

Performance of zebu donor cows in vitro production of embryos**Desempenho de vacas doadoras zebu na produção in vitro de embriões**

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ABSTRACT

In vitro production (IVP) of embryos is a biotechnology capable of increase bovine production. Thus, the performance of Brahman, Gir, and Nellore donor cows in IVP was evaluated. Data from 2012 to 2017 were collected from donors subjected to ovum pick up (OPU) for IVP at the In Vitro Acre Company, Rio Branco, Acre, Brazil. The numbers of aspirated oocytes, viable oocytes, viable embryos, and pregnancy per OPU, and the cleavage, viable embryo, and pregnancy rates were analyzed considering the breed, location, time, number of OPU/day, and number of OPU/donor. The numbers were compared by the Student t test, and the rates by the chi-square test. Brahman donors

presented higher rates of cleavage, and viable embryos, followed by Nelore, and Gir ($p < 0.05$), and lower number of pregnancies ($p < 0.05$). Donors raised in the state of Acre, Brazil, had higher numbers of aspirated oocytes, viable oocytes, and viable embryos ($p < 0.05$), whereas those raised in Peru had higher cleavage and pregnancy rates ($p < 0.05$). Pregnancy rates were higher in the rainy season ($p < 0.05$). Cleavage rates were higher when more than 20 OPU/day were performed ($p < 0.05$), and intermediate when performing 11-20 OPU/day. The numbers of aspirated and viable oocytes of donors subjected to one OPU were higher than those of donors subjected to four to seven OPU ($p < 0.05$). Cleavage rates of donors subjected to two OPU were higher than those of donors subjected to more than three OPU ($p < 0.05$). The breed, location, number of OPU/day, and number of OPU performed in the same donor affected, with different intensities, the performance variables of Brahman, Gir, and Nelore donors in IVP.

Keywords: Bovine; Biotechniques; IVF; ovum pick up.

RESUMO

A produção *in vitro* de embriões (PIVE) é uma biotecnologia capaz de incrementar os resultados da produção de bovinos. Nesse contexto, avaliou-se o desempenho de doadoras Brahman, Gir e Nelore na PIVE, onde dados de 2012 a 2017 foram coletados de doadoras submetidas à ovum pick up (OPU) visando PIVE, na In Vitro Acre, em Rio Branco-AC. Avaliou-se a média por OPU de oócitos aspirados, oócitos viáveis, embriões viáveis e gestação, taxas de clivagem, de embriões viáveis e de gestação, considerando raça, local da propriedade, época do ano, número de OPU/dia e número OPU/doadora. As médias foram testadas pelo teste t de Student, e as taxas pelo Qui-quadrado. Doadoras Brahman apresentaram maiores taxas de clivagem e de embriões viáveis, seguidas pela Nelore e Gir ($p < 0,05$), e menor média de gestação ($p < 0,05$). Doadoras mantidas no Acre apresentaram maior número de oócitos aspirados, oócitos viáveis e embriões viáveis ($p < 0,05$), enquanto que as no Peru apresentaram maior taxa de clivagem e de gestação ($p < 0,05$). A taxa de gestação foi superior na época das águas ($p < 0,05$). A taxa de clivagem foi maior quando ocorreu mais de 20 OPU/dia em relação a até 10 OPU/dia ($p < 0,05$), e intermediária quando se realizaram de 11-20 OPU/dia. A média de oócitos aspirados e de viáveis foi maior nas doadoras submetidas a uma OPU, quando comparada as submetidas de quatro a sete OPUs ($p < 0,05$). A taxa de clivagem foi maior em doadoras submetidas até duas OPUs, que nas submetidas a mais de três ($p < 0,05$). Nas condições desse estudo, concluí-se que as variáveis atribuídas ao desempenho de doadoras Brahman, Gir e Nelore em PIVE sofreram influência, em diferentes graus, da raça, da localização da propriedade onde as doadoras são mantidas, do número de OPU realizada por dia e do número de OPU realizada na mesma doadora.

Palavras-chave: Bovinos; Biotécnicas; FIV; OPU.

1 INTRODUCTION

In vitro production (IVP) of embryos is the third generation of assisted reproduction biotechnology after artificial insemination, and cryopreservation of gametes, superovulation, and embryo transfer (Bertolini and Bertolini, 2009). Brazil is the world's largest producer of *in vitro* embryos, accounting for 57.7% of all cattle *in vitro* embryo production (Viana et al., 2017).

