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Effect of selected factors on the time of ossification of the epiphyseal cartilage of the proximal end of the proximal phalanx in Icelandic horses

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Abstract

Background: The time required for certain skeletal elements to ossify completely may be influenced by several factors, as demonstrated in studies of Thoroughbreds, purebred Arabians, Anglo-Arabians, and Huculs. Admittedly, studies on ossification have also been conducted on Icelandic horses, but only those kept in Nordic climates. The aim of the study was, therefore, to analyze the impact of a wide range of 21 factors grouped into five clusters, as well as the effect of the foal's sex and the sire on the time of ossification of the epiphyseal cartilage of the proximal end of the proximal phalanx in Icelandic foals reared in Eastern Europe (Poland).

Results: Ossification of the proximal epiphysis of the proximal phalanx in Icelandic foals reared in Poland occurred between 168 and 294 days of age, which was generally not comparable to the corresponding values for foals reared in Iceland. The greater body mass and biometric measurements of the foals at birth resulted in a significant prolongation of the ossification time by an average of 32.11 days. The opposite situation was true for a faster rate of growth in body weight and body measurements, associated with a significant acceleration of ossification by 31.47 days. A significant reduction in the time of ossification was associated with dam's milk or, more specifically, with higher fat, lactose and dry matter contents. The study observed no effect of body measurements at six months of age, the factors related to the mare's reproduction, and the foal's sex and the sire on the time of ossification.

Conclusions: The age at which the complete disappearance of the cartilage under study occurred was determined by body weight and body measurements at birth, the growth rate during the first six months of life, and the fat, lactose and dry matter contents of the dams' milk during that period. Generally, in foals that are smaller at birth, ossification of the proximal epiphysis of the proximal phalanx should proceed more rapidly than in larger newborns. This increased growth may be attributed to the milk of their dams, which was richer in fat, lactose and dry matter.

Keywords: Icelandic horses, foals, ossification, proximal phalanx, factors

Introduction

A horse's skeletal age is significantly correlated with its somatic maturity. This measurable characteristic should be the primary factor considered when assessing the stage of an animal's body development [1].

Skeletal age is most often determined radiologically in the region of the epiphyseal cartilages of long bones. In this case, the time required for complete ossification of the epiphyseal cartilage of a specific long bone is considered [2]. Bone maturation occurs when the primary ossification center merges with the secondary ossification center [3]. The process of ossification of the epiphyseal cartilage begins in its central part and then progresses towards the periphery. On X-ray images, this can be seen as a gradual disappearance of the radiolucent line on the growth plate [4, 5]. The epiphyseal cartilage exhibits the characteristics of a transitional structure and is a remnant of the final stage of development, just like articular cartilage, which, however, unlike epiphyseal cartilage, is found in animals throughout their lives [6-8]. A study by Dzierzęcka et al. [4] demonstrated that the closer a bone is to the axial skeleton, the longer the time required for its epiphyseal cartilage to ossify.

The time required for complete ossification of the epiphyseal cartilage may be influenced by breed, sex, husbandry conditions, season of birth, training intensity or nutrition, as demonstrated by Łuszczyński [1] in a study on Thoroughbreds, purebred Arabians, Anglo-Arabians and Huculs. As early as the second half of the 20th century, researchers recognized that the period from a foal's birth to six months of age is the most intensive phase for growth in withers height as well as in the circumference of the cannon bone and chest [9]. A study by Łuszczyński and Pieszka [10] showed that the radiographic closure time of the distal radial metaphyseal growth plate in Thoroughbreds was most strongly influenced by the season of birth, with the ossification process in foals born in spring being more intense than in those born in winter. A significant impact was also shown for the foals' sire, the breeder, and the year of birth. Another important factor was found to be hormonal balance, specifically the levels of testosterone, estrone and estradiol in horses' blood plasma [11].

It was also demonstrated that nutrients in milk and feed influence the normal course of growth and maturation in foals, including the ossification process [12]. The mare's diet, age and condition, as well as husbandry conditions, have a significant impact on the composition of colostrum and the subsequent chemical composition of milk as the foal's primary source of food. The most important components of mare's milk, essential for the proper development of foals, include protein, fat, lactose, and macro- and micronutrients. Mare's milk is also rich in biological components that modulate developmental processes,

e.g., lysozyme, ferritin, EFAs, and vitamin C. Special importance in the development of newborn foals is attributed to colostrum, which is characterized by much higher dry matter and protein contents [13].

