



Short Communication

Effect of breed and corpus luteum on pregnancy rate of bovine embryo recipients

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ABSTRACT - The objective of this study was to evaluate pregnancy rates of recipients of different breed groups (Nellore and crossbred), as well as the effects of size and type of the corpus luteum (CL) on plasmatic concentrations of progesterone and pregnancy rates of embryo recipients. A total of 152 heifers were synchronized with progesterone implants and on the day of embryo transfer, previously obtained by superovulation and frozen in ethylene glycol, the diameter and type of the corpus luteum (cavitary and compact) was measured and blood was collected for progesterone measurement. The pregnancy rate was 44.1%, with a diameter of corpus luteum higher in recipients that became pregnant (2.03 ± 0.41) compared with non-pregnant ones (1.86 ± 0.34 cm). Plasmatic concentrations of progesterone did not differ between pregnant (1.50 ± 1.05) and non-pregnant (1.31 ± 0.91 ng/mL) animals. The type of corpus luteum did not influence the pregnancy rates. Only Angus and crossbred Marchigiana differ among themselves in pregnancy rates (33.3 and 59.2%, respectively). The pregnancy probability was affected only by CL diameter, but not by P4 plasmatic concentration. Selection of the corpus luteum size at the time of embryo transfer is an important factor to increase pregnancy rates in recipients, and compact and cavitary corpora lutea do not influence the pregnancy rates of bovine embryo recipients. Nellore recipients have pregnancy rates that are satisfactory and comparable to crossbred (*Bos taurus* × *Bos indicus*) recipients.

Key Words: cavitary corpus luteum, Nellore, progesterone, ultrasound

Introduction

Embryo transfer is a biotechnology that accelerates genetic improvement to increase the number of descendants of a donor of superior genetics in a short period of time. Among the factors to consider in embryo transfer programs, recipients are noted for being the determining factor to the success of biotechnology (Spell et al., 2001). The cost of maintenance of non-pregnant crossbred *Bos indicus* × *Bos taurus* as embryo recipients, coupled with low utilization rates, raises the cost of biotechnology. Today, some producers have used Nellore as recipients, but the data in the literature is scarce and with controversial results. Such data are critical because of the mandatory use of zebu genetic recipients with embryo transfer and in the process of *in vitro* fertilization for the breeds Brahman, Cangaian, Indubrasil, Nellore and Sindi since 2014 (ABCZ, 2010).

The establishment and maintenance of pregnancy involve complex interaction between the embryo and

the uterine environment and the corpus luteum (Mann et al., 1995). The pregnancy rates of embryos from superovulation and *in vitro* fertilization are lower than those obtained by artificial insemination or natural mating (Peterson & Lee, 2003). These results may be associated with underdevelopment of the embryo, the embryo-uterine asynchrony and the poor quality of the corpus luteum receptor (Sreenan & Diskin, 1987), resulting in gaps in maternal recognition and maintenance of pregnancy. Cavities of corpus luteum are found in 40-80% of the estrous cycles of cows and heifers treated with progesterone (P4) or prostaglandin, and P4 concentrations are not influenced by the presence of the cavity (Spell et al., 2001; Marques et al., 2002). However, the use of corpus luteum with cavity has generated controversy (Grygar et al., 1997; Marques et al., 2002; Looney et al., 2006; Siqueira et al., 2009a).

Baruselli et al. (2001) found increase in the concentration of P4 in accordance with the corpus luteum increase in crossbred recipients and according to Binelli et al. (2001),

larger corpus luteum in recipients may increase pregnancy rates in embryo transfer programs. However, Nogueira et al. (2004) found an opposite result, in which recipients that received doses of 400 and 600 IU of equine chorionic gonadotrophin showed higher concentrations of P4 and higher corpus luteum, but lower pregnancy rates than those not treated or given 200 IU of equine chorionic gonadotrophin.

The objective of this study was to compare pregnancy rates of Nellore and crossbred (*Bos taurus* × *Bos indicus*) recipients and to evaluate the effects of size and type of the corpus luteum on plasmatic concentrations of P4 and pregnancy rates of bovine embryo recipients.

