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Genetic parameters of *in vitro* production of Nellore and Senepol embryos

Parâmetros genéticos da produção *in vitro* de embriões das raças Nelore e Senepol

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Resumo: Este estudo teve por objetivo estimar parâmetros genéticos para características de Produção *in vitro* de Embriões - PIVE das raças Nellore e Senepol. Foram utilizados dados de 1.247 rodadas de fertilização *in vitro* (1.029 Nellore, 218 Senepol), no total de 148.311 oócitos (116.972 Nellore, 31.339 Senepol), 47.301 embriões (38.722 Nellore, 8.579 Senepol) e 6.323 prenhezes (5.534 Nellore e 789 Senepol). Foram analisadas as variáveis: porcentagem de oócitos viáveis (Pooc), porcentagem de embriões clivados (Pcliv); porcentagem de embriões produzidos (Pemb); porcentagem de prenhezes (Ppren) por rodada/touro; média de oócitos viáveis por doadora (MOD), média de embriões produzidos por doadora (MED) e média de prenhezes por doadora (MPD) de dados fornecidos por empresa parceira entre os anos de 2019 a 2022. Foi utilizado o programa SAS para análise dos efeitos fixos e Correlação Linear de Pearson. Os componentes de variância para cálculo das herdabilidades foram calculados por meio do programa MTDFREML. Foram obtidos valores de MOD, MED e MPD para as raças Nellore de 29,94; 10,01; 2,53 e Senepol de 30,12; 8,17; 2,34. De modo geral, a raça Nellore proporcionou melhor produção de embriões em relação à raça Senepol. As estimativas de herdabilidades foram de baixa a média magnitude, sendo para Pcliv (0,16 e 0,04), Pemb (0,14 e 0,08), Ppren (0,02 e 0,15), MED (0,07 e 0,02) e MPD (0,05 e 0,00) para as raças Nellore e Senepol. Porém, indicando a presença de variabilidade genética e possibilidade de seleção. Conclui-se que há variabilidade genética para as características PIVE, para ambas as raças, indicando que podem ser utilizadas como critérios de seleção por serem herdáveis e que a raça Nellore apresenta melhor desempenho para as características de PIVE em relação à raça Senepol.

Palavras-chave: Características Reprodutivas; Efeitos fixos; Herdabilidade.

Abstract: This study aimed to estimate genetic parameters for traits of *in vitro* embryo production (IVEP) of Nellore and Senepol cattle. Data from 1,247 rounds of *in vitro* fertilization (1,029 Nellore, 218 Senepol) were used, totaling 148,311 oocytes (116,972 Nellore, 31,339 Senepol), 47,301 embryos (38,722 Nellore, 8,579 Senepol), and 6,323 pregnancies (5,534 Nellore, 789 Senepol). The variables

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percentage of viable oocytes (Pooc), percentage of cleaved embryos (Pcleav), percentage of produced embryos (Pemb), percentage of pregnancy (Ppreg) per round per bull, mean number of viable oocytes per donor (MOD), mean number of embryos produced per donor (MED), and mean number of pregnancies per donor (MPD) were analyzed from data provided by a partner company between the years 2019 and 2022. The SAS program was used to analyze fixed effects and Pearson linear correlation. The components of variance for heritabilities were calculated using the MTDFREML program. MOD, MED, and MPD values of 29.94, 10.01, and 2.53 were obtained for Nellore and 30.12, 8.17, and 2.34 for Senepol, respectively. In general, Nellore provided better embryo production compared to Senepol. Heritability estimates showed low to medium magnitude, with values Nellore and Senepol of 0.16 and 0.04 (Pcleav), 0.14 and 0.08 (Pemb), 0.02 and 0.15 (Ppreg), 0.07 and 0.02 (MED), and 0.05 and 0.00 (MPD), respectively. However, it indicates the presence of genetic variability and the possibility of selection. Therefore, there is genetic variability for IVEP traits in both breeds, indicating that they can be used as selection criteria because they are heritable and that Nellore presents better performance for IVEP traits compared to Senepol.

Keywords: Reproduction traits; Fixed effects; Heritability.

