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Logistic regression analysis of pregnancy rate following transfer of *Bos indicus* embryos into *Bos indicus* × *Bos taurus* heifers

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Abstract

Factors affecting pregnancy rate of 5627 Zebu embryos in crossbred females with unknown proportions of Holstein and Zebu breeding were examined. After evaluation for developmental stage, quality, and viability, embryos were immediately transferred to recipients. Pregnancy diagnosis was conducted approximately 53 d after transfer; pregnancy rate was coded as a binomial event and analyzed using logistic regression models. Maximum likelihood methodology and the likelihood ratio statistic were used to estimate regression coefficients and test hypotheses. Explanatory variables were year of transfer (1992–1999), season of transfer (summer, autumn, winter and spring), breed of the embryo (Guzerat, Gyr or Nellore), stage of the embryo (morula, early blastocyst, blastocyst, expanded blastocyst, and hatching blastocyst), quality of the embryo (excellent, good or regular), and donor–recipient synchrony (estrus in the recipient occurred 2–3 d before, 1 d before, the day of, 1 d after, or 2–3 d after estrus in the donor). Average pregnancy rate was 63.7%. Pregnancy rates were not significantly affected by breed of embryo. The best multiple-logistic model to explain the pregnancy result included the effects of year and season of transfer, embryo stage and quality, and estrous synchrony between donor and recipient ($P \leq .01$). High pregnancy rates occurred when transfers occurred in autumn, early blastocysts or morulae were transferred, and excellent quality embryos were chosen. In addition, pregnancy rates were highest when estrus in the recipient began 1 d earlier than that of the donor.

Keywords: MOET; Embryo transfer; Pregnancy rate; Zebu cattle; Logistic regression

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1. Introduction

Most multiple ovulation and embryo transfers (MOET) in Brazil utilize Zebu (*Bos indicus*) embryos and Zebu × *Bos taurus* crossbred recipients (preferred because of their availability and resistance to tropical environmental stresses). Many factors are critical to the success of embryo transfer [1–6]; apparently the most important factors are technician experience, breed and age of donors and recipients, time interval between estrus of the donor and embryo recovery, estrous synchrony between the donor and the recipient, embryo stage and quality, and month or season of transfer.

Pregnancy rates following embryo transfer have ranged from 35% to 72% worldwide [1–3,7–10], but few studies in Brazil have examined factors affecting

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the success of MOET. In a study on transfer of Blonde d'Aquitaine embryos to Holstein recipients, the pregnancy rate (72%) was similar to that obtained when embryos and recipients were the same breed [2]. Furthermore, there were no differences in pregnancy rates with transfer of fair, good or excellent embryos. In another study, however, a reduced pregnancy rate was obtained with fair embryos [11]. There was an effect of development stage of the embryo; pregnancy rates were worst and best with transfer of morulae/expanded morulae and early to expanded blastocysts, respectively [2]. The effects of year, month, embryo stage and quality, donor–recipient synchrony (as well as some interactions between these factors) significantly affected pregnancy rate (71.3%) in a Holstein embryo transfer program [3].

Concerning donor–recipient synchrony, pregnancy rates decreased and were maximal when recipients were in estrus 36 h after and 12 h before the donors, respectively [4]. It was suggested that matching embryo stages with donor–recipient estrous synchrony was advantageous and that slightly negative asynchrony (recipient in estrus before donor) could be desirable for the transfer of low-quality embryos [3]. However, exact donor–recipient estrous synchrony does not seem essential [3,4,12]; pregnancy rates were similar for exact donor–recipient estrous synchrony and those with asynchrony of 1 d [3,4].

Two questions have been frequently raised: to what extent is asynchrony of reproductive cycles tolerated, and what pharmacological methods can be successfully used for synchronizing reproductive cycles? Furthermore, the influence of many factors on pregnancy rates in MOET programs, such as the reutilization of recipients, location and number of corpora lutea in recipients must be studied [1,4,5,13]. No investigations have been conducted on factors affecting pregnancy rate of crossbred recipients in MOET programs. Thus, the objectives of the present study were to evaluate the effects of various variables on pregnancy rate of Holstein–Zebu recipients of Zebu embryos.

