# Technologies for *ex situ* conservation of farm animal species

Collection of epididymal semen

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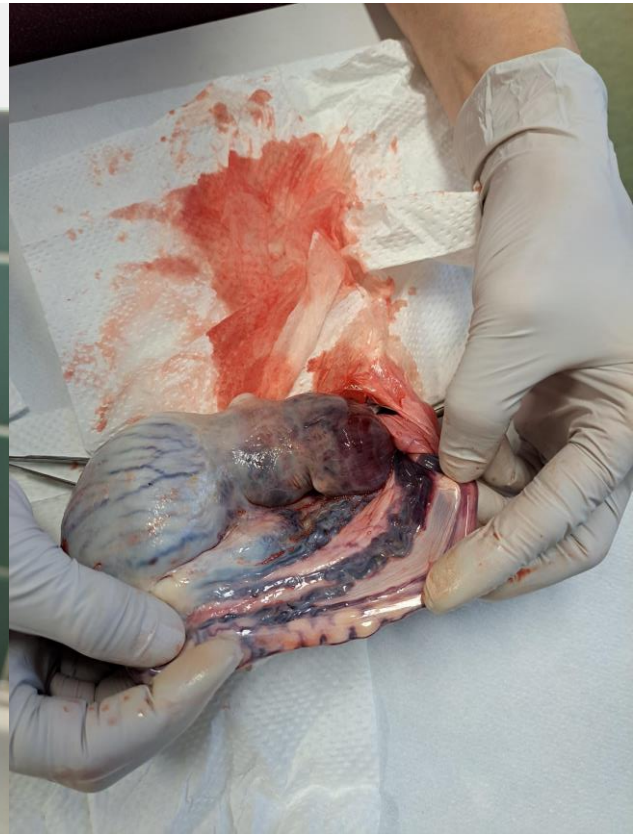
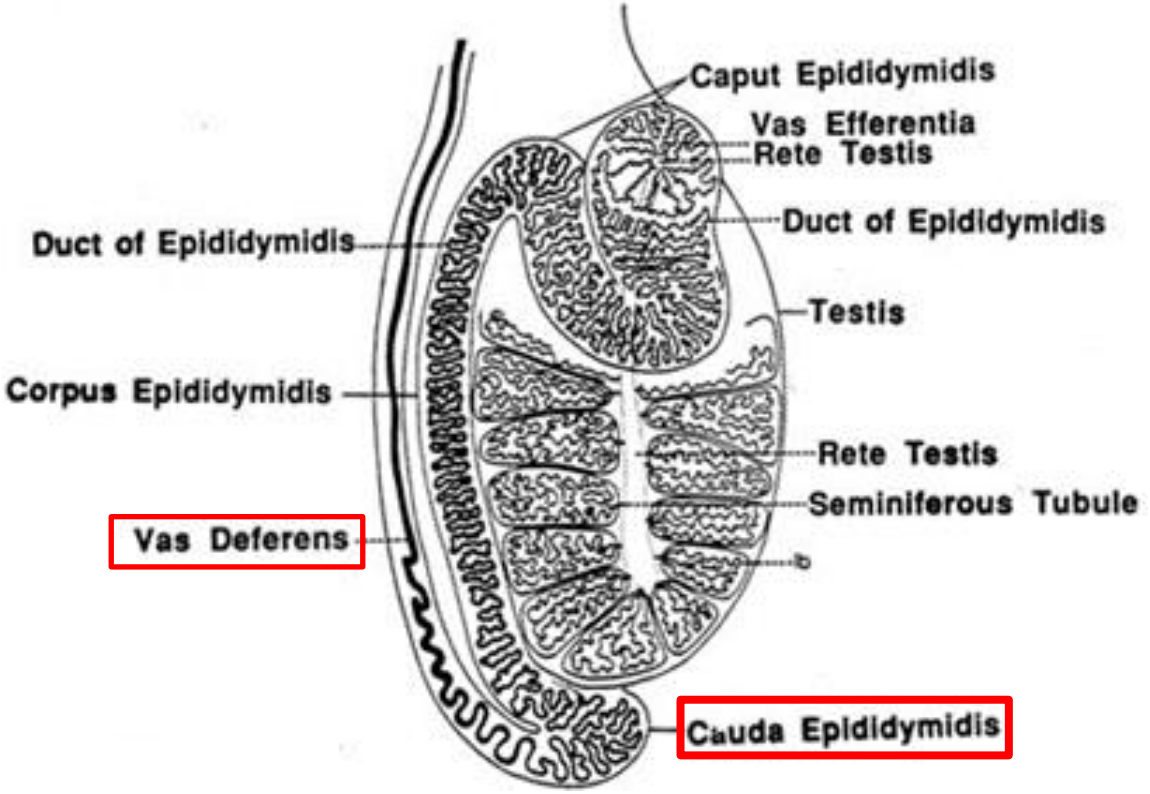
# Collection of viable epididymal semen / Heli Lindeberg

