

STUDY REGARDING EFFECT OF TRISS-BASED AND CANIPLUS EXTENDERS ON SEVERAL SPERM PARAMETERS IN MEDIUM-LARGE BREED OF DOGS

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Abstract

The study was carried out in different breeds of dogs owned by breeders in the city of Cluj Napoca. Mostly medium-large breeds were used and all the participating animals have been in good health during the time of acquiring the samples. The purpose of the study was to make a comparison between our own Tris-based extender and the commercial extender CANIPLUS CHILL in accordance with semen parameters with particular regards to motility, progressive motility, and length of survival of the spermatozoa. The evaluation has been done using the Computer Aided Sperm Analysis (CASA) system at the Faculty of Veterinary Medicine of Cluj-Napoca.

We have found that in medium large size breeds the commercia CaniPlus extender have shown better results on the majority of the parameters over the Tris-based extender and has the ability to preserve the integrity is spermatozoa more efficiently over time compared to Tris extender. Furthermore we identified an improvement in all parameters when comparing samples on the day of collection between large breed dogs and medium-large breed dogs in both extender types.

Additionally, we had results shown increase values of most parameter in tris extender when comparing it with CaniPlus extender in medium-large size breeds on the day of collection.

Key words: Semen Extender, canine, Artificial Insemination, CASA, CANIPLUS CHILL, TRIS

Introduction

AI is widely applied to a wide range of species. Furthermore, AI needs fresh or well-preserved semen, and the majority of AI is accomplished using preserved semen. (Raheja N. *et al.*, 2018; Malik A., *et al.*, 2018) Thus, an optimum medium is needed to maintain its adequate quality. Accordingly, it is necessary to develop and evaluate semen extenders used to preserve semen during chilling or cryopreservation. Semen extenders were discovered and developed to protect sperm from harmful factors such as cold and osmotic shock, oxidative stress (Mousavi S.M., *et al.*, 2019), and cell injury by ice crystals. Semen extenders preserve sperm by stabilizing its properties, including sperm morphology, motility, viability, and membrane, acrosomal, and DNA integrity. Semen extenders need to have a favorable pH (Liu C.H., *et al.*, 2016), provide energy (Mohamed M.Y., *et al.*, 2019); adenosine triphosphate, anti-cooling and anti-

freeze shock properties (Amirat-Briand L., *et al.*, 2010; Tariq, A. *et al.*, 2020) and antioxidant activity to keep the quality of the sperm high enough for fertilization.

In the initial stages of semen preservation, early formulations encompassed uncomplicated solutions such as milk (Filho, I.C.B., *et al.*, 2018), saline, or egg yolk (Chaudhari D.V., *et al.*, 2015), which offered a degree of protection but yielded restricted efficacy. (Layek S.S., *et al.*, 2021) Over the course of time, researchers have made improvements to these compositions by integrating a range of additives, antioxidants, cryoprotectants (Johnston, S.D., *et al.*, 2012), and antibiotics in order to augment the viability and reproductive capacity of sperm (Schulze *et al.*, 2020).

Tris(tris(hydroxymethyl)aminomethane) exhibited enhanced buffering capacity, thereby effectively sustaining the requisite pH levels

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conducive to the viability of sperm. This significant advancement resulted in enhanced semen preservation techniques, enabling the successful transportation of sperm samples over long distances (Bustani & Baiee, 2021).

Today, semen extenders are an irreplaceable instrument in modern reproduction and have highly sophisticated formulations, tailored to the specific needs of different animal species (Alm-Kristiansen & Dalen, 2018). They have been extensively studied and optimized for factors such as osmolality, pH regulation, energy sources, and antimicrobial properties. Cryopreservation techniques have improved, and the addition of cryoprotectants like glycerol or dimethyl sulfoxide (DMSO) has made semen extenders even better for long-term storage and artificial insemination (Watson P.F., 2000)

The compatibility of semen extenders may vary among different animal species and between individuals of the same species, with some showing enhanced responses when exposed to extenders containing animal proteins, while others may demonstrate comparable performance (Bencharif D., *et al*, 2012) The cost and availability of semen extenders can also vary, depending on the brand and geographical proximity. In summary, the selection between CaniPlus and TRISS depends on factors such as the specific requirements of sperm cells, the intended species, scientific substantiation, cost-effectiveness, and accessibility of the extenders.

