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THE EFFECT OF THE AGE OF DOGS ON THEIR EJACULATE

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Abstract

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The effect of the age of dogs on quantitative and qualitative parameters of the ejaculate was explored. We evaluated 43 dogs; from each dog we collected three samples on three successive days. The dogs were divided into three age categories: 1.5–2 years; 2–3 years; and 3–5 years. The maximum ejaculate volume was collected from dogs of 2–3 years of age; the average volume of ejaculate from 3 collections was 9.1 ml. Microscopic examinations of the ejaculate of this age category of dogs showed that the highest average sperm concentration was $153.62.10^3.mm^{-3}$. In older dogs the ejaculate volume decreased considerably with increasing frequency of collections (8 ml > 5 ml > 2 ml at the 3rd collection). The amount of motile sperm was the highest in 3 to 5-year-old dogs (76%) and was related with the highest proportion of morphologically normal sperm (66.8%). However in the 3rd collection of this age category we saw a significant ($P < 0.05$) increase in the amount of pathologically changed sperm (29.5 < 28.8 < 41.2%), mostly in the structure of the flagellum. Significant differences in the quantity and quality of the ejaculate were discovered not only among the age categories of the dogs but also among the individual collections.

dog ejaculate, volume of ejaculate, sperm activity, sperm concentration

The number of breeders involved in dog breeding is relatively high. However if the owner of the dog is to be a successful breeder his dog must produce a sufficient amount of good-quality ejaculate capable of fertilisation. The fertility of dogs can be evaluated in two ways. The breeder may wait to see if the bitch will conceive after mating or if she gives birth to pups. However, such an approach is unprofessional; for reproduction purposes the breeder should use only dogs whose qualitative and quantitative parameters of the ejaculate had been examined. The birth of pups should be taken as the final confirmation of the fertility of the dog. DEIBEL *et al.* (1976), DUNPHY (1989), RIGAU *et al.* (2001) evaluated the basic qualitative parameters of the canine ejaculate. According to KUTZLER (2005) the optimal room temperature for ejaculate collection is 20 °C; the colour, volume, motility, concentration and morphological structure of the ejaculate should be evaluated. GUNAY *et al.* (2003) evaluated the sperm fraction of the ejaculate of seven German shepherd dogs. On one day the ejaculate was collected twice within 60 minutes. Between the first and the second collection the authors discovered statistically significant ($P < 0.05$) differences in the volume of the ejaculate and sperm concentration, but not in the motility, live sperm count and amount of morphological changes in the sperm structure. SCHAFER *et al.* (1997) collected canine ejaculates twice a week over a period of 6 months and they discovered significant changes only in the volume of the ejaculate. VĚŽNÍK *et al.* (2003) evaluated the quality of ejaculate of dogs of ages ranging from 2 to 6 years; they discovered that sperm motility was at a 74% level, and that the ejaculate contained 81% of live sperm. Pathological changes were discovered in 42.5% of the sperm. According to PŘINOSILOVÁ *et al.* (2005) the sperm concentration in fresh canine ejaculate was 198.5. 10^6.ml. The ejaculate was kept in liquid nitrogen and after thawing the concentration

was 99.4. 10^6.ml. ENGLAND (1999) stated that the volume of the sperm fraction in German shepherd dogs was 4.1 ml and sperm motility was 65%. VERSTEGEN *et al.* (2005) evaluated sperm motility in the course of 27 days from the collection of the ejaculate kept at a temperature of 4 °C. Up to day 10 after the collection the sperm motility was more than 90% and then the sperm motility rapidly decreased.

