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Increase of the motility of buck semen with Andromed® extender and the addition of HTF

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ABSTRACT

Objective: To determine if HTF (Human Tubal Fluid) can protect buck sperm cells, increasing semen motility when used by itself or combined with the Andromed® commercial extender.

Design/Methodology/Approach: The semen of eight bucks of the Boer breed was used (10 ejaculates per male). Each ejaculate was diluted in three treatments (5 repetitions per treatment): 1) only Andromed®; 2) only HTF; and 3) Andromed® + HTF. The following variables were evaluated: mass motility (MM) and progressive motility (MP) in fresh (37 °C) and refrigerated (5 °C) semen. The differences between treatments were detected, based on the MM and MP in fresh and refrigerated semen variables, using the GLM procedure (SAS V9). In the case of the MM evaluated in three different periods, the measures repeated over time were analyzed using the Proc Mixed procedure (SAS V9).

Results: Regarding the quality of fresh spermatozoa, MM and MP recorded similar values ($p>0.05$) for the three treatments. On the one hand, MM registered 97.1% for Andromed®, 95.8% for HTF, and 97.5% for Andromed® + HTF; its highest value was achieved with the Andromed® + HTF combination, followed by Andromed® and HTF. On the other hand, the highest MP percentage was observed with the combination of Andromed® + HTF (91.0%), followed by Andromed® (90.4%), and HTF (89.0%). Regarding the quality of the sperm refrigerated at 5 °C, the combination of Andromed® + HTF had a higher MP value ($p<0.05$) than other treatments.

Study Limitations/Implications: In all the evaluated treatments, the motility of sperm decreased over time (0 to 24 hours after cooling); however, the treatments with Andromed®, and Andromed® + HTF maintained a high motility.

Findings/Conclusions: The combination of Andromed® and HTF can be used to dilute both fresh and cooled semen.

Keywords: extenders, HTF, Andromed®, goat.

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INTRODUCTION

Regarding artificial insemination (AI), highlighting the importance of several factors that affect the desired level of fertility is imperative. In this sense, Allai *et al.* (2018) emphasize the importance of preserving ovine semen as one of the determining factors to be considered during the implementation of an AI program. Consequently, extenders play a fundamental role in the preservation and prolongation of sperm viability before and during AI. Currently, there is no ideal extender that provides the desired sperm quality, because each species needs different conditions.

HTF (Human Tubal Fluid) is an alternative that contains commercial extenders that contribute to the embryonic maturation process. It is a highly nutrient-rich medium, as a result of the compounds (*e.g.*, sodium chloride, potassium chloride, dehydrated calcium chloride, and hepta-hydrated magnesium sulfate) that it contains. The composition of the HTF is based on the compounds of the human oviduct and it has been widely used in the manipulation of gametes and embryos in humans, rats, mice (Aoto *et al.*, 2011; Cossiello *et al.*, 2012; Kleijkers *et al.*, 2016), and more recently in horses (Arroyo-Salvo *et al.*, 2019). The effect of HTF on sperm cells is directly based on sperm capacitation and changes in the levels of reactive oxygen species (ROS), which have been studied in humans but not yet in domestic species (Shih *et al.*, 2016; Hernandez *et al.*, 2021). Therefore, it may be acceptable as a reliable medium in the processing of domestic animal semen. Meanwhile, the development of new *in vitro* technologies —such as the CASA system (Computer Assisted Sperm Analysis System)— enables the study of multiple functional and morphological characteristics of spermatozoa, which are used to predict their fertilizing capacity (Quintero-Moreno *et al.*, 2011). Likewise, this reliable method is the most frequently used tool for the study of fresh or frozen diluted semen (Vicente-Fiel *et al.*, 2013) and it provides the bases for the hypothesis of the present work. There is at least one increase in MM and/or MP between treatments. Therefore, the objective of this study was to test if HTF can protect buck sperm cells by itself or combined in a certain ratio with the Andromed® commercial extender, in order to achieve better fertility in the AI.

MATERIALS AND METHODS

A total of eight Boer bucks (2-4 years old in average) were used to obtain 10 ejaculates per male during the reproductive season. The semen was collected twice a week until the total number of ejaculates per male was completed. For this purpose, the males were induced to ejaculate by means of the presence of a doe in heat and an artificial vagina. Prior to the application of the treatments, the ejaculates were qualitatively evaluated following the procedure described by Clemente-Sánchez *et al.* (2017) and those that did not show favorable qualitative characteristics were discarded and no longer considered in the treatments. Each ejaculate obtained was subjected to a particular treatment (Andromed®, HTF, and Andromed® + HTF); each treatment included five repetitions per treatment and their dilution rate was 1:5. The statistical analysis of the treatments was based on a total of 400 observations. The treatments (T) were made up as follows: Andromed® (T1); HTF medium (T2); 2/3 of Andromed® + 1/3 of HTF (T3).

