In vitro Embryo Production in Calves

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Genetic Improvement

In 1987, the concept of MOET programs was introduced by the University of Guelph.

Genetic Change = Sel. Int. x Acc. x $h^2$

Generation Interval

*Shortening generation intervals will impact genetic gain

What role will genomics play?
IVF Terminology

OPU - Ovum pick-up, or ultrasound-guided oocyte aspiration
LOPU – Laparoscopic ovum pick-up
IVM - In vitro maturation
IVF - In vitro fertilization
IVC - In vitro culture
IVP/IVEP - In vitro production of embryos
The effect of follicle size on OPU/IVF results

Figure 1. Relation between the percentage of transferable embryos (morula or blastocysts of Quality 1 or 2) produced after 7 or 8 days of in vitro culture vs follicle diameter of the majority of the follicles (> 2/3) at the time of OPU. (n = number of OPU sessions).

Blondin and Vigneault, 2013
Treatment with gonadotrophins for OPU

50 mg P4
2.5 mg EB

Treatments with FSH

Progestin device

Coasting 40 hours

OPU

Blondin et al., 2012   Lonergan and Fair 2008
In vitro embryo production in calves

Background:

1) Attempts to produce “in vivo” embryos from calves have been unsuccessful
   - small percentage of superstimulated follicles ovulated
   - only unfertilized ova were recovered
2) In 1990’s, IVF with calf oocytes was attempted with variable success
   - initial efforts produced rather poor results
   - low developmental capacity (<10%)
   - wasn’t possible to predict breeding value at such a young age
   - technology was abandoned
4) Today OPU/IVF technology and culture conditions has improved greatly
5) Reproducing animals at early ages now possible through genomic testing
   - development of genetic markers that allow the prediction of the
     production phenotype of calves
6) However, hormonal regimes applied to calves must be tested and improved
   - must recover oocytes that are competent for full development
   - Age and gonadotropin stimulation would seem to be important
General procedures in calves
(2 to 6 months of age)

- Insert small ruminant vaginal device
- Initiate FSH treatments after 2 days (dose?)
- Twice daily FSH treatments for 48 – 72 hours
- Coasting periods range from 12 to 36 hours
- LOPU after coasting period
- After LOPU, wash ovaries with warm saline
- Suture or glue holes in abdomen
- Repeat in approximately 2 weeks
Preparation for Laparoscopy (LOPU)

1) Fasting  
2) Anaesthesia  
3) Clipping and surgical prep.  
4) Laparoscopic equipment  
5) Closure of incisions  
6) Recovery from anaesthesia
Transvaginal OPU in prepubertal calves

➢ Standing position:
➢ Infrastructure – squeeze chute - very important
➢ Epidural! – good epidural
➢ Operator – arm and hand size – smaller is better!
➢ Little manipulation as possible – little space in the rectum for manipulation
➢ Needle, tubing, flow rate... same as for adult animals!
➢ Handle for ultrasound probe and needle guide - special size for calves

Adult animals ➔

Calves ➔
Quantity and quality of follicles in *Bos taurus* and *Bos indicus*
AFC and ovarian response in prepubertal calves

In adult cattle, the number of follicles recruited into successive follicular waves is consistent within individuals;

Among individuals, however, the ovarian response to gonadotropin stimulation is highly variable

**Objective:** to compare the ovarian response to superstimulation in calves with high vs low AFC at wave emergence
Numbers of follicles by antral follicle counts

Total number of follicles ≥3 and ≥6 mm were greater in High than Low AFC group. High AFC group had a greater number of 6-8 mm follicles at oocyte collection than Low AFC group.
Plasma anti-Müllerian hormone as a predictive endocrine marker to select *Bos taurus* (Holstein) and *Bos indicus* (Nelore) calves for in vitro embryo production

E.O.S. Batista\textsuperscript{a,\*}, B.M. Guerreiro\textsuperscript{a}, B.G. Freitas\textsuperscript{a}, J.C.B. Silva\textsuperscript{b}, L.M. Vieira\textsuperscript{a}, R.M. Ferreira\textsuperscript{c}, R.G. Rezende\textsuperscript{a}, A.C. Basso\textsuperscript{d}, R.N.V.R. Lopes\textsuperscript{e}, F.P. Rennô\textsuperscript{f}, A.H. Souza\textsuperscript{g}, P.S. Baruselli\textsuperscript{a}
Impact of age on cleavage and development to the blastocyst stage of oocytes collected from calves of 2-6 months of age.