MATERIAL AND METHODS

We evaluated the quality of ejaculate from 43 dogs of 8 breeds (Malinois, Tervueren, German Spitz, Poodle, German shepherd, Australian shepherd, Irish setter, Weimaraner). The dogs were divided into 3 age categories (1: 1.5–2 years; 2: 2–3 years; 3: 3–5 years of age). Ejaculates were collected by manual manipulation in plastic tubes. Three ejaculates were obtained from each dog (once a day, three days running). A total of 129 ejaculates were analysed. Immediately after collection the ejaculates were subjected to macroscopic examination – the volume was measured in calibrated containers; also the motility of sperm was immediately evaluated subjectively. We evaluated the percentage of sperm with a progressive direct movement after the head. Recorded were 3 fields of view in a microscope at 200–300x magnification. Sperm concentration was evaluated haemocytometrically and the number of sperm was counted in Bürker's chamber. Morphological evaluation of the ejaculate was conducted on preparations stained using the method of Farelly (SEVERA *et al.*, 2010). In each preparation 200 sperm were evaluated and if they had no defect they were evaluated as "morphologically normal sperm" (PEÑA MARTÍNEZ, 2004). Next we evaluated the total pathology which was characterised in greater detail by changes on the head, acrosome, attachment of the head to the flagellum, changes in the flagellum, immature and degenerated sperm. Morphological examination of the sperm was conducted at 1000x magnification using immersion oil. Data were statistically analysed using the statistical package STATISTICA 9.0, by means of variance analysis:

$$y_{ijk} = \mu + A_i + C_j + e_{ijk,}$$

that

A ... age categories (1.5–2 years; 2–3 years; 3–5 years of age),

C.... time of collection (once a day, three days running),

e .... residuum.

Tukey's HSD test was used to determine the statistically significant differences.

RESULTS AND DISCUSSION

The average volume of the first collection of ejaculate was 8.35 ml; the volume of the ejaculate from dogs of the age of 1.5–2 years was 6.00 ± 1.48 ml. The volume was significantly ($P < 0.05$) the highest in age category 2–3 years (11.05 ± 4.31 ml). From 3 to 5 year-old dogs we obtained 8.00 ± 2.35 ml of ejaculate in the first collection (Tab. I). In this age category the volume of the ejaculate of the following collections decreased considerably (2nd collection 5.00 ± 2.08, 3rd collection 2.00 ± 0.38 ml). Statistically highly significant differences ($P < 0.01$) were recorded between the volume of the ejaculate at the first collection and at the third collection. A similar trend, i.e. reduced volume of ejaculate, was also discovered in the second age category where the sperm amount decreased from 11.05 ml to 8.44 ± 4.62 ml and to 7.82 ± 2.35 ml at the second and third collection, respectively. The opposite was seen in dogs of the age of 1.5–2 years where the semen volume in the second collection increased more than twofold ($P < 0.05$) (12.50 ± 5.39 ml). The volume of the ejaculate collected on the third day was almost the same (6.93 ± 2.68 ml) as the volume of the first collection. ENGLAND (1999) reported that the volume of the second fraction of ejaculate from a German shepherd was 4.1 ± 0.9 ml. On the other hand DOSTAL *et al.*, 2001) reported volumes of canine ejaculate comparable with our results (7.0 ± 0.95 ml). The highest sperm motility was monitored in dogs of 3–5 years of age; the estimated motility of the first and second collection was at a level of 80% and in the third collection the amount of motile sperm dropped to 70 ± 10%. In dogs of 1.5–2 years of age the motility of sperm was the highest in the first collection (79 ± 6%); in the second collection the number of motile sperm decreased to 67 ± 14%. In the third collection the proportion of motile sperm again increased (73 ± 5%). The worst sperm motility was detected in dogs of 2–3 years of age. In the first collection the motility of sperm in the ejaculate was at a level of 68% with a relatively high variability of 23%. In further collections the proportion of motile sperm decreased (67 ± 17% and 59 ± 10% in the second and third collection, respectively). JOHNSTON *et al.* (2001) reported that the optimal motility of sperm in the dog ejaculate was 70–90%. In dogs of 1 to 11 years of age PŘINOSILOVÁ *et al.* (2005) discovered a relatively wide range in sperm motility, i.e. between 35 and 95%; the average was at a comparable level of 77 ± 13%. Sperm motility is very closely connected with sperm concentration in the ejaculate. The surprisingly highest sperm count was seen in the third collection of the oldest age category of dogs (218.00 ± 40.19. $10^3.mm^{-3}$). In the first collections the sperm count was the highest in ejaculates of dogs of 1.5–2 years of age (195.91 ± 38.27. $10^3.mm^{-3}$).

