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THE EFFECT OF CRUDE GLYCEROL ON CANINE SPERMATOZOA MOTILITY

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Abstract
The target of this study was to analyse the effect of different crude glycerol concentrations: GA – 0.11 ml.ml^{-1}, GB – 0.027 ml.ml^{-1}, GC – 0.0069 ml.ml^{-1}, GD – 0.0017 ml.ml^{-1} on the canine spermatozoa motility parameters after an *in vitro* cultivation at 5°C. Semen samples diluted with physiological solution were used as the control. Individual motility parameters were recorded at five time periods: 0, 1, 2, 3, 4 hours and evaluated using Computer Assisted Semen Analyzer (CASA) system. A significantly lower ($p<0.001$) values of spermatozoa motility were detected at the beginning of cultivation only in the samples GA. At times of 1, 2, 3, 4 significant decrease ($p<0.001$) of spermatozoa motility was observed in all experimental groups compared to the control. Spermatozoa progressive motility followed the tendency of spermatozoa motility. Progressive motility in the control sample ranged between 68.80 and 81.46%. In the experimental groups, progressive motility ranged from 0 to 72.39%. The parameter of velocity curved line showed significantly ($P<0.001$) decreasing values between experimental samples and the control ($P<0.001$) from time 1 to the end of cultivation to. Results of this work clearly describe the negative effect of high glycerol concentrations on canine spermatozoa motility parameters.

Key words: dog, spermatozoa, motility, CASA, glycerol

Introduction

Glycerol ($CH_3H_8O_3$), a highly permeable polyhydric alcohol, is the cryoprotector most frequently used in semen freezing in different species (*Silva et al.*, 2003). However, this cryoprotector exhibits toxic effects on spermatozoa, such as physicochemical alterations that can lead to rupture of the plasma membrane or removal of important membrane proteins, as well as cause acrosomal damage, which will be reflected in reduced fertility (*Curry, 2000; Holt, 2000*). Since glycerol has a relative high molecular weight (92 kDa) and does not readily penetrate into the spermatozoa membrane, it causes tremendous osmotic stress during the thawing procedure, because it is easily removed from the cell membranes (*Squires et al.*, 2004).

The ideal concentration of glycerol in the extender represents a balance between its toxicant and protecting effects; high concentrations can also affect the fertilizing capacity of the spermatozoa (*England, 1993; Silva et al., 2003*). However, the sensitivity of spermatozoa to glycerol is highly species dependent (*Schäfer-Somi et al.*, 2006).

The aim of this study was to analyse the influence of different crude glycerol concentrations on canine spermatozoa motility parameters during short *in vitro* cultivation.

Material and Methods

In this study semen was obtained from sexually mature male of five large dog breeds: Boxer, Border collie, Rottweiler, Greyhound and Husky. Semen was diluted in a ratio of 1 part of semen and 10 parts of physiological solution (Sodium chloride 0.9% Braun, B. Braun Melsungen AG, Melsungen, Germany) – Control sample C. In the same ratio the semen was diluted with four different concentration of crude glycerol solution: GA – 0.11 $ml.ml^{-1}$, GB – 0.027 $ml.ml^{-1}$, GC – 0.0069 $ml.ml^{-1}$, GD – 0.0017 $ml.ml^{-1}$ diluted in the physiological solution. Samples were cultured at 5 °C and recorded at five time periods: 0, 1, 2, 3, and 4 hours.

Characteristic used glycerol:

Crude glycerol (G) – content at least 80.06 %; NaCl content 7.15 %; water 8.0 %; methanol 0.001 %; Cd < 0.01 $mg.l^{-1}$; Pb < 0.1 $mg.l^{-1}$; Cu < 0.04 $mg.l^{-1}$; Mn < 0.03 $mg.l^{-1}$; Zn – 2.5 $mg.l^{-1}$; Fe – 15 $mg.l^{-1}$; Ni – 2.5 $mg.l^{-1}$; Co – 12.5 $mg.l^{-1}$; Cr – 7.5 $mg.l^{-1}$.