For example, in Icelandic horses aged approximately three years, ossification of the epiphyseal cartilages was observed in most long bones [5]. It is not known, however, how the ossification process proceeds in this breed when reared outside the climate of the Nordic countries, nor whether any factors are significant in modelling this process. No such studies have been conducted to date, and the topic appears important, as Icelandic horses are becoming increasingly popular worldwide, and their husbandry in various countries differs significantly from that under the climatic and nutritional conditions prevailing in Iceland. Since the end of the 10th century, when the import of horses to the island was banned, there is no evidence that any crossbreeding with other breeds has occurred within the Icelandic horse population [14-16]. Thanks to this isolation, a uniform breed has been developed and, at the same time, protected from diseases that might threaten it. According to Björnsdóttir et al. [17], the isolation of animals in Iceland has resulted in the horses bred there being free from diseases common worldwide, such as rhodococcosis in foals, *Streptococcus equi*, arteritis and inflammation of the nasal mucosa and lungs.

The severe topographical and climatic conditions of the region, genetic isolation and limited access to feed have contributed to the development of a strong, hardy and resilient breed of horse, which, for a very long time, was used primarily for agricultural work and as the main means of transporting goods. These horses are currently used mainly for riding due to their stamina, gentle nature, calm disposition, and distinctive gaits [18].

It was assumed that the biometric characteristics of foals do not influence the radiographic closure time of the proximal epiphyseal cartilage of the first phalanx in Icelandic foals. However, the foal's sex and age, parity, the composition of the dam's milk, and the sire's lineage do have such an effect. In line with this assumption, the aim of the study was to analyze the impact of 23 factors on the timing of ossification of the aforementioned cartilage.

Methods

Horses

The study included 24 Icelandic foals born at the "Punktur" Icelandic Horse Stud Farm in Dębowy Gaj (Poland, GPS: 51.0751299607843N, 15.6345452941176E). The stud farm applied a pasture-based system of horse maintenance, allowing both the dams and their foals, as well as the weanlings and young horses, to remain in the pasture 24 hours a day. They had free access to pasture grass and a water point. Mineral supplements were usually provided via salt (NaCl) licks. During the breeding season, stallions were allowed access to specific groups of mares at the pasture (harem mating). The foals were weaned from their dams at approximately six months of age.

For the study, foals showing no signs of illness or visible physical abnormalities were selected from the group of all foals born successively in May. Therefore, on the last day of the month, the study group comprised twelve colts and twelve fillies. These foals were born to mares aged between 4 and 15 years (6.33±2.08), and sired by four stallions. Once a day, the mares and their foals were herded from the pastures to a shelter at the stud farm to be fed meadow hay, and this time was used to carry out the study.

Ethical statement

All procedures within this study were carried out in accordance with the Act of 15 January 2015 (as amended on 17 November 2021) on the protection of animals used for scientific or educational purposes. Pursuant to this Act, research requires approval if it "leads to pain, suffering, distress or permanent damage to the body, to a degree equal to or more intense than a needle prick." Measurements and weighing of horses, milking of mares, as well as radiographic examinations are not considered experimental procedures and do not meet the criteria described above. Moreover, they were performed at the request and with the consent of the all animal owners as routine breeding procedures carried out at the stud farm. The animals used in this study were neither slaughtered nor subjected to any special treatment. Due to the non-invasive nature of the study, approval from the ethics committee was deemed unnecessary.

Research methods

Biometric measurements

The tests were performed during the first six months of the foals' lives at intervals of 30-31 days. The first measurements were usually performed on the third day after birth, as this was the time when the mare began to allow people near her offspring.

The foal's body weight was determined using an OHAUS T32XW electronic platform scale. The biometric measurements were conducted according to the methodology developed by Komosa and Purzyc [31]. During the measurements, the foals were kept on a hard, level surface. Immobilization of the foals was achieved using a foal restraint technique, namely holding the animal's neck with one hand and bending the tail over the rump with the other. Withers height was measured, using a measuring stick, from the highest point of the withers to the ground. A measuring tape was used to measure the chest circumference, the left front cannon bone circumference, and the longitudinal body circumference. The chest circumference was measured along the girth line, i.e., along the tangent to the rear end of the shoulder. The cannon bone circumference was measured at the narrowest point of the left forelimb, below the carpus. The longitudinal body circumference was measured across the external surfaces of the shoulder joints and points of the buttock. The accuracy of the measurements of the withers height, chest circumference and longitudinal body circumference was 1 cm, and for the circumference of the left front cannon bone it was 0.25 cm.