1. Introduction

Bovine embryo production has increased worldwide over the years, and Brazil has a prominent position, mainly due to the use of the IVEP technique. Brazil became the world's second-largest embryo producer in 2019.[1]

Genetic multiplication of cattle in the 1980s was exclusive to males through natural breeding or, to a lesser extent, the reproductive technique of artificial insemination. However, bovine female genetics began to be used on a large scale with the emergence of reproduction techniques for producing *in vivo* and *in vitro* embryos.[2]

IVEP allowed greater dissemination of female genetics in genetic improvement, contributing to a higher number of offspring from a single donor.[3] However, there are still some limiting factors in the application of this biotechnique, including those related to the donor,[8,9] such as quality and quantity of oocytes per donor, and also extrinsic factors such as cultivation conditions,[6,7] the technician experience,[3,10] the bull effect,[4,5] the semen effect,[4,10], the year[4] and season effects,[4,10] and nutritional conditions.[5] These factors lead to low reproduction rates in the production of oocytes, embryos, and pregnancies.

In addition to information on production performance, genetic parameters related to production in IVEP are important to support genetic improvement strategies in donor and breeding selection programs.

Regarding genetic components, some reports in the literature show that zebu animals have better results for IVEP traits than taurine ones. Therefore, studies on the methodologies that consider the selection of IVEP traits are necessary for possible inclusion in future genetic evaluations.

In this sense, there is a need for studies that include the estimation of genetic parameters aiming to evaluate the genetic variability of these traits for Nellore and Senepol cattle, enabling the selection of breeders for higher efficiency in this biotechnique.

The literature also has few studies regarding variance components and other genetic parameters related to the production of oocytes, embryos, and pregnancies in IVEP for Nellore and Senepol cattle in the Amazon biome. In this context, this study aimed to estimate genetic parameters for the *in vitro* production of embryos in Nellore and Senepol cattle in the State of Acre, Brazil.

2. Material and methods

2.1 Data structure

This study was submitted and approved by the Ethics Committee on the Use of Animals of the Federal University of Acre (UFAC) (CEUA/UFAC No. 23107.018598/2021-33). The IVEP data for Nellore and Senepol cattle for the years 2019 to 2022 of this study were provided by the company *In Vitro*, Acre.

The animals used in IVEP mating, donors and bulls, are purebred and registered with the Brazilian Association of Zebu Breeders (ABCZ) and the Brazilian Association of Senepol Cattle Breeders (ABCB Senepol). Data from 1,247 rounds of *in vitro* fertilization (1,029 Nellore, 218 Senepol) were used, totaling 148,311 oocytes (116,972 Nellore, 31,339 Senepol), 47,301 embryos (38,722 Nellore, 8,579 Senepol), and 6,323 pregnancies (5,534 Nellore, 789 Senepol). Even though the sample size is different between breeds, the volume of information is expected to be sufficient for the statistical inference intended in the present study.

2.2 IVEP technique procedure

Oocytes were collected using the Ovum Pick-Up (OPU) follicular aspiration technique, sent to the laboratory for *in vitro* maturation, and maintained in an incubator at of 38.5 °C with an atmosphere of 5% CO2 for 20 to 22 hours. Sexed and conventional semen obtained from semen marketing centers was used in the *in vitro* fertilization. The protocol was based on the centrifugation technique through the discontinuous Percoll gradient, with a sperm concentration of $5x10^6$ sperm/mL.

Incubation (oocytes and sperm) was carried out for a period of 18 to 21 hours in an oven with an atmosphere of 5% CO2 and a temperature of 38.5 °C. Cumulus cells were taken from the zygotes and maintained in an incubator at 38.5 °C and an atmosphere of 5% CO2 for *in vitro* cultivation. Cleavage assessment was performed on day three (D3) after *in vitro* fertilization, while inovulation was performed on day 17.

The protocol for preparing the recipients for inovulation began on day zero (D0) with the insertion of the intravaginal device with 1 g progesterone (P4) (Repro Neo®, Biogénesis) and the application of 2 mL estradiol benzoate (EB) (Bioestrogen®, Biogénesis Bagó). The implant was removed on day eight (D8) by applying 2 mL prostaglandin (PGF2) (Croniben® D-cloprostenol, Biogénesis Bagó), 300 IU (1.5 mL) equine chorionic gonadotropin (eCG)

(Ecegon®, Biogénesis Bagó), and 2 mL estradiol cypionate (EC) (Croni-cip®, Biogénesis Bagó). The embryos were inovulated on day seventeen (D17). Pregnancy diagnosis was performed 30 and 60 days after inovulation, using transrectal ultrasound (Mindray/DP10 Vet).