IVP has been used by farmers to accelerate the multiplication of females of high genetic and zootechnical value (Pontes et al., 2011). Bovine females can be subjected to a follicular aspiration process called ovum pick up (OPU) from two to six months of age (Currin et al., 2017). This process

can be performed twice a week for long periods (Jin et al., 2016), with promising results, without compromising the animals' health (Currin et al., 2017; Jin et al., 2016).

The IVP process for cattle involves several steps, from the oocyte collection in the farms to the laboratory. Moreover, factors inherent or not inherent to the donor, such as race, age, estrous phase, nutrition, seasonality (Mello et al., 2016), hormone stimulation (Silva et al., 2017), number of OPU sessions (Gimenes et al., 2015), embryo culture media (Guemra et al., 2013), and semen used for in vitro fertilization (IVF) (Fernández-Gonzalez et al., 2008) can affect the results.

Considering the variables that affect the production of viable in vitro-produced embryos, the selection of donors and recipient females should be carefully conducted to generate positive results during pregnancy (Honorato et al., 2013).

In this context, the objective of this study was to evaluate the in vitro production (IVP) of embryos of zebu cows of the Brahman, Gir, and Nellore breeds.

2 MATERIAL AND METHODS

The data of IVP of cows of the Brahman, Nellore, and Gir breeds used in this study were collected from 2012 to 2017 by the In Vitro Acre Company, Rio Branco, Acre, Brazil; 496 donor cows (84 Brahman, 327 Nellore, and 105 Gir) underwent 887 OPU sessions, with production and collection of 33,339 Cumulus Oophorus complexes (oocytes), which resulted in 6,983 in vitro-produced (IVP) embryos, in properties located in Brazil (state of Acre) and in Peru.

The numbers of aspirated oocytes in each OPU performed in the donors, viable oocytes, cleaved embryos, viable embryos, pregnancy, and the cleavage rate (number of embryos cleaved / number of viable oocytes), and viable embryo rate (number of viable embryos / numbers of viable oocytes) were evaluated (Gimenes et al., 2015). In addition, the pregnancy rate from IVP embryos transferred to the recipient cows was evaluated; these cows were previously synchronized using a hormonal protocol (Vieira et al., 2016).

The effects of the breed (Brahman, Nellore, and Gir), location of the farms where they were raised (state of Acre in Brazil, and Peru), time of year (rainy season: October to March; and dry season: April to September), number of OPU per day (up to 10 OPU/day, 11 to 20 OPU/day, and more than 20 OPU/day), and number of OPU performed in each donor cow (1, 2, 3, and 4 to 7) on the variables related to the donor cows were also evaluated.

The evaluation of cow breed, location, time of year, and number of OPU/day were based on data from the 582 OPU performed in the 887 OPU sessions for the present study.

The data were subjected to analysis of variance; the numbers were compared by the Student t test. Pregnancy, cleavage, and viable embryo rates were compared by chi-square, and the results were

expressed as percentages. The SAS statistical package was used for the analyses, considering a 5% significance level.

3 RESULTS

The numbers of aspirated oocytes, viable oocytes, and viable embryos, and pregnancy rates of donor cows of the Brahman, Gir, and Nelore breeds presented no significant differences ($p>0.05$) (Table 1).

Table 1. Performance of donor cows of the Brahman, Gir, and Nelore breeds subjected to ovum pick up (OPU) for in vitro production of embryos

Variable / OPU	Breed			p value
	Brahman	Gir	Nelore	
Number of aspirated oocytes	25.61±2.45	19.17±1.99	23.47±2.66	0.07
Number of viable oocytes	19.72±2.07	15.11±1.68	18.70±2.24	0.12
Cleavage rate (%)	86.73%	76.12%	83.28%	<0.001
	(2289/2639) ^a	(1065/1399) ^c	(7136/8568) ^b	
Number of viable embryos	8.40±1.09	5.29±0.88	6.95±1.18	0.07
Viable embryo rate (%)	45.77%	33.52%	39.66%	<0.001
	(1208/2639) ^a	(469/1399) ^c	(3398/8568) ^b	
Number of pregnancies	1.34±0.48 ^b	3.47±0.45 ^a	3.61±0.53 ^a	<0.01
Pregnancy rate (%)	33.45%	715.57%	43.67%	>0.05
	(294/879)	(198/262)	(1080/2473)	

^{a,b,c} Means followed by different letters in the same row differ ($p<0.05$)

Donor Brahman cows presented higher ($p<0.05$) cleavage, and viable embryo rates, followed by Nelore cows, which had higher ($p<0.05$) means than Gir cows. However, the mean number of pregnancies per OPU of Brahman donors (1.34±0.48) was lower ($p<0.05$) than those of Nelore (3.61±0.53), and Gir (3.47±0.45) donors, which had similar means ($p>0.05$) (Table 1).