After the dilution, the sperm characteristics (Mass Motility (MM) and Progressive Motility (MP)) were first evaluated in diluted fresh semen (37 °C) and, subsequently, in refrigerated semen (5 °C). Additionally, the spermatozoa MM was evaluated three times (at 0, 12, and 24 h) after they had been refrigerated at 5 °C. The evaluations were carried out using the Sperm Vision CASA (Computer Assisted Sperm Analysis System) from MOFA Global Company. Finally, the differences between treatments (along with the behavior of each treatment) were determined according to the variables evaluated. The MM and MP variables were analyzed using the Proc GLM procedure (SAS V9), followed by Tukey's multiple range test ($p<0.05$). The MM evaluated at three different times was subjected to a repeated measures analysis over time using the Proc Mixed procedure (SAS V9).

RESULTS AND DISCUSSION

In terms of the MM and MP variables, the fresh sperm quality results obtained from the comparison of treatments did not show any differences between treatments ($p>0.05$): Andromed® + HTF (97.5% and 91.0%), Andromed® (97.1% and 90.4%), and HTF (95.8% and 89.0%) (Table 1). Mortimer (1997) mentions that the use of computerized systems such as CASA leads to changes in the sperm flagellum, causing a decrease in motility; these changes were not observed in the fresh semen values, because the numbers obtained provided appropriate motility values for use in AI. However, the CASA System has the advantage that all the readings are standardized, avoiding the sampling or evaluator effects.

Most of researches about ruminant semen consider MM as the main variable to determine semen quality. However, the evaluation by MM as the only semen quality parameter is not enough to predict the fertilizing capacity of semen (Grasa *et al.*, 2005). Clemente-Sanchez *et al.* (2012) developed a linear model to determine semen quality in white-tailed deer, known as the Deer Semen Quality Index (SQID), which in addition to MM considers individual motility, sperm concentration, percentage of live spermatozoa, and percentage of normal spermatozoa. The result of this model provides a criterion of semen quality that is closer to reality and its use is recommended as a standardization tool in the deer semen evaluation process. Likewise, it is a useful tool in conducting researches where evaluating the semen is necessary or for treatments such as those developed in this study. However, although this study only considered the mass and progressive motility variables, all the sperm cell variables that the CASA system includes must be taken into consideration, including curvilinear velocity, progressive velocity, linear velocity, linearity

Table 1. Evaluation of mass motility (MM) and progressive motility (MP) in the spermatozoa of diluted fresh semen of buck.

Tratamientos	Motilidad	
	MM (%)±EE	MP (%)±EE
Andromed®	97.1[a]±2.3	90.4[a]±2.4
HTF	95.8[a]±1.7	89.0[a]±2.6
Andromed® + HTF	97.5[a]±1.9	91.0[a]±2.0

[a] Values with the same letter within columns are not different ($p<0.05$).
±ES (±EE)=Standard Error.

index, straightness index, oscillation index, and mean amplitude of lateral head displacement (Hernández-Corredor *et al.*, 2013). These variables provide objective parameters for the evaluation of semen diluted in different extenders to facilitate the standardization of the morphometric characteristics of spermatozoa (Vicente-Fiel *et al.*, 2013). Other reports describe the evaluation of buck semen, including a study by Evans and Mawell (1990), who describe the procedure for accepting or rejecting the ejaculate to be used in AI, based on a series of parameters, such as MM, MP, ejaculate volume, sperm vigor, sperm concentration, vital coloration, sperm morphology, and endosmosis test.

Regarding the MM and MP of sperm refrigerated at 5 °C (Table 2), the combination of Andromed® + HTF showed a significant difference ($p<0.05$) in the MM variable with respect to the other treatments. This may be the result of the combination of Andromed® and HTF: once the semen is subjected to cold temperatures, it maintains a high motility, as a consequence of the large amount of nutrients provided jointly by these two substances. However, spermatozoa tend to suffer damage and deterioration when they are diluted and preserved at low temperatures. Therefore, it is necessary to use extenders —especially those based on soybean lecithin, and which are free of egg yolk, given their great cryopreservation capacity to preserve the viability, membrane integrity, motility, and progressivity of spermatozoa when buck semen is frozen (Hernández-Corredor *et al.*, 2017). In this regard, Singh *et al.* (2012) found that using the Andromed® extender increases the spermatic motility of semen stored at 5 °C. MM and MP values above 80% for buck semen refrigerated at 5 °C are both considered excellent for later use in assisted reproduction programs. Therefore, the results of this study indicate that the evaluated treatments — particularly the Andromed® + HTF combination— can be used as extenders. This result is similar to that reported by Roof *et al.* (2012), who corroborated that the composition of the extender is crucial for the protection of spermatozoa against cryopreservation (Jiménez-Rabadan *et al.*, 2012), because the structural changes that take place at cellular level during the cryopreservation process cause irreversible damage to spermatozoa and affect motility (Dorado *et al.*, 2009).