MATERIAL AND METHOD

This study compares two semen extenders, CaniPlus chill - a commercially available product containing vegetable proteins, and TRISS - an extender containing animal proteins. The composition and ingredients of each product are crucial when analyzing their properties and characteristics. CaniPlus chill is a commercially available semen extender that incorporates vegetable proteins, while TRISS includes animal proteins, which are typically obtained from egg yolk or milk-derived products.

The method used to analyze the semen samples was through Computer Cssisted Sperm Analysis (CASA) that allows wide range of function and programs that allow detailed analysis of all the important parameters of sperm.

The samples were collected by manual stimulation from 16 dogs, all in good state, clinically healthy and fully mature between the age

of 2 and 7 years old. The breeds that have taken place in the research are 1 Bull Terrier, 5 Tibetan Mastiff, 2 Cane Corso, 1 Rottweiler and 7 Central Asian Shepherds.

The semen samples were collected in special "sperm friendly" tubes, which was pre-heated by friction to reduce the shock on spermatozoa by the abrupt change in temperature. The sample collected was split in 2 equal parts. Next, they were diluted separately with each type of extender.

Dilution for both extenders was performed at 1:3 parts, 1 part being the semen sample and 3 parts the extender, additionally the dilution was made at the same temperature- 38° C (the extenders were preheated to this temperature in a marine bath). The dilution was chosen due to the official recommendation of the producer, and it was done the same for both extenders used. After dilution, the sampling tubes with each extender were differentiated and placed in a water-bath at 37° C, slides were heated to 37°C as well. With the help of micropipette, a drop of the sample was placed on the pre-heated slide and examined to the microscope that is connected to the CASA system.

RESULTS AND DISCUSSIONS

The results obtained by comparison of the effect of the Triss -based extender and CaniPlus Chill extender on the time of collection show overall improvements by the commercial CaniPlus Chill extender in all parameters of motility, progressive motility, non-progressive motility, and total immotile spermatozoa.

While the values concerning concentration, motility have shown small difference between the two extenders used, differences were registered in the progressive motility, non-progressive motility, and immobile cells.

The average results for the medium-large breeds, after 24h from of collection showed a difference for the two extenders with the motility of the spermatozoa being more noticeable, and for the other parameters studied maintaining their values in the CaniPlus extender compared to the Triss -based extender.

In the evaluation of progressive motility for medium large breeds, on the day of collection, we registered a difference: at CaniPlus extender we registered a progressive motility with 15.29% higher compared with the one diluted with Triss based extender. For non-progressive motility the difference

registered was minimal, the CaniPlus showing a value with only 0.51% higher compared to Triss extender and for the parameter of mobility there was a difference of 16.00% in favor of CaniPlus extender.

The results of rapid velocity registered a value of 13.48% for Triss -based extender and a value of 29.38% for the CaniPlus, thus a 15.89% difference. For medium velocity we have a value of 12.51% for Triss -based extender and a value of 11.73% for the CaniPlus, thus a 0.77% difference. For slow velocity we have a value of 28.23% for Triss -based extender and a value of 29.11% for the CaniPlus thus a 0.88% difference.

The results in percentage after 24h hours of collection in medium large breeds registered for progressive motility a difference of 6.54% in favor of the CaniPlus extender and for non-progressive motility, we have a 15.40% difference in favor of CaniPlus extender. For motility we observed a difference of 21.77% with Triss having the lower motility value.