- Testes can be collected *post mortem* at slaughterhouse or after castration
- Testes temperature is allowed to drop gradually to RT during transportation
- In the lab, the processing of testes is started when the testis have reached RT

# Equine testes





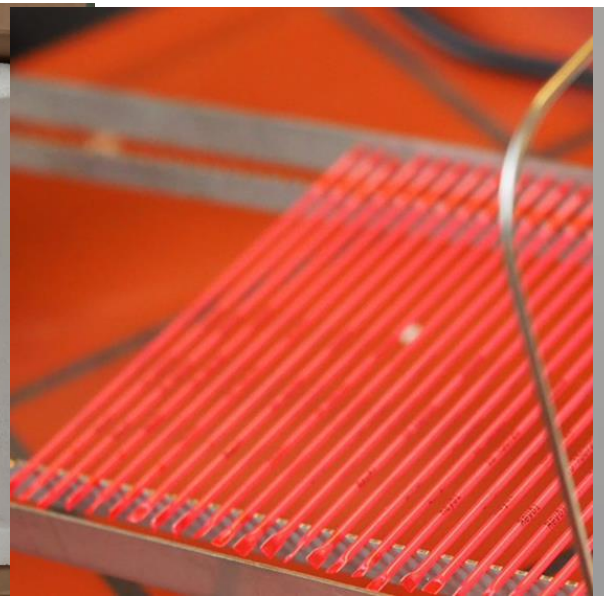
# What is needed for collections?

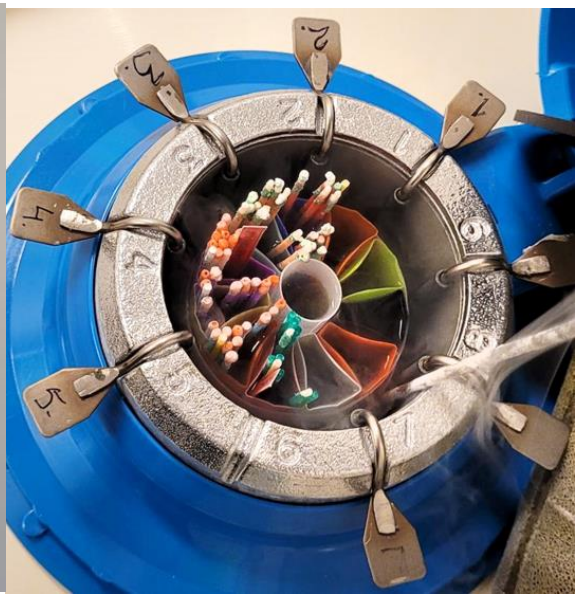
- Box with lid (big enough for transport of testes)
- Thermometer for measurement of testes temperature
- Scissors for dissection testes from skin
- Surgical blades for dissection of epididymides from testes (WWR International)
- Forceps for flushing the epididymides (fine tipped, WWR International)
- Commercial semen extender
    - Cattle: Steridyl, from Minitube, Germany (strorage +4/-20°C)
    - **Horse: for centrifugation BotuSemen (Nidacon, Sweden) and cushion (Cushion Fluid, Minitube, Germany); for cryopreservation Botucrio (Nidacon, Sweden) (storage -20°C)**
- Rubber-free syringes (5-10 ml)
- Blunt needles (self-made from Terumo 20 G needles)
- Large petri dishes (Nunc)
- Finnpipettes + tips (200 µl - 1 ml and 0.5 - 10 µl)
- 15 ml glass tubes (with 0,1 ml scale graduated, WWR International)
- Cell counting chamber (e.g., Bürker, WWR International)
- Objective and cover slides (WWR International)
- Heating plate for slides (Minitube)
- Microscopes (stereo and phase contrast, Olympus)



