

Comparison of seminal characteristics of Aberdeen Angus, Holstein and Nelore bulls before and after cryopreservation

Comparaç o das caracter sticas seminais de touros das raças Aberdeen Angus, Holand es e Nelore antes e ap s a criopreservaç o

Comparaci n de las caracter sticas seminales de toros Aberdeen angus, Holand es y Nelore antes y despu s de la criopreservaci n

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Diego Andr  Costa Saranholi

ORCID: <https://orcid.org/0000-0002-2740-537X>
Universidade Estadual do Norte do Paran , Brazil
E-mail: diego.saranholi@gmail.com

Rafael Rocha de Paula

ORCID: <https://orcid.org/0000-0002-0181-8045>
Central Bela Vista Pecu ria Ltda, Brazil
E-mail: rrocha_vet@yahoo.com.br

Edmilson Pytilak

ORCID: <https://orcid.org/0000-0002-5513-1943>
Central Bela Vista Pecu ria Ltda, Brazil
E-mail: edmilson.ppytilak@gmail.com

Fabiola Afonso

ORCID: <https://orcid.org/0000-0002-1898-8286>
Central Bela Vista Pecu ria Ltda, Brazil
E-mail: fabiola.zzafonso@gmail.com

Luis Felipe Canela

ORCID: <https://orcid.org/0000-0003-3798-6865>
Central Bela Vista Pecu ria Ltda, Brazil
E-mail: luis_fcanela@hotmail.com

Ana Beatriz Marques de Almeida

ORCID: <https://orcid.org/0000-0003-2659-4786>
Universidade Estadual de Londrina, Brazil
E-mail: biamarquesvet30@gmail.com

Myrian Megumy Tsunokawa Hidalgo

ORCID: <https://orcid.org/0000-0003-4650-029X>
Universidade Estadual de Londrina, Brazil
E-mail: myrianhidalgo@hotmail.com

Maria Isabel Mello Martins

ORCID: <https://orcid.org/0000-0001-8416-2450>
Universidade Estadual de Londrina, Brazil
E-mail: imartins@uel.br

Wanessa Blaschi

ORCID: <https://orcid.org/0000-0002-6161-6576>
Universidade Estadual do Norte do Paran , Brazil
E-mail: wblaschi@uenp.edu.br

Thales Ricardo Rigo Barreiros

ORCID: <https://orcid.org/0000-0001-8735-5901>
Universidade Estadual do Norte do Paran , Brazil
E-mail: thalesrigo@uenp.edu.br

Abstract

The aim of this study was to evaluate parameters indicative of sperm quality of fresh and post-thawed semen of Aberdeen Angus, Holstein and Nelore bulls. Thirty-nine bulls were used: Aberdeen Angus (n=13), Holstein (n=13) and Nelore (n=13). The ejaculate collects were performed twice a week using artificial vagina, totaling 792 semen collections, 307 for Aberdeen Angus, 225 for Holstein and 260 for Nelore bulls. After collection, fresh semen was evaluated and semen freezing was performed. After freezing, the batches were thawed and progressive motility was determined. The analysis of fresh semen showed that there was no difference ($P = 0.053$) between the Aberdeen Angus and Nelore breeds, while ejaculates from Holstein bulls showed a statistical difference ($P = 0.024$). As well, a difference ($P < 0.001$) was identified in the sperm concentration of the three breeds. In the samples evaluated after thawing, a statistical difference was observed between Holstein and Nelore breeds ($P < 0.001$), while the values of the

Angus breed were similar to the other two breeds. The difference in motility of fresh and post-thawing semen showed that Nelore and Angus bulls showed greater variation in values between the analyzes ($26.0 \pm 8.9\%$ and $25.3 \pm 8.4\%$, respectively) showing a significant difference ($P < 0.001$) in relation to Holstein bulls ($20.6 \pm 9.3\%$) that obtained the smallest difference. The analysis of fresh and post-thawing semen did not show any significant difference ($P = 0.13$) between breeds. In conclusion, the semen cryopreservation process causes a decrease in the physical parameters of the semen and these quality losses suffer interference according to the breeds.

Keywords: Freezing; Bovine; *Bos taurus*; *Bos indicus*.