Graph showing embryo development (%):
- Cleavage
- Blastocysts/Oocytes
- Blastocysts/Cleaved

Legend:
- ■ <100 Days
- □ 100 to 130 Days
- □ >130 Days

AB - P<0.01

Baldassarre et al., 2018
Normal fertilization and polyspermy rates among groups
Normally Fertilized = 2 polar bodies and 2 pronuclei. Polyspermic = 3 or more pronuclei. Unfertilized = Metaphase II.  
A,B - P<0.05.
Pregnancy rate of calf embryos produced in vitro (2015)

<table>
<thead>
<tr>
<th>Cycling Heifers (2 year)</th>
<th>29/56</th>
<th>51.8%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heifer Calves (2 to 4 months)</td>
<td>13/30</td>
<td>43.3%</td>
</tr>
</tbody>
</table>
Progesterone priming effect on oocyte quality and in vitro embryo production of prepubertal Nelore heifers
1) Group **Negative Control** (prepubertal heifers; n=10)

D0

2) Group **Short P4 primer** (n=11)

D0

3) Group **Short and Long P4 primer** (n=11)

D0 - D7 - D14

4) Group **Positive Control** (cycling cows; n=10)

D0 - D24 - D31
BLASTOCYST RATE/VIABLE OOCYTE (%)

P < 0.001

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blastocyst Rate (%)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>15%</td>
<td>10</td>
</tr>
<tr>
<td>Short P4 primer</td>
<td>18%</td>
<td>10</td>
</tr>
<tr>
<td>Short &amp; long P4 primer</td>
<td>29%</td>
<td>11</td>
</tr>
<tr>
<td>Positive control</td>
<td>26%</td>
<td>8</td>
</tr>
</tbody>
</table>
NUMBER OF BLASTOCYSTS/ OPU

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total (N)</th>
<th>N=11</th>
<th>N=10</th>
<th>N=10</th>
<th>N=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>5.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short P4 primer</td>
<td>4.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short &amp; long P4 primer</td>
<td>4.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>4.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P = 0.732
Effect of FSH treatment in OPU programs in prepubertal Holstein heifers

Bruna Martin s Guerreiro
Pietro Sampaio Baruselli
2015
Experimental Design

- **CONTROL** (n = 20 puberal heifers)
  
  EB (2mg) + 2mL PROSTAGLANDIN

  P4 Device

  D0

  D7am

- **FSH 160 mg** (n = 20 puberal heifers)
  
  EB (2mg) + 2mL PROSTAGLANDIN

  FSH (46mg)  FSH (46mg)  FSH (34mg)  FSH (34mg)

  P4 Device

  D0

  D4am  D4pm  D5am  D5pm  D7am

  Coasting 36 hours
### Effect of FSH treatment in OPU programs in prepubertal Holstein heifers

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Follicles aspirated, n</td>
<td>9,0</td>
<td>25,6</td>
</tr>
<tr>
<td>Recovery rate, n</td>
<td>70,0</td>
<td>40,0</td>
</tr>
<tr>
<td>Total oocytes recovered, n</td>
<td>6,5</td>
<td>10,2</td>
</tr>
<tr>
<td>Viable oocytes, n</td>
<td>3,3</td>
<td>5,8</td>
</tr>
<tr>
<td>Percentage viable, %</td>
<td>51,3</td>
<td>57,4</td>
</tr>
<tr>
<td>Cleaved embryos, n</td>
<td>2,5</td>
<td>5,2</td>
</tr>
<tr>
<td>Cleavage rate, %</td>
<td>55.1</td>
<td>84.4</td>
</tr>
<tr>
<td>Percentage blastocysts, %</td>
<td>33.3</td>
<td>58.6</td>
</tr>
<tr>
<td>Blastocysts, n</td>
<td>1.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

1 (No. Oócitos totais/folículos aspirados)*100
2 (No. oócitos viáveis/oócitos totais)*100
3 (No. oócitos clivados/Oócitos viáveis)*100
4 (No. blastocistos/Oócitos viáveis)*100
Quality of oocytes and in vitro embryo production from Nelore heifers

3 mo old heifers - P4 (n=8)

3 mo old heifers - P4+FSH (n=8)

Puberal heifers Positive control (n=8)

LOPU (laparoscopy)

Evaluation of oocyte quality, IVEP
Treatment groups and experimental design

1. Group heifers with **P4** (n = 8)

   - D0
   - CIDR®
   - D7

   ![CIDR® with P4 treatment](chart)

