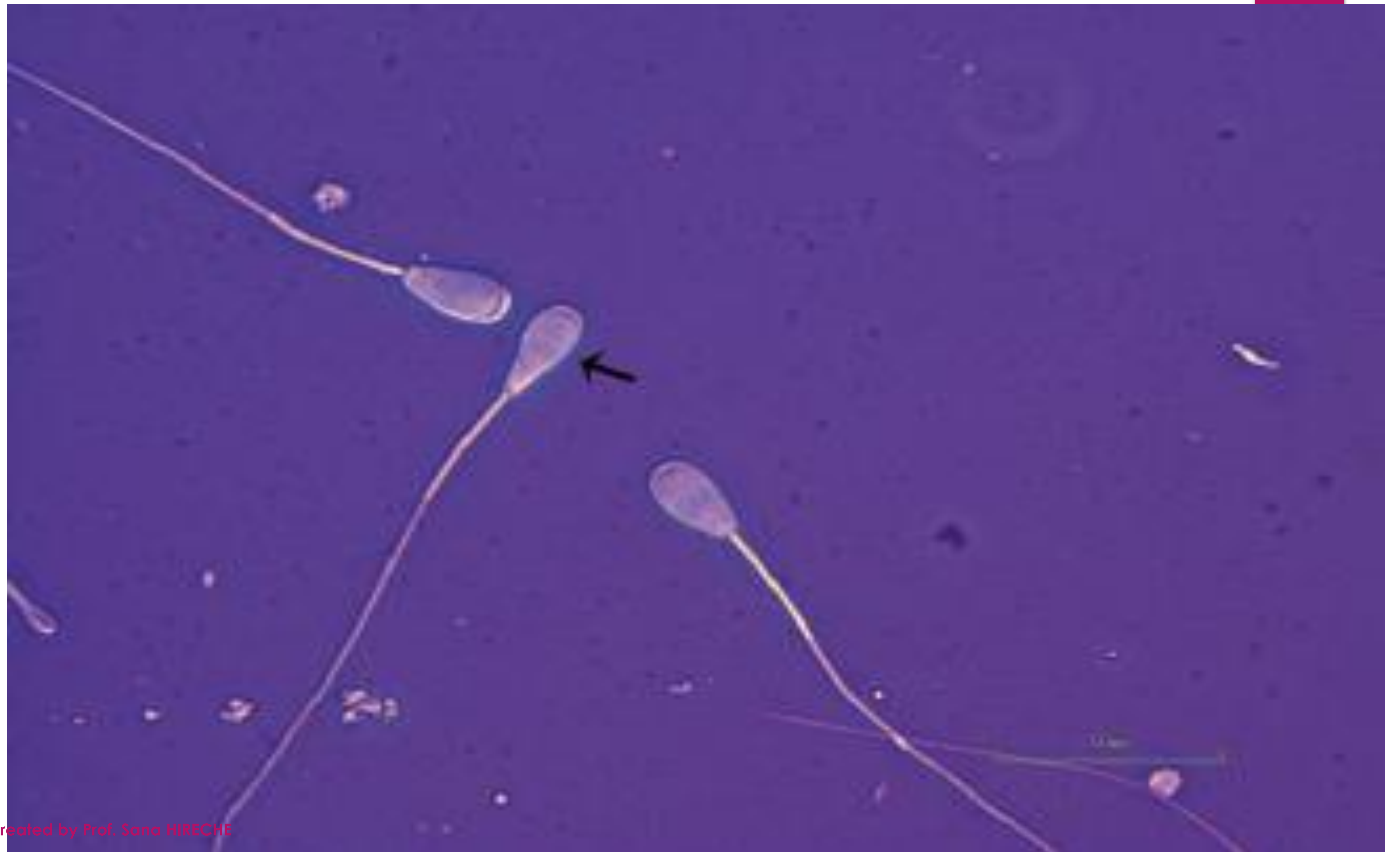
Head abnormalities

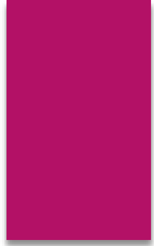
- Sperm with irregularity in head shape and size generally have good motility







