EQUINE SEMEN FREEZING

State of the art and future developements

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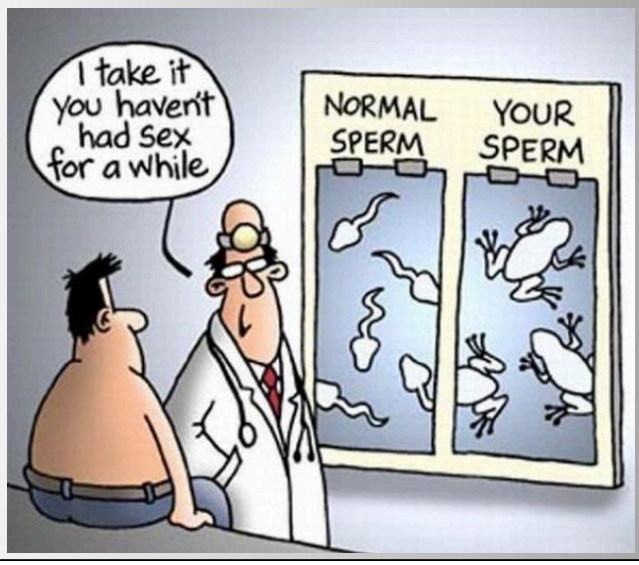
1. Introduction

- Equine semen freezing:
  - ✓Doses available worldwide
  - ✓Any time available doses
  - ✓Sanitary safe semen
- 20% of stallions with unfreezable semen
  - ✓Economic and genetic losses for owner and clients
- Aim of presentation:
  - Review of spermatozoa physiology with implications in semen freezing
  - Review of freezing process and future improvements

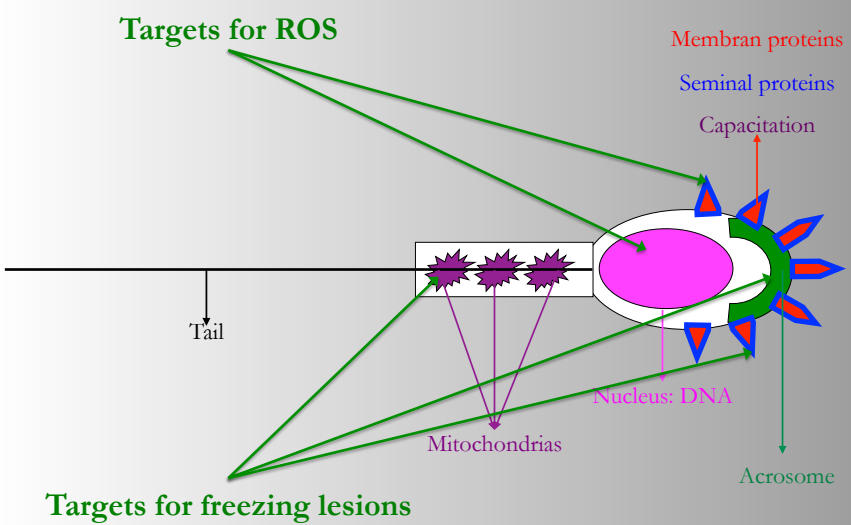
2. From spermatogenesis to ejaculation

- In the testicle: 57d to form a spermatozoon:
  - Proliferation (mitosis ➔ meiosis)
  - Differentiation (round cell ➔ differentiated cell)
- In epididymis: 12d to mature a spermatozoon:
  - From head to tail:
    - Motility & Fertility acquisition
    - Cytoplasmic droplet elimination
- Seminal plasma: prostate + vesicular + bulbo urethral grands
  - Volume of ejaculate: sexual arousal effect on volume & concentration
  - Total sperm number per ejaculate 8-10x10^9spz

