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Age determination of the Arctic Fox (*Vulpes lagopus*) using the pulp cavity–tooth width ratio

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Abstract

The aim of this study was to ascertain the suitability of an age determination method which uses the ratio between the width of the pulp cavity and the width of the tooth itself. The measurements were obtained from X-ray pictures of individual teeth using an intraoral sensor. We found that with age, the ratio decreased, and that this change was more prominent in the maxillary canines (104 and 204) than the mandibular (304 and 404). The Arctic fox heads used in this study were sourced from a private fur farm from Poland, and were deep-frozen until the research began.

The results suggest that the method we used is reliable for determining the age of an individual, however, it can be inaccurate due to the fact it doesn't consider three-dimensional changes occurring in the dental cavity and the shape of the tooth. On the other hand, this method is far more accessible and simpler than, for example, one utilising three-dimensional imaging, since most veterinary practices are equipped with basic X-ray equipment.

Running title: Determining the age of a fox by its teeth

Keywords: age determination, pulp cavity, radiography

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Introduction

Teeth are one of the strongest tissues in a living organism, which is directly tied to their purpose: food intake and grinding. Their resilience to wear, exposure to various agents (chemical, physical, thermal) and hormonal or nutritional fluctuations makes them the most suitable to be used in determining the age of an individual [1,2]. The most suitable teeth for this type of research are canines, because they are the least susceptible to dietary fluctuations [1-3] and have a high level of longevity compared to other teeth [1].

Having a reliable method of determining an animal's age is an essential tool in the veterinary profession, especially when working with animals whose exact date of birth is not known, such as wild or stray animals. Knowing even the approximate age of an individual allows a better understanding of the cause of a possible condition, which in turn allows for more accurate diagnosis and treatment, or to determine the population structure, which is important especially when working with wild animals.

Morris [4] highlights the following methods for age determination:

- based on the dimensional growth,
- based on the structural degradation,
- assessment of age derivatives based on changes in growth,
- determination of actual age based on growth lines (growth rings).

Methods for determining and estimating the age of mammals can furthermore be divided into so-called "field" and laboratory methods, living and post mortem.

The choice of age determination method depends on the needs and technical and financial possibilities. Some methods of age determination require a histological preparation of the tooth [5,6], others use computed tomography to create a 3D model of the tooth [7]. These methods are time-consuming and expensive, which means that not every veterinarian can afford to use them in their work. Another method that is often used in pet dogs is to assess the attrition of cusps present on the teeth, however, this method only works for young individuals, because once all cusps have worn away, the age of the individual can no longer be determined [8]. In addition, this method of age determination is not universal across carnivore species or even between individuals of the same species, as the degree of tooth wear also depends on diet (especially mineral content), physiological state, chewing pattern or dental occlusion pattern [9,10].

According to Wood, tooth wear is a very good indicator of an animal's age, with tooth loss and fracture also being age-dependent [11]. The Teeth Condition Index (TCI) is one method of age determining, whereby teeth are assessed in groups (incisors, canines or premolars/molars).

They are assigned a corresponding numerical value – the more worn teeth in a group, the greater the value. In summary, the TCI is the sum of these numerical values and ranges from 0 (teeth in excellent condition) to 9 (very worn teeth). This one is the method that has been used to determine the age of living polar foxes [12].

A far more versatile way of determining an animal's age is to use radiological methods – equipment for such an examination is available in most veterinary practices, and there is no long wait for measurements.

Changes in the dimensions of the dental cavity, associated with the appositional growth of secondary dentin [1,3,8-10,13-15] are a good way to determine the age of an animal.

The aim of this study was to verify the suitability of changes in the dental cavity metric parameters of the maxillary (104 = right canine, 204 = left canine) and mandibular (304 = left canine, 404 = right canine) canines determined by X-ray to determine the age of Arctic foxes. The age of individuals was known and based on breeding records. Applications of the research method are classified according to Morris [4] into a method of assessing age derivatives based on changes in growth.

Material and methods

The study was conducted on 15 Arctic foxes from a private fur farm from Poland. Information on the age of the animals was obtained from the breeding records and included the year of birth. The age of the animals studied ranged from 1 to 9 years. The skinned heads of 11 females and 4 males were collected for the study. Material was deep-frozen until the study and was thawed 3 days before the first analyses.

