DEVELOPMENT OF RESEARCH IN METHODOLOGIES THAT IMPROVE CAPACITY OF PRESERVED CANINE SEMEN

Preliminary results

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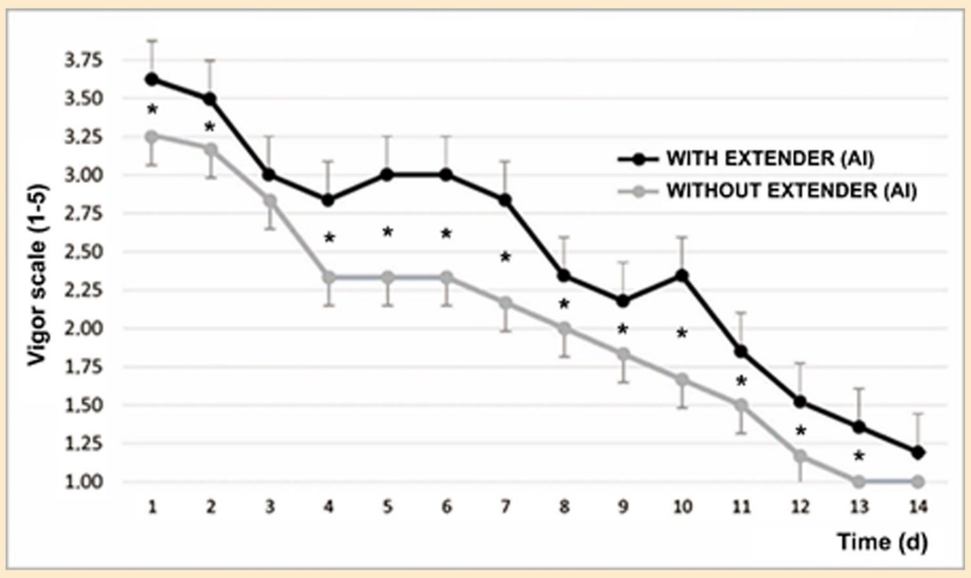
INTRODUCTION

During the storage process of semen, for limited the damages caused by drop temperature, and to provide energy, maintain pH and osmolarity, reduce oxidation, preserve plasma, acrosomal and mitocondrial membrane integrity, etc., an appropriate diluent must be used (extender) with sperm [1]. Storage of refrigerated semen at 4-8 °C induces a transition in the sperm plasma membrane from the cooled crystalline to the gel phase. Use of supplementation of activators of insemination (SA) whose basic composition is formulated by easily metabolized carbohydrates that provide the mitochondria of the spermatic neck with a fast energy substrate to maximize their metabolism [2-3]. The objective of this experiment was to evaluate the motility and survival of the spermatozoa under protocol with refrigeration for 14 days, corroborating the effect of SA during the whole process.

MATERIALS AND METHODS

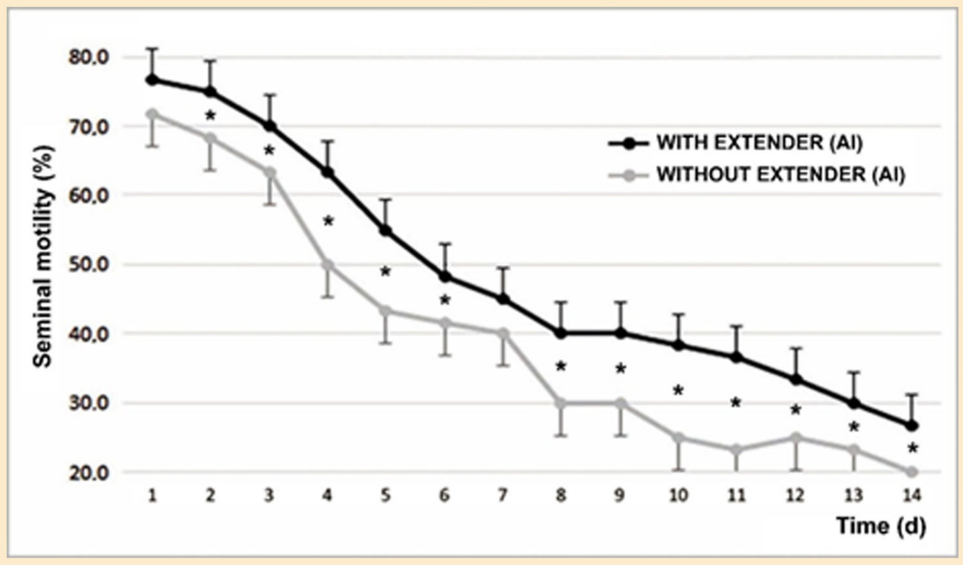
Six Napolitan Mastiff male dog with an average weight 88 ± 12 kg (MED ± SEM) and age 30.5 ± 3.5 months respectively. Confirmed healthy based on history, clinical examination including full andrological evaluation and ultrasound examination of the prostate and testis. Semen was manually collected and promptly examined the volume of the sperm rich fraction, pH, sperm morphology, vitality and total sperm concentration. Two aliquots pre-tempered at 37 ºC with and without SA, were evaluated in motility (%) and vigor (1-5) at different times. Farelly staining was used for morphology and for membrane stability Hyposmolarity Test (Simplified Host –Host-s-) [4]. The individual semen samples were refrigerated at 4 ºC and evaluated every 24 hours for 14 days. The data were analyzed by ANOVA and T-test using SPSS® 21.0 ($p \leq 0.05$).

