QUALITATIVE ASPECTS OF OOCYTES FROM NELORE AND SENEPOL BREEDS REARED IN A TROPICAL REGION

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SUMMARY: The objective of this work was to evaluate the influence of the tropical climate on donor races on the quantity and quality of oocytes aspirated in Nellore and Senepol cows by means of follicular aspiration, destined to the *in vitro* production of embryos. The experimental design was in randomized blocks, considering each bovine female as a plot and each farm being a block totaling 14 different properties, and the number of cows used were 238 for the Nelore breed and 267 for the Senepol breed totaling 505 bovine females. The evaluation of the total number of oocytes and the quality by the quality classification of oocytes aspirated in Grade I, II, III, atresic and degenerate. The results obtained in this work, we can conclude that among the breeds evaluated, the Nelore breed was superior in total oocyte quantities and in the classification of GI and GII, being superior in the quantities of viable oocytes compared to the Senepol breed.

Keywords: Physiology. Classification of oocytes. Follicular development.

ASPECTOS QUALITATIVOS DE OÓCITOS DE MATRIZES DAS RAÇAS NELORE E SENEPOL CRIADAS EM REGIÃO TROPICAL

RESUMO: Objetivou-se avaliar neste trabalho a influência do clima tropical em raças doadoras quanto a quantidade e qualidade dos oócitos aspirados em vacas da raça Nelore e Senepol, por meio da aspiração folicular, destinados à produção *in vitro* de embriões. O delineamento experimental foi em blocos ao acaso, considerando cada fêmea bovina como uma parcela e cada fazenda sendo como bloco totalizando 14 propriedades diferentes, e o número de vacas utilizadas foram 238 para raça Nelore e 267 para a raça Senepol totalizando 505 fêmeas bovinas. A avaliação do número total de oócitos e da qualidade foi feita pela classificação do *Complexo Cumulus* Oócitos (CCOs) em Grau I, II, III, atrésicos e desnudos ou (degenerado). Os resultados obtidos no presente trabalho, podemos concluir que entre raças avaliadas, a raça Nelore foi superior na quantidade total de oócitos e na classificação de GI e GII, sendo superior na quantidade de oócitos viáveis comparara a raça Senepol.

Palavras-chave: Fisiologia. Classificação dos oócitos. Desenvolvimento folicular.

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INTRODUCTION

The development of bovine breeding biotechnologies has maintained a consolidated growth in Brazil with the use of techniques such as artificial insemination, embryo transfer and *in vitro* embryo production, which is a fundamental tool in the genetic improvement of herds (VIANA, 2010). With this, the use of bovine breeds of high genetic aptitude is being employed in order to improve and increase animal production (MELLO *et al*., 2016).

Although the technique of *in vitro* embryo production in laboratories dates back to 1978, with the birth of Louise Brown, the "first test specimen baby", the growth and wide use of the technique in animal reproduction occurred in the latter (STEPTOE; EDWARDS, 1978; GONÇALVES *et al*., 2007; MELLO *et al*., 2016). The improvement and development of the techniques made it possible to collect, cryopreserve and transfer embryos, being able to repeat the procedure without interfering in the amount of oocytes (VARAGO; MENDONÇA; LAGARES, 2008). The optimization of the technique provided alternatives that increased the potential of females with higher genetic value, avoiding early disposal due to inability to reproduce naturally (BUENO; BELTRAN, 2008).

The oocyte, female germ cells produced in the ovaries, is involved by granular cells, forming the *cumulus oopohorus* complex (AGOSTINHO; LÉGA, 2009). Cumulus cells include follicle coordination and development and are essential in oocyte maturation, fecundation and embryonic development (GONÇALVES; FIGUEIREDO; FREITAS, 2002). Therefore, the existence of this cell complex is an important quality factor in oocyte maturation *in vitro* (AGOSTINHO; LÉGA, 2009).

