Correlation between hypoosmotic swelling test and breeding soundness evaluation of adult Nelore bulls

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Abstract

This study aimed at evaluating the relationship between physical and morphological semen features with the hypoosmotic swelling (HOS) test in raw semen of adult Nelore bulls classified as sound and unsound for breeding. Two hundred and six Nelore bulls aging from 3 to 10 years of age were subjected to breeding soundness examination. After physical and morphological semen examination, HOS test was done. After the breeding soundness examination, 94.2% of the bulls were classified as sound and unsound for breeding. There was no difference between the average scrotal circumference of bulls classified as sound and unsound for breeding (P>0.05), but there was difference between all semen physical and morphological aspects of bulls classified as sound and unsound for breeding (P>0.05), and no difference resulted in the mean percentage of reactive spermatozoa to HOS test results both for sound (38.4±17.9) and unsound animals (39.5±16.4; P>0.05), with no Pearson correlation between the HOS test and variables. According to these results HOS test can not be used alone to predict the reproductive potential of adult Nelore bulls.

Materials and methods

A total of 206 adult Nelore bulls were examined for breeding soundness evaluation, according to the criteria established by the Brazilian College of Animal Reproduction (CBRA, 1998). The bulls were aged from 3 to 10 years, with good body condition, raised in pastures, predominately Brachiaria decumbens and Colionão (Panicum maximum), with mineral salt and water ad libitum. The farms were located at São Paulo (located at latitude 20-21° South and longitude 50-51° West, with an average temperature of 24°C and annual rainfall of 1189 mm) and Mato Grosso do Sul (at latitude 20-21° South and longitude 55-56° West, with an average temperature of 21°C and annual rainfall between 1000 and 1400 mm) States, Brazil.

After palpation of the vesicular gland (evaluation of size, symmetry, lobulation and consistency to detect possible changes) and testis size (scrotal circumference, testicular length and width), sperm was collected by electroejaculation method. The semen was subjected to physical and morphological evaluation, and the HOS test. The physical evaluation of the semen was done by analyzing the following aspects: mass motility (0-5), progressive sperm motility (0-100%) and the spermatic vigor (0-5). One drop of semen (10 μL) was placed on a slide preheated at 38°C and 20x magnification for mass motility evaluation. Progressive sperm motility and spermatid vigor were evaluated with the deposition of 10 μL of semen between slide and coverslip also preheated to 38°C, at a 400x magnification by optical microscopy (CBRA, 1998).
In a tube containing 1 mL of saline formaldehyde-buffered solution (Hancock, 1957) was placed an aliquot of the ejaculate sufficient to muddy the solution for the analysis of sperm morphology by wet preparation and use of phase contrast microscopy in a 1000X magnification (under a drop of immersion oil). Four-hundred cells per ejaculate were counted, and sperm defects were measured as a percentage according to the classification criteria adopted by Blom (1973) and recommended by the CBRA (1998).

The classification recommended by CBRA (1998) in which the reproductive potential is predicted by means of values recorded for the physical and morphological sperm features, and the limits of 70% for progressive sperm motility, major sperm defects below 15%, and total sperm defects, less than 30% of abnormalities, resulting in two classes of animals: animals sound for breeding and animal unsound for breeding. Groups of unsound animals used were classified only as to its spermiogram not used by other animals deemed unsound for reproductive clinical finding, such as testicular asymmetry, shell or angulation problems, low scrotal circumference, vesiculythis, among others.

The HOS test was conducted with an amount of semen from 20 to 50 μL in 1 mL of a hypoosmotic solution, depending on the aspect of semen (creamy, milky, cloudy or watery). It consisted in incubating of 20 to 50 μL of semen for 1 h at 37°C in 1 mL of hypoosmotic solution with 150 mOsm/kg, according Revel and Mrode (1994). After the incubation period were added 0.5 mL of saline formaldehyde-buffered solution for fixation of sperm and then made the analysis in phase contrast microscopy. A total of 100 sperm were counted in at 1000X magnification and obtained the percentage of sperm that had their scurvy curving along the expanded membrane, and then calculating the percentage of spermatozoa reactive subtracting the percentage of tail defects recorded in raw semen (Melo and Henry, 1999).

