Age estimation of Arabian mares by incisors morphometry and dentition changes

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Abstract
Accurate estimation of a horse's age based on the condition of the tooth status is necessary as a scientific and artistic technique, which has not been performed so far in pure Arabian horses of Khuzestan (southern west of Iran). This study aimed to investigate the age-dependent changes in the morphology and morphometry of the incisors in Arabian mares of Khuzestan to estimate age and to compare the estimated dental age and actual age. In this study, 78 Arabian mares of Khuzestan were examined in several equestrian clubs. Then, images were taken with a digital camera from the vestibular and occlusal surfaces of the lower incisors. Parameters of deciduous and permanent teeth eruption and their number, occlusal surface changes in the lower incisors including appearance and disappearance of the cup, enamel spot, dental star and appearance, and changes of Galvayne’s groove in the upper corner incisor were investigated. Comparison of the clinical crown length of incisors in each group showed that first, second and third incisors (I1, I2, and I3) had the maximum to minimum crown length, respectively. The correlation between actual age and clinical crown length was strong in I1 (r = 0.73, p ≤ 0.001), I2 (r = 0.8, p ≤ 0.001), and I3 (r = 0.81, p ≤ 0.001) in the Arabian mares of Khuzestan. The correlation of estimated dental age with actual age in the Arabian mares of Khuzestan was very strong (r = 0.992, p ≤ 0.001). Therefore, the dental age estimation in the Arabian mares of Khuzestan appears to be very close to the actual age of the animal.

Keywords Age estimation · Arabian mares · Dentition · Incisors

Introduction

Many people regard horses as beautiful and magical animals having dignity, sensitivity, and excellent athletic ability. In Asia, the cradle of horse breeding in the world, two major horse breeds have been identified, including the Aryan and Mongolian breeds. The Aryan breed includes genuine Iranian (Arabian) and Bakhtiari, Qashqai, and Darreh-Shuri horses. The Mongolian breed also includes Turkmen and Kurdish horses. The breeds known to be genuine Iranian are Turkmen, Kurdish, Arabian, and Caspian (Sadeghi et al. 2019). Researchers have emphasized the importance of accurately estimating the age of a horse, largely based on the teeth (Muylle et al. 1996). The ability to estimate age can be useful when purchasing livestock and predicting useful sports life, helping to determine disease prognosis and insurance coverage (Muylle et