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THE INFLUENCE OF EXTERNAL AND INTERNAL FACTORS ON THE QUALITY OF SEMEN COLLECTION AND QUALITATIVE INDICATORS OF SEMEN IN THE DOG (CANIS FAMILIARIS)

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Abstract

VÁGENKNECHTOVÁ, M., HOŠEK, M., MÁCHAL, L., CHLÁDEK, G.: *The influence of external and internal factors on the quality of semen collection and qualitative indicators of semen in the dog (Canis familiaris).* Acta univ. agric. et silvic. Mendel. Brun., 2011, LIX, No. 6, pp. 00–00

The influence of external and internal factors on the course of semen collection and quality of ejaculate has been evaluated in this work. This observation included 30 dogs of various breeds, each of which we collected 3 ejaculates from within one week (first, third and fifth day). The internal factors included the weight of the dog, order of semen collection and the age of the dog. The external factors included type of housing, utilization of the dog, point of the collection and type of nutrition. Average length of preparation of a dog for a collection and onset of sexual reflex was 95.7 sec. The observations showed that none of the evaluated internal factors had any influence on the length of preparation for the semen collection. The largest volume of ejaculate was collected from the first collection (10.5cm^3), at the second collection it was 8.9 cm^3 and the third one the volume was the smallest – with only 6.6 cm^3. The average volume of 8.7 cm^3 was found in the whole set of observed dogs. No statistically conclusive difference was found among groups. The average activity of sperm in the observed set was 68.8%. Activity declined from the first to the third collection from 73% to 65.5%. The difference in sperm activity was not statistically conclusive in any indicator. Sperm concentration in whole set of dogs was 143.6 10^3.mm^{-3} and was not significantly affected by any monitored internal factors.
The longest preparation for the collection was found in dogs housed in pens (134.4 sec), dogs living in households (84.7 sec) and the shortest preparation was needed in dogs with freedom of movement. The longest onset of sexual reflexes was found in Police forces dogs, then family dogs and sport dogs (130.0 sec, 97.8 sec and 68.3 sec). In dogs collected in laboratory, the emergence of sexual reflex was longer (110.7 sec) than in dogs collected in their own natural environment (80.7 sec). The average length of the whole ejaculation was 396.0 sec in the whole set of dogs. The longest ejaculation was found in dogs held home (468.0 sec), the shortest in dogs with freedom of movement (290.0 sec). Family dogs had the longest ejaculation time, Police forces dogs had the shortest. The highest volume of semen was found in dogs kept in households (11.0 cm^3), the smallest volume was in dogs with freedom of movement (4.2 cm^3). Dogs used in sport, family dogs and Police forces dogs had the volume of semen 12.7 cm^3, 6.3 cm^3 and 5.7 cm^3. Statistically highly significant differences in the activity of sperm was found between dogs with freedom of movement (35.0%) and dogs kept in pens and household of the breeder (77.8%, respectively 77.0%), between Police dogs (49.4%) and sport dogs (76.7%) and family dog (77.8%), as well as in the place of collection ($P \leq 0.01$). The lowest sperm concentration was found in dogs kept in households (124.10^3.mm^{-3}), in dogs kept in pens (152.10^3.mm^{-3}), the highest sperm concentration was in dogs with freedom of movement (180.10^3.mm^{-3}). Police, sport and family dogs had the concentration of 175.10^3.mm^{-3}, 126,5.10^3.mm^{-3}, respectively 135,0.10^3.mm^{-3}. Dogs collected in household had the average sperm concentration 102.7.10^3.mm^{-3}, compared to the sperm concentration of dogs collected in the laboratory (184.5.10^3.mm^{-3}).

dog semen, semen collection, time of collection, semen volume, activity, concentration

In the last two decades the interest of breeders in insemination has risen. For the insemination it is necessary to successfully collect the semen. Semen samples may be collected not only for the purposes of insemination, but for diagnosis and long-term sperm preservation (Kutzler, 2005). This reduces the necessity of long distance animal transportation for purposes of reproduction. It makes the reproduction possible in animals with limited possibilities of natural mating. There is also a possibility to store the semen for future use, both for a period of infertility of a living stud and after his death (Rijsselaere *et. al.*, 2002).

