Arq. Bras. Med. Vet. Zootec., v.58, n.4, p.567-574, 2006

Effects of environmental factors on multiple ovulation of zebu donors

[*Efeitos de fatores ambientes sobre a ovulação múltipla de doadoras zebuínas*]

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ABSTRACT

Data on 1,294 superovulations of Brahman, Gyr, Guzerat and Nellore females were used to evaluate the effects of: breed; herd; year of birth; inbreeding coefficient and age at superovulation of the donor; month, season and year of superovulation; hormone source and dose; and the number of previous treatments on the superovulation results. Four data sets were considered to study the influence of donors' elimination effect after each consecutive superovulation. Each one contained only records of the first, or of the two firsts, or three firsts or all superovulations. The average number of palpated *corpora lutea* per superovulation varied from 8.6 to 12.6. The total number of recovered structures and viable embryos ranged from 4.1 to 7.3 and from 7.3 to 13.8, respectively. Least squares means of the number of viable embryos at first superovulation were 7.8 ± 6.6 (Brahman), 3.7 ± 4.5 (Gyr), 6.1 ± 5.9 (Guzerat) and 5.2 ± 5.9 (Nellore). The numbers of viable embryos of the second and the third superovulations were not different from those of the first superovulation. The mean intervals between first and second superovulations were 91.8 days for Brahman, 101.8 days for Gyr, 93.1 days for Guzerat and 111.3 days for Nellore donors. Intervals between the second and the third superovulations were 134.3, 110.3, 116.4 and 108.5 days for Brahman, Gyr, Guzerat and Nellore donors, respectively. Effects of herd nested within breed and dose nested within hormone affected all traits. For some data sets, the effects of month and order of superovulation on three traits were importants. The maximum number of viable embryos was observed for 7-8 year-old donors. The best responses for *corpora lutea* and recovered structures were observed for 4-5 year-old donors. Inbreeding coefficient was positively associated to the number of recovered structures when data set on all superovulations was considered.

Keywords: cattle, Zebu, superovulation, environmental effects, MOET nucleus

RESUMO

Informações de 1.294 superovulações de fêmeas das raças Brahman, Gir, Guzerá e Nelore foram estudados para avaliar a influência de: raça; rebanho; ano de nascimento, coeficiente de endogamia e idade da doadora; mês, estação e ano da superovulação; e dose do hormônio utilizado; e número de tratamentos prévios sobre o desempenho reprodutivo na Ovulação Múltipla e Transferência de Embriões (MOET). Quatro arquivos de dados foram usados para estudar o efeito de eliminação das doadoras após cada superovulação consecutiva, cada um contendo apenas registros da primeira, ou duas primeiras, ou três primeiras ou de todas as superovulações. O número médio de corpos lúteos palpados por superovulação variou de 8,6 a 12,6, e o número total de estruturas recuperadas e de embriões viáveis variou, respectivamente, de 4,1 a 7,3 e de 7,3 a 13,8. A média dos quadrados mínimos do número de

Recebido em 8 de junho de 2005
Aceito em 7 de abril de 2006
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embriões viáveis na primeira superovulação foi de 7,8 ± 6,6 (Brahman), 3,7 ± 4,5 (Gir), 6,1 ± 5,9 (Guzerá) e de 5,2 ± 5,9 (Nelore). As médias dos quadrados mínimos na segunda e terceira superovulações não foram diferentes daquelas obtidas na primeira superovulação. Os intervalos médios entre a primeira e a segunda superovulação foram de 91,8 dias (Brahman), 101,8 dias (Gir), 93,1 dias (Guzerá) e 111,3 dias (Nelore). Os intervalos médios entre a segunda e a terceira superovulações foram de, respectivamente, 134,3; 110,3; 116,.4 e 108,5 para doadoras Brahman, Gir, Guzerá e Nelore. Os efeitos de rebanho aninhado dentro de raça e dose aninhada dentro de hormônio influenciaram as três características. Em alguns arquivos, mês e ordem da superovulação foram importantes fontes de variação para as três características. O número máximo de embriões viáveis foi observado em doadoras entre sete e oito anos de idade. Os maiores números de corpos lúteos e de estruturas recuperadas foram observados em doadoras entre quatro e cinco anos de idade. O coeficiente de endogamia foi positivamente associado ao número de estruturas recuperadas, quando se analisou o arquivo contendo todas as superovulações.

