Animal welfare, etológia és tartástechnológia

Animal welfare, ethology and housing systems

Volume 9 Issue 3

Különszám/Special Issue

Gödöllő
2013

THE EFFECT OF THE AGE, BODY WEIGHT AND LABOUR UTILIZATION OF DOGS TO THE QUALITATIVE AND QUANTITATIVE PARAMETERS OF THEIR EJACULATE

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Abstract

In this study the quality of ejaculate from 20 male dogs of 8 different breeds was evaluated. The dogs were divided into groups according to the age, body weight and labour utilization. We focused on these parameters: volume of ejaculate, sperm activity, concentration of sperm, morphologically normal sperm, total number of sperm with changes on the head and changes on the flagellum. Based on the results can be stated that, in case of the quantitative and qualitative parameters of dog ejaculate, the age and body weight had the greatest influence. In case of the age, as well as body weight, was found statistically highly significant difference ($P \leq 0.01$) in these follows evaluated parameters: morphologically normal sperm and total number of sperm with changes on the head. Another, significant difference ($P \leq 0.05$) was proved in volume of ejaculate and concentration of sperm. Between sperm activity as well as total number of sperm with changes on the flagellum was not found statistically significant differences ($P \geq 0.05$) with measured parameters. Effect of labour utilization is shown by worst results of police dogs in all evaluated parameters.
Key words: dog ejaculate, volume, sperm activity, concentration of sperm, age, body weight

Acknowledgement
The research was supported by IGA TP 2/2013

Introduction

In the Czech Republic, there are approximately 3 million dogs of different breeds bred. The most important assumption for successful breeding work becomes as quality control of their reproduction. 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. Thanks to 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 sperm in the ejaculate, their activity and concentration. 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. The results of these tests should primarily serve breeders as feedback for their objective assessment of availability of dog breeding.

Material and methods

We evaluated the 117 samples of ejaculate from 20 male dogs of 8 different breeds (Australian Shepherd, Belgian Shepherd Groenendael, Belgian Shepherd Malinois, Belgian Shepherd Tervueren, Border Collie, Irish Setter, German Shepherd, Spitz) and 2 representatives of crossbreeds. The dogs were divided into groups according to the age (A: < 2 years; B: 2 - 6 years; C: > 6 years), body weight (A: < 10 kg; B: 11 - 25 kg; C: > 25 kg) and labour utilization (A: family dogs; B: sports-used dogs; C: police forces). Ejaculate were collected by manual manipulation in plastic tubes. Immediately after collection macroscopic examination was made for all samples, which included to find out volume of ejaculate, sperm activity and concentration of sperm. Volume of ejaculate was measured using the calibration cup 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 a progressive direct movement after the head. Concentration of sperm was evaluated by the haematocytometry method using Bürker chamber. Next, we evaluated the total number of morphologically normal sperm and the total number of pathology sperm, which were characterised in greater detail by changes on the head and changes on the flagellum. Monitored characteristics were expressed in weighted average and standard deviation.

Results and discussion

Table 1 shows the evaluation of the effect of the age, body weight and labour utilization of the individual on semen quality. More precisely to its macroscopic parameters, which are: volume of ejaculate, sperm activity and concentration of sperm.

Table 1: **The effect of the age, body weight and labour utilization of dogs to the volume of ejaculate, sperm activity and concentration of sperm in their ejaculate.**

MONITORING FACTOR			n	Volume of ejaculate *(ml)*		Sperm activity *(%)*		Concentration of sperm *(10^3. mm^{-3})*	
				$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
TOTAL AVERAGE			117	9.16	6.93	74.18	11.68	141.72	98.33
Age	*A*	*< 2 years*	17	11.35^{b}	5.49	68.23	9.51	88.24^{b}	20.30
	B	*2 - 6 years*	77	8.22^{a}	6.75	75.71	12.71	152.19^{a}	107.57
	C	*> 6 years*	23	10.67	8.04	73.47	7.75	146.19	89.94
Weight	*A*	*< 10 kg*	18	5.83^{C}	1.51	80.00	5.15	100.00^{c}	52.13
	B	*10 - 25 kg*	57	8.76^{c}	7.96	73.07	13.18	138.54	72.79
	C	*> 25 kg*	42	11.12^{Ab}	6.26	73.21	10.97	163.93^{a}	133.07
Utilization	*A*	*Family*	44	8.90	6.24	73.63	8.72	100.79^{BC}	54.06
	B	*Sport*	52	10.17^{c}	8.17	76.05	13.69	152.53^{A}	77.44
	C	*Police*	21	7.16^{b}	4.15	70.71	11.32	200.71^{A}	163.89

