COMPARISON OF OOCYTE RECOVERY RATES AND MORPHOLOGY OBTAINED BY OPU AFTER DIFFERENT HORMONAL TREATMENTS IN CATTLE

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The development of efficient methods of ovum pick-up (OPU) and in-vitro production (IVP) systems in recent years, has allowed the cattle industry to have an alternative method of producing embryos than the traditional superovulation and in-vivo production of embryos (Van Wagendonk-De Leeuw 2006). However, the commercial application of IVP systems is greatly dependant on the number and quality of oocytes obtained from a highly valuable donor in a OPU session (Merton et al 2003). Synchronization of follicle wave emergence by ultrasound-guided follicle aspiration has been shown to be an effective way of improving the efficiency of OPU in cattle, and protocols for OPU of donor cows every three to four days have been developed (Merton et al 2003). However, this routine is not very practical for on-farm OPU, especially in countries where farms are separated by long distances. Although some hormonal regimes have been studied to increase oocyte recovery and quality that could be applied in an on-farm situation, there is still controversy about the benefit of synchronizing the emergence of a follicular wave and the use of gonadotropin treatments prior to OPU (Merton et al 2003). A series of experiments were designed to evaluate the effect different treatments of follicle wave synchronization and superstimulation on the number and quality of oocytes aspirated per OPU session in beef cattle.

Synchronization of follicular wave emergence.
In superovulation and in-vivo embryo production programs, one approach to controlling the time of follicular wave emergence involves transvaginal ultrasound-guided ablation of all follicles ≥ 5 mm (Bergfelt et al 1997) or the two largest follicles present in the ovaries (Baracaldo et al 2000). Gonadotropin treatments are initiated at the time of follicular wave emergence, 1 to 2 days later. Another approach to the synchronization of follicular wave emergence for superstimulation is the treatment with estradiol and progesterone (P4). Gonadotropin treatments initiated 4 days after estradiol and P4 have resulted in comparable or higher number of embryos than that of donors superstimulated beginning 8 to 12 d after observed estrus (Bo et al 2002).

A series of experiments were designed to evaluate the effect different treatments of follicle wave synchronization and superstimulation on the number and quality of oocytes aspirated per OPU session in beef cattle. Experiment 1 was designed to test the hypothesis that synchronizing the emergence of follicular wave using estradiol benzoate (EB) and P4 would improve the number and quality of oocytes aspirated by OPU. Brangus (n = 10) and Angus (n = 10) cows were randomly allocated in two treatment groups and treated two times in a cross-over design (all cows received both treatments and all treatments were represented in each OPU session). On Day 0, donors in Group 1 received 2.5 mg EB (Zoovet, Argentina) and 50 mg P4 (Syntex, Argentina) intramuscularly (im); whereas, donors in Group 2 (Control) did not receive any hormonal treatment. On Day 6, OPU was performed by ultrasound-guided follicular aspiration and COCs were classified based on cytoplasm appearance and cumulus cell numbers as described by Chaubal et al (2007). Data was analyzed by ANOVA. The mean number of follicles aspirated on Day 6, total number of oocytes retrieved and the number of viable oocytes were higher (P < 0.05) in cows in Group 1 than is those in Group 2 (table 1).

Table 1. Mean numbers of aspirated follicles, retrieved and viable oocytes in beef donors treated or not with EB+P4.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total Follicles</th>
<th>Total oocytes</th>
<th>Viable Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - EB+P4</td>
<td>20</td>
<td>12.9 ± 1.1b</td>
<td>7.9 ± 1.3b</td>
<td>52 ± 0.9b</td>
</tr>
<tr>
<td>2 - Control</td>
<td>20</td>
<td>7.3 ± 0.7*</td>
<td>3.2 ± 0.5*</td>
<td>21 ± 0.4*</td>
</tr>
</tbody>
</table>

* Means in the same column differ significantly (P<0.05).