Preparation for the radiographs consisted of cleaning the gingival area around the canines and opening the temporomandibular joints to facilitate access to the teeth during the radiographs.

The study included measurements of the width of the dental cavity and the width of the maxillary and mandibular canines at the cementum-enamel border (neck). The diagnostic veterinary X-ray system ZEN-PX4 Vet* and the PortView intraoral sensor was used to obtain the measurements. After taking the images, the width of the entire tooth, as well as the dental cavity, was measured at the level of the neck in the PortView computer programme.

From the collected measurement data, the coefficient (ratio) of the dental cavity to the width of the tooth was calculated [3,8,10,13,15,16].

Results

The results of measurements of the width of the teeth (canines) of the studied male foxes (**Fig. 1,2**) are presented in **table 1** and of the females (**Fig. 3-5**) in **table 2**, and the dental cavity of the males in **table 3** and of the females in **table 4**. The values of ratios of the width of the dental cavity to the width

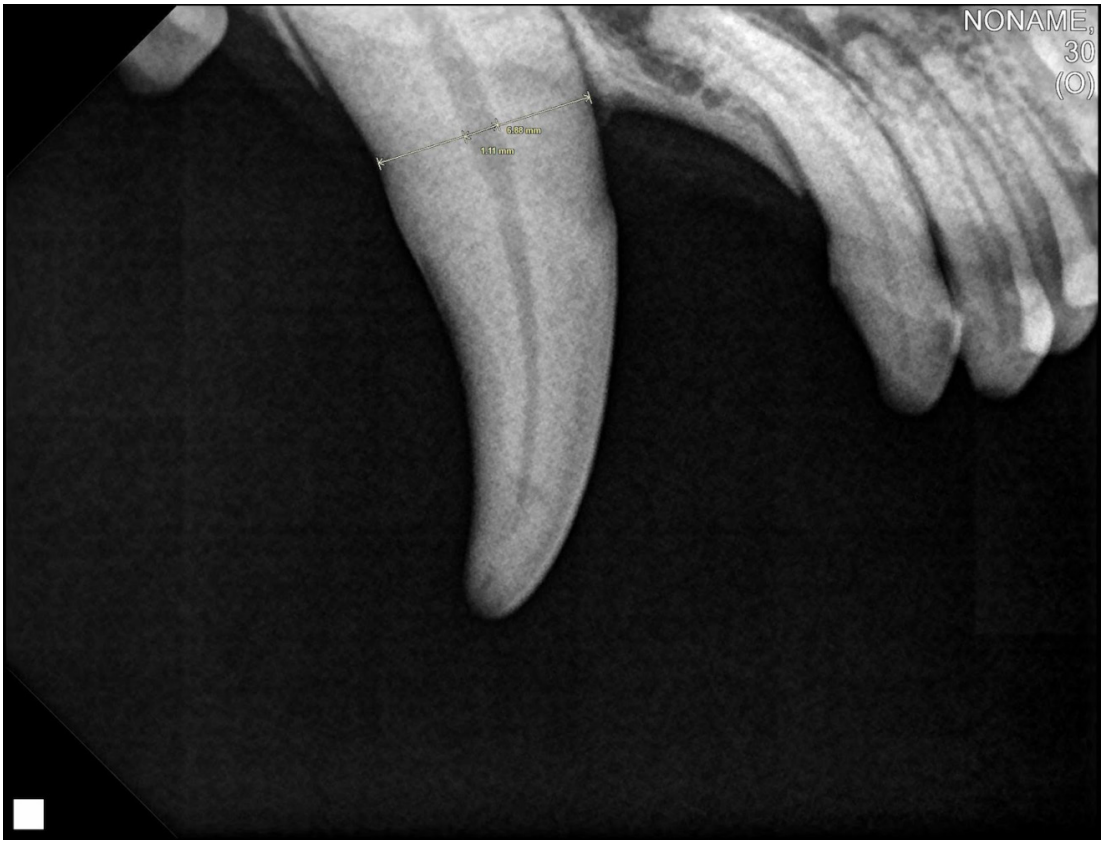

FIGURE 1 Tooth 104 of a 3-year-old male. Tooth width: 6.88 mm, tooth cavity width: 1.11 mm, tooth cavity width to tooth width ratio: 0.161

