The use of steroid hormones in superovulation of Nelore donors at different stages of estrous cycle


**Abstract**

The objective of the present study was to evaluate the superovulatory response and ova/embryo recovery from Nelore donors following treatment with a controlled internal drug releasing device (CIDR-B program) at different stages of the estrous cycle. The control group (TI; n = 40) received a standard superovulation protocol with females of this group being between days 9 and 12 of the estrous cycle (estrus = day 0). The donors that received a CIDR-B program containing 1.9 g progesterone and an intramuscular injection of estradiol benzoate (2 mg) were at day 0 (TII; n = 30), between days 2 and 6 (TIII; n = 30), days 7 and 12 (TIV; n = 30), days 13 and 16 (TV; n = 30) and days 17 and 20 (TVI; n = 30) of the estrous cycle. Superovulation was induced with 400 IU of p-FSH, divided into eight decreasing doses (80/80; 60/60; 40/40; 20/20) at intervals of 12 h. The donors received PGF2α (Cloprostenol) 48 h after beginning the treatment and CIDRs were removed 12 h later. Artificial inseminations (AI) were performed 12 and 22 h after the initiation of estrus and embryos were collected 7 days after AI. The mean numbers (±SEM) of total ova and embryos, viable (transferable) and degenerated embryos were 14.2±11.3, 7.4±6.9 and 3.2±3.5 (TI), 13.3±10.4, 7.1±6.2 and 3.3±4.3 (TII), 13.5±7.0, 8.1±6.7 and 2.3±3.0 (TIII), 17.4±9.9, 9.4±6.9 and 4.0±4.4 (TIV), 16.9±8.8, 9.8±8.1 and 2.7±2.5 (TV) and

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13.0 ± 7.8, 7.2 ± 6.9 and 2.3 ± 2.5 (TVI), with no significant differences (\( P \geq 0.05 \)) among groups. Pregnancy rates of 67.1% (TI; \( n = 86/128 \)), 60.8% (TII; \( n = 59/97 \)), 62.5% (TIII; \( n = 73/115 \)), 64.1% (TIV; \( n = 84/131 \)), 72.3% (TV; \( n = 81/112 \)) and 60.6% (TVI; \( n = 63/104 \)) were obtained with embryos transferred from these collections and did not differ significantly (\( P \geq 0.05 \)) among groups. The results of the present study allow us to conclude that a combination of steroid hormones may be used prior to superovulation in Nelore donors, at any stage of the estrous cycle without affecting the efficiency of embryo transfer programs.

1. Introduction

Superovulation is an efficient technique for obtaining progeny from genetically valuable females. The ovarian response of each female depends on the number of gonadotropin-sensitive follicles present at the time that treatment is initiated. Identification of the number of such follicles in each female would improve the efficacy of superovulation, by allocating potential non-responders to groups where other techniques are used for superovulation. One of the main factors influencing response to superovulation is stage of the follicular wave when gonadotropin treatments are given. Treatment in the absence of a dominant follicle generally enhances the response to superovulation. The development of practical approaches to achieve enhanced responses to superovulation protocols still require further research (Driancourt, 2001).

Superovulation in cattle is a factor, which may limit the efficiency of embryo transfer and must be initiated on the day prior (Adams, 1994), or on the day, of follicular wave emergence (Adams, 1994; Bo et al., 1995b), before the subordinate follicles begin the process of atresia (Bo et al., 1995b). Differences in the stage of follicle and oocyte maturation are often observed at the beginning of superovulatory treatments (Vos et al., 1994), which may result in the presence of follicles at different stages of development at the time of the preovulatory LH surge.

