Performance of Senepol females as oocyte donors

Desempenho de fêmeas Senepol como doadoras de oócitos

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ABSTRACT
The goal of this study was to evaluate the effect of the donor and the months of the year on the oocyte recovery rate in Senepol donors. Data on 604 follicular aspirations guided by ultrasound, performed on 33 healthy cows aged between two and seven years were evaluated. Data were made available through individual spreadsheets by a commercial company. All donors belonged to the same batch and breeding farm and were kept under the same food management. Viable and degenerate oocyte recovery rates and the effect of the month of collection and the individual factor of the donor were analyzed. Data were tested by analysis of variance (ANOVA) followed by Tukey's test, with a significance level of 5%. A significant number of total (27.13 ± 14.38) and viable (23.17 ± 12.94) oocytes was observed. The number of grade I oocytes was high among the donors, and the number of total and grade II and III oocytes were the least altered between the months evaluated. There was an excellent number of oocytes recovered, with variation observed according to the donor and the month of the year evaluated.

Keywords: cattle, *in vitro* maturation, oocytes.

RESUMO
O objetivo deste estudo foi avaliar o efeito da doadora e dos meses do ano sobre a taxa de recuperação oocitária em doadoras de Senepol. Foram avaliados dados de 604 aspirações foliculares guiadas por ultrassom, realizadas em 33 vacas saudáveis com idade entre dois e sete anos. Os dados foram disponibilizados em planilhas individuais por uma empresa comercial. Todos as doadores pertenciam ao mesmo lote e fazenda de criação e eram mantidos sob o mesmo manejo alimentar. Foram analisadas as taxas de recuperação oócitos viáveis e degenerados e o efeito do mês de coleta e do fator individual da doadora. Os dados foram testados por análise de variância (ANOVA) seguida do teste de Tukey, com nível de significância de 5%. Observou-se um número significativo de ovócitos totais (27,13 ± 14,38) e viáveis (23,17 ± 12,94). O número de oócitos grau I foi elevado entre as doadoras, sendo que o número de oócitos totais e graus II e III foi o que menos se alterou entre os meses avaliados. Houve um excelente número de oócitos recuperados, com variação observada de acordo com a doadora e o mês do ano avaliado.

Palavras-chave: bovinos, maturação *in vitro*, oócitos.

1 INTRODUCTION

The Senepol breed, originated from crosses between Taurine breeds RedPoll (British) and N'Dama (African), was introduced in Brazil in 2000, coming from the United States and the Virgin Islands. Brazilian breeders propagated their genetic quality, making Brazil a world reference for the breed (ASSOCIAÇÃO BRASILEIRA DOS CRIADORES DE BOVINOS SENEPOL, 2020).

Its characteristics are, mainly, destined to meat production, presenting early finish and meat quality. It also exhibits attractive reproductive characteristics, such as the first heat in females at 14 months of age, in addition to having good maternal ability at birth (MARTINS, 2018).

Due to the diverse positive characteristics of the breed, which highlights it in *in vitro* fertilization programs (FARIAS et al., 2016), an increasingly growing search for biotechnologies has resulted, which aim to continue genetic improvement and advance the production of animals with high genetics in the country (DA SILVA et al., 2018).

Among the techniques already established, there is the selection of donors with high reproductive efficiency for the *in vitro* production of embryos, with the aim of aspiration of quality oocytes, with good morphological and developmental characteristics (PINTO NETO et al., 2018). Oocytes are female germ cells, produced in ovaries, present inside the follicles, where granulosa cells and teak cells can be found, which assist in oocyte maturation (BONI, 2012). According to Chagas et al. (2014), oocyte quality is of paramount importance in the success of assisted reproductive technologies.

Ultrasound-guided transvaginal follicle aspiration (OPU) is a method that allows the collection of oocytes from a donor and, in the next step, makes the embryo fertilization technique possible in the laboratory (MARIANO et al., 2015). Since Brazil has significant global representativeness in the area of reproduction, being the largest *in vitro* embryo producer in the world (LUSTOSA et al., 2018), as well as the growing interest of breeders in increasing the reproductive efficiency of Senepol animals, the present study aimed at evaluating oocyte recovery rates from Senepol donors after ultrasound-guided follicular aspiration (OPU) and *in vitro* maturation (MIV).

2 MATERIAL AND METHODS

The present project was exempted from evaluation by the Animal Use Ethics Committee (CEUA) of the Instituto Federal Goiano because it is a study of data collection from the Laboratory's archive of animal breeding.