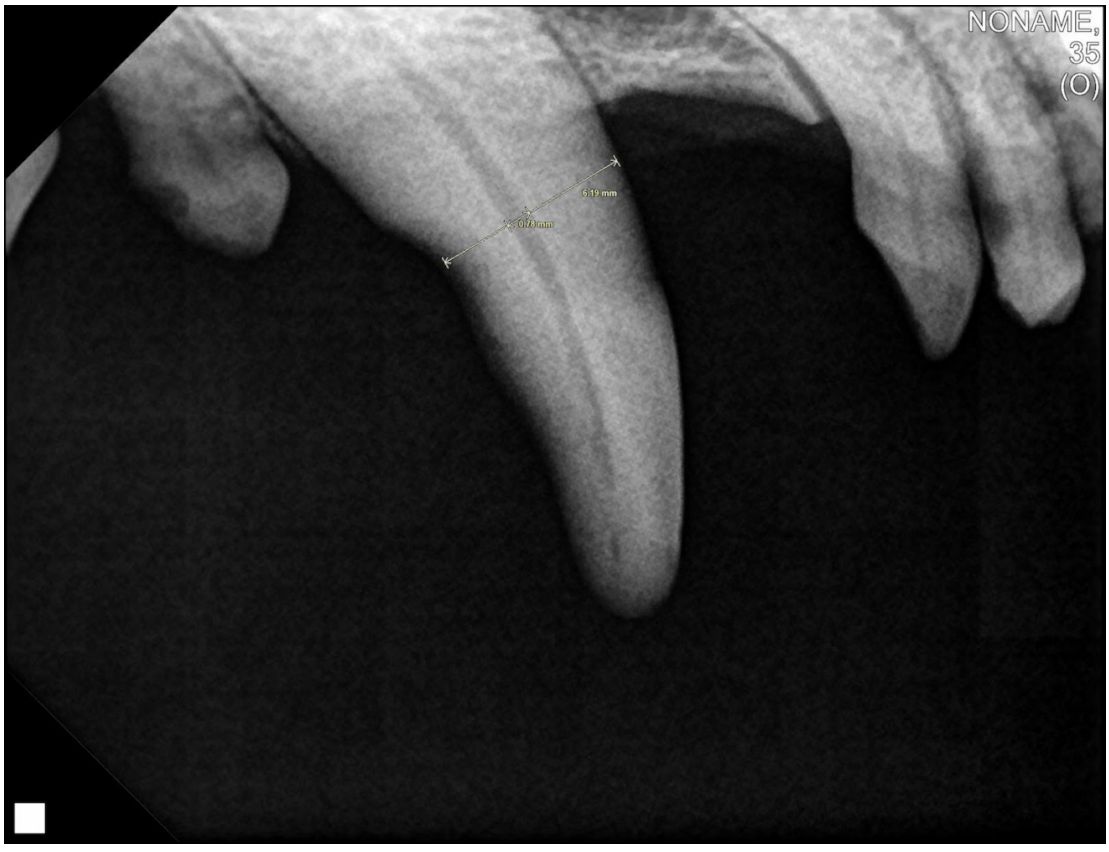

FIGURE 2 Tooth 104 of a 9-year-old male. Tooth width: 6.19 mm, tooth canal width: 0.78 mm, ratio of cavity width to tooth width: 0.126

TABLE 1 Tooth widths of males given in millimetres. Each tooth is described according to the Modified Triadan system. 104 – right upper canine. 204 – left upper canine. 304 – left lower canine. 404 – right lower canine

		TOOTH WIDTH (MM)			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
3	male	6,88	6,27	4,59	5,56
4	male	6,05	5,59	3,96	4,69
5	male	6,92	6,18	5,41	5,12
9	male	6,19	5,7	4,66	4,43

