RELATIONSHIP BETWEEN BLOOD SERUM PROGESTERONE LEVELS IN CATTLE DONORS AND THE YIELD AND QUALITY OF EMBRYOS

FRANTIŠEK NOVOTNÝ, JAROSLAV HAJURKA AND VLADIMÍR MACÁK

Clinic of Animal Obstetrics, Gynaecology and Andrology, University of Veterinary Medicine, 041 81 Košice, Slovakia
e-mail: fnovotny@uvm.sk

Received for publication July 21, 2004.

Abstract

Blood serum progesterone (P₄) levels in donor cows and heifers at donor selection, superovulation, insemination and embryo recovery were investigated. Significant differences in P₄ levels in the investigated donors during superovulatory oestrus insemination were observed. The cows with low levels of P₄ at insemination (5.8 ± 1.7 nmol/l) showed a better yield and quality of embryos (P<0.01) compared to donor cows with high P₄ levels at insemination. A negative correlation between P₄ levels and superovulatory response (r = -0.70), total recovered embryos (r = -0.47), and transferred embryos (r = -0.44), i.e. lower P₄ levels on the day of superovulatory oestrus resulted in an increased yield and quality of embryos. Similarly, a significant difference was observed between donor heifers and cows with high P₄ levels on the day of superovulatory oestrus (P<0.01). No significant differences in the yield and quality of embryos (P>0.05) were observed between heifers and cows with low levels of P₄ at insemination.

Key words: donor cows, embryos, superovulation, progesterone.

The objective of superstimulatory treatment in the cow is to obtain a maximum number of transferable embryos with a high probability of producing pregnancies (2). The efficiency of breeding technology, i.e. the breeding progress and costs, depends to a large extent on the results of superovulatory treatment and artificial insemination (11). Despite a new technology in producing ova for embryo transfer, such as ovum pick-up and in vitro production, superovulation is still widely used in cow embryo transfer programs. Human menopausal gonadotrophin (hMG), equine chorionic gonadotrophin (eCG) and follicle stimulating gonadotrophin (FSH) are used, among others, for the induction of superovulation, also in connection with ovarian follicle status at time of superovulatory treatment (7, 11, 15). Concentrations of progesterone in blood or milk have been used for the identification of potential causes of poor response and embryo quality or exclusion of potentially unsuitable donors (1, 5).

Controversial results have been obtained concerning correlation between progesterone levels and superovulatory responses. It has been accepted in general that irregular progesterone profiles in donors correlate negatively with the superovulatory response and yield and quality of embryos (4, 8). Herrier et al. (9) reported the importance of progesterone levels at the beginning of superovulatory treatment and determined threshold progesterone levels applicable for prediction of superovulatory response. They indicated that these was high probability of impaired ovarian response at progesterone levels below 8 ng/ml in milk and 3 ng/ml in the plasma. While a considerable recent progress in the field of bovine reproductive physiology has been observed. However, factors inherent to the donor animals, which affect superovulatory response are still only partially understood. The aim of our study was firstly to compare the embryo yield and quality and secondly to compare blood serum progesterone profile in FSH treated cows and evaluate the differences in embryo yield and quality in relation to differences in progesterone profiles.

Material and Methods

The investigations were carried out on 56 cows of the Slovak spotted breed, 3-7-year-old, weighing 490-680 kg, and on 36 heifers of the same breed, 18-20 months of age. All the animals were cycling normally and gynaecological examination showed no abnormalities. The superovulatory treatment consisted of administration of Follicotropin inj. a.u.v. (Spofa, Czech Republic) in decreasing doses for 4 d in 12-h intervals, starting with 80 IU on days 1-2 and ending with 40 IU on days 3-4. PGF₂ alpha (Cloprostenol, Oestrophan inj. a.u.v., Léčiva, Czech Republic) was
administered on day 3 at a dose of 250 µg twice in 12-h intervals. The donors were inseminated three times during two days using double doses of semen. Embryos were flushed on days 7-8 after the insemination using a common non-surgical procedure. The quality of superovulatory response was assessed by palpation and ultrasonography with regard to the number of corpora lutea in the ovaries. Searching for and evaluation of embryos were carried out on Petri dishes under a stereomicroscope at 20-fold magnification according to Pivko and Grafenau (12). Blood samples were taken from the jugular vein of the donors at the time of their selection, at application of FSH and PGF$_2$ alpha, during insemination and flushing of embryos. The sera obtained were stored frozen until further processing. Blood serum progesterone (P$_4$) levels were determined by a RIA method, without extraction, using commercial Ria-test-Prog kits produced by the Institute of Radioecology and Exploitation of Nuclear Technique in Košice. The respective standards and samples were examined in duplicate. Determinations included total activity and non-specific and specific bound activity at nil concentration of P$_4$.