2.3 Evaluated variables

The variables percentage of viable oocytes (Pooc), percentage of cleaved embryos (Pcleav), percentage of produced embryos (Pemb), percentage of pregnancies (Ppreg) per round per bull, mean number of viable oocytes per donor (MOD), mean number of embryos produced per donor (MED), and mean number of pregnancies per donor (MPD) were considered for statistical analysis. The following equation calculated the variable Pooc:

$$\text{Pooc} = \frac{\text{Nooc}}{\text{Noa}} \cdot 100$$

where Pooc is the percentage of viable oocytes, Nooc is the number of viable oocytes, and Noa is the number of aspirated oocytes. The following equation calculated the variable Pcleav:

$$\text{Pcleav} = \frac{\text{Ncleav}}{\text{Nooc}} \cdot 100$$

where Pcleav is the percentage of cleaved embryos, Nclaev is the number of cleaved embryos, and Nooc is the number of viable oocytes. The following equation calculated the variable Pemb:

$$\text{Pemb} = \frac{\text{Nemb}}{\text{Nooc}} \cdot 100$$

where Pemb is the percentage of produced embryos, Nemb is the number of produced embryos, and Nooc is the number of viable oocytes. The following equation calculated the variable Ppreg:

$$\text{Ppreg} = \frac{\text{Npreg}}{\text{Nooc}} \cdot 100$$

where Ppreg is the percentage of pregnancies, Npreg is the number of pregnancies, and Nooc is the number of viable oocytes. The following equation calculated the variable MOD:

$$\text{MOD} = \frac{\text{Nooc}}{\text{Nd}}$$

where MOD is the mean number of oocytes per donor, Nooc is the number of viable oocytes, and Nd is the number of donors. The following equation calculated the variable MED:

$$\text{MED} = \frac{\text{Nemb}}{\text{Nd}}$$

where MED is the mean number of embryos per donor, Nemb is the number of produced embryos, and Nd is the number of donors. The following equation calculated the variable MPD:

$$\text{MPD} = \frac{\text{Npren}}{\text{Nd}}$$

where MPD is the mean number of pregnancies per donor, Npreg is the number of pregnancies, and Nd is the number of donors.

2.4 Statistical analysis

Initially, data consistency analysis was carried out by discarding incomplete or inconsistent information, such as discrepant pregnancy values. Subsequently, descriptive statistics was performed to obtain the means, standard deviations, and maximum and minimum values. The generalized least squares method was used to compose the data set using the PROC GLM procedure of the Statistical Analysis System program[11] assuming a 5% statistical significance level to verify the significance of non-genetic effects (fixed effects) that affect IVEP. The Tukey test was applied to the traits that had a statistical difference in the analysis of variance. Data normality was analyzed using the Shapiro-Wilk test at a 5% significance level.

The following fixed effects were evaluated: season (dry – corresponding to the months June to September, and rainy – corresponding to the months October to May), year (2019, 2020, 2021, and 2022), and type of semen (conventional and sexed).

Correlations were obtained through Pearson linear correlation analyses using the SAS PROC CORR procedure. The mixed model methodology by Henderson (1953) was used for genetic analysis, and the animal model was adopted. Estimates of variance and heritability components were obtained by the restricted maximum likelihood (REML) method, using the MTDFREML – Multiple Trait Derivative Free Restricted Maximum Likelihood program,[12] adopting the single-character animal model, according to:

$$Y_{ij} = \mu + EF_i + \propto_{ij} + e_{ij}$$

in which Y_{ij} is the mean of *in vitro* production parameters for each animal j belonging to fixed effect i , μ is the overall mean, *EFi* is the fixed effect considered in the genetic evaluation for the in vitro production traits, $\propto_{ij}$ is the direct additive genetic effect of animal j belonging to fixed effect i , and e_{ij} is the residual effect.

The contemporary groups were formed according to the combination of significant fixed effects, based on the analysis of variance using PROC GLM from SAS. The contemporary group for evaluating the variables Pcleav, Pemb, Ppreg, MED, and MPD was formed by combining the fixed effect of year, season, and semen. The model used for data analysis is represented matricially by:

$$y = X\beta + Z\alpha + e$$

in which y is the vector of observations for each evaluated trait, β is the vector of fixed effects, α is the vector of random effects of additive genetic values of animals, e is the vector of random environmental effects/errors, and X and Z are the matrices corresponding to the

observations for fixed effects and additive genetic random effects of animals, respectively, for which assumes:

$$\begin{bmatrix} y \\ a \\ e \end{bmatrix} \sim N\left\{ \begin{bmatrix} X\beta \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} ZGZ' + R & ZG & R \\ GZ' & G & \emptyset \\ R & \emptyset & R \end{bmatrix} \right\}$$

in which *G* is the matrix of variances and covariances of the random effects of vector α, and *R* is the matrix of residual variances and covariances. The matrices *G* and *R* are described as:

$$G = A \otimes G_0$$

where *A* is the matrix that indicates the degree of kinship between individuals, and G_0 is the matrix of additive genetic variances and covariances between the traits that make up the observations, and ⊗ is the direct product operator between matrices, and:

where I is the identity matrix of order equal to the line dimension of y, R_0 is the matrix of variances and residual covariances between the traits that make up the observations, and ⊗ is the direct product operator between matrices.