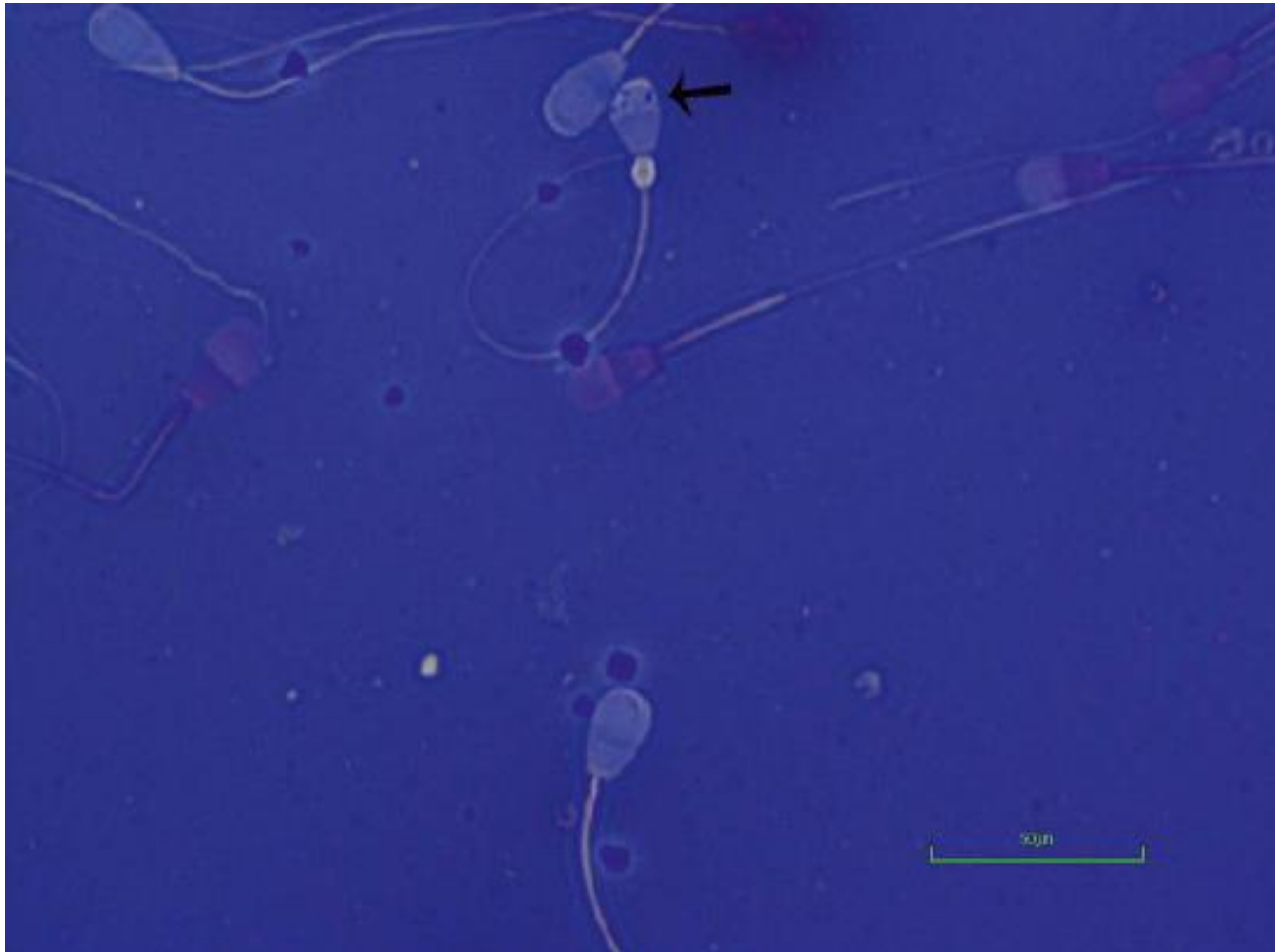




Created by Prof. Sana HIRECHE

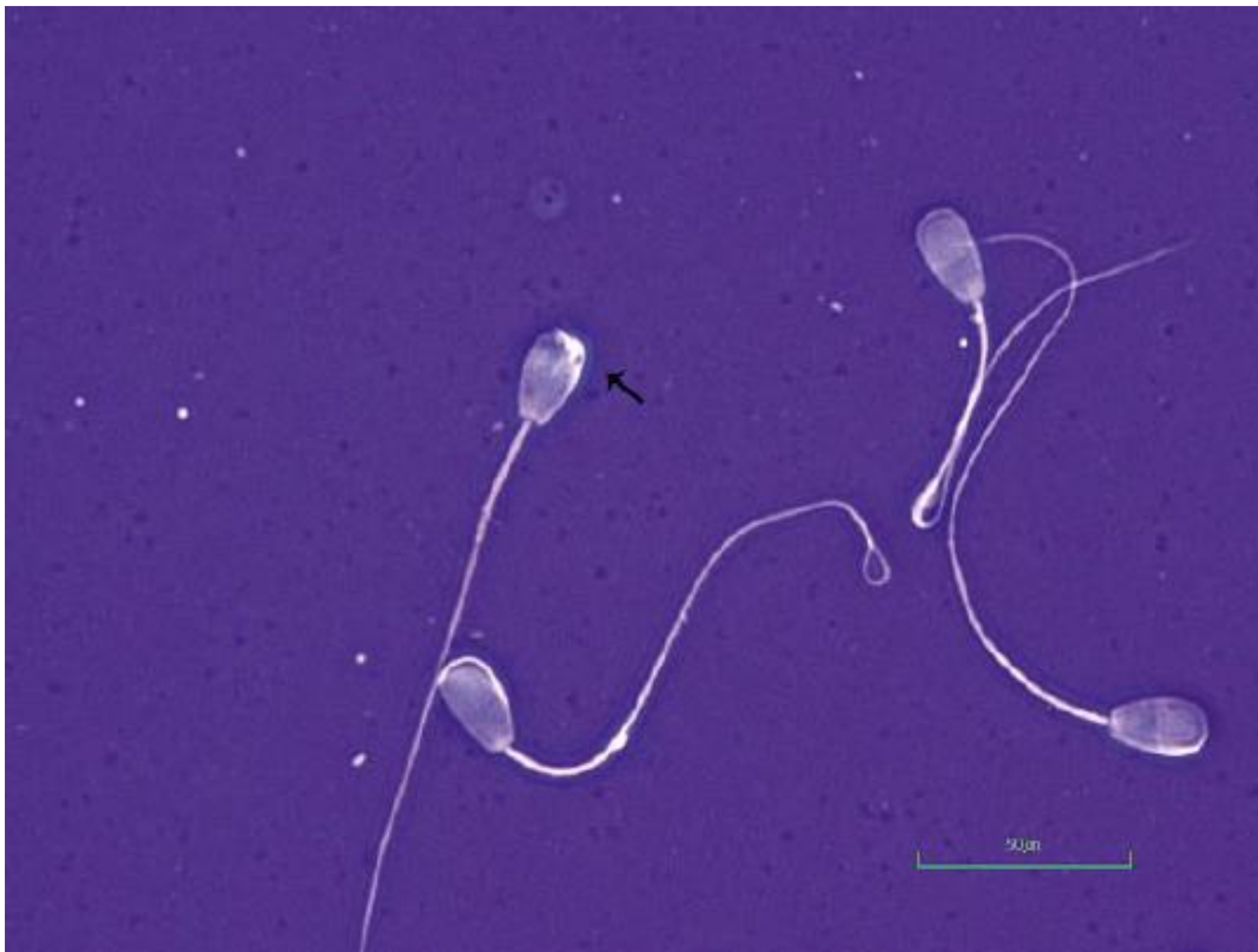
Pyriform head in bull as indicated by arrow also note the proximal droplet and terminally coiled tail

Created by Prof. Sana HIRECHE

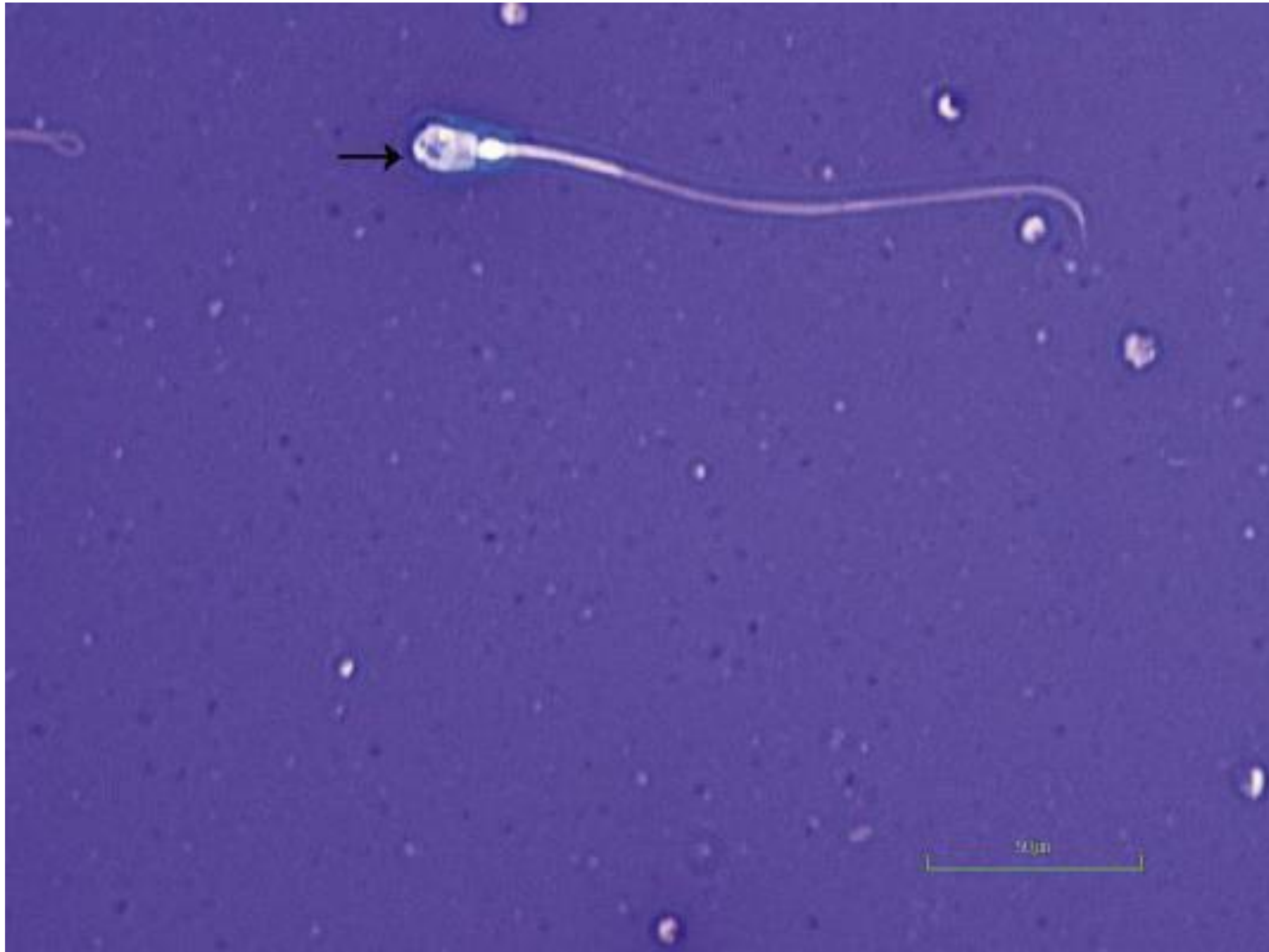


Nuclear vacuoles as indicated with arrow also note the proximal droplet (eosin–nigrosin, 1000×)