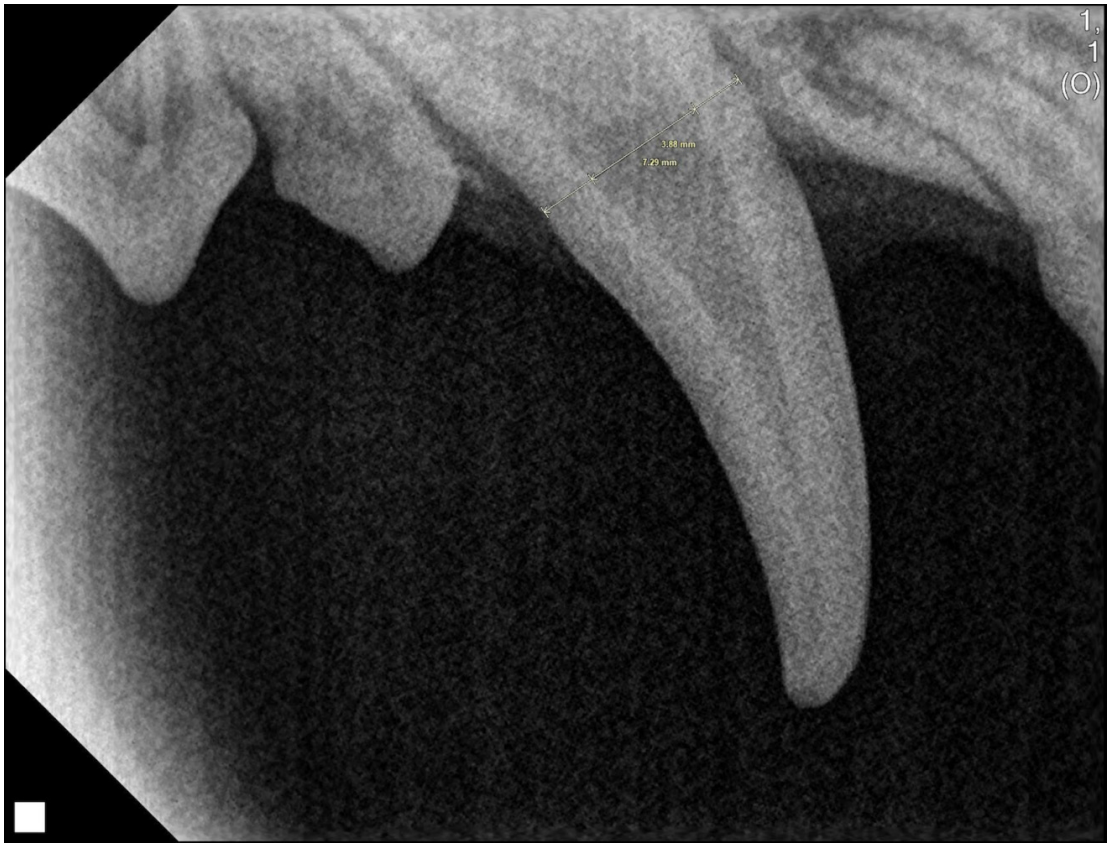

FIGURE 3 Tooth 104 of a 1-year-old female fox. Tooth width: 7.29 mm, tooth cavity width: 3.88 mm, tooth cavity width to tooth width ratio: 0.532

of the tooth of the males are presented in **table 5** and of the females in **table 6**. One of the 7-year-old females was discarded from the statistics due to the large measurement error burdening her results.

The small size of the study sample of males precluded a statistical analysis of the obtained results of measurements and calculations. Hence, they were limited to a tabular summary only. The values of measurements of males' teeth summarised in **table 3** show a clear difference in the size of the dental cavity in younger males – 3 and 4 years old – compared to older males – 5 and 9 years old. Consequently, this is reflected in the values of the tooth cavity to tooth width ratios (**Tab. 5**). For this reason, all statistical measurements were made on a mixed group of females and males.

The slightly more numerous group of females represented animals aged 1, 3, 5, 6 and 7 years. In the youngest females – one year old – the width of the dental cavity clearly dominated (**Tab. 4**) over the value of this measurement in animals from the other age groups (**Tab. 4**). This regularity was reflected in the values of the ratio of the width of the dental cavity to the width of the tooth (**Tab. 6**).

The mean values of canine width and canine width ratio related to tooth width for females at 1, 3, 5 and 7 years of age confirmed the regularities shown above.