The quality of ejaculate is affected by many internal and external factors. To the most important internal factors affecting the quality of semen belongs the order of collections which is the overall factor of collection frequency and the level of dog's experience. The order of the collection may significantly affect some of the qualitative indicators (Michael *et al.,* 2008). The live weight of a dog plays the indispensable role as well. The weight is a significant factor that must be taken into account when analyzing the quality of dog semen because e.g. total number of sperm depends of the size of the testicles and the size of the testicles depends on the weight of the dog (Kutzler, 2005). Also age may play an important role in the quality of semen produced by a particular stud. There was a decrease of sperm production found in six year old dogs, with decrease of the activity of sperm and the number of morphologically normal sperm as well (Schafer *et al.*, 1997).

The quality of semen can also be influenced by external factors which primarily include type of housing, use or working utilization of dog and last, but not least place of semen collection (Lopes *et al.*, 2009). Effect of nutrition is sometimes overlooked, but deficiencies in nutrition such as malnutrition or severe obesity may have a significant impact on the quality of the ejaculate. Effect of nutrition as one of the indicators of the semen quality was observed by e.g. Linde-Forsberg *et al.*, 1993, Michael *et al.*, 2008, etc.

MATERIALS AND METHODS

During the two months period from March 1st to April 30th, 2010 ejaculates of 30 dogs of 13 various breeds were collected by digital stimulation. In all dogs three collections of ejaculate were performed always within one week (first, third and fifth day). Total 90 samples of semen were collected. All collections were performed by the same technique, the same laboratory technician and under constant conditions.

The collections were performed at: 1) Department of the animal reproduction laboratory, Institute of Animal Breeding, Faculty of Agronomy of the Mendel University in Brno, 2) Veterinary Laboratory, Police of the Czech Republic, Hradec Králové, 3) households of observed dogs.

The effect of external and internal factors on course of collection and quality of semen was evaluated in dogs. Among the internal factors were included weight of the dog, order of collection and age of the dog. Dogs were assigned to two groups by age with first group of younger dogs aged 12 to 30 months and second group of older dogs from 36 to 60 months. By weight, dogs were assigned to three groups (small dogs 1–10 kg, medium dogs 11–25 kg and large dogs over 25 kg). An evaluation of quality of ejaculate was performed by the order of collection (1–3).

Among the external factors were included type of housing, dog utilization, place of collection and type of nutrition. By type of housing dogs were assigned to three groups – dogs housed in pens, in a flat or free on the plot (with freedom of movement). Dogs included into the observation had three different uses – family dogs, dogs for sports and Czech Police dogs. By the type of nutrition, dogs were assigned to two groups – first group was fed exclusively by commercially manufactured dry pet-food, second was fed by commercially manufactured dry pet-food with the addition of raw meat.

During the collection both emergence of sexual reflexes (from the first hand contact with dog's penis to the first jet of semen) and the length of the ejaculation from the first ejaculation spray to the end of ejaculation were observed. The whole ejaculate was collected (all three fractions).

Immediately after the collection volume of the ejaculation, activity and concentration of sperm were set. The volume was determined using the calibration cup. Sperm activity was found using subjective method at 200 times magnification and sperm concentration by hemo cytometric method.

Statistical evaluation was performed by the analysis of variance. Observed characteristics were expressed by mean value and standard deviation.

RESULTS AND DISCUSSION

Table I shows the influence of internal factors on the length of the collection and quality of semen. The average length of preparation of dogs to the collection and onset of the sexual reflexes was 95.7 sec. In terms of the order of collection it was found that dogs needed significantly longer preparation in the first collection, when the sexual reflexes onset more slowly, with the average of 171.0 sec. In the second collection the average length of preparation was already reduced to 72.0 sec and in the third collection to average of 44.0 sec. Despite the significant reduction of preparation time, no statistically significant difference was found between the first and the third collection, see Table I. Older dogs needed longer time for preparation to collection, 103.9 sec, compared to only 83.3 sec in younger dogs, yet even here no statistically significant difference was found. When examining the influence of weight on the onset of