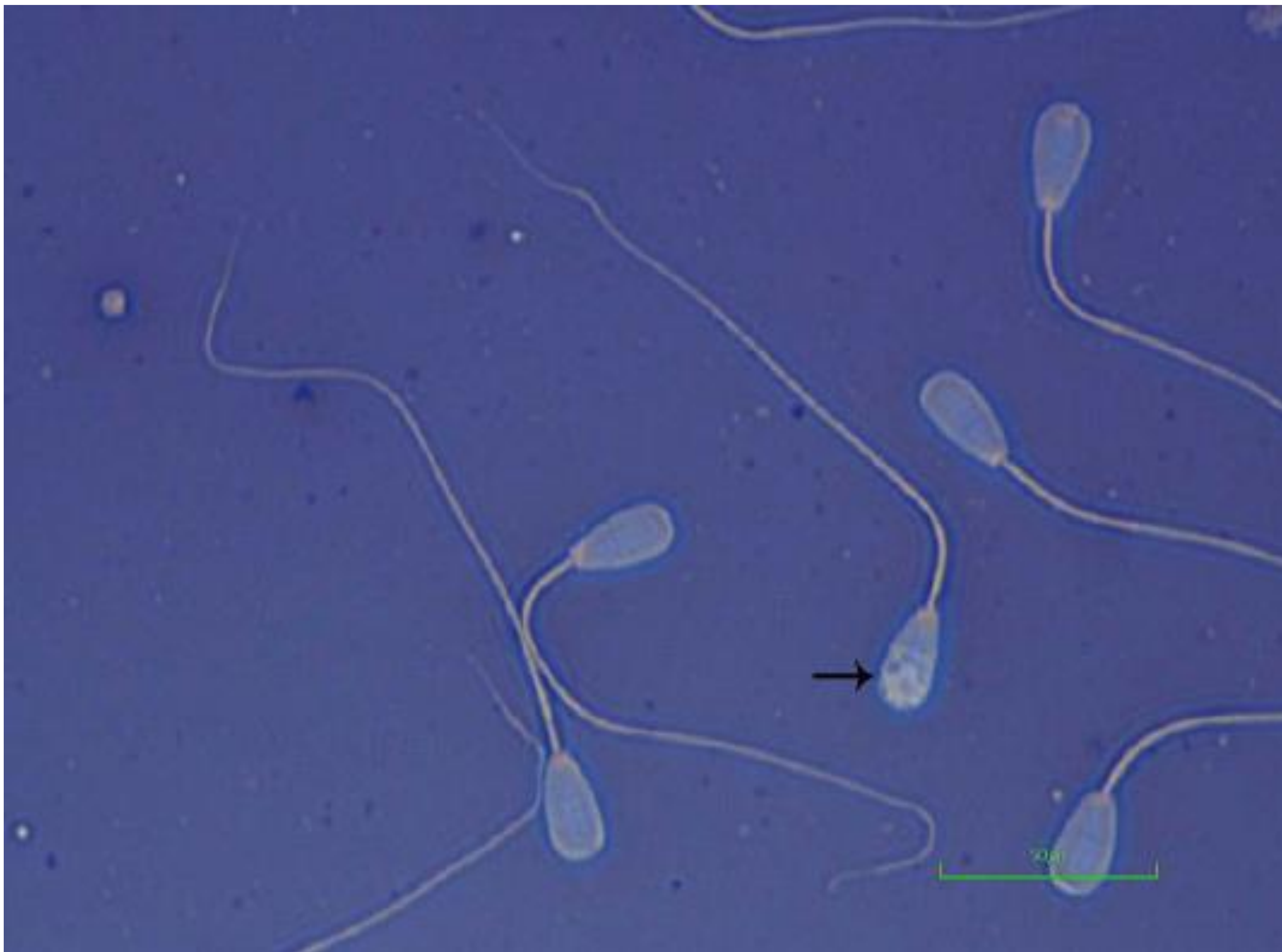
Created by Prof. Sana HIRECHE



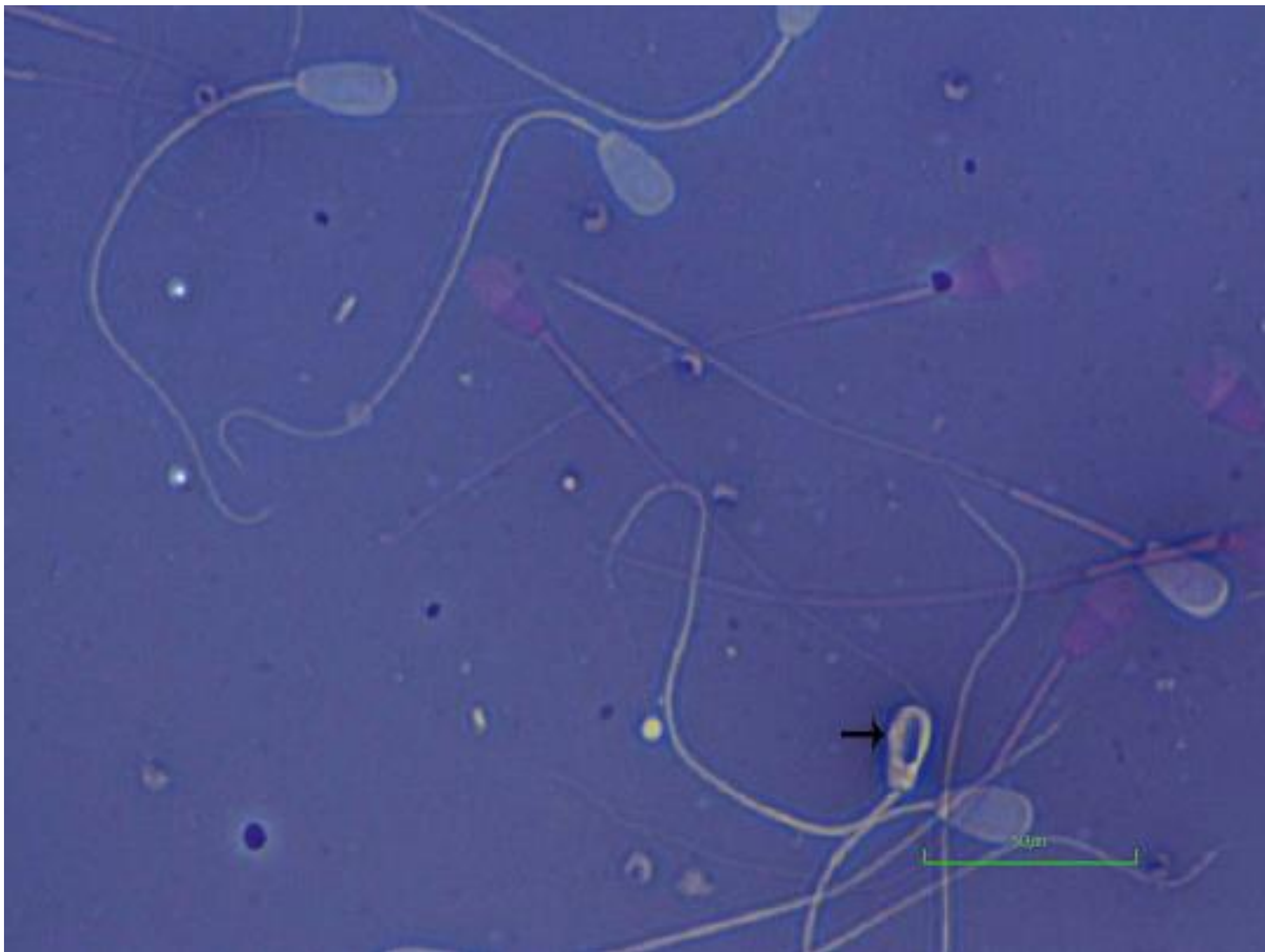
Small vacuoles in head of sperm as indicated with arrow (eosin–nigrosin, 1000×).



Nuclear vacuoles in a bull also note the proximal droplet (eosin–nigrosin, bull, 1000×).

