In vitro production of equine embryos and genetic testing

In Vitro Production of Equine Embryos and Preimplantation Genetic Testing

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In Vitro Fertilization (FIV)

- Oocytes from OPU or slaughterhouse ovaries
- Sperm sample
- In vitro maturation
- Sperm preparation
- 22-24h in microdroplets of IVFm under mineral oil
- CO₂ incubator

Methodology

In Vitro Maturation of oocytes

- TCM199 + NaHCO₃ + FSH + EGF + IGF + 10% SFB
- 38° C
- Saturated humidity
- 5% CO₂ in air for 24 - 30 hs

Methodology

MII oocytes

SEMEN

Sperm cells
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Methodology

In Vitro culture for 6 to 8 days
5% CO2, 6% O2

ICSI: Factors affecting the success rate

- Donor mare
  - Type of infertility
- Sperm
  - Stallion
  - Sample
- Laboratory setup
  - Strict control of conditions
- Operator

Clinical application of ICSI

- Subfertile stallions
- Subfertile mares
- Semen samples of poor quality, unsuitable for AI
- Limited amount of semen samples
- Mare or stallions that die unexpectedly
- Mares in training
- Young mares

Expected results

10 oocytes
7 mature oocytes
4.5 cleaved embryos
0.9 blastocysts
0.54 pregnancies

70% IVM
65% cleaved after ICSI
20% blastocyst rate
60% pregnancy rate

Clinical application of ICSI
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Ovum Pick Up (OPU) – Intracytoplasmic Sperm Injection (ICSI).

- OPU-ICSI is performed throughout the year, every 14 days
- Only one straw of semen can be used to fertilize more than 100 oocytes
- Each straw can be thawed, diluted x 100 and re-frozen
- Equine embryos produced by ICSI can be successfully cryopreserved

Preimplantation Genetic Testing: Methodology

1) Obtain a result after the genetic analysis, starting from a very small sample.
2) Maintain the viability of the embryo after biopsy and cryopreservation.

Materials and Methods

Biopsy
In vitro production of equine embryos and genetic testing

**Embryo biopsy vs. blastocoele fluid**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Amplified</th>
<th>% Amplified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryo biopsy</td>
<td>13</td>
<td>11</td>
<td>84.50%</td>
</tr>
<tr>
<td>Blastocoele fluid</td>
<td>34</td>
<td>29</td>
<td>85.20%</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>40</td>
<td>85.10%</td>
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</tbody>
</table>

- The collection of blastocoele fluid can be used in combination with vitrification of equine large embryos

**Preimplantation Genetic Diagnosis (PGD)**

- Results reported in:
  - Argentina
  - Colombia
  - Germany
  - Spain
  - Sweden
  - USA
- Main application:
  - Embryo sex determination
- Other applications:
  - Detect mutations associated with genetic disorders
  - Select genes linked to certain phenotypes (e.g., coat color)

**WHY?**

Transfer only embryos of the desired sex or carrying the desired genetic trait.

Significantly reduce the number of recipient mares.

Avoid the need to abort pregnancies of the undesired sex.

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**Pregnancy rates at 25 days of gestation**

<table>
<thead>
<tr>
<th></th>
<th>Embryos</th>
<th>Time before ET</th>
<th>n</th>
<th>Pregnancy rate (%)</th>
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</thead>
<tbody>
<tr>
<td>Biopsied</td>
<td>1 – 2 h</td>
<td>58</td>
<td>34</td>
<td>58 / 58 (58.62)</td>
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<tr>
<td></td>
<td>6 – 10 h</td>
<td>46</td>
<td>29/46 (63.04)</td>
<td></td>
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<tr>
<td>Non-Biopsed</td>
<td>1 – 2 h</td>
<td>35</td>
<td>23/35 (65.71)</td>
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</tr>
<tr>
<td></td>
<td>6 – 10 h</td>
<td>6</td>
<td>3/6 (50)</td>
<td></td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>89/145 (61.37)</td>
<td></td>
</tr>
</tbody>
</table>

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**SRY**

Sex determination in males
429 bp (males)

**AMELOGENIN**

Protein involved in teeth enamel
184 bp (female)
160 y 200 bp (male)

**PRIMER**

<table>
<thead>
<tr>
<th>SEQUENCE</th>
<th>REFERENCE</th>
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<tbody>
<tr>
<td>SRY-R</td>
<td>Hasegawa et al, 2000</td>
</tr>
<tr>
<td>AMEL-F</td>
<td>Herrera et al, Theriogenology 2014</td>
</tr>
<tr>
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</tr>
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100 embryos
50 female 50 male

PCR (85%)

42.5 female 42.5 male

42.5 + 35 = 57.5 ETs

100 ETs

THANK YOU