3. Results and discussion

Table 1 shows the phenotypic means and respective standard deviations for Nellore and Senepol cattle. The main percentage means obtained for Nellore and Senepol were 84.66 and 81.14 for viable oocytes, 33.89 and 27.50 for produced embryos, and 8.98 and 6.92 for pregnancies, respectively, per round of IVEP battery.

Table 1 Phenotypic means and standard deviations of Nellore and Senepol cattle.

Variable	Nellore	Senepol	p-value
Pooc	84.66 ± 9.82	81.14 ± 10.75	<0.0001*
Pcleav	65.72 ± 21.83	61.74 ± 20.92	<0.0102*
Pemb	33.89 ± 16.83	27.50 ± 13.92	<0.0001*
Ppreg	8.98 ± 7.97	6.92 ± 5.66	<0.0247*
MOD	29.94 ± 15.24	30.12 ± 19.55	0.8644ns
MED	10.01 ± 7.41	8.17 ± 6.46	<0.0005*
MPD	2.53 ± 2.26	2.34 ± 2.11	0.4776ns

** = significant (P<0.05), and ns = not significant (P>0.05). Pooc = percentage of viable oocytes; Pcleav = percentage of cleaved embryos; Pemb = percentage of produced embryos; Ppreg = percentage of pregnancies; MOD = mean number of viable oocytes per donor; MED = mean number of embryos produced per donor; MPD = mean number of pregnancies per donor.*

No statistical difference p>0.05 was observed for MOD and MPD when comparing the two breeds. However, the variables Pooc, Pcleav, Pemb, Ppreg, and MED showed a significant difference (p≤0.05). In general, Nellore cattle provided better mean phenotypic values for IVEP traits than Senepol cattle.

Nogueira et al.[10] also found effects between genetic groups on IVEP when evaluating and compiling data from a commercial company in Brazil, with superiority in the production of embryos/OPU for the zebu breed (Nellore = 7.83), followed by synthetic breeds (Girolando = 6.73 and Brangus = 4.78) and finally the taurine breeds (Holstein = 2.84 and Senepol = 2.53).

Moschini et al.[13] developed research with records from the company ABS Pecplan to evaluate taurine (Holstein and Senepol) and zebu (Nellore and Gir) donors and found variation between genetic groups for the mean number of cleaved embryos (taurine = 16 and zebu = 20.4) and mean embryos (taurine = 4.4 and zebu = 8.3).

Research has been conducted aiming to clarify the mechanisms involving variations between taurine and zebu breeds. These differences are mainly related to the breed's physiology and genetics and are modulated by several factors such as management, nutrition, heat stress, conditions, and climate.[14,15,16]

The mean production values of oocytes, embryos, and pregnancies per donor for both breeds are consistent with research in several Brazilian regions, demonstrating that the IVEP technique has been well developed in Acre.

The IVEP technique is widely disseminated and consistent for commercial production in Nellore cattle.[17] The literature shows mean values of oocytes per donor ranging from 23.35 to 30.74, mean embryos per donor ranging from 7.83 to 10.09, and mean pregnancies per donor ranging from 2.71 and 3.03.[8,10,13,18,19] The means observed in this study corroborate the values found for these groups. The mean number of oocytes per donor in this research was 29.94, the mean number of embryos per donor was 10.01, and the mean number of pregnancies per donor was 2.53.

Senepol cattle showed quite satisfactory mean production values for oocytes and embryos. IVEP data in the literature on Senepol cattle is still scarce, but the mean values for oocyte production in recent research on the breed were 23.17 and 31.5 and the mean values for embryo production per donor were 2.53 and 8.0.[10,13,20] In this study, the means are within these values, in which the mean number of oocytes per donor in this research was 30.12, the mean number of embryos per donor was 8.17, and the mean number of pregnancies per donor was 2.34.