Resumo

O objetivo deste estudo foi avaliar parâmetros da qualidade espermática de sêmen fresco e após a descongelação de touros Aberdeen Angus, Holandês e Nelore. Foram utilizados 39 touros: Aberdeen Angus ($n=13$), Holandês ($n=13$) e Nelore ($n=13$). A colheita de sêmen foi realizada por vagina artificial duas vezes/semana, totalizando 792 colheitas de sêmen, sendo 307 para touros Aberdeen Angus, 225 para touros Holandês e 260 para touros Nelore. Após colheita, o sêmen fresco foi avaliado e em seguida realizada a congelação. Após a congelação, as partidas foram descongeladas e foi determinada a motilidade progressiva. A análise do sêmen fresco demonstrou que não houve diferença ($P = 0,053$) entre as raças Aberdeen Angus e Nelore, enquanto, que ejaculados de touros Holandês observou-se diferença estatística ($P = 0,024$). Assim como, foi identificado diferença ($P < 0,001$) na concentração espermática das três raças. Nas amostras avaliadas pós-descongelação, observou-se diferença estatística entre touros Holandês e Nelore ($P < 0,001$), enquanto, que os valores de touros Angus foram semelhantes às outras duas raças. A diferença da motilidade do sêmen fresco e pós- descongelação evidenciou que touros Nelore e Angus apresentaram maior variação dos valores entre as análises ($26,0 \pm 8,9\%$ e $25,3 \pm 8,4\%$, respectivamente) apresentando diferença significativa ($P < 0,001$) em relação aos touros Holandês ($20,6 \pm 9,3\%$) que obtiveram a menor diferença. A Análise do sêmen fresco e pós- descongelação não apresentou diferença significativa ($P = 0,13$) entre as raças. Em conclusão, o processo de criopreservação do sêmen reduziu os parâmetros físicos do sêmen e estas perdas de qualidade sofrem interferência de acordo com as raças avaliadas.

Palavras-chave: Congelamento; Bovino; *Bos taurus*; *Bos indicus*.

Resumen

El objetivo de este estudio fue evaluar los parámetros de calidad del esperma de semen fresco y después de la descongelación de toros Aberdeen Angus, Holandés y Nelore. Se utilizaron 39 toros: Aberdeen Angus ($n = 13$), Holandés ($n = 13$) y Nelore ($n = 13$). La recolección de semen se realizó por vagina artificial dos veces por semana, totalizando 792 recolecciones de semen, 307 para toros Aberdeen Angus, 225 para toros holandeses y 260 para toros Nelore. Después de la cosecha, se evaluó el semen fresco y luego se congeló. Después de la congelación, se descongelaron las dosis y se determinó la motilidad progresiva. El análisis de semen fresco mostró que no hubo diferencia ($P = 0.053$) entre las razas Aberdeen Angus y Nelore, mientras que los eyaculados de toros Holandés mostraron una diferencia ($P = 0.024$). Además, se identificó diferencia ($P < 0,001$) en la concentración de esperma de las tres razas. En las muestras evaluadas después de la descongelación, hubo diferencia entre los toros Holandés y Nelore ($P < 0.001$), mientras que los valores de los toros Angus fueron similares a las otras dos razas. La diferencia en la motilidad del semen fresco y post-descongelación mostró que los toros Nelore y Angus presentaron mayor variación en los valores entre los análisis ($26,0 \pm 8,9\%$ y $25,3 \pm 8,4\%$, respectivamente) mostrando una diferencia ($P < 0,001$) en comparación a los toros holandeses ($20,6 \pm 9,3\%$) que tuvieron la menor diferencia. El análisis de semen fresco y posterior a la descongelación no mostró diferencias ($P = 0,13$) entre razas. En conclusión, el proceso de criopreservación de semen redujo los parámetros físicos del semen y estas pérdidas de calidad sufren interferencia según las razas evaluadas.

Palabras clave: Congelación; Toro; *Bos taurus*; *Bos indicus*.

1. Introduction

Reproductive biotechnologies are used to accelerate production, increase reproductive efficiency, and promote genetic improvement in the herd. In Brazil, artificial insemination (AI) has been standing out, both in the beef herd and in the dairy herd, reflecting an increase of 30.1% in sales and 32% in the production of doses in the year 2020 (ASBIA, 2020).

