Fertility of cooled and transported semen based on field studies and how to optimize the storage of equine semen

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Outline:

Semen collection & sperm quality

Fresh and cooled-transported semen

Equine semen cryopreservation:

i. Inter- and intraindividual variation of semen quality and cryosurvival

ii. Centrifugation processing for sperm selection and removal of seminal plasma

iii. Composition of the extender used, for primary dilution as well as cryopreservation

iv. Cooling and freezing protocols

Insemination strategies

Assisted Reproductive Technologies in the German Riding Horse Industry.

Preparation prior to freezing

season

semen collection

- technique

- interval

Development of Reproductive Technologies in the German Riding Horse Industry.

Updated 2017; ref.: BMELV / TGDDEU / LWK Niedersachsen

Equine Reproductive Biotechnologies in Germany
Regulations and specifications for collecting equine sperm

- semen collection
  - technique
    - closed AV
    - fractional
    - open AV
    - Equidame™
  - mounting
    - by mating
    - dummy
    - mare
    - ground (standing)
    - pharmacologically induced
  - sort and preparation of collection devices
    - safety
    - preparation before collection
      - temperature
      - coating (siliconized), rinsed by ext.
      - preload of extender
  - seminal plasma

- semen collection & fertility

- food supplementation:
  - adapted from DEER 2014 & DEER 2016
  - Hoogewijs et al.: Equi Sperfor Plus®
    - whey protein, Tribulus terrestris, Lepidium meyenii,
      Vaccinium macrocarpon, omega-3 fatty acids,
      vitamins, amino acids, antioxidants
  - Blomfield et al.: antioxidant diet
  - Stowers et al.: FiberProtect®
  - Burger et al.: Equi-Strath®
  - how to proceed
    - consult nutritionist
    - need for supplementation?
      - omega-3 fatty acids (omega-3 DHA > omega 6 DPA)
      - polyamines: spermine, spermidine
      - vitamin C, E
      - antioxidants eg L-Carnitin
  - for review:

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Insemination strategies

fresh and cooled transported semen

storage at roomtemp. ~17°C vs storage at at +5°C

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>DEER 2014</th>
<th>DEER 2016</th>
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<tbody>
<tr>
<td>NaCl</td>
<td>95 mM</td>
<td>5,552 g/l</td>
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<tr>
<td>KCl</td>
<td>4.7 mM</td>
<td>0.35 g/l</td>
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<tr>
<td>CaCl₂</td>
<td>1.7 mM</td>
<td>0.25 g/l</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.2 mM</td>
<td>0.163 g/l</td>
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<tr>
<td>MgSO₄</td>
<td>1.2 mM</td>
<td>0.296 g/l</td>
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<tr>
<td>NaHCO₃</td>
<td>25 mM</td>
<td>2.1 g/l</td>
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<tr>
<td>D-Glucose</td>
<td>5.6 mM</td>
<td>1.009 g/l</td>
</tr>
<tr>
<td>Natrium-Pyruvat</td>
<td>275 µM</td>
<td>0.03 g/l</td>
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<tr>
<td>Natrium Lactat Sirup 60%</td>
<td>3.7 µl/ml</td>
<td>3.7 ml/l</td>
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<tr>
<td>Hepes</td>
<td>20 mM</td>
<td>4.766 g/l</td>
</tr>
<tr>
<td>Penicillin</td>
<td>50 U/ml</td>
<td>P3032-1MU</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>250 µg/ml</td>
<td>G1914-250MG</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>50 µg/ml</td>
<td>S6501-5G</td>
</tr>
<tr>
<td>Polyvinylalkohol 0.1% (w/v)</td>
<td>10 g/l</td>
<td>P8136-250G</td>
</tr>
<tr>
<td>L-Carnitin</td>
<td>50 mM</td>
<td>8.059 g/l</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>50 µM</td>
<td>17.871 mg/l</td>
</tr>
</tbody>
</table>

fresh and cooled transported semen

- long-term liquid semen preservation
- nucleophilic Thiol: d-Penicillamine

storage at room temp.

storage at +5°C

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3. Composition of the extender used, for primary dilution as well as cryopreservation
4. Cooling and freezing protocols

Insemination strategies

I. Inter- and intra-individual variation of semen quality and cryosurvival

- Tischner (1979)
  - good = 20%; fair = 60%; poor = 20%
  - pms: good = >40%; fair = 20-40%; poor <20%
- Vidament et al. (1997)
  - ≥35%; >33% ejaculates = acceptable;
  - 51% of stallions passed
  - variations: freezing extender, cooling rate, package size, thawing rate
- Loomis (1999)
  - variations: freezing extender, cooling rate, package size, thawing rate
  - 330/348, 96%
- Vidament (2005)
  - ≥35% rapid sperm, VAP ≥30 or 40 µm/s, >33% ejaculates selected