Experiments and field studies performed during the 1980s concluded that superovulatory treatments initiated 9–10 days after estrous detection resulted in a greater superovulatory response as compared with those initiated 2–6 or 12–13 days after estrous detection (Lindsell et al., 1986). This is due to the occurrence of two to three (and even four in zebu) follicular waves during the estrous cycle and, in the majority of these animals, the second follicular wave begins between days 9 and 10 of the estrous cycle (Huhtinen et al., 1992; Bungarts and Niemann, 1994; Bo et al., 1996). In Nelore cows, Figueiredo et al. (1997) reported a predominant pattern of two ovarian follicular growth waves. The synchronization of follicular wave emergence is achieved by the removal of the suppressive effect of the dominant follicle over the growth of the next follicular wave (Bo et al., 1995b). One of the most promising strategies is the use of hormone treatments to synchronize the follicular wave so that it begins at the start of superovulation (Barros, 2000). Administration of steroid hormones has been used to promote the emergence of a new follicular wave at a specific time (Bo et al., 1993, 1994, 1996; Carriere et al., 1995), because these hormones are capable of promoting the emergence of a new follicular wave by causing regression of antral follicles.
The steroid hormones also have a luteolytic action, particularly when estradiol valerate is used (probably as a result of increasing prostaglandin secretion), as well as acting directly on the CL, making it more susceptible to luteolysis (Kesler and Favero, 1995).

Combinations of progesterone/estrogen have gained special attention in protocols for superovulation in cattle (Bo et al., 1995b, 1996; Broadbent et al., 1995; Andrade et al., 2002a,b,c; Oliveira et al., 2002). When progesterone is used in combination with estradiol benzoate, the emergence of a new follicular wave occurs around 5 days after treatment (Caccia and Bo, 1998). The progesterone/estrogen combination used in the cattle superovulatory protocol has enhanced the efficiency of the embryo transfer program, minimizing the routine problem of control of the estrous cycle in large herds (Andrade and Oliveira, 1998; Andrade et al., 2002a,b,c; Oliveira et al., 2002).

In recent years, a number of studies determined the efficiency of steroid hormones in the superovulatory protocols of Nelore cattle (Andrade and Oliveira, 1998; Andrade et al., 2002a,c). However, because the administration of steroid hormones was performed without control of estrous cycle stage, it remains necessary to determine if the effectiveness of this treatment is equivalent at all stages of the estrous cycle (Andrade et al., 2002b). Thus, the aim of the present study was to observe the efficiency of steroid hormones on the superovulatory response of Nelore donors when administered at different stages of the estrous cycle. The superovulatory response, as measured by ova/embryo recovery and number of transferable embryos was the variable used to evaluate the effect of a progesterone/estrogen combination administered before gonadotrophin treatment.

2. Materials and methods

Superovulation treatments were analyzed in females \( n = 180 \) of the Nelore breed. Animals had presented regular estrous cycles and were between 5 and 10 years of age with body condition scores ranging from 3 to 4 on a scale of 1–5 (Edmonson et al., 1989). All animals were submitted to clinical-gynecological examinations before the experiment. Animals were kept on pasture, where they grazed *Braquiaria brasanta* grass and had free access to water and mineral salts.

The 180 donors, submitted to a daily control of estrous cycle using sterilized males equipped with a chin ball-marking device, were equally distributed into six groups according to the stage of their estrous cycle. Donors between the 9th and 12th day \( n = 30 \) of their cycle \( (\text{estrus} = \text{day} 0) \) formed the control group (TI), while the remaining 150 donors \( (30 \text{ per group}) \), at all other stages of the estrous cycle, were distributed into five treatment groups. Donors at day 0 (TII), between days 2 and 6 (TIII), days 7 and 12 (TIV), days 13 and 16 (TV), as well as between days 17 and 20 (TVI) of their estrous cycle were treated using a protocol which has been reported to synchronize emergence of a new follicular wave. The donors treated with the CIDR-B program (InterAg, Edvet HD) received an intravaginal device containing 1.9 g of progesterone and an intramuscular injection of 4 mg estradiol benzoate (Estrogin, Farmavet Veterinary Products LTDA) administered 24 h after the introduction of the CIDR device.

