

Some factors influencing canine sperm motility

Fatores que influenciam a motilidade espermática canina

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Abstract

This study aimed to evaluate whether sperm motility (MOT) in dog semen is influenced by dog age, breed, or number of sperm collections. The study group consisted of four Shih Tzus and five Border Collies, aged 1–8 years. Sperm was collected from each dog once every seven days, for a total of six weeks. Sperm motility did not differ ($P > 0.05$) based on age and breed. Mean MOT at the first collection ($67.4\% \pm 6.9\%$) was lower ($P < 0.05$) than that at the third, fifth, and sixth collections (86.2% ; 88.9% ; $89.1\% \pm 6.9\%$, respectively). Increased MOT was observed after the third collection, demonstrating a positive correlation with the number of sperm collections and sperm motility ($P < 0.05$). Accordingly, our results indicate that sperm processing efficiency is increased after the collection of three ejaculates.

Keywords: Breed. Age. Number of collections. Sperm.

Resumo

Este estudo teve como objetivo avaliar se a motilidade espermática (MS) é alterada em relação a idade do animal, ao número de coletas seminais e as raças de cães. Foram utilizados nove cães com 1 a 8 anos de idade (quatro da raça Shih Tzu e cinco da raça Border Collie), sendo realizadas 6 coletas de cada reprodutor, com intervalo de 7 dias entre as coletas. A motilidade espermática não apresentou diferença estatística em relação a idade e raça dos animais ($P > 0,05$). A média da MS na 1ª coleta ($67,4 \pm 6,9\%$) foi inferior a 3ª, 5ª, e 6ª. ($86,2$; $88,9$; $89,1 \pm 6,9\%$, respectivamente) ($P < 0,05$). Um aumento na MS



foi identificado após a 3ª. rotina, apresentando correlação positiva ($P < 0,04$) em relação ao número de coletas. Pode-se verificar que o número de coletas de ejaculados influencia a motilidade espermática de cães, sugerindo que para um melhor aproveitamento do ejaculado, o mesmo seja utilizado a partir da terceira coleta.

Palavras-chave: Raça. Idade. Número de coletas. Sêmen.

Introduction

International trade in dog semen and the establishment of genetic studies of dogs have increased interest in the development of new technologies for assisted reproduction for dogs (Farstad, 1996; Linde-Forsberg, 2001). As biotechnology research related to dog sperm processing increases, it represents a potentially broad research field that still needs to be explored (De Los Reyes, 2004).

Breeders select individuals in order to produce a line with desired genetic and reproductive traits. One important reproductive trait to be considered is a dog's production of fertile sperm. Quality evaluation of sperm, therefore, must be extremely reliable to prevent negative consequences for reproductive performance (Rodriguez-Martinez et al., 1997). The use of a particular animal is optimized when its reproductive merit is expressed by a high sperm quality (Saacke, 2006).

Compared to other species, dogs exhibit certain peculiarities with regard to variations in female reproductive cycle and male reproductive patterns. Therefore, applied research is still necessary to clarify a few aspects related to dog reproductive physiology (Gavrilovic et al., 2008).

Sperm motility is one of the most important parameters analyzed in a breeding soundness evaluation because it reflects the ability of sperm to migrate through the female reproductive tract (Bukowska et al., 2011). Sperm motility is also an essential parameter to consider when evaluating the efficiency of extenders, cryoprotectants, and freezing protocols (Rijsselaere et al., 2005).

The objective of this study was to evaluate the effects of age, breed, and number of dog semen collection on sperm motility.

Material and methods

Animals/Samples

The study subjects consisted of nine dogs between the ages of 1 and 8 years old (four Shih Tzus and five Border Collies), which were maintained under adequate feeding and housing conditions. Each dog was submitted to six semen collections at 7-day intervals, totaling 54 collections.

The dogs were stimulated by a vaginal swab collected from a bitch in estrus. Semen samples were collected by penis massage (Ortega-Pacheco et al., 2006). Only the second portion of the ejaculates (the spermatozoa-rich fraction) was used for processing. The project was approved by the Animal Experimentation Ethics Committee of the University Federal of Pelotas (UFPel) (number 1946).

Ejaculate evaluation

Sperm motility (SM) was analyzed using a binocular phase-contrast microscope at 200× magnification, using a heated (37 °C) slide with cover slip (Christiansen, 1986).