To evaluate spermatozoa motility parameters Computer Assisted Semen Analyzer (CASA) system – SpermVision program (Minitube, Tiefenbach, Germany) equipped with a microscope Olympus BX 51 (Olympus, Japan) was used. Each sample was placed into Makler Counting Chamber (depth of 10µm, Sefi – Medical Instruments, Germany) and then placed in a microscopic field. Using the canine specific set up the total motile spermatozoa (MOT) and progressively motile spermatozoa (PRO) were evaluated.

Obtained data were statistically analysed with the help of the PC program Excel and a statistics package SAS 9.1 (SAS Institute Inc., USA) using Student's t–test and Scheffe's test. Statistical significance was indicated by P values of less than 0.05; 0.01 and 0.001.

Results and Discussion

Results of the spermatozoa motility are shown in *Figure 1*. The percentage of total spermatozoa motility (MOT) cultured at 5 °C in the control sample (C) was in range from 71.75 to 84.10%. In the experimental groups motility ranged from 0 to 75.95%. A significantly lower ($p<0.001$) values were detected at the beginning of cultivation only in the samples GA with concentration of glycerol 0.11 $ml.ml^{-1}$. In times of 1, 2, 3, 4 significantly decrease ($p<0.001$) of spermatozoa motility were observed in all experimental groups compared to the control group.

Spermatozoa progressive motility (PRO) followed the tendency of spermatozoa motility. Progressive motility in the control sample ranged between 68.80 and 81.46%. In the experimental groups, motility ranged from 0 to 72.39%. Statistically significant difference ($p<0,001$) was detected at the time 1 hour only between sample C and GA, where the highest progressive motility was recorded in sample C. When compared the experimental groups to the control sample, significantly lower values ($p<0,001$) were observed after 1, 2, 3, 4 hours of culture in all samples (*Figure 2*).

Analysis of velocity curved line (VCL) showed no significant differences between samples GB, GC, GD and the control at the beginning of *in vitro* cultivation (*Figure 3*). Only in the sample GA significantly lower ($P<0.001$) value was observed. However, other cultivation times showed a significant decrease ($P<0.001$) of this parameter in all experimental samples when compared to the control.