The development and, consequently, the quality of the oocytes are directly associated with the physiological conditions of the donor, such as the estral cycle, follicular growth, among others (LONERGAN; FAIR, 2008). In addition, climatic factors such as high temperature and humidity may affect the quality and development of oocytes, due to the higher caloric stress especially in taurine cows with lower adaptability to tropical conditions (WARD *et al*., 2000). Although zebu cows also suffer from heat stress, they are more adapted and may have normal oocyte development (ROCHA *et al*., 1998). Thus, the quality of oocytes is compromised and fertility decreased when sensitive breeds are subjected to adverse weather conditions such as heat and high temperatures (OZAWA *et al*., 2005).

The greater adaptation of zebu breeds in tropical regions, characterized by warm climate, low quality fodder and high parasitic load, usually presents higher productivity compared to taurine breeds. However, zebuins breeds present smaller ovaries and corpus luteum in comparison with taurine females, resulting in fewer follicles per ovary and lower oocyte quality,

mainly during dry months (LEAL *et al*., 2009). Thus, regardless of genetics, the higher number of oocytes with higher quality are obtained during the rainy season, resulting in higher conception rate (FERNANDES *et al*., 2001).

The objective was to evaluate the influence of donor breeds on the quantity and quality of oocytes aspirated, in Nelore and Senepol cows through follicular aspiration, intended for in vitro production of embryos in places of tropical climate.

MATERIAL AND METHODS

The oocyte samples were collected from properties located in the following municipalities of Mato Grosso, Brazil: Cáceres, Comodoro, Pontes e Lacerda, Porto Esperidião, Santo Afonso, Rio Branco, Vila Bela da Santíssima Trindade, Chapada dos Guimarães, Cuiabá, Jangada, Santo Antônio do Leverger, Barra do Garças and Rosário Oeste. The collections took place during the rainy season (January to February and September to December/2016). The climatic classification, according to Köppen's (1928) criteria, is predominantly of type Aw, that is, tropical climate with rainy season in summer and dry in winter. The average temperature varies between 22-25°C and annual rainfall between 1200 mm and 1800 mm (MARCUZZO, CARDOSO; FARIA, 2012).

In each property the matrices were kept in marandu grass (*Urochloa brizantha* cv. Marandu) supplemented with mineral salt *ad libitum* and protein supplement plus cyanocobalamin (vitamin B12).

All the matrices used were Pure of Origin (PO), non-lactating, of the breeds Nelore (*Bos taurus indicus*) and Senepol (*Bos taurus taurus*), with an average body score of 3.0 on a scale of 0 to 5 (LOWMAN, SCOTT; SOMERVILLE, 1976), and body weight between 350 to 450 kg in both breeds.

The matrices were subjected to the following hormonal protocol: Day 0 = administration of 2mL of intramuscular Estradiol Benzoate (IM) and placement of the subcutaneous auricular progestogen implant containing 3 mg of Norgestomet (Crestar® Intervet); Day 4 = application of 2mL of follicle stimulating hormone (FSH) (IM) from (Folltropin®-V); Day 5 = application of 2mL of FSH (IM) from (Folltropin®-V); Days 6 = removal of the progestogen implant and performing the follicular aspiration.

Follicular aspiration was performed by the *ovum pick-up* method (OPU), by the vaginal fornix, performed with the aid of ultrasound equipment (Mindray DP-20 VET) with a Bivolt transducer, coupled to a transvaginal follicular aspiration guide. The suctions were performed with a hypodermic disposable needle (40×9 mm long, Becton Dickson, Brazil) connected to a 50

mL conical tube (Corning, USA) by a 0.8 m internal diameter silicone tube of 2 mm and suction system using a vacuum pump (BV-003D WTA-Watanabe) with adjustable vacuum pressure.

The ovaries were positioned on the puncture line indicated on the ultrasound screen and the vacuum pump was triggered by starting the aspirations of the follicles, which were stored immediately in a 50 mL falcon tube plus phosphate saline buffer (PBS; Dulbecco®) and heparin (50 IU/ml; Liquemine®).

The collected material, with the proper identification of the animal, was immediately transported to a mobile laboratory mounted on the property. The aspirated liquid was washed with the same storage solution (PBS and heparin), passing through a 75 micron nylon screen filter (WTA-Watanabe), washing the collected material until the content of the filter became translucent, to finally be classified.