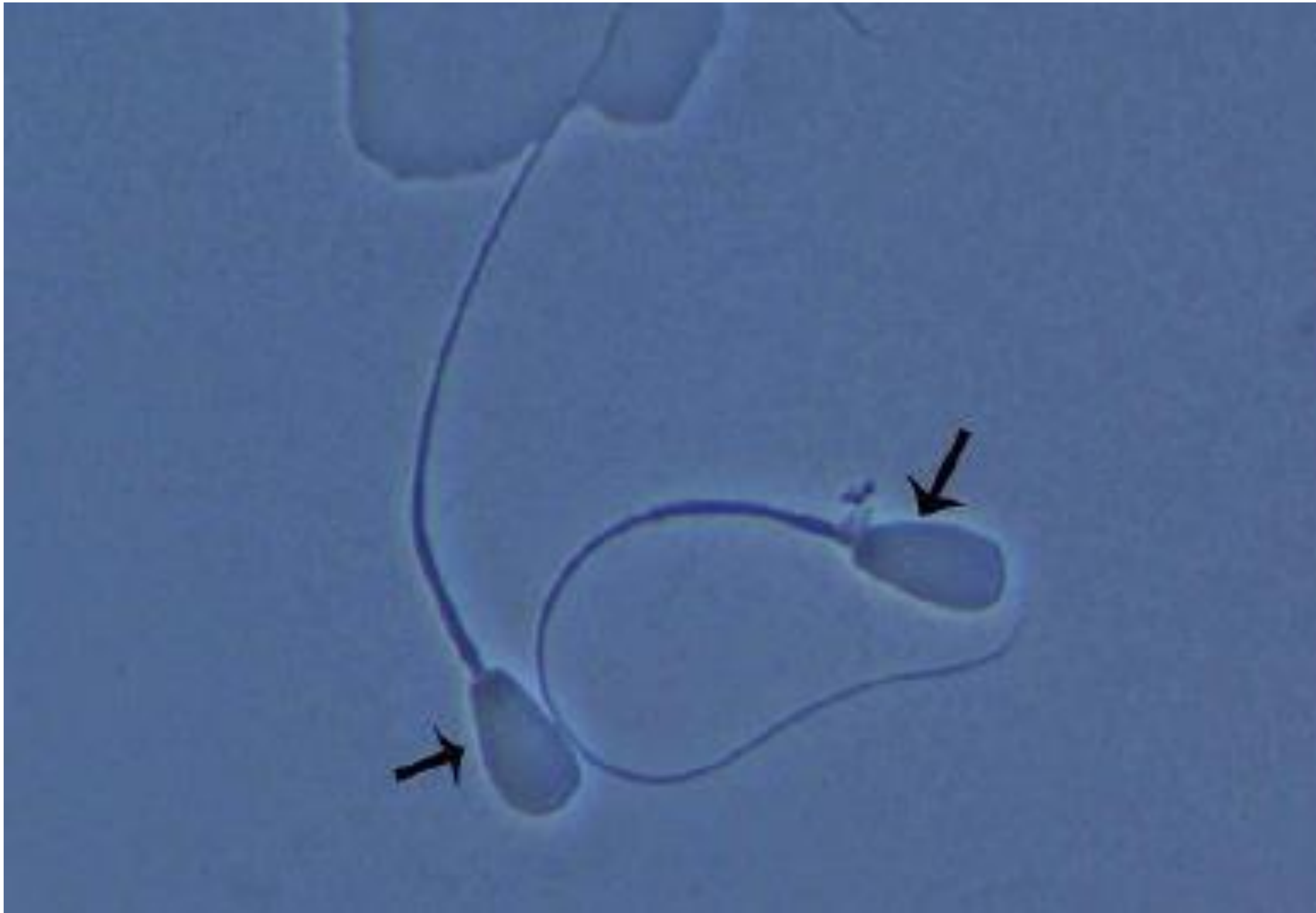


Multiple small nuclear vacuoles in the head of a sperm from a bull (eosin–nigrosin, 1000×).

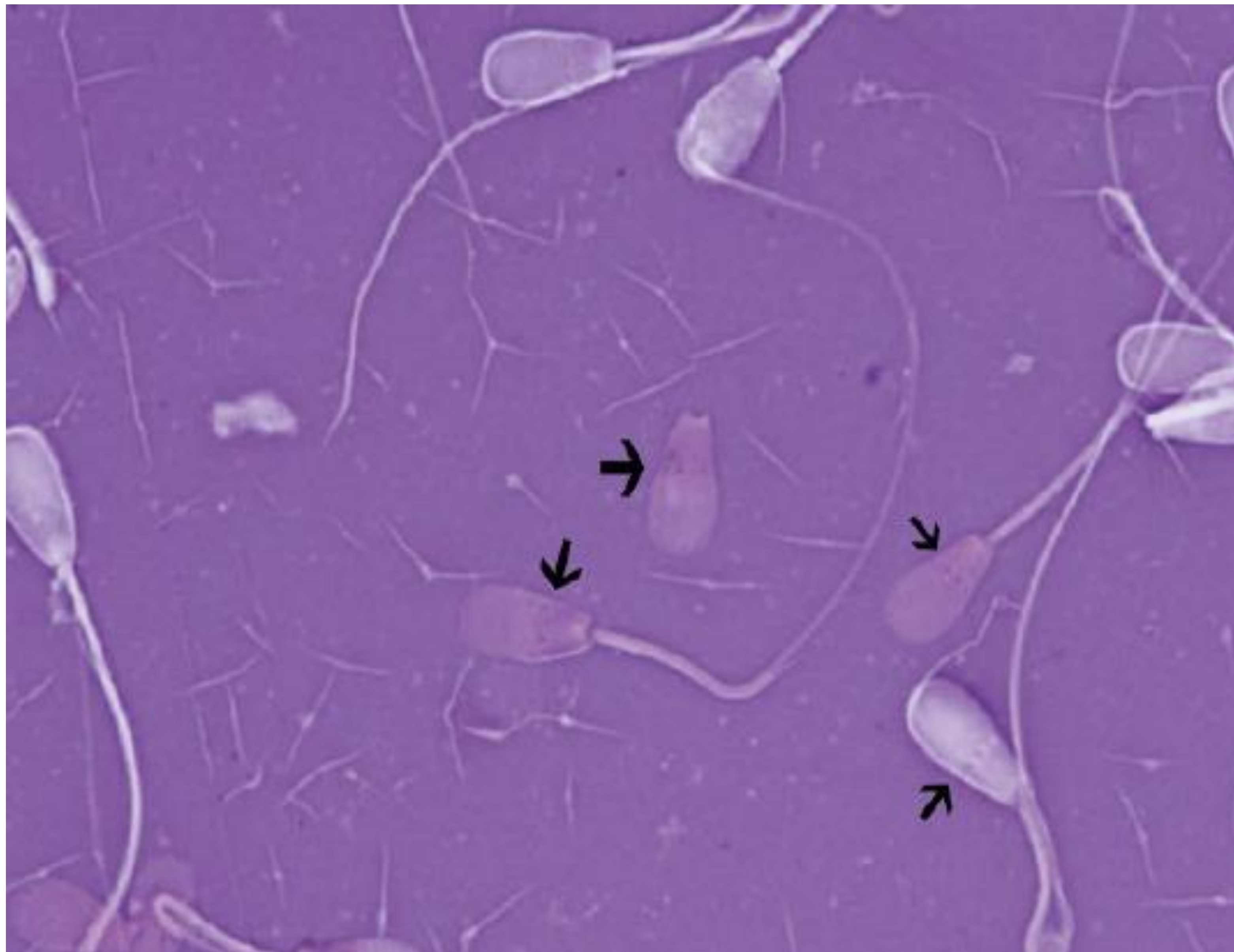


Large confluent nuclear vacuole from a bull (eosin–nigrosin, 1000×).

