FACTORS THAT INFLUENCED MULTIPLE OVULATION RESULTS OF ZEBU DONORS

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INTRODUCTION
Despite Multiple ovulation and embryo transfer technique (MOET) has spread all over the country, aspects influencing MOET efficiency were little evaluated. It become more important when considered the breeding and conservation programs in MOET nucleus (Nicholas and Smith, 1983). Different factors have been pointed as responsible for MOET results (Monniaux et al., 1983; Callesen et al., 1986; Lamberson and Lambeth, 1986; Walton and Stubbings, 1986; Bastidas and Randel, 1987; Armstrong, 1993; Woolliams et al., 1995). The complexity of superovulation response variation, according to authors, is evident. Thus, the objective of this study was to verify the influence of breed, herd, year of birthday, age at superovulation, age at 1st calving, month, season and year of superovulation, flushing order, drug, dose, number of inseminations and interactions on the number of corpora luteous (CL), total of structures per flushing (SF) and number of viable embryos (VE).

MATERIAL AND METHODS
Data from 1294 superovulations of 598 Zebu donors (27 Brahman, 22 Gir, 74 Guzerá and 475 Nelore) from 61 herds of different regions of Brazil were analyzed. Donors aged 1.5 to 19.5 years. The MOET program was carried out in CENATTE Embryos Inc., in SE region, under standardized management. Donors were superovulated when in a fair nutritional and health conditions, after presenting 2 consecutive heats in an 18-24 d interval. FSH was applied in 8-12 d after 1st signals of estrus. Doses at 1st superovulation were 320 UI to Brahman and 380 UI to the others and individually adjusted in subsequent superovulation accordingly 1st superovulation response. Consecutive superovulations happened after donors had presented 2 heats in 18-24 d interval and, at least, 60-d superovulation interval. Some donors went back to the herd according to 1st flushing results or the owner interest. Descriptive analyses of data were carried out to eliminate doubtful observations. Data were analyzed considering only 1st flushings, two 1st flushings, three 1st flushings and all consecutive flushings to verify the effect of donors’ elimination after consecutive flushings. Each flushing result was used as an individual observation and no adjustment was made to correlation among observations of the same donor. The general model was $Y = Xb + e$, where, $Y$= vector of observations ; $b$= unknown vector of fixed effects ; $X$= known matrix relating observations in $Y$ to parameters in $b$ and $e$= random vector of residuals. Fixed effects studied were : breed, herd, herd nested into breed, year of birth, age at superovulation, age at 1st calving, month, season and year of superovulation, drug, dose, dose nested into drug, flushing order, number of inseminations, inbreeding coefficient and interactions. The age at superovulation and the inbreeding coefficient were included as covariates. Low frequent doses were grouped to the nearest high frequent doses. It was assumed normal distribution for all traits to proceed hypothesis test and
variance component analyses by the least squares means methodology. Through derivation of quadratic functions, it was obtained the values of ‘X’ where the response (Y) was maxim.

RESULTS AND DISCUSSION
Produced embryos per flushing and flushing intervals (Table 1) indicate that 2 1st flushings were efficient in number and time for breeding purposes (Penna, 1998). The discrepancy in results obtained with Gir donors could be attributed to the small size of these donors in the sample.

Table 1. Interval between 1st and 2nd (1-2) and between 2nd and 3rd (2-3) flushings and means of viable embryos (VE) in the three 1st flushings according to Zebu breed

<table>
<thead>
<tr>
<th>Zebu Breeds (n)*</th>
<th>Flushing</th>
<th>1-2</th>
<th>2-3</th>
<th>Means p/ flushing</th>
</tr>
</thead>
<tbody>
<tr>
<td>* n = number of donors.</td>
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<tr>
<td>Brahman (27)</td>
<td>91.8±47.7</td>
<td>101.8±39.0</td>
<td>93.1±23.8</td>
<td>111.2±36.9</td>
</tr>
<tr>
<td>Gir (22)</td>
<td>101.8±39.0</td>
<td>110.3±76.9</td>
<td>116.4±68.8</td>
<td>108.5±39.3</td>
</tr>
<tr>
<td>Guzerá (74)</td>
<td>93.1±23.8</td>
<td>116.4±68.8</td>
<td>111.2±36.9</td>
<td>108.5±39.3</td>
</tr>
</tbody>
</table>