3. Spermatozoon



3. Spermatozoon



4. Freezing procedures

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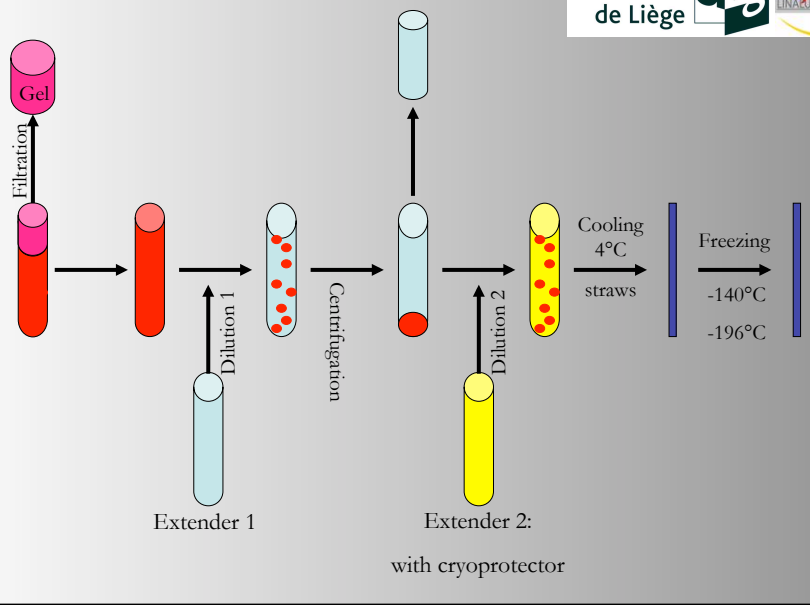
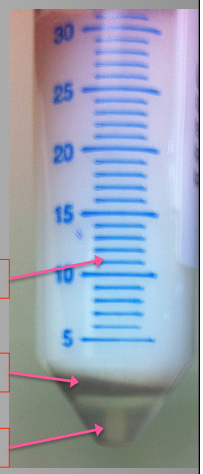


4. Freezing procedures

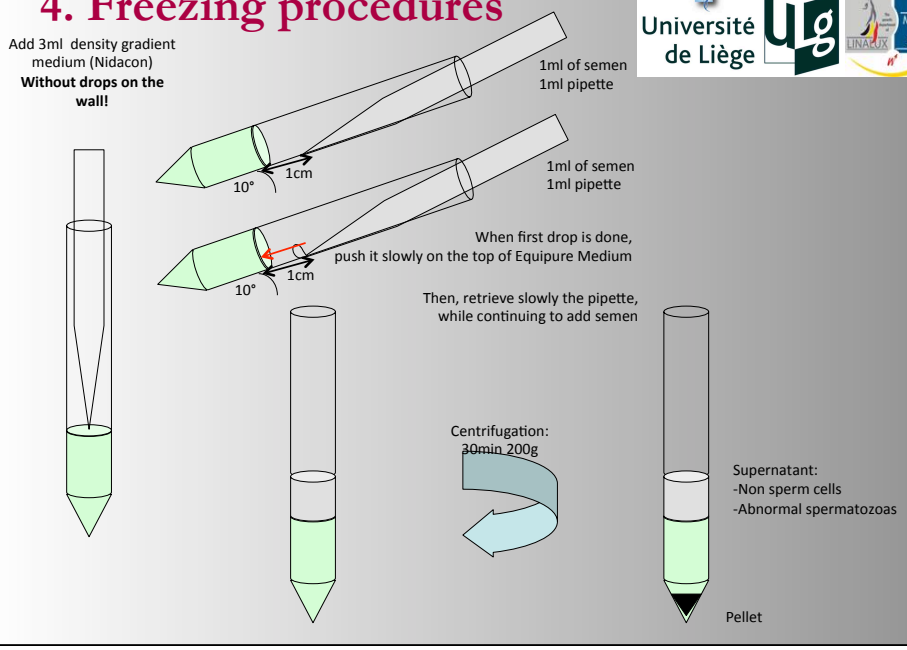
- New process: Cushioned centrifugation
  - g force and duration increased
    - No spermatozoa lesions
  - Recovery rate increased:
    - Conventional protocol 600g 10 min: RR=60-70%
    - Cushioned protocol 1000g 20min: RR=85-95%



4. Freezing procedures

- New process: Spermatozoa selection
  - Gradient density centrifugation:
    - Nidacon™ Equipure®
  - Morphology & specific gravity selection
    - Isopicnotic point
  - Selection of motile and normal spermatozoa but also selection of non fragmented DNA spermatozoa