Created by Prof. Sana HIRECHE

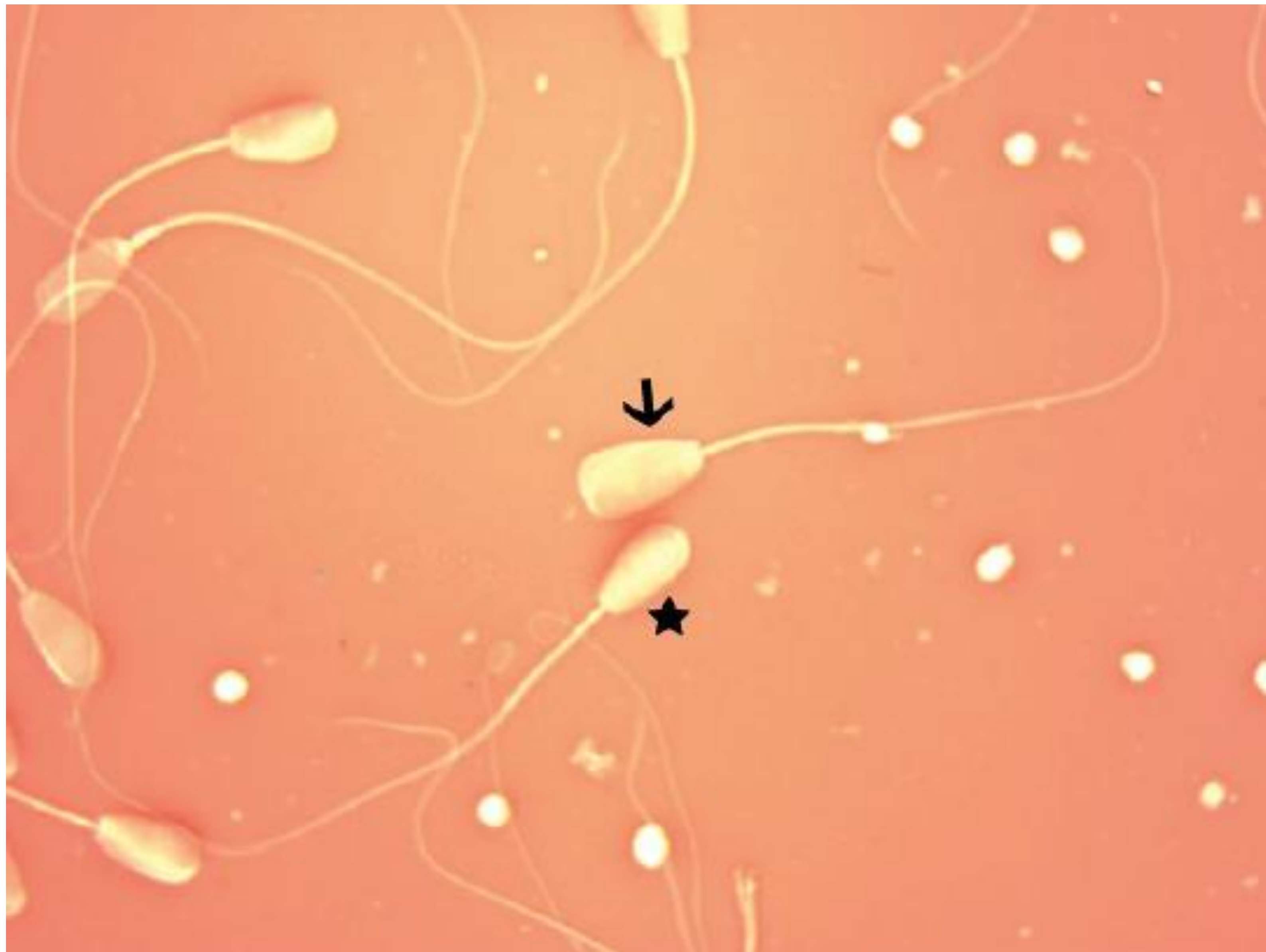


Diadem defects in a bull (phase contrast wet mount, 1000×).

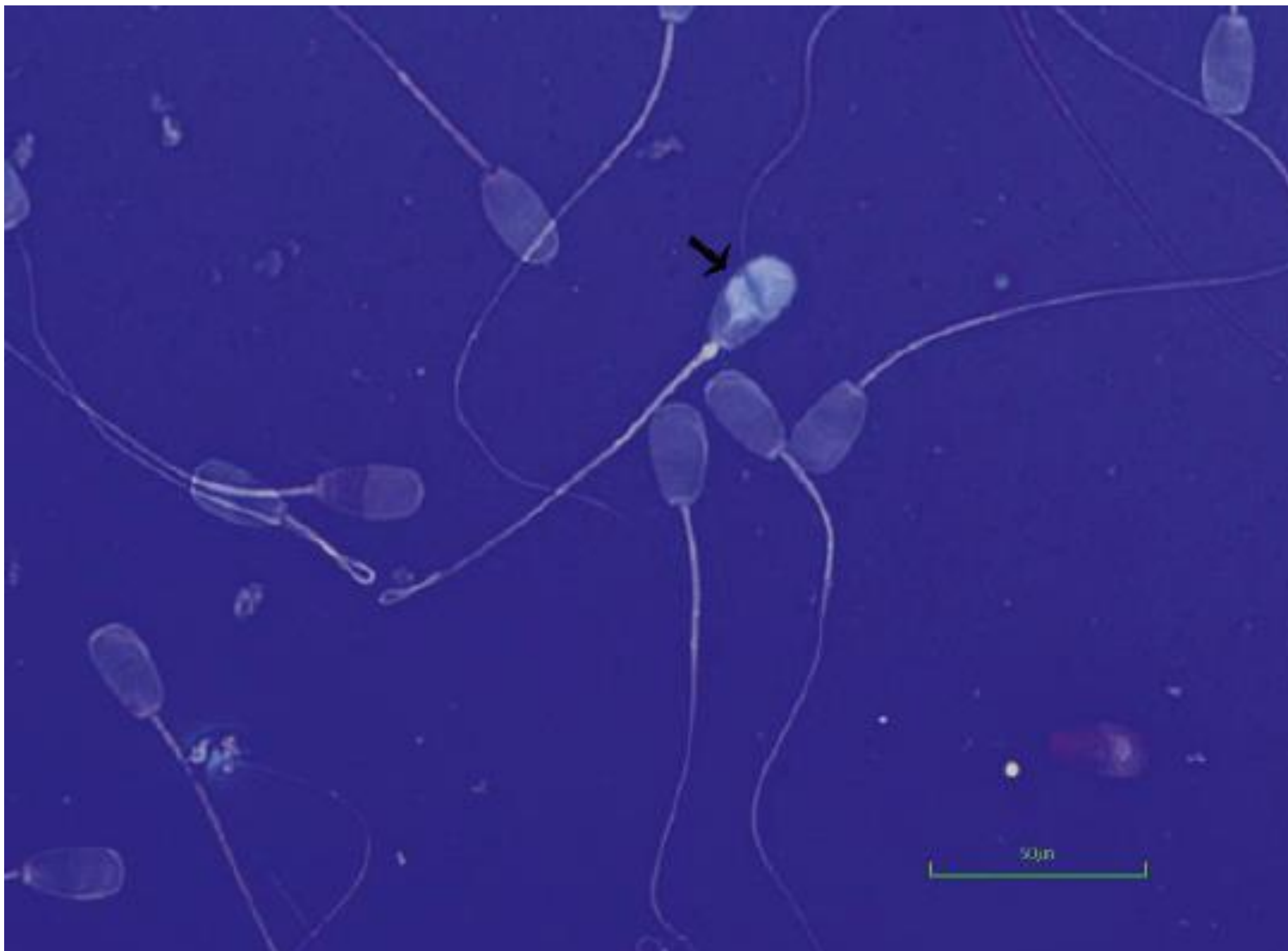


Multiple diadem defects indicated by arrow (eosin–nigrosin, bull, 1000×).

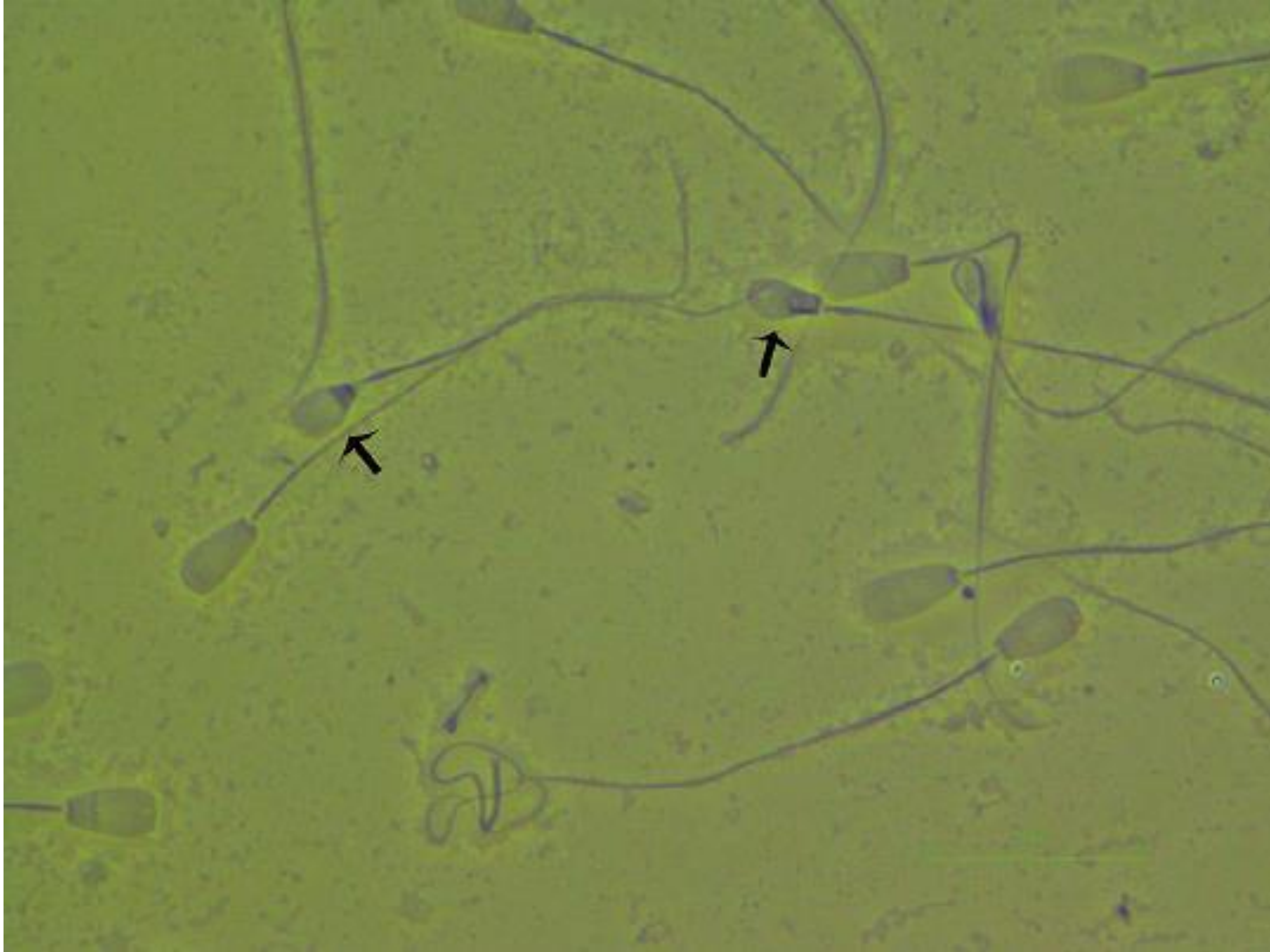
Created by Prof. Sana HIRECHE



Macrocephalic sperm head in a bull indicated by arrow next to a normal sperm indicated by star (eosin–nigrosin, 1000×).



