DOI: 10.5216/cab.v11i1.4521

ANALYSIS OF EMBRYO PRODUCTION IN VITRO FERTILIZATION AND EMBRYO TRANSFER TO NELLORE DONORS

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ABSTRACT

A probability density function for the number of viable embryos produced after an in vitro fertilization program in Nelore donors was adjusted through data provided by the Brazilian Association of Zebu Breeders. Results were based on 20,619 donors, 71,602 aspirations and the total of 509,643 embryos. The probability density function of the number of viable embryos was modeled using exponential distribution. Parameters fitting were carried out for the maximum likelihood using a non-linear gradient method. The precision level was RMSE = 0.040 and R2 = 0.98 for the representation of probability of number of viable embryos produced by Nellore donors by in vitro fertilization technique. To analyze probability density of embryo recovery (Beltrame, 2006) with in vitro fertilization adjust was used the curve comparison test through F test (Silva and Azevedo, 2002). There were no differences between the curves. These results suggest that there may be a unique and restricting factor that affects biologically the embryo production on the embryo transfer and in vitro fertilization techniques.

KEY WORDS: Data bank, probability density, donors, simulation.

RESUMO

ANÁLISE DA PRODUÇÃO DE EMBRIÕES NA FERTILIZAÇÃO IN VITRO E TRANSFERÊNCIA DE EMBRIÕES PARA DOADORAS NELORE

Uma função de densidade probabilidade para o número de embriões viáveis produzidos após Fertilização In Vitro em doadoras da raça Nelore foi ajustada a partir de dados fornecidos pela Associação Brasileira de Criadores de Zebu (ABCZ). Os resultados partiram da análise referente a 20.619 doadoras, 71.602 aspirações e um total de 509.643 embriões. A densidade probabilidade do número de embriões viáveis foi modelada utilizando a função exponencial. A determinação dos parâmetros foi executada utilizando a máxima verossimilhança em um método de gradiente não linear. O nível de precisão obtido foi de RMSE = 0,040 e R2 = 0,98 para a representação da probabilidade do número de embriões viáveis produzidos por doadoras Nelore na técnica de Fertilização In Vitro. A comparação destes resultados com a curva de densidade probabilidade da recuperação embrionária determinada em Beltrame 2006, foi realizada através da técnica de comparação de curvas pelo teste F segundo o descrito por Silva & Azevedo (2002). Não foram encontradas diferenças entre as curvas do número de embriões viáveis obtidos após coleta e produzidos após aspiração de doadoras na raça Nelore. Ainda sugere-se a existência de um fator único limitante que afete biologicamente a produção de embriões nas técnicas de transferência de embriões e fertilização in Vitro.

PALAVRAS-CHAVES: doadoras, densidade probabilidade, simulação, banco de dados.

INTRODUCTION

In national bovineculture, many advancements have occured In Vitro Fertilization (IVF), Embryo Transfer (ET), and Spermatozoom Sexing. However, the uncertainty about the results is constant, what generates inaccurate simulated answers compared to observed results. Thses distinctions have been minimized by the implementation of appropriate simulation methodologies (Beltrame, 2006; Beltrame, 2007; Barioni, 2007).

During the IVF process of embryos coming from any donor, it is impossible to predict the exact number of oocytes that will be picked up neither the number of viable embryos that will be preduced. Even though many different decisions concerning IVF and ET depend on some information (number of recipients to be syncronized, cost, number of embryos to be frozen, used material, time, etc.), in some animal reproduction biotechniques, it is necessary to make predictions based on historical data (Beltrame et al., 2007). Thus, the demand for a deeper study of a set of random variables is evident, with the purpose of determining their behavior in simulated settings, not as determinists variables.

Once a random variable is defined, the interest in calculationg the probability values associated to this particular varible arises. The set of variables and corresponding probabilities is named probability distribution, i.e. {(xi,p(xi), I=1,2,…n}. In this case, the probability density function is the one that associates to each value assumed by the random variable the probability of the corresponding event, such as, P(X=xi)= P(Ai), i=1,2,…,n (FREITAS FILHO, 2001; LEVINE et al., 2005).