Based on the results obtained, the growth rate of the foals during the first six months of life was calculated using the following formula:

$$T=((W2–W1)/((W2+W1)/2))*100$$

where:

T - growth rate at specified time [%]

W1 - initial measurement [cm]

W2 - final measurement [cm]

These measures enabled the comparison of the growth process among foals, regardless of their body measurements [32].

Analysis of mare's milk composition

During the period when the foals stayed with their dams, from birth until weaning, milk samples of approximately 60 mL were collected from the mares at monthly intervals. The samples were frozen immediately after collection and, after the tests were completed, they were thawed and analyzed using a Milko Scan FT120 analyzer. The analysis involved determining the percentage contents of protein, fat,

lactose, and dry matter. Based on the results obtained, the average percentage contents of the individual milk components during lactation were also calculated.

X-ray examinations

The X-ray examinations were performed by the veterinarian who daily looks after the horses at the stud farm, using an Orange 8016 HF X-ray apparatus (65 kV, 1 mAs). When taking the images, care was taken to ensure that the X-ray film cassette was in contact with the caudal surface of the limb and that the central beam passed horizontally. A radiographic image of the area of the proximal epiphysis of the proximal phalanx of the left forelimb of each horse was taken in the cephalocaudal direction. The first X-rays were taken when the foals were five months old (first examination), and the subsequent ones were taken 30 days later (second examination). If the radiolucent line (representing the epiphyseal cartilage) was found to be visible in the second examination, X-rays were repeated every two weeks until the complete ossification of the epiphyseal cartilage (subsequent examinations).

The age at ossification of the cartilage under study was reported in days, counting from the foal's date of birth until the moment when complete ossification of the cartilage was observed on the radiograph. To this end, the classification developed by Strand et al. [5] was used, assuming that the epiphyseal cartilage is "fully open", i.e., there is no ossification (Photo 1), when the radiolucent line was very distinct on the radiograph, and covered the entire growth plate. When the radiolucent line could be seen as intermittent within the growth plate region, the epiphyseal cartilage was considered to be "in the process of closing," i.e., partially ossified (Photo 2). As in the first case, this resulted in another X-ray being taken on the next scheduled date. The radiolucent line, which was not visible within the growth plate, provided grounds for concluding that the epiphyseal cartilage was "fully closed", indicating complete ossification (Photo 3).



Photo 1. The epiphyseal cartilage is “fully open”: no ossification



Photo 2. The epiphyseal cartilage is “in the process of closing”: partial ossification



Photo 3. The epiphyseal cartilage is “fully closed”: complete ossification

Statistical methods

191 The statistical analysis was performed using Statistica (StatSoft) version 13.0 (TIBCO Software Inc.,
192 Palo Alto, CA, USA). The normal distribution of the variables was assessed using the Shapiro-Wilk and
193 Lilliefors tests. In order to investigate the relationship between the age at disappearance of the
194 epiphyseal cartilage of the proximal end of the proximal phalanx and selected factors, these factors
195 were grouped into the following clusters (Table 1).

196 Table 1. A list of factors grouped into clusters

A cluster of factors	Grouped factors
A (a cluster of body weight and body measurements at birth)	body weight at birth, withers height at birth, chest circumference at birth, cannon bone circumference at birth, longitudinal body circumference at birth
B (a cluster of body weight and body measurements after six months)	body weight at six months of age, withers height at six months of age, chest circumference at six months of age, cannon bone circumference at six months of age, longitudinal body circumference at six months of age
C (a cluster of body weight and body measurements growth rate)	body weight growth rate at six months of age, withers height growth rate at six months of age, chest circumference growth rate at six months of age, cannon bone circumference growth rate at six months of age, longitudinal body circumference growth rate at six months of age
D (a cluster of dam's milk)	% of fat, % of protein, % of lactose, % of dry matter
E (a cluster of factors related to a mare's reproduction)	dam's age, parity