Table 2 shows the mean values and respective standard deviations for the fixed effects of season, year, and type of semen. The fixed effects were mostly significant ($p \leq 0.05$) for IVEP traits, demonstrating that the different seasons, the variability between years, and the type of semen are environmental factors that explain part of the variability of traits assessed in IVEP.

Table 2 Means and standard deviations according to the fixed effects of season, year, and type of semen.

Effect	Variable	Pcleav	Pemb	Ppreg	MED	MPD
Season	Rainy	70.60±26.05	33.72±16.62	8.71±7.91	9.10±6.50	2.30±2.01
	Dry	65.91±24.97	32.44±16.70	8.79±7.61	10.70±8.11	2.75±2.46
	PR > F	<0.0016*	0.1821ns	0.8857ns	<0.0001*	<0.0066*

Ano	2019	70.43±19.20a	35.32±16.31a	10.44±8.67a	10.39±8.02a	2.77±2.34a
	2020	70.58±21.76a	33.66±16.63a	9.77±6.79a	9.07±6.33b	2.52±1.81ab
	2021	62.04±22.73b	29.68±16.52b	6.98±7.44b	8.98±6.66b	2.12±2.14b
	2022	59.97±20.23b	34.26±16.02a	8.83±7.26a	10.64±8.03a	2.89±2.55a
	PR > F	<0.0001*	<0.0001*	<0.0001*	<0.0038*	<0.0021*
Type of semen	Sexed	57.47±19.97	25.10±12.09	6.67±6.01	8.16±6.23	1.93±1.66
	Conventional	65.95±21.75	33.69±16.75	8.98±7.91	9.88±7.38	2.57±2.29
	p-value	<0.0001*	<0.0001*	<0.0166*	<0.0101*	<0.0214*

** = significant (P<0.05), and ns = not significant (P>0.05); abc = means followed by different letters on the same row differ from each other (p<0.05). Pcleav = percentage of cleaved embryos; Pemb = percentage of produced embryos; Ppreg = percentage of pregnancies; MED = mean number of embryos produced per donor; MPD = mean number of pregnancies per donor.*

The effect of season showed no significance (p>0.05) for Pemb and Ppreg, but a significant effect (p≤0.05) was observed for the variables Pcleav, MED, and MPD. The variables most susceptible to seasonal effects in IVEP were oocyte, which depends on the donor, and pregnancy, which depends on the recipient category. Both are more influenced by climate fluctuations. Considering these variables, the best means found for oocytes occurred in the rainy season. A possible explanation for this result is that the animals have a higher supply of food and better quality of forage during this period and, consequently, a higher number of viable oocytes, which does not occur during the dry season. In contrast, the variable pregnancy showed no significant effect (p>0.05).

Nogueira et al.[10] also found the influence of season when evaluating factors that affect IVEP using data from a commercial program. Donors aspirated in the spring/summer seasons resulted in a higher number of viable oocytes compared to autumn/winter, but seasonality did not influence cleavage and blastocyst rates.

Cordeiro et al.[21] found that high temperature and humidity index values induced low conception rates in Nellore embryos in the State of Acre. Also, Becher et al.[22] evaluated IVEP of Brahman, Gir, and Nellore cows and obtained a higher pregnancy rate in the rainy season (46.92%) compared to the dry season (40.08%).

The effect of year had a significant effect (p≤0.05) for all variables assessed in IVEP. It may be the result of the effect of the technician, the specific seasonal period, current laboratory logistics, weather, or even a consequence of the pandemic that occurred in 2020. In this sense, the effect of year was used as a fixed effect to be adjusted in the genetic assessment model, as it simultaneously corrects all specific random events that occurred throughout the year.

Peixoto et al.[23] also observed a significant effect of year when using data from a MOET company with information on zebu embryos (Guzerá, Gir, or Nellore), transferred into crossbreed recipients with unknown proportions of Holstein and zebu between the years 1992 to 1999. These differences were mainly attributed to environmental conditions (air, temperature, and humidity), procedures (type and dose of drugs), or breeds (taurine and

zebu). Moreover, Pinheiro et al.[19] found differences in embryo production between 2015 and 2018 in Nellore cattle. A significant effect was observed on the total number of oocytes, the total number of cleaved embryos, the total number of produced embryos, and the total number of pregnancies.

The effect of semen also affected the efficiency of IVEP, resulting in a significant effect ($p \leq 0.05$). Conventional semen presented better mean values than sexed semen for all variables.