Palavras-chave: bovino, Zebu, efeitos de meio, núcleo MOET, superovulação

INTRODUCTION

Multiple ovulation and embryo transfer technique (MOET) has been carried out in central units (CU) located in several regions of Brazil. In CU, potential donor cows are evaluated and standardised, and MOET costs are minimised. According to Brazilian Society for Embryo Transfer (SBTE), since 1995, the number of transferred and frozen embryos has increased by 20% and 60%, respectively. As MOET spread all over the country, the factors that influence its results need to be analysed. With the introduction of breeding and conservation MOET nucleus schemes (Nicholas and Smith, 1983), particularly in Brazilian Zebu cattle (Penna et al., 1998), these studies became more important.

The reduction in generation interval when selection is carried out in MOET nucleus is important to genetic gain (Nicholas and Smith, 1983). MOET practice allows the birth of a broad number of progenies and the sire genetic evaluation earlier than usually obtained. MOET results, however, are largely variable and unpredictable. Monniaux et al. (1983) and Armstrong (1993) described the limitations of the application of MOET to animal breeding schemes. According to Lerner et al. (1986), there is broad variation in consecutive superovulation responses within and between donors. When considering Zebu and crossbred donors, means of 1.7 to 5.8 viable embryos per superovulation were found (Coelho and Azevedo, 1991; Penna et al., 1998; Evangelista and Sousa, 1999).

Variation in MOET results has been attributed to many factors. Hasler et al. (1981) and Lerner et al. (1986) found a negative effect of the donor's age on response to superovulation. Younger donors produced more viable embryos than the older ones, as a consequence of the decrease in ovulation and fertilisation rate in older cows. Hasler et al. (1987) observed that neither very young nor very old donors had produced adequate number of embryos. In addition, MOET results can be influenced by the effects of breed, ovarian condition, lactation period, hormone source, superovulatory treatment, method of recovering, year and season of treatment, nutritional conditions, number of consecutive superovulations, interval between consecutive superovulations and the health of the donor during the program (Lamberson and Lambeth, 1986; Callesen et al., 1996).

In Zebu cattle, some authors have reported a decrease in the number of recovered structures and viable embryos as the number of consecutive superovulations increases. These results contrast with those verified by Lamberson and Lambeth (1986) in which carrying out successive superovulations had no effect on the results. Concerning the season of the year, Bastidas and Randel (1987) showed the reduction in the number of viable embryos of Zebu donors in winter. Woolliams et al. (1995) described environmental aspects as critical to superovulation response.

The objective of this study was to evaluate the influence of breed, herd, year of birth, age at superovulation, month, season and year of superovulation, superovulation order, superovulatory hormone source and dose, number of inseminations and their interactions on the number of *corpora lutea*, the number of recovered structures and the number of viable embryos of Zebu donors.

MATERIAL AND METHODS

A total of 1,294 superovulation records of 598 Zebu females (27 Brahman, 22 Gyr, 74 Guzerat and 475 Nellore), was collected from 1989 to 1999 in a private MOET company (Cenatte Embryos Inc.), located in Minas Gerais State, Southeast of Brazil. Females came from 63 herds, from different regions of the country and ranged from 1.5 to 19.5 year-old at the time of the superovulatory treatment and 3, 25, 30, 23, and 19% of the donors were <3, 3-5.9, 6-8.9, 9-11.9 and >12 year-old, respectively.

The MOET program was carried out under standardised management. Prior to superovulation, donors were examined for evidence of reproductive disorders and observation of at least two consecutive oestrus cycles in a period of 18 to 24 days. Two commercial sources of porcine follicle-stimulating hormone (p-FSH; FSH® and Pluset®) were used for superovulation. The p-FSH treatments were given at 12-h intervals, starting 8-12 days after the onset of oestrus. The total dose of p-FSH used for the first superovulation were 320IU for Brahman and 380IU for the other breeds. After first superovulation, subsequent superovulations were induced after donors were detected in oestrus on at least two occasions, 18-24 days apart, and at least 60 days after the previous superovulation, and p-FSH doses were individually adjusted accordingly to the previous superovulation response. Superovulated cows were artificially inseminated three to four times, after palpation *per rectum* of ovaries, in order to assess the number of *corpora lutea* . Some donors returned to their herds after first superovulation, according to the owner's interest.