After washing, the material was deposited in Petri dishes and with the aid of a magnifying glass (NIKON, model C-DS, Japan) the search and selection of oocytes and subsequent classification were performed.

The oocytes were classified according to the quality proposed by Lonergan *et al*. (1994), in degree I, II, III, naked and atretic (Table 1) based on the morphological aspect of the *cumulus oophorus* complex (COC) and the ooplasm. After classification, the oocytes were placed in cryotubes where they contain a means of maturation of the oocytes that are gasified, identified and stored inside the mini tubes where their temperature is around 37.5ºC to 38ºC and are transported to the laboratory where the fecundation process took place.

Table 1. Morphological classification of oocytes as a function of *cumulus oophorus* complex (COC) and ooplasma.

Classification	*Cumulus* cells	*Ooplasma*
Grade I (GI)	Compact CCO > 3 layers of *cumulus* cells	Homogenous
Grade II (GII)	Compact CCO < 3 layers of *cumulus* cells	Heterogenic (slightly)
Grade III (GIII) or partially naked	Removal of less than 1/3 of the *cumulus* cells	____
Naked or Degenerate	No cells in the zona pelúcida	Retroaction and vaccination
Cumulus expanded or atresic	Expansion	____

Source: adapted from Lonergan *et al*. (1994)

The experimental design was in complete unbalanced blocks, considering the animal as parcel and the region as block. The blocks were composed of oocytes collected in 166, 40 and 32 Nelore cows, 60, 133 and 74 Senepol cows in regions 1, 2 and 3, respectively, making a total of 238 Nelore cows and 267 Senepol cows, totaling 505 cows experimental units.

He statistical model used was:

$Y_{ijl(j)} = \mu + r_j + b_{l(j)} + R_i + \varepsilon_{ijl(j)}$,

In which: *Yijl*(*j*) = observed value of race i in repetition j of block l; μ = overall mean effect; rj = random effect of repetition j in block l, *j* = 1, 2, ..., r); *bj* = random effect of block l, l = region 1, 2 and 3; *Ri* = fixed effect of race *i*, *i* = Nelore and Senepol; *εij* = random error associated with each observation, assuming εij ~NID(0, Iσ2ε) and Iσ2ε as the matrix of variance and covariance.

The counting data (Poisson distribution) were transformed into (x+1) and analyzed using the method of mixed models with special parametric structure in the covariance matrix, using the MIXED procedure of the SAS statistical software (SAS Studio, v. 9.4) (LITTELL *et al*., 2006) using the method of maximum restricted likelihood (REML). For the choice of the covariance matrix, the Akaike information criterion (AIC) was used (WOLFINGER, 1993) and the correction of the degrees of freedom was done using the method of Kenward and Roger (1997) (DDFM = KR). Treatment means were estimated by the mean of least squares (LSMEANS) and the comparison was made using the probability of difference (PDIFF) of Student's t test ($P < 0.05$).

RESULTS AND DISCUSSION

The classification of GI oocytes did not differ between the races (P=0,1401), presenting on average 0.28 oocytes per animal and a total of 139 aspirated oocytes (Table 2). However, GII differed between breeds (P=0,0063), in which the total number of oocytes and per animal in the Nelore breed was 50 and 68% higher than Senepol, respectively.

The GIII degree of maturity was affected by the breed (P=0,0089; Table 2). Nelore presented a total and mean number of oocytes 9% and 22% higher than Senepol, respectively. In this degree of maturation are 91% of viable oocytes (GI, GII, and GIII oocytes). Thus, the viable oocytes were affected by the race (P=0,0191), in which Nelore was 13% higher in total and 26% higher in the average oocytes per animal.

The oocytes classified as atresic (P=0,7953) and denuded (P=0,2022) were not affected by the race (Table 2). On average, the animals presented 1.25 atresic oocytes and 3.38 denuded oocytes. Thus, the total number of non-viable oocytes (atresic and denuded) did not differ between the breeds (P=0,3555), presenting a total of 2338 and a mean of 4,63 non-viable oocytes per cow. The number of non-viable oocytes was ~14% of the total oocytes aspirated.

