OPU in the mare
How to do it & what to expect
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Why use OPU?
- Chronic sub-fertility in mares
  Unable to recover normal embryos
- Mares in sport
- Mare – euthanasia / death
  – genetic salvage
- Too few viable sperm available
  – Dead stallion (epididymal sperm?)
  – Sexed-sorted sperm (?)
  – Sub-fertile stallion (acquired)
- Host cytoplast for cloning by SCNT

In vitro embryo production
- In vitro maturation
- ICSI
- SCNT
- In vitro embryo culture
- Not trivial procedures!
  – Laboratory expertise & experience
  – Transport to lab?

Oocyte recovery from live mares
- Flank aspiration
  – Pre-ovulatory follicle
- Tranvaginal aspiration
  – Pre-ovulatory follicle
  – Immature follicles > 8 mm
  – Oocytes from very small follicles aren’t capable of yielding a blastocyst

Transvaginal OPU
Double lumen needle: 12g ‘Scrape’; not just aspirate

SBS-AVANTEA 2004-9
123 Donors 290 OPU

\[
\begin{align*}
4845 & = 4845 \\
3150 (65\% & = 10.9/OPU) \\
2029 (64.4\%) & = 2029 \\
1189 (58.6\%) & = 1189 \\
171 (14.4\% & = 0.6/OPU)
\end{align*}
\]

- 145 ETs → 81 pregnancies (56%)
  - Pregnancy loss < day 50 = 20 (25%)
  - Pregnancy loss > day 50 = 9 (11%)
  - 1 ET – no feedback
  - 51 foals born (i.e. 36% pregnancy loss)
Mare preparation

- Enough follicles (8-35mm) ≥12?
  Spring / autumn
- Out-patient <1 h
- Disease tests? CEM/EIA
- Sedation: Epidural or intestinal relaxation
- NSAIDs / antibiotics (sport mares – doping)
- Well tolerated – post-OPU discomfort
- No permanent ovarian damage
  (Velez et al, 2012)

Pre-ovulatory follicle

- 20-35h after hCG / GnRH
- High chance of oocyte recovery (>70%)
- More likely to yield blastocyst
- Only 1-2 per cycle

Not ideal for transport; less resistant to damage

Immature follicles

Sept 2014 – July 2017

- 597 commercial OPUs
- ET flush medium + heparin (20000 IU/L) / oocyte recovery medium
- Flush each follicle 5-10 times
- Mean no. Follicles: 25 (6-82)
- Oocyte recovery: 13.7 (2-56)
  – Efficiency = 55% (20-100%)
- Mare (age) effect (Claes et al 2016)
Transport of equine ovaries for assisted reproduction

- Transport of ovaries for <7h
  - No effect on oocyte competence
- Transport of ovaries for 7h
  - No effect on IVM (%MII)
  - Reduced blastocyst %
- Transport of ovaries for 20h
  - Compromised MII & blastocyst %

Follicle aspiration / scrape

- Incise follicle, scrape with bone curette, flush
- High recovery rate (65-100%)

After euthanasia or death

- Remove connective tissue
- Clean in sterile PBS / Lactated Ringer’s
- Embryo flush medium

Oocyte searching

- Filter, wash, transfer to petri dish
Oocyte collection:

Packaging for transport

- H-SOF or embryo holding medium

Packaging:

- Organ transport box

All materials pre-incubated at 25°C

Packaging: Organ transport box

- For < 24h - oocytes arrive at >20°C
- Delay (48h) – 23 OPUs / 11 embryo's (0.48)

Other transport systems

Equitainers

- Work well at warm ambient temperature
- Didn’t maintain 20°C for 24h when it was cold (MII but blastocysts)
**MicroQ iQ1E**

- Program temp (22°C)
- 96h battery life
- Blastocysts after 48h
- Fewer blastocysts - 24h?

**Transporting oocytes?**

- Transport at 22°C for < 24h
  - Good for GV (immature) oocytes
  - MII oocytes do not tolerate holding well
- Pre-ovulatory follicles
  - 37°C in IVM medium (Foss et al, 2008)
- Holding at 4°C OK? (Dini et al, 2016)
- Our experience - drop below ~18°C
  - No effect on IVM / reduced blastocysts
- Oocyte cryopreservation
  - MII spindle damage / few blastocysts

**Effect of transport on results?**

<table>
<thead>
<tr>
<th></th>
<th>On-site immediate IVM</th>
<th>Oocytes shipped at 20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPUs (mares)</td>
<td>202 (82)</td>
<td>158 (102)</td>
</tr>
<tr>
<td>Oocytes</td>
<td>1923 (9.5 per OPU)</td>
<td>2058 (13 per OPU)</td>
</tr>
<tr>
<td>Degenerated</td>
<td>216 (11.2%)</td>
<td>556 (27.0%)</td>
</tr>
<tr>
<td>MII</td>
<td>1400 (72.8%)</td>
<td>1210 (58.8%)</td>
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*Utrecht University and Avantea (Galli et al, 2016: ISEET)*

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<tr>
<td>Cleaved</td>
<td>960 (68.6%)</td>
<td>853 (70.5%)</td>
</tr>
<tr>
<td>Day 6-8 blastocysts (cryopreserved)</td>
<td>238 (17%)</td>
<td>182 (15%)</td>
</tr>
<tr>
<td>Degenerated</td>
<td>216 (11.2%)</td>
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<tr>
<td>Pregnancies</td>
<td>46/66 (69.7%)</td>
<td>28/46 (60.9%)</td>
</tr>
</tbody>
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**Embryo production: 728 OPUs**

- Day 6-8 blastocysts: 741
  - mean = 1.02/13.2% of injected
- Embryos per OPU
  - 0 – 324 x (46%)
  - 1 – 193 x (27%)
  - 2 – 103 x (14.5%)
  - 3 – 60 x
  - ≥4 – 31 x (max. 10)
  - ~1.87 per success
  (Excludes delayed shipments / post-mortem)

**Factors affecting blastocyst production**

- Stallion
  - Individual (4-27%)
  - in vivo fertility
  - Straw (Galli et al, 2016: ISSR)
- Mare
  - Individual (0-41%: 0-3.3 per OPU)
  - Follicle number
  - Fertility status
- Active endometritis
  (Claes et al, 2016: ISEET)
### Pregnancies

<table>
<thead>
<tr>
<th>Year</th>
<th># transfers</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>2015</td>
<td>66</td>
<td>65%</td>
</tr>
<tr>
<td>2016</td>
<td>195</td>
<td>53%</td>
</tr>
<tr>
<td>2017</td>
<td>137</td>
<td>75%</td>
</tr>
<tr>
<td>All</td>
<td>398</td>
<td>62%</td>
</tr>
</tbody>
</table>

**2015-16**
- 18% embryonic death / 48.5% (32) foals
- 24/32 foals (75%) were colts!

### Sex of Foals

- **In-vitro embryos** (n=103)
  - Colt: 72%
  - Filly: 28%
- **In-vivo embryos** (n=126)
  - Colt: 58%
  - Filly: 42%

### Conclusions

- **OPU / ICSI**
  - works well with transported oocytes
  - Sub-fertile & competing mares
  - Stallions – sub-fertile / expensive semen
- **Areas for improvement**
  - Ovarian transport / storage
  - Longer term oocyte storage
  - More embryos of better quality
- **fewer pregnancy losses**

### KWPN stallion show champion 2018!

Thank you for your attention!