Viable embryo rate, and number of pregnancies per OPU of donor cows raised in Brazil and in Peru were similar ($p>0.05$) (Table 2).

Table 2. Effect of the location of the farm on the performance of donor cows of the Brahman, Gir, and Nellore breeds subjected to ovum pick up (OPU) for in vitro production of embryos

Variable / OPU	Peru	Acre, Brazil	<i>p</i> value
Number of aspirated oocytes	17.32±3.12 ^b	28.18±2.37 ^a	0.03
Number of viable oocytes	12.99±2.63 ^b	22.70±2.00 ^a	0.02
Cleavage rate (%)	87.42% (1849/2115) ^a	82.37% (8641/10491) ^b	<0.001
Number of viable embryos	4.48±1.38 ^b	9.28±1.05 ^a	0.03
Viable embryo rate (%)	40.47% (856/2115)	40.21% (4219/10491)	0.083
Number of pregnancies	3.41±0.64	2.20±0.46	0.22
Pregnancy rate (%)	48.90% (311/636) ^a	42.34% (1261/2978) ^b	<0.001

^{a,b}Means followed by different letters in the same row differ ($p < 0.05$)

Donors raised in Brazil, had higher number of aspirated oocytes, viable oocytes, and viable embryos ($p < 0.05$) (Table 2), whereas those raised in Peru had higher cleavage, and pregnancy rates ($p < 0.05$) (Table 2).

Although presenting higher number of aspirated oocytes, and viable oocytes, donor cows raised in Peru, which were more distant from the laboratory (approximately 2300 km from Rio Branco, Acre, Brazil), had higher cleavage and pregnancy rates than those raised in Brazil, closer to the laboratory (Acre) ($p < 0.05$) (Table 2).

The time of year did not affect the numbers of aspirated oocytes, viable oocytes, viable embryos, and pregnancy, and cleavage and viable embryo rates of the Brahman, Gir, and Nellore donors ($p > 0.05$) (Table 3). However, a higher pregnancy rate was found when the embryos were produced in the rainy season ($p < 0.05$) (Table 3).

Table 3. Effect of the time of year on the performance of donor cows of the Brahman, Gir, and Nellore breeds subjected to ovum pick up (OPU) for in vitro production of embryos

Variable/OPU	Time of year		<i>p</i> value
	Rainy season	Dry season	
Number of aspirated oocytes	23.77±1.63	21.73±1.61	0.35
Number of viable oocytes	18.93±1.37	16.76±1.35	0.24
Cleavage rate (%)	82.32% (3497/4248)	83.67% (6993/8358)	0.79
Number of viable embryos	7.49±0.72	6.27±0.71	0.21
Viable embryo rate (%)	40.51% (1721/4248)	40.12% (3354/8358)	0.68
Number of pregnancies	3.16±0.34	2.45±0.32	0.11
Pregnancy rate (%)	46.92% (626/1254) ^a	40.08% (946/2360) ^b	<0.001

^{a,b}Means followed by different letters in the same row differ ($p < 0.05$)

The numbers of aspirated oocytes, viable oocytes, viable embryos, and pregnancies were not affected by the number of OPU performed on the same day ($p>0.05$) (Table 4).

Table 4. Effect of the number of ovum pick up (OPU) performed on the same day on the performance of donor cows of the Brahman, Gir, and Nellore breeds subjected to ovum pick up (OPU) for in vitro production of embryos

Variable/OPU	Number of OPU/day			p value
	Up to 10	11 to 20	More than 20	
Number of aspirated oocytes	24.15±1.33	23.82±1.65	20.29±2.51	0.39
Number of viable oocytes	18.92±1.12	18.66±1.39	15.95±2.12	0.45
Cleavage rate (%)	82.56% (7149/8659) ^b	84.10% (2645/3145) ^{ab}	86.78% (696/802) ^a	<0.05
Number of viable embryos	7.68±0.59	6.49±0.73	6.47±1.11	0.27
Viable embryo rate (%)	41.66% (3608/8659) ^a	36.59% (1151/3145) ^b	39.40% (316/802) ^b	<0.05
Number of pregnancies	3.05±0.28	3.10±0.33	2.27±0.52	0.36
Pregnancy rate (%)	38.26% (1028/2687) ^c	61.07% (455/745) ^a	48.90% (89/182) ^b	<0.05

^{a,b,c}Means followed by different letters in the same row differ ($p<0.05$)

The cleavage rate was higher when performing more than 20 OPU/day when compared to that when performing up to 10 OPU/day, and intermediate when using 11 to 20 OPU/day ($p<0.05$) (Table 4).