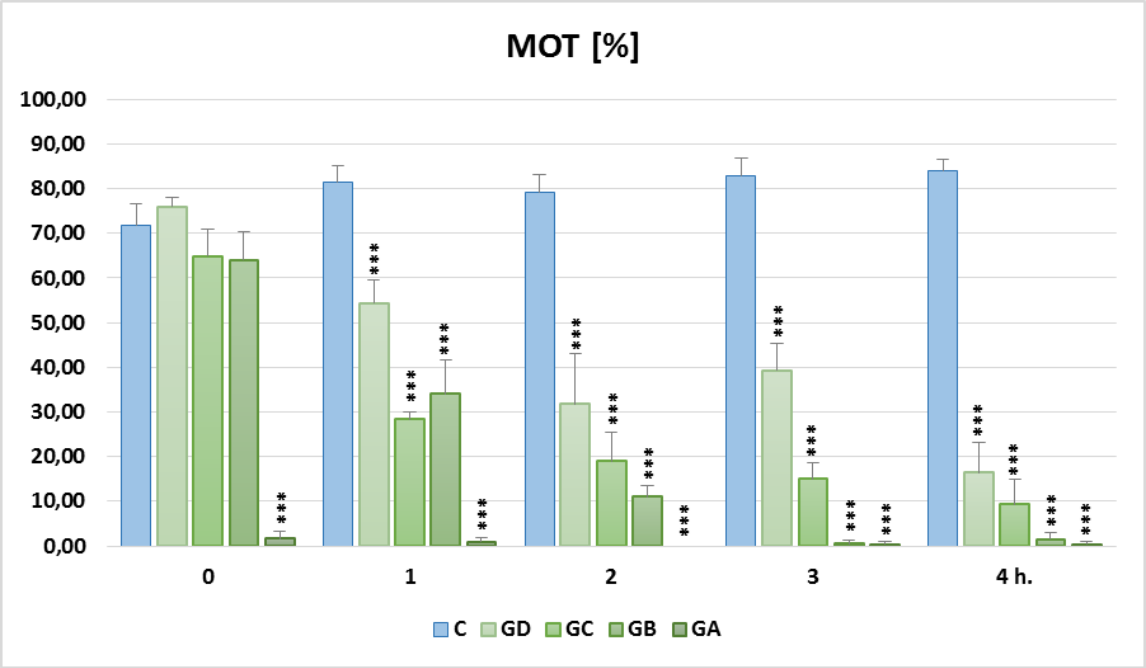

Figure 1:
Spermatozoa motility [%] in groups with different concentrations of glycerol and time periods [hours].

Significant differences *p<0.05; **p<0.01; ***p<0.001.

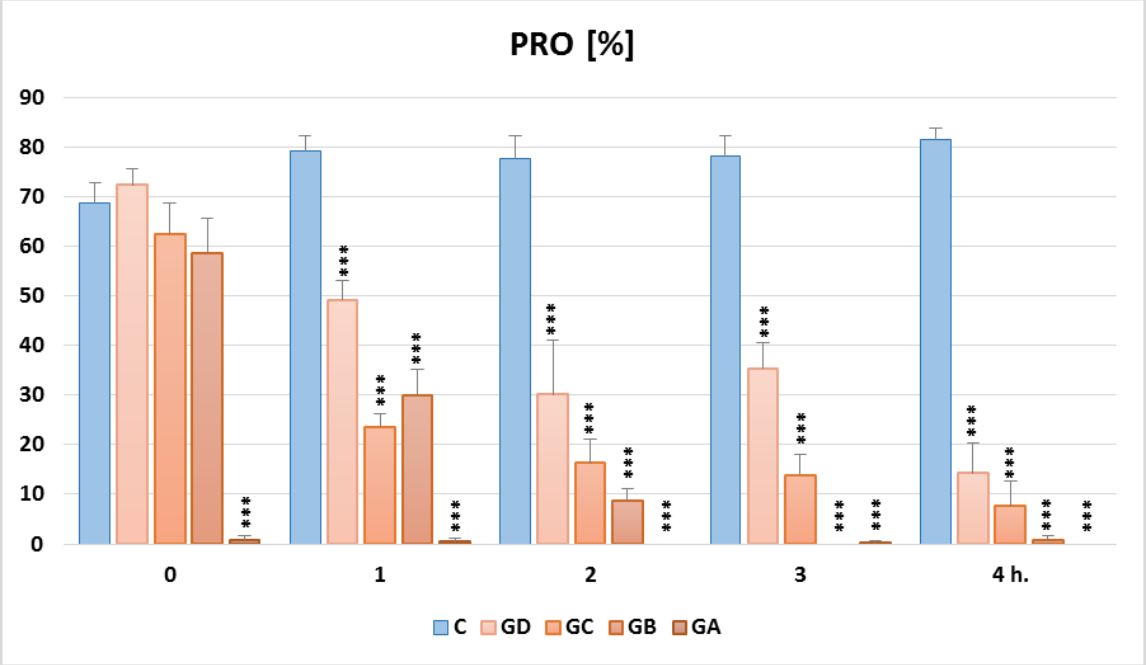

Figure 2:
Spermatozoa progressive motility [%] in groups with different concentrations of glycerol and time periods [hours].

Significant differences *p<0.05; **p<0.01; ***p<0.001.