# What is needed for cryopreservation (Minitube)?

- Styrox box and freezing rack for straws
- Liquid nitrogen
- Straws
  - Preferably labelled with all necessary information about the donor
- Sealing method for straws:
  - Glass/metal balls
  - Heating device
- Goblets for packing of straws
- Liquid nitrogen container for storage

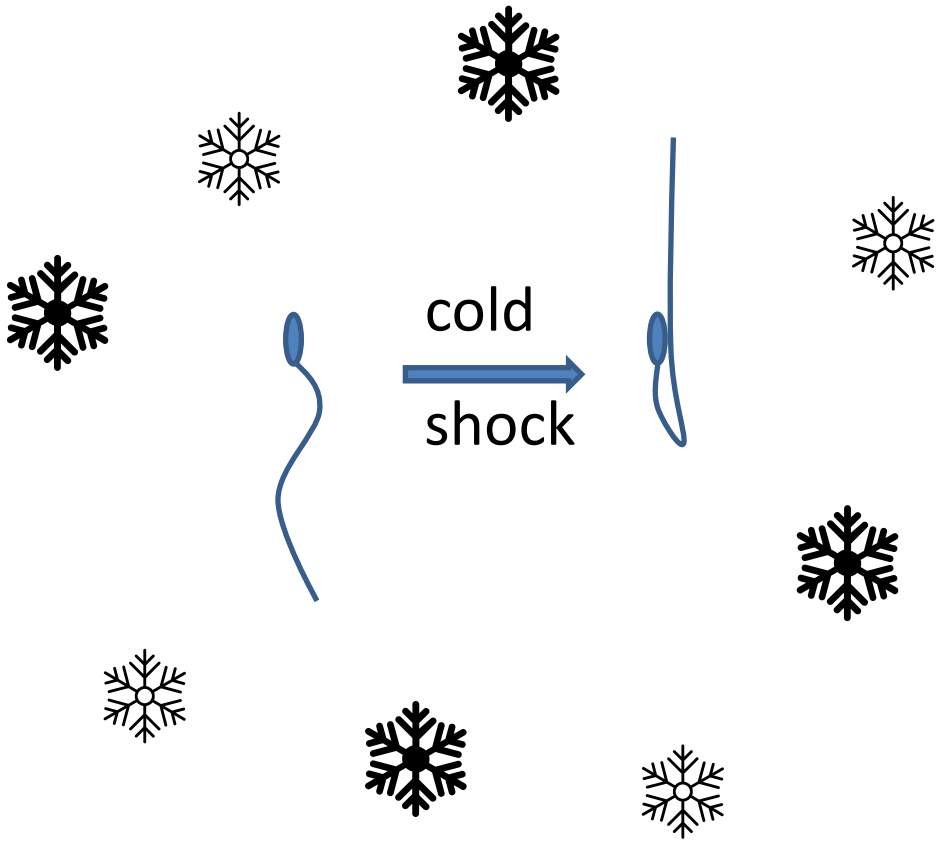