The value registered for rapid velocity at 24 hours after collection were 6.53% for Triss -based extender and 15.04% for the CaniPlus. For medium velocity similar values were registered for both extenders used (7.8%). For slow velocity there is a difference of 13.32% for the CaniPlus extender (26.66% vs 13.32%).

An experimental sample for the comparison of the two types of extenders in the day of the collection, 72 hours after and on day five was made to see the progress further than 24 hours.

For the Triss -Based extender we observed the following: progressive motility was reduced from 22% from day 1 to 11.66% after 72 hours. Non-progressive motility went from 47.4% on day 1 to 30.04% in 72 hours. Motility decreased from 70.38% on day 1 to 41.7% after 72 hours. Rapid velocity was reduced from 11.31% - day 1, to 10.09% after 72 hours. Medium velocity was reduced from 13.82% to 4.48% after 72 hours. Slow velocity was reduced from 45.24% to 27.13%. Finally, immotile spermatozoa where increased from 29.62% to 58.3% after 72 hours. On day 5 there was no motility identified and as such it was considered 100% mortality of the spermatozoa.

For the CaniPlus extender the following values were obtained: Progressive motility decreased from day 1 to 72 hours to day 5 from 44.01%, to 16.63% and 2.3% respectively. Non-progressive motility had a decrease from day 1 to 72 hours and day 5 from 36.09%, to 34.03% to 25.96% respectively. Motility decreased from 80.1% on day 1 to 50.67% after 72 hours and further decrease of 18.26% on day 5. Immotile spermatozoa increased from day 1 to 72h and 5 days after collection from 19.9% to 49.33% and 81.74% respectively. Rapid velocity decreased from day 1 to 72 hours to day 5 from 41.99% to 14.72% and 1.42% respectively. Medium velocity was increased from 4.48% on day 1 to 12.24% after 72 hours and decreased to 1.24% after 5 days. Slow velocity was decreased from 1 day on to 72 hours and day 5 from 33.73% to 30.98% and 15.6% respectively.

CONCLUSIONS

Results on the comparison on the effect of CaniPlus Chill extender and Triss extender day 1 and 24 hours.

In the group of medium-large size breeds on day of collection we have satisfactory results in the samples with the CaniPlus extender in all fields with an improve of 15.29% on progressive motility, which is one of the most important factors taken in consideration in semen evaluation.

A minimal difference was noted for non-progressive motility with a 0.51% in favor of CaniPlus and finally a difference of 16.002% in the parameters of motility and immobility.

After 24 hours the re-evaluation revealed the superiority of CaniPlus in all fields researched. A reduced difference is showed for the progressive motility with a difference of 6.54%, while the difference increased in non-progressive motility and motility/immobility with values 15.40% and 21.77% respectively, all the values in the advantage of CaniPlus over Triss based extender.

Overall CaniPlus has given improved result over Triss-based on day one and 24 hours later with the value of overall motility having the greatest significance in values with 18.89% overall improvement, followed by overall progressive motility of 10.91% and

finally 7.96% overall improvement in non-progressive motility.

For the sample evaluated on the day of collection, 72 hours and 5 days later the results showed at 72 hours an insignificant percentage loss of 1.32% in progressive motility in favor of CaniPlus, a higher improvement by a difference of loss of percentage 12.68% by CaniPlus and a 13.02% difference of loss of percentage in the parameter of motility in favor of the CaniPlus showing a moderate overall improvement over the Triss-based extender.

Loss of progressive motility at 72 hours to day 5 for the CaniPlus was 14.33%, for non-progressive motility was 8.07% and for motility was 13.02%. The Triss extender sample showed no viability after 5 days, thus we considered to be inefficient in this sample.

This fairly constant improvement between the 2 extenders could indicate a significance in the individual, genetic or environmental influence since it contradicts the results of comparison on the day of collection by the medium-large size breeds between the 2 extenders.

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