Data were submitted to analysis of variance considering four groups of data (first superovulation only, two first superovulations, three first superovulations, and all superovulations) in order to study the effect of donors' elimination after each superovulation. Each superovulation result was used as an individual observation and no adjustment was made to correlate the observations of the same donor. The general model was Y = Xb + e, where Y is the vector of observations (*corpora lutea*, recovered structures and viable embryos); b is the unknown vector of fixed effects; X is the known matrix relating observations in Y to parameters in b and e is the random vector of residuals.

Normality test showed the evidence of quasi-normal distribution of the data, therefore normal distribution was assumed for all dependent variables to proceed hypothesis testing and variance component analyses by least squares methodology (SAS, 1995). Dependent variables were the number of *corpora lutea*, the total number of recovered structures and the number of viable embryos. Tested fixed effects were: breed, herd, year of birth, age at superovulation (or age at first and inbreeding coefficient of the donor, month, season and year of superovulation, hormone source and dose, number of previous superovulations, number of inseminations, the nested effects of herd within breed and dose within hormone source, and interactions among these factors. Doses at low frequency were grouped with those of the highest frequency and of the closest value. Age at superovulation and inbreeding coefficient were included in the model as covariates.

RESULTS AND DISCUSSION

Table 1 shows the number of superovulations carried out and the observed averages for dependent variables, despite of the recovering order. The viable embryos average values ranging from 4.1 to 7.3, can be considered excellent for Zebu cattle, compared to 1.7 to 5.8 viable embryos per superovulation reported by Herrera-Alvarez et al., 1987; Coelho and Azevedo, 1991; Penna et al., 1998; Evangelista and Sousa, 1999. According to Penna et al. (1998), at least four viable or transferred embryos would be necessary to allow a minimum value for accuracy of genetic evaluations of animals in a MOET nucleus scheme. These results suggest that selection in Zebu MOET nuclei can be successful.

Table 1. Observed means (X) and standard deviation (SD) of *corpora lutea,* recovered structures and viable embryos, according to breeds

Breed	Number of superovulations	*Corpora lutea*		Recovered structures		Viable embryos	
		Number	$\overline{X}$ ±SD	Number	$\overline{X}$ ±SD	Number	$\overline{X}$ ±SD
Brahman	51	641	12.6±5.4	702	13.8±8.7	374	7.3±5.7
Gyr	48	414	8.6±4.7	350	7.3±5.9	198	4.1±3.9
Guzerat	159	1,516	9.5±5.4	1,580	9.9±7.9	901	5.7±5.4
Nellore	1,036	11,142	10.8±5.4	10,551	10.2±7.8	5,267	5.1±5.3
Total	1,294	13,713	10.6±5.4	13,183	10.2±7.9	6,740	5.7±5.3

The period necessary to get a satisfactory number of viable embryos is important to Moet nucleus scheme (Nicholas and Smith, 1983). Intervals among consecutive superovulations and number of viable embryos produced in each superovulation (Table 2) indicated that two superovulations would be successful in reaching a sufficient number of embryos to carry out genetic evaluation and to maximise annual genetic gain in Zebu cattle MOET nuclei (Nicholas and Smith; 1983; Penna et al., 1998). The wide interval between the first and the second superovulation in Gyr cattle could be a limitation to MOET nucleus efficiency. Nevertheless, the interpretation of this result needs to be careful because of the small sample size. The best first superovulation response of Brahman donors could be related to previous selection for reproductive traits in the breed.

The small variation in viable embryos means from the first to the second superovulation (Table 2) was similar to that presented by Lamberson and Lamberth (1986), but different of the decrease observed in *Bos indicus* (Bastidas and Randel, 1987; Coelho et al., 1989). Individual adjustment of doses after the first superovulation could explain the smaller variation observed n this study, since differences in superovulation response among females were cancelled when low-response donors received from 380 to 510IU, whereas high-response donors received 320IU.