This study was developed by collecting data from follicular aspiration sessions guided by ultrasound (OPU) during 2013. All data were provided by the Reproduction Biotechnology Laboratory of the commercial company Samvet Embriões®, located in Morrinhos, state of Goiás, through individual spreadsheets of PIV maintained at Fazenda Córrego do Meio, in Neropólis, state of Goiás. All animals came from the same breeding and were part of the same batch.

The evaluated data refer to 604 sessions of OPU, carried out in 33 Senepol cows, healthy and aged between two and seven years. All OPUs were performed on the same farm of origin of the animals, and donors were not subjected to any type of hormonal stimulation before follicular aspirations. As criteria for the inclusion of animals in the study, we decided to use only donors with a regular reproductive history (age, number of aspirations, rate of production of oocytes and embryos), free of reproductive problems and without any genital anomaly, which went through a previous gynecological evaluation and who had a frequency equal to or greater than 10 aspirations in the year of the study. Only donors with a body condition score between 3.0 and 4.0 were used, with a scale ranging from 1.0 to 5.0 (FERREIRA, 2010).

All donors were kept under equal management and feeding conditions, which consisted of semi-confinement with mineralization and water ad libitum, pasture and supplementation. The vaccination scheme adopted included vaccines against rabies and foot-and-mouth.

OPU sessions did not have a specific frequency routine, with, on average, one aspiration session every 40 days. However, during January, May and July 2013, this procedure was not performed due to problems in the supply of electricity that interrupted the work routine.

Oocyte were evaluated for total oocytes recovered (mean ± standard error), viable oocytes (total viable, in relation to those recovered) in grades I, II and III (mean ± standard error) and non-viable oocytes (total number of degenerated oocytes, in relation to those recovered). The effect of the month of collection and the individual donor factor on the oocyte recovery rates was also analyzed.

Analysis of variance (ANOVA) was applied using the PROC GLM procedure of SAS® software (STATISTICAL ANALYSIS SYSTEM, 2008), assuming a 5% statistical significance level ($P<0.05$). For the characteristics with statistical difference in the analysis of variance, Tukey's test was applied.

3 RESULTS AND DISCUSSION

Considering the large number of procedures performed, the general results indicate a huge possibility of multiplication of animals of high genetic value by applying OPU techniques in Senepol donors, as well as the possibility of selecting the matrices with the best oocyte production, representing an alternative technique to increase the reproductive efficiency of national herds.

There was great variability in the recovery rates after OPU and MIV of oocytes in Senepol donors (Table 1), with a significant number of total and viable oocytes.

Table 1. Mean total number of non-viable, viable oocytes, grades I, II and III and % viable oocytes after maturation *in vitro* (MIV) by follicular aspiration guided by ultrasound (OPU) in Senepol donors

Parameters	Minimum	Maximum	Mean	CV (%)
Total oocytes	3	75	27.13 ± 14.38	53.00
Non-viable oocytes	0	11	3.69 ± 2.28	61.86
Viable oocytes	1	66	23.17 ± 12.94	55.85
Grade I	0	15	2.07 ± 2.76	133.68
Grade II	0	30	10.13 ± 6.12	60.45
Grade III	0	35	10.61 ± 7.76	73.09
% viable after MIV	0	66	23.14 ± 13.14	56.81

CV, coefficient of variation.

This variability in parameters of oocytes recovered between one donor and another, characterized by the CV (greater than 50%), and by its range, corroborates Pontes et al. (2011), who state that individuals of the same breed also show variations in oocyte recovery, which may be due to differences in physiological and endocrine parameters related to management, age, nutrition and environmental conditions, among other factors.

However, the average of total oocytes in the present study (27.13 ± 14.38 oocytes per aspiration), 23.17 ± 12.94 viable, is satisfactory and within the ranges found in several Brazilian cattle breeds. According to Silva et al. (2017), in a study carried out to evaluate the average number of oocytes of cattle submitted to OPU of different breeds raised in Brazil, observed that the number and viability of oocytes varied significantly, with the Nellore (34.82 ± 17.61) and Girolanda (34.31 ± 12.44) breeds producing the largest number, however, Nellore animals showed the largest share of viable oocytes (27.63 ± 14.61), similar to what was observed in the present study.

In addition, the variation in oocyte MIV may also be related to the management of the OPU technique (PONTES et al., 2010). In this sense, there was an excellent number of oocytes recovered and suitable for cultivation in different breeds *Bos taurus*, *Bos indicus* and *indicus-taurus*, probably due to the experience of technicians responsible for OPU sessions.

