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

Acknowledgments: The research was supported by IGA FA MENDELU TP 2/2013.

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 basic 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. Secondarily, 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 ± 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 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 ± 0.65 % to 81.67 ± 2.17 %

with an overall average of around 78.83 ± 0.79 % (Tab. 1). The highest value of sperm activity (81.67 ± 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 ± 0.87 %) and the lowest value (71.25 ± 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 ± 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 ± 0.73 %). And the lowest value was reached by the group of oldest dog (78.25 ± 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. 10^3. mm^{-3} to 800. 10^3. mm^{-3}, while the total number of spermatozoa in the ejaculate should contain 300. 10^6 to 32 000. 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 ± 0.29 .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 ± 0.23 .10^9) and the lowest total number of spermatozoa was achieved by the group of oldest dog (1.97 ± 0.23 .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 (n=24)		B: 2–5 years (n = 48)		C: 5–6.5 years (n = 18)		r
		L.S.M.	S.E.M.	L.S.M.	S.E.M.	L.S.M.	S.E.M.	
Sperm activity	*(%)*	**81.67^{C}**	2.17	**79.50^{c}**	0.87	**71.25^{A,b}**	0.65	**- 0.44****
Sperm cell viability	*(%)*	**87.83^{C}**	2.31	**86.85^{c}**	0.73	**78.25^{A,b}**	1.09	**- 0.33***
Total number of sperm.	*(.10^9)*	**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.*

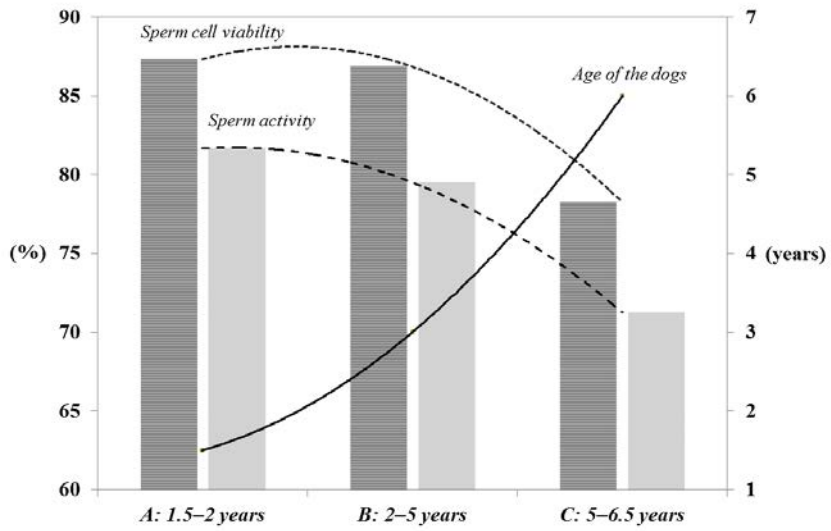

Fig. 1 The negative correlation between the age category of dogs, sperm activity and sperm cell viability.

CONCLUSIONS

Based on the 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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Sperm motility and semen ejaculate volume declined at the age of 43 years and 45 years respectively [43]. Another study conducted in China examined the semen analysis of 20â€“60 years old men and showed that age was negatively correlated with progressive motility, vitality, and percentage of normal sperm.Â Spermatozoa earmarked for elimination escape at ejaculation in what is called abortive apoptosis and contribute to poor sperm quality. This is largely due to the presence of excess cytoplasm present in morphologically abnormal sperm [80-82].Â Ageing male and its effect on the functional capacity of the sperm as measured by phosphatidyl serine expression have been reported [94]. Sperm are male reproductive cells present in the ejaculate. A doctor can tell someone their sperm count using a test called semen analysis. The average sperm count is between 40 million and 300 million sperm per milliliter. This number can vary between testing centers, with the above range coming from Resolve, a nonprofit fertility association. A doctor may recommend a semen analysis for many reasons, such as to test for possible underlying causes of infertility, how well the reproductive organs are working, or whether a surgical procedure for sterility was successful. If your sperm count is abnormal, work with your doctor to determine the cause. Your doctor can also help you understand your options for having a child if fertility is a concern.Â The number, shape, and mobility of sperm are important for testing for male factor infertility. Your doctor may recommend testing up to three samples of sperm at different visits to get an accurate analysis. At-home tests only test for the number of sperm. Talk to your doctor if you are interested in a full analysis. Semen analysis results table. a. Human sperm are highly pleomorphic in the sense that a large number of cells in the ejaculate display a great variety of morphological forms. In contrast, the proportion of mouse sperm with morphological variations is rather small. b. Humans deposit the ejaculate in the vagina, in contrast to mice that ejaculate in the uterus (Kawano et al., 2014).Â f. In vitro incubation under capacitating conditions for human sperm ranges from 3 to 24 h. As a result, a great variability of results is reported in the literature. In contrast, most studies in mouse sperm are performed using 1â€“1.5 h of incubation under capacitating conditions. g. Based on non-human data, the oviductal epithelium is considered a sperm reservoir that regulates binding and release of sperm toward the site of fertilization.