197

198 For each cluster of factors, including age at disappearance of the epiphyseal cartilage of the proximal
199 end of the proximal phalanx, a cluster analysis was performed to identify study groups. The cluster
200 analysis employed Ward's agglomerative clustering method and the Euclidean distance metric.
201 For each factor within each cluster, tests were conducted to determine the significance of
202 differences between the groups identified through cluster analysis. In addition, the effect of the
203 foal's sex and the sire on the age at disappearance of the cartilage under study was analyzed. For
204 variables that did not show a normal distribution (parity, cannon bone circumference at birth, body

205 weight at six months of age, cannon bone circumference at six months of age, withers height growth
206 rate at six months of age, % contents of fat, lactose and dry matter in dam's milk), the Mann-
207 Whitney U test was employed. The other variables, which showed a normal distribution, were
208 analyzed using the t-test. For both tests, significance levels of $p \leq 0.05$ and $p \leq 0.01$ were adopted. The
209 data are presented as means and standard deviations.

210 **Results**

211 For information purposes, Table 2 presents descriptive statistics on the age at disappearance of the
212 epiphyseal cartilage of the proximal end of the proximal phalanx, as well as on the body weight,
213 body measurements, and growth rates in Icelandic foals.

214 Table 2. Body measurements and growth rate of Icelandic foals, and the age at disappearance of the
215 epiphyseal cartilage

Parameter	Mean	Min	Max	SD
Age at disappearance of the epiphyseal cartilage [days]	227.04	168.00	294.00	37.71
Parameters at birth				
Body weight [kg]	42.91	32.20	57.80	6.42
Withers height [cm]	86.48	78.00	91.00	3.33
Chest circumference [cm]	77.63	69.00	85.00	4.32
Cannon bone circumference [cm]	11.83	11.00	13.00	0.60
Longitudinal body circumference [cm]	159.33	144.00	176.00	8.09
Parameters at six months of age				
Body weight [kg]	163.53	147.00	187.80	12.37
Withers height [cm]	112.29	107.00	117.00	2.39
Chest circumference [cm]	123.46	114.00	130.00	4.20
Cannon bone circumference [cm]	14.58	13.50	15.50	0.55
Longitudinal body circumference [cm]	261.67	247.00	275.00	8.68
Growth rate parameters during the first six months of life				
Body weight [%]	116.96	95.69	131.52	9.71
Withers height [%]	26.01	19.10	40.00	3.97
Chest circumference [cm]	45.63	32.65	56.57	5.94

Cannon bone circumference [cm]	20.87	11.32	30.77	4.63
Longitudinal body circumference [%]	48.64	34.74	58.82	5.52

At birth (factor cluster A), the mean values for body weight and body measurements, obtained in the groups following cluster analysis, differed significantly (P≤0.01) in the case of body weight, withers height, chest circumference and longitudinal body circumference (Table 3). However, the mean ages at disappearance of the epiphyseal cartilage differed significantly (P≤0.05). At six months of age (factor cluster B), there were fewer differences between the mean values. At P≤0.01, these were noted for body weight, chest circumference and longitudinal body circumference. The withers height differed between the groups at P≤0.05, whereas no significant differences between the mean values in the groups were noted for the cannon bone circumference and the age at cartilage disappearance. The average growth rates between the groups during the first six months (factor cluster C) differed significantly in terms of body weight, withers height, chest circumference, and longitudinal body circumference. No significant differences in the cannon bone circumference were observed. However, the average age at disappearance of the epiphyseal cartilage differed between the groups at P≤0.05.

Table 3. Body measurements and growth rate of Icelandic foals, and the age at disappearance of the epiphyseal cartilage

Cluster analysis		Body weight and body measurements at birth (cluster A)										Age at disappearance of the epiphyseal cartilage [days]	
		Body weight [kg]		Withers height [cm]		Chest circumference [cm]		Cannon bone circumference [cm]		Longitudinal body circumference [cm]			
Group	n	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd
1	15	39.46 A	3.85	85.00 A	3.16	75.13 A	2.92	11.53 A	0.48	155.80A	7.04	215.00a	31.68
2	9	48.67 B	5.75	88.94 B	1.84	81.78 B	2.77	12.33 B	0.43	165.22B	6.24	247.11b	40.09
Cluster analysis		Body weight and body measurements at six months of age (cluster B)										Age at disappearance of the	
		Body weight [kg]		Withers height		Chest circumferen		Cannon bone		Longitudinal body			