4. Freezing procedures

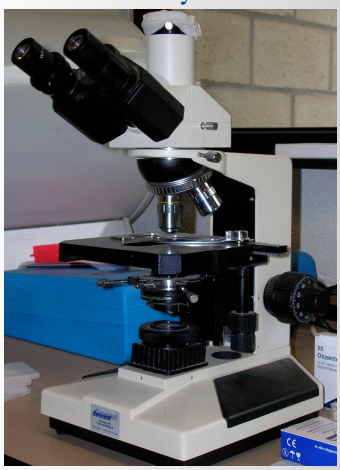


5. Quality control

- Requirements for frozen semen:
  - French National studs: $140x10^6$ progressive spz
  - World Breeding Federation: $250x10^6$ progressive spz
  - >35% progressive spz
- Concentration: according producer definition
  - For AI doses with 8 straws of 0.5ml:
    - 0.35 x 0.5 x 8 x 100 = 140
  - For AI doses with 4 straws of 0.5ml:
    - 0.35 x 0.5 x 4 x 200 = 140

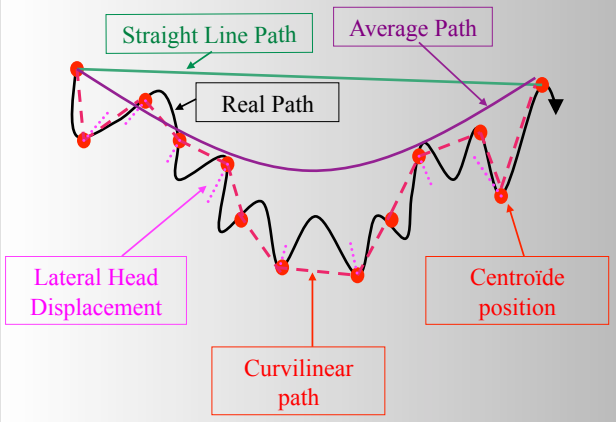
5. Quality control

1. Motility



5. Quality control

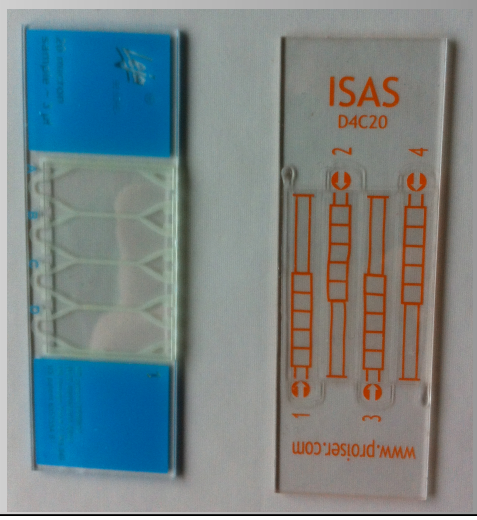
1. Motility



Definition	
VCL	Velocity Curvilinear Path
VSL	Velocity Straight Line Path
VAP	Velocity Average Path
LIN	VSL/VCL
STR	VSL/VAP
WOB	VAP/VCL

