

## EFFECT OF AGE ON THE SPERM ACTIVITY, SPERM CELL VIABILITY AND TOTAL NUMBER OF SPERMATOZOA IN THE EJACULATE OF DOGS

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### ABSTRACT

The aim of this study was to evaluate the effect of age on sperm activity, sperm cell viability and total number of spermatozoa in the ejaculate of dogs. We evaluated 90 semen samples. The dogs were divided into groups according to the age (A: 1.5–2 years, B: 2–5 years, C: 5–6.5 years). Semen samples were collected by manual manipulation into the glass beaker. Immediately after collection of macroscopic examination was made for all samples, which included find out volume of ejaculate, sperm activity, concentration and sperm cell viability. Volume was measured using the graduated cylinder. Concentration was evaluated by haematocytometry method using Bürker chamber and activity by subjective method according to the percentage of motile sperm in the native ejaculate. We evaluated the percentage of sperm with progressive direct movement after the head. Viability eosin-nigrosin stain method was performed for evaluation. In this case, we evaluated the total number of alive and dead sperms. Monitored characteristics were expressed in weighted average and standard error. Based on the results we can state that, in case of monitoring factors, the age of dogs had the significant influence. In case of activity, as well as viability, statistically highly significant differences ( $P < 0.01$ ) were observed between group of youngest dogs (A: 1.5–2 years) and oldest dogs (C: 5–6.5 years). Statistically significant difference ( $P < 0.05$ ) was proved between dogs from group B (2–5 years) and dogs from group C (5–6.5 years). In conclusion, the negative correlation of age, in case of activity ( $r = -0.44$ ;  $P < 0.001$ ) and even viability ( $r = -0.33$ ;  $P < 0.01$ ), was demonstrated. With increasing age, the values of both factors were reduced. In case of total number of spermatozoa, this phenomenon was not observed ( $r = 0.01$ ;  $P > 0.05$ ).

**Key words:** dog ejaculate, sperm activity, sperm cell viability, total number of spermatozoa, age

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## INTRODUCTION

The dog is actually the oldest domesticated animal at all and goes along with a human for more than 14.000 years. The role of dogs in human society is diverse, and so the dogs find application in several directions. In the past they were widely used in the hunt, herding of cattle or protection of property. At present time the dogs find more and more often application in the integrated rescue system or as an integration element returning to the normal life of people with visual or mobility impairments. In the Czech Republic, there are approximately 3 million dogs of different breeds bred. Věžník Z. *et al.* (2004) states, on the basis of their long-time studies, that 20–25 % of breeding dogs does not fulfill level basic requirements of successful reproduction. So, the most important precondition for successful breeding work becomes as quality control of their reproduction function (Linde Forsberg C. *et al.* 1999). Thanks to reproduction, insemination and cryopreservation, it is possible to preserve file of required properties to the coming years. Be it in the form of progeny, overflowing with these properties from some individuals, or in the form of preserved genetic materials. Through modern methods used in the evaluation of ejaculate, we can determine semen quality, and thus, to some extent affect the chance of successful fertilization. Special attention should be given to the total number of spermatozoa in the ejaculate, their activity, concentration and viability. This examination should precede the stud and it would be advisable to perform it after long pause in reproduction, before re-inclusion of the dog in reproduction (Eilts B.E. 2005). The results of these tests should primarily serve to breeders as a feedback for their objective assessment of availability of dog breeding. Secondly, as the information, which way is possible further use ejaculate of the individual dogs. If it is possible to cool it and to use for insemination of female dogs, or, if it is so quality, that would be appropriate to freeze it and thus enable its use for several years even after the death of sire.

## MATERIAL AND METHODS

We evaluated 90 samples of ejaculate from 15 male dogs. The dogs were divided into 3 groups according to the age (A: 1.5–2 years, B: 2–5 years, C: 5–6.5 years). Semen samples were collected by manual manipulation into the pre-warmed glass beaker to  $39 \pm 1$  °C. Immediately after collection macroscopic examination was performed for all samples, which included finding volume of ejaculate, sperm activity, concentration of spermatozoa and sperm cell viability. Volume of ejaculate was measured using the graduated cylinder. Concentration of spermatozoa was evaluated by haematocytometry method using Bürker chamber (Věžník Z. *et al.* 2004) and sperm activity then by subjective method according to the percentage of motile sperm in the native ejaculate. We evaluated the percentage of sperm with progressive direct movement after the head (Filipčík R. *et al.* 2010). To evaluation sperm cell viability eosin-nigrosin stain method of dried smears was performed. In this case, we evaluated the total number of live sperms and the total number of dead sperms. The heads of live sperms remains uncolored, while heads of dead sperms were pink, because their plasmatic membranes were damaged, which resulted in the intrusion eosin inside (WHO 1999). The total number of spermatozoa was found by simple calculation of the concentration of spermatozoa per  $\text{mm}^3$  and a total volume of ejaculate. Monitored characteristics were expressed in weighted average and standard error.