# Collection of epididymal semen from a stallion

Testes at arrival
to laboratory

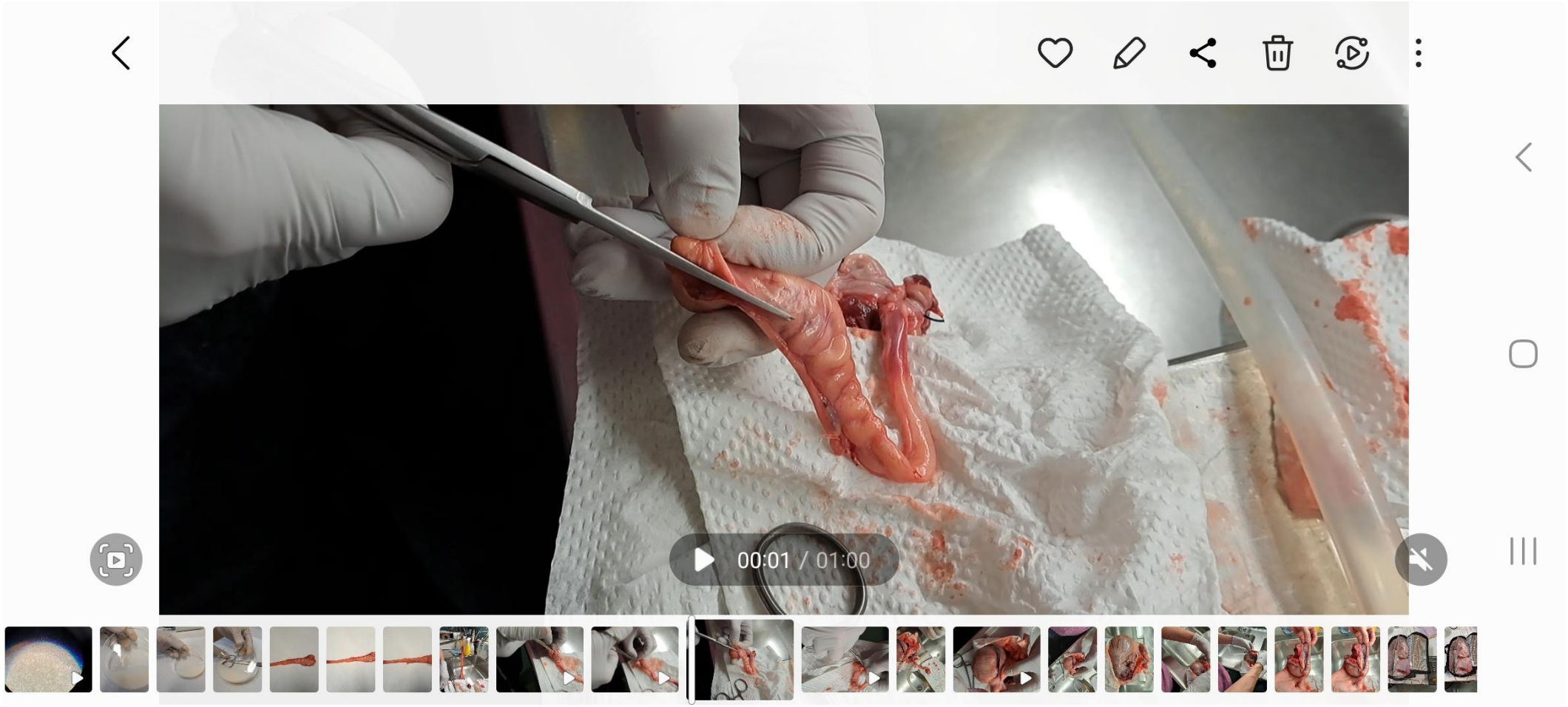
# Temperature and sperm quality



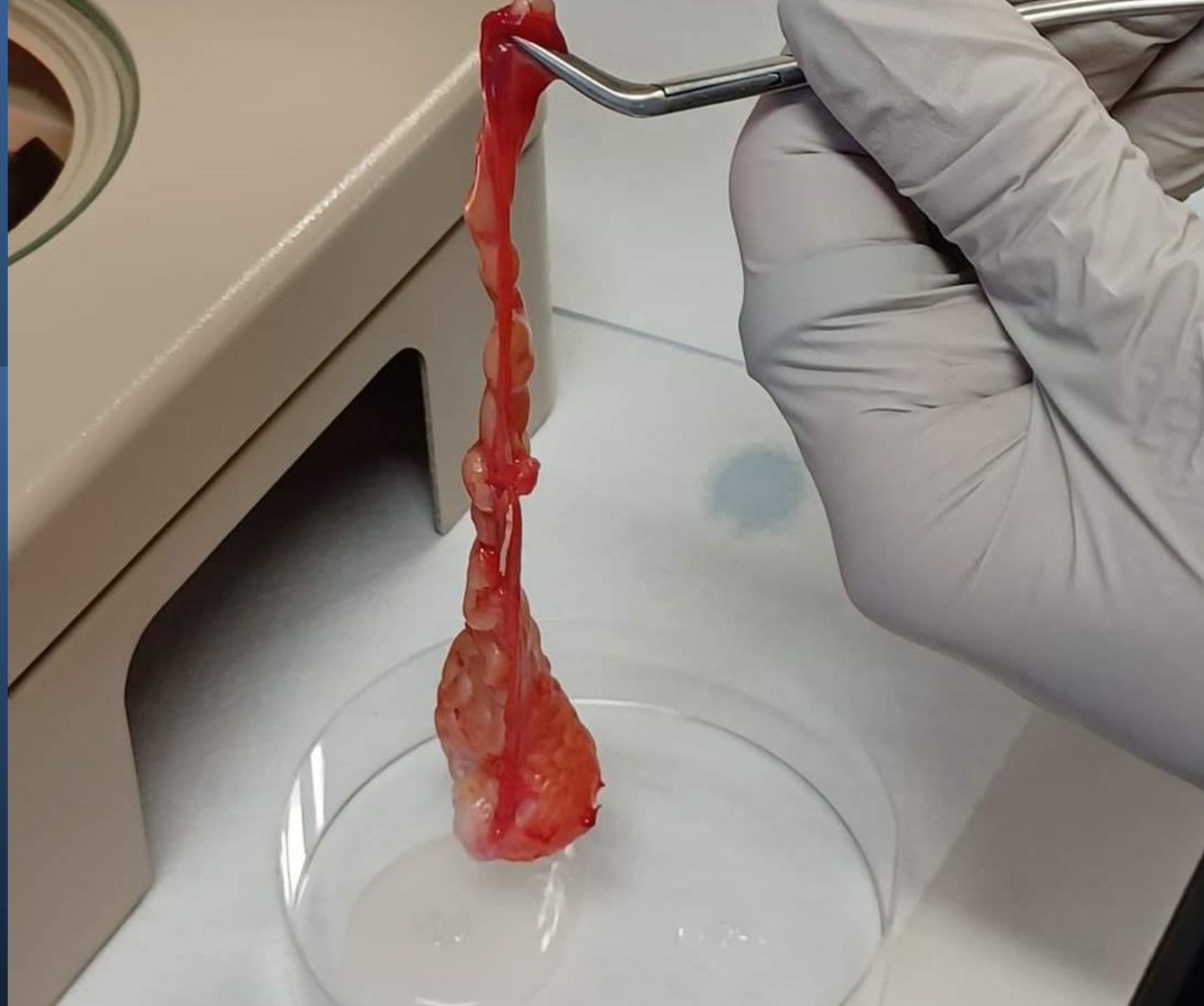
# Stallion epididymis

# Stallion epididymis preparation

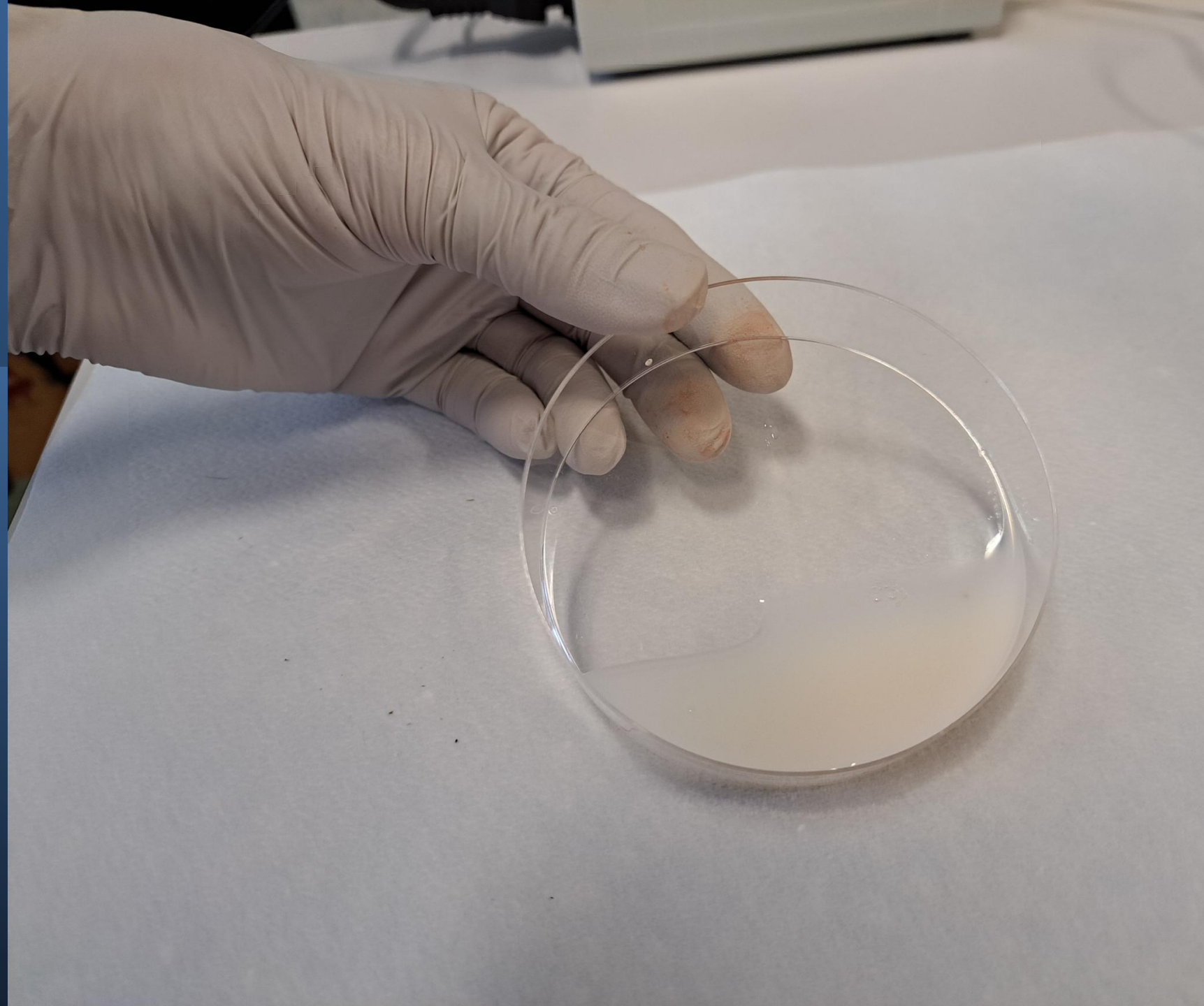
# Preparation of epididymis



# Stallion epididymal semen collection



Fresh stallion epididymal semen