Table 2. Intervals between the first and the second (1-2) and between the second and the third superovulations (2-3), and means of viable embryos in the first three superovulations (1, 2 or 3), according to breeds

Interval between superovulations	Brahman (27)	Gyr (22)	Guzerat (74)	Nellore (475)
	Interval (days)			
1-2	91.8±47.7	101.8±39.0	93.1±23.8	111.2±36.9
2-3	134.3±87.4	110.3±76.9	116.4±68.8	108.5±39.3
	Means			
Viable embryos (1)	7.78±6.59 (27)	3.74±4.50 (22)	6.06±5.89 (74)	5.18±5.88 (475)
Viable embryos (2)	7.94±4.11 (15)	2.92±3.20 (7)	5.41±5.63 (45)	5.32±5.18 (290)
Viable embryos (3)	4.92±3.29 (8)	4.43±3.60 (6)	4.87±4.46 (21)	4.59±4.47 (137)

( ) = number of donors.

Analyses of variance are presented in Table 3, 4 and 5. Overall low R^2 values indicated that the variation in superovulation response was not completely explained by the fitted models. Peixoto (2001) found genetic effect as an important source of variation for superovulatory response in Zebu donors. It is possible that absence of genetic factors in the models, e.g. sire, not included due to insufficient number of replicates in sub classes, was responsible for the lack of fitness of studied models. In addition, these low values of R^2 can be also a consequence of other factors not fitted, such as inbreeding coefficient of the embryo, donor's weight or body condition at superovulation and many others (Lerner et al.; 1986; Walton and Stubbings, 1986; Bastidas and Randel, 1987). The high CV observed, mainly for recovered structures and viable embryos, can be attributed to the instability of variables involving hormonal response (Sampaio, 1998).

Table 3. Analyses of variance of number of *corpora lutea* in different data sets

Source of variation	Superovulations							
	1st		1st and 2nd		1st, 2nd and 3rd		All	
	DF	P>F	DF	P>F	DF	P>F	DF	P>F
Breed	3	0.3196 ns	3	0.0591 ns	3	0.3791 ns	3	0.1663 ns
Herd (breed)	59	0.1872 ns	60	0.0042*	60	0.0041*	60	0.0010*
Hormone source	1	0.4605 ns	1	0.2417 ns	1	0.1832 ns	1	0.2016 ns
Dose (hormone)	8	-	10	0.0004*	10	0.0001*	10	0.0001*
Month	11	0.0197*	11	0.0371*	11	0.1406 ns	11	0.0572 ns
Year	10	0.2842 ns	10	0.4182 ns	10	0.8368 ns	10	0.8443 ns
Order	-	-	1	0.2249 ns	2	0.1015 ns	6	0.1401 ns
Age (linear)	1	0.3620 ns	1	0.2065 ns	1	0.1374 ns	1	0.3953 ns
Age (quadratic)	1	0.0565 ns	1	0.0089*	1	0.0044*	1	0.0201*
Inbreeding coefficient	1	0.3248 ns	1	0.5974 ns	1	0.9377 ns	1	0.6765 ns
Error	561		902		1,063		1,209	
Error variance	28.2		26.5		26.4		25.5	
CV/R^2	47.1/23%		46.2/21%		46.8/19%		47.0/13%	

*Significant effect (P<0.05); ns = not significant (P>0.05).

Table 4. Analyses of variance of the total of recovered structures in different data sets

Source of variation	Superovulation							
	1st		1st and 2nd		1st, 2nd and 3rd		All	
	DF	P>F	DF	P>F	DF	P>F	DF	P>F
Breed	3	0.1288 ns	3	0.6774 ns	3	0.6644 ns	3	0.6858 ns
Herd (breed)	59	0.0350*	60	0.0002*	60	0.0001*	60	0.0001*
Hormone source	1	0.1654 ns	1	0.1540 ns	1	0.2115 ns	1	0.2458 ns
Dose (hormone)	8	-	10	0.0047*	10	0.0033*	10	0.0002*
Month	11	0.4382 ns	11	0.2179 ns	11	0.2472 ns	11	0.0451*
Year	10	0.5436 ns	10	0.1416 ns	10	0.1629 ns	10	0.0611 ns
Order	-	-	1	0.0019*	2	0.0002*	6	0.0018*
Age (linear)	1	0.8550 ns	1	0.1982 ns	1	0.1404 ns	1	0.1921 ns
Age (quadratic)	1	0.3948 ns	1	0.0120*	1	0.0074*	1	0.0077*
Inbreeding coefficient	1	0.1325 ns	1	0.2065 ns	1	0.1183 ns	1	0.0459*
Error	561		902		1,063		1,209	
Error variance	69.4		59.9		57.1		53.3	
CV/R^2	72.7/24%		70.6/22%		70.8/21%		70.7/22%	

*Significant effect (P<0.05); ns = not significant (P>0.05).