I: *Effect of internal factors on the collection and quality of semen in observed dogs*

Factor		Number of collections	Emergence of sexual reflexes (sec)		Length of ejaculation (sec)		Volume of ejaculation (cm^3)		Sperm activity (%)		Sperm concentration ($10^3.mm^{-3}$)	
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
Total average		90	95.7	108.7	396.0	174.6	8.7	6.8	68.8	19.8	143.6	106.0
Order of collection	1.	30	171.0	161.5	396.0	142.3	10.5^{AB}	9.5	73.0	18.1	133.0	87.2
	2.	30	72.0	28.0	456.0	152.4	8.9^{AB}	5.2	68.0	16.4	150.0	150.1
	3.	30	44.0	13.8	336.0	206.4	6.6^{AB}	4.5	65.5	24.1	147.8	64.6
Age	12–30 mon.	36	83.3	73.1	435.0	197.4	8.8	5.1	74.6	9.4	131.3	90.9
	31–60 mon.	54	103.9	127.1	370.0	154.1	8.6	7.8	65.0	23.8	151.8	115.0
Weight	1–10 kg	18	71.7^{AB}	26.8	440.0	126.9	5.7	1.5	80.0	5.1	100.0	52.1
	11–25 kg	27	97.8^{AB}	86.6	480.0	230.6	11.8	10.1	78.3	6.8	152.0	87.3
	25–45 kg	45	104.0^{AB}	137.4	328.0	119.0	8.0	4.9	58.7	23.4	156.0	127.2

A – a statistically highly significant differences ($P \leq 0.01$) have been found between values with same letters in column in each section,
a – a statistically significant differences have been found ($P \leq 0.05$) between values with same letters in column in each section

sexual reflexes, a statistically significant difference ($P \leq 0.01$) was found.

Despite the significant reduction in preparation time and onset of sexual reflexes, no difference between the first and the third collection was found statistically significant, see Table I. Older dogs needed longer preparation time (103.9 sec) compared to 83.3 sec needed for younger dogs, yet even here no statistically significant difference was found. When examining the influence of the weight of dogs on the emergence of sexual reflexes, a highly significant difference ($P \leq 0.01$) was found in every observed group: the smallest dogs (1–10 kg) had the shortest time of onset of sexual reflexes (71.1 sec), medium sized dogs (11–25 kg) needed average of 97.8 sec and dogs over 25 kg needed 104.0 sec in average for onset of sexual reflexes. Vágenknechtová *et al.* (2010) in her work show the average length of the onset of the sexual reflexes of 222.0 sec which is significantly longer time than we found in this observation. In terms of dogs' weight a similar trend was only noticed in small dogs (72.0 sec), medium sized dogs (19–22 kg) needed 135.0 sec and large breeds needed 66.0 sec.

The observation showed that none of the evaluated internal factors had any influence on the length of the collection itself. We observed the longest collection time in the second collection (456.0 sec), with younger dogs having longer average collection time (435.0 sec) compared to 370.0 sec in older dogs. Medium sized dogs had the longest collection time (480.0 sec) compared to small dogs (440.0 sec) and large dogs (328.0 sec). Vágenknechtová *et al.*, (2010) state a value similar to the one we found in our observation. In their work the average length of the manual collection of the entire ejaculate of 384.0 sec was found. In their work the length of collection (438.0 sec) in small dogs is almost identical with our findings, while in medium sized and large dogs they observed the opposite trend (310.0 sec, respectively 402.0 sec).