In the other age categories the sperm counts in the first collections were virtually balanced (111.36 ± 44.57. $10^3.mm^{-3}$ and 100.00 ± 39.42. $10^3.mm^{-3}$, respectively). The sperm count in the ejaculate was the same also in the second collection from dogs of the age of 1.5–2 years (73.33 ± 4.92. $10^3.mm^{-3}$) and dogs of the age of 3–5 years (70.00 ± 2.14. $10^3.mm^{-3}$). The sperm count increased relatively markedly in dogs

I: *The effect of age and frequency of collection on the quality of canine ejaculate*

Indicator		collection	Age (1.5–2 years) (A)	Age (2–3 years) (B)	Age (3–5 years) (C)
			$\mu \pm s_x$	$\mu \pm s_x$	$\mu \pm s_x$
			n = 11	n = 22	n = 10
Ejaculate volume (ml)		1	$6.00^{b2} \pm 1.48$	$11.05^{a} \pm 4.31$	$8{,}00^{3} \pm 2.35$
		2	$12.50^{bC13} \pm 5.39$	$8.44^{ac} \pm 4.62$	$5.00^{Ab} \pm 2.08$
		3	$6.93^{C2} \pm 2.68$	$7.82^{C} \pm 2.35$	$2.00^{AB1} \pm 0.38$
Sperm motility (%)		1	79 ± 6	68 ± 23	80 ± 19
		2	67 ± 14	$67^{c} \pm 17$	$80^{b} \pm 10$
		3	$73^{b} \pm 5$	$59^{a} \pm 10$	70 ± 10
Concentration of sperm ($10^3.mm^{-3}$)		1	$195.91^{BC23} \pm 38.27$	$111.36^{A2} \pm 44.57$	$100.00^{A23} \pm 39.42$
		2	$71.07^{B1} \pm 29.62$	$195.93^{AC1} \pm 61.51$	$70.00^{B13} \pm 25.14$
		3	$110.00^{C1} \pm 18.59$	$153.57^{c} \pm 51.33$	$218.00^{Ab12} \pm 40.19$
Morphologically normal sperm (%)		1	$73.09^{b} \pm 5.54$	$64.19^{a} \pm 19.95$	$70.50^{3} \pm 4.65$
		2	67.43 ± 10.42	59.41 ± 18.11	71.17 ± 12.54
		3	64.75 ± 24.45	60.93 ± 20.36	$58.75^{1} \pm 21.27$
Pathological changes	Changes on the head (%)	1	$5.82^{23} \pm 3.16$	7.05 ± 3.23	$4.00^{3} \pm 2.16$
		2	$10.36^{1} \pm 2.17$	8.52 ± 4.31	6.33 ± 2.25
		3	$11.50^{1} \pm 5.22$	8.36 ± 3.23	$8.50^{1} \pm 3.32$
	Changes on the acrosome (%)	1	2.00 ± 0.77	1.82 ± 0.52	$1.25^{3} \pm 0.50$
		2	1.57 ± 0.99	$2.22^{c} \pm 0.65$	$0.50^{b3} \pm 0.08$
		3	$1.00^{c} \pm 0.13$	2.57 ± 0.41	$3.00^{a12} \pm 0.90$
	Attachment of the flagellum (%)	1	3.64 ± 0.95	2.91 ± 0.71	3.50 ± 0.91
		2	2.86 ± 0.21	3.15 ± 0.23	3.33 ± 0.08
		3	3.83 ± 0.38	3.11 ± 0.28	4.50 ± 0.04
	Changes on the flagellum (%)	1	$9.82^{B} \pm 3.49$	$16.11^{Ac} \pm 2.98$	$10.75^{b} \pm 3.20$
		2	$12.50^{b} \pm 4.38$	$20.32^{ac} \pm 6.00$	$12.33^{b} \pm 4.52$
		3	$10.75^{b} \pm 3.31$	$18.79^{a} \pm 4.41$	16.00 ± 5.60
	Immature sperm (%)	1	5.18 ± 1.96	$4.63^{c} \pm 1.14$	$9.75^{b2} \pm 2.35$
		2	4.86 ± 1.77	6.91 ± 1.37	$3.33^{13} \pm 0.25$
		3	6.92 ± 1.93	$4.61^{c} \pm 1.05$	$9.75^{b2} \pm 3.40$
	Degenerated sperm (%)	1	$0.45^{b3} \pm 0.19$	$1.59^{ac} \pm 0.74$	$0.25^{b3} \pm 0.05$
		2	$0.43^{b3} \pm 0.05$	$1.15^{a} \pm 0.38$	$0.67^{3} \pm 0.05$
		3	$1.25^{c12} \pm 0.36$	1.68 ± 0.52	$2.00^{b12} \pm 0.41$
	Total pathology (%)	1	$26.91^{b3} \pm 5.54$	$35.81^{a} \pm 10.95$	$29.50^{3} \pm 4.65$
		2	32.57 ± 8.42	$40.59^{c} \pm 12.11$	$28.83^{b3} \pm 5.54$
		3	$35.25^{1} \pm 4.45$	39.07 ± 11.36	$41.25^{12} \pm 13.27$

