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Use of Andromed® and OviXcell® diluents in the processing of sheep semen with the addition of HTF

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ABSTRACT

Objective: To assess the effect of two ovine semen diluents, Andromed® and OviXcell®, on the mass motility, vitality, and acrosomal integrity of spermatozoa processed at different temperatures and with the addition of post-thawed HTF (human tubal fluid).

Design/Methodology/Approach: Semen from 2 Dorper ram was used. Four ejaculations per male were collected and diluted with Andromed® and Ovixcell®. There were four treatments with Ovixcell®: 1) fresh Ovixcell®, 2) refrigerated Ovixcell®, 3) post-thawed Ovixcell®, and 4) post-thawed Ovixcell® + HTF. Four treaments with Andromed® were also carried out: 1) fresh Andromed®, 2) refrigerated Andromed®, 3) post-thawed Andromed®, and 4) post-thawed Andromed® + HTF. Mass motility, vitality, and acrosomal integrity were performed with a CASA computer system and statistically analyzed with the GLM procedure of the SAS software.

Results: When the effect of Ovixcell® mass motility was assessed, the following results were obtained, with differences ($p<0.05$) between treatments: 87% in fresh semen, 72% refrigerated semen, 55% in post-thawed semen, and 68% in post-thawed semen + HTF. A clear difference ($p<0.05$) was observed when HTF was added to post-thawed semen (13%). Andromed® behaved in the same way as Ovixcell® ($p<0.05$) and a 18% recovery was observed with the addition of HTF. A high percentage of live spermatozoa with intact acrosome was observed for fresh semen (97.8%), while it diminished ($p<0.05$) as the temperature of refrigerated and frozen semen gradually decreased.

Study Limitations/Implications: More experimental units should be used, despite the increase in maintenance costs per animal.

Findings/Conclusions: The use of Andromed® and OviXcell® diluents was satisfactory in relation to the mass motility, vitality, and acrosomal integrity in spermatozoa processed at different temperatures (fresh, refrigerated, and post-thawed). Furthermore, the addition of HTF increased post-thawed mass motility.

Keywords: Diluents, Andromed®, OviXcell®, HTF, sheep.

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INTRODUCTION

Semen conservation plays an important role in artificial insemination (AI) programs for sheep; however, its process faces several disadvantages. For example, pregnancy percentage ranges between 30-50% with refrigerated semen, as a consequence of the difficulties related to the sperm motility of the semen used (Yaniz *et al.*, 2005). Similarly, when frozen semen is used, the fertilization capacity of spermatozoa shows a significant decrease. This situation is related to damage to sperm cell caused by the use of diluents, which do not protect the spermatozoa during the preservation (refrigeration and freezing) processes (Canizales, 2013). In addition, the use of inadequate preservation methods caused a low fertilizing capacity of both refrigerated and frozen semen (Aitken and Clarkson, 1988). Therefore, choosing the most appropriate diluent is of great importance.

The addition of diluent is an important step in the semen preservation process, as it provides nutrients and volume to the semen sample (Gadea, 2003). Diluents should preserve and extend sperm life and guarantee an adequate nutrient environment for the spermatozoa, during storage and upon thawing for AI (Allai *et al.*, 2018).

The Andromed® and OviXcell® diluents pose no risk of microbiological contamination, because they are free of animal proteins, which increases fertility rates. These diluents contain soy lecithin, which, given its plant origin, has a greater protective effect during the cooling process (Fukui *et al.*, 2008), consequently improving sperm viability and fertility (Canizales, 2013).

Human Tubal Fluid (HTF) is a product used in the embryo maturation process and is an alternative that, used in conjunction with the diluent, increases semen motility during the cooling and post-thawing process. This product contains compounds such as sodium chloride, potassium chloride, calcium chloride dihydrate, and magnesium sulfate heptahydrate (Quinn *et al.*, 1985), which make it a highly nutrient-rich medium. In addition, HTF contains energy sources such as pyruvate, lactate, and glucose, as well as albumin as the main protein supplement (Gimeno *et al.*, 2006). HTF has been shown to improve embryonic development and gestation rate after *in vitro* fertilization and it can be used to process semen from all kind of domestic animals (Conaghan *et al.*, 1998). Therefore, the objective of this study was to assess the effect of two semen diluents, Andromed® and OviXcell®, on the mass motility, vitality, and acrosomal integrity of spermatozoa processed at different temperatures, with the addition of post-thaw HTF to protect sheep sperm cells.