2. Materials and methods

Data from 6806 transfers of Zebu (*B. indicus*) embryos conducted from 1992 to 1999 by a private MOET company (Cenatte Embryos Inc.) located in Minas Gerais state, southeastern Brazil were analyzed (Table 1). Following recovery, embryos were cleaned and evaluated under stereoscopic magnification for embryonic stage and quality. All embryos considered viable, i.e., in morula, early blastocyst, blastocyst, expanded blastocyst or hatching blastocyst stages, and graded as excellent, good or fair [14] were loaded in a straw and immediately transferred to recipients using a surgical method [15]. Recipients were crossbred heifers, varying from 3/4 to 1/2 Holstein–Zebu. The recipient animals were kept in a good nutritional and management conditions and treated with prostaglandin F-2α (Ciosin®, Schering-Plough Coopers, São Paulo, SP, Brazil) to synchronize estrous expression. Pregnancy diagnosis (transrectal palpation) was conducted approximately 53 d after embryo transfer.

Table 1
Transfer of Zebu embryos to *B. taurus* × *B. indicus* recipients, according to breed of the donor (all viable embryos were transferred)

Breed (no. of donors)	No. of structures recovered	No. of embryos transferred	No. of pregnant (%)
Nellore (1095)	11110	5510	3289 (59.7)
Guzerat (169)	1612	916	577 (63.0)
Gyr (61)	415	224	153 (68.3)
Total (1325)	13137	6650	4019 (63.7)

Pregnancy was modeled as a binomial event, with the probability P_i of the ith recipient becoming pregnant (success) and the probability $Q_i = 1 - P_i$ of the ith recipient not becoming pregnant (failure) after embryo transfer. Logistic methodology was applied [16,17] using the CATMOD procedure in SAS® (Statistical Analysis System Institute, Cary, NC, USA) to evaluate the pregnancy event. Maximum likelihood methodology was used to estimate regression coefficients and to test the effects on pregnancy rate of the explanatory variables, using iterative methods and Newton–Raphson algorithm for solving non-linear equations. The following expression was used:

$$P_i = \frac{1}{1 + \exp(-\text{linear model})},$$

where $i = 1, 2, \ldots, n$ (the number of recipients on which pregnancy data were available).

The *linear model* in the expression above was:

$$\beta_0 + \sum_{j=1}^{k} \beta_j X_{ij}$$

where β_0 is the intercept, k the number of explanatory variables and β_j is the regression coefficients associated with the jth explanatory variable (X_{ij}). Under the logistic model, the last observed level of each explanatory variable was considered "phantom" and its regression coefficient equaled the negative of the sum of the regression coefficients of the other levels of that same variable.

The explanatory variables studied were year (1992–1999) and season (summer, autumn, winter and spring) of transfer, breed of the embryo (Nellore, Guzerat and Gyr), stage and quality of the embryo, donor–recipient synchrony and all two-way interactions. All explanatory variables were assumed to be categorical for CATMOD procedures. For this analysis, transfer season was categorized into four classes corresponding to variation in temperature and rainfall, from the mostly rainy and hot to the mostly dry and cold periods, as follows: summer (1 = from December to February of the following year), autumn (2 = from March to May), winter (3 = from June to August), and spring (4 = from September to November). The stage of each embryo was categorized according to its developmental phase [10–14] as late morula (4), early blastocyst (5), blastocyst (6), expanded blastocyst (7), or hatching blastocyst (8). Following literature recommendations [10–14], embryo quality was categorized as excellent (1), good (2) or fair (3). Donor–recipient synchrony was categorized as follows: recipients were in estrus 2–3 d before donors (+2), recipients were in estrus 1 d before donors (+1), recipients were in estrus on the same day as donors (0), recipients were in estrus 1 d after donors (−1), or recipients were in estrus 2–3 d after donors (−2).