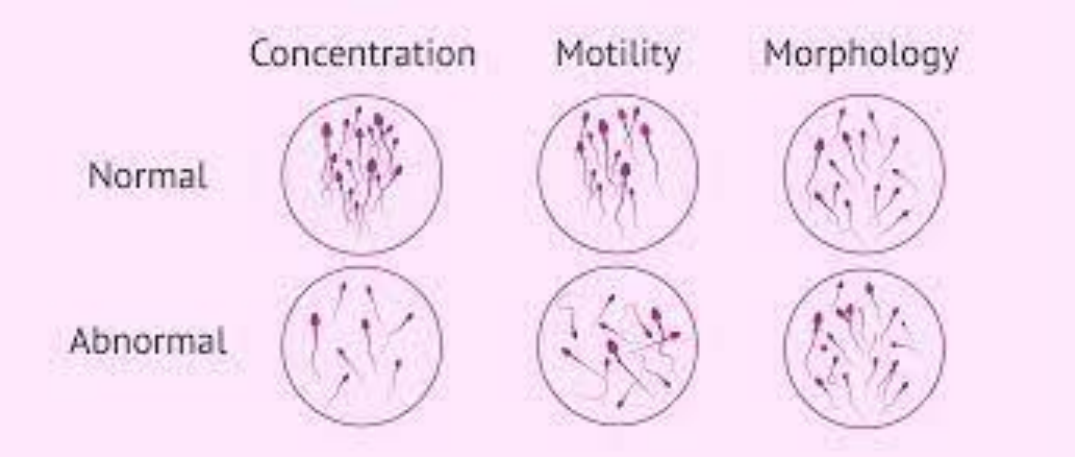


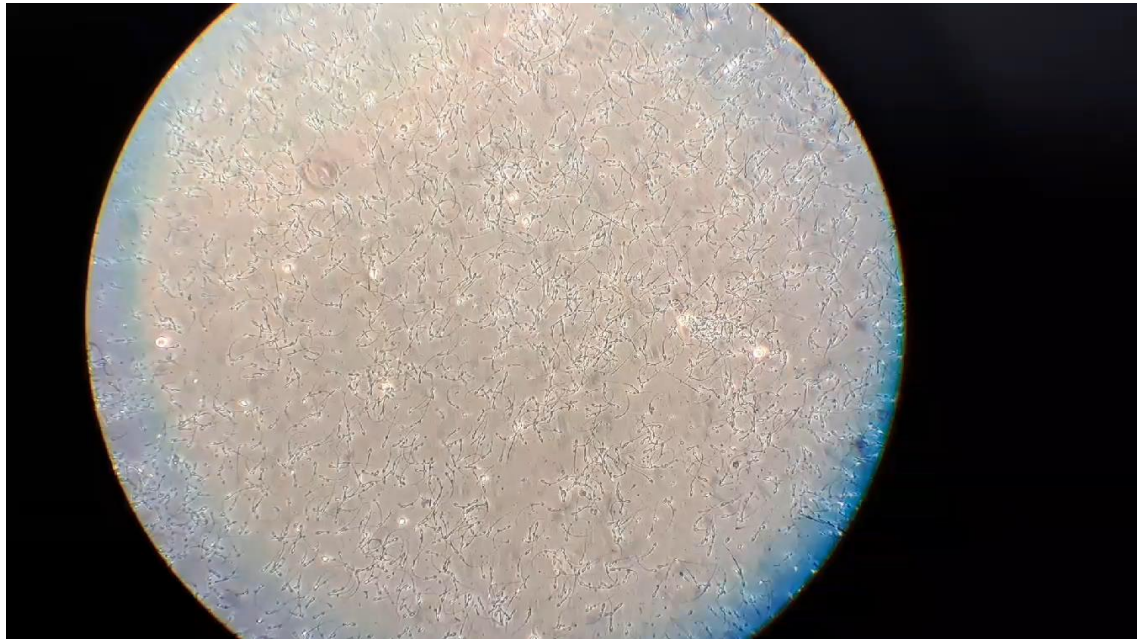
# After successful collection of semen

Measures for

- total volume (ml, semen + BotuSemen)
- concentration and total sperm count (sperm/ml)
- progressive motility (%)
- possible malformations (type, %)

# Fresh sperm quality



# Stallion epididymal semen: 2-step cryopreservation protocol

- Flushing of epididymal semen with BotuSemen (10-20 ml)

  => viability (eosin-nigrosine staining)

  => progressive motility

  => centrifugation @ 600-1000 g (RT) to remove BotuSemen
- Addition of BotuGrio to dilute the semen (very carefully!)

# Loading of straws

What is needed :

- Prelabelled straws
- 1 ml syringe with silicone tubing  / 250 µl pipet
- Powder/balls/heating device to seal the straws