Material and Methods

The trial was conducted on Band 'alta Farm, located in Corumbá - MS, Brazil (19° 7' 26.39"S latitude and 57° 32' 42.14" W longitude) in the period from January to April 2008. Initially, 201 *Bos indicus* and crossbred *Bos indicus* × *Bos taurus* cows and heifers were synchronized in three periods, at intervals of 40 days. The sires were Nellore and crossbred Angus, Caracu and Marchigiana. Data from crossbred Simmental and Brown Swiss were grouped and analyzed together in the group called mestizo. The animals had regular body condition score ≥ 3 , on a scale of 1 to 5 (Ferreira, 1991). Animals were kept on pasture grass with *B. brizantha* and *B. decumbens*, receiving mineral salt (80 g P/kg) and water *ad libitum*.

For synchronization, the animals received intravaginal device, containing 1 g of P4 (Primer®, Tecnopec, Brazil) and 2 mg of oestradiol benzoate (Estrogin®, Farmavet, Brazil), intramuscularly on a random day of the estrous cycle (day 0) after previous gynecological evaluation to assess uterine tone and the presence of structures indicative of ovarian cyclicity (corpus luteum or follicle >10 mm). The device was removed on the eighth day in the morning and 0.150 mg d-cloprostenol (PGF2) (Prolise®, Tecnopec, Brazil) and 10 IU of FSH (Follicle Stimulating Hormone-Folltropin®, Tecnopec, Brazil) were given intramuscularly.

One milligram of oestradiol benzoate was administered (Estrogin®, Farmavet, Brazil) on the ninth day in the morning, with observation of estrus on day 10. On day 17 after insertion of implants, recipients were evaluated using an ultrasound device with 8.0 MHz linear probe (Falcon 100 - Pie Medical®), to verify the response to the protocol and measure the diameter and type of corpus luteum (Figure 1). On the same day, embryo transfer was carried out, by non-surgical method, by the same veterinary with embryo quality grade 1 and 2, according to IETS (1998), previously

frozen and thawed in ethylene glycol "one-step", from 12 Nellore donors superovulated with FSH injection. On the day of embryo transfer, samples of blood were collected via the coccygeal vein into tubes containing heparin and stored at a temperature of 5 °C. The plasma samples were placed into microtubes identified and stored at -20 °C, after centrifugation at 1700 g for 10 minutes.

Plasma samples were subjected to radioimmunoassay (RIA) for measurement of plasmatic concentrations of P4, performed at FMVA Araçatuba-UNESP Campus, Laboratory of Endocrinology, Department of Field Support, Animal Production and Health (DAPSA), using the commercial kit (Coat-a-Count Diagnostic Products Corporation, CA, USA). In the assay, sensitivity was 0.01 ng/mL, and the intra-assay coefficient 2.4%.

The recipients were examined with Falcon 100 ultrasound (Pie Medical®) with 8.0 MHz transrectal transducer 30 days after the ET for the diagnosis of pregnancy.