The results obtained were processed statistically by the $\chi^2$ test using computer software and parameters such as arithmetic mean and standard deviation were calculated too. Significance of differences was determined by the t-test. The relationship between the yield and quality of embryos and P$_4$ levels was evaluated by correlation analysis and respective correlation coefficients were computed.

**Results**

According to P$_4$ levels at insemination, the cows were divided into 2 groups. The first group (Group 1) included donor cows (n=28) with mean P$_4$ levels reaching $5.8 \pm 1.7$ nmol/l. The second group (Group 2) comprised donor cows (n=28) with mean P$_4$ levels equal to $10.2 \pm 4.15$ nmol/l (Table 1). As can be seen from the Table the superovulatory response and the number and quality of recovered and transferred embryos in the Group 1 were significantly higher in comparison with the Group 2.

The results obtained indicate that at the time of donor selection no significant differences (P>0.05) between donor cows with higher and lower blood serum levels of P$_4$ and the superovulatory response were observed. No significant difference (P>0.05) was recorded between production of embryos and the level of P$_4$ in the donors. The number of transferred embryos in donors with higher level of P$_4$ showed a moderate but insignificant (P>0.05) decrease.

At the time of superovulation no significant differences (P>0.05) between donors with lower and higher P$_4$ levels were observed and no correlation was detected (r = 0.26).

When evaluating the levels of P$_4$ and the results of embryo transfer in donors at insemination, the results obtained were significantly different (P<0.05) in all parameters observed and a relatively close negative correlation suggested that higher levels of P$_4$ in donors were associated with lower superovulatory responses ($r = -0.70$), lower embryo yields ($r = -0.47$), and decreased number of transferred embryos ($r = -0.44$).

Table 2 presents mean values of P$_4$ concentrations in heifers at the time of their selection, superovulatory treatment, and insemination together with the superovulatory response assessed with regard to the number of recovered and transferred embryos. Significant differences were observed between P$_4$ levels at insemination (P< 0.01) and the number of recovered (P<0.01) and transferred (P<0.01) embryos when comparing heifers and cows with higher level of P$_4$ at insemination (P<0.01).

The yield and quality of embryos as well as the superovulatory response of donor cows from group 1 with low P$_4$ levels at insemination did not differ significantly from those of donor heifers. The group 2 of donor cows with high P$_4$ levels at insemination yielded significantly less embryos of lower quality and their superovulatory response was inferior in comparison with heifer donors (P<0.01).

<p>| Table 1 |
|---|---|---|---|---|---|---|---|
| <strong>Mean values of blood serum P4 (nmol/l) in donor cows at selection (DS), superovulatory treatment (SOV) and insemination and the results of superovulation (n=56)</strong> |</p>
<table>
<thead>
<tr>
<th></th>
<th>DS</th>
<th>SOV</th>
<th>Insemination</th>
<th>Superovulatory response</th>
<th>Recovered embryos</th>
<th>Transferred embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.1</td>
<td>25.8</td>
<td>5.8i</td>
<td>5.1a</td>
<td>5.8a</td>
<td>8.8a</td>
</tr>
<tr>
<td>±SD</td>
<td>4.36</td>
<td>4.3</td>
<td>1.7</td>
<td>0.99</td>
<td>1.08</td>
<td>4.29</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12.6</td>
<td>32.3</td>
<td>10.2i</td>
<td>3b</td>
<td>3.28b</td>
<td>3.85b</td>
</tr>
<tr>
<td>±SD</td>
<td>10.9</td>
<td>5.2</td>
<td>4.15</td>
<td>1</td>
<td>0.48</td>
<td>1.67</td>
</tr>
<tr>
<td><strong>LO</strong></td>
<td><strong>RO</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.06a</td>
<td>2.96</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The results indicate that at the time of donor selection no significant differences (P>0.05) between donor cows with higher and lower blood serum levels of P$_4$ and the superovulatory response were observed. No significant difference (P>0.05) was recorded between production of embryos and the level of P$_4$ in the donors. The number of transferred embryos in donors with higher level of P$_4$ showed a moderate but insignificant (P>0.05) decrease.