A, B, C – between values with different letters in a column in each section were proved statistical highly significant differences ($P \leq 0.01$)
a, b, c – – between values with different letters in a column in each section were proved statistical evidential differences ($P \leq 0.05$)

The volume of the ejaculate is not itself an indicator of sperm quality of males. However, its measuring is an essential part of the evaluation of the quality of dog ejaculate (*Root Kustritz*, 2007). *Kvapil and Kvapilova* (2007) report that physiological amounts of dogs ejaculate range from 1 to 40 ml, and the average value is about 7 ml. *Dolezel et al.* (2001) is in his publication something specific. They argue that the average volume of ejaculate from them examined dogs ranged from 4.5 to 8.5 ml. Our measured values mostly exceed this range, because the volume of ejaculate moved in variation range from 5.83 ± 1.51 ml to 11.35 ± 5.49 ml. Between a group of dogs from 10 to 25 kg and group of dogs over 25 kg was found a statistically evidential difference ($P \leq 0.05$). This difference, however, was not as significant as difference found between the groups of dogs less than 10 kg and dogs over 25 kg. Here the statistical difference was highly significant ($P \leq 0.01$).

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 et al*, 2001). *Root Kustritz* (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 individuals, the activity of their sperm was moving in variation ranging from 70.71 ± 11.32 % to 80.00 ± 5.15 %. The only exception was group of dogs under two years old, who achieved only 68.23 ± 9.51 %. This fact was most likely caused by the stress from the first collection in life, or other disturbing influences from the surroundings. The highest activity of sperm (80.00 ± 5.15 %) we registered in the group of dogs weighing less than 10 kg. These dogs also produced the smallest average amount of ejaculate from all evaluated groups (5.83 ± 1.51 ml). The second lowest activity was found in a group of dogs from 10 to 25 kg (73.07 ± 13.18%). This phenomenon was probably due to the fact that to this group were included most of service dogs, their results belonged generally to the worst. Statistically highly significant difference ($P \leq 0.01$) of this factor in their work demonstrated *Vagenknechtova et al.* (2011), when they examinated service dogs achieved activities only 49.4 %. We, however, this difference failed to substantiate statistically.

Dolezel et al. (2001) reported that the concentration of sperm in a healthy dog should be $300.\ 10^3.\ mm^{-3}$ to $800.\ 10^3.\ mm^{-3}$, while the total ejaculate should contain $300.\ 10^6$ to $32\ 000.\ 10^6$ sperm (*Kvapil and Kvapilova*, 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* (2004) with his arguing that the total number of sperm 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. *Root Kustritz* (2007) stated that rather than belonging to a breed, the sperm production, volume and total number mainly related with the size of the testes of the individual. As the total number of sperm in the ejaculate, is directly proportional to the size of tested tissue. So, in general, breeds of large dogs have a total number of sperm in the ejaculate bigger than small breeds (*Kvapil and Kvapilova*, 2007). This fact was also proved in our study, that between the group of dogs to 10 kg and a group of dogs over 25 kg is a statistically significant difference ($P \leq 0.05$). The average concentration of sperm of examined individuals proportionally increased with increasing of ejaculate quantity and body weight of individuals ($100.00.\ 10^3.\ mm^{-3} < 138.54.\ 10^3.\ mm^{-3} < 163.93.\ 10^3.\ mm^{-3}$). Lowest values again reached the group of dogs under two years old ($88.24.\ 10^3.\ mm^{-3}$) and the best results the dogs in reproductive peak, i.e. group of dogs from two to six years old ($152.19.\ 10^3.\ mm^{-3}$). Even among these two groups was found significant difference ($P \leq 0.05$). *Root Kustritz* (2007) reported the age of six years as the maximum limit of high reproduction ability, because already in advanced age the sperm production is decreasing and the percentage of moving sperm miss reducing. In our experiment,

this tendency, although slightly, managed to record. On the other hand, even in a group of dogs older than six years, then individuals over the reproductive peak, we have measured the concentration of sperm over the overall average (146.19. 10^3. mm^{-3}) and sperm activity values above the minimum required (73.47 ±7.75 %), which is certainly pleasing result for their owner. Eventually, highly statistically significant difference ($P \leq 0.01$) was found in the factor of labour utilization, where family dogs reached values of 100.79. 10^3. mm^{-3}, sports-used dogs 152.53. 10^3. mm^{-3} and dogs from group of police forces 200.71. 10^3. mm^{-3}.