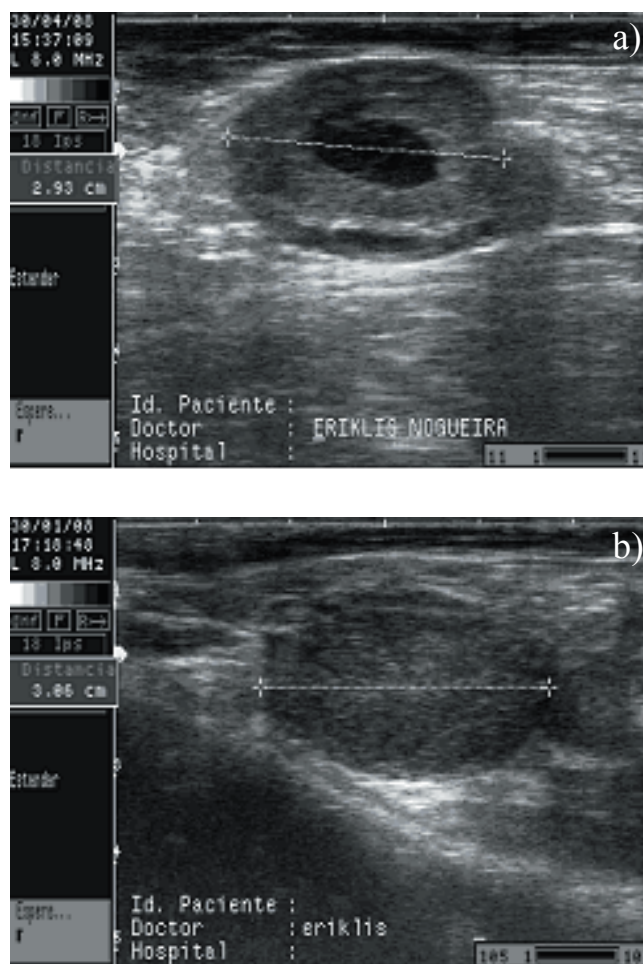


Figure 1 - Evaluation of ovarian by ultrasound on the day of embryo transfer with visualization of the cavitory corpus luteum (a) and compact corpus luteum (b).

Analysis of variance was performed using the Tukey test for comparison of means ($P < 0.05$). For the analysis of pregnancy rates, the frequencies were analyzed by Chi-square and correlations were evaluated via the Spearman test. The probability of pregnancy was modeled by means of logistic regression analysis, according to the independent variables: size of corpus luteum and P4 concentration. The probability of pregnancy was determined assuming a binomial distribution (1 = pregnant, 0 = no pregnant), and logit link. The analyses were performed employing software SAS (Statistical Analysis System, version 9.2), adopting $\alpha = 0.05$.

Results and Discussion

The recipients synchronized with intravaginal device containing P4 associated with the application of FSH showed satisfactory utilization rates of 75.6% (152/201), and pregnancy rates of 44.1% (67/152), similar to other studies in the literature, e.g., Barreiros et al. (2006), who found similar results in the ultrasound assessment to estimate the rate of utilization of recipients treated with P4 and PGF2 α , resulting in utilization of 72.8% and 79%, respectively.

There were no differences between corpus luteum diameter and plasmatic P4 between the sires evaluated (Table 1). Crossbred Marchigiana recipients presented higher pregnancy rates than crossbred Angus ones. The other breeds showed intermediate results, with no differences between each other (Table 1).

Gonzales et al. (1993) reported differences in the reproductive performance of animals with different genotypes. The same author found that those who had higher *Bos indicus* genetic fraction showed greater efficiency in the use of feed, greater adaptability to adverse conditions and tend to have better reproductive rates in tropical conditions. However, these observations appear to be conflicting with other studies, in which animals with high genetic *Bos taurus* fraction showed greater reproductive efficiency, particularly when used in embryo transfer programs as recipients. Teodoro & Madalena (2002) stated that, depending on the management type, crossbred *Bos taurus* \times *Bos indicus*

may show results of production and reproduction equal or superior to *Bos taurus* animals.

It is noteworthy that the region had high temperatures (maximum and minimum of 32.56 and 23.60 °C, respectively) and relative humidity (85.35%) during the months preceding the experiment, for being located in the Pantanal (tropical wetland) region of Mato Grosso do Sul (Soriano, 1999). The pregnancy rates from artificial insemination or natural mating fall dramatically due to heat stress (Badinga et al., 1993). Also, Ealy et al. (1993) have shown a deleterious effect of temperature on the embryo in studies conducted *in vivo*. Therefore, the high temperatures and humidity may have promoted local thermal discomfort in animals and may have influenced the reduction of pregnancy rates, especially in animals with darker hair color as the case of Angus cross. However, further studies on the influence of local temperature and humidity in the pregnancy rate of these animals are necessary for this hypothesis to be confirmed.