   - **LOPU Laparoscopy (AM)**

2. Group heifers with **P4 + FSH** (n = 8)

   - D0
   - CIDR®
   - D5am, D5pm, D6am, D6pm, D7am

   ![CIDR® with P4 + FSH treatment](chart)

   - **FSH (30mg), FSH (30mg), FSH (20mg), FSH (20mg)**
   - **LOPU Laparoscopy (AM)**

3. Group puberal heifers **Positive Control** (n = 8)

   - D0
   - D7

   ![CIDR® with Positive Control](chart)

   - **OPU Transvaginal (AM)**
<table>
<thead>
<tr>
<th>Item</th>
<th>Calves without FSH</th>
<th>Calves with FSH</th>
<th>Cycling heifers</th>
<th>P value&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total follicles visualized</td>
<td>19.7 ± 4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.3 ± 5.9&lt;sup&gt;y&lt;/sup&gt;</td>
<td>47.1 ± 6.3&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>Total COCs retrieved</td>
<td>13.5 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.9 ± 5.1&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>29.9 ± 5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.04</td>
</tr>
<tr>
<td>Recovery rate (%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>68.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>COCs cultured</td>
<td>4.7 ± 1.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.3 ± 4.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.1 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>COCs cultured rate (%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>35.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>Cleavage rate (%)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>47.0</td>
<td>52.2</td>
<td>50.3</td>
<td>0.41</td>
</tr>
<tr>
<td>Blastocysts produced</td>
<td>1.7 ± 0.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.3 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blastocyst rate (%)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>12.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>30.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>d</sup>Data with different superscripts in the same line differ with P ≤ 0.05 (a ≠ b ≠ c) or P ≤ 0.06 (x ≠ y ≠ z). <sup>e</sup>Total number of COCs/number of follicles aspirated. <sup>f</sup>Number of COCs cultured/number of follicles aspirated. <sup>g</sup>Number of cleaved zygotes/ number of COCs. <sup>h</sup>Number of blastocyst/number of COCs.
In vitro embryo production of Holstein calves

Heifers 2-4 mo (n=24)

Puberal heifers 13-16 mo (n=8)

LOPU - Laparoscopy (calves)

OPU - Transvaginal guided OPU (puberal heifers)

IVEP
1. Group heifers with **P4** (n = 8)

   - **CIDR®**
   - D0 to D7
   - LOPU Laparoscopy (AM)

2. Group heifers with **P4 + FSH** (n = 8)

   - **CIDR®**
   - D0 to D7
   - FSH (30mg) at D5am, D5pm
   - FSH (30mg) at D6am
   - FSH (20mg) at D6pm
   - LOPU Laparoscopy (AM)

3. Group puberal heifers **Positive Control** (n = 8)

   - OPU Transvaginal (AM)
   - D0 to D7
Table 2. Number of visualized follicles, COCs and blastocysts (mean ± SEM) after LOPU-IVP in *Bos taurus* (Holstein) donor calves and after OPU - IVP in *Bos taurus* (Holstein) cycling heifers.

<table>
<thead>
<tr>
<th></th>
<th>Calves without FSH</th>
<th>Calves with FSH</th>
<th>Cycling heifers</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total follicles visualized</td>
<td>22.7 ± 4.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>54.3 ± 9.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.9 ± 3.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>Total COCs retrieved</td>
<td>11.7 ± 2.4&lt;sup&gt;bx&lt;/sup&gt;</td>
<td>22.4 ± 5.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.2 ± 1.7&lt;sup&gt;cy&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Recovery rate (%)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>51.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>41.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>COCs cultured</td>
<td>3.6 ± 1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>12.3 ± 3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.7 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>COCs cultured rate (%)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>30.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>37.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>51.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>Cleavage rate (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>17.8</td>
<td>30.5</td>
<td>26.1</td>
<td>0.47</td>
</tr>
<tr>
<td>Blastocyst produced</td>
<td>0.4 ± 0.2</td>
<td>0.7 ± 0.4</td>
<td>0.5 ± 0.3</td>
<td>0.78</td>
</tr>
<tr>
<td>Blastocyst rate (%)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.9</td>
<td>2.0</td>
<td>4.3</td>
<td>0.60</td>
</tr>
</tbody>
</table>