The viable embryo rate was higher when using up to 10 OPU/day ($p<0.05$) when compared to those from 11 to 20 OPU/day and more than 20 OPU/day, which were similar ($p>0.05$) (Table 4).

Greater numbers of aspirated and viable oocytes were found when performing one aspiration of oocytes per donor, when compared to the results found when performing four to seven aspirations in the same donor ($p<0.05$) (Table 5); two or three aspirations per donor resulted in intermediate results ($p>0.05$) (Table 5).

Table 5: Effect of number of ovum pick up (OPU) performed in the same animal on the performance of donor cows of the Brahman, Gir, and Nellore breeds subjected to ovum pick up (OPU) for in vitro production of embryos

Variable/OPU	One OPU	Two OPU	Three OPU	Four to seven OPU	<i>p</i> value
Number of aspirated oocytes	40.10± 1.33 ^a	35.36 ±2.12 ^{ab}	35.69± 3.06 ^{ab}	31.31± 2.93 ^b	0.02
Number of viable oocytes	21.44±0.68 ^a	18.96±1.09 ^{ab}	19.07±1.57 ^{ab}	17.16±1.51 ^b	0.02
Cleavage rate (%)	85.34% (9077/10636) ^a	84.34% (3119/3698) ^a	81.59% (1463/1793) ^b	79.84% (1398/1751) ^b	<0.05
Number of viable embryos	8.34±0.33	7.40±0.53	7.59±0.77	6.75±0.74	0.16
Viable embryo rates (%)	38.89% (4137/10636)	39.02% (1443/3698)	39.82% (714/1793)	39.34% (689/1751)	>0.05
Number of pregnancies	2.65±0.14	2.41±0.23	2.47±0.33	2.48±0.32	0.81
Pregnancy rate (%)	42.36% (1260/2974)	40.80% (459/1125)	43.59% (228/523)	42.09% (253/601)	>0.05

^{a,b}Means followed by different letters in the same row differ ($p < 0.05$)

The cleavage rate was higher in donor cows subjected to one or two OPU when compared to those subjected to three or more OPU ($p < 0.05$), which had similar results ($p > 0.05$) (Table 5).

The numbers of viable embryos/OPU of the donors evaluated were similar, regardless of the number of OPU to which they were subjected ($p > 0.05$) (Table 5).

4 DISCUSSION

IVP is a biotechnology often used in reproductive programs, however, the breed of the animals directly affects the success of this technique. Donor cows of the taurine and zebu races can present significant hormonal differences, and ovarian function, even when raised under the same environmental conditions (Sartori *et al.*, 2016). Zebu donors have better performance in IVP programs (Sales *et al.*, 2015). Moreover, considering the breeds used in the present study, differences in the performance of donors in the IVP program were found even among the zebu breeds, which could share some of the characteristics presented by Sartori *et al.* (Sartori *et al.*, 2016).

Gir donor cows presented a lower cleavage rate compared to Brahman, and Nellore. However, even lower values (59.1% to 68.4%) have been found for Gir donors (Viana *et al.*, 2004). It is

expected that donors of dairy breeds present lower reproductive performance than meat breeds, even when they are in the same genetic group (zebu) (Watanabe *et al.*, 2017). This could explain the result found in the present study, in which Gir donors had lower performance than Brahman and Nellore donors.

The variability in IVP results for different breeds may be associated with the donor nutritional status (Kouamo *et al.*, 2014), estrous cycle phase (Reis *et al.*, 2006), reproductive stage (Landry *et al.*, 2016), age, number of deliveries (Su *et al.*, 2012), and even gene sequence of each animal (Biase *et al.*, 2008), which were not evaluated in the present study.

A study found variation in the number of aspirated oocytes, with means of 15.7 to 24.9 oocytes per OPU for the Gir breed (Watanabe *et al.*, 2017), which were similar results to those found in the present study.

Nellore donors present considerable variability in oocyte production, with means varying from 18.4 to 30 oocytes per OPU, and of 8.13 viable embryos (Pontes *et al.*, 2011). These were similar results to those found in the present study. However, a study showed means of 46.18 oocytes and 10.09 embryos per OPU (22), which were higher than those found in the present study (Table 1).