The system simulation demands the use of value sequencies of certain random variables. There are three ways of obtaining such sequencies: the use of sequencies that come from previously made observations; randomly generated sequencies from empirical distributions constituted with previously made observations;random generated sequencies from classical distributions, whose patterns were estimated based on previously made observations (PERIN FILHO, 1995).

In the first case, the process is long and requires much memory, being its application inadequate for system simulation. The other two alternatives depende on a random number generator, which allows to obtain random variable values with the desireable distributions.

The determination of the probality density curve of the variable number of viable embryos has as objective its realistic projection in simulation studies. Thus, estimates the number of pregnancies, number of recipients and donors to be used, technique cost, development of projects and financial viability calculation can be carried out with greater precision, minimizing the risk (BELTRAME, 2006).

In this context, the objective of this work was to determine the probability density curve of the number of viable embryos produced by IVF for Nellore donors, comparing it to the probability density curve of the ET determined by BELTRAME in 2006.

METHODS AND MATERIALS

The present work is based on data given by the Brazilian Association of Zebu Breeders (Associação Brasileira de Criadores de Zebu - ABCZ) relative to IVF in Nellore donors. The frequency of number of viable embryos obtained after ET described by BELTRAME in 2006 was also used in this work.

The ABCZ database was composed by "txt" electronic files with data about IVF embryos. The files presented the events in the lines, and the donor´s code, the bull´s code, the Federal State where the farm is located, the pick-up date, the transfer date, produced embryos, transfered embryos, and lost embryos in the columns.

The information related to the IVF was organized in Microsoft Excel 2003 spreadsheets and hence analyzed by the SAS tool for Windows 1999. Data about 73,121 pick-ups were available. Once the data were in the SAS tool, all the incoherent data and the null values were eliminated. Only the pick-ups carried out between 2000 and 2007 were considered. The pick-ups whose number of obtained and/or lost embryos was superior than 35, as well as the ones with inconsistent date and/or values in blank were withdrawn

The restriction application resulted in the discard of 1,519 pick-ups. Consequently, 20,619 donors, 71,602 pick-ups, and 509,643 embryos were analyzed.

The selection of relevant data was carried out by the ´proc freq` function of the SAS for Windows 1999. In this work specific case, this function returned the summation of the pick-up observed number for each value of number of produced viable embryos. The total number of pick-ups that generated null viable embryo, until the summation of pick-ups that generated 35 viable embryos, maximum number of viable embryos per donor restrict by the database were obtained by this "command".

A graphic comparison and the numerical analysis based on the frequency observed and estimated from the selected distribution (or distributions) model were carried out. It was decided that the null frequency would be estimated as similar to the viable embryo obtainment frequency, and then an adjustment would be operated.

The data graphic behavior is essential for a preliminary diagnosis of a distribution choice according to the demonstration in Figure 1.

The graphic disposition of the various existent distributions, and the behavior of the observed data suggest a proximity to a negative exponential distribution [EX(λ)] (BENJAMIN & CORNELL, 1970). For this reason, a numerical methodology was used to identify the best values for the patterns and compare these models adjustment to the observed data..

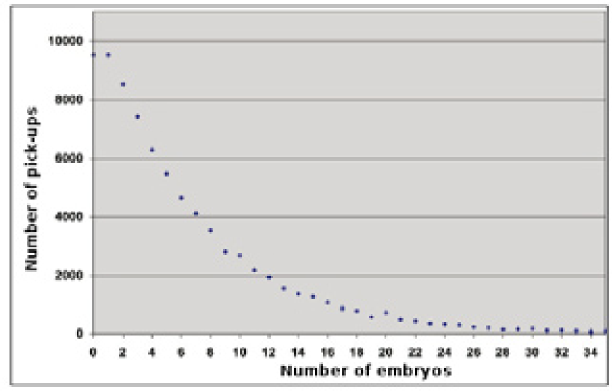

Figure 1 – Observed frequency of the embryo production regarding the number of pick-ups carried out for the in vitro fertilization technique.

The distribution patterns estimation and its adjustment, to efficiently represent the variance and minimize total error, were carried out by the "maximum likelihood estimation" method and its estimator (KOSTINA, 2004). The method GRG2 with Microsoft Excel 2003® Solver was applied as an optimization tool.