# Setting up freezing

Equilibration of straws at **+5°C**

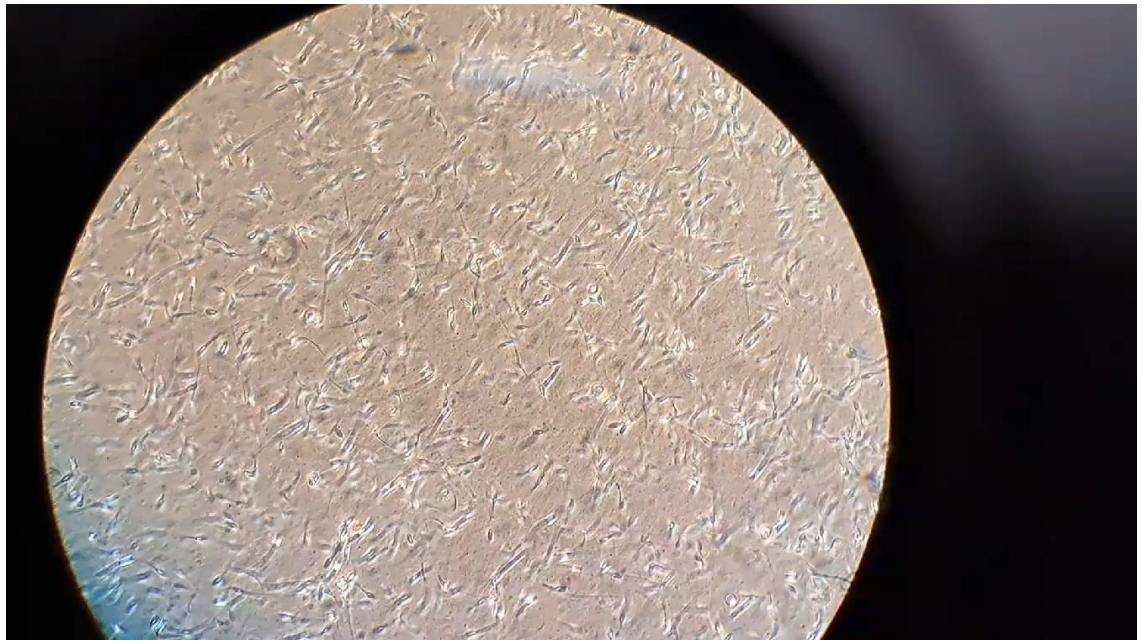
- 2 h for bulls
- 20 min for stallions

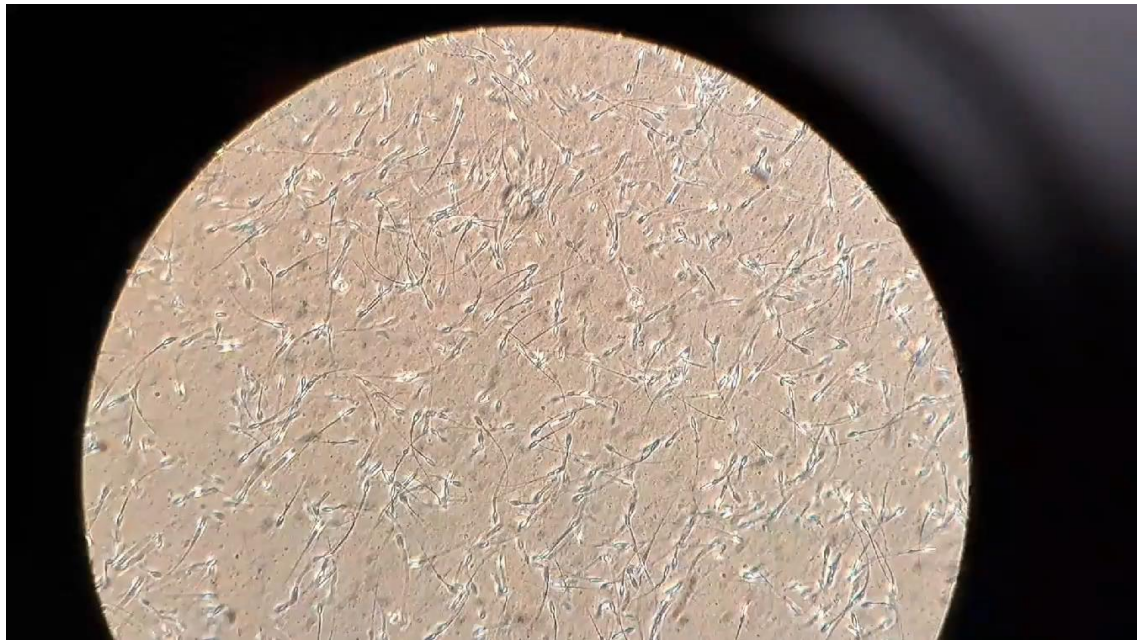
On a rack, 4 cm above liquid nitrogen level

- 20 min for bulls and stallions

=> Plunged in liquid nitrogen for storage

# Fresh => Frozen-thawed





# The use of ejaculated and epididymal semen

**Ejaculated semen**

- Expensive infrastructure
- Few bulls/stallions
  - Breeding schemes
  - Genetic diversity ↓
- Repeated collections
  - Large number of doses
  - X and Y sorted semen
- Sanitary status of bulls/stallions is already well defined

**Epididymal semen**

- Cheap infrastructure
- Many bulls/stallions
  - Conservation schemes
  - Genetic diversity↑
- Single collection
  - Limited number of doses
- Sanitary status of bulls/stallions needs to be defined

# Legal considerations

- Sanitary regulations depend on EU and national regulations.
- If samples are collected in an emergency, without knowledge of sanitary status of animals, they must be stored separately from samples of known sanitary status.
- In scheduled collections, donors should be tested the same way as the ones going to collections at AI stations?
- In Finland horses (2 x CME, 1 x EVA & EIA):
  - Contagious Equine Metritis (CME, swab sample)
  - Equine Viral Arteristis (EVA, bood sample)
  - Equine Infectious Anemia (EIA, blood sample)
- In Finland cattle (negative test results needed before collection/storage):
  - BVD

# NordFrost Hands-on Workshop

Faculty of Veterinary Medicine, NMBU

20 April 2023

**Aim of the workshop:**

- To become familiar with collection and cryopreservation of epididymal semen using bovine model.

**Program of the day (9 am – 5 pm):**

- Presentation about the applied protocol.
- During the workshop, the participants learn how:
  - testis are collected after castration and slaughter.
  - to transport testis to laboratory.
  - to dissect epididymis.
  - to collect semen from epididymis.
  - to process the semen for cryopreservation.
  - to freeze and thaw semen.
  - to evaluate post-thaw sperm motility.