Influence of length of p-FSH treatment prior to ovum pick-up on ovarian response and *in vitro* embryo production in Holstein heifers

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**INTRODUCTION**

- Ovarian follicle stimulation with exogenous FSH prior to ovum pick-up (OPU) in *Bos taurus* females is a common practice to increase *in vitro* embryo production (IVP).
- The optimal stimulatory period length for OPU-IVP, however, has not been definitively ascertained.

**OBJECTIVE**

The objective of the present study was to determine the effect of length of the superstimulatory treatment period prior to OPU on ovarian response and IVP in Holstein heifers.

**METHODS**

- Non-pregnant heifers (n = 57) 13.8 ± 0.2 months of age with a body condition score of 3.0 ± 0.1 (scale 1 to 5) were assigned in a completely randomized design to one of the following experimental groups (Figure 1):
  - FSH2d – 200 mg of FSH (Folltropin®V, Vetoquinol) distributed in four injections (60, 60, 40, and 40 mg) of FSH 12 h apart
  - FSH3d – 200 mg of FSH distributed in six injections (40, 40, 40, 40, 20 and 20 mg) of FSH 12 h apart
- Superstimulatory treatments were initiated 36 h after dominant follicle removal
- An intravaginal progesterone (P4) implant (1.38 g P4 CIDR®, Zoetis) was inserted at the time of the first p-FSH injection and removed at the time of OPU
- OPU was performed in all heifers 44 h after the last p-FSH injection
- Follicle numbers were determined at OPU and classified as small (< 6 mm), medium (6–10 mm) or large (> 10 mm)
- Oocytes from different size follicles were pooled by heifer at OPU and then classified and subjected to IVP procedures
- Differences between treatment groups were evaluated using generalized linear mixed models (SAS 9.4)

**RESULTS**

**Figure 1. Treatment schedule for heifers superstimulated during 2 or 3 days**

**Figure 2. Proportion of small (<6 mm), medium (6–10 mm), and large (>10 mm) follicles at OPU**

**Table 1. Follicle numbers by size category in heifers superstimulated during 2 or 3 days with 200 mg of p-FSH**

<table>
<thead>
<tr>
<th>Size Category</th>
<th>FSH2d (n = 28)</th>
<th>FSH3d (n = 29)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small Follicles (&lt;6 mm)</td>
<td>5.9 ± 0.6</td>
<td>5.7 ± 0.8</td>
<td>0.83</td>
</tr>
<tr>
<td>Medium Follicles (6–10 mm)</td>
<td>17.0 ± 2.4</td>
<td>12.9 ± 1.6</td>
<td>0.18</td>
</tr>
<tr>
<td>Large Follicles (&gt; 10 mm)</td>
<td>2.5 ± 0.5ª</td>
<td>4.5 ± 0.6ª</td>
<td>0.01</td>
</tr>
<tr>
<td>Total Follicles</td>
<td>25.4 ± 2.6</td>
<td>23.1 ± 1.8</td>
<td>0.60</td>
</tr>
</tbody>
</table>

ª, b Means within a row with different superscripts differ (P < 0.05).

**Figure 3. Oocyte production in heifers superstimulated during 2 or 3 days with 200 mg of p-FSH**

**Table 2. Oocyte recovery rate, percent viable oocytes, cleavage and blastocyst rate in heifers superstimulated during 2 vs. 3 days**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Recovery rate (%)</th>
<th>Viable Oocytes (%)</th>
<th>Cleavage rate (%)</th>
<th>Blastocyst rate (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH2d</td>
<td>62.6 ± 3.7</td>
<td>85.0 ± 2.4</td>
<td>54.7 ± 5.7</td>
<td>20.6 ± 4.0</td>
<td>0.26</td>
</tr>
<tr>
<td>FSH3d</td>
<td>56.9 ± 3.1</td>
<td>88.0 ± 3.0</td>
<td>54.1 ± 5.7</td>
<td>22.4 ± 3.7</td>
<td>0.31</td>
</tr>
</tbody>
</table>

**Figure 4. Blastocyst number in heifers superstimulated during 2 or 3 days with 200 mg of p-FSH**

**DISCUSSION**

In conclusion, lengthening the period of FSH treatment by 1 d increases the number of large follicles (> 10 mm) at OPU, however, does not improve overall ovarian response, oocyte recovery nor embryo production.