At the time of superovulation no significant differences (P>0.05) between donors with lower and higher P$_4$ levels were observed and no correlation was detected (r = 0.26).

When evaluating the levels of P$_4$ and the results of embryo transfer in donors at insemination, the results obtained were significantly different (P<0.05) in all parameters observed and a relatively close negative correlation suggested that higher levels of P$_4$ in donors were associated with lower superovulatory responses ($r = -0.70$), lower embryo yields ($r = -0.47$), and decreased number of transferred embryos ($r = -0.44$).

Table 2 presents mean values of P$_4$ concentrations in heifers at the time of their selection, superovulatory treatment, and insemination together with the superovulatory response assessed with regard to the number of recovered and transferred embryos. Significant differences were observed between P$_4$ levels at insemination (P< 0.01) and the number of recovered (P<0.01) and transferred (P<0.01) embryos when comparing heifers and cows with higher level of P$_4$ at insemination (P<0.01).

The yield and quality of embryos as well as the superovulatory response of donor cows from group 1 with low P$_4$ levels at insemination did not differ significantly from those of donor heifers. The group 2 of donor cows with high P$_4$ levels at insemination yielded significantly less embryos of lower quality and their superovulatory response was inferior in comparison with heifer donors (P<0.01).
Table 2
Mean values of blood serum P₄ (nmol/l) in donor heifers at selection, superovulatory treatment and insemination (n = 36)

<table>
<thead>
<tr>
<th></th>
<th>DS</th>
<th>SOV</th>
<th>Insemination</th>
<th>Superovulatory response</th>
<th>Recovered embryos</th>
<th>Transferred embryos</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LO</td>
<td>RO</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>±SD</td>
<td>±SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>14.9</td>
<td>27.7</td>
<td>4.5i₃</td>
<td>4c</td>
<td>5.28c</td>
<td>9.8c₁</td>
</tr>
<tr>
<td></td>
<td>3.12</td>
<td>7.3</td>
<td>1.4</td>
<td>0.7</td>
<td>1.2</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Denotations for Tables 1 and 2: ±SD - standard deviation; LO - left ovary; RO - right ovary; NS - non-significant; a - b = r = - 0.70, P< 0.05; a₁ - b₁ = r = - 0.47, P< 0.05; a₂ - b₂ = r = - 0.44, P< 0.05; a - c = NS; a₁ - c₁ = NS; a₂ - c₂ = NS; b - c = P< 0.05; b₁ - c₁ = P< 0.01; b₂ - c₂ = P< 0.01; i₁ - i₃ = NS; i₂ - i₃ = P< 0.01

Discussion
Evaluation of P₄ levels in blood serum of potential donors during their preparation for embryo transfer together with their superovulatory response was the subject of study of a number of authors. They concluded that both the total number of embryos and their quality increased with increasing levels of P₄ at 5 d after the superovulatory oestrus (13, 14). Insufficient superovulatory response, lower yield and capability of transfer of embryos in lactating cows compared to the heifers were associated with lower concentrations of P₄ at dioestrous and before and after the superovulatory treatment (10). Desaulniers et al. (6) observed that a disturbed pattern of follicular development and of endocrine events in cows could lead to follicles of low steroidogenic capacity and to low competence of oocytes. These disturbances were associated with significantly lower P₄ levels during the superovulatory treatment in cows compared to heifers, which were observed also in our study, however, the differences in the levels of P₄ between heifers and cows during the superovulatory treatment were insignificant. The difference observed between heifers and lactating cows in P₄ concentrations, the yield and quality of embryos might be related to the nutritional and metabolic status of donors before, during, and after the superovulatory treatment and recovery of embryos (3).

In the present study two groups of donor cows and one group of donor heifers was evaluated. We observed that donor cows differed significantly in the level of progesterone at insemination. The cows with lower P₄ yielded more embryos of higher quality and did not differ significantly from donor heifers. The yield and quality of embryos of cows with high serum levels of progesterone at insemination was significantly lower compared to the heifers.

Acknowledgments: This work was supported by grant VEGA 1/0590/03.

References