				[cm]		ce [cm]		circumference [cm]		circumference [cm]		epiphyseal cartilage [days]	
Group	n	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd
1	8	149.33A	2.68	110.75a	2.41	118.75A	2.60	14.63	0.74	252.63A	4.37	224.25	37.31
2	16	170.63B	8.36	113.06b	2.41	125.81B	2.48	14.56	0.44	266.19B	6.41	228.44	39.04

Cluster analysis		Growth rates during the first six months of life (cluster C)										Age at disappearance of the epiphyseal cartilage [days]	
		Body weight [%]		Withers height [%]		Chest circumference [%]		Cannon bone circumference [%]		Longitudinal body circumference [%]			
Group	n	x	sd	x	sd	x	sd	x	sd	x	sd	x	sd
1	14	122.86A	5.44	27.88A	4.00	49.23A	3.91	22.01	4.77	51.83A	3.42	213.93a	32.59
2	10	108.72B	8.29	23.40B	2.03	40.60B	4.45	19.26	4.12	44.19B	4.81	245.40b	38.18

Means denoted by different letters differ significantly at P≤0.01: uppercase letters, and P≤0.05: lowercase letters.

Table 4 provides descriptive statistics on the composition of milk from mares that gave birth to Icelandic foals at six months of lactation.

Table 4. Descriptive statistics on the composition of milk from Icelandic mares at six months of lactation

Parameter	Mean	Min	Max	SD
Fat [%]	1.45	0.93	1.92	0.25
Protein [%]	2.07	1.79	2.30	0.14
Lactose [%]	6.16	4.76	6.94	0.49
Dry matter [%]	10.78	9.28	11.88	0.62

The average content of milk components in mares at six months of lactation differed between the groups identified through cluster analysis for fat, lactose and dry matter (Table 5). In each case, the differences appeared to be highly significant at P≤0.01. No differences in the protein content were

noted. However, the average age at disappearance of the epiphyseal cartilage differed between the groups at P≤0.05.

Table 5. Average milk composition in mares at six months of lactation and the age at disappearance of the epiphyseal cartilage

Cluster analysis		A component of milk from mares-dams (cluster D)								Age at disappearance of the epiphyseal cartilage [days]	
		Fat [%]		Protein [%]		Lactose [%]		Dry matter [%]			
Group	n	x	sd	x	sd	x	sd	x	sd	x	sd
1	13	1.34A	0.15	2.09	0.12	5.84A	0.38	10.35A	0.41	240.77a	35.83
2	11	1.57B	0.28	2.05	0.16	6.54B	0.28	11.30B	0.37	210.82b	34.57

Means denoted by different letters differ significantly at P≤0.01 (uppercase letters) and P≤0.05 (lowercase letters).

In factor cluster E, no significant differences in terms of the age at disappearance of the epiphyseal cartilage between the groups identified through cluster analysis were noted (Table 6). However, the dam's average age and parity differed significantly.

Table 6. Dam's age and parity and the age at disappearance of the epiphyseal cartilage in foals

Cluster analysis		Factors (cluster E)				Age at disappearance of the epiphyseal cartilage [days]	
		Dam's age [years]		Parity			
Group	n	x	sd	x	sd	x	sd
1	11	8.55A	2.66	4.82A	1.25	230.36	33.60
2	13	4.46B	0.52	1.38B	0.51	224.23	42.03

Means denoted by different letters differ significantly at P≤0.01.

No significant differences were noted between the groups with regard to the impact of the foal's sex and sire (Table 7).

Table 7. Foal's sex and sire, and the age at disappearance of the epiphyseal cartilage in foals

Factor		n	Age at disappearance of the epiphyseal cartilage [days]	
			x	sd
Sex	mare	12	214.83	34.525
	stallion	12	239.25	38.158
Sire	A	9	228.22	39.87
	B	9	221.25	43.25
	C	7	212.14	36.22
	D	4	256.25	21.56

No significant differences were noted between the means.