a, b, c, and/or A, B, C = significant differences among age categories of dogs ($P < 0.05$), or ($P < 0.01$);
1, 2, 3 = significant differences ($P < 0.05$) among the individual collections

of 2–3 years of age; in the second collection the sperm count increased to 195.93 ± 61.51. $10^3.mm^{-3}$). It was the opposite in the third collection when the sperm count in the ejaculate of the other age categories increased (110.00 ± 18.59. $10^3.mm^{-3}$ in the age category 1.5–2 years and 218.00 ± 40.19. $10^3.mm^{-3}$ in the age category 3–5 years), the sperm concentration decreased to 153.57 ± 51.33. $10^3.mm^{-3}$. Significant differences ($P < 0.01$) in the concentration of sperm in canine ejaculate were discovered among the individual age categories of dogs and also among the individual collections ($P < 0.05$). VÁGENKNECHTOVÁ *et al.* (2010) evaluated the effect of the weight of the dog on the sperm concentration in the ejaculate. Even though they discovered that the sperm count varied greatly among the respective groups of dogs (65.8–205.0. $10^3.mm^3$) they did not prove a significant correlation between the weight of the dog and the sperm concentration. When evaluating the morphological structure of the sperm the highest per cent of morphologically normal spermatozoa was found in the first collections of all