The influence of the individual factor of Senepol donors on the number of total, non-viable, grade I, II and III oocytes and % viable oocytes after *in vitro* maturation (MIV) during 2013 was significant (Table 2), being significant the rate of viable oocytes

after OPU, the parameter with the greatest range of variation among donors and the level of grade I oocytes after OPU, the aspect with the least variation.

Table 2. Total number of ultrasound-guided follicular aspiration (OPU) sessions per donor and the mean number of recovered and viable oocytes per donor

		Parameters						
Donors	Number of aspirations	Total oocytes	Non-viable	Viable	Grade I	Grade II	Grade III	% viable oocyetes after MIV
1	16	27.18^{cdef}	4.37^{abcd}	22.18^{cdefgh}	0.93^{b}	10.43^{bc}	11.43^{abcdefgh}	23.81^{cdef}
2	12	25.91^{cdef}	3.50 ^{bcd}	22.41^{cdefgh}	2.53^{ab}	9.50^{c}	10.33^{abcdefg}	21.08^{cdef}
3	10	14.90^{f}	2.10^{d}	12.80^{gh}	2.00^{ab}	5.90c	4.90^{g}	12.90^{f}
4	11	16.54^{ef}	2.20^{d}	13.27^{gh}	1.72^{b}	6.72^{c}	4.81^{g}	12.81^{f}
5	13	22.92^{cedf}	5.36^{abc}	16.38^{defgh}	0.69^{b}	6.69^{c}	9.00^{cdefg}	18.23^{def}
6	12	31.91^{bcde}	3.27^{bcd}	27.66^{cdef}	1.08^{b}	11.33^{bc}	15.25^{abcde}	28.41^{bcde}
7	13	29.00^{cdef}	4.07^{abcd}	24.92^{cdefgh}	3.46^{ab}	10.69^{bc}	10.76^{abcdefg}	23.00^{cdef}
8	14	45.57^{ab}	3.64^{abcd}	43.53^{ab}	3.71^{ab}	19.40^{a}	18.14^{ab}	40.21^{ab}
9	14	23.92^{cdef}	3.50^{bcd}	20.42^{cdefgh}	1.57^{b}	8.50^{c}	10.35^{abcdefg}	20.07^{cdef}
10	11	25.45^{cdef}	3.90^{abcd}	20.72^{cdefgh}	3.00^{ab}	8.27^{c}	9.45^{bcdefg}	21.36^{cdef}
11	15	33.26^{bcd}	3.71^{abcd}	28.86^{cdef}	1.66^{b}	10.66^{bc}	16.53^{abc}	28.46^{bcde}
12	14	53.50^{a}	6.00^{ab}	45.84^{a}	5.30^{a}	17.92^{ab}	18.91^{a}	48.78^{a}
13	16	25.31^{cdef}	2.93^{cd}	22.37^{cdefgh}	2.00^{ab}	8.81^{c}	11.56^{abcdefg}	20.18^{cdef}
14	13	23.76^{cdef}	2.15^{d}	21.61^{cdefgh}	2.15^{ab}	9.84^{c}	9.61^{bcdefg}	20.15^{cdef}
15	12	25.75^{cdef}	3.16^{bcd}	22.58^{cdefgh}	1.83^{ab}	9.16^{c}	11.58^{abcdefg}	20.75^{cdef}
16	15	26.20^{cdef}	3.93^{abcd}	22.26^{cdefgh}	1.20^{b}	10.13^{c}	10.93^{abcdefg}	22.20^{cdef}
17	13	20.46^{cdef}	4.23^{abcd}	16.23^{efdh}	0.61^{b}	8.46^{c}	7.15^{efg}	16.76^{ef}
18	13	27.07^{cdef}	2.50^{cd}	23.76^{cdefhg}	2.07^{ab}	11.33^{abc}	8.46^{cdefg}	21.84^{cdef}
19	14	26.71^{cdef}	3.78^{abcd}	22.92^{cdefgh}	3.14^{ab}	9.78^{c}	10.00^{abcdefg}	24.50^{cdef}
20	14	15.28^{f}	3.35^{bcd}	11.92^{h}	0.21^{b}	5.78^{c}	5.92^{fg}	12.50^{f}
21	12	35.50^{bc}	3.90^{abcd}	29.83^{bcde}	2.91^{ab}	12.16^{abc}	14.75^{abcdef}	32.83^{bc}
22	10	25.80^{cdef}	2.44^{cd}	22.20^{cdefgh}	3.30^{ab}	9.00^{c}	9.90^{bcdefg}	21.70^{cdef}
23	12	35.50^{bc}	4.16^{abcd}	31.33^{bc}	2.15^{ab}	12.75^{abc}	16.33^{abcd}	31.75^{bcd}
24	16	25.06^{cedf}	3.31^{bcd}	21.75^{cdefgh}	2.18^{ab}	10.62^{bc}	8.93^{cdefg}	22.87^{cdef}