Discussion

The epiphyseal cartilages of the distal end of the radial bone have been used for a long time as indicators of skeletal maturity in Thoroughbred racehorses and standard-bred horses [10, 19-21]. The ossification of the epiphyseal cartilages of the proximal phalanx, which initiates the process of skeletal ossification [4], has not attracted as much attention, particularly with regard to the Icelandic horses that are the subject of the present study. It is worth emphasizing that, according to a study conducted by Strand et al. [5], no ossification of the epiphyseal cartilage of the proximal end of the proximal phalanx was observed in Icelandic horses between 247 and 259 days of age. However, the methodology adopted by those authors did not allow them to precisely determine the exact timing

of ossification of this cartilage or the factors that might influence the rate at which this process occurs. In the current study, the epiphyseal cartilage ossified between 168 and 294 days of the foals' lives. Therefore, the actual time span for the ossification of this cartilage (126 days) covered a wider range than the results of the cited study indicate. Furthermore, the present study found that ossification usually occurred more rapidly. The causes of the differences in the results of these two studies are not yet known. The impact may be due to entirely different factors that have not been investigated. Additionally, it cannot be definitively stated whether the differences in ossification of the cartilage under study correspond to a later age of skeletal maturity. Therefore, results are regarded as merely illustrative and as a starting point for further research in this area.

Let us now move on to an analysis of the impact of the examined factors. It was found that the age range of the dams, although quite broad, did not affect the time of disappearance of the cartilage under study. No such effect was observed for the factor of parity, either. Therefore, the cluster of factors relating to mare's reproduction is considered to be irrelevant and, thus, to have been incorrectly identified at the hypothesis stage.

A similar situation, i.e., the elimination of the influence of a particular factor, occurred in the case of the sire and the foal's sex. At this stage of the research, it is certainly not possible to conclude that the trait under study is clearly not heritable, as the foals were sired by only four stallions. The result in this respect may, therefore, be regarded at most as a subtle suggestion for the direction of future research, particularly in the context of the results published by Łuszczyński and Pieszka [10]. What is also intriguing is the lack of significant differences within the sex factor, as this is inconsistent with the results obtained by Łuszczyński et al. [20], who noted a significantly faster disappearance of the cartilage in fillies compared to colts. This discrepancy may be due to the fact that these authors studied ossification in a different part of the skeleton and in a different horse breed.

Another issue is the body measurements of Icelandic foals, which are often of interest. It was decided to check whether they are similar to those obtained by other researchers. Satoła et al. [22], when developing a formula to determine the body weight of Icelandic horses, indicated that at 1-30

290 days/151–180 days of age, the withers height was 88.5 ± 5.0 /110.3 ± 2.7 cm, the chest
291 circumference was 80.9 ± 7.3/ 121.3 ± 4.7 cm, the cannon bone circumference was 12.0 ± 0.6/ 14.3 ±
292 0.5 cm, the longitudinal body circumference was 166.9 ± 15.7/ 254.0 ± 6.9 cm, and the body weight
293 was 49.9 ± 14.3/ 150.4 ± 15.3 kg. These results were, therefore, similar to those obtained in the
294 present study. However, given that the study was carried out on horses kept at the same stud farm
295 several years earlier, it can only be inferred that the population maintained there had not undergone
296 any significant changes during the breeding process. The study also examined the biometric
297 measurements of foals bred in Iceland. According to a study conducted by Thorvaldur and Thorkell
298 [23] on 6-month-old Icelandic horses, their withers height was 110.4±4.04 cm, chest circumference
299 was 23.9± 6.08 cm, and the cannon bone circumference was 15.0± 0.84 cm. The current study
300 obtained results comparable to those cited, even though the publication was written over 30 years
301 ago. This suggests that the lack of differences in the results may be linked to the consistent
302 maintenance of the Icelandic horse breed standard during their growth and maturation. It is also
303 likely that the climate at the breeding site has no impact on this phenomenon. It is not known,
304 however, how these horses develop in later stages of life, as Söderroos et al. [24] found differences
305 in body measurements between Icelandic horses kept in Sweden and Iceland. The results obtained
306 did not usually influence the overall performance value of these animals. However, in horses used
307 for recreational purposes, withers height, which differed significantly between these populations,
308 was positively correlated with rideability, i.e., a trait of great importance to horse breeders and
309 riders [25].
310 However, in the field of biometric measurements, the study yielded interesting results regarding the
311 relationship between the measurements and the age at disappearance of the cartilage under study.
312 It appears that in foals that were heavier and larger at birth, the time of disappearance of the
313 cartilage was significantly longer. In the case of the horses under study, the difference in body
314 measurements and body weight was highly significant, and amounted to almost 9 kg for the body
315 weight, 4 cm for the withers height, over 6 cm for the chest circumference, and just under 1 cm for