The total number of oocytes aspirated was affected by the breed (P=0,1401). Nelore was 25% higher than Senepol in the number of oocytes aspirated per cow. Nelore presented a difference (896 oocytes) 11% higher than Senepol.

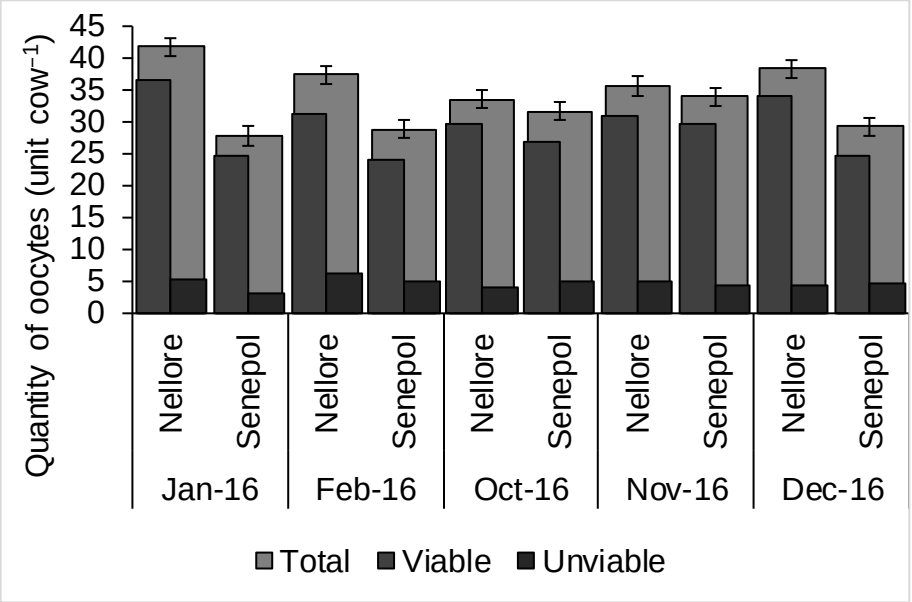
Table 2. Classification of oocysts distributed according to quality grade GI, GII and GIII (viable), atresic and denuded (non-viable), aspirated in donor cows of the Nelore and Senepol breeds in Mato Grosso.

Variables	Breeds						*P*-value
	Nelore			Senepol			
	Means[a]±EPM[b]	Means[c]	Sum[d]	Means[a]±EPM[b]	Means[c]	Sum[d]	
GI	1,12±0,03 a	0,40	95	1,06±0,03 a	0,16	44	0.1401
GII	1,79±0,07a	2,84	675	1,50±0,07 b	1,69	451	0,0063
GIII	5,23±0,23 a	29,26	6963	4,80±0,22 b	23,88	6376	0,0089
Viables	5,48±0,24 a	32,49	7733	4,96±0,22 b	25,73	6871	0,0191
Atrésicos	1,40±0,08 a	1,19	283	1,37±0,08 a	1,31	349	0,7953
Naked	2,02±0,09 a	3,79	903	1,90±0,08 a	3,01	803	0,2022
Unpredictable	2,29±0,11 a	4,98	1186	2,16±0,09 a	4,31	1152	0,3555
Total	5,89±0,25 a	37,47	8919	5,36±0,23 b	30,05	8023	0,0209

[a]Transformed mean ($\sqrt{x+1}$) ±; [b]Standard error of the mean; [c]Real mean (number of oocytes aspirated per cow); and [d] Sum of oocytes aspirated in each breed. Averages with different letters on the line are different by Student's t test (P <0,05). Source: Prepared by the authors themselves, 2016.

Comparing the breeds over the months of collection (Figure 1), we observed that, regardless of the month, the Nelore breed presented higher quantities of viable and total oocytes than Senepol. In the months of January, February and December, Nelore was 50, 30 and 31% higher than Senepol in the total number of oocytes aspirated, respectively. This total is composed mainly of viable oocytes, which in January corresponded to 88% of the total, in which Nelore was 48% higher than Senepol. In October and November, months with the smallest differences between the breeds, Nelore was 5 and 6% in total and 10 and 4% higher than Senepol in viable oocytes.