25	12	18.83^{def}	2.33^{d}	16.50^{defgh}	2.08^{ab}	8.00^{c}	6.41^{efg}	14.91^{ef}
26	13	22.23^{cdef}	2.92^{cd}	19.30^{cdefgh}	1.07^{b}	9.76^{c}	8.46^{cdefg}	20.15^{cdef}
27	14	30.00^{cdef}	3.92^{abcd}	26.07^{cdefg}	2.57^{ab}	12.21^{abc}	11.28^{abcdefg}	25.07^{cdef}
28	13	22.30^{cdef}	4.69^{abcd}	17.61^{cdefgh}	0.86^{b}	8.07^{c}	8.69^{cdefg}	19.53^{cdef}
29	14	35.14^{bc}	5.07^{abcd}	30.07^{bcd}	3.00^{ab}	12.28^{abc}	13.07^{abcdefg}	28.57^{bcde}
30	10	31.50^{bcde}	6.55^{a}	24.20^{cdefgh}	2.20^{ab}	12.70^{abc}	9.30^{bcdefg}	26.70^{bcdef}
31	12	22.16^{cdef}	4.41^{abcd}	17.75^{cdefgh}	1.33^{b}	7.91^{c}	8.50^{cdefg}	18.75^{cdef}
32	11	25.27^{cdef}	3.45^{bcd}	21.81^{cdefgh}	3.09^{ab}	10.09^{c}	8.63^{cdefg}	20.81^{cdef}
33	12	17.91^{def}	2.58^{cd}	15.33^{fgh}	1.33b	6.66^{c}	7.33^{defg}	14.66^{ef}

* Same letters in the same column do not differ. ** Different letters in the same column differ by Tukey's test at the level of significance of 5%.

The individual variation in the number and quality of the obtained oocytes evidenced in all characteristics of the oocyte recovery rates analyzed, was a relevant factor, and this behavior was expected, as according to Nibart et al. (1995), animals respond differently to follicular aspiration, and this can be attributed to several factors such as age, health, food and nutritional management and genetics, or may be related to the particularities of the herd under study, including physiological conditions of the animals, reproductive status, hormonal stimulation, frequency and procedures of follicular aspiration and climate (SENEDA et al., 2002).

Age may be related to the influence of aging on reproductive functions, since older donors have diminished ovarian reserve. Heifers recruit a greater number of follicles per wave of follicular growth, due to the greater hormonal peaks they present (LONERGAN; FAIR, 2016). Younger females can also recruit a higher number of follicles per wave of follicular growth and, consequently, produce a greater number of recovered and viable oocytes per OPU session (MALHI et al., 2008).

Welfare aspects offered to donors can also influence reproductive performance. It is known that animals that suffer from thermal stress decrease their food intake, which causes changes in their entire physiology, affecting even their reproductive management, through negative influence on their cyclicity, establishment of pregnancy and fetal development (HASEN, 2009).

There was an effect of the months of 2013 on the mean number of oocytes recovered after OPU in Senepol donors (Table 3), with the parameters of non-viable

oocytes, those with the widest range of monthly variation and the number of total oocytes and grades II and III, the least altered.

Table 3. Influence of the month on the mean number of total, non-viable, viable oocytes, grade I, grade II, grade III and % viable oocytes after *in vitro* maturation (MIV) of Senepol donors