The MM results of the spermatozoa evaluated over time are shown in Figure 1. There were no differences ($p>0.05$) at zero (0) h; however, the highest percentage of motile spermatozoa was observed in the Andromed® + HTF (92.4%). At 12 h, MM decreased in all treatments, with differences between them ($p<0.05$). The HTF treatment showed 15% reduction in mass motility. Just like the HTF, it greatly reduced mass motility

Table 2. Evaluation of mass motility (MM) and progressive motility (MP) in the spermatozoa of diluted and refrigerated (5 °C) semen of buck.

Tratamientos	Motilidad	
	MM (%) ±EE	MP (%) ±EE
Andromed®	86.0^{a}±3.2	84.9^{a}±2.3
HTF	84.1^{a}±2.6	84.6^{a}±2.0
Andromed® + HTF	90.1^{b}±2.1	86.3^{a}±1.6

[a, b] Values with different letter within columns are different ($p<0.05$).
±SE (±EE)=Standard Error.

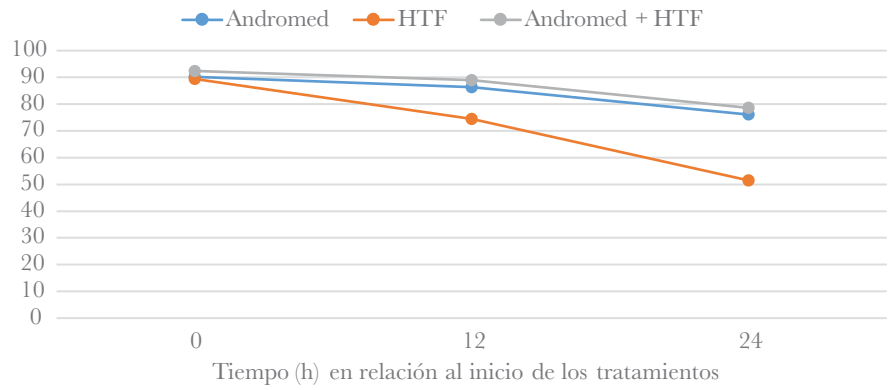

Figure 1. Mass motility (MM) of spermatozoa in semen diluted in three mediums (treatments), evaluated at 0, 12, and 24 h after refrigeration at 5 °C.

(22.9%), twenty-four hours after refrigeration. Likewise, it was different than the other two treatments ($p<0.05$).

Both Andromed® and the Andromed® + HTF combination recorded 50 to 75% motility values, which are desirable in the refrigerated semen stored for up to 24 h that will be used in AI. According to Hernandez *et al.* (2014), the use of animal protein-free extenders (*e.g.*, Andromed®) provides a better response, because its phospholipid content is obtained from soybean extract and spermatozoa are not affected by the phospholipase used in other extenders. Paulenz *et al.* (2002) evaluated an extender over time (0, 6, 12, and 24 h), and found that motility remains acceptable (70-80%) for a long period. Diaz *et al.* (2015) also evaluated sperm quality over time (0, 2, 4, 6, and 24 h) with different extenders and found that the best MM is recorded 4 to 24 h after the semen has been refrigerated at 5 °C. Because there is scarce information about the parametric evaluation of buck sperm using the CASA system (Hernández-Corredor *et al.*, 2013), it would be advisable to highlight that this system represents an innovative technology that determines the values of the behavior of spermatozoa stored under fresh, refrigerated, or frozen conditions (Quintero-Moreno *et al.*, 2011). Coupled with an adequate selection of an extender for the conservation of buck semen (Hernández-Corredor *et al.*, 2013), this system is a practical alternative for the conservation of the germplasm that is used in species improvement programs.

CONCLUSIONS

With respect to the MM and MP of spermatozoa from fresh and refrigerated semen, the highest percentage was found in the combination of extenders (Andromed® + HTF). Consequently, Andromed®, in combination with other and by itself serves as an appropriate medium to maintain and improve the characteristics of fresh and refrigerated semen. Regarding sperm quality over time, the Andromed® + HTF combination recorded the highest percentage in mass motility among the three compared extenders. Therefore, we conclude that Andromed® can be combined with HTF to dilute both fresh and refrigerated semen, maintaining an acceptable quality for up to 24 h. However, when using only HTF as an extender, we advise using it only in fresh semen; if it is used to dilute refrigerated semen, the sperm quality will record a considerable fall.

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