A remarkable difference was observed between results from the first superovulation data set and the three others. In the first superovulation, few effects influenced the results: month, in the case of *corpora lutea* and herd within breed for recovered structures. This can be attributed to some aspects of the first superovulation donors, as the standardisation of body conditions and hormone doses, in addition to the variation in ovarian and hormonal conditions (Saumande et al., 1978; Lamberson and Lamberth, 1986; Armstrong, 1993). Estimated least square means were similar (Table 6), whatever the data set considered. This result can be explained by the tendency to early elimination of donors with the worst and best superovulation responses.

Breed did not influence the variables studied, whatever the data set analysed. The effect of herd nested within breed on the three traits was significant, except for *corpora lutea* and viable embryos at the first superovulation. The importance of this effect on subsequent superovulations suggested individual genetic and environmental, as well as management, differences among herds more than Zebu breed differences (Woolliams et al., 1995). Probably, at first superovulation, differences in response for *corpora lutea* and viable embryos were minimised due to previous donors standardisation at the Central and good ovarian and uterine conditions of females chosen for donors.

Table 5. Variance analyses of the number of viable embryos in different data sets

Source of variation	Superovulation							
	1st		1st and 2nd		1st, 2nd and 3rd		All	
	DF	P>F	DF	P>F	DF	P>F	DF	P>F
Breed	3	0.2823 ns	3	0.7372 ns	3	0.6912 ns	3	0.7465 ns
Herd (breed)	59	0.1529 ns	60	0.0010*	60	0.0003*	60	0.0001*
Hormone source	1	0.7092 ns	1	0.2440 ns	1	0.3037 ns	1	0.3564 ns
Dose (hormone)	8	-	10	0.0148*	10	0.0143*	10	0.0049*
Month	11	0.0667 ns	11	0.1154 ns	11	0.1691 ns	11	0.1085 ns
Year	10	0.8584 ns	10	0.8155 ns	10	0.7615 ns	10	0.6953 ns
Order	-	-	1	0.7578 ns	2	0.1881 ns	6	0.7617 ns
Age (linear)	1	0.7059 ns	1	0.1025 ns	1	0.0376*	1	0.0167*
Age (quadratic)	1	0.4439 ns	1	0.0206*	1	0.0055*	1	0.0011*
Inbreeding coefficient	1	0.4289 ns	1	0.3127 ns	1	0.1588 ns	1	0.1033 ns
Error	561		902		1,063		1,209	
Error variance	34.4		30.2		28.5		26.7	
CV/R^2	105.8/19%		99.0/18%		98.0/17%		97.0/17%	

*Significant effect (P<0.05); ns = not significant (P>0.05).

Table 6. Least square means and respective standard errors for MOET traits, according to analysed data sets

Superovulation	*Corpora lutea*	Recovered structures	Viable embryos
1st	11.39±5.36	11.52±8.38	5.54±5.85
1st and 2nd	11.17±5.15	10.97±7.75	5.55±5.49
1st, 2nd and 3rd	11.00±5.14	10.67±7.56	5.45±5.33
All	10.75±5.05	10.33±7.30	5.32±5.17

Hormone dose was not included as source of variation for the first ovulation because it was the same for all donors. The effect of dose nested within hormone was significant on *corpora lutea,* recovered structures and viable embryos for the three other data sets. After the first superovulation, doses of hormones sources were individually adjusted according to the first superovulation response; therefore differences in the response can be explained, among others, by differences in the number of ovarian receptors and ovarian conditions during hormonal treatment (Walton and Stubbings, 1986; Armstrong, 1993). The relationship between dose and response was different for each hormone source (Fig. 1 and 2).