MATERIALS AND METHODS

Description of the study area

The current study was conducted at the Rancho Universitario of the Universidad Autónoma de Ciudad Juárez, located in Ciudad Juárez, Chihuahua. This region has a predominantly hot desert (BWh) climate, with an average annual temperature of 16° to 18 °C, an average altitude of 1,100 m.a.s.l., and an average annual rainfall of 244 mm (INEGI, 2017).

Management of experimental units

The study was carried out with semen from 2 to 4-year old Dorper rams. During the reproductive season (from November to February), four samples from each male were collected by ejaculation in an artificial vagina. These ejaculates were subjected to four treatments with each diluent (Andromed® and Ovixcell®). The four Ovixcell® treatments were: 1) fresh Ovixcell®, 2) refrigerated Ovixcell®, 3) post-thawed Ovixcell®, and 4) post-thawed Ovixcell® + HTF. Meanwhile, the four Andromed® treaments were: 1) fresh Andromed®, 2) refrigerated Andromed®, 3) post-thawed Andromed®, and 4) post-thawed Andromed® + HTF. Dilution in all treatments was performed 1:5, following the recommendations of the manufacturer. After the diluents were added, mass motility (%), vitality (%), and acrosomal integrity (%) were assessed at the different treatment temperatures (fresh, frozen, and refrigerated). The tests were carried out by means of the CASA (Computer-Assisted Sperm Analysis) procedure (Amann and Wabersky, 2014).

Experimental design

The study lasted two months (November and December) and the experiment consisted of adding two types of nutrient media (diluents) and HTF to the ejaculates. Finally, differences between treatments were assessed for each diluent, based on the mass motility, vitality, and acrosomal integrity variables.

Semen sample collection

Seminal samples were obtained by means of the artificial vagina (Wulster-Radcliffe *et al.*, 2001) and electroejaculation (Morillo *et al.*, 2012) techniques.

Diluted fresh semen

Eight ejaculates from two males were used. One ejaculate was assigned to be diluted with a specific diluent and processed at a specific temperature (fresh, refrigerated, and frozen). Ten replicates were performed for each diluted ejaculate. In total, 80 observations were made among the 4 study treatments for each diluent, as described below.

The four treatments that used the Ovixcell® diluent were: fresh Ovixcell®, refrigerated Ovixcell®, post-thawed Ovixcell®, and post-thawed Ovixcell® + HTF. Meanwhile, 4 treatments used the Andromed® diluent: fresh Andromed®, refrigerated Andromed®, post-thawed Andromed®, and post-thawed Andromed® + HTF.

Semen evaluation

The mass motility, vitality, and acrosomal integrity seminal parameters were assessed for each treatment.

Mass motility: 5 μL of each replicate per treatment were placed on a slide at 35 °C on top of a thermal plate (Clemente *et al.*, 2012), for their observation under the microscope using CASA.

Vitality: To assess the sperm vitality, 5 μL of each replicate per treatment were placed in a counting chamber for their analysis at 35 °C. Subsequently, they were observed using CASA, to determine the number of live spermatozoa.

Acrosomal integrity: The same amount of semen was used to determine sperm viability. This parameter was observed with CASA, in which the acrosomal shape of the spermatozoa was analyzed, checking that it had an oval head, a straight neck, and a long and thin tail without folds (Clemente *et al.*, 2012).

Diluted refrigerated semen

Once the diluted fresh semen was analyzed, it was stored at 4 °C for 2 hours. Afterwards, mass motility, vitality, and acrosomal integrity were assessed, following the same procedure as in the fresh diluted semen.