Statistical analysis

After checking for normality using the Shapiro-Wilk test, sperm motility was compared across ages, breeds, and number of sperm collections through analysis of variance with repeated measures. Comparisons of means were performed using the Tukey test. Correlations among the responses of interest were determined using the Pearson coefficient. All analyses were performed in Statistix 9.

Results

Sperm motility did not significantly differ ($P > 0.05$) for dogs at distinct ages (Table 1).

Sperm motility was significantly lower in the first collection than in the third, fifth, and sixth collections ($P < 0.05$; Table 2). Motility in the second and the fourth collections did not significantly differ ($P > 0.05$; Table 2).

No significant difference in sperm motility ($P > 0.05$) was observed between breeds: $82.0\% \pm 11.4\%$ for Shih Tzus and $79.4\% \pm 17.6\%$ for

Border Collies. The latter group exhibited wide variation in sperm motility (20–90%).

Sperm motility was positively correlated ($P < 0.04$) with age ($r = 0.31$) and the number of semen collections performed ($r = 0.43$).

Discussion

Older dogs appeared to have improved motility over time, based on the positive correlation between age and sperm motility. However, dogs

Table 1 - Age \times sperm motility (means \pm SD) obtained from sperm-rich fractions collected by penis massage

Age (years)	Motility (%)
1	79.9% \pm 6.1%
3	83.8% \pm 6.1%
4	73.0% \pm 6.1%
5	85.9% \pm 6.1%
6	72.8% \pm 6.1%
8	85.2% \pm 6.1%

Table 2 - Collection number \times sperm motility (Means \pm SEM) obtained from sperm rich fraction collected by penis massage

Collection	Motility (%)
1 st	67.4 \pm 6.9% ^b
2 nd	72.3 \pm 6.9% ^{ab}
3 rd	86.2 \pm 6.9% ^a
4 th	76.7 \pm 6.9% ^{ab}
5 th	88.9 \pm 6.9% ^a
6 th	89.1 \pm 6.9% ^a

Note: ^{a,b} Different superscript letters indicate statistically significant differences among the values in the column.

more than 8 years old may produce semen that once cryopreserved, results in a lower fertility rate (Thomassen et al., 2006). In addition, Foote (1978) reported a decline in semen quality in older males of other species such as cattle, sheep, pigs, and horses. In humans, decreased sperm motility is one of the most remarkable findings observed in ejaculates of aged donors (Sloter et al., 2006). Furthermore, higher rate of nuclear degeneration in human spermatozoa is observed in men older than 41 years (Silva et al., 2012). The maintenance of acceptable sperm motility for a relatively long time (8 years) in dogs has been suggested to be attributed to the conditions related to reduced semen quality, such as decreased testosterone secretion and the occurrence of testicular tumors, which generally affect animals older than 8 years (Lowseth et al., 1990; Peters et al., 2000).

That sperm motility was improved after three collections may be relevant when in vitro tests are used for the evaluation of breeding soundness. Therefore, after a single semen collection, the results of analyses of dog semen quality may be biased, labeling sound breeders as sub fertile, especially after a period of sexual rest. Our results corroborate the findings of another study (Boucher et al., 1958) that observed an increase in sperm motility with increased frequency of collections. However, that study did not indicate a minimum number of semen collections prior to the achievement of a sample with acceptable sperm quality, and it did not address the fact that an excessive number of collections may deplete sperm reserves, resulting in the reduction of spermatozoa concentration in subsequent collections (England et al., 1999). Unfamiliar environments and lack of sexual experience may also affect the libido of the dog and its sperm quality (Olar et al., 1983). Thus, it would be important to ensure at least three sperm collections in order to obtain a reliable estimate of dog semen quality.

The lack of effect of breed on sperm motility may result from the large variation observed among the breeds evaluated in the present study, especially in the case of Border Collies. We recommend the establishment of specific standards for sperm quality for each breed, because a single standard for all breeds might be biased and unreliable. The sperm quality of a given dog must not be

evaluated according to standards established for other breeds (Rota et al., 2005; Dunbar, 1975). Several studies (Dunbar, 1975; Dahlbon et al., 1997; Thurston et al., 2001; Rota et al., 2005) have observed differences not only between breeds but also among individuals.

In conclusion, dog sperm motility is only associated with the number of collections, with no significant effect observed in relation to the dog's age and breed.

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