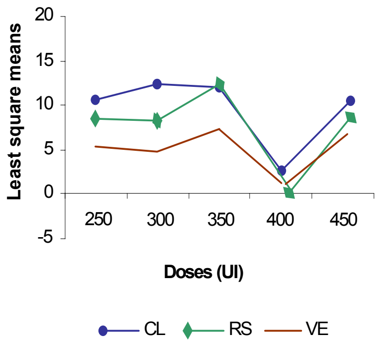

Figure 1. Least square means of the number of *corpora lutea* (CL), total of recovered structures (RS) and number of viable embryos (VE), according to the dose of FSR®.

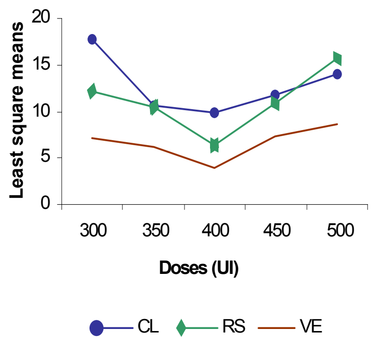

Figure 2. Least square means of the number of *corpora lutea* (CL), total of recovered structures (RS) and number of viable embryos (VE), according to the dose of Pluset®.

The linear and quadratic effects of donor's age were not significant on the first superovulation response, whatever the data set. On the other hand, when considering consecutive superovulation data sets, the linear effect of age was significant only on viable embryos and the quadratic effect was significant for the three traits.

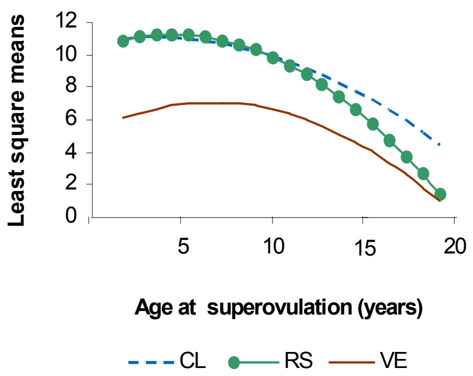

Figure 3. Least square means of the number of *corpora lutea* (CL), total of recovered structures (RS) and number of viable embryos (VE) in function of donor's age.

Response of *corpora lutea* and recovered structure decreases as age increases (Fig. 3). Regarding these two traits, the maximum response to superovulation was derived and the best response was obtained between 4 and 5 years of age.

The maximum value of viable embryos was obtained by donors around eight years old. The superiority of middle-aged cows for this trait is probably related to ovarian maturity (Callesen et al., 1996). Hasler et al. (1981) have addressed the disadvantages of the maintenance of expensive females in MOET nuclei, beyond the age they would usually be discarded in their herds. Accordingly to these authors, this practice could have negative consequences to breeding program efficiency since the genetic superiority of younger individuals is presumed in populations under selection.

The effect of inbreeding coefficient of the donor was important for recovered structures when data of all superovulations were analysed. The linear regression coefficient of the trait on inbreeding coefficient was positive. It must be emphasised, however, that the sample studied consisted of highly chosen donors considering, among others, their previous reproductive performance. Therefore, the undesirable effects of inbreeding depression on reproductive traits could not have been observed.

CONCLUSIONS

Superovulation results are influenced by individual and environmental differences. Dose of hormone and age of superovulation must be considered in the superovulation scheme, at the moment of defining procedures and routines with Zebu donors in order to obtain the desirable results. The procedure of successive treatments and recoveries seems not to damage the results, so this practice can be used as a tool to increase the number of embryos. The effect of inbreeding coefficient needs further studies, since the number of inbred donors in the data was small. These evaluations points out to the viability of breeding schemes based in Selection MOET nuclei of Zebu cattle, since the superovulations of Zebu donors can reach the minimum number of viable embryos to proceed to accurate genetic evaluation and selection.

ACKNOWLEDGEMENTS

The authors kindly thank CENATTE Embryos Inc., especially Dr. Múcio Túlio Teixeira Alvim, by cession of data and EMBRAPA – Milho e Sorgo, especially Dr. Antônio Fernandino de Castro Bahia, by the disposability of computational resources.

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