Likelihood ratio statistics were used to apply a goodness of fit test to selected models. This statistic is described as $\lambda(B) = -2 \log_e[L(B)/L(P)]$, where $L(B)$ equals the maximum likelihood value associated with the selected (reduced) model and $L(P)$ equals the maximum likelihood value associated with the complete (full) model. According to Myers [18], $\lambda(B)$ is the log of the ratio of the maximized value of the likelihood function for the model that completely satisfies variation in the dependent variable over the maximized value of the likelihood function for the simpler model. Following the procedures of Bergmann and Hohenboken [17], individual regression models for each explanatory variable as well as multiple regression models including combinations of explanatory variables were fit in order to verify their contribution to variation in pregnancy outcome. For multiple regression models, all two-way interactions involving stage of the embryo, quality of the embryo and donor–recipient synchrony in the presence of the main effects were unimportant ($P > .05$). In addition, contrasts were designed to test logit functions of the estimated parameters in the model in order to compare differences in the probability of pregnancy associated with each level of the explanatory variables.

3. Results and discussion

The overall pregnancy rate was 63.7%, ranging from 59.7% for Nellore to 68.3% for Gyr embryos (Table 1). That these results seemed higher than previous reports for Zebu females [7–9] may be attributed to the surgical method of embryo transfer and utilization of crossbred recipient heifers.

The ranges in observed pregnancy rates for each explanatory variable (Table 2) were 53.5% in 1998 to 68.3% in 1994; 60.2% for embryo transfers in summer to 66.2% for autumn transfers; 61.2% when hatching blastocysts were transferred to 65.7% when early blastocysts were transferred; 52.6% when fair embryos were transferred to 66.2% when excellent embryos were transferred; 45.8%, with −2 and −3 recipients (recipients in estrus 2–3 d before donors) to 69.5% with −1 recipients.

Table 2
Pegnancy rate in *B. taurus* × *B. indicus* recipients of Zebu embryos at each level of each explanatory variable

Explanatory variable	Level (code)	Pregnancy rate (%) (no.)
Year of transfer	1992	67.6 (230)
	1993	67.5 (224)
	1994	68.3 (468)
	1995	65.2 (584)
	1996	66.4 (558)
	1997	67.1 (890)
	1998	53.5 (472)
	1999	60.6 (384)
Season of transfer	Summer (1)	60.2 (785)
	Autumn (2)	66.2 (1081)
	Winter (3)	64.4 (995)
	Spring (4)	65.2 (949)
Embryo stage	4	64.1 (312)
	5	65.7 (702)
	6	64.9 (1683)
	7	62.4 (1039)
	8	61.2 (74)
Embryo quality	1	66.2 (2459)
	2	62.2 (1186)
	3	52.6 (165)
Donor–recipient synchrony	+2	60.4 (200)
	+1	69.5 (1343)
	Exact	64.8 (1700)
	−1	55.5 (496)
	−2	45.8 (721)

Embryo stage: 4 = morula, 5 = early blastocyst, 6 = blastocyst, 7 = expanded blastocyst, and 8 = hatching blastocyst. Embryo quality: 1 = excellent, 2 = good, and 3 = fair. Donor–recipient synchrony: +2 = recipient estrus was 2–3 d before donor, +1 = 1 d before donor, exact = same day as donor, −1 = 1 d after donor, and −2 = 2–3 d after donor.

Table 3
Multiple-logistic regression models to evaluate pregnancy rate in *B. taurus* × *B. indicus* recipients of Zebu embryos, using different combinations of explanatory variables

Models	d.f.	Maximum likelihood ratio	$P > \chi^2$
1. Y + Se + Es + Eq + Si	893	1265.3	.0039
2. Y + Se + Es + Si	486	722.9	<.0001
3. Y + Se + Eq + Si	340	563.7	<.0001
4. Y + Se + Es + Eq	325	485.5	<.0001
5. Y + Se + Si	132	238.4	<.0001
6. Y + Se + Es	131	214.4	<.0001
7. Y + Se + Eq	83	161.3	<.0001
8. Es + Eq + Si	50	60.7	.1428
9. Es + Si	15	25.2	.0470
10. Eq + Si	8	8.2	.4169
11. Es + Eq	7	13.2	.0679

Y, year of transfer; Se, season of transfer; Es, embryo stage; Eq, embryo quality; Si, donor–recipient synchrony.