## RESULT AND DISCUSSION

Progressive moving forward to the head is one of the most important indicators of fertilization ability and is a functional indicator of biological full value of the sperm (Louda F. *et al.* 2001). Root Kustritz M.V. (2007) states, that the normal percentage of motile sperm in the ejaculate of normal dog should be 70.00 % or more. This condition was fulfilled by most of our collected dogs, the activity of their sperm was moving in variation ranging from  $71.25 \pm 0.65$  % to  $81.67 \pm 2.17$  %

with an overall average of around  $78.83 \pm 0.79$  % (Tab. 1). The highest value of sperm activity ( $81.67 \pm 2.17$  %) we registered in the group of the youngest dogs (1.5–2 years). The second highest sperm activity was found in a group of dogs from 2 to 5 years ( $79.50 \pm 0.87$  %) and the lowest value ( $71.25 \pm 0.79$  %) was achieved by the group of oldest dogs (5–6.5 years). Between this group and group of youngest dogs a highly statistically significant difference ( $P < 0.01$ ), was found. In case of group of dogs from 2 to 5 years, statistical difference was only significant ( $P < 0.05$ ). Between age and sperm activity, the negative correlation ( $r = -0.44$ ;  $P < 0.001$ ) was demonstrated (Fig. 1). Rijsselaere T. *et al.* (2007) observed the same phenomenon in their study. The highest sperm cell viability ( $87.83 \pm 2.31$  %) was demonstrated in group of the youngest dogs. The lower sperm cell viability was found in a group of dogs from 2 to 5 years ( $86.85 \pm 0.73$  %). And the lowest value was reached by the group of oldest dog ( $78.25 \pm 0.79$  %). Between this group and group of youngest dogs, the highly statistically significant difference ( $P < 0.01$ ), was proved. Only statistically difference ( $P < 0.05$ ), in case of group of dogs from 2 to 5 years, was found. Finally, even between age and sperm cell viability, the negative correlation ( $r = -0.33$ ;  $P < 0.01$ ), was observed. Svoboda M. *et al.* (2001) reported that the concentration of spermatozoa of a healthy dog should be  $300 \cdot 10^3 \cdot \text{mm}^{-3}$  to  $800 \cdot 10^3 \cdot \text{mm}^{-3}$ , while the total number of spermatozoa in the ejaculate should contain  $300 \cdot 10^6$  to  $32\,000 \cdot 10^6$  sperms (Kvapil R., Kvapilová R. 2007). The exact range of the total number of spermatozoa in the dog ejaculate is not specified, but any number less than 100 million sperm in a semen sample usually means that the dog has health issues that are affecting his fertility (Eldredge D.M. *et al.* 2007). The most important factors affecting the value considered: herd affiliation, age and sexual activity of dog. The list of these factors is further supplemented Peña Martínez A.I. (2004) with his arguing that the total number of spermatozoa in the ejaculate can also be negatively affected by a lack of sexual stimulation in the absence of female dog, stress or pain due to sampling. The highest total number of spermatozoa ( $2.89 \pm 0.29 \cdot 10^9$ ) was noted in the group of dogs from 2 to 5 years. The second highest value was found in a group of youngest dogs ( $2.67 \pm 0.23 \cdot 10^9$ ) and the lowest total number of spermatozoa was achieved by the group of oldest dog ( $1.97 \pm 0.23 \cdot 10^9$ ). In case of this monitoring factor, statistically significant difference as well as correlation ( $r = 0.01$ ;  $P \geq 0.05$ ), was not proved.

Tab. 1 The effect of the age of dogs on qualitative parameters of their ejaculate.

MONITORING FACTORS		A: 1.5–2 years		B: 2–5 years		C: 5–6.5 years		r
		(n=24)		(n = 48)		(n = 18)		
		L.S.M.	S.E.M.	L.S.M.	S.E.M.	L.S.M.	S.E.M.	
Sperm activity	(%)	81.67 <sup>C</sup>	2.17	79.50 <sup>c</sup>	0.87	71.25 <sup>A,b</sup>	0.65	- 0.44 <sup>**</sup>
Sperm cell viability	(%)	87.83 <sup>C</sup>	2.31	86.85 <sup>c</sup>	0.73	78.25 <sup>A,b</sup>	1.09	- 0.33 <sup>*</sup>
Total number of sperm.	(.10 <sup>9</sup> )	2.67	0.23	2.89	0.29	1.97	0.23	0.01

A, B, C – among values with different letters were proved statistical highly significant differences ( $P < 0.01$ ); a, b, c – among values with different letters were proved statistical evidential differences ( $P < 0.05$ ); L.S.M. – weighted average; S.E.M. – standard error; r – correlation, \*\* =  $P < 0.001$  and \* =  $P < 0.01$ .