We observed significant differences in evaluation of volume of entire ejaculate depending on the order of collection. The highest volume of ejaculate came from the first collection (10.5 cm^3), the second collection produced lower volume (8.9 cm^3) and in the third collection the volume was the lowest with only 6.6 cm^3. Statistically highly significant differences were found in the order of semen collection ($P \leq 0.01$). Both younger and older dogs had the similar average volumes of the second semen collection with younger of 8.8 cm^3 and older of 8.6 cm^3. The total average volume of ejaculate in the whole set of observed dogs was 8.7 cm^3. Although a difference was found in volume of ejaculate between small, medium and large dogs, there was no statistically conclusive difference between groups, see Tab I. Filipčík *et al.* (2011) found similar volumes of ejaculate in three age groups, especially for dog aged 2–3 years the volumes were almost identical (11.05; 8.44 respectively 7.82 cm^3). Yet compared to our observations, they found significantly lower volume of the whole ejaculate in older dogs – depending on the order of collection (8.00, 5.00, resp. 2.00 cm^3). England *et al.* (1999) found the volume of the second ejaculate fraction of 4.1 cm^3 in German Shepherds which corresponds to the volumes of the whole ejaculate observed by us. But in other groups of medium sized and large breeds they found a volume of the second ejaculate fraction only of 1.5 cm^3, when this value corresponds to ejaculate volume only observed in the group of small dogs or the third order of collection (5.7 cm^3, respectively 6.6 cm^3). Also Rijsselaere *et al.* (2002) state the volume of the two first ejaculate fractions of 3.9 ± 1.16 cm^3 in medium sized dogs which is the lower limit of our observed values for the entire volume of ejaculate.

The average sperm activity in ejaculate was 68.8%. Similarly as the time needed for preparation of ejaculate collection decreased or as the ejaculate volume between the first and the third collection decreased, the sperm activity in the second and the third collection compared to the first collection decreased, too (73.0% vs. 68.0%, resp. 65.5%, see Table I.). Younger males had higher sperm activity by almost 10% (74.6%), compared to older dogs (only 65.0%). In terms of weight, the lowest sperm activity was found in large dogs (only 58.7%) while the sperm activity in medium sized and small dogs was significantly higher (80.0%, respectively 78.3%). The difference in sperm activity between various orders of collection, age groups or between weight groups was not statistically conclusive. Similar values of sperm activity we found in our observation are stated also by e.g. Filipčík *et al.* (2011), who also noticed the identical trend of sperm activity depending on the order of ejaculate collection. Also Rijsselaere *et al.* (2002) or England *et al.*, (1999) similarly state high percentage of sperm with progressive activity (78.7; respectively 64.6–71.5%). On the other hand, Kojima *et al.* (2001) found in Beagles who were collected by digital stimulation and electroejaculation in anesthesia sperm activity of only 42.5%, respectively 36.8%, so the values significantly lower than values observed by us. In medium sized and large dogs the sperm activity at the level of 20–30% can normally be observed, as illustrated by Vágenknechtová *et al.*, (2010).

Sperm concentration was not significantly affected by the order of collection and ranged from 133,0.10^3.mm^{-3} at the first collection to 150,0.10^3.mm^{-3} at the second collection. In the group of older males a higher concentration was found to 151,8.10^3.mm^{-3}, compared to 131,3.10^3.mm^{-3} in the group of younger dogs. In terms of weight of the dogs, the highest concentration of sperm was in a group of large dogs, 156.10^3.mm^{-3}, medium sized dogs had an average concentration of 152.10^3.mm^{-3} and small dogs only 100.10^3.mm^{-3}. However, even here no statistically conclusive difference was found. Similar sperm concentration which we found in our observation is stated in Vágenknechtová *et al.*, (2010) or Filipčík *et al.* (2011), when especially the second named author observed the most balanced values of sperm concentration in dogs in the age category of 2–3 years olds, as well as with the others qualitative ejaculate indicators. Significantly lower concentration of sperms collected under anesthesia by electroejaculation was found in Beagles, e.g. Kojima *et al.*, (2001). Our findings in sperm concentration in the whole ejaculate were comparable to those which give Rijsselaere *et al.* (2002), with ours observed in the second, sperm-rich fraction. On contrary, England *et al.* (1999) present a sperm concentration of 302,1–423,6.10^3.mm^{-3}, in the second ejaculate fraction in medium sized dogs which is significantly higher value than our observed data, but given the volume of ejaculate received a comparable total number of sperm as in our observations. In another work England *et al.* (2010) state that from 48 retrievers at the age of 16–28 months evaluated in heritability of semen parameters only 9 males had sperm concentration up to 300.10^3.mm^{-3}, 20 males had a concentration between 310–690.10^3.mm^{-3}, and 19 males had a concentration higher than 700.10^3.mm^{-3}, which are values significantly higher than values found in our observations.