Mello et al.[4] reported similar results when evaluating the effect of sexed and conventional semen on the Sindi breed with data provided by a commercial IVEP company, with better cleavage and blastocyst rates observed for conventional than sexed semen. The percentages of cleavages and blastocysts for conventional semen were 76.42% and 27.50%, respectively, and for sexed semen 58.89% and 23.13%, respectively.

Nascimento et al.[24] compared the blastocyst rate produced with sexed and conventional semen in *in vitro* fertilization and found a difference. Conventional semen (31.06%) showed better blastocyst production than sexed semen (21%). Also, Barrozo et al.[25] used breeding season data from an embryo company in the years 2021 and 2022 and found a significant effect on embryos. They achieved better embryonic conversion for conventional semen (53.55%) compared to sexed semen (30.64%) in Nellore bulls.

In general, part of these variations between sexed and conventional semen is due to the sexing process. Sexed semen, unlike conventional semen, goes through the sexing process using flow cytometry. This process involves more than 20 sub-processes that cause exposure to chemicals, resulting in damage to sperm and a disadvantage compared to conventional semen.[26] Part of these variations occur because some bulls are more susceptible to the sexing process in an IVEP program.[27]

Table 3 shows the phenotypic means, genotypic values, and respective standard deviations of the genetic groups of Nellore and Senepol cattle.

Table 3 Phenotypic means, genotypic values, and standard deviations of Nellore and Senepol genetic groups.

Variable	Phenotypic value	Genotypic value	
		Nellore	Senepol
Pooc	84.32 ± 10.85	0.61 ± 10.43	−2.87 ± 9.64
Pcleav	68.63 ± 25.69	0.93 ± 25.15	−4.35 ± 23.00
Pemb	33.18 ± 16.66	1.19 ± 16.40	−5.59 ± 13.87
Ppreg	8.75 ± 7.77	0.20 ± 7.79	−1.63 ± 5.53
MOD	30.00 ± 16.08	−0.08 ± 14.87	0.30 ± 19.20
MED	9.77 ± 7.26	0.30 ± 7.21	−1.44 ± 6.48
MPD	2.51 ± 2.24	0.02 ± 2.22	−0.13 ± 2.10

Pooc = percentage of viable oocytes; Pcleav = percentage of cleaved embryos; Pemb = Percentage of produced embryos; Ppreg = percentage of pregnancies; MOD = mean number of viable oocytes per donor; MED = mean number of embryos produced per donor; MPD = mean number of pregnancies per donor.

The results obtained in this study demonstrate that, for most of the analyzed variables, Nellore cattle had better production than Senepol cattle for IVEP traits. However, high standard deviation values were observed, in some cases higher than the mean genetic values, indicating genetic variability in these traits within the breed, which could be explored in an eventual selection process.

Genotypic values for traits related to reproduction biotechniques relative to the effect of breed are very scarce in the literature. However, some studies have compared the phenotypic values between zebu and taurine breeds and significant differences have been found.[10,13]

According to Baruselli et al.,[14] zebu breeds present better results in the production of oocytes, embryos, and pregnancies. These differences are mainly attributed to reproduction physiology such as the estrous cycle, follicular development, and postpartum anestrus.

Phenotypic results obtained from IVEP in taurine cattle have varied from 10.9 to 24.7 for mean oocytes and 1.1 to 3.89 for mean embryos.[20,28,29,30,31] Phenotypic IVEP results in zebu cattle have varied from 7.1 to 30.74 for mean oocytes, 3.89 to 10.09 for mean embryos, and 3.03 to 3.61 for mean pregnancy.[18,19,22,28,29,30,32,33]

Regarding genetic components, there is evidence that zebu animals also have higher genetic variability in the production of oocytes and embryos than taurine cattle.[34] In the literature, some studies have reported heritability for traits related to reproductive biotechniques in zebu and taurine breeds, indicating that zebu breeds respond better to the selection process for these traits than taurine breeds.

Heritability in taurine breeds, represented by Holstein, has varied from 0.09 to 0.25 for oocyte production and 0.03 to 0.14 for number of embryos.[15,35,36,37,38,39] In contrast, zebu breeds, represented by Guzerá, Gir, and Nellore, showed heritability varying from 0.08 to 0.38 for oocyte production, 0.10 to 0.65 for embryo production, and a value of 0.24 for the total number of pregnancies.[19,40,41,42,43,44]

Table 4 shows the estimates of variance and heritability components of bulls according to IVEP traits, obtained using the restricted maximum likelihood (REML) method.