RESULTS and DISCUSSION



Graph Nº 1 - Variation of seminal vigor after cooling and timing[1]. Mean ± SEM * P <0.05
[1]The graphed evaluations correspond to 1 minute after adding the SA

Graph. Nº 2 - Motility with and without the effect of seminal activator during the 14 d of the evaluation of refrigerated semen[1]. Mean ± SEM * P <0.05
[1]The graphed evaluations correspond to 1 minute after adding the SA.



The volume of the second fraction of the ejaculate was 3.9 ± 1.6 mL with a pH of 6.2 ± 2.8. The sperm concentration was 417.3 ± 170.4 million sperm per mL. The initial seminal motility was 85.83 ± 5.23%. After one minute of AS, motility increased to 89.92 ± 5.18% (P <0.05).

After 14 days of refrigeration, with a practically linear decrease, a 19.47% lower value was found, with an average reduction of 1.5%/day, according to the Farelly staining analysis method. It should be noted that distinguishing between the affected area, tail changes increase by 49%, while in the intermediate part they increase by 122% and finally, problems in the sperm head grow by 216% (P<0.001). Regarding the stability of the membrane, at d0 85.89 ± 4.76% of spermatozoa were found to be normal, subtracting 14.11 ± 4.76% of those with permeability integrity defects. To analyze the changes in membrane stability and sperm morphology, the times were divided into three periods, according to the viability of the seminal extensors that are normally divided into short, medium and long-term. Thus, T1 (short term) is used for the period between d0 and d5, T2 (medium term) for the period between d6 and d10 and finally T3 (long term) between d11 and d14 (Table Nº 1). This technique of chilling preservation is a promising alternative to conventional semen cryopreservation, and is easily adapted for clinical use. It is particularly useful for the shipment of semen where the cost of procedures and materials are high [5]. Thus semen could be collected a few days after the detection of pro-estrus in bitch and then processed, transported and stored at the location of its intended use. During our evaluation, in 14 d, either the partial results of the use of chilling extender using motility and vigor as indicators of vitality and Host-s as a test of plasma membrane stability, showed very promising results. In the 14 days of evaluation, the morphologically normal sperm barely reached 70 % at d10, reaching 65% at d14. Our results showed a stability up to d5 of 85%, then it decreased almost linearly until d14 reaching 65%, decreasing approximately 2% per day.

CONCLUSIONS

The use of SA extender enhancers significantly improves the quality parameters at any time, after tempering the refrigerated sample, which would improve the fertility and prolificacy results of the obtained litters.

	T1 (%)	CV (%)	T2 (%)	CV (%)	T3 (%)	CV (%)
Normal Morphology[1]	81,97 ± 0,89[a]	2,67	72,62 ± 1,06[b]	3,28	67,62 ± 0,94[c]	2,78
Membrane Stability[2]	85,43 ± 0,13[d]	0,38	79,71 ± 1,45[e]	4,06	69,40 ± 1,81[f]	5,22

Table Nº 1 - Comparison between sperm morphology and membrane stability test with distinction of storage times by refrigeration. **T1** = d0-d5; **T2** = d6-d10; **T3** = d11-d14.
[1] Refers to Farelly's Vital Staining subjective assessment.
[2] Corresponds to normal sperm according to Host-s. Different letters in the same row p <0.001

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