II: *The influence of external factors on the collection and quality of semen in observed dogs*

Observed factor		Number of collection	Onset of sexual reflexes (sec)		Length of ejaculation (sec)		Volume of ejaculate (cm^3)		Sperm activity(%)		Sperm concentration (10^3.mm^{-3})	
			$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x	$\bar{x}$	s_x
Total average		90	95.7	108,7	396.0	174.6	8.7	6.8	68.8	19.8	143.6	106.0
Type of housing	Pen	27	134.4^{AB}	174.1	346.7^{A}	149.2	7.7^{AB}	3.1	77.8^{A}	4.9	152.0	61.9
	Household	45	84.7^{A}	65.2	468.0^{AB}	188.3	11.0^{AB}	8.5	77.0^{B}	9.6	124.0^{A}	75.7
	Freedom of movement	18	65.0^{B}	27.7	290.0^{B}	65.9	4.2^{AB}	2.3	35.0^{AB}	16.5	180.0^{A}	188.6
Utilization	Family	27	97.8^{AB}	79.0	493.3^{AB}	192.9	6.3^{A}	3.2	77.8^{A}	5.9	135.0^{A}	95.1
	Sport	36	68.3^{AB}	42.6	375.0^{A}	170.5	12.7^{AB}	8.7	76.7^{B}	9.8	126.5^{B}	53.1
	Police forces	27	130.0^{AB}	171.7	326.7^{B}	112.0	5.7^{B}	3.2	49.4^{AB}	25.0	175.0^{AB}	155.3
Place of collection	Household	45	80.7^{A}	38.6	404.0	128.2	8.0^{a}	4.5	67.0^{A}	20.5	102.7^{A}	44.7
	Laboratory	45	110.7^{A}	148.1	388.0	212.4	9.3^{a}	8.6	70.7^{A}	19.3	184.5^{A}	131.5
Type of nutrition	Dry pet-food	45	120.0	144.9	416.0	192.5	9.5^{A}	8.5	61.3	24.7	170.3	133.4
	Dry pet-food and meat	45	71.3	41.3	376.0	154.2	7.8^{A}	4.6	76.3	8.6	116.9	58.9

A – a statistically highly conclusive differences ($P \leq 0.01$) have been proved between values of the same letter in a column in each section

a – a statistically conclusive differences ($P \leq 0.05$) have been proved between values of the same letters in a column in each section

The influence of external factors on the semen collection and quality is shown in Table II. Regarding the type of housing, the longest preparation and onset of sexual reflexes was observed in dogs housed in pens (134.4 sec) and the shortest in dogs with freedom of movement on plot (65 sec). In dogs housed in breeder's households the length of preparation was 84.7 sec. A type of housing showed highly conclusive effect ($P \leq 0.01$) on the length of preparation for a semen collection. Also the utilization had a highly conclusive effect ($P \leq 0.01$) on the onset of sexual reflexes. The longest onset of sexual reflexes (130.0 sec) was found in dogs of Police forces compared to family dogs (97.8 sec) and dogs for sport activities (68.3 sec). A place of collection also showed a highly conclusive effect ($P \leq 0.01$) on the onset of sexual reflexes with 110.7 sec for dogs collected in laboratory compared to dogs collected in households with 80.7 sec. Dogs with nutrition consisted of dry pet-food and raw meat had faster onset of sexual reflexes (71.3 sec) than dogs fed exclusively by dry pet-food. The onset of sexual reflexes in these dogs was longer (120.0 sec), yet influence of nutrition on the onset of sexual reflexes was not found statistically conclusive. Vágenknechtová *et al.* (2010) found the same trend, but in dogs fed only by commercial dry pet-food observed the average length of preparation for a collection of 366.0 sec compared to combined dry pet-food and meat (84.0 sec) and home made food (72.0 sec).