Gonadotrophin treatment began between on days 9 and 12 of the estrous cycle in those animals undergoing the standard superovulatory protocol (TI) and 5 days after the insertion
of the CIDR-B device in the other groups (TII, TIII, TIV, TV, TVI). In all groups, superovulation was induced by intramuscular injections of 400 IU of p-FSH (Pluset, Serono Pharmaceutical Products LTDA) administered in eight decreasing doses (80/80; 60/60; 40/40; 20/20) at intervals of 12 h. Forty-eight hours after the beginning of this treatment, donors received 1 mg Cloprostenol (PGF2α, Ciosin, Coopers, Mallinckrodt Vet LTD) via intramuscular injection and, 12 h later, CIDR devices were removed from groups TII, TIII, TIV, TV and TVI.

Two trained technicians performed the artificial inseminations (AI) with commercial semen from three bulls, equally distributed into six experimental groups, at 12 and 22 h after detection of estrus by sterilized males equipped with a chin ball-marking device. Donors that did not display estrus behavior within 54 h after the 1 mg of PGF2α injection administered intramuscularly were treated intramuscularly with 0.02 mg buserelin acetate (Conceptal, Hoechst Roussel Vet) and inseminated 6, 12 and 18 h later.

Embryos were collected 7 days after the first AI and were then evaluated and classified according to development and morphology (Robertson and Nelson, 1998). Only blastocyst of grade I were transferred to recipients by the transcervical method and the others viable embryos which were not transferred were frozen. The recipient estrous synchrony, obtained using PGF2α administration, was between (−) 2 days before and (+) 2 days after the time of insemination.

Data were analyzed using the PROC GLM option of Statistical Analysis Systems (1988). The numbers of total ova, viable (transferable) embryos, degenerate embryos and unfertilized ova were compared using the Kruskal–Wallis test since data were not distributed normally (verified using the Shapiro–Wilks test). Pregnancy rates were analyzed using the contingency table for the χ²-test. A confidence interval of 5% or less was considered to be statistically significant.

3. Results

Data from the females that displayed or did not display behavioral estrus, mean numbers of total ova and embryos, viable and degenerate embryos and unfertilized ova are presented in Table 1, differences among the six groups were not detected (P ≥ 0.05). The females that

<table>
<thead>
<tr>
<th>Groups</th>
<th>Donors (n)</th>
<th>Total embryos/ova</th>
<th>Embryos</th>
<th>Unfertilized ova</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viable</td>
<td>Degenerate</td>
</tr>
<tr>
<td>TI</td>
<td>30</td>
<td>14.2 ± 11.3</td>
<td>7.4 ± 6.9</td>
<td>3.2 ± 3.5</td>
</tr>
<tr>
<td>TII</td>
<td>30</td>
<td>13.3 ± 10.4</td>
<td>7.1 ± 6.2</td>
<td>3.3 ± 4.3</td>
</tr>
<tr>
<td>TIII</td>
<td>30</td>
<td>13.5 ± 7.0</td>
<td>8.1 ± 6.7</td>
<td>2.3 ± 3.0</td>
</tr>
<tr>
<td>TIV</td>
<td>30</td>
<td>17.4 ± 9.9</td>
<td>9.4 ± 6.9</td>
<td>4.0 ± 4.4</td>
</tr>
<tr>
<td>TV</td>
<td>30</td>
<td>16.9 ± 8.8</td>
<td>9.8 ± 8.1</td>
<td>2.7 ± 2.5</td>
</tr>
<tr>
<td>TVI</td>
<td>30</td>
<td>13.0 ± 7.8</td>
<td>7.2 ± 6.9</td>
<td>2.3 ± 2.5</td>
</tr>
</tbody>
</table>
Table 2
Mean (±S.E.M.) numbers of total embryos/ova, viable and degenerated embryos and the total number of unfertilized ova in the females which did not display estrus after superovulation, using the standard protocol (TI) or using a steroid hormone combination before superovulation (TII, TIII, TIV, TV, TVI)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Donors (n)</th>
<th>Total embryos/ova</th>
<th>Embryos</th>
<th>Unfertilized ova</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Viable</td>
<td>Degenerate</td>
</tr>
<tr>
<td>TI</td>
<td>7</td>
<td>6.5 ± 7.2</td>
<td>3.5 ± 3.7</td>
<td>1.1 ± 1.2</td>
</tr>
<tr>
<td>TII</td>
<td>3</td>
<td>11.7 ± 10.8</td>
<td>7.2 ± 7.4</td>
<td>2.3 ± 1.8</td>
</tr>
<tr>
<td>TIII</td>
<td>3</td>
<td>11.9 ± 8.2</td>
<td>9.7 ± 8.3</td>
<td>1.8 ± 1.5</td>
</tr>
<tr>
<td>TIV</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TV</td>
<td>2</td>
<td>15.1 ± 12.2</td>
<td>10.1 ± 8.1</td>
<td>3.4 ± 3.8</td>
</tr>
<tr>
<td>TVI</td>
<td>2</td>
<td>10.3 ± 9.3</td>
<td>6.4 ± 5.6</td>
<td>2.1 ± 2.4</td>
</tr>
</tbody>
</table>