Post-thawed semen

To assess its characteristics, post-thawed semen was processed after it had been refrigerated at 4 °C for 2 hours. Subsequently, 0.25-mL straws were filled with semen and the freezing process was initiated, following the liquid nitrogen vapour method described by Jerez *et al.* (2016). The straws were placed 7 cm above the level of the liquid nitrogen (LN, −140 °C) for 10 min; they were then directly immersed in the LN (−196 °C) and stored until further analysis. Semen was analyzed 24 hours later, for the post-thaw Ovixcell® and post-thaw Andromed® treatments, thawing the straw by immersion in water at 37 °C for 50 seconds. Similarly, once semen was thawed, HTF was added to both diluents (post-thawed Ovixcell® + HTF and post-thawed Andromed® + HTF), before the assessment of mass motility, vitality, and acrosomal integrity.

Statistical analysis

The mass motility, vitality, and acrosomal integrity variables were analyzed with the GLM procedure in SAS V9 (SAS, 2004), followed by Tukey's multiple comparison test (α=0.05).

RESULTS AND DISCUSSIONS

Mass motility

Mass motility (MM) recorded the following percentages when Ovixcell® was used. with some differences ($p<0.05$) between treatments: 87% (fresh semen), 72% (refrigerated semen), 55% (post-thawed semen), and 68% (post-thawed semen + HTF). Such behavior could be the result of the oxidative stress induced by cryopreservation, which has adverse effects on post-thawed semen quality (Jiménez-Aguilar *et al.*, 2021). Mass motility follows a downwards trend, according to the gradual decline in temperature (changing from fresh to refrigerated semen and subsequently frozen). A 13% increase in MM was also observed when HTF was added to post-frozen semen (Figure 1). For their part, Mata-Campuzano *et al.* (2015) mention that, at the time of cryopreservation, spermatozoa are exposed to physical and chemical impacts that hinder viability, decrease motility, damage the acrosome, and decrease fertility.

Results from the assessment of the effect of the Andromed® diluent on the quality of fresh, refrigerated, and post-thawed semen and of Andromed® + HTF on the quality of

post-thawed fresh sperm indicate 92, 69, 53, and 71% MM, respectively, with differences ($p<0.05$) between fresh, refrigerated, and post-thawed semen.

Andromed® evidently induces excellent motility levels in fresh semen, although this characteristic diminishes considerably as the storage temperature decreases (refrigerated and frozen); however, an 18% increase in MM was observed when HTF was added at the thawing stage (Figure 1). Berkovich *et al.* (2013) mention that the addition of antioxidants to diluents during semen freezing improves post-thawing characteristics, since they protect the polyunsaturated fatty acids of the spermatozoa, consequently preventing oxidative stress damage.

Finally, during the assessment of differences between diluents, both Andromed® and Ovixcell® show the same effect on mass motility, at different storage temperatures and despite the addition of HTF ($p<0.05$).

Ovixcell® maintained the motility characteristics of spermatozoa that were processed in fresh, refrigerated, and frozen conditions, as well as their quality characteristics for AI. Combining the diluent with HTF improved MM; this result matches the findings of Khalifa and Lymberopoulos (2013), who used the Ovixcell® diluent and observed a higher sperm motility than with the use of an egg yolk and milk-based diluent.

This result may be caused by the combination of Ovixcell® and HTF; after semen has been subjected to cold temperatures, it maintains high motility, as a result of the large amount of nutrients that these two substances provide together. Values above 80% for MM in semen refrigerated at 4 °C are considered excellent; therefore, the results of the current study indicate that the assessed treatments meet the requirements for their use as diluents and, to a greater extent, for the use of the Ovixcell® + HTF combination.

When artificial insemination is performed, semen should have many live spermatozoa with high motility percentages (>50%), ensuring high pregnancy rates. According to the results of the current study, both diluents can be used in any of the treatments tested to process semen at refrigeration temperatures (4 °C).

The use of Andromed® + HTF improved the motility of post-thawed spermatozoa (71%); in comparison, the MM of spermatozoa post-thawed only recorded 53% motility ($p<0.05$) with Andromed®.