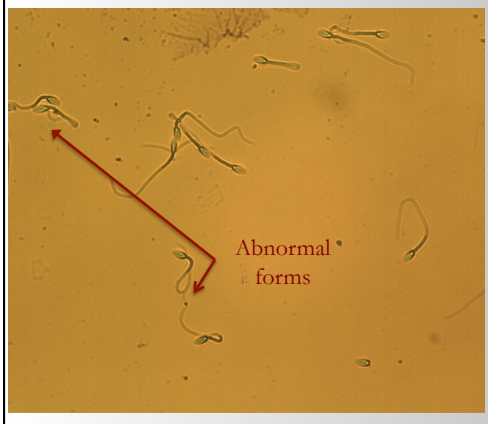
5. Quality control

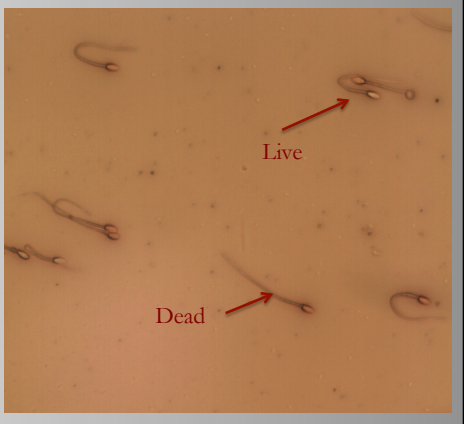
1. Motility



5. Quality control

2. Stainings for microscopic exam

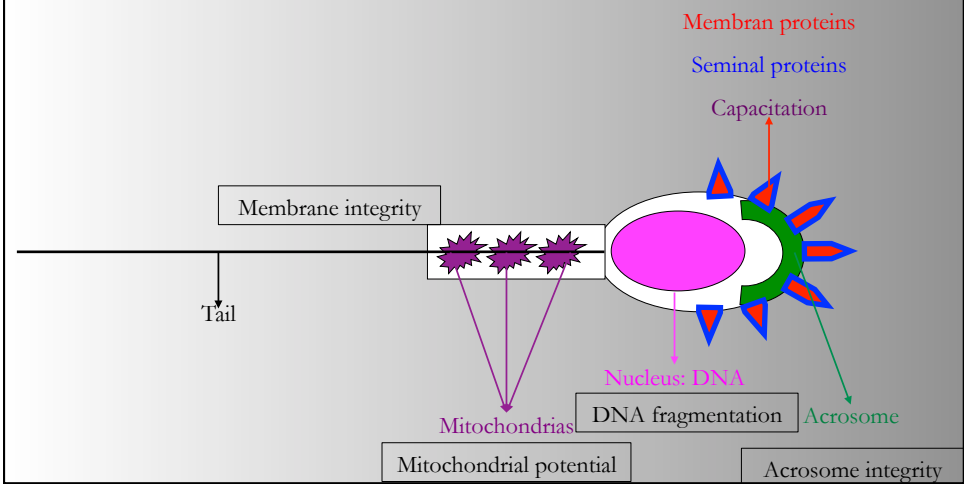




Diff-Quick **Eosin-Nigrosin**

5. Quality control

3. New quality controls: flow cytometry



5. Quality control

3. New quality controls: flow cytometry

6. Limits, results and future

1. Freezability prognosis
   - In the human:
     - Membrane fluidity
     - GSH concentration & GPX transcription
     - Membrane integrity & symetry: Annexin V
   - In the equine:
     - Apoptotic factors (by flow cytometry)

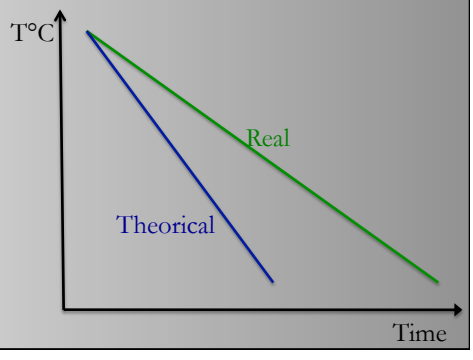
6. Limits, results and future

2. Extender improvement
   - Cryoprotective agents
     - Reducing glycerol concentration (3,5%)
     - Lipids and Amides cryoprotective effects to replace glycerol (Botucryo®)
   - Physicochemicals and nutritive characteristics
     - Cholesterol
     - Isolation of LDL (INRA Freeze®)
     - Animal protein free media

6. Limits, results and future

3. Freezing process improvement

- Cooling?
  - After centrifugation
  - Time can be increased
- Freezing?
  - Curves?
  - Vitrification?



6. Limits, results and future

4. Reactive oxygen species and semen freezing

- Effects on motility, membrane integrity, DNA fragmentation but necessary for some functions
- Origin:
  - Intrinsic pathway
  - Extrinsic pathway
    - MPO?
- Anti-oxidant therapy?
  - Broad spectrum anti-oxidants (vit E & C): +/-
  - Specific MPO inhibitors: ++

6. Conclusions

- Spermatozoa: motility, but also: acrosome, DNA, mitochondria,…
- Equine frozen semen: harmonization of thresholds
- Future:
  - Selection of good freezers on fresh semen basis
    - Treatment of bad freezer
    - Better media to freeze
    - New processes?

Questions?