Average total length of ejaculation was 396.0 sec in whole observed set of dogs. In terms of housing, the ejaculation was longest in dogs kept in households (468.0 sec). The shortest time of ejaculation was observed in dogs with freedom of movement (290.0 sec). Dogs kept in pens ejaculated 346.7 sec) in average. The type of housing showed a statistically highly significant difference ($P \leq 0.01$). In terms of utilization the longest ejaculation time was found in family dogs, the shortest time in Police forces dogs, see Table II. A statistically highly conclusive difference ($P \leq 0.01$) was found in the length of ejaculation also in terms of utilization, unlike collection points and type of nutrition. Vágenknechtová *et al.* (2010) state that the type of nutrition did not have any effect on the length of semen collection, when the length of collection in dogs fed by commercial dry pet-food was 348.0 sec, in dogs fed by combined dry pet-food plus meat nutrition it was 432.0 sec and in dogs fed by home prepared food it was 402.0 sec. A statistically conclusive difference was found only in the influence of number of dogs in a household, when they observed trend of reduction of ejaculation length with increased number of dogs in the household. The largest volume of semen was found in dogs kept in household (11 cm^3), by contrast, dogs with freedom of movement had the lowest volume of ejaculate, only 4.2 cm^3, see Table II. A statistically highly significant difference ($P \leq 0.01$) was found in the volume of ejaculate in terms of housing. Similarly such difference ($P \leq 0.01$) was found in volume of ejaculate between dogs for sports (12.7 cm^3) and family and Police forces dogs (6.3 cm^3, respectively 5.7 cm^3). Statistically conclusive ($P \leq 0.05$) and highly conclusive ($P \leq 0.01$) difference was found in volume of ejaculate in terms of collection point, respectively type of nutrition, see Table II. Rijsselaere *et al.* (2002) state the volume of the second fraction of ejaculate of 3.9 ± 1.16 cm^3, in dogs housed in pens with access to yard, which is a value comparable to our findings in dogs with freedom of movement. Also Vágenknechtová *et al.* (2010) state similar values, especially for the type of nutrition.

Statistically highly conclusive difference ($P \leq 0.01$) in sperm activity was found between a group of dogs with freedom of movement (35.0%) and dogs housed in pens and households (77.8%, respectively 77.00%). A similar difference in sperm activity was found also in type of utilization, where Police forces dogs had the percentage of 49.4% compared to 76.7% in sport dogs, respectively 77.8% in family dogs, see Table II. In terms of point of collection, a highly conclusive difference ($P \leq 0.01$) was found between dogs collected in households and dogs collected in laboratory. Type of nutrition had no conclusive effect on the sperm activity. These findings do not match the values stated by Vágenknechtová *et al.* (2010) where they found a conclusive difference ($P \leq 0.05$) between feeding by commercial dry pet-food and homemade feeding. Both Rijsselaere *et al.* (2002) and Kojima *et al.* (2001) state higher sperm activity in dogs fed exclusively by commercial dry pet-food compared to finding of our observation, whereas England *et al.* (1999) and Filipčík *et al.* (2011) both report values of sperm activity comparable with our results. In another work England *et al.*, (2010) report that among 48 evaluated retrievers 8 males had low sperm activity (up to 60%), 25 dogs had medium sperm activity (65–75%) and 15 dogs had high sperm activity (over 80%). This is consistent with our findings when in none of evaluated factors an average 80% limit in sperm activity was exceeded.

The lowest sperm concentration was found in dogs kept in households ($124.10^3.mm^{-3}$), dogs kept in pens had $152.10^3.mm^{-3}$ sperm concentration and dogs with freedom of movement had $180.10^3.mm^{-3}$. A statistically highly conclusive difference ($P \leq 0.01$) was found between group of dogs kept in households and dogs with freedom of movement. Similarly, a difference was found in a sperm concentration in Police forces dogs ($175.10^3.mm^{-3}$) and sport and family dogs ($126.5.10^3.mm^{-3}$, resp. $135.0.10^3.mm^{-3}$).