The use of frozen bovine semen represents the main reproductive biotechnique for animal genetic improvement (Freitas et al., 2009; Leite et al., 2011). However, the cryopreservation process can cause damage to sperm, which reflect a decrease of approximately 50% of sperm motility (Celeghini et al., 2007; Thomas et al., 1997). Although the negative effects on sperm after thawing are notable, such as decreased motility due to changes in mitochondrial function, damage to the plasma membrane and acrosome (Layek et al., 2016; Ntenka et al., 2016), sperm cryopreservation has the advantage of storage for indefinite periods and providing worldwide distribution (Layek et al., 2016). In order to obtain good results in the use of

artificial insemination, rigorous quality processes are necessary, from the management of breeding bulls to the stages of collection and cryopreservation of the semen, taking into account that the process of the semen cryopreservation causes a drop in sperm quality (Abud et al., 2014; García-Álvarez et al., 2014). Furthermore, it is essential to select breeding bulls capable of producing cryopreservation-resistant ejaculates and maintaining high sperm viability and fertilizing capacity after thawing (Ntenka et al., 2016; Queiroz et al., 2015; Ram et al., 2017).

However, not all bulls are able to produce frozen semen, since numerous intrinsic variables (age, bull's health, libido) and extrinsic (season, diet, management) are involved in this process. (El-Harairy et al., 2011; Queiroz et al., 2015). Another important factor to be considered is the difference in semen quality between the bovine breeds and the respective aptitudes of the animals, which even lacking scientific studies, it is speculated that dairy bulls produce ejaculates with higher quality compared to beef bulls (Morrell et al., 2018).

Thus, this study aimed to evaluate and compare the sperm characteristics of fresh and cryopreserved semen between Aberdeen Angus, Holstein and Nelore bulls breeds.

2. Methodology

2.1 Ethical aspects

This study was approved by the Research Ethics Committee under number 04/2016. All procedures followed federal law No. 11,794 of October 8, 2008.

2.2 Location and animals

The study was conducted at Central Bela Vista, located in Botucatu, State of São Paulo, in September and October 2020. Retrospective data from andrological examinations and semen freezing of 39 bulls of the breeds were analyzed; Nelore (n=13), Holstein (n=13) and Aberdeen Angus (n=13). All bulls were managed in individual paddocks and received a diet based on corn silage, *Tifton* hay (*Cynodon spp.*), soybean meal, minerals twice a day and water *ad libitum*.

2.3 Collection, freezing and semen analysis

Semen collections were performed on alternate days, twice a week using the artificial vagina method (IMV Technologies, France), totaling two jumps per day. A total of 792 semen collections were carried out, 307 for Aberdeen Angus bulls, 225 for Holstein bulls and 260 for Nelore bulls. The samples were identified with the name of the bull and the respective jump and sent to the andrology laboratory, located in the same center. In the laboratory, the samples remained in a water bath at 35°-37°C until macroscopic analysis, to classify the appearance and volume of the ejaculate. Two aliquots of semen were removed for concentration and sperm morphology analysis. Then, the tube with the ejaculate was weighed on a precision scale to measure the volume and based on the weight, add an antibiotic solution with the intention of avoiding external contamination. This antibiotic solution was made daily and consisted of 0.375 ml of Tylan® 200 (Elanco, USA), 3.75 ml of Pangram® (Virbac, France), 4.5 ml of Linco Spectin (Zoetis, Brazil) and 6.375 ml of ultrapure water, totaling 15 ml of antibiotic solution, aliquots of which were used according to the ejaculate, in the proportion of 0.02 ml of antibiotic for each ml of semen. To determine the sperm concentration, a dilution was made with 0.27 ml of semen in 9.90 ml of a solution based on sodium phosphate, sodium hydroxide and octylphenol ethoxylate (Reagent s100®, ChemoMetec, Denmark). This solution promotes a lysis of the sperm cell membrane making the nuclei susceptible to staining. After that, an aliquot of 0.06 ml was deposited in an SP1-Cassette™ chamber (ChemoMetec, Denmark) which has inside a fluorescent material, propidium iodide (PI), which in contact with the sample dissolves and stains sperm nuclei. Then the SP1-Cassette™ was placed in the Core Counter® SP-100™ equipment (ChemoMetec, Denmark) which read and calculated the concentration of the ejaculate.

For morphological analysis, dilution was performed with an aliquot of 0.1 ml of semen in 0.5 ml of formalin-saline solution. After dilution, a drop of this sample was placed on a slide and covered with a cover slip, and 100 cells of each ejaculate were evaluated. The reading of the slide was performed under a phase contrast microscope (Olympus® BX53, Japan) with a magnification of 100x. Sperm defects were classified as major and minor, and only samples with less than 30% of total defects were cryopreserved (Bloom, 1973).