Tabela 4 Estimates of components of additive genetic variance (σ_a^2), environmental variance (σ_e^2), phenotypic variance (σ_p^2) and coefficients of heritability (h^2) for Nellore and Senepol cattle.

Variable	Nelore				Senepol			
	σ_a^2	σ_e^2	σ_p^2	h^2	σ_a^2	σ_e^2	σ_p^2	h^2
Pcliv	104,07	546,59	650,66	0,16	15,02	413,18	428,20	0,04
Pemb	39,61	235,57	275,19	0,14	11,91	142,87	154,79	0,08
Ppren	1,12	60,37	6149	0,02	4,83	26,53	31,37	0,15
MED	3,89	48,73	52,62	0,07	0,80	41,47	42,27	0,02
MPD	0,21	4,42	4,63	0,05	0,00	4,58	4,58	0,00

Pcleav = percentage of cleaved embryos; Pemb = percentage of produced embryos; Ppreg = percentage of pregnancies; MED = mean number of embryos produced per donor; MPD = mean number of pregnancies per donor.

The heritability estimates found in this study for Pcleav (0.16 and 0.04), Pemb (0.14 and 0.08), Ppreg (0.02 and 0.15), MED (0.07 and 0.02), and MPD (0.05 and 0.00) in Nellore and Senepol cattle, respectively, were of low to medium magnitude, indicating the presence of genetic variability and the possibility of selection for these traits.

The relevant heritability values obtained in this study were observed for the traits Pcleav and Pemb in Nellore and Ppreg in Senepol, with moderate magnitude, demonstrating that there is genetic variability and the possibility of selection for these traits in the population. Therefore, genetic selection of animals that perform well to improve these traits is possible, and the heifer will inherit these traits and, consequently, tend to produce more embryos and more pregnancies.

The heritabilities estimated in this study follow a similar pattern to those reported in the literature. Perez et al.[42] estimated variance components to produce oocytes and embryos in Guzerá donors and found heritability for the number of cleaved embryos and number of transferable embryos of 0.16 and 0.14, respectively. Furthermore, Perez et al.[43] estimated heritability of 0.13 to 0.19 for the number of cleaved embryos and 0.10 to 0.20 for the number of transferable embryos in Guzerá cattle with IVEP data in Brazilian regions.

Merton et al.[15] investigated the genetic factors that influence the outcome of IVEP in Holstein donors and found moderate heritabilities for embryos. The estimated heritabilities were 0.19 for the number of embryos cleaved on day four, 0.21 for the total number of embryos on day seven, and 0.16 for the number of transferable embryos.

Peixoto et al.[40] found higher heritability estimates for embryo traits in Nellore donors in the MOET program. Estimated values ranged from 0.20 to 0.65 for viable embryos. Pinheiro et al.[19] estimated genetic parameters of IVEP in Nellore cattle and also found moderate heritabilities of 0.33 and 0.24 for the total number of embryos and the total number of pregnancies, respectively.

Heritability for the traits Ppreg, MED, and MPD in Nellore and Pcleav, Pemb, MED, and MPD in Senepol ranged from zero to 0.08, indicating that additive genetic variation is low even when present, as most phenotypic variation is a consequence of environmental variation. These traits are difficult to select, showing positive and favorable correlations with other IVEP traits with higher heritability (Table 5). Thus, the indirect selection of these traits or even the use of multivariate methods can be chosen.

Other studies have also reported similar results for the traits Pcleav, Pemb, and Ppreg with low magnitude. Tonhati et al.[35] estimated heritability of 0.03 for transferable embryos when studying the relative effects of genetic and phenotypic factors on the effectiveness and efficiency of superovulation in Holstein cows raised in Brazil.

Pinheiro et al.[19] estimated heritability of 0.04 in Nellore donors for cleavage rate and 0.05 for the percentage of oocyte conversion to pregnancy. Moreover, Konig et al.[37] estimated variance components for traits related to embryo transfer in Holstein donors and observed

heritabilities of 0.10 for the number of transferable embryos and 0.10 for the percentage of transferable embryos.

The variables MED and MPD represent the success of IVEP after several stages, which are influenced by several environmental factors, masking the possible existing genetic variation, which may explain their low heritabilities. Thus, environmental improvements may be more significant in the short term to increase embryo production and pregnancies per donor.

In general, heritability estimates obtained in this study indicate that there are important genetic components to be considered in selection programs even if IVEP traits undergo strong environmental influence.