Figure 1. Number of oocytes aspirated during the months of collection in the Nelore and Senepol breeds in Mato Grosso.



Source: Prepared by the authors themselves, 2016.

In both races, the largest collection of non-viable oocytes occurred in February (Figure 1), when Nelore was 25% higher than Senepol. In this sense, the greatest difference was recorded for Nelore in January, 73% higher than Senepol. In October and December, the Senepol breed had 24 and 5% more oocytes than Nelore.

The ability of oocytes to develop *in vitro* and their viability depends exclusively on the cells of the cumulus, which is coated directly to the cytoplasm (ooplasm), allowing the transport of nutrients, the signals that control the metabolism, and the nuclear maturation, and cytoplasm (FERNANDES *et al*., 2001). The classification allows a visual evaluation of the oocytes and the quantity and appearance of cumulus cells and the uniformity of the ooplasm, in ways to select quality oocytes being viable for *in vitro* development (COSTA *et al*., 1997).

Compared to the total number of oocytes produced, the Senepol breed has low reproductive capacity in nelores, because the climatic conditions in the region are of transition from the spring-summer season, in rainy periods, hot with high temperatures and high humidity. We have shown that taurine breeds are few resistant to tropical climate, affecting the reproductive part of the animal. Zebu breeds obtain greater ability in thermo regulation of body temperature with lower metabolic rate and a greater ability in body heat loss than breeds of European origin (HANSEN, 2004). Another explanation for the resistance of high temperatures is that they contain greater amounts of sweat glands, with a coat that increases heat dissipation by solar radiation. (HUTCHINSON; BROWN, 1969; HANSEN, 2004)

Results obtained indicate that zebu breeds produce more total oocytes related to taurines, so that it was found for the Nelore breed that reached a statistically different amount than the

Senepol breed. Work performed by Cruz *et al*. (2009), quantified the average number of oocytes aspirated from female zebuins (Nelore) and taurines (Devon) bred under pasture conditions and concluded that female zebuins provided the highest number of recovered oocytes, due to variability of the recovered extructures, and by the damage to oocyte integrity caused by puncture and excessive time from aspiration to vitrification process.

Due to the higher amount of oocytes produced by the Nelore matrices, it reflected in the higher capacity to produce good quality oocytes in GI, GII and GIII, meaning that there will be a higher amount of aspirated oocytes that will be viable for *in vitro* fertilization. The classification of degrees refers to oocyte morphology based on the coverage of cumulus cells to develop into a blastocyte, being found in degrees of atrophy or naked losing their ability to mature (LEIBFRIED; FIRST, 1979; WARD *et al*., 2000).

Senepol females produced smaller quantities of viable oocyte quality, and similar in the production of non-viable ones. This result demonstrates the low capacity of oocyte development. According to some authors, GI oocytes, GII to GIII, have a similar capacity to develop cumulus, only for naked morphology or (degeneration) that has its potency compromised to develop *in vitro* (LEIBFRIED; FIRST, 1979; LOOS *et al*., 1989; WARD *et al*., 2000).

Thus, the increase in body temperature or hyperthermia may compromise cell function resulting in physiological changes, compromising oocyte and embryo culture (HANSEN, 2004) reducing the rate of fertilization when in hot periods of the year such as summer (SARTORI *et al*., 2002). Probably it is related to the fact that heat is harmful affecting the steroidogenic capacity of follicles and their follicular dynamics, affecting the beginning of the antral stage of development and causing the reduction of dominance of the selected follicle (OZAWA *et al*., 2005; ROCHA *et al*., 2012).

Regarding the aspirations, after the months of January and February in (Figure 1), the animals go through a period of rest and soon after enters the winter and dry period, where the matrices go through seasonal periods with low quality fodder, being only supplemented by the energetic protein base. And when starting again between September and November the oocyte production is low compared to the months of January and February when the pastures are of better quality.

CONCLUSION

The results obtained in this work, we can conclude that among the breeds evaluated, the Nelore breed was superior in total oocyte quantities and in the classification of GI and GII, being superior in the quantities of viable oocytes compared to the Senepol breed.

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