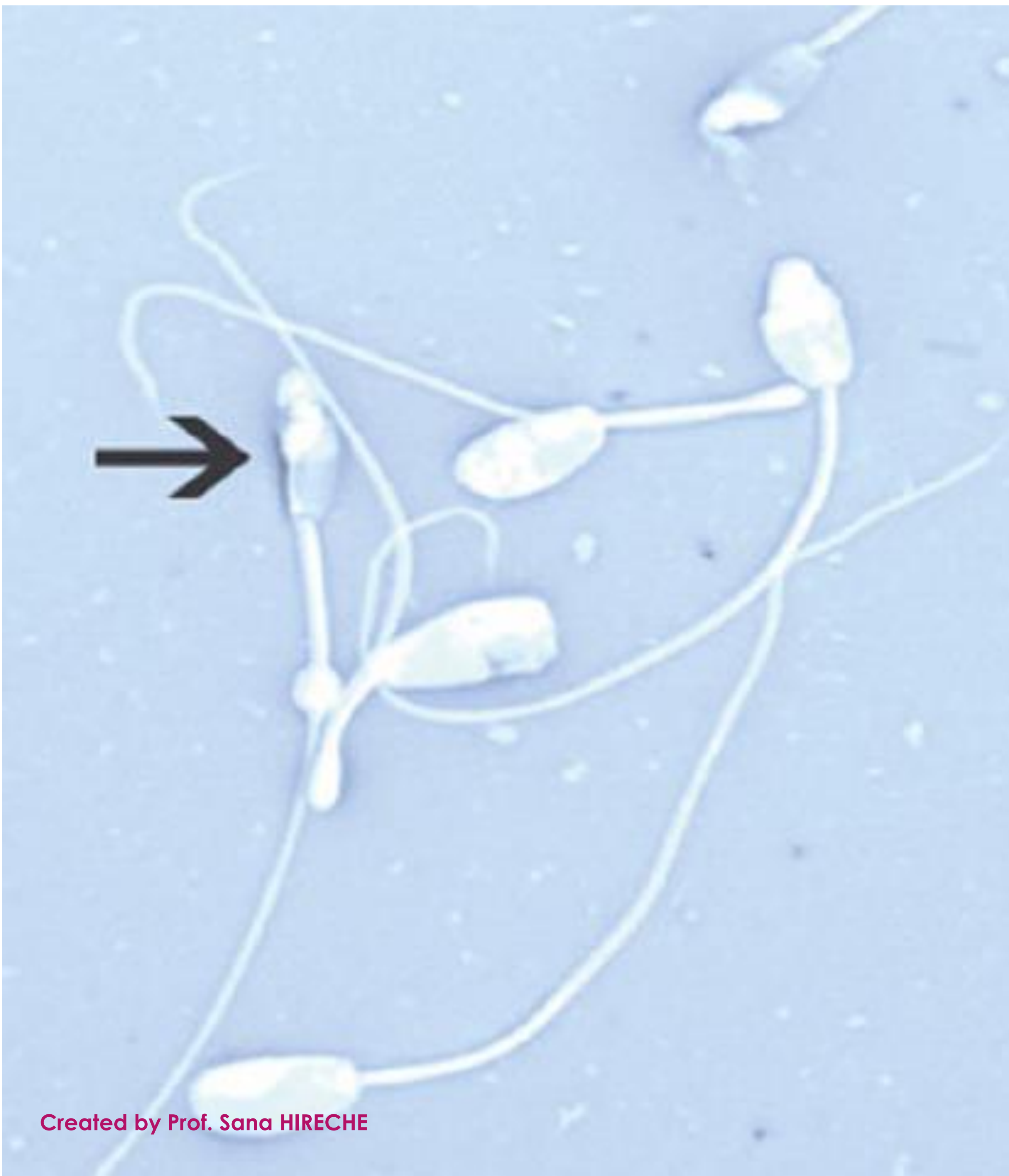
Macrocephalic head with proximal droplet in a bull (eosin–nigrosin, 1000×).



Microcephalic heads in a bull as indicated by arrows (phase contrast wet mount, 1000×).

Rolled Head–Nuclear Crest–Giant Head Syndrome

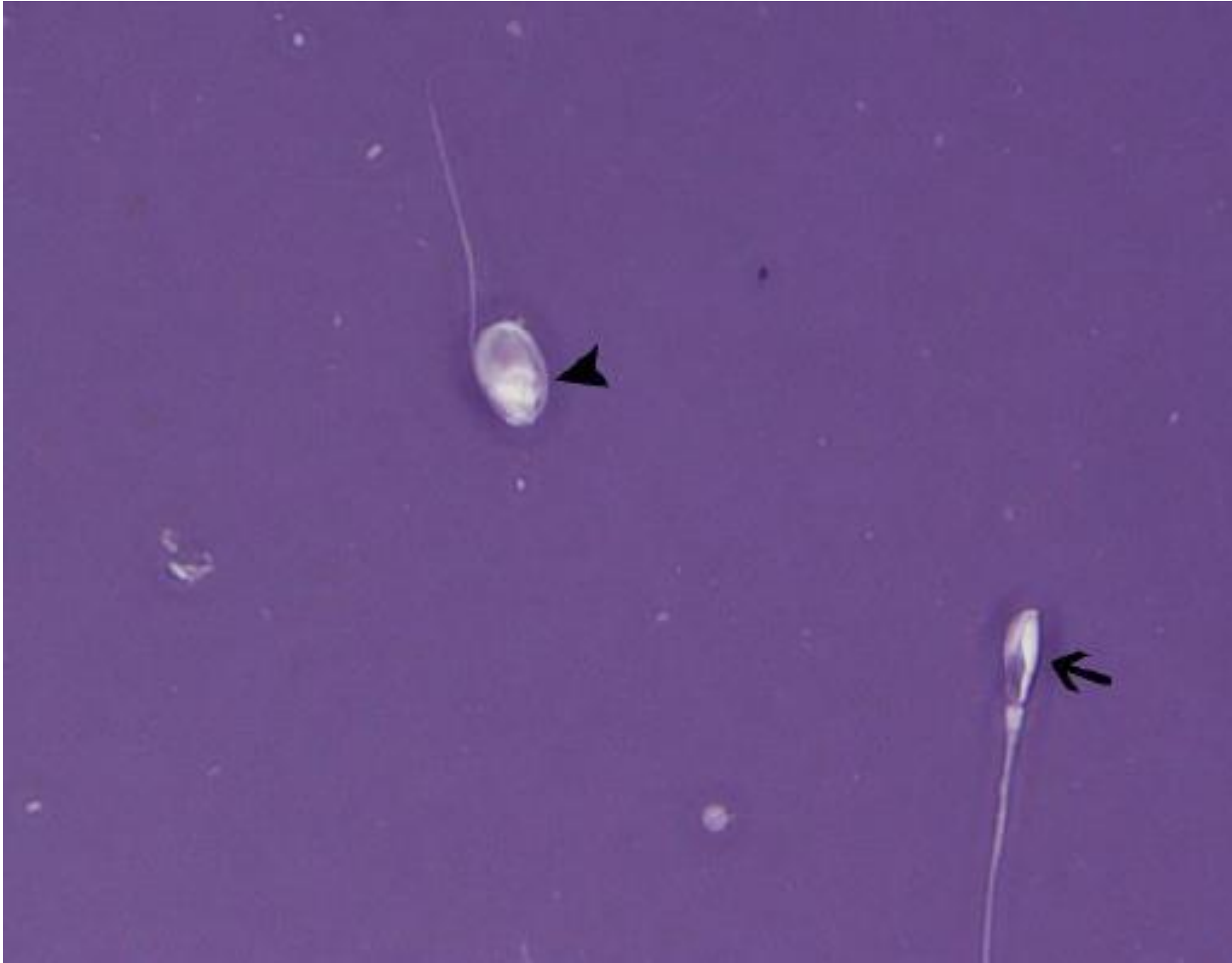
- Rolled heads, nuclear crests, or giant head syndrome are rare defects that have been reported in Brown Swiss, South Devon, and Friesian bulls and are thought to have a genetic basis



Rolled head (arrow):

Rolled heads are often noted with the roll along the long axis of the head of the sperm

(eosin–nigrosin, 1000×)



Rolled head in a bull indicated by arrow also note **the teratoid indicated by the arrowhead** (eosin–nigrosin, 1000×).