316 the cannon bone circumference. The differences indicated were due to differences in ossification
317 time, which ranged up to 32 days. At this stage of the research, this suggests that, based on
318 newborns' body measurements, it is possible to predict a shorter or longer time to the
319 disappearance of epiphyseal cartilage. It may also be suggested at this point that the reason for the
320 faster ossification of cartilages in smaller and lighter foals may be analogous to that discussed in the
321 case of humans. This is because positive correlations have been demonstrated between body
322 weight, the BMI index, and epiphyseal ossification in female adolescents [26].
323 However, predictions regarding the timing of ossification cannot be made on the basis of an analysis
324 of the body weight and body measurements of foals at six months of age. Although the differences
325 between the mean values (apart from the cannon bone circumference) proved to be highly
326 significant or significant, no such difference in the groups characterized by the time of cartilage
327 disappearance was observed. This situation may be due to growth compensation, which is
328 frequently observed in horses [27]. These measurements, therefore, proved useful for identifying
329 factors that influence the ossification process, particularly regarding growth rate, which
330 characterizes the aforementioned compensation. In foals with lower gains in body weight and body
331 measurements, the epiphyseal cartilage of the proximal end of the proximal phalanx ossified
332 significantly more slowly than in those with greater gains. It is most likely that the growth rate was
333 higher in smaller newborns, suggesting consistency between the current study's findings at birth and
334 those on growth rate. Therefore, the results obtained partially refute the study's hypothesis.
335 The proper development of foals is also influenced by the composition of the milk they consume [28,
336 29]. The basic composition of mare's milk that was recorded is comparable to that obtained by other
337 authors in studies conducted on mares of various breeds [13]. Furthermore, the current results
338 indicate that higher fat, lactose, and dry matter contents were associated with a significantly shorter
339 time to cartilage disappearance. At this stage of the research, it can be assumed that higher-quality
340 mare's milk helps compensate for growth and maturation, thereby accelerating ossification. At this
341 point, therefore, the study obtained results consistent with the hypothesis. Most likely, if smaller

foals had been fed poor-quality milk, their growth would have been impaired, potentially prolonging ossification. It should also come as no surprise that there are no significant differences in the protein content, which is so valuable for the body's development, between the groups identified through cluster analysis. In this case, very low standard deviation values within this component were obtained, which may indicate that the protein content in milk is only slightly differentiating Icelandic mares. Similar results were obtained by Boranbayeva et al. [30] in a study of Kazakh breed horses.

Conclusion

The ossification of the proximal epiphysis of the proximal phalanx in Icelandic horses occurred between 168 and 294 days of age. The age at the complete disappearance of the cartilage under study was determined by body weight and body measurements at birth, the growth rate during the first six months of life, and the contents of fat, lactose and dry matter in the milk of the dams during the specified period. In general, in foals that are smaller at birth, ossification of the proximal epiphysis of the bone under study should proceed more rapidly than in larger newborns. However, the shorter ossification time was likely due not to lower body weight and body measurements at birth, but to a higher, compensatory rate of growth in these parameters during the first six months of life. This compensation appears to be greater due to the milk of foals' dams, which was richer in fat, lactose and dry matter.

Ethics approval and consent to participate

All procedures within this study were carried out in accordance with the Act of 15 January 2015 (as amended on 17 November 2021) on the protection of animals used for scientific or educational purposes. Pursuant to this Act, research requires approval if it "leads to pain, suffering, distress or permanent damage to the body, to a degree equal to or more intense than a needle prick." Measurements and weighing of horses, milking of mares, as well as radiographic examinations are not considered experimental procedures and do not meet the criteria described above. Moreover, they were performed at the request and with the consent of the all animal owners as routine breeding procedures carried out at the stud farm. The owners of the animals were prior informed about the study and then consent was obtained. The animals used in this study were

neither slaughtered nor subjected to any special treatment. Due to the non-invasive nature of the study, approval from the ethics committee was deemed unnecessary.

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