<sup>d</sup>Data with different superscripts in the same line differ with P ≤ 0.05 (a ≠ b ≠ c) or P ≤ 0.06 (x ≠ y ≠ z). <sup>e</sup>Total number of COCs/number of follicles aspirated. <sup>f</sup>Number of COCs cultured/number of follicles aspirated. <sup>g</sup>Number of cleaved zygotes/ number of COCs. <sup>h</sup>Number of blastocyst/number of COCs.
Oocyte quality and IVEP in prepubertal and pubertal heifers
Experimental design

• Nelore heifers

Prepubertal
(8 to 12 mo)
n=24

Prepubertal
(18 to 22 mo)
n=20

Pubertal
(22 to 26 mo)
n=25
Table 4. Number of visualized follicles, COCs and blastocysts (mean ± SEM) after OPU - IVP in *Bos indicus* (Nelore) prepubertal and pubertal and heifers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Prepubertal heifers (8 - 12 month) (n = 24)</th>
<th>Prepubertal heifers (18 - 22 month) (n = 20)</th>
<th>Pubertal heifers (22 - 26 month) (n = 25)</th>
<th>P value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ciclicality&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total follicles visualized</td>
<td>19.7 ± 2.1</td>
<td>41.3 ± 5.28</td>
<td>34.0 ± 3.3</td>
<td>&lt;0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Total COCs retrieved</td>
<td>13.4 ± 1.7</td>
<td>30.8 ± 5.8</td>
<td>22.6 ± 3.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total COCs cleaved</td>
<td>5.6 ± 0.8</td>
<td>14.8 ± 2.5</td>
<td>13.3 ± 1.9</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>COCs cultured</td>
<td>7.6 ± 1.0</td>
<td>16.8 ± 2.7</td>
<td>15.1 ± 2.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>COCs cultured rate (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>57.0</td>
<td>54.0</td>
<td>60.0</td>
<td>0.13</td>
<td>0.45</td>
</tr>
<tr>
<td>Cleavage rate (%)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>73.0</td>
<td>88.0</td>
<td>84.0</td>
<td>&lt;0.0001</td>
<td>0.25</td>
</tr>
<tr>
<td>Blastocysts produced</td>
<td>1.5 ± 0.3</td>
<td>4.7 ± 0.9</td>
<td>7.2 ± 1.2</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Blastocyst rate (%)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>20.2</td>
<td>28.1</td>
<td>47.0</td>
<td>0.05</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>a</sup>Effect of evaluated group.  
<sup>b</sup>Effect of age in the prepubertal group (8-12 month vs. 18-24 month).  
<sup>c</sup>Effect of cyclicity (cyclic vs. non cyclic).  
<sup>d</sup>Number of viable oocytes/number of total oocytes.  
<sup>e</sup>Number of cleaved oocytes/number of cultured oocytes.  
<sup>f</sup>Number of blastocysts/number of cultured oocytes.
In vitro embryo production of prepubertal Holstein donors

Prepubertal heifers
8-10 m (n=32)

Pubertal heifers
12-14 m (n=32)

Lactating cows
(n=32)

Non-lactating cows
(n=32)

OPU + US
Oocyte quality and IVF
Table 3. Number of aspirated follicles, oocytes and embryos produced after OPU-IVP in prepubertal and pubertal heifers, and in lactating and non-lactating cows from the Holstein breed. Data is presented as mean ± standard error mean.

<table>
<thead>
<tr>
<th>Item</th>
<th>Heifer</th>
<th>Cows</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Prepubertal</td>
<td>Pubertal</td>
<td>Lactating</td>
</tr>
<tr>
<td>N</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Total follicles aspirated</td>
<td>18.3 ± 2.1</td>
<td>17.3 ± 1.2</td>
<td>14.0 ± 1.0</td>
</tr>
<tr>
<td>Total COC retrieved</td>
<td>14.2 ± 2.2</td>
<td>13.1 ± 1.1</td>
<td>9.8 ± 1.1</td>
</tr>
<tr>
<td>COCs cultured</td>
<td>10.5 ± 1.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.3 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.5 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cleavage rate (%)&lt;sup&gt;2&lt;/sup&gt;</td>
<td>68.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blastocysts produced</td>
<td>0.5 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1 ± 0.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Blastocysts rate (%)&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>COC - cumulus oocyte complex. <sup>2</sup>Number of cleaved embryos/viable COCs. <sup>3</sup>Number of blastocysts/viable COCs. <sup>a,b,c</sup>Different letters within rows indicate statistical difference (P < 0.05).
Impact of follicle size in calves on the developmental capacity of oocytes collected from follicles larger and smaller than 5 mm diameter.