II. Centrifugation processing for sperm selection and removal of seminal plasma

- semen conc. & seminal plasma during storage
  - ≥3:1 dilution rate, seminal plasma ≥25%
  - 25 to 50 µl sperm/mL
  - 400 to 600 g for 10 to 15 minutes resulting in a 75% sperm recovery
  - retain 5 to 20% seminal plasma
- centrifugation
  - dense solution was layered below the extended semen, glucose-EDTA cushion
  - egg yolk containing extender cushion supplemented with 4% glycerol
  - high-speed cushioned centrifugation, 20 min at 1500 g + cushion fluid
  - small volume (30 µl) of cushion solution is placed at the bottom of a special designed nipple centrifugation tube, 400 x g for 20 min
  - protective effects of iodixanol during bovine sperm cryopreservation
  - seminal plasma removal by SpermFilter® (Ramires-Neto et al. 2008)

References

II. Centrifugation processing for sperm selection and removal of seminal plasma

- "high-speed-cushioned-centrifugation"
- NIPPLE tubes
  - Pesce Lab Sales, Kennett Square, PA, USA
  - 40-ml capacity glass nipple-bottom centrifugation tubes
- CONICAL tubes
  - Corning Life Sciences, Lowell, MA, USA
  - 50-ml capacity polypropylene conical-bottom centrifuge tubes


III. Composition of the extender used, for primary dilution as well as cryopreservation

- primary dilution
  - centrifugation-ext.: saline+sugars: glucose-EDTA, Sucrose, Citrat-EDTA (modif.) SKME(E), D11, commercial extenders
  - dilution ratio: 1+1; 50 million sperm/ml

III. Composition of the extender used, for primary dilution as well as cryopreservation

- cryoprotective agents (CPAs):
  - are not equally effective in cryopreserving
  - can be penetrating / non-penetrating
  - do not have similar toxicity
  - concentrations can be reduced by addition of other solutes that affect ice formation
  - e.g. sugars, dextrans, PVP, PEG, anti-freeze proteins,
  - ice-blockers (non protein synthetic polymers)

IV. Composition of the extender used, for primary dilution as well as cryopreservation

- permeating cryoprotectants (cpa):
  - interplay between cpa concentration and cooling rate

III. Composition of the extender used, for primary dilution as well as cryopreservation

- BotuCrio®
  - http://www.nidacon.com/animal/botucrion
- Equi Plus CryoGuard®
- French system: 37Milk/22E2
- INRA-Freeze Package®
- SBS-system

III. Composition of the extender used, for primary dilution as well as cryopreservation

- selection of spermatozoa
  - migration
  - migration-sedimentation
  - filtration
  - glass wool
  - glass beads
  - Sephadex
  - density colloid centrifugation
    - Percoll
    - EquiPureTM and EquiPureTM Pro
    - Single Layer Centrifugation (SLC)
  - Androcoll®
  - Iodixanol


III. Composition of the extender used, for primary dilution as well as cryopreservation

- Iodixanol

Stuhtmann et al. (2012); Anim. Reprod. Sci. 133: 184–190
III. Composition of the extender used, for primary dilution as well as cryopreservation

- protective agents in extender:
  adopted from ISER 2014, ISSR 2016
  - Gibb et al.: Pyruvate, L-Carnitine
  - Cheek et al.: N-Acetylcysteine
  - Lisboa et al.: Carnitine
  - Aspland et al.: plant-based ingredients
  - Oldenhof et al.: impermeable protectants
  - Rodgers et al.: LowDensityLipoproteins (LDL)
  - Ramires-Neto et al.: motility stimulators
  - Duan et al.: Soybean Lecithin

addition at 22°C
- two-step dilution technique
- slow (drop wise) addition of extender
- final glycerol concentration ~2-2.5%

III. Composition of the extender used, for primary dilution as well as cryopreservation

processing methods
- final sperm concentration
  - 100-200 x 10^6/ml
  - >250 x 10^6/ml straw (AI dose splitting)

packaging
- 0.5 cc straw
- 0.25 cc straw
- Macrotüb®

IV. Cooling and freezing protocols

- freezing rate
  - automatic
    - +20°C -> +5°C: 10°C/min.
    - +5°C -> -15°C: 25°C/min.
    - -15°C -> -120°C: 25°C/min.
    - or: +4°C -> -140°C: 60°C/min.
  - alternatives
    - directional freezing
      - www.coredynamics.com
    - UFT Unique Freezing Technology
      - www.supachillausa.com
    - HHP High Hydrostatic Pressure
      - www.cryoinnovation.com
    - cryoprotectant-free vitrification
      - https://www.minitube.de

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Insemination strategies

- Fresh, cooled (10^6 x 10^7) semen
- Cooled-transported (10^6 x 10^7) semen
- Frozen-thawed (10^6 x 10^7) semen


Insemination strategies

- Fresh, cooled-transported semen
- Cooled-thawed (pre-inj.) semen
- Cooled-thawed (post-inj.) semen


Insemination strategies

- First AI
- Second AI


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