Table 3
Pregnancy rates with embryos collected from Nelore cows submitted to a standard superovulatory protocol (TI) or submitted to a treatment with a steroid hormone combination prior to superovulation (TII, TIII, TIV, TV, TVI)

<table>
<thead>
<tr>
<th>Group</th>
<th>Embryos</th>
<th>Pregnancy, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viable (n)</td>
<td>Transferred (n)</td>
</tr>
<tr>
<td>TI</td>
<td>213</td>
<td>128</td>
</tr>
<tr>
<td>TII</td>
<td>203</td>
<td>97</td>
</tr>
<tr>
<td>TIII</td>
<td>225</td>
<td>115</td>
</tr>
<tr>
<td>TIV</td>
<td>252</td>
<td>131</td>
</tr>
<tr>
<td>TV</td>
<td>238</td>
<td>112</td>
</tr>
<tr>
<td>TVI</td>
<td>221</td>
<td>104</td>
</tr>
</tbody>
</table>

did not display estrus (Table 2), total embryos/ova, viable embryos, degenerated embryos and unfertilized ova also did not differ (P ≥ 0.05) among groups. Large variations in the number of viable embryos were observed both among animals of the same group and among animals in different groups; these ranged from 0 to 33 (TI), 0 to 31 (TII), 0 to 28 (TIII), 0 to 29 (TIV), 0 to 26 (TV) and 0 to 30 (TVI).

Some embryos were transferred fresh and others were frozen for transfer at a later time (Table 3). There were no differences in pregnancy rates with embryos collected from the six superovulatory groups.

4. Discussion

The data from the present study indicate that the combination of progesterone/estrogen treatment can be effectively used in superovulatory protocol in Nelore donors probably as a consequence of the synchronization of follicular wave emergence. Previous authors (Bo et al., 1996; Andrade et al., 2002a,b,c) have hypothesized that the steroid hormones promote the emergence of a follicular wave after treatment at a previously known time, a situation, which occurs naturally in animals between the days 9 and the 12 after detection of estrus. Another relevant aspect of the superovulatory protocol using the progesterone/estrogen
combination, is the fact that it eliminates the need to know the exact stage of the estrous cycle of each animal at the start of gonadotropin treatments, a factor that must be calculated when using the conventional superovulation protocol. Thus, this technique allows the superovulation of a large number of donors in a reduced period of time, making it convenient for the veterinarian and the farmer. Furthermore, these methods may allow the collection and transfer of embryos in large herds in a practical manner since they may be implemented on separate properties almost simultaneously. As a consequence, the costs of an embryo transfer program can be reduced due to the reduced time involved. In addition, the interval between embryo collections in the same donor may be decreased because the protocol does not depend on the time of the previous estrus (Andrade and Oliveira, 1998).