Table 5 shows the phenotypic correlations above the diagonal and the genetic correlations below the diagonal for IVEP traits of Nellore and Senepol cattle.

Table 5 Phenotypic (above the diagonal) and genetic (below the diagonal) correlations between Nellore and Senepol cattle.

	Variable	Pcleav	Pemb	Ppreg	MED	MPD
NELLORE	Pcleav	1.00	0.61*	0.35*	0.31*	0.27*
	Pemb	0.71*	1.00	0.46*	0.62*	0.39*
	Ppreg	0.44*	0.45*	1.00	0.15*	0.74*
	MED	0.40*	0.63*	0.25*	1.00	0.55*
	MPD	0.18*	0.32*	0.61*	0.52*	1.00
SENEPOL	Pcleav	1.00	0.66*	0.49*	0.34*	0.45*
	Pemb	0.73*	1.00	0.69*	0.53*	0.60*
	Ppreg	0.41*	0.45*	1.00	0.31*	0.75*
	MED	0.36*	0.42*	0.19*	1.00	0.75*
	MPD	0.28*	0.25*	0.73*	0.52*	1.00

** = significant (P<0.05), and ns = not significant (P>0.05). Pcleav = percentage of cleaved embryos; Pemb = percentage of produced embryos; Ppreg = percentage of pregnancies; MED = mean number of embryos produced per donor; MPD = mean number of pregnancies per donor.*

The phenotypic and genotypic correlations in this study were positive and significant for all traits in Nellore and Senepol cattle. Overall, genetic correlations follow the same pattern as phenotypic correlations and have low, moderate, and high magnitudes, ranging from 0.15 to 0.75.

The phenotypic correlation obtained between Pcleav and Pemb had a high magnitude, with values of 0.71 and 0.73 for Nellore and Senepol cattle, respectively. However, phenotypic correlations are less precise because there is still environmental variation in phenotypic expression, while genetic variations present what is in fact heritable. The genetic correlation obtained for Pcleav and Pemb reached 0.61 and 0.66 for Nellore and Senepol cattle, respectively, with a high magnitude, indicating that indirect selection for Pcleav tends to increase embryo production.

Few studies have reported phenotypic and genetic correlations for traits related to reproduction biotechniques. However, Vega et al.[45] obtained a phenotypic correlation of 0.65 for the number of cleaved embryos and the number of transferable embryos and 0.50 for the proportions of cleaved embryos and transferable embryos.

Merton et al.[15] worked with Holstein donors and obtained phenotypic and genetic correlations of 0.67 for the number of cleaved embryos and 0.85 for the total number of embryos, respectively. Also in this study, a phenotypic and genetic correlation of 0.63 was obtained for the percentage of cleaved embryos and 0.45 for the percentage of transferred embryos, respectively. Perez et al.[41] evaluated the genetic aspects of Guzerá donors in three Brazilian regions and obtained a correlation of 0.68 between viable oocytes and viable embryos, suggesting that selection for the number of oocytes can increase the total number of produced embryos.

The IVEP technique aims to increase the number of pregnancies. For this purpose, the factors that interfere with production need to be identified to increase improvements. Therefore, correlation is one of the important strategies to predict the relationship between pregnancy and traits that can be quickly measured in IVEP, aiming at indirect selection.

Phenotypic and genetic correlation with moderate to high magnitude related to pregnancy was observed between Pcleav and Ppreg, with values of 0.35 and 0.44 in Nellore and 0.49 and 0.41 in Senepol, and between Pemb and Ppreg, with values of 0.46 and 0.45 in Nellore and 0.69 and 0.45 in Senepol, respectively. It suggests that indirect selection for Pcleav and Pemb leads to a higher percentage of pregnancies.

Pinheiro et al.[19] used data from Nellore cattle and estimated a phenotypic correlation of 0.70 between the number of embryos and the total pregnancy and a genetic correlation of 0.71 between the number of embryos and the total pregnancy. The phenotypic and genetic correlations related to MED and MPD showed low, moderate, and high magnitudes. This variation occurs because the complexity of the various steps of IVEP affects the expression of the trait.

4. Conclusion

Genetic variability was observed for the IVEP traits in Nellore and Senepol cattle, indicating that they can be used as selection criteria because they are heritable. The fixed effects of season, year, and type of semen influence the expression of IVEP traits and should be considered in genetic evaluations. The Nellore breed presents better performance for IVEP traits than the Senepol breed.

Declaration of conflict of interest

The authors declare no conflict of interest.

Author contributions

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