For the statistical analysis SAEG version 9.1 software was used (SAEG, 2007). Descriptive analysis regarding the average and standard deviations were made for all features. The Lillifors test was used for verification of data normality of variables. The homogeneity of variance was studied, using the Cochran normality of variables. The homogeneity of Lilliefors test was used for verification of data analysis regarding the average and standard software was used (SAEG, 2007). Descriptive Henry, 1999).

Then calculating the percentage of spermatozoa reactive curving along the expanded membrane, and then made the analysis in phase contrast microscopy. A total of 100 sperm were counted then making the analysis in phase contrast buffered solution for fixation of sperm and were added 0.5 mL of saline formaldehyde-solution with 150 mOsm/kg, according Revel 1957) was placed an aliquot of the ejaculate for sound and unsound bulls (P<0.05; Table 1). The average scrotal circumference (38.5±2.4 cm) was considered very good, according to the criteria established by the Brazilian College of Animal Reproduction (1998), similar to those observed by Santos et al. (2004) with 37.9 cm in adult Nellore bulls pre-selected for the breeding season and those of Chacón et al. (1999) which reported 36.2±4 cm using 598 adult zebu bulls with an average age of 4.2 years. Lopes et al. (2009) working with 14 adult Nellore bulls pre-selected for natural mating system with mean age of 50.5±15.7 months, recorded a 37.3±1.5 cm scrotal circumference.

There was differences between the mean mass motility, progressive sperm motility and spermatic vigor of the ejaculate from sound and unsound bulls (P<0.05; Table 1). The averages of the physical aspects were similar to those reported by Santos et al. (2004) which observed 74.5% of progressive sperm motility and 3.1 of spermatic vigor. Chacón et al. (1999) observed average below this experiment (61±21%) in adult Nellore bulls. Lopes et al. (2009) observed 74.2±12.3% of progressive sperm motility, 2.5±1.3 of mass motility, and 3.0±0.9 of spermatic vigor, values similar to this experiment.

Differences resulted as well between the means of all the morphological features for sound and unsound bulls (P<0.05; Table 1), one of the main criteria for the soundness evaluation (CBRA, 1998). Santos et al. (2004) and Lopes et al. (2009) found 13.8% and 9.5 % of total defects in adult Nelore bulls sound for breeding, respectively, and the means are inferior to this experiment. But when analyzing only the bulls for breeding the averages are similar. The major sperm defects were folded tail and tucked tail with distal cytoplasmic droplets (2.6±2.4 and 0.8±1.7 to 15.4±13.9 for sound and 8.5±9.0 for unsound animals), confiming the findings of Chácón et al. (1999).

No difference was observed between the mean percentage of reactive spermatozoa to the HOS test for sound and unsound bulls to natural mating regime (P>0.05). The average percentage of reactive spermatozoa to the HOS test was very low (Table 1), comparing with other studies using the HOS swelling test in raw semen of Zebu bulls. Vera-Munoz et al. (2009) using adult crossbred bulls for insemination center had a mean of 68.1% reactive sperm HOS test in raw semen and 48.8% in frozen / thawed semen, showing damage to the plasma membrane by the cryopreservation process. Martins et al. (2011) using 6 adult Nelore bulls found 60.3% of reactive spermatozoa to the HOS test in raw semen and 30.8% for frozen semen, showing damage to the plasma membrane by the cryopreservation process.

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### Results and discussion

After the breeding soundness evaluation, 94.2% (194/206) of animals were classified as sound and 5.8% (12/206) unsound for breeding. There was no difference between the mean testicular biometry between the groups (P>0.05; Table 1). The average scrotal circumference (38.5±2.4 cm) was considered very good, according to the criteria established by the Brazilian College of Animal Reproduction (1998), similar to those observed by Santos et al. (2004) with 37.9 cm in adult Nellore bulls pre-selected for the breeding season and those of Chacón et al. (1999) which reported 36.2±4 cm using 598 adult zebu bulls with an average age of 4.2 years. Lopes et al. (2009) working with 14 adult Nellore bulls pre-selected for natural mating system with mean age of 50.5±15.7 months, recorded a 37.3±1.5 cm scrotal circumference.