Month	Parameters						
	Total oocytes	Non-viable	Viable	Grade I	Grade II	Grade III	% viable after MIV
February	37.16^{a}	4.88^{a}	32.50^{a}	1.63^{bc}	12.57^{a}	17.08^{a}	30.48^{a}
March	35.79^{a}	4.82^{a}	30.50^{a}	1.15^{c}	13.42^{a}	15.30^{a}	31.09^{a}
April	32.12^{a}	4.27^{ab}	27.21^{ab}	1.45^{bc}	12.04^{a}	13.72^{a}	27.04^{ab}
June	24.81^{b}	3.06^{bcd}	21.75^{bc}	4.69^{a}	10.27^{ab}	6.28^{b}	20.90^{bc}
August	20.19^{b}	2.93^{cd}	17.25^{c}	2.96^{b}	8.61^{b}	5.67^{b}	18.02^{c}
September	20.09^{b}	3.42^{bcd}	16.66^{c}	1.61^{bc}	7.42^{b}	7.61^{b}	18.02^{c}
October	21.61^{b}	3.82^{abc}	17.74^{c}	1.41^{bc}	6.97^{b}	9.41^{b}	18.88^{c}
November	20.91^{b}	2.89^{cd}	16.88^{c}	1.35^{c}	7.42^{b}	8.21^{b}	18.63^{c}
December	21.93^{b}	2.32^{d}	19.61^{c}	1.41^{bc}	8.16^{b}	9.16^{b}	18.38^{c}

Same letters in the same column do not differ. ** Different letters in the same column differ by Tukey's test at the level of significance of 5%.

Senepol donors produced more recovered and viable oocytes per session of OPU and % viable oocytes after MIV in the rainy season (February and March). In this period, although the temperature in the region is high, the rainfall is also high, which has a beneficial effect on food availability by increasing the quality of forages, and makes the animals present better nutritional condition and consequently, they produce a higher number of oocytes in PIV (STRADA et al., 2020).

In the present study, seasonal variations on pasture characteristics were not directly measured. However, this effect on tropical pastures is well documented. As the management commonly used for Zebu breed donors depends on obtaining nutrients from pastures, it is subjected to great variations, since the supplementation of forage and concentrate is less intense. This could explain the greater proportion of viable oocytes obtained during the rainy season, since tropical forages, at this time of year, show an increase in the digestibility of nutrients, which contributes to obtaining better PIV indices (AL-KATANANI, 2002).

Fernandes et al. (2001) reported the effect of seasonality on the number of viable and degenerate oocytes in Nellore donors, which is an indication that important changes in ovarian function, based on the production and quality of oocytes, can be estimated

between seasons. In the present study, there was an effect of seasonality on the production and viability of oocytes, probably due to the variation in the donor's body condition and, thus, OPU in Senepol females cannot be performed all year, with the same efficiency of production, regardless of the rainy or dry season.

In turn, the number of grade I oocytes was higher in June, a period prior to the beginning of the dry season in the central-west region of Brazil, where temperatures are slightly lower than the rest of the year and the rainfall regime it is also inferior. Nevertheless, it is believed that the rate of grade I oocytes was higher in this respective month, due to the climatic characteristics that could still be in the intermediate stage of the seasons, showing mild temperatures and pastures of reasonable nutritional value, which may have contributed to the production of this type of oocyte (WEBB et al., 2004).

The combination of the effect of months of OPU completion in 2013 with the individual influence of the evaluated donors, significantly interfered with the number and quality of oocytes analyzed (Table 4), with a wide variation observed in the recovery rate after OPU and *in vitro* maturation (MIV), for both factors evaluated.

Table 4. Relationship of total, non-viable, viable oocytes, grade I, II, III and % viable oocytes after *in vitro* maturation (MIV) evaluated in Senepol donors during 2013

Characteristics	Source of variation	P-value*	R^2 of the model
Total oocytes	Month	<0. 0001	0.55
	Donor	<0. 0001	
Non-viable oocytes	Month	<0. 0001	0.34
	Donor	<0. 0001	
Viable oocytes	Month	<0. 0001	0.55
	Donor	<0. 0001	
Grade I	Month	<0. 0001	0.37
	Donor	<0. 0001	
Grade II	Month	<0. 0001	0.39
	Donor	<0. 0001	
Grade III	Month	<0. 0001	0.47
	Donor	<0. 0001	
% viable oocytes after MIV	Month	<0. 0001	0.52
	Donor	<0. 0001	

*Statistically significant when P<0.05.

The relationship between the evaluated variables was consistent and the estimated coefficient was positive and moderate. Thus, the oocyte recovery rate varied according to the donor and the month of the year evaluated. This situation suggests the need to adopt strategies to direct the PIV of oocytes from Senepol donors according to the time of year in the central-west region of Brazil, given the consecutive effect of climatic variation on

the quality and availability of food to animals, which directly affect aspects of metabolism, physiology and reproductive performance in cows (SILVA, 2000).

4 CONCLUSION

Oocyte recovery rates from Senepol donors showed satisfactory results, with significant variation among donors and between the months of the year.

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