BREEDING SOUNDNESS OF THE STALLION
Past – Present – Future

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1. Libido

- Erection within 5-10 min
- Ejaculation after 6-8 movements
- Influences:
  - Previous natural mating
  - Puberty
  - Pain
  - Education
  - Breed

Semen collection

Natural courtship

VS
2. Physical examination

• General exam:
  – Clinically normal (weight!)
  – Orthopedic exam (lameness, wobbler syndrom)

• Penis examination (dolor, semen contamination):
  – Paraphymosis?
  – Lesions of penis?
    • Tumors (sarcoïds vs squamous cell carcinoma)
    • EHV3
    • Habronema spp.

2. Physical examination

• Testicular examination:
  – Two testis!!
  – Testicular volume:
    • 5-6 (h) x 5-6 (ł) x 8-10 (L) cm
    • 15-20 x 10⁶ spz/g/day
  – Daily Sperm Output (DSO):
    – Testicular volume (TV) = 0.5233 x Ł x ł x h (cm)
    – DSO = (0.024 x TV) – (0.76 to 1.26)
    – Seasonnal variations of DSO & TV
2. Physical examination

• Testicular examination:
  – Consistency & temperature:
    • Soft: inflammation (hot) vs degeneration (cold)
    • Irregular: local (Tumors?) => US
  – Orientation:
    • Tail of epididymis: caudal
      – Soft round zone (1.5 to 2cm diameter)
    • Deviation of orientation
      – Strangulation (>180°) > pain: colics
      – Not strangulated (<180°) > vascular effect?

• Sanitary aspects (EU only):
  – Contagious equine metritis (CEM): *Taylorella equigenitalis*
  – Equine viral arteritis (EVA)
  – Equine Infectious Anemia (EIA)
3. Ultrasonography – doppler

- **Testis envelop:**
  - (Parietal & visceral vaginal tunic)
  - Hydro-hématocele
- **Testis:**
  - Measures (only testis)
  - Echogenicity (homogenous inside & between)
  - Varicoceles (doppler)
- **Epididymis:**
  - Dilatated - swollen
- **Accessory glands**

4. Semen analysis

- **Volume:**
  - **Seminal plasma secretion:**
    - Vesicular glands, prostate and bulbo-urethral glands
    - Long excitation > Increased secretion of seminal plasma
    - Increased volume
    - <20ml for a well educated stallion during production
    - >80ml for a stallion excited for a long time with many mount attempt before ejaculation
  - **Color:**
- **Concentration:**
  - Grey translucent (<100x10^6 spz/ml) to creamy white
  - (>250x10^6 spz/ml)
  - Color: yellow, pink…
4. Semen analysis

• **Concentration:**

  - Dilution of semen in inactivator (formol)
  - Count of spz within cell (HC) or part of cell (TC)
  - Concentration = nbr x10^6

  ![Haemocytometer, Thoma cell](image1)

  ![Nucleo Counter](image2)

  ![Photometry](image3)

  - Dilution of semen in detergent solution
  - PI (Propidium Iodide) enters in all spz
  - Count of nuclei stained by PI
  - Concentration

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4. Semen analysis

• **Concentration:**

  – **No cut-off value:**

  • Volume depends on previous excitation
  • Dilution of Total Sperm Number by seminal plasma
  • From <5x10^6spz/ml to >600x10^6spz/ml
  • Effect of epididymal storage
4. Semen analysis

- **Total Sperm Number:**
  - = volume x concentration
  - 4 to 12x10⁹ spz, stability for a stallion
- **Flush of epididymal storage:**
  - Storage of mature spermatozoa in tail of epididymis
  - Old spermatozoa: death but stored (no elimination)
  - First collection: high TSN but low motility & viability
    => 2-3 collections before analysis
- **Variations:**
  - Individuals, season…

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<table>
<thead>
<tr>
<th>Observed &gt; Estimated</th>
<th>Observed &lt; Estimated</th>
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<tbody>
<tr>
<td>End of flush?</td>
<td>Testicular pathology?</td>
</tr>
<tr>
<td>Epididymal storage</td>
<td>• Decreased production by testicular tissue</td>
</tr>
<tr>
<td>Collection repetition?</td>
<td>Obstruction of sperm output?</td>
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<tr>
<td>• Too much collections (Distal droplets)</td>
<td>• Ampullae</td>
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</tbody>
</table>
4. Semen analysis

• Non sperm cells:
  – Diff Quick staining (not for concentration)
  – Neutrophils:
    • Leukospermia? (5 \texttimes 10^6/ml)
    • Testis & epididymis – Accessory glands
  – Red Blood Cells:
    • Origin: Testis & epididymis – Accessory glands – Penis
  – Epithelial debris:
    • Effect on freezability?