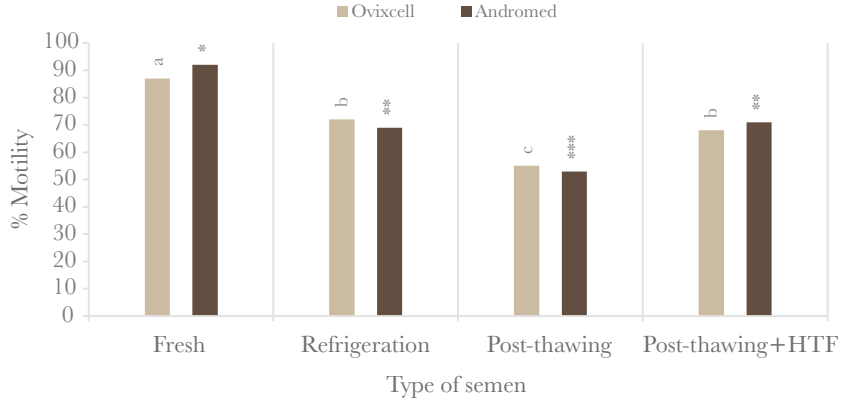

Figure 1. Mass motility (MM) of Dorper sheep spermatozoa processed at different temperatures (fresh, refrigerated, and post-thawed), with 2 diluents and post-thawed HTF.

Perhaps once semen has been subjected to cold temperatures with the dilution of Andromed® and HTF, it maintains a high motility given the large amount of nutrients provided by the combination of these two substances. El-Keraby *et al.* (2010) and Singh *et al.* (2012) determined that using Andromed® diluent increased the sperm motility of sheep semen stored at 4 °C (>5%). The effect of HTF on sperm cells is directly based on sperm capacitation and on changes in reactive oxygen species (ROS) levels, which have been studied in humans, but not yet in domestic species (Shih *et al.*, 2016; Hernández *et al.*, 2021).

According to Hernandez *et al.* (2014), the use of animal protein-free diluents (*e.g.*, Andromed®) show a better response, since their phospholipid content is derived from soybean extract and spermatozoids are not affected by the phospholipase used in other diluents.

Acrosome vitality and integrity

With the Andromed® diluent, a high percentage of live spermatozoa with intact acrosome was observed in fresh semen (97.8%), as well as a reduction ($p<0.05$) as temperature gradually increased (refrigerated and frozen semen). As for spermatozoa without acrosome, no differences were found between treatments ($p>0.05$), although differences in the percentage of dead spermatozoa were recorded ($p<0.05$).

The use of Andromed® preserved the motility, vitality, and acrosomal integrity of spermatozoa processed at different storage temperatures (fresh, refrigerated, and frozen), either alone or combined with HTF. These results match the findings of Arifiantini and Yusuf (2010), who reported viability values of 84.2% when semen was diluted with Andromed®. In this sense, Velez *et al.* (2014) asserted that the average (normal) vitality is >60%; therefore, the results found in this study are quite satisfactory in terms of vitality.

Table 1. Vitality and acrosomal integrity of Dorper sheep spermatozoa processed at different temperatures (fresh, refrigerated, and post-thawed), with Andromed® diluent and post-thawed HTF.

Treatment	Vitality and acrosome classification		
	Lsia (%)	Lswa (%)	Dead sperm (%)
Fresh semen	97.8a	1.3a	0.9a
Refrigerated semen	88.8b	0.7a	10.5b
Post-thawed semen	85.6b	1.1a	13.3b
Post-thawed + HFT	87.1b	1.3a	11.6b

Lsia: live sperm intact acrosome; Lswa: live sperm without acrosome. a,b Different literals between columns are different (Tukey; $\alpha=0.05$).

CONCLUSIONS

Similar efficient performance regarding the mass motility, vitality, and acrosomal integrity of sperm processed at different temperatures (fresh, refrigerated, and post-thawed) was observed with the use of the Andromed® and OviXcell® diluents. Finally, adding HTF to both diluents increased post-thawed mass motility with respect to post-thawed sperm motility.

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