age categories and the ejaculate of the youngest age categories of dogs contained most of the developmentally sound sperm (73.09 ± 5.54%). The smallest proportion of normal sperm was detected in dogs of 2–3 years of age (64.19%) as well as a relatively high variability (19.95%) among the individual ejaculates. In further collections we saw an insignificant ($P > 0.05$) and almost linear decrease in the number of morphologically normal sperm in the dog ejaculates; in the third collection the proportion of non-pathological sperm was 64.75% (age group 1.5–2 years), 60.93% (age group 2–3 years) and 58.75% (age group 3–5 years). Statistically significant ($P < 0.05$) differences were discovered in the first collections between dogs of the age of 1.5–2 years and 2–3 years, i.e. 73.09 and 64.19%, respectively. Significant differences in the numbers of morphologically normal sperm were monitored in dogs of 3–5 years of age in the first (70.50%) and third (58.75%) collections. VĚŽNÍK *et al.* (2003) reported that the proportion of normal sperm in fresh ejaculate was 57.5 ± 12.8% and the proportion of pathological sperm was 42.5 ± 12.85%. Dogs of 2–3 years of age had the highest proportion of pathologically changed sperm (38.49%); in the first collection 35.81 ± 10.95% of pathological changes were detected and in the second and third collections we saw that the proportion of abnormal sperm increased significantly ($P > 0.05$) to 40.59 ± 12.11% and 39.07 ± 11.3,6%, respectively. The proportion of pathological changes was the lowest in the youngest age category of dogs; in the ejaculate from the first collection we detected 26.91 ± 5.54% of pathologically changed sperm; in the second and third collections this proportion increased to 32.57 ± 8.42% and 35.25 ± 4.45%, respectively. This trend was the same in the oldest age category of dogs as well; between the first and second collection no significant increase in the proportion of pathological changes of sperm were detected, but in the third collection the proportion of total pathological changes increased significantly ($P < 0.05$) (41.25 ± 13.27%). KODERLE *et al.* (2009), SCHÄFER-SOMI *et al.* (2006) reported a proportion of morphologically changed sperm in canine ejaculate comparable with our conclusions (26.3 ± 10.8% and 26.6 ± 8.1%). The worst results in the morphological structure of sperm of dogs of 2–3 years of age were caused mainly by the pathological structure of the flagellum; the first collection showed 16.11 ± 2.98% of pathological changes, in the second collection the proportion increased to 20.32 ± 6.00% and in the third collection we saw a slight decrease in the proportion of changes on the flagellum (18.79 ± 4.41%). DOSTAL *et al.* (2001) published comparable data on the number of changes on the flagellum (17.4 ± 6.81%). By contrast NÖTHLING *et al.* (2007) reported that the proportion of changes on the flagellum in fresh ejaculate was 5.8 ± 1.9%. The occurrence of pathological changes on the flagellum was also seen in dogs of 1.5–2 years of age; in the first collection we detected 9.82 ± 3.49% of changes in the structure of the flagellum, in the second collection it increased to 12.50 ± 4.38% and in the third collection it decreased to 10.75 ± 3.31%. A linear growth ($10.75 < 12.33 < 16.00\%$) in the number of changes on the flagellum was seen in the third age group of dogs. In the first collections highly significant differences ($P < 0.01$) were detected in the number of changes in the structure of the flagellum between the first (1.5–2 years) and second (2–3 years) age categories of dogs; significant differences ($P < 0.05$) were also detected in the number of pathological changes on the flagellum between sperm collected from dogs of 2–3 and 3–5 years of age. In the second and third collections significant differences ($P < 0.05$) were detected in the number of changes on the flagellum between dogs of 1.5–2 and 2–3 years of age. The number of sperm with a pathologically attached flagellum to the head was relatively balanced in all the age categories of dogs and ranged between 2.86% (dogs of 1.5–2 years of age) and 4.50% (dogs of 3–5 years of age). Most of the changes in the structure of the sperm head were detected in the youngest dogs; in the ejaculate from the first collection we detected 5.82 ± 3.16% of defects. In the second and third collections they increased significantly ($P < 0.05$) to 10.36 ± 2.17% and 11.50 ± 5.22%, respectively. In dogs of 2–3 years of age the number of changes on the sperm head in the individual collections was relatively balanced and ranged between 7.05 and 8.52%. In the oldest age category of dogs significant ($P < 0.05$) differences were detected in the number of changes on the sperm head between the first and third collection; i.e. 4.00% and 8.50%, respectively. Among the individual age categories of dogs no statistically significant ($P < 0.05$) differences were discovered among the individual collections. Apart from the pathological shapes of the sperm heads we evaluated separately the changes on the acrosome. The number of defects in the development of the acrosome ranged between 0.50% (in the second collection from dogs of the age of 3–5 years) and 3.00% (in the third collection from dogs of the same age). NÖTHLING *et al.* (2005) reported relatively numerous changes on the acrosome; on the basis of analyses of ejaculates of 8 dog breeds they reported a 10.75% proportion of acrosome abnormalities. The number of immature sperm was relatively stable in the individual collections, only in the oldest age category significant ($P < 0.05$) differences were detected in the number of immature sperm between the 1st and 2nd and the 2nd and 3rd collections. In dogs of this age category the number of immature sperm was the highest ($P < 0.05$) when compared with the younger age categories where the proportion of incompletely developed sperm ranged between 5 and 7%. Out of the total number of pathological defects in the formation of sperm the lowest number was that of the so-called degenerated sperm. This defect ranged between 0.25 and 2.00% and was the highest ($P < 0.05$) in 2–3 year old dogs. In this parameter the correlation between the number of degenerated sperm and sequence of collection was

confirmed; in the ejaculate of dogs of 1.5–2 years of age and 3–5 years of age significant differences were monitored between the first two collections and the third collection where the number of degenerated sperm increased significantly ($P < 0.05$).

CONCLUSION

The reproduction capacity of the dog is basically influenced by its age. A correlation was confirmed between the age of the dog and the volume of the ejaculate; dogs of 2–3 years of age had the largest volume of sperm and highest sperm concentration. By contrast sperm motility of this age category was the lowest. The ejaculate of dogs of 3–5 years of age contained the highest number of motile sperm and with it related highest proportion of morphologically normal sperm. However, in this age category the amount of pathologically changed sperm increased significantly in the 3rd collection. This parameter was evaluated as the best in dogs of the youngest age category. The highest number of pathological changes in the structure of canine sperm was connected with the formation of the flagellum; relatively frequent was the occurrence of torsions of the flagellum. On the other hand the least frequently occurring were degenerated sperm. We can conclude that with increasing age of the dog the volume of the ejaculate decreases and with an increasing frequency of collections the occurrence of pathological changes in the structure of sperm increases significantly.

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