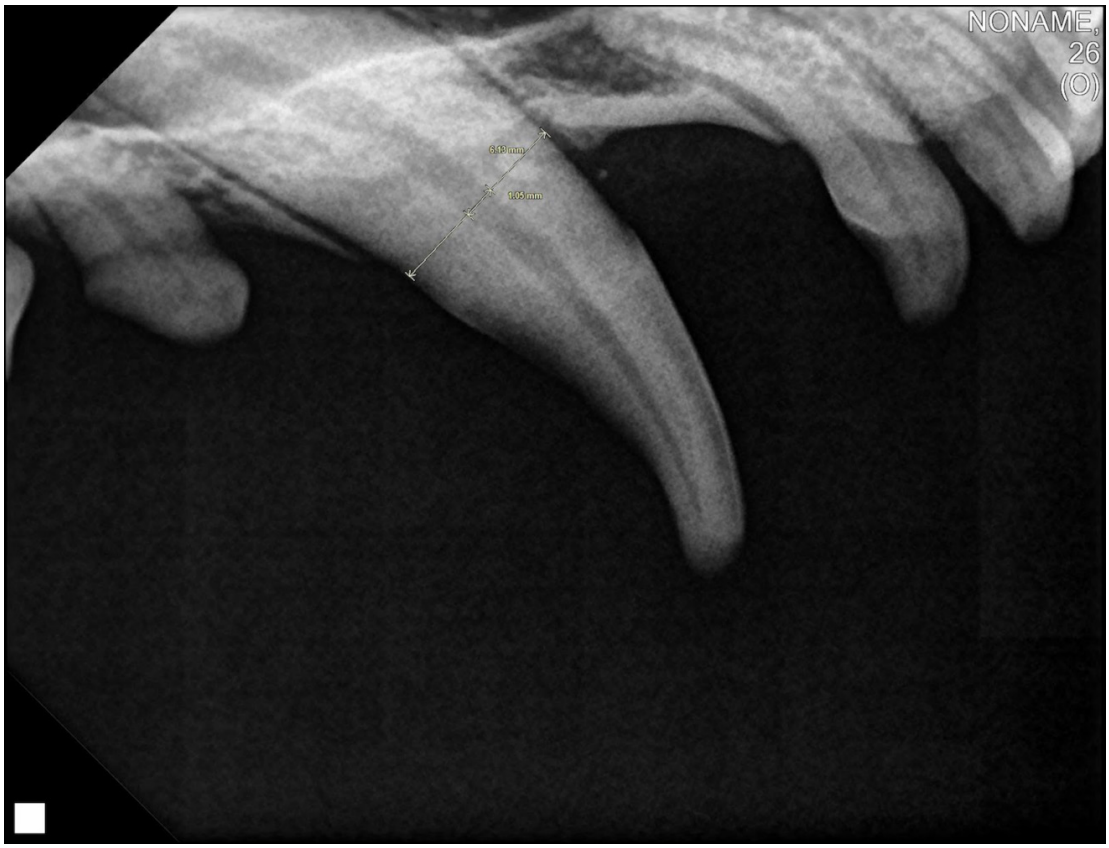

FIGURE 4 Tooth 104 of a 3-year-old female. Tooth width: 6.13 mm, tooth cavity width: 1.05 mm, tooth cavity width to tooth width ratio: 0.171