AB, CD - P<0.05

Baldassarre et al., 2018
Effect of FSH treatment on follicle sizes in prepubertal Holstein heifers

![Graph showing the effect of FSH treatment on follicle sizes in prepubertal Holstein heifers. The graph compares the proportion of follicles in three size categories: Fols < 6 mm, Fols 6-10 mm, and Fols > 10 mm. The FSH group has a higher proportion of smaller follicles (39.6%) and fewer larger follicles compared to the control group (88.9% for Fols < 6 mm).]
Effect of length of FSH treatment in heifer calves on the number and size of follicles available for aspiration.

<table>
<thead>
<tr>
<th>FSH Treatment</th>
<th>Average Follicles Aspirated per donor/per LOPU session</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>No Treatment</td>
<td>19.8±9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Short Treatment (36 h)</td>
<td>20.7±11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Long Treatment (72 h)</td>
<td>16.9±10&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results in the same column with different superscript differ significantly (P < 0.05).

Currin et al., 2017
Effect of length of gonadotropin stimulation on the number of oocytes recovered

A, B - $P < 0.05$

Currin et al., 2017
Cleavage and in vitro embryo development by treatment. Morula and Blastocyst rates calculated as a function of total number of oocytes in IVC

A, B - P<0.05

Currin et al., 2017
Short (4 days) vs Long (7 days) protocol

- Extending the gonadotropin treatment from 4 to 7 days in pubertal cattle resulted in:
  - Greater number of follicles
  - 2.5 times more transferable embryos per animal

*Dias et al., 2013*

**Objective:** to examine dose and duration of superstimulatory treatment in 6-month-old prepubertal calves.

---

**TREATMENTS**

<table>
<thead>
<tr>
<th>Duration</th>
<th>200mg</th>
<th>350mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 days (Short)</td>
<td>n=6 25mg/ inj</td>
<td>n=6 44mg/ inj</td>
</tr>
<tr>
<td>7 days (Long)</td>
<td>n=6 14mg/ inj</td>
<td>n=5 25mg/ inj</td>
</tr>
</tbody>
</table>

*Krause et al., ICAR 2016*
Number of follicles according to FSH dose

Higher FSH dose resulted in a greater number of follicles ≥6 and ≥9 mm

Krause et al., ICAR 2016
Number of follicles and diameter of the five largest follicles by duration of FSH treatment

"Number of follicles ≥6 mm"

"Number of follicles ≥9 mm"

"Diameter of 5 largest follicles (mm)"

✓ Longer duration of gonadotropin treatment resulted in a greater number of follicles and larger mean follicle diameter

**Conclusion:** Extending the period of treatment from 4 to 7 days with a total dose of 350mg FSH was able to induce a greater number of large follicles at the end of the superstimulatory treatment.
Impact of eCG in a gonadotropin treatment protocol on cleavage and development to blastocyst of oocytes collected from 2 to 6 months old calves.

AB - P<0.05

Baldassarre et al., 2018
Summary

➢ In at least one study the LOPU procedure was safe after repeating 8 times before females were size-suitable for ultrasound-guided OPU
➢ However, transvaginal ultrasound-guided OPU is possible in calves
➢ AFC are repeatable in calves and correlate with AMH circulating levels
➢ The number of follicles at the beginning of a wave is predictive of the number recruited into subsequent waves in prepubertal calves
➢ Calves with a high AFC had more follicles available for oocyte collection after superstimulation than calves with a low AFC
➢ Cyclicity increases COC recovery and blastocyst production in heifers
➢ Transfer of embryos derived from calf oocytes to adult recipients resulted in pregnancies and development to term comparable with those from adults, but there are still exceptions
➢ Progesterone priming improves blastocyst production in prepubertal heifers
Summary (continued)

➢ Longer gonadotropin stimulation of 2-6 month old Holstein calves resulted in a higher proportion of larger follicles available for aspiration, a larger proportion of usable oocytes and greater blastocyst rates.

➢ Higher FSH doses increase numbers of follicles 6-10 mm in diameter, cleavage rates, blastocyst rates and number of blastocysts.

➢ The addition of eCG improved ovarian superstimulation for COC recovery and blastocyst production.

➢ These protocols repeated every 2 weeks could result in 10-30 transferable embryos before donors are 6 months of age.

➢ This could translate into a potential for production of 5-15 offspring that will be born before the donor calf reaches breeding age.
Thank you

Obrigado