Abnormal DNA Condensation

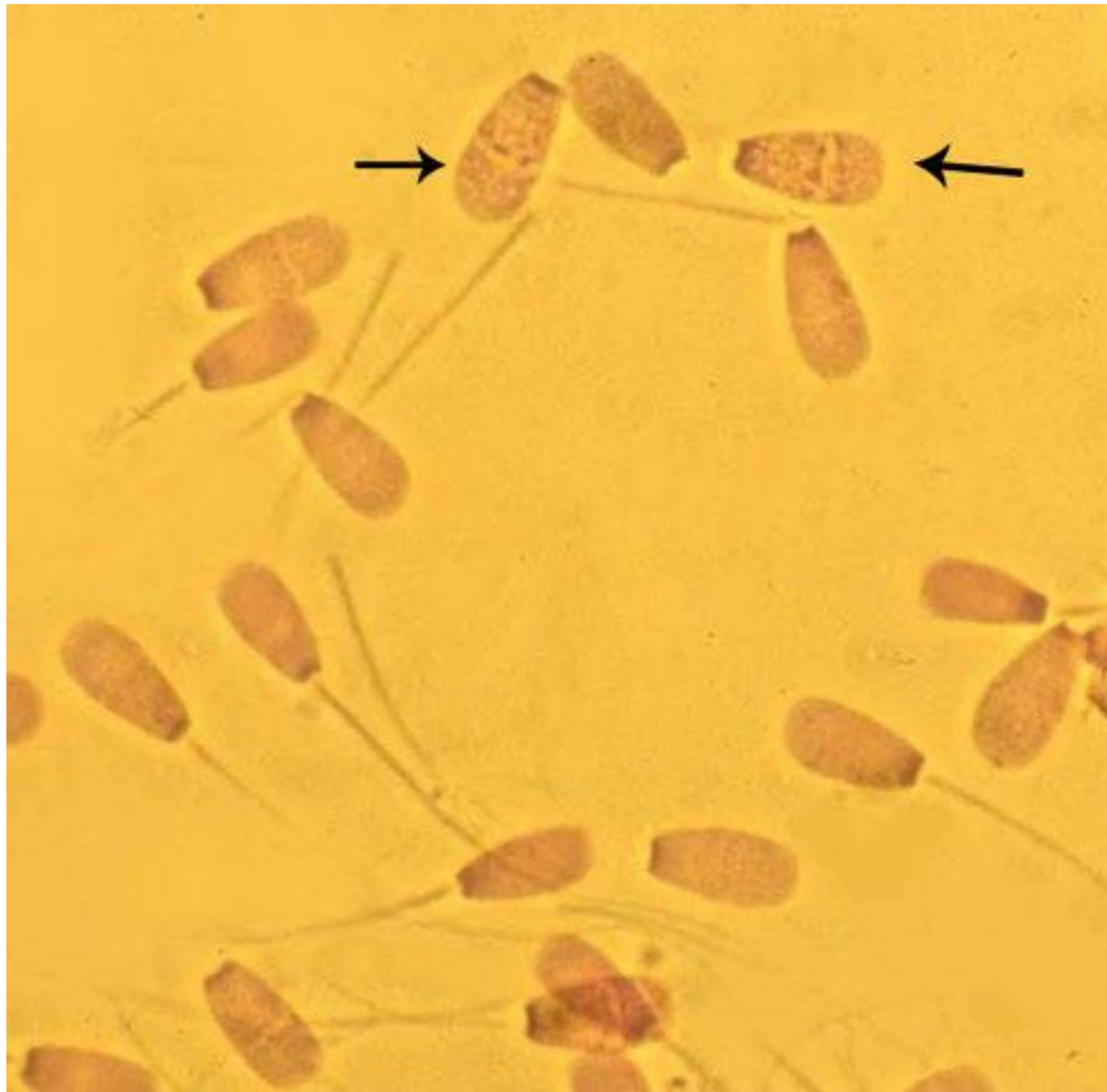
- Abnormal DNA condensation cannot be detected by standard light microscopic examination of unstained semen preparations or in routinely stained preparations that do not specifically identify chromosomal material or DNA
- When infertility is observed in healthy animals with adequate semen quality, libido, and mating ability, abnormal DNA condensation may explain fertilization failure.

Abnormal DNA Condensation

- SCSA involve acridine orange staining and assessment by flow cytometry to measure susceptibility of sperm chromatin to acid-induced denaturation
- Feulgen staining allows visual microscopic detection of abnormal DNA condensation

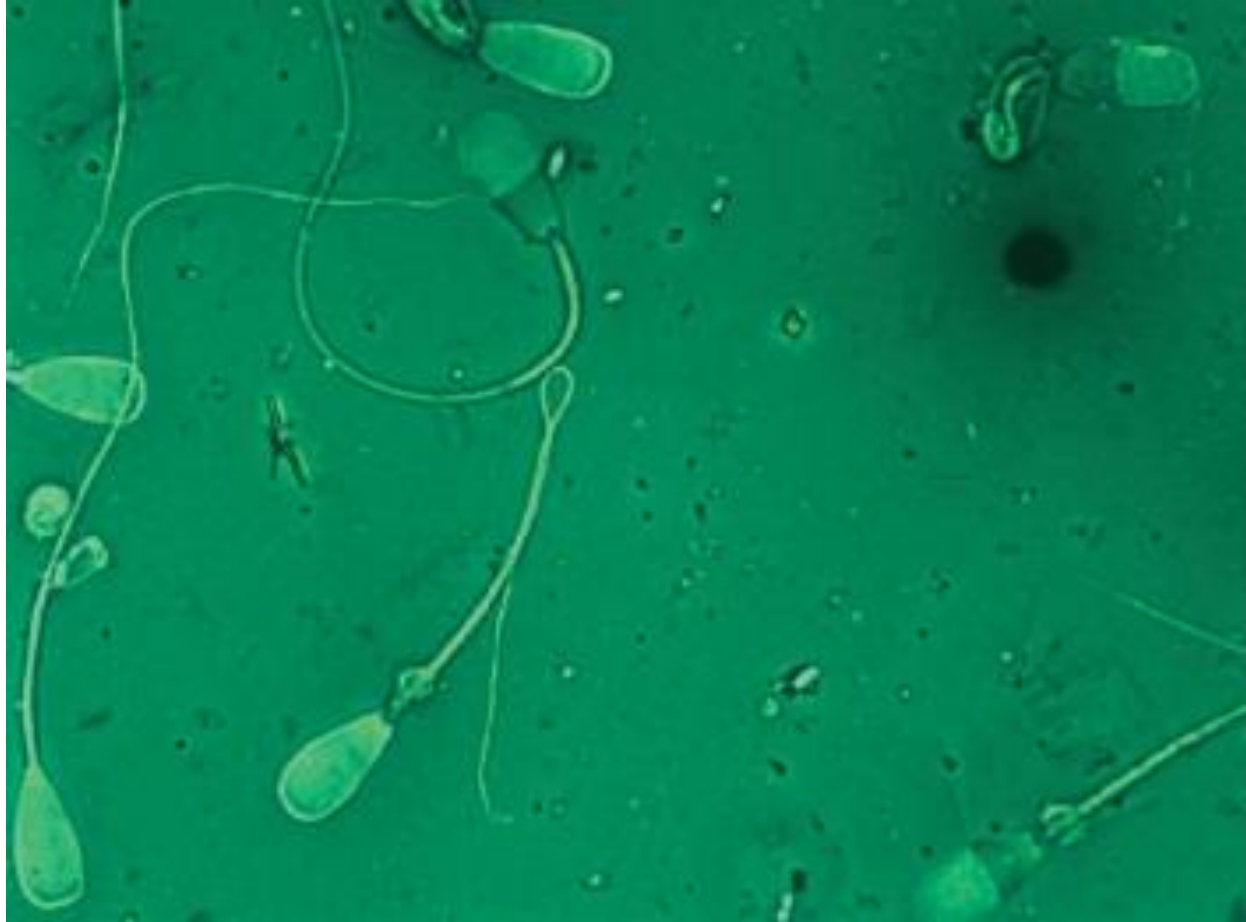
Abnormal DNA Condensation

- In normal bulls, the number of sperm with abnormal DNA condensation is **<5%**
- A few bulls with >80% normal sperm when evaluated on an eosin-nigrosin- stained smear had 100% of nuclei with abnormal chromatin condensation when a Feulgen- stained smear was evaluated