Ejaculates were diluted with the medium for freezing bovine semen (Optixcell 2®, IMV, France) in a ratio of 1:1 (v:v). Sperm motility was measured subjectively, ranging from 0 to 100%, and force of rectilinear and progressive movement, rated from 1 to 5, after evaluating 5 fields (CBRA, 2013). For the evaluation, a drop of semen and a drop of freezing medium were deposited on a slide and covered with a pre-warmed cover slip at 37°C, being visualized under an optical microscope (Olympus® BX53, Japan) with 10x magnification. Only samples that showed motility above 60% and a minimum vigor of 3 were selected for cryopreservation (CBRA, 2013).

After the evaluations, the last semen dilution was carried out with the Optixcell 2® diluent, according to the volume and concentration of the ejaculate. After dilution, the semen was submitted to a cooling curve for 3 hours, at a temperature of 4° C, in which it remained until the straws were filled by an system automated IMV-MRS4® (IMV Technologies, France). Semen freezing was performed in a Digitcool® equipment (IMV Technologies, France) with a freezing curve of 12.4 oC/min until reaching a temperature of -145°C.

After undergoing the refrigeration and freezing process, one match of each bull jump was evaluated. Sperm parameters were determined using computerized semen analysis (CASA-Computer Assisted Sperm Analysis, Hamilton Thorne II, USA). For analysis, the semen batches were thawed in a water bath at 35° C to 37° C for 30 seconds, then the slides were prepared (Leja®, IMV Technologies, France) and placed for reading. In this analysis, samples should have a minimum of 60% viable cells, 30% progressive motility and vigor ≥ 3 .

2.4 Statistical analysis

Initially, the parameters evaluated before semen freezing were compared between breeds. Motility and vigor were compared before and after freezing, as well as the difference between them. Data were subjected to analysis of variance and compared by Tukey test with 95% confidence interval using Minitab 19 software.

3. Results

Regarding the sperm characteristics evaluated in fresh semen, it was possible to observe that for the motility parameter there was no significant difference between the bulls breeds ($P = 0.053$). The vigor values showed similarities between the Aberdeen Angus and Nelore breeds, while in the ejaculates of Holstein bulls there was a statistical difference ($P = 0.024$); (Table 1).

When evaluating the sperm concentration, it was observed that this parameter was relevant, showing a difference between the three evaluated breeds ($P < 0.001$).

Table 1 - Analysis of the parameters evaluated in fresh semen samples from Aberdeen Angus, Holstein and Nelore bulls (Mean \pm SD).

Parameter (mean \pm sd)	Breed			P Value
	Angus N=307	Holstein N=225	Nelore N=260	
Age (months)	52.6 \pm 23.5 ^a	37.2 \pm 15.0 ^b	45.8 \pm 23.7 ^c	<0.001
Motility (%)	75.6 \pm 3.8	76.8 \pm 3.4	76.0 \pm 3.4	0.053
Vigor (1 a 5)	4.9 \pm 0.3 ^a	4.8 \pm 0.3 ^b	4.9 \pm 0.2 ^a	0.024
Concentration (x 10 ⁶ spztz/mL)	818.2 \pm 367.1 ^a	973.8 \pm 343.5 ^b	1.173.7 \pm 516.3 ^c	<0.001
Major defects (%)	5.3 \pm 2.5	5.7 \pm 2.5	5.8 \pm 2.4	0.35
Minor defects (%)	7.1 \pm 2.1	6.8 \pm 2.0	6.5 \pm 1.2	0.16
Total defects (%)	12.5 \pm 2.8	12.5 \pm 2.2	12.4 \pm 2.5	0.97

a, b and c - Values followed by different letters within the same line differ statistically (P < 0.05). Source: Authors.

Three jumps of Angus bulls and seven jumps of Holstein and Nelore bulls were excluded from the analysis for presenting inadequate motility after cryopreservation with values between zero and 23%. The post-thawing semen parameters are summarized in Table 2. It was possible to observe a difference for the sperm motility variable between the Holstein and Nelore breeds, with the averages for the Holstein breed being higher than the means for the Nelore breed bulls (P<0.001). On the other hand, it was possible to observe that there was no difference in the values of spermatic vigor between the evaluated breeds (P = 0.516).

Table 2 - Motility and vigor analysis after thawing of semen from Aberdeen Angus, Holstein and Nelore bulls (Mean \pm SD).