The pregnancy rate of Nelore was 41.1% (Table 1), and did not differ from the other breeds ($P > 0.05$), demonstrating that this breed can be used as a recipient of bovine embryos, showing satisfactory results of pregnancy, since parameters for such (synchronization of the day of the estrous cycle and age of the embryo, the size of the corpus luteum, health and nutrition) are met.

The plasmatic concentrations of P4, corpus luteum diameter and pregnancy rates did not differ ($P > 0.05$) according to the corpus luteum type (Table 2), which is agreement with the results obtained by Spell et al. (2001). This demonstrates that the ability to produce P4 and maintain pregnancy is equivalent in the two types of corpus luteum, differing only in morphology. Barreiros et al. (2006), in a trial in Parana with crossbred recipients, found 22.7% of the cavity corpus luteum when the animals were synchronized with P4. These values were consistent with those found in this study (27.6%) and did not influence the P4 plasmatic concentration by the presence of the cavity, as also observed by Siqueira et al. (2009a).

Another aspect observed by Kastelic et al. (1990) was the loss of the cavities of corpus luteum in pregnant heifers. Such loss of cavity in the ultrasound examination

Table 1 - Plasmatic concentration of P4, corpus luteum diameter and pregnancy rate of recipients according to breed group

	Marchigiana	Caracu	Nelore	Mestizo	Angus	P
N	27	32	34	29	30	
P4 plasmatic concentration, ng/mL ¹	1.58 \pm 1.30	1.50 \pm 1.11	1.48 \pm 1.05	1.41 \pm 0.89	1.07 \pm 0.40	0.666
Corpus luteum diameter, cm ¹	2.10 \pm 0.29	1.88 \pm 0.39	1.92 \pm 0.44	2.10 \pm 0.42	1.96 \pm 0.33	0.915
Pregnancy rate, % ²	59.2a	40.6ab	41.1ab	48.3ab	33.3b	0.353

¹ Values do not differ ($P > 0.05$).

² Pregnancy rate - a \neq b ($P < 0.05$) by Chi-square.

demonstrated that this finding in the beginning of pregnancy is not important for the maintenance of pregnancy. Additionally, other studies show no significant difference in P4 concentration with cavitory or compact corpora lutea at the time of embryo transfer (Spell et al., 2001; Marques et al., 2002).

Plasmatic concentrations of P4 at the time of embryo transfer did not differ between recipients that became pregnant and non-pregnant ($P>0.05$) (Table 3), which is in disagreement with Siqueira et al. (2009a). Probability of pregnancy was not influenced ($P>0.05$) by increasing the concentration of plasmatic P4 (Figure 2). Marques et al. (2009) also found no difference in P4 concentrations on embryo transfer from recipients that became pregnant and non-pregnant, with values of 2.51 and 2.42 ng/mL, respectively. The optimal P4 plasmatic concentration for establishment of pregnancy varies from 2.0 to 5.0 ng/mL (Niemann et al., 1985). However, Spell et al. (2001) showed that the P4 concentration required to establish and maintain pregnancy may be less than the previously described. In the study, there was no difference in pregnancy rates when the concentrations of P4 were lower than 0.58 ng/mL, or exceeded 16 ng/mL. Hasler et al. (1980) also found that 8 of 177 pregnant recipients had concentrations of P4<0.5 ng/mL on days 10, 11 and 12 of the estrous cycle.

There were differences ($P<0.05$) in corpus luteum diameter between pregnant and non-pregnant recipients, with a larger diameter of corpus luteum in the former (Table 3). Some authors have found positive correlation between corpus luteum area and its ability to produce P4. In this study, there was a positive correlation between the diameter of the corpus luteum and plasmatic concentrations of P4, with value of 0.32 ($P<0.05$), as also observed by Siqueira et al. (2009b). The probability of pregnancy was influenced by the size of corpus luteum ($P<0.05$) (Figure 3).