Dogs collected at home had an average concentration of $102.7.10^3.mm^{-3}$, compared to dogs collected in the laboratory with concentration of $184.5.10^3.mm^{-3}$. A highly significant difference ($P \leq 0.01$) was found between collections performed at homes and in the laboratory. Type of nutrition had no influence on sperm concentration. Similar concentrations are reported also in Vágenknechtová

et al. (2010) or in Filipčík *et al.* (2011) when the first one found no significant differences in sperm concentration for the form of feeding dogs. A lower sperm concentration in dogs housed in pens with access to yard and fed by the commercial dry pet-food was found by Rijsselaere *et al.* (2002). Although they report comparable value of sperm concentration as we do in our observations, it is a value found only for the second – sperm-rich fraction, whereas our findings are for the whole volume of ejaculate, including the first and the third fraction. Significantly lower sperm concentration in Beagles report Kojima *et al.* (2001) when they found only $20.6.10^3.mm^{-3}$sperm concentration in dogs collected by electroejaculation under anesthesia. In contrast England *et al.* (2010) state that out of 48 retrievers observed for heritability of semen parameters, only 9 had sperm concentration up to $300.10^3.mm^{-3}$, 20 dogs had a concentration between $310–690.10^3.mm^{-3}$, and 19 had a concentration higher than $700.10^3.mm^{-3}$ that is significantly more than in our observation.

CONCLUSION

In conclusion, we can say that the external factors had greater influence on the quality of ejaculate than internal ones. Housing and utilization of dogs had the greatest influence as we found statistically significant influence of them both on the monitored reproduction parameters. The type of nutrition had a statistically significant impact only on the volume of ejaculate. Among the internal factors, the influence of the order of collection on the volume of ejaculate has been proved as well as the influence of the weight of a dog on the onset of sexual reflexes. The age of dogs had no conclusive influence on the monitored parameters. It can therefore be recommended to breeders to pay an increased attention to the quality of housing and the utilization of dogs; sport activity of dog had a positive impact on the quality of ejaculate in observed dogs.

SUMMARY

Average length of preparation of dogs for the collection and onset of sexual reflexes was 95.7 sec. At the first collection dogs needed longer time for preparation because of later onset of sexual reflexes (171.0 sec). At the second and third collection the average length of preparation was reduced to 72.0 sec, respectively to 44.0 sec. when despite a significant reduction of preparation time and of the onset of sexual reflexes no statistically conclusive difference was found between the collections. Older dogs needed longer time for preparation for the collection (103.9 sec) compared to younger dogs (83.3 sec). Even here, no statistically conclusive difference was found. In terms of weight of dogs a highly significant difference ($P \leq 0.01$) in onset of sexual reflexes was found between all observed groups with smallest dogs having the shortest time (71.1 sec), medium sized dogs needing average of 97.8 sec and large dogs needing 104.0 sec for the onset of reflexes.

The observation showed that none of the evaluated internal factors had any affect on the length of the collection itself. The second collection required the longest time (456.0 sec), younger dogs had longer average collection time (435.0 sec) compared to older ones (370.0 sec). Medium sized dogs had the longest average of semen collection (480.0 sec) compared to small (440.0 sec) and large dogs (328.0 sec). The highest volume of the semen came from the first collection (10.5 cm^3), the volume from the second collection was lower (8.9 cm^3) and the third collection gave only 6.6 cm^3 of ejaculate, a highly significant difference ($P \leq 0.01$) was thus found in the order of collection. Young dogs' ejaculate volume was 8.8 cm^3, older dogs' was 8.6 cm^3, average volume of semen in the whole set of observed dogs was 8.7 cm^3. Among groups there was no statistically conclusive difference.

The average sperm activity in the whole observed set was 68.8%. The activity of sperms declined from the first to the third collection from values 73.0% to 65.5%. The activity was higher in younger dogs almost in 10 percent (74.6%) compared to older dogs (65.0%). In terms of weight of dogs we noticed the lowest activity in large dogs (58.7%), whereas in small and medium sized dogs it was significantly higher (80.0%, respectively 78.3%). The difference in activity of sperms was not statistically conclusive in any indicator.