Table 4
Logistic analysis of some variables on pregnancy rate in *B. taurus* × *B. indicus* recipients of Zebu embryos (Model 1)

Source of variation	d.f.	χ^2	$P > \chi^2$
Intercept	1	8.32	.0039
Year of transfer	7	63.77	<.0001
Season of transfer	3	19.32	.0002
Embryo stage	4	27.75	<.0001
Embryo quality	2	31.99	<.0001
Donor–recipient synchrony	4	83.03	<.0001
Maximum likelihood ratio	908	1289.89	<.0001

Results of 11 logistic multiple regression models including combinations of the effects of year and season of transfer, embryo stage and quality and donor–recipient synchrony are presented (Table 3). Comparing the maximum likelihood ratio and probability level resulting from multiple regression models including year and season of transfer (Models 1–7) to those resulting from models not including these factors (8–11), it was clear that year and season of transfer, mostly represented by environmental changes, were the most important factors affecting pregnancy rate following the transfer of Zebu embryos.

For Models 9–11, the multiple-regression model including embryo stage and donor–recipient synchrony (Model 9) was the most important model to evaluate pregnancy rate, indicating that these variables were adequate to predict and obtain better pregnancy rates. In the analyses with the absence of year and season of transfer effects, there was a tendency for the joint effects of embryo stage and embryo quality to cancel out each other, probably because of collinearity.

The first seven models in Table 3 including year and season of transfer effects, plus combinations of embryo–donor–recipient characteristics, were well-fitted to pregnancy results. When sorted by the maximum likelihood ratio, there was a decreasing precision from Models 1–7. Model 1, which explained most of the variance in pregnancy results, was therefore selected for evaluating pregnancy rates and further interpretation (Table 4). Effects of year and season of transfer, embryo stage, embryo quality and donor–recipient synchrony were important ($P < .005$). These factors have also been reported to affect pregnancy rate of purebred recipient females [3]. Effect of embryo breed was not important ($P > .05$), in contrast with other reports [3,5–7], probably due to the lack of improvement for reproduction traits in the Brazilian Zebu breeds (in addition to their common and relatively recent evolutionary origin).

Estimates of regression coefficients for each level of each explanatory variable from the selected model are presented (Table 5). The level of significance of these estimates was related to the last level for each regressor (phantom). Since nutrition at the MOET station had been standardized over the years, differences among years may be mostly attributable to variation in average environmental conditions (e.g. air, temperature and humidity), differences during MOET procedures (e.g. type and dosage of pharmaceuticals used), as well as in the *B. Taurus*–*B. indicus* proportions of recipients. Other authors have considered the contribution of these factors to differences among years [2]. Higher pregnancy rates (14.9%, $P < .002$) were obtained with transfers in autumn; we inferred that this may have been due to lower relative humidity, cool temperatures, and better nutrition (grazing animals were supplemented during this season). Therefore, MOET activities in southeastern Brazil should ideally be conducted during autumn, with spring as a second choice.

Embryos in the early blastocyst stage (5) (Table 5) produced the highest increase in the pregnancy rate (24.76%, $P = .0007$) taken as a deviate from the hatching blastocysts (8), the extreme transferred stage (phantom). However, linear contrasts among estimates indicated the coefficient for early blastocysts (5) was similar to those from morula (4) ($P \geq 0.90$); therefore, the use of a morula or blastocyst lead to an increase of nearly 25% in the probability of pregnancy. Although many technicians have recommended the blastocyst stage (6) as the ideal stage embryo for transfer, in the present study, the use of stages (4) or (5) (morula or early blastocyst) produced the highest pregnancy rate ($P < .001$).