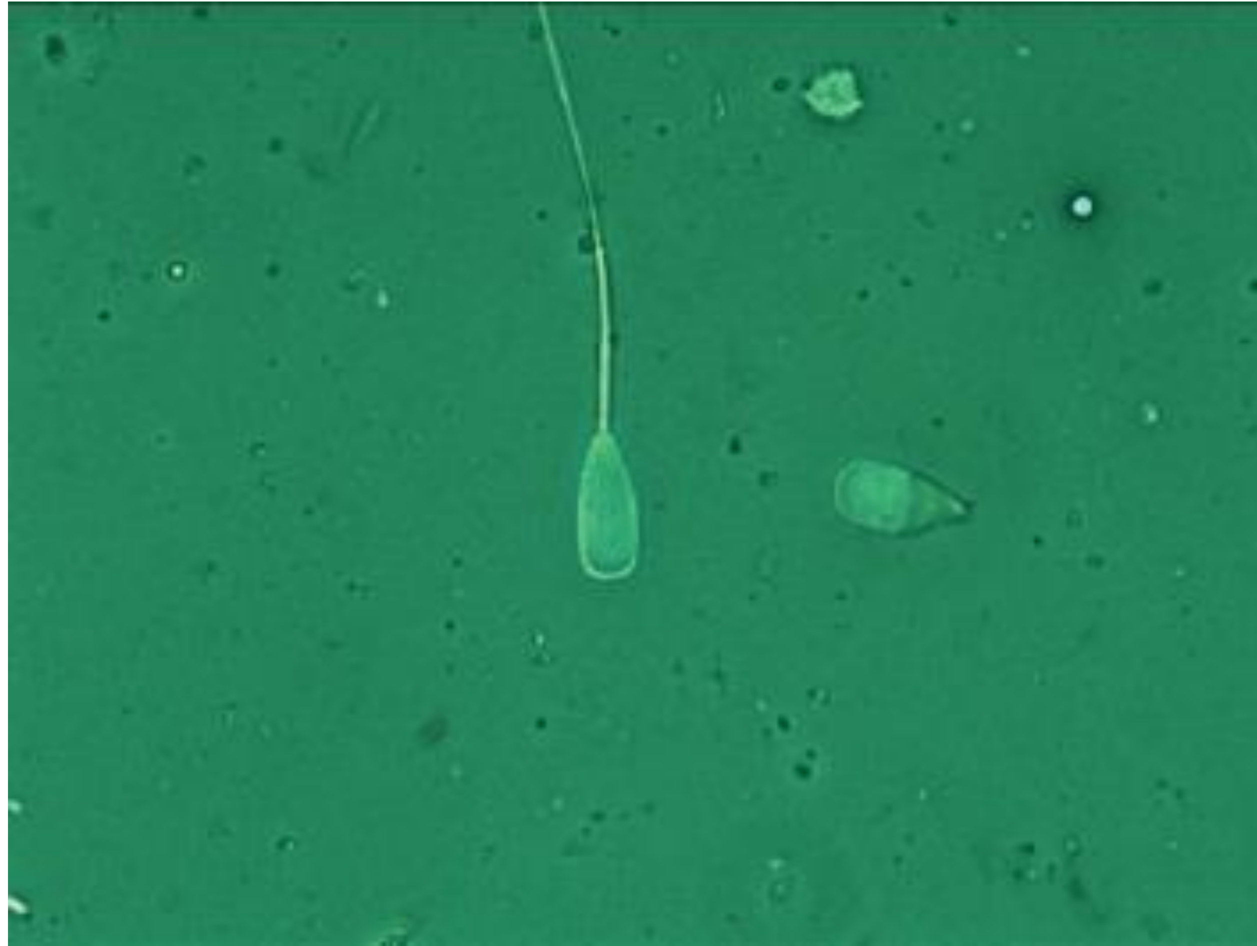


Abnormal chromatin condensation in a bull as shown by arrows (Feulgen, 1000×).

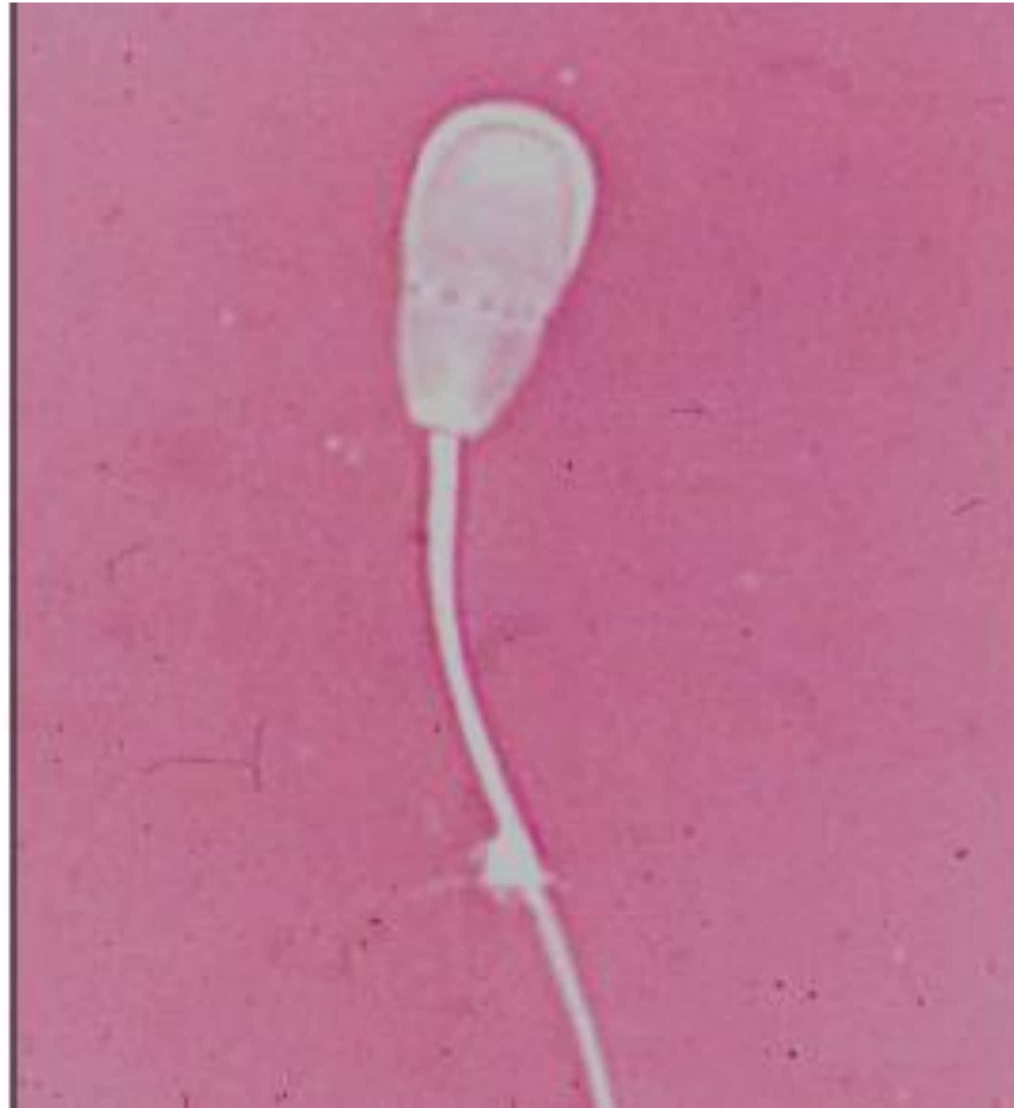
Created by Prof. Sana HIRECHE



A sperm with a bowed midpiece and a sperm displaying hypotonic shock (looped tail)



Tapered head and a detached, pyriform head



Sperm with diadem vacuoles

Sperm with diadem vacuoles

Sperm with confluent vacuoles and sperm with distal midpiece reflexes