The larger the area of luteal tissue, the more positive the influence on conception rates in bovine embryo recipients (Tribulo et al., 2000). According to Binelli et al. (2001), obtaining greater corpora lutea in receptors may increase the pregnancy rate in embryo transfer programs; however, Spell et al. (2001) showed that the fluctuation of P4 reflects a combination of different rates of development of the corpus luteum and secretion of P4 during the early stages of the estrous cycle. Looney et al. (2006) indicate that corpora lutea of at least 10 mm are acceptable for embryo recipients to become suitable for embryo transfer. The same authors have observed differences in pregnancy rates when using recipients with corpus luteum greater than 10 mm. In this study, the mean diameter of corpus luteum was 20 mm.

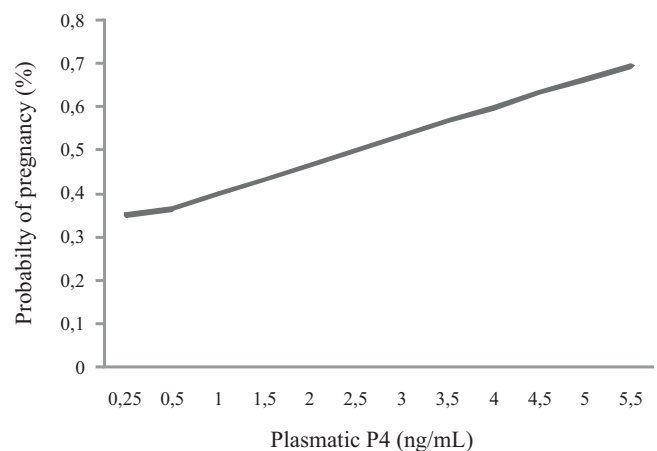


Figure 2 - Probability of pregnancy in recipients in function of the P4 concentration ($P = 0.092$).

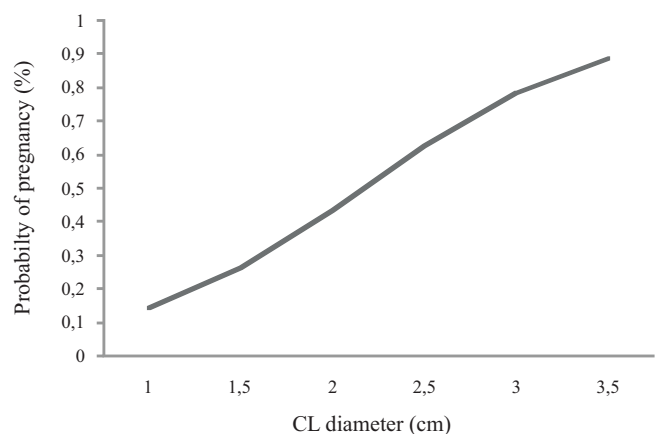


Figure 3 - Probability of pregnancy in embryo recipients on the basis of corpus luteum (CL) diameter ($P = 0.006$).

Table 2 - P4 plasmatic concentration, corpus luteum diameter and pregnancy rate of recipients according to the corpus luteum classification

	Compact	Cavitory	P
N	110	42	
P4 plasmatic concentration, ng/mL	1.58±1.23	1.43±0.96	0.602
Corpus luteum diameter, cm ¹	2.00±0.29	2.04±0.34	0.380
Pregnancy rate, % ²	45.4	42.9	0.254

¹ Values do not differ ($P>0.05$).

² Values did not differ ($P>0.05$) by chi-square.

Table 3 - P4 plasmatic concentration, CL diameter of recipients according to the pregnancy

	Pregnant	Non-pregnant	P
N	67	85	
P4 plasmatic concentration, ng/mL	1.50±1.05	1.31±0.91	0.385
Corpus luteum diameter, cm ¹	2.03±0.41a	1.86±0.34b	0.042

¹ a ≠ b ($P<0.05$).

Conclusions

Nellore Recipients have pregnancy rates that are satisfactory and comparable to crossbred (*Bos taurus* × *Bos indicus*) recipients. The selection of the size of the corpus luteum at the time of embryo transfer is an important factor to increase pregnancy rates in recipients of bovine embryos. Type of corpus luteum (compact or cavitary) do not influence the pregnancy rates of bovine embryo recipients.

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