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EFFECT OF TWO COMMERCIAL DILUENTS USED TO CRYOPRESERVE SEMEN OF RODEO BULLS ON SPERM MOTILITY AND VIABILITY

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Abstract: Jaripeo, the mexican equivalent of rodeos, also uses untamed bulls, which have taken on great importance in Mexico due to their cultural value. In order to maintain their genetic material, semen cryopreservation is utilized. In the present work, two commercial diluents that have not been tested in jaripeo bulls were used, to evaluate the motility and viability of sperm after the cryopreservation process. In the study, 6 healthy bulls of undefined breeds, ranging in age from 2 to 5 years, were used, and three ejaculate samples were obtained and evaluated. The freezing protocol was according to the diluent of each group; samples were loaded in 0.25ml straws, at a concentration of 20 x 106 cells/ml. The straws were left for 4 hours at 4 ° C for equilibration. Then freezing was carried out exposing the straws to nitrogen vapors for 12 minutes and, finally, they were immersed in liquid nitrogen. Properly identified straws were put in containers and were stored in a tank with liquid nitrogen. Thawing was carried out by removing the straws and exposing them to room temperature for 10 seconds and then they were placed in a water bath at 37°C for 30 seconds. For the analysis of the data, one-way ANOVA was used, for sample comparison, Dunnett's multiple test was used. Post-thawed viability of the sperm that were diluted with Triladyl® was 58.44% while in those diluted with AndroMed® 47.72% viability was obtained. In regard to post-thawed motility of the sperm with the diluent AndroMed® a motility of 67.22% vs the diluent Triladyl® of 69.16% ($P \leq 0.05$), i.e., there was no significant statistical difference between sperm from the two media. It is concluded that the frozen/thawed semen of jaripeo bulls had the same motility, although samples diluted with Triladyl® medium exhibited better sperm viability than those diluted with AndroMed® medium.

Keywords: Bull, jaripeo, cryopreservation, Triladyl®, AndroMed®

INTRODUCTION

Due to the great boom that the jaripeos in Mexico today, as well as the cultural value that they represent for some people, it is important to preserve the genetic material of the animals that show desirable characteristics to carry out this type of activity (Molano and Lombada, 2021). Cryopreservation of semen is a biotechnology of great importance, which aims to keep the genetic material viable indefinitely. With the help of this technology, genetic banks can be generated, which preserve biodiversity and preserve the physical and productive characteristics of a species (Álvarez, 2005).

In freezing protocols, the use of media for the dilution of semen aims to increase the volume of the ejaculate, as well as to maintain the viability and fertilizing capacity of sperm (Caraballo et al., 2009).

There are several types of commercial diluents, among these we can mention Triladyl®, AndroMed®, Optidyl and Bioexcel, among others.

Triladyl® is a concentrate to which egg yolk is added for reconstitution. Normally 250 g yield 1250 ml of diluent. It consists of Tris, citric acid, sucrose, pH buffers, glycerin, tyrosine and antibiotics (gentamicin, spectinomicin and lincomycin). To reconstitute it, distilled or bidet distilled water must be used at a ratio of 1:8 (Caraballo et al., 2009).

AndroMed® contains Tris, phospholipids, citric acid, glycerin and antibiotics (tyrosine, gentamicin, spectinomicin and lincomycin). Its main feature is that no egg yolk is added, which reduces the likelihood of bacterial contamination (Caraballo et al., 2009).

Currently, jaripeo bulls have taken on great importance and the application of biotechnologies is required for the cryopreservation of genetic material in an efficient way; however, the information available about it is scarce. Here, two commercial diluents were used, which have not been tested on jaripeo bulls of defined breed, to evaluate the survival of sperm after the cryopreservation process. The study aims to determine which diluent promotes better sperm survival upon thawing.

MATERIAL AND METHODS

Evaluation of semen: macroscopically and microscopically, they were evaluated before and after cryopreservation. 6 healthy bulls of of undefined breeds were used, between 2 and 5 years of age, located in the state of Morelos; of which 3 ejaculate samples were obtained and evaluated. The semen samples were kept at a temperature of 37° C for evaluation and until they were processed. Sperm concentration, progressive motility and viability were evaluated (Pérez, 2008)

Once these parameters were evaluated, the diluent was added, the samples were divided into 2 groups: 1) half Triladyl® and 2) half AndroMed®.

The freezing protocol was carried out according to the diluent of each group; processed samples were introduced into 0.25ml straws, at a concentration of 20 x 106 cells/ml. The straws were left for 4 hours at 4 ° C for balancing, then the freezing was carried out exposing the straws to nitrogen vapors for 12 minutes and, finally, they were immersed in liquid nitrogen. Properly identified straws were distributed in containers and stored in a tank with liquid nitrogen. Thawing was carried out by removing the straws and exposing them to room temperature for 10 seconds and then they were placed in a water bath at 37°C for 30 seconds.

Upon thawing, evaluation of sperm motility and viability was performed. To evaluate motility, 1 drop (10 µL) of the diluted semen is taken with a pipette, placed on a slide at a temperature of 37ºC and protected with a cover slip. It was observed under the

microscope at 400 magnifications. A field was observed and sperm that moved in a progressive rectilinear way were subjectively detected. These being the ones that crossed the observation field. Sperm rotating in circles or advancig in an oscillatory way, were considered to have abnormal movements. The percentage indicated is that of sperm with progressive rectilinear movement of the total number of accepted sperm, with the minimum acceptable value of 50% (Palacios, C.J., 2005). The viability was evaluated with eosin-nigrosin staining (Selles et. al., 2003).