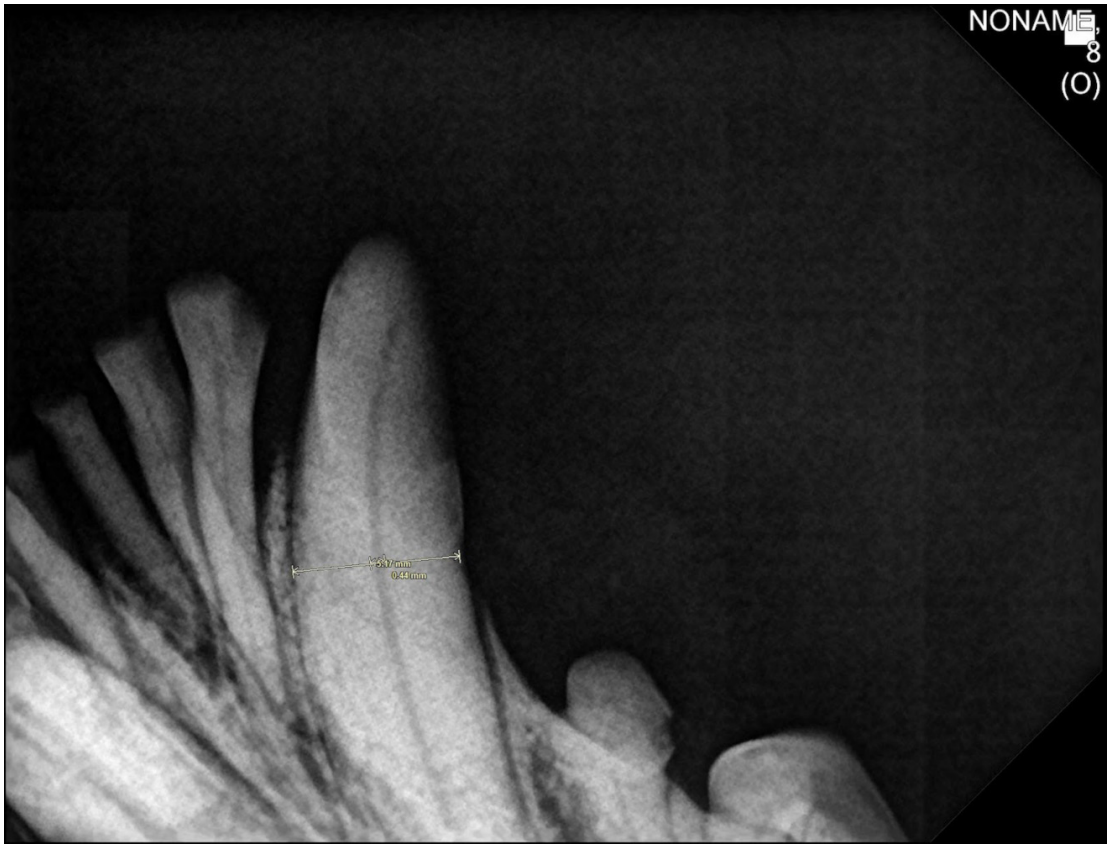

FIGURE 5 Tooth 304 of a 7-year-old female. Tooth width: 5.17 mm, tooth cavity width: 0.44 mm, tooth cavity width to tooth width ratio: 0.085

TABLE 2 Tooth widths of females given in millimetres

		TOOTH WIDTH (MM)			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
1	female	7,29	7,22	4,79	5,44
1	female	6,69	5,25	4,71	4,55
3	female	6,16	5,97	5,28	4,56
3	female	6,13	4,89	4,59	4,5
3	female	6,36	5,26	5,38	5,48
5	female	7,1	6,66	5,23	5,53
5	female	6,71	6,41	4,61	4,49
6	female	6,15	6,39	4,58	4,94
7	female	5,98	5,81	5,17	4,85
7	female	6,24	6,23	5,03	3,92
7	female	6,48	4,28	4,69	4,6

TABLE 3 The width of the dental cavity of the corresponding teeth of males given in millimetres. A value written in bold indicates an oversized value

		PULP CAVITY WIDTH (MM)			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
3	male	1,11	0,98	0,67	0,52
4	male	2,14	2,4	0,58	1,2
5	male	0,86	0,87	0,57	0,42
9	male	0,78	0,86	**2,54**	0,46

TABLE 4 The width of the dental cavity of the corresponding teeth of females given in millimetres

		PULP CAVITY WIDTH (MM)			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
1	female	3,88	2,69	1,51	2,31
1	female	3,11	2,05	1,55	1,46
3	female	1,15	1,03	1,27	2,01
3	female	1,05	0,66	0,91	0,58
3	female	0,86	1,17	0,72	0,83
5	female	1,18	1,18	0,62	0,52
5	female	0,83	0,85	1,13	1,16
6	female	1,07	1,14	0,93	1,17
7	female	0,68	0,7	0,44	0,49
7	female	0,59	0,73	0,43	0,43
7	female	2,74	1,19	1,15	0,96