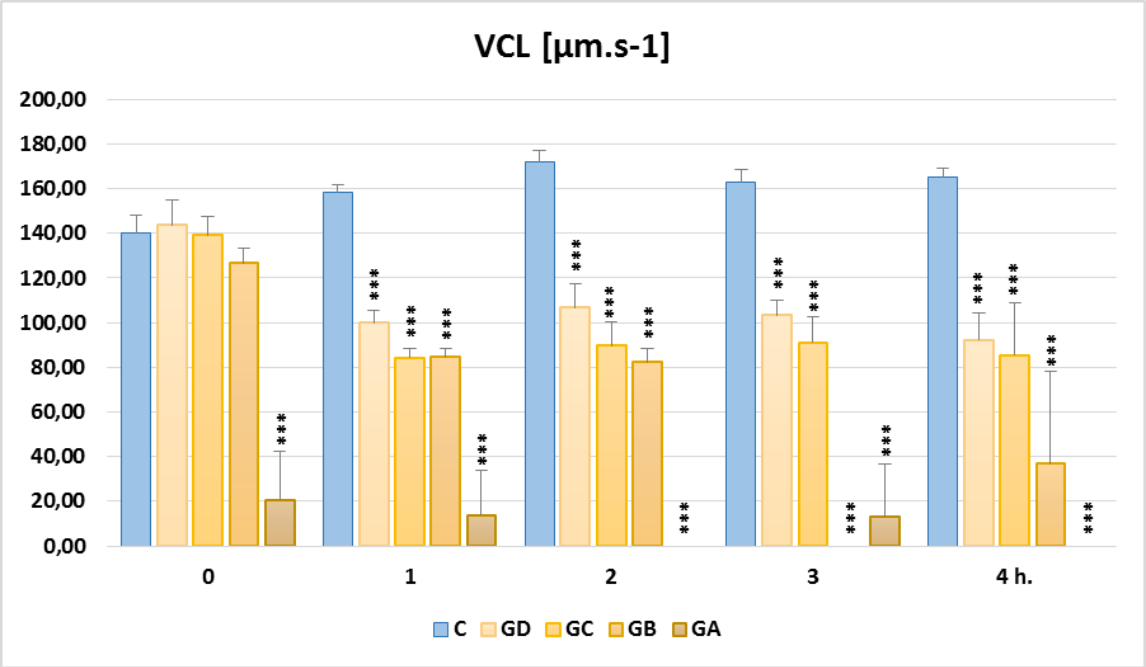

Figure 3:
Velocity curved line [µm.s^{-1}] in groups with different concentrations of glycerol and time periods [hours].

Significant differences *p<0.05; **p<0.01; ***p<0.001.

A similar tendency was detected also in other species. *Slanina et al.* (2012) evaluated the effect of crude and pure glycerol on rabbit spermatozoa motility parameters in vitro. The total spermatozoa motility and progressive motility in experimental groups, diluted with crude glycerol and pure glycerol were significantly lower compared to control group.

Glycerol has also negative effect on the velocity curved line parameter, where samples with addition pure glycerol showed a significant decrease values compare to control (*Kováčik et al., 2013*). The negative effect of high concentrations of crude glycerol on the rabbit spermatozoa motility detected also *Qoja et al.* (2011). Results of their study show some interesting outcomes – the initial addition of glycerol in higher concentration decreased immediately the spermatozoa motility, but later only a negative effect of higher crude glycerol concentration was found.

However, results of studies *Province et al.* (1984) showed that addition of glycerol to specific extenders had not negative effect on canine and equine spermatozoa motility. The inclusion of 6% glycerol depressed (P<0.05) motility of canine spermatozoa, but there was no effect (P>0.05) of glycerol concentration on the percentage of motile equine spermatozoa. For both species, the interaction of glycerol level and extender was not significant.

Conclusion

The target of this study was to analyse the effect of different crude glycerol concentrations on the canine spermatozoa motility parameters. The selected spermatozoa motility parameters (MOT, PRO and VCL) were significantly decreased in all analyzed samples from 1 hour of cultivation. This results confirmed negative effect of crude glycerol on canine spermatozoa mobility in *in vitro* conditions at 5°C.

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