Studies of IVP commonly report that laboratories are often far from the site where the donors are raised (Loiola *et al.*, 2014). This increases the time of transport of oocytes from the farm to the laboratory, and of embryos in the opposite direction, compromising the quality and viability of gametes and embryos (Cavalieri *et al.*, 2015). However, studies reported that the transport of embryos for 48 hours, and terrestrial transport of aspirated oocytes through up to 2000 km between farm and laboratory do not affect IVP results (Loiola *et al.*, 2014; Cavalieri *et al.*, 2015).

However, although donor cows raised in Peru, in a more distant location (approximately 2300 km) from the laboratory in Rio Branco, Acre, Brazil, had a higher number of aspirated oocytes and viable oocytes, they had higher cleavage and pregnancy rates when compared to those raised in farms closer to the laboratory ($p < 0.05$) (Table 2), indicating that the distance between the sites where the cows are raised and the laboratory affected the results found.

The performance of the donor cows could have been also affected by other factors related to the animal itself (Reis *et al.*, 2006; Landry *et al.*, 2016; Su *et al.*, 2012; Biase *et al.*, 2008) management (Kouamo *et al.*, 2014), seasonality (Watanabe *et al.*, 2017), hormonal protocol used (Silva *et al.*, 2017), and number of OPU performed in the same donor (Gimenes *et al.*, 2015).

Regarding the effect of time of year on the IVP (Table 3), a study in the Southeastern Region of Brazil, found similar results, with no differences in the number of recovered structures, and viable embryos by OPU, in Gir donor cows raised in winter or summer times (Dourado *et al.*, 2012).

A study with Sindi zebu animals found that the proportion of viable oocytes was higher in the rainy season, and non-viable oocytes was higher in the dry season (Mello *et al.*, 2016). However, the time of year had no influence on the number of viable or non-viable oocytes of the donors in the present study (Table 3).

The availability of grazing in adequate quantity and quality for animal nutrition is important for beef cattle breeds in extensive systems, in which pasture is the main or the only source of nutrients and energy for the animals (Carvalho *et al.*, 2010). Air temperature and rainfall increases in the rainy season, which benefits pasture development, resulting in greater food availability for the animals (Mello *et al.*, 2016). This could explain the higher pregnancy rate found in the present study, after the transference of the IVP embryos produced in the rainy season (Table 3); the greater pasture availability could have resulted in better nutritional status of the recipient cows, favoring the establishment of pregnancy (Torres *et al.*, 2015).

The waiting time of the donors in the corral affects negatively the IVP results, since the contact with humans and the corral environment can cause stress and release of cortisol, which affects follicular maturation, and reproductive hormone dynamics (Costa e Silva *et al.*, 2010). This could explain the results presented in Table 4, since a greater number of OPU performed on the same day would lead to an increase in waiting time in the corral, affecting negatively the IVP results.

Contrastingly to the results in Table 5, a study found no difference in number of viable oocytes when performing one to six OPU in the same donor at intervals shorter than 15 days (Pontes *et al.*, 2011). Moreover, a study with Holstein, Nellore, and buffalo heifers, and OPU performed every 14 days found that the number of OPU only affected the performance of zebu cows, with a decrease in the number of viable oocytes, and cleavage rate (Gimenes *et al.*, 2015), as observed in the Brahman, Gir, and Nellore donors evaluated in the present study.

The number of OPU sessions in the donors and the interval between them can affect the number of aspirated follicles and, consequently, the number of recovered oocytes (Gimenes *et al.*, 2015). Thus, the number of OPU/donor, and other factors such as interval between OPU could have affected the results found (Table 5).

Cows with higher oocyte production present a higher risk of ovarian damage due to the greater number of perforations by OPU; and the number of oocytes decreases along with increases in the number of blastocysts per OPU (Monteiro *et al.*, 2017). This effect was not observed in the present study; the number of viable embryos per OPU of the donors was similar, regardless of the number of OPU performed in each donor ($p>0.05$) (Table 5).

However, a decrease in the cleavage rate of donors subjected to more than three OPU was observed, which could indicate a decrease in the quality of the aspirated oocytes (Merton *et al.*, 2003).

Considering the conditions of the present study, the variables related to the performance of donor cows of the of Brahman, Gir, and Nelore breeds in *in vitro* production (IVP) of embryos were affected, with different intensities, by their breed, location of the farms where they were raised, number of ovum pick up (OPU) performed per day, and number of OPU performed in the same donor. However, some variables still need to be studied to overcome some obstacles for IVP, since animals of the same race, and raised under the same conditions have different performances in IVP.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this repor.

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