For the analysis of the data using one-way ANOVA, the Dunnett multiple test was used for the comparison of the means. The motility and viability averages between fresh semen and commercial means for freezing semen were analyzed, Group 1: Triladyl® medium and Group 2: AndroMed® medium; using GraphPad Prism Version 5 (GraphPad Software, Inc., La Jolla, CA, USA). As already mentioned above, the bulls lack a defined breed, so the effect of the bull could not be considered in the statistical analysis. Significant differences between averages with values of $P \leq 0.05$ were considered.

RESULTS AND DISCUSSION

Motility of each fresh ejaculate was 77.66%. The motility of fresh semen was significantly higher than frozen/thawed samples with both diluents ($P \leq 0.05$). There was no statistical difference in motility between the two means used for the cryopreservation of semen (Figure 1).

Although cryopreservation has been used as a routine technique for the preservation of bovine semen, it is known to damage sperm in different ways, both functionally and structurally (Mostek et al., 2017, Martínez et al., 2006). Approximately 50% of sperm become immobile and those that remain mobile are compromised, which leads to a decrease in fertilization capacity: that is, between 40 and 50% of sperm do not survive the freezing-thawed process, so the efficiency of cryopreserved semen is low in relation to fresh samples (Mostek et al., 2017, Martínez et al., 2006). Our results show a percentage above the aforementioned 50% after thawing, finding a better percentage of motility after the cryopreservation process obtaining with the AndroMed® diluent a motility of 67.22%, while in the Triladyl® diluent the motility was 69.16% ($P \leq 0.05$). Sperm motility is essential for sperm to reach the uterine environment and the fertilization site, and it is the most important criterion for evaluation of sperm before and after cryopreservation (Siqueira et al., 2007).

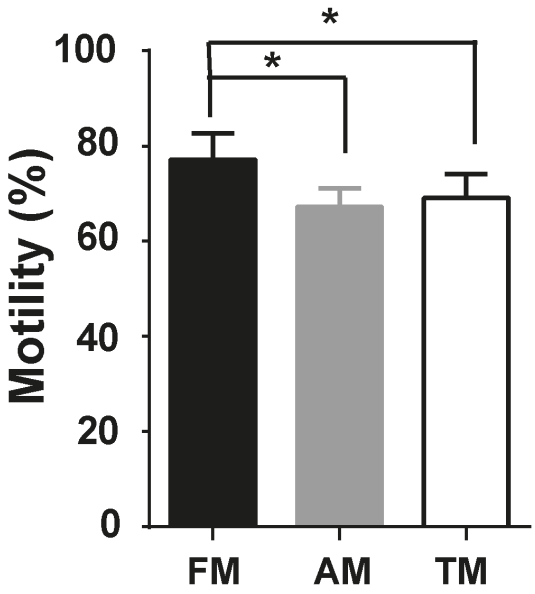

Figure 1. Motility of the sperm of jaripeo bulls after the cryopreservation process FM: Fresh motility 77.66%, AM: AndroMed® motility 67.22%, TM: Triladyl® motility 69.16% ($P \leq 0.05$).

As for viability, fresh semen samples had 86.67% 95% live sperm. The viability of fresh semen was greater than the frozen/thawed samples with both diluents ($P \leq 0.05$). The viability of the samples was evaluated after the cryopreservation process, there was no

statistical difference between the two means used for the freezing process of the semen sample ($P \leq 0.05$) (Figure 2). However, a better post-thawed sperm viability was observed with the Triladyl® 58.55% medium compared to the AndroMed® 47.72% medium ($P \leq 0.05$). In this regard, Argudo et al., 2019 mentions that, when evaluating Creole cattle the effect of these diluents on some seminal parameters through the CASA system, the best cryopreservation option was with the medium containing egg yolk (Triladyl). Carballo et al. (2009) and Nuñez y Rubio (2015) studied the effect of different diluents with and without egg yolk, concluding that those containing egg yolk presented better results to the post-thawed evaluation, while Galarza (2013) found no significant differences between the two diluents, except for the sperm vitality that was higher with the diluent containing egg yolk.

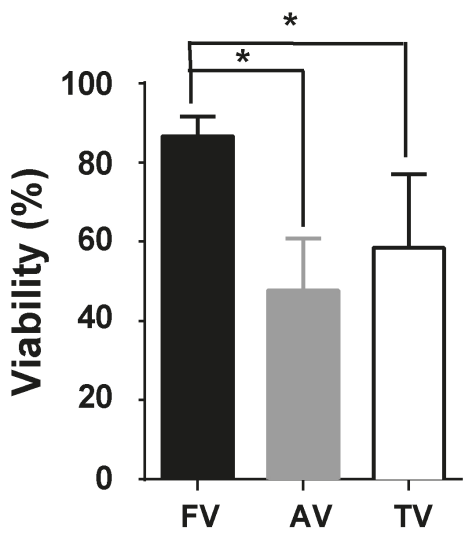

Figure 2. Percentage of viability of the sperm of jaripeo bulls after the cryopreservation process VF: Fresh Viability 86.67%, AV: AndroMed® viability: 47.72%, TV: Triladyl® viability: 58.44% ($P \leq 0.05$).

CONCLUSIONS

The frozen/thawed semen of jaripeo bulls diluted with the Triladyl® medium had the same motility and sperm viability as the semen diluted with the AndroMed® medium, although samples diluted with Triladyl® medium exhibited better sperm viability than those diluted with AndroMed® medium.

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