TABLE 5 Pulp cavity – tooth width ratio in males. A value written in bold indicates an oversized value

		PULP CAVITY–TOOTH WIDTH RATIO			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
3	male	0,1613	0,1563	0,146	0,0935
4	male	0,3537	0,4293	0,1465	0,2559
5	male	0,1243	0,1408	0,1054	0,082
9	male	0,126	0,1509	**0,5451**	0,1038

TABLE 6 Pulp cavity – tooth width ratio in females

		PULP CAVITY–TOOTH WIDTH RATIO			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
1	female	0,5322	0,3726	0,3152	0,4246
1	female	0,4649	0,3905	0,3291	0,3209
3	female	0,1867	0,1725	0,2405	0,4408
3	female	0,1713	0,135	0,1983	0,1289
3	female	0,1352	0,2224	0,1338	0,1515
5	female	0,1662	0,1772	0,1185	0,094
5	female	0,1237	0,1326	0,2451	0,2584
6	female	0,174	0,1784	0,1031	0,2368
7	female	0,1137	0,1205	0,0551	0,101
7	female	0,0946	0,1172	0,0855	0,1097
7	female	0,4228	0,278	0,2452	0,2087

TABLE 7 Descriptive statistics on the canines studied. In order to increase the study groups for statistics, foxes were divided into 2 groups: old (o) and young (y)

	104		**204**		**304**		**404**	
	o	**y**	**o**	**y**	**o**	**y**	**o**	**y**
VALID	7	7	7	7	7	7	7	7
MISSING	0	0	0	0	0	0	0	0
MEAN	0.132	0.286	0.145	0.268	0.198	0.216	0.141	0.259
STD. DEVIATION	0.028	0.163	0.025	0.125	0.165	0.082	0.074	0.142
MIN	0.095	0.135	0.117	0.135	0.085	0.134	0.082	0.093
MAX	0.174	0.532	0.178	0.429	0.545	0.329	0.258	0.441

The width of the canines in all Arctic foxes studied regardless of sex and age showed similar values. This is illustrated in **table 7** and **table 8**.

The measurements showed that the ratio of the width of the canine cavity to the whole tooth decreased with age, and that this change progressed

TABLE 8 Results of a nonparametric significance test of canine measurements. 104 – right upper canine. 204 – left upper canine. 304 – left lower canine. 404 – right lower canine. Mann-Whitney U test

	W	p
104	5.000	0.011
204	8.000	0.038
304	16.000	0.318
404	11.000	0.097

TABLE 9 Average tooth width for each age group for females given in milometers

		AVERAGE TOOTH WIDTH (MM)			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
1	female	6,99	6,235	4,75	4,995
3	female	6,2167	5,3733	5,0833	4,8467
5	female	6,905	6,535	4,92	5,01
7	female	6,11	6,02	5,1	4,385

TABLE 10 Average width of dental cavity for each age group for females given in millimetres

		AVERAGE PULP CAVITY WIDTH (MM)			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
1	female	3,495	2,37	1,53	1,885
3	female	1,02	0,9533	0,9667	1,14
5	female	1,005	1,015	0,875	0,84
7	female	0,6350	0,715	0,4350	0,46

TABLE 11 Average pulp cavity – tooth width ratio for each group of females

		AVERAGE PULP CAVITY–TOOTH WIDTH RATIO			
AGE (YEARS)	**SEX**	**104**	**204**	**304**	**404**
1	female	0,4986	0,3816	0,3222	0,3728
3	female	0,1644	0,1766	0,1909	0,2404
5	female	0,1450	0,1549	0,1818	0,1762
7	female	0,1042	0,1189	0,0853	0,1054

more clearly in the maxillary teeth. This is more evident in females, as shown in **tables 9-11**, than in males, although this may be due to differences in the size of the study groups.

In order to perform a more detailed statistical analysis the entire study group was divided into young (up to and including 4 years) and old individuals (older than 4 years). Both groups consisted of 7 individuals. The old individuals were marked as ‘o’, the young as ‘y’. Descriptive statistics and the Mann-Whitney U test were chosen. The level of statistical significance was 5%.