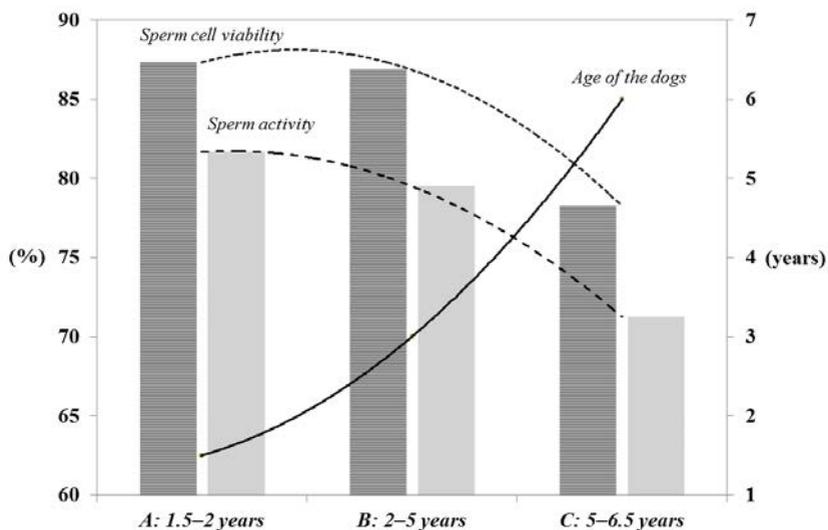


Fig. 1 The negative correlation between the age category of dogs, sperm activity and sperm cell viability.

## CONCLUSIONS

Based on our results, we can state that, in case of the sperm activity, sperm cell viability and the total number of spermatozoa, the age had a statistically significant influence. Because, the values of all observed parameters decreased with increasing age. In conclusion, the negative correlation of age, in case of sperm activity as well as sperm cell viability, was proved. With increasing age, the values of both monitoring factors were reduced. In case of the total number of spermatozoa, this phenomenon was not observed.

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Purpose: To determine if spermatozoa are present in the preejaculatory penile secretion, originating from Cowper's gland. Design: Prospective clinical and laboratory study. Setting: Andrology and Sex Counseling Unit, Department of Obstetrics and Gynecology, Academic Teaching Hospital. Patients: Five patients referred for premature ejaculation, three for excessive fluid secreted during foreplay and four normal healthy volunteers. Intervention: Glass slide smears of preejaculatory Cowper's gland secretion obtained during foreplay from at least two different occasions, and semen samples

A spermatozoon is a motile sperm cell, or moving form of the haploid cell that is the male gamete. A spermatozoon joins an ovum to form a zygote. (A zygote is a single cell, with a complete set of chromosomes, that normally develops into an embryo.) Sperm cells contribute approximately half of the nuclear genetic information to the diploid offspring (excluding, in most cases, mitochondrial DNA). In mammals, the sex of the offspring is determined by the sperm cell: a spermatozoon bearing an X Sperm cell diseases or defects are more common than one may think. They occur in the structure of the sperm cell and can lead to male infertility, thereby preventing fertilization from taking place. Teratozoospermia is a common abnormality that affects sperm morphology, that is, the shape of the sperm cells is altered (for example, sperm cells with 2 heads). Defects can be found either in the head, neck, tail, or in several parts simultaneously. Spermatozoa will be stored in the epididymis until they are expelled with ejaculation. When ejaculation starts, sperm travel through the vasa deferentia and mingle with the seminal fluid that originates in the secretory glands, creating what we all know as semen. Sperm, male reproductive cell, produced by most animals. In higher vertebrates, especially mammals, sperm are produced in the testes. The sperm unites with (fertilizes) the ovum (egg) of a female to produce a new offspring. Mature sperm have two distinguishable parts, a head and a tail. Sperm, also called spermatozoon, plural spermatozoa, male reproductive cell, produced by most animals. With the exception of nematode worms, decapods (e.g., crayfish), diplopods (e.g., millipedes), and mites, sperm are flagellated; that is, they have a whiplike tail. In higher vertebrates, especially mammals, sperm are produced in the testes. The sperm unites with (fertilizes) an ovum (egg) of the female to produce a new offspring. Mature sperm have two distinguishable parts, a head and a tail.