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No difference was observed between the mean percentage of reactive spermatozoa to the HOS test for sound and unsound bulls to natural mating regime (P>0.05). The average percentage of reactive spermatozoa to the HOS test was very low (Table 1), comparing with other studies using the HOS swelling test in raw semen of Zebu bulls. Vera-Munoz et al. (2009) using adult crossbred bulls for insemination center had a mean of 68.1% reactive sperm HOS test in raw semen and 48.8% in frozen / thawed semen, showing damage to the plasma membrane by the cryopreservation process. Martins et al. (2011) using 6 adult Nelore bulls found 60.3% of reactive spermatozoa to the HOS test in raw semen and 30.8% for frozen semen, showing damage to the plasma membrane by the cryopreservation process.

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### Table 1. Scrotal circumference, percentage of reactive spermatozoa to the hypoosmotic swelling test and physical and morphology aspects of raw semen of adult Nelore bulls, classified as sound and unsound for breeding.

| Parameters        | Sound bulls | Unsound bulls | General |
|-------------------|-------------|---------------|---------|
| Number of animals | 194         | 12            | 206     |
| Scrotal circumference, cm | 38.5±2.4    | 39.1±2.6       | 38.5±2.4 |
| Hypoosmotic swelling test, % | 38.4±17.9   | 39.5±16.4    | 38.5±17.8 |
| Physical aspects  |             |               |         |
| Sperm progressive motility, % | 74.6±8.6    | 50.0±23.3     | 73.2±11.5 |
| Spermatic vigor, 0-5 | 3.1±0.4     | 2.6±0.5       | 3.1±0.4  |
| Mass motility, 0-5 | 2.1±2       | 0.9±1.1       | 2.0±1.1  |
| Morphological aspects |             |               |         |
| Tail defects, %   | 2.6±2.4     | 15.4±13.9     | 3.3±5.0  |
| Major defects, %  | 10.4±5.7    | 55.1±18.7     | 11.6±9.1 |
| Minor defects, %  | 4.5±2.7     | 7.8±6.1       | 4.7±3.1  |
| Total, %          | 14.9±6.1    | 42.9±21.4     | 16.5±10.1 |

Different small letters on the same line indicate difference (P<0.05) by F test at 5%; A,B,Cdifferent capital letters in the same column indicate difference (P<0.05) by Wilcoxon 5%.
explained by the prolonged sexual rest. The tail of the epididymis serves to keep the sperm viable and defective phagocyte, the dead release enzymes that may interfere with the viability of which will remain in the epididymis (Jones, 2004), which cause damage to the plasma membrane of spermatozoa (Marengo, 2008), and justifies the mean low sperm HOS test reactivity to this experiment.

The results of the HOS test did not correlate with any physical or morphological appearance of the semen, in disagreement with Martins et al. (2011) which reported average and positive correlations with the HOS test, sperm progressive motility and spermatic vigor after thawing ($r=0.38$ and $r=0.34$, respectively). Corroborating this study, these authors also found no correlation between the results of the HOS test in raw semen and the physical and morphological semen aspects. Siqueira et al. (2007) using frozen semen of adult Nelore bulls reported an average and positive correlation ($r=0.21$), being lower than the findings of Vera-Munoz et al. (2009) with a high positive correlation ($r=0.96$).

The functional integrity of sperm plasma membrane should be used routinely in the evaluation of frozen/thawed semen, and has relationships with the high fertility of bovine frozen semen samples in artificial insemination programs (Revell and Mrode, 1994; Correa et al., 1997; Bacinoglu et al., 2008) is also other information on spermogram, another aspect of quality of the plasma membrane when compared to tests of physical integrity. It is not possible to measure the true potential of fertilization of frozen samples and marketed, but the identification of samples of low quality is possible using additional tests (Rodriguez-Martinez, 2006; Mocé and Graham, 2008).

Conclusions

The HOS test can not be used alone to classify adult Nelore bulls as sound and unsound for breeding. Although it is an important complementary test in the evaluation of frozen semen, it can not be used routinely in breeding soundness evaluation, because there are no established standards of the percentage of reactive spermatozoa to the classification of reproductive status of adult Nelore bulls.

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