For canines 104, 204 and 404, the average ratio of cavity width to tooth width in young foxes is high-

er than in old foxes. This feature does not apply to tooth 304, probably due to the high number of outliers in old foxes.

A p-value was also calculated for all teeth. For teeth 104 and 204, the p-value is less than 0.05, so the result is statistically significant. There was a lack of statistical significance between the right and left teeth of both the maxilla (p=0.862) and the mandible (p=0.891).

Due to the small number of males (4 individuals) compared to females (10 individuals), statistics were not performed on the differences between the sexes.

Discussion

The pulp cavity–tooth width ratio decreases with age due to successive deposition of secondary dentin. It results in a decrease of volume, surface and width of the pulp and dental cavity mainly due to ageing. This physiological process begins after the tooth root is fully developed [14,15]. It is considered an indicator for determining age using various dental imaging techniques [17]. Secondary dentin deposition can be also caused by tooth abrasion, erosion or changes in osmotic pressure in the dental cavity [3].

The teeth structure and properties, including their resistance to temperature and chemicals, as well as changes associated with the deposition of secondary dentin, make them a good indicator of age in humans and others [18].

The development of dental cavity and secondary dentin layers is species-specific. In carnivores, one layer of dentin is deposited each year. Counting these layers is accepted as another form of determining the age of carnivorous mammals [7].

For estimating the age of Arctic foxes, their canines (maxillary and mandibular) were used because they have only one root and the largest dental cavity, making the analysis easier than for the other teeth. Moreover, they are also present in older specimens and they show a lesser susceptibility to erosion and the effects of diet than other teeth [1,3].

Dental cavity volume decline is not a linear process. In humans, there is a rapid decrease in volume at the beginning of life and in old age, it stabilises in middle age [1]. In dogs, the decrease in the volume of the dental cavity of the upper and lower canines occurs most rapidly during the first 24 months of life, this process slows down thereafter [16]. In present study, this mechanism was shown to be more universal and also applies to Arctic foxes. The thematic literature reports that this phenomenon is universal to prey species (Carnivora) and has been described in coyote [19], spotted Lycaon [20], dingo dogs [13], the red fox [6] and the American mink [7], the domestic marten [21], the cat [3] and in the African lion [22].

Dental changes are also used to determine the age of dead animals (e.g., dogs), which is important in the context of research in archaeozoology, including the history of animal domestication [16]. Observing these changes also has applications in forensic medicine when determining the age and identity of crime victims [18].

Radiographic techniques are very useful in determining the age of animals. They are characterised by simplicity and do not require destruction or extraction of teeth. They are also inexpensive and require publicly available diagnostic equipment. They also provide ease of measurement, all of which makes them practical for working with live animals [16].

Despite the favourable results, this method may be considered controversial because it doesn't consider the three-dimensional morphological changes occurring in the dental cavity and the three-dimensional shape of the tooth. Another disadvantage of this method is the possibility of overlapping structures but also, to some extent, magnification and distortion of the obtained image [1,23].

Volumetric measurement is insensitive to deformation, making age estimation more accurate. However, this method can only be used on fully extracted teeth or with three-dimensional imaging techniques (e.g., CT scans), so two-dimensional imaging techniques, such as X-rays, are more commonly used in veterinary practice [8,15].

For qualitative analysis of canine teeth, there are no significant differences between right and left teeth [8,17]. There is also no statistically significant effect of sex on age determination when calculating the width ratio [8,14]. Age estimation based on changes in the dental cavity only works well for individuals in which the process of closure of the tooth root apices, which is necessary for secondary pulp deposition to begin, has ended [17]. In dogs over 12 months of age, the root apices of all teeth are already closed [24]. This method of age determination has the lowest accuracy for adult dogs (24-59 months), which it can identify as older [16].

Conclusions

Confirmed in present study in Arctic foxes, the phenomenon of tooth cavity shrinking with age, as expressed by the coefficient of tooth cavity width related to the width of the canine, provide arguments about the validity of the study being conducted. The demonstrated regularity can be useful as a principle of the method of age determination in animals of this species.

Ethical approval
The conducted research is not related to either human or animal use.

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Conflict of interest statement
The authors declare no conflict of interest.

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