Table 2 shows the evaluation of the effect of the age, body weight and labour utilization of the individual on the morphological status of dog sperm.

Table 2: **The effect of the age, body weight and labour utilization to the morphological status of dog sperm**

MONITORING FACTOR			n	Normal sperm *(%)*		Changes on the head *(%)*		Changes on the flagellum *(%)*	
				$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
TOTAL AVERAGE			117	76.44	12.18	4.64	3.45	10.87	7.30
Age	*A*	*< 2 years*	17	76.29^{C}	19.22	8.58^{C}	4.06	8.64	3.93
	B	*2 – 6 years*	77	73.74^{C}	10.31	4.88	2.90	12.01	8.06
	C	*> 6 years*	23	85.60^{AB}	5.77	0.91^{A}	0.54	8.69	5.70
Weight	*A*	*< 10 kg*	18	71.16^{B}	8.86	6.50	3.91	12.55	7.41
	B	*10 – 25 kg*	57	80.93^{AC}	9.54	2.14^{C}	1.80	10.12	7.26
	C	*> 25 kg*	42	72.62^{B}	14.42	7.29^{B}	5.90	10.90	7.35
Utilization	*A*	*Family*	44	80.50^{BC}	10.32	3.32	1.19	9.84^{c}	6.19
	B	*Sport*	52	75.03^{A}	13.17	5.78	1.09	10.33	6.61
	C	*Police*	21	71.42^{A}	11.03	4.57	2.08	14.38^{a}	9.99

A, B, C – between values with different letters in a column in each section were proved statistical highly significant differences (P≤ 0.01)
a, b, c – between values with different letters in a column in each section were proved statistical evidential differences (P≤ 0.05)

Johnston et al. (2001) reported that dogs ejaculate should have a minimally 80.00 % of morphologically normal sperm. The effect of the age has been shown by highly statistically significant difference ($P \leq 0.01$) between the results of the groups of dogs older than six years (85.60 ± 5.77 %), group of dogs from two to six years of age (73.74 ± 10.31 %) and group of dogs younger than two years (76.29 ± 19.22 %). Also, in assessing the impact of body weight on the percentage number of morphologically normal sperm, was found highly statistically significant difference ($P \leq 0.01$), and that between group of dogs from 10 to 25 kg against to the two remaining groups. Finally, we should mention even the effect labour utilization, which was demonstrated by highly statistically significant difference ($P \leq 0.01$), between family dogs and dogs from group of police forces in this case.

Pathological changes in the shape of sperm heads are the most common abnormalities in disorders of spermatogenesis. In most cases, accompanying the emergence of degenerative and inflammatory processes in the testes. By a healthy individual, quantum of these anomalies can´t

be a bigger than 5.00 % *(Louda et al*, 2001*)*. Best result was found in the group of dogs over six years of age (0.91 ± 1.04 %). This value showed a highly significant difference ($P \leq 0.01$) with results of dogs from the group of dogs to two years of age. In fact, results these dogs (8.58 ± 17. 06 %) were the worst of all evaluated groups.

The largest number of defects in the formation of sperm cells was demonstrated in the construction of flagellum. The proportion of these changes were moving in a variation range from 8.64 ± 3.93 % to 14.38 ± 9.99 %, with an overall average of around 10.87 ± 7.30 %. Significant difference ($P \leq 0.05$), was detected only in effect of labour utilization, and that between the family dogs (9.84 ± 6.19 %) and a group of police dogs (14.38 ± 9.99 %).

Conclusion

Our results show that, in case of the quantitative and qualitative parameters, the age and body weight had the greatest influence. Volume of ejaculate increased with increasing body weight observed dogs. A similar tendency we have registered even at average concentration of sperm, when the concentration was well balanced, with the exception of the youngest dogs and dogs weighing less than 10 kilograms body weight. The low quality of ejaculate was proved in a group of dogs from the youngest age, their results most likely reflected the stress of the first sample in life. Effect of the labour utilization is shown by worst results of police dogs in all evaluated parameters.

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