The described adjustment procedures were summerized and they can be observed in Table 1, for the use of exponential distribution [*EX(λ)*].

Table 1 – Adjustment procedures of negative exponential distribution of the number of viable embryos in Vitro Fertilization technique.

N. embryoes (1)	Obs. Freq. (2)	Estimated Freq. (3)	Pred. Error (4)	Error (5)
0	0,118	$F(x) = 1 - e^{-1x}, x>0$	(3)-(2)	(4)
1	0,118	$F(x) = 1 - e^{-1x}, x>0$	(3)-(2)	(4)
2	0,105	$F(x) = 1 - e^{-1x}, x>0$	(3)-(2)	(4)
...	...	...		Σ*error*

(1) Number of viable embryoes per pick-up; (2) Observed frequency in the collected data; (3) Estimated frequency from probability distribution; (4) Prediction error; (5) Total error.

Statiscally, after patterning the model, F test was carried out and the determination coefficient was calculated. This way, it was determined how much of the obtained variance could be explained by the model as well as of the data significance by the new distribution.

The best choice criteria relative to the distribution that adjusts to the observed data were evaluated according to the importance, respectively, the sum of squared of error (SQ error) and the error prediction

$$\Sigma \text{ EP} = \frac{\text{Observed Frequency} - \text{Estimated Frequency}}{\text{Observed Freqency}} x \ 100$$

Models comparison

In BELTRAME (2006) the obtained ET database was adjusted by a database management application. In this work a free software modeled by the IBAdmin 4.0 tool was choosen, in which 26,767 donors, 61,928 collections, and 451,322 embryos were analyzed.

Considering the exposed, in BELTRAME (2006), the ET adjustment was carried out through negative exponential distribution. Then, using the same model and two (or more) data sets (ET and IVF), the objective of this work was to know if these data sets would behave in the same way.

Thus, in order to compare the statistical significance of the difference between the models (probability curves of ET, ajusted by BELTRAME, in 2006, and of IVF, in this work) a new statistical analysis was applied. The likelihood test was represented by the following equation (Silva & Azevedo, 2002):

$$F = \frac{[SRQ_{(comb)} - (SRQ_{ET} + SRQ_{IVF}]/1}{(SRQ_{ET} + SRQ_{IVF}) / 35},$$

In the equation F is the distribution value corresponding to the ratio of the difference between the sum of residual squares of the combined curve; SRQ_{ET} is the sum of the residual squares of the ET probability curve; SRQ_{IVF} is the sum of residual squares of the IVF probability curve; $SRQ_{(comb)}$ is the sum of the residual squares of the probability resulting from the adjustment with the data from the ET and IVF curves combined.

The divisors 1 and 35 represent the respective degrees of freedom of the numerator and of the denominator used in the calculation of the ratio between the residual mean squares of each pair of campared curves. The critical level of probability stablished to judge the significance of differences was 5%, which, in this case, corresponded to the value of F=4.13 for 1 and 34 degrees of freedom in the numerator and denominator, respectively.

RESULTS AND DISCUSSION

The observed frequency corresponds to the demonstration of the specific event in the past. The obtained data represent the summation of the number of collections with viable embryos varying from 0 to 35. Such information render possible the determination of the probability density curve. Table 2 presents these data.

Tablel 2 – Observed frequency of the number of viable embryos per pick-up for Nellore breed.

Viable embr.	Total of pick-ups	Obs. Freq. (%)
0	448	0,63
1	9.531	13,31
2	8.542	11,93
3	7.431	10,38
4	6.300	8,8
5	5.494	7,67
6	4.660	6,51
7	4.125	5,76
8	3.548	4,96
9	2.820	3,94
10	2.704	3,78
11	2.194	3,06
12	1.955	2,73
13	1.573	2,2
14	1.376	1,92
15	1.292	1,8
16	1.098	1,53
17	878	1,23
18	789	1,1
19	589	0,82
20	742	1,04
21	507	0,71
22	438	0,61
23	369	0,52
24	338	0,47
25	314	0,44
26	241	0,34
27	220	0,31
28	161	0,22
29	176	0,25
30	200	0,28
31	124	0,17
32	136	0,19
33	108	0,15
34	81	0,11
35	100	0,14

As it can be observed in Table 2, the frequency of null embryos is presented as an atypical datum in the table (f(0)=0.63). Although it can occur, it can be suggested that such datum comes from miscommunication among veterinarians and farmers about the pick-ups that present null number of viable embryos, showing, therefore, extremely low frequency.