Table 5
Logistic coefficients (β) estimated for each level of variables influencing pregnancy rate in *B. taurus* × *B. indicus* recipients of Zebu embryos (Model 1)

Source of variation	Level	β	Standard error	χ^2	P
Intercept year of transfer	–	0.1933	0.0670	8.32	.0039
	1992	0.1263	0.1074	1.38	.2393
	1993	0.1952	0.1080	3.27	.0706
	1994	0.1803	0.0787	5.25	.0517
	1995	−0.0383	0.0690	0.30	.9315
	1996	0.0215	0.0704	0.09	.4823
	1997	0.1209	0.0603	3.98	.0480
	1998	−0.4885	0.0664	51.85	<.0001
	1999	0.0246	–	–	–
Season of transfer	Summer	−0.1798	0.0491	13.24	.0001
	Autumn	0.1493	0.0472	9.85	.0021
	Winter	−0.0424	0.0469	0.78	.1839
	Spring	0.0729	–	–	–
Embryo stage	4	0.2324	0.0972	5.72	.0168
	5	0.2476	0.0729	11.55	.0007
	6	0.0222	0.0581	0.15	.7030
	7	−0.2258	0.0662	11.62	.0007
	8	−0.2764	–	–	–
Embryo quality	1	0.3199	0.0557	31.90	<.0001
	2	0.0810	0.0519	2.37	.1240
	3	−0.4009	–	–	–
Donor–recipient synchrony	+2	0.1486	0.1014	2.10	.1470
	+1	0.4702	0.0586	62.73	<.0001
	Exact	0.2155	0.0542	15.31	.0001
	−1	−0.1746	0.0683	6.26	.0123
	−2	−0.6597	–	–	–

Embryo stage: 4 = morula, 5 = early blastocyst, 6 = blastocyst, 7 = expanded blastocyst, and 8 = hatching blastocyst. Embryo quality: 1 = excellent, 2 = good, and 3 = fair. Donor–recipient synchrony: +2 = recipient estrus was 2–3 d before donor, +1 = 1 d before donor, exact = same day as donor, −1 = 1 d after donor, and −2 = 2–3 d after donor.

Embryo quality was closely related to pregnancy rate (Table 2); as expected, the better the quality of transferred embryos, the greater the probability of pregnancy. When compared to fair embryos, those classified as excellent produced increases of almost 32.0% ($P < .0005$) for scenarios including the worst and the best combinations of levels of all other explanatory variables (Table 5).

Regarding synchrony, there was a higher pregnancy rate for the +1 recipients, followed by similar results for the +2 and exact recipients (Table 5). Associated increases in pregnancy rates varied from 47.0% for +1 recipients to 14.9% for +2 recipients, depending on the condition examined. Regression coefficient estimates and predicted pregnancy rates associated with exact synchrony were similar to those associated with negative (+2) synchrony ($P < .0001$). Therefore, optimum uterine conditions for Zebu embryo implantation occur when estrus of the crossbred recipient was earlier than that of the donor [2]. It is not clear, however, whether the phase of the reproductive cycle of the recipient is an important aspect of embryo transfer and whether the recipient's phase of cycle must precisely correspond to that of the donor, as recommended by some technicians [5].

Models did not include variable proportions of *B. taurus* and *B. indicus* inheritance of the crossbred recipients, because these percentages were not known. Consequently, differences related to physiological and immunological aspects of recipients and donors were not considered [19,20]. In addition, an unknown Holstein–Zebu heterosis effect might be important for pregnancy rate, as for other reproductive traits [21].

In conclusion, pregnancy rate in a MOET scheme using Zebu donors and crossbred *B. taurus* × *B. indicus* recipient females was significantly affected by year and season of transfer, embryo stage, embryo quality, and donor–recipient synchrony. In general, the highest pregnancy rates in an MOET program in southeastern Brazil were obtained when transfers occurred in autumn, when early blastocysts and morula were used, when the embryos were in excellent condition, and when estrous in the recipient occurred 1 d before that of the donor. Therefore, these factors should be taken into consideration in MOET programs.

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