R² for CL, SF and VE were low (±21%), probably the variation in superovulation response was not completely explained by the tested sources in the models. According to Bastidas and Randel (1987), it can be assigned to the lack of knowledge about the factors influencing superovulation response in Bos indicus up to this moment. The high CV (±50) indicated the great dispersion around the mean. SF and VE involve hormonal response and, therefore, are very unstable, resulting in high CVs (Sampaio, 1998). It could be affecting the precision of fixed effects evaluation, but, accordingly author, significant variation sources are in fact very much important due to the strength of hypothesis test when error variance is high. The results suggest nevertheless the necessity of carrying out studies about distribution patterns of those characteristics. Concerning data set analyzed, remarkable differences were noticed between the 1st flushing and consecutive flushings data sets. When analyzed 1st flushing data set, few effects were identified as influencing superovulation results. Differences found can be attributed to aspects such as : standardization of doses and ovarian and hormonal conditions at 1st superovulation. Least squares means are similar (Table 2), whatever the data set considered.

Table 2. Least square means and respective standard errors for MOET traits according to analyzed data set

<table>
<thead>
<tr>
<th>DATA</th>
<th>CL</th>
<th>SF</th>
<th>VE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st flushing</td>
<td>11.39±5.36</td>
<td>11.52±8.38</td>
<td>5.54±5.85</td>
</tr>
<tr>
<td>1st and 2nd flushings</td>
<td>11.17±5.15</td>
<td>10.97±7.75</td>
<td>5.55±5.49</td>
</tr>
<tr>
<td>1st, 2nd and 3rd flushings</td>
<td>11.00±5.14</td>
<td>10.67±7.56</td>
<td>5.45±5.33</td>
</tr>
<tr>
<td>All flushings</td>
<td>10.75±5.05</td>
<td>10.33±7.30</td>
<td>5.32±5.17</td>
</tr>
</tbody>
</table>
It can be justified by early discard tendency of the worst and best superovulation response donors. The extreme discard of donors did not bring damage to the sample. The effect of herd nested into breed on CL, SF and VE was significant, except for CL and VE at 1st flushing. Probably, at 1st flushing, differences in superovulation response due to previous donors standardization in Central and good ovarian and uterine conditions at 1st flushing were minimized (Saumande et al., 1978; Lerner et al., 1986; Armstrong, 1993). Breed and herd were confounded in the sample studied, so herd was nested into breed. The nested effect of herd was significant. It suggests genetic and environmental differences more than breed ones (Woolliams et al., 1995). Dose was fixed at the 1st flushing and, so, it was not tested with 1st flushing data set. Dose nested into drug was significant on CL, SF, and VE when considered consecutive flushings data set. After 1st flushing, doses of drugs were individually adjusted according to the 1st superovulation response and differences in the response can be explained by the differences in the number of ovarian receptors, ovarian conditions at hormonal treatment, among others (Monniaux et al., 1983; Walton and Stubbings, 1986; Armstrong, 1993). The behavior of nested effect of dose varied as it was increased accordingly the origin of administrated drug. Month, year and order of flushing, generally, not influenced CL, SF and VE. The linear and quadratic effects of age of donor were not significant on 1st superovulation response, whatever the data set. When considered consecutive flushing data set, on the other hand, the linear effect of age was significant on VE and quadratic effect were significant on CL, SF and VE. It was verified decreasing superovulation response as age increased (Figure 1).

![Figure 1. Least square means of CL, SF and VE in function of age of donor, considering consecutive flushing data](image)

The maximum point for VE was obtained between 7 and 8 year-old females. Regarding CL and RE, the best response were obtained between 4 and 5 year-old ones. Those results indicated that aged donors, in spite of not responding satisfactorily to superovulation, produce more VE than younger donors (Callesen et al., 1986). Hasler et al. (1983), however, have warned against the maintenance of high value females beyond the age they would usually be discarded. Accordingly authors, this practice could bring negative consequences to breeding program efficiency since that, in populations under selection, it is presumed the genetic superiority of younger individuals. With respect to inbreeding coefficient (IC), it was found significant effect
on SF when data of consecutive flushings were analyzed. The regression coefficient for IC was positive, the higher the IC the higher the superovulation response. It would be in disagreement with the theory (Falconer, 1989), but, besides the sample size and the small number of inbreeding donors, must be emphasized that the sample studied is constituted of highly selected sample and discard after each flushing can mask undesirable inbreeding effects.

CONCLUSION
From these results, it was shown that dose of drug and age of superovulation had influenced CL, RS and VE and must be considered in the definition of superovulation procedures and routines of Zebu donors.

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REFERENCES