In the analysis process of the collected data for use in a stochastic analysis simulator, one of the main activities is the identification of a theorical probability distribution that better represents the variable behavior (FREITAS FILHO, 2001).

For IVF, the mean of viable embryos per pick-up revealed by the observed frequency was of 6.43 + 5.5. This mean obtention presupposed that the number of pick-ups which resulted null and in one embryo was similar. The adjustment pattern of the IVF model was k=0.148 for the negative exponential distribution. The adjustment was considered highly significant (P<0.0001; Table 3 and Figure 2).

The determination coefficient can also be considered high (R2=0.98), indicating that the distribution explains most of the variance in the observed data. The standard regression error was of 0.040.

One try to predict the efficiency of the IVF programs by measurement in relation to the number of blastocysts obtained from the number of oocyte cultivated and fertilized with certain semen (FUENTES, 2006). However, these variables behavior is not known in the IVF technique.

The mean of viable embryos obtained by pick-up in this work is close to what is reported in the literature. NONATO JR. et al (2006) compared four OPU protocols in Nellore cows. The number of produced embryos varied from 6.1 + 1.0 to 12.0 + 2.6, being the former with the use of progestagens.

MACHADO´s et al group (2006), with the purpose of studying the variability in vitro production, analyzed the hypothesis that lower variability would occur between identical twins than when non-related individuals were worked. Difference (P<0.05) was found in the number of blastocyts produced between the compared groups.

CHAUBAL et al (2007) compared hormonal treatments in an attempt of promoting ovarian stimulation and of increasing the embryos production through the IVF technique. Although a smaller number of embryos had been produced with the utilized treatments, the variability in the embryos production persisted. The importance of the knowledge about the variable behavior and its projections in simulation systems is evident. Figure 2 demonstrates that there is one characteristic behavior of the analyzed variable regardless of the system being studied. This behavior is decreasing and proportional to the increase of the number of produced embryos, being ajustable to specific situations. Moreover, this work results indicate that the number of viable embryos produced by pick-ups from Nellore donors can be estimated by exponential distribution.

Table 3 – Characters of the variance analysis for the exponintial distribution ajusted to the number of viable embryos per pick-up.

VS	FD	SQ	MQ	F	P<
Regression	1	0,04023	0,0402	2510,9	0.0001**
Error	34	0,00062	0,000016	-	-
Total	35	0,04085	-	-	-
R^2	0,98471		-	-	-

VS=Variation source; FD= Freedom degree; SQ= Sum of the squared error; MQ= Mean square; F= Significance Test; ** = probability P<0.0001.

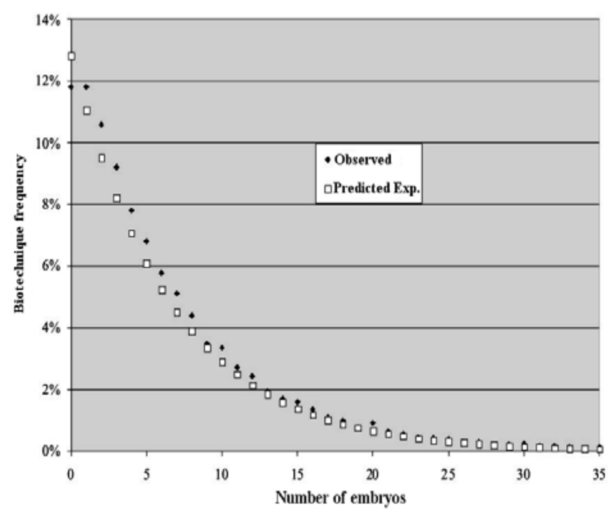

Figure 2 – Comparison between the observed frequency and the estimated frequency of the number of viable embryos per pick-up obtained by the exponential distribution.

Models comparison

Figure 3 and the results obtained from the evaluation of the models comparison indicate the non-significance of the tested hypothesis, i.e., there is no difference (P>0.05) beteween the ET and IVF analyzed curves. The statistical demonstration of the result can be observed in Table 4.