Table 1. Mean numbers of aspirated follicles, retrieved and visible oocytes in beef donors treated or not with EB+P4

Synchronization of follicle wave emergence and gonadotropin treatments. Experiment 2 was designed test the hypotheses that: 1) induction of luteolysis three days prior to aspiration (to avoid the presence of the CL at the time of OPU) and 2) treatment with equine chorionic gonadotropin (eCG) (to stimulate follicle development), would improve the number and quality of oocytes aspirated by OPU. Brangus (n = 20) cows were randomly allocated into three treatment groups and treated three times in a cross-over design. On Day 0, all donors received 2.5 mg EB and 50 mg P4 im. On Day 4, donors in Group 1 received 150 µg D(+)-cloprostenol (PGF, Ciclar, Zoovet, Argentina) and 50 mg P4 (Syntex, Argentina) intramuscularly (im); whereas, donors in Group 2 (Control) did not receive any hormonal treatment. On Day 7, OPU was performed and COCs were classified as in Experiment 1. The number of follicles aspirated on Day 7, total number of oocytes retrieved and the number of viable oocytes were higher (P < 0.05) in cows in Group 1 than in those in Groups 2 and 3 (table 2).
Table 2. Mean number of aspirated follicles, retrieved and viable oocytes of beef donors treated at different synchronization treatments

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total Follicles</th>
<th>Total Oocytes</th>
<th>Viable Oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 - PC</td>
<td>20</td>
<td>18.3±1.4b</td>
<td>11.4±1.2b</td>
<td>7.9±1.1b</td>
</tr>
<tr>
<td>2 - PG + eCG</td>
<td>19</td>
<td>11.8±1.4a</td>
<td>7.5±1.4a</td>
<td>4.5±1.0a</td>
</tr>
<tr>
<td>3 - Control</td>
<td>20</td>
<td>12.7±0.8a</td>
<td>7.1±0.8a</td>
<td>4.7±0.7a</td>
</tr>
</tbody>
</table>

*Means in the same column differ significantly (P<0.05).

**Experiment 3**, was designed to compare the effect of: 1) dominant follicle removal (DFR) by ultrasound guided follicle aspiration or EB+P4, to control follicular wave emergence, and 2) treatment with eCG or FSH, to superstimulate follicle growth, on the number and quality of oocytes obtained by OPU. Brangus and Angus donors (n=12) were treated four times by four treatments (2 x 2 factorial) in a cross-over design. Donors in Group 1 and Group 2 received 2.5 mg EB and 50 mg P4 im on Day 0; whereas donors in Groups 3 and 4 were subjected to DFR on Day 3. DFR was performed by aspirating all follicles > 8 mm in diameter. All cows received PGF on Day 4, and those in Groups 1 and 3 also received 800 IU eCG (Novormon, Syntex) im; whereas donors in Groups 2 and 4 received 160 mg Folltropin-V (Bioniche Animal Health, Canada) in twice daily equal doses for 2 days (i.e. Days 4 to 6). On Day 7, OPU was performed and COCs were classified as in Experiment 1. No significant effect of follicle wave synchronization treatment (DFR vs EB+P4) was detected in the total number of follicles aspirated (14.8±1.2 vs 14.5±1.4), number of oocytes retrieved (8.3±0.9 vs 7.8±1.0) and number of viable oocytes (5.3±0.8 vs 4.8±0.8). However, the mean number of follicles aspirated on Day 7, total number of oocytes retrieved and the number of viable oocytes were higher (P< 0.05) in cows treated with FSH (18.2±1.1; 9.7±1.0; 6.8±0.8) than is those treated with eCG (11.2±1.0; 6.3±0.8; 3.3±0.5).

**Experiment 4** was designed to evaluate the effect of inserting a progesterone releasing device (CIDR) during the supertimulation treatment on the number and quality of oocytes obtained by OPU. Brangus donors (n = 16) were superstimulated by two treatments in a cross-over design. On Day 0, all donors were subjected to DFR and received PGF and those in Group 1 also received a CIDR device (Pfizer Animal Health, Argentina) at the same time. On Day 1, all cows received 160 mg Folltropin-V in twice daily equal doses for 2 days and OPU was performed on Day 4. The mean number of follicles aspirated on Day 4 (14.26 ± 1.15 vs 15.28 ± 1.14), total number of oocytes retrieved (8.32 ± 1.02 vs 8.72 ± 0.97) and the number of viable oocytes (5.71 ± 0.77 vs 6.0 ± 0.75) were not different between groups (DFR+CIDR vs DFR).

**Summary and conclusions:** Results from these studies supported the notion that synchronizing the emergence of follicular wave increases the number and quality of oocytes obtained by OPU. The two methods of synchronizing follicular wave emergence, EB+P4 or DFR, were equally efficacious. The number and quality of oocytes were positively affected by the induction of luteolysis prior to OPU and the superstimulation treatment with FSH; but not by treatment with eCG. Finally, the addition of a CIDR device during treatment had no effect. These treatments would permit the successful on-farm collection of oocytes for the production of embryos in-vitro.

**Key words:** oocyte, follicular wave emergence, gonadotropin, estradiol.

**References:**