The concentration of sperms in the whole set was $143.6\ 10^3.mm^{-3}$ and was not significantly affected by any of observed internal factors which proves there was no statistically conclusive difference found between groups.

In terms of housing we found the longest preparation and onset of sexual reflexes was in dogs housed in pens (134.4 sec) and the shortest in dogs with freedom of movement (65 sec). Dogs kept at homes of breeders needed preparation time of 84.7 sec. The longest emergence of sexual reflexes (130.0 sec) was found in Police forces dogs compared to family dogs (97.8 sec) and sport dogs (68.3 sec). Dogs collected in laboratory had longer onset of sexual reflexes (110.7 sec) than dogs collected at homes (80.7 sec). Dogs fed by combined diet of dry pet-food and meat had shortest onset of sexual reflexes

(71.3 sec) than dogs fed exclusively by dry pet-food (120.0 sec). Type of housing, utilization of dogs and place of collection had a highly conclusive effect ($P \leq 0.01$) on the length of preparation of dogs for semen collection.

The average length of ejaculation of whole ejaculate was 396.0 sec in the whole observed set. In terms of housing the ejaculation was longest in dogs kept in households (468.0 sec), followed by dogs housed in pens (346.7 sec) and with the shortest in dogs with freedom of movement (290.0 sec). A statistically highly conclusive difference was found between types of housing ($P \leq 0.01$). In terms of utilization of dogs we found the longest ejaculation time in family dogs and the shortest in Police forces dogs. For ways of utilization of dogs we found a statistically highly conclusive difference ($P \leq 0.01$) in the length of ejaculation, unlike the place of collection and type of nutrition.

The highest volume of semen was found in dogs kept in households (11 cm^3), whereas dogs with freedom of movement had the lowest volume (4.2 cm^3). Dogs used for sports, family dogs and Police forces dogs had the semen volume of 12.7 cm^3, 6.3 cm^3, respectively 5.7 cm^3. Among the ways of housing and utilization of dogs we found a statistically highly conclusive difference ($P \leq 0.01$) for the volume of ejaculate. Statistically conclusive ($P \leq 0.05$) and highly conclusive ($P \leq 0.01$) difference was found in volume of ejaculate for the place of collection, respectively type of nutrition.

Statistically highly significant difference ($P \leq 0.01$) in the activity of sperm was found between a group of dogs with freedom of movement (35.0%) and dogs housed in pens or the owner's household (77.8%, respectively 77.0%). Similarly as in utilization of dogs where the Police forces had the sperm activity of 49.4% compared to 76.7% and 77.8% in sports dogs and family dogs respectively.

In terms of points of collection, a highly conclusive difference ($P \leq 0.01$) was found between dogs collected at home and dogs collected in the laboratory. The type of nutrition had no conclusive affect on the sperm activity.

The lowest sperm concentration was found in dogs kept at homes ($124.10^3.mm^{-3}$), followed by dogs housed in pens ($152.10^3.mm^{-3}$) and dogs with freedom of movement ($180.10^3.mm^{-3}$). Police forces dogs, sport dogs and family dogs had the sperm concentration of $175.10^3.mm^{-3}$, $126,5.10^3.mm^{-3}$, respectively $135,0.10^3.mm^{-3}$. Dogs collected at homes showed the average concentration of $102,7.10^3.mm^{-3}$, in contrary to those collected in the laboratory ($184,5.10^3.mm^{-3}$). Statistically highly significant differences ($P \leq 0.01$) was found between dogs kept in households and dogs with freedom of movement, between Police forces dogs, sportive dogs and family dogs and between dogs collected at homes and in the laboratory. The type of nutrition had no effect on the sperm concentration.

External factors showed greater impact on the quality of ejaculate with greatest effect of the type of housing and utilization of a dog that both affected all monitored parameters. In contrast, the type of nutrition conclusively affected only the volume of ejaculate. Among the internal factors, the influence of the order of collection on the volume of ejaculate has been proved as well as the influence of the weight of a dog on the onset of sexual reflexes. The age of dogs had no conclusive impact on monitored parameters.

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