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To cite this version:

Alessandra Paganotto, Camille Langlade, Emilie Rosset, S. Buff. Dispelling myths on external factors affecting post-thawing motility of canine spermatozoa: a retrospective study on 1242 samples. 9th International Symposium on Canine and Feline Reproduction, ICAR 2022 Satellite & 24th EVSSAR Congress, Jun 2022, Milano, Italy. hal-03720243

HAL Id: hal-03720243

https://vetagro-sup.hal.science/hal-03720243

Submitted on 15 Jul 2022

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Dispelling myths on external factors affecting post-thawing motility of canine spermatozoa: a retrospective study on 1242 samples

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Introduction

Although the characteristics of canine ejaculates have been the subject of a number of studies, the literature on the **influence of donor traits and external factors on post-thawing outcome** remains scarce [1], in particular regarding the possibility of **repeating semen collection** twice in the same day. The purpose of this retrospective study was to analyse characteristics influencing post-thawing motility.

Material and methods

This analysis is based on 1,242 ejaculates frozen at VetAgro Sup between 1995 and 2021. Records included:

DONOR TRAITS AND EXTERNAL PARAMETERS

- Spermatozoa **concentration**, **motility** and **morphology** evaluated at the time of collection and 4 weeks after, **age** and dog **weight**, straw **size**, whether the collection was the **first or second** of the day and **season**.
- Depending on spermatozoa concentration, the ejaculate was divided into 0,25 or 0,5 mL straws.
- All samples were cryopreserved with a tris-egg yolk and 6% glycerol extender and stored following the same protocol [2]. Thawing control was carried out by placing the straw in a water bath (37°C) for 45 s and motility was analysed after 5 min by at least 2 different clinicians.

STATISTICAL ANALYSIS

- The influence of external parameters on motility loss was evaluated with ANOVA and Pearson's r for group differences and variable relationships respectively.

Data Analysis

The number of collections performed on the same day was only found significantly correlated with the **number of spermatozoa** ($p<0.001$), with a lower presence for the second ejaculate [See Figure 1]. However:

A. the collection repetition was not significantly correlated with post-thawing motility loss. In fact, motility loss did not differ among any of the analysed donor-related and external parameters, ie. age, weight, season, straw size

B. Among the most represented races (N>30), the ones with significatively higher motility loss were the Leonberger and the Great Dane.

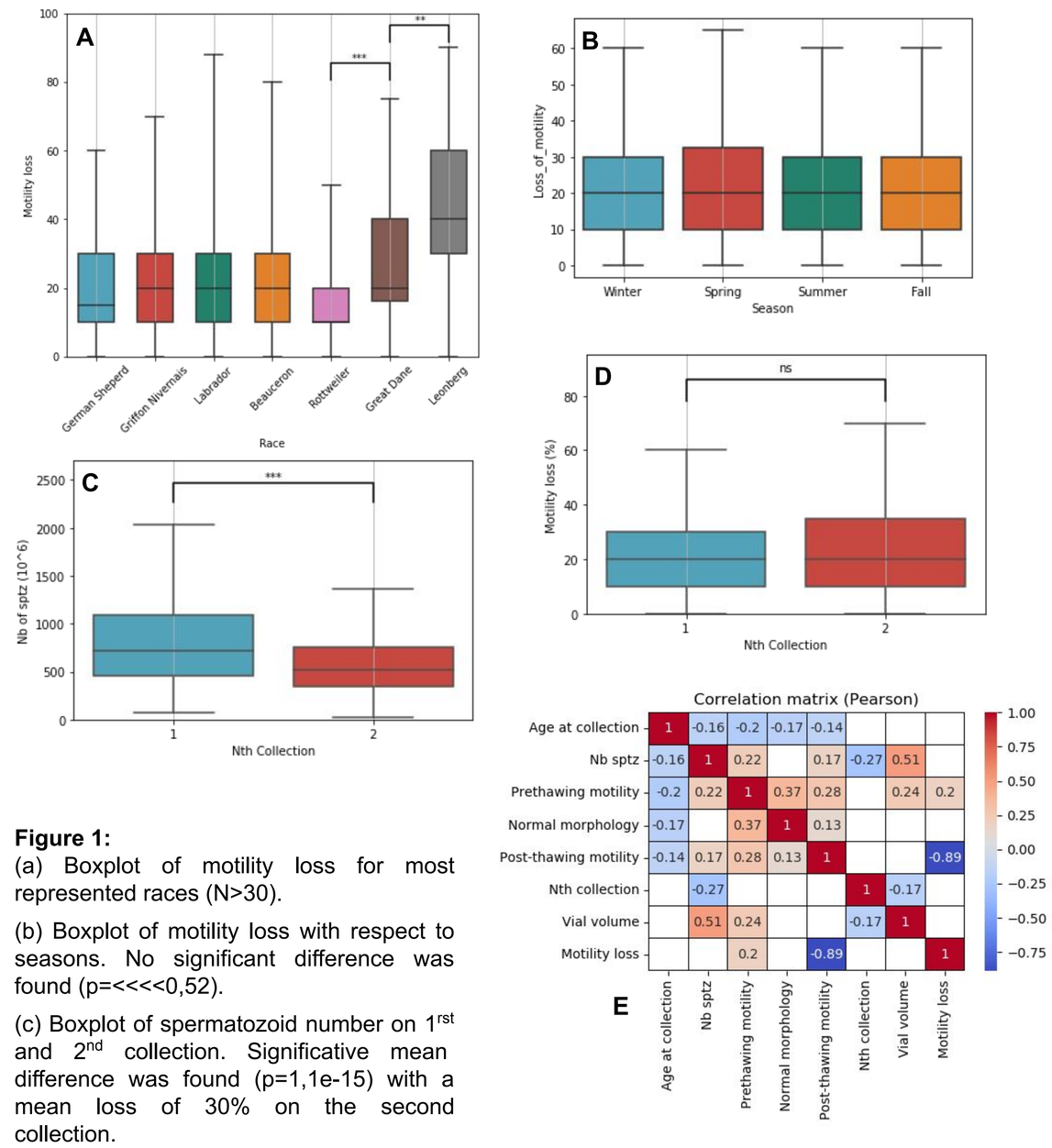

Figure 1:
(a) Boxplot of motility loss for most represented races (N>30).

(b) Boxplot of motility loss with respect to seasons. No significant difference was found (p=<<<<0,52).

(c) Boxplot of spermatozoid number on 1rst and 2nd collection. Significative mean difference was found (p=1,1e-15) with a mean loss of 30% on the second collection.

(d) Boxplot of motility loss on 1rst and 2nd collection. No significant difference was found (p=0,39).

(e) Pearson's correlation coefficients (p-value < 0,005) between pairwise variables.

*** p-value < 0,0005. ** p-value < 0,005

DISCUSSION

Recent work on ejaculates collected at short intervals for one cryopreservation process noted that their combination allows a growth in quantity without compromising semen quality [3].

We further observed in our study that the **second ejaculate** can also be utilised independently **without a significant change in post-thawing motility**. In addition, we found that neither age, time of the year, dog's weight, straw size or sperm morphology influenced post-thawing motility loss. Semen cryopreservation in dogs can therefore be performed successfully without these parameters' influence on the outcome.

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European Veterinary Society For Small Animal Reproduction
XXIV International Congress – Milan (IT) – June 30th to July 2nd, 2022