The use of steroid hormones prior to superovulation of Nelore donors does not harm the efficiency of embryo transfer program because the number of degenerated embryos and unfertilized ova are not increased (Andrade and Oliveira, 1998; Andrade et al., 2002a,b,c; Oliveira et al., 2002). Results of the present study agree with the previous data and it is important to emphasize that pregnancy rates obtained are comparable with those reported by Azevedo and Coelho (1991), Andrade and Oliveira (1998) and Andrade et al. (2002a,b,c) and support the hypothesis that the synchronization of follicular wave emergence, with a combination of progesterone/estrogen, does not interfere with the morphology of the embryo, nor with its developmental capacity in vivo.

Cavaliere et al. (1997) suggested that both progesterone and estrogen can regulate the emergence of the ovulatory follicle and prevent the ovulation of dominant ovarian follicles present at the time of treatment in Bos indicus cows. These results are consistent with the findings of others who have used either estrogen or progesteron to regulate ovarian folliclar development in Bos taurus females (Anderson and Day, 1994; Rajamahendran and Manikkan, 1994; Bo et al., 1995a).

The ability of the combination of progesterone and estrogen to promote the regression of the dominant follicle is based on the fact that lesser mean concentrations of LH and greater mean concentrations of FSH are detected 24 h after treatment (Cavaliere et al., 1997). As dominant follicles are thought to be critically dependent on LH for continued survival (Campbell et al., 1995), increasing LH pulse frequency can prolong the life of dominant follicles whereas a reduction in LH pulse frequency is associated with the induction of atresia among dominant follicles (Savio et al., 1993).

Cavaliere et al. (1997) reported a greater effect of progesterone compared with estrogen in suppressing LH pulse frequency, whilst Hutz et al. (1990) reported that estrogen functions directly at the ovarian follicle to cause atresia. These findings are consistent with those of the current study where treatments occurred at different stages of the estrous cycle in animals presenting different follicle categories. The treatment efficiently promoted the regression of dominant follicles and the start of a new follicle wave so that the superovulatory treatment was started a time when no dominant follicle was present, resulting in acceptable embryo recovery in all females at different stages of follicle development.

As seen in the present study, the use of a protocol employing the combination of progesterone and estrogen at different controlled stages of estrous cycle did not interfere with the recovery of viable embryos. This finding demonstrates the efficiency of estrogen in promoting the synchronous emergency of a new follicular wave, independently of the status of follicular development at the start of treatment. This result corroborates those reported
Another relevant aspect of the protocols utilizing combinations of steroid hormones is the fact that those females that did not display estrus after treatment produced a similar number of viable embryos to those that demonstrated estrus. However, this outcome is not observed in donors treated using the conventional superovulation protocol. The percentage of females which did not display estrus was less than the percentages previously reported by Coelho and Azevedo (1991) and Tahira and Hackett (1993a,b), but was similar to those obtained by Butzke and Cardoso (1996) and Andrade et al. (2002a,b,c). This result was expected, since the ability of estradiol benzoate to induce behavioral estrus in cattle is independent of ovarian follicular events.

A problem with regard to superovulation is the large degree of variation in superovulatory responses among individuals of the same species (Bowen and Pineda, 1989; Mapletoft et al., 1991). Despite the variation in responses between donors observed in the present study, results demonstrate that the CIDR-B program makes Nelore (Bos indicus) embryo transfer programs viable, a theory previously defended by Bo et al. (1995b), Broadbent et al. (1995) and Bo et al. (1996) with females of European races (Bos taurus).

Pregnancy rates obtained are compatible with those reported by Azevedo and Coelho (1991), Andrade and Oliveira (1998), Andrade et al. (2002a,b,c) and support the hypothesis that the superovulatory protocol using a combination of progesterone and estrogen, does not interfere with the morphology of the embryo, nor with its developmental capacity in vivo.

The results of the present study allow us to conclude that a combination of steroid hormones may be used before superovulation in Nelore donors, at any stage of the estrous cycle without affecting efficiency of embryo transfer programs.

References