• Viability:
  
  **Eosin-nigrosin**
  
  - Live: white: Not stained by eosin
  - Dead: pink Stained by eosin

  **Nucleo Counter**
  
  - Dilution of semen in PBS
  - PI enters in damaged spz
  - Concentration of dead spz
  - Viability = (1 - concentration dead) / concentration tot

Flow cytometry
4. Semen analysis

• Motility:

- Concentration
- Individual effect?

<table>
<thead>
<tr>
<th>Definition</th>
<th>Calculation</th>
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<tr>
<td>VCL</td>
<td>Velocity Curvilinear Path</td>
</tr>
<tr>
<td>VSL</td>
<td>Velocity Straight Line Path</td>
</tr>
<tr>
<td>VAP</td>
<td>Velocity Average Path</td>
</tr>
<tr>
<td>LIN</td>
<td>VSL/VCL</td>
</tr>
<tr>
<td>STR</td>
<td>VSL/VAP</td>
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<tr>
<td>WOB</td>
<td>VAP/VCL</td>
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4. Semen analysis

• Motility:

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<thead>
<tr>
<th></th>
<th>Direct filling</th>
<th>Capillarity filling</th>
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<tbody>
<tr>
<td>Total Motility</td>
<td>• VAP &gt;10-15µm/s</td>
<td>• VAP &gt;15µm/s</td>
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<tr>
<td></td>
<td>• VAP &gt;20µm/s</td>
<td></td>
</tr>
<tr>
<td>Progressive Motility</td>
<td>• VAP &gt;10-15µm/s &amp; STR &gt;100%</td>
<td>• VAP &gt;30µm/s &amp; STR &gt;50%</td>
</tr>
<tr>
<td></td>
<td>• VAP &gt;40µm/s &amp; STR &gt;80%</td>
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4. Semen analysis

• Motility:
  – Before conclusions: assess end of flush of epididymal storage
  – Cut-off values (?):
    • Total motility: >70%
    • Progressive motility: >50-60%
  – Conclusions:
    • End of epididymal storage?
    • 1 dose of fresh semen = 500x10^6 progressive spz
    • Needs?

4. Semen analysis

• Morphology:
  – Smearing & Diff Quick
  – Inactivation & Droplet
  – Count 200spz
  – Abnormal forms

• Abnormal forms
4. Semen analysis

• Morphology:
  – Primary abnormalities:
    • Abnormalities of spz formation inside testis

– Secondary abnormalities:
  • Abnormalities of:
    – Maturation
    – Collection
    – Manipulation (cold chock)

– Case of distal droplets:
  • Maturation of spz in epididymis:
    – Proximal > Distal > No droplet
    – If too much collections: recruitment of immature spz in epididymis
    – Correlate these data with observed and estimated DSO
4. Semen analysis

• Flow cytometry

4. Semen analysis

• Assays in semen:
  – Alkaline Phosphatase:
    • Secreted by testis and epididymis
    • If no spz in semen:
      – AP high: real aspermia
      – AP low: obstruction of semen output
  – Creatinine – Urea: suspicion of urinary contamination
  – Assay in urine:
    • Suspicion of retrograde ejaculation
    • Urinary catheterism, centrifugation and pellet exam
5. Endoscopy:
- Inflammation of vesicular glands:
  - Endoscopy of genital tract
  - Cathetersim of excretion way of vesicular glands
  - Flush, cytology and bacteriology

6. Biopsy:
- Lesions in hemato-spermatic bareer > risk of infertility
- Fine needle aspiration: easy but interpretation = ?
- Real biopsy: surgery but diagnosis & prognosis

7. Hormonal assays:
- Testosterone assay: hCG stimulation
- Oestrone sulfate?

8. Conclusions

VS other observed clinical data
VS required activity of stallion
Flush of epididymal storage
Semen of 3-6 collections
Conclusions
Questions?