Table 4 – Characters of the variance analysis for combined models of the number of viable embryos produced after collection and pick-up**.**

VS	FD	SQ	MQ	F	P>
Regression	1	0,0853	0,0853	0,81	0,05^{ns}
Error	34	0,0024	0,0000715	-	-
Total	35	0,0877	-	-	-
R^2	0,9723		-	-	-

VS=Variation source; FD= Freedom degree; SQ= Sum of the squared error; MQ= Mean square; F= Significance Test; ns = probability P>0.05 – non significant.

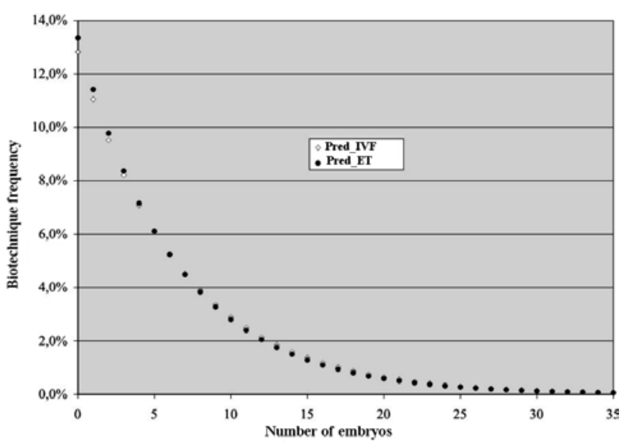

Figure 3- Frequency predicted by the exponential distribution of viable embryos produced after embryo tranfer and in vitro fertilization.

It is important to emphasize how incipient the study is at the animal reproduction realm. Although an accurate result can be reached mathematically, the biological explanation of the curves similarities encompasses a series of suppositions.

By the presente results, it can be initially assumed that there is a common process, which limits the number of viable embryos in both techniques. It could be the consequence of factors that inflence the follicular dynamic at the donors´ ovulatory response, that affect the fertilization and the embryo vialability, and that are realated to the manegement program as described by PEIXOTO et al (2002).

Considering the follicular dynamic a limiting factor to the embryo production, the construction of mathematical models which could depict individually the follicular growth evolution, excluding the population behavior, could be useful to more accurately estimate the number of viable embryos obtained per collect. Nevertheless, studies whose object is such modeling are complex, and they have not been described in Veterinary Medicine yet.

With regards to the curves similarities, one aspect that could be cotated is that, considering the projection number of viable embryos in ET or IVF, the number of embryos generated by a random process will have the same evolution capacity. In this case, the time dynamic should be altered. The animal´s genetical improvement acceleration becomes evident by the time (repetition), not by the technique used. In similar situations, in which the genetical and environmental effect are not considered, the embryo production projected in a simulation model could be the same.

Also, with regards to the IVF and ET comparison, the results allow the identification of the biotechniques as auxiliary, not as conflicting. In this case, the inefficiencis of one of them could be compensated by the benefits of the other. Animals which do not respond to the ET technique could be used for IVF. Similarly, animals that underwent consecutive ET can be inseminated and at the beginning of the pregnancy they can be submitted to IVF. Thus, a greater selection speed could be reached. However, other batabase should be tested in order to confirm the results obtained in this study.

It is emphasized in this work that more attention should be given to the biotechnique indices. Although no differences between the ET and IVF embryo production curves have been demonstrated, some variables such as pregnancy rates, consecutive use of donors, and possibility of embryo frozen could be crucial for specifically choosing one of the biotechniques.

CONCLUSIONS

The results presented lead to the folowing conclusions:

1) The distribution of exponensial probability density is more adequate to the adjustment to the observed frequency for the purpose proposed (use in simulation) in the IVF biotechnique.

2) There is great proximity of the number of viable embryos obtained after collecting and pick-up between the tested ET and IVF curves

ACKNOWLEGMENTS

To the Associação Brasileira dos Criadores de Zebu (Mr. Carlos Henrique Cavalari) for the concesson and permission for data use. To CNPq for the scholarship concession for the first author.

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Submitted on: 14 August 2008. Accepted on: 21 December 2009.