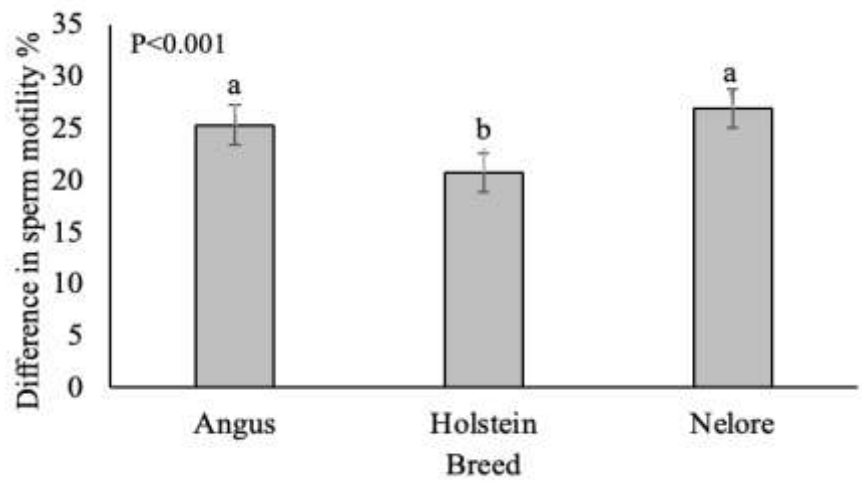
Parameter (mean \pm dp)	Breed			P Value
	Angus N=304	Holstein N=218	Nelore N=253	
Motility (%)	50.4 \pm 9.3 ^a	53.6 \pm 9.8 ^b	49.3 \pm 9.1 ^a	<0.001
Vigor (1 to 5)	3.8 \pm 0.3	3.8 \pm 0.3	3.9 \pm 0.2	0.26

a and b - Values followed by different letters within the same line differ statistically (P < 0.05). Source: Authors.

Regarding the percentage difference in motility in the analysis of fresh and post-thawed semen (Figure 1), it was observed that Nelore and Angus bulls presented greater variation in values between the analyzes (26.0 \pm 8.9% and 25.3 \pm 8.4%. respectively) showing difference (P<0.001) in relation to Holstein bulls that obtained the smallest difference between the analyzes (20.6 \pm 9.3%).

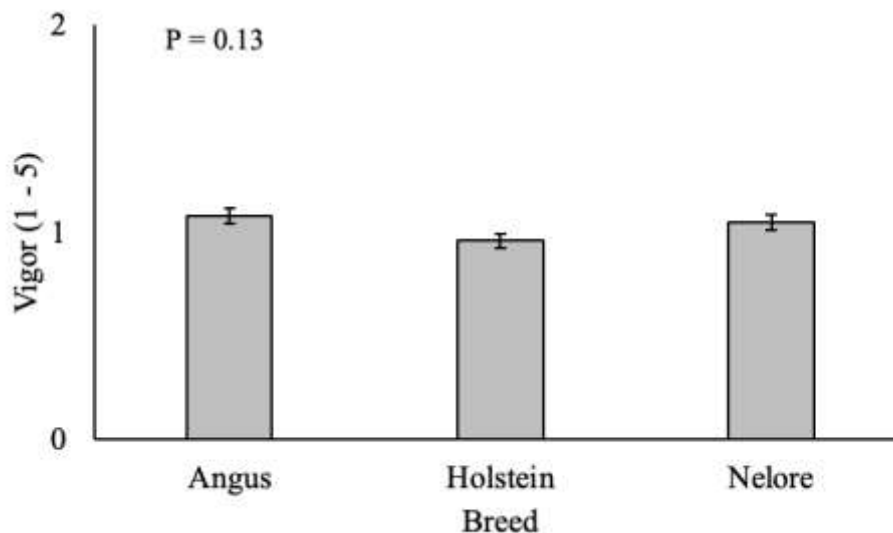
The difference in vigor values between fresh and post-thawing semen analysis (Figure 2) did not show any difference ($P= 0.13$) between the breeds. The mean variation of vigor between the breeds was: Aberdeen Angus bulls $1.0\pm 0.5\%$. Holstein bulls $0.9\pm 0.4\%$ and Nelore bulls 1.0 ± 0.3 .

Figure 1 - Difference in sperm motility percentage values between fresh and post-thaw semen analysis of Angus, Holstein and Nelore bulls.



Source: Authors.

Figure 2 - Difference in vigor values between fresh and post-thawed semen analysis of Angus, Holstein and Nelore bulls.



Source: Authors.

4. Discussion

It is known that sperm motility is considered the most reliable method for selecting the ejaculate, since this parameter has shown a positive correlation with *in vivo* and *in vitro* conception rates (Bergstein et al., 2014; Zhang et al., 1998). In this study, sperm motility was also used as a criterion for selecting ejaculates for freezing.

Data from this study show that there was no significant difference in fresh semen for the motility parameter between races. Similar results were also found by Brito et al. (2002), who evaluated the effect of seasonality on the semen production of *B. taurus* and *B. indicus* over a period of two consecutive years and concluded that factors such as temperature, humidity and seasonality did not significantly affect semen production, as well as quality. Motility did not show statistical difference between genetic groups, in the first (*B. indicus* 58.8% versus *B. taurus* 60%) and second year (*B. indicus* 58% versus *B. taurus* 59.3%). As for Chacur et al. (2012), who, when comparing the seminal quality of zebu and taurine bulls in the four seasons, did not identify motility differences between the subspecies.

On the other hand, a study by Koivisto et al. (2009), *Bos indicus* bulls showed superior sperm motility and vigor when compared to *Bos taurus* bulls in the autumn and summer seasons. The progressive motility of *B. indicus* bulls was $56.9 \pm 0.5\%$ and $56.5 \pm 0.5\%$ in the summer and autumn seasons, respectively, while the progressive motility of *B. taurus* bulls was $51.1 \pm 0.7\%$ and $53.7 \pm 0.3\%$ in the same periods. Vigor for *B. indicus* samples showed values of 4 ± 0.04 in the summer and autumn seasons, while for *B. taurus*, it was 3.8 ± 0.04 in both seasons. The authors attributed these results to the better adaptation of Zebu bulls compared to Taurus bulls to higher climatic temperatures. In the present study, inferiority of the parameters of *B. indicus* bulls was noted in relation to *B. taurus* in equivalent seasonal periods, which can be explained by the climatic difference in the region where the studies were carried out and by the welfare conditions of each location.

The sperm concentration values presented in the study showed differences between the three breeds evaluated, indicating that Nelore bulls have higher sperm concentrations compared to other breeds. In a study carried out by Morrel et al. (2018), the authors observed a similar result in the concentration values, comparing semen from dairy bulls (Swedish Red and Holstein) and beef bulls (Limousin, Charolais, Simmental, Hereford, Angus and Blonde D'Aquitaine) in which the mean sperm concentration was $88 \pm 20 \times 10^6$ spz/mL for beef bull samples and $55 \pm 19 \times 10^6$ spz/mL for dairy bulls ($P < 0.001$).

Although the sperm motility of fresh semen was similar between species, in the post-thaw sample a difference was detected in the percentage of motility in which Nelore bulls ($26.0 \pm 8.9\%$) and Angus ($25.3 \pm 8.4\%$) showed greater variation and reduced motility compared to Holstein bulls ($20.6 \pm 9.3\%$). Morrell et al. (2018), comparing thawed semen from beef bulls (Limousin, Charolais, Simmental, Hereford, Angus and Blonde D'Aquitaine) and dairy bulls (Swedish Red and Dutch) obtained progressive motility values of $58 \pm 13\%$ for beef bulls and $55.6 \pm 14\%$ for dairy bulls, demonstrating that there was no statistical difference ($P > 0.05$) in this parameter between groups.

Li et al. (2016), in a study that evaluated groups of bulls with ejaculates with high progressive motility values and groups with ejaculates with low progressive motility, obtained results that demonstrated the greater *in vitro* fertilization capacity of samples with high motility values compared to samples with lower values for motility. With these results, it can be deduced that sperm motility is of great relevance in the fertility of cows submitted to artificial insemination, being considered as a good parameter in the selection of semen samples for use in biotechniques applied to animal reproduction.

Although the results of the present study are not in agreement with some data already published, it is believed that Holstein bulls showed a lower motility difference and a supposedly better semen viability after cryopreservation is due to cellular factors associated with the breed. Morrell et al. (2018) reported indices such as sperm plasma membrane integrity to be higher in dairy bulls compared to beef bulls ($46 \pm 8\%$ versus $40 \pm 11\%$, respectively), as well as superiority in relation to

mitochondrial potential in which the proportion of sperm with high mitochondrial activity was $52\pm 12\%$ in dairy bulls and $36\pm 10\%$ in beef bulls.

5. Conclusion

The semen cryopreservation process causes a decrease in the physical parameters of the semen and these quality losses, suffer interference according to the breeds, genetic groups evaluated and the system in which the animal is inserted. However, further studies are needed in order to improve gamete cryopreservation protocols and, in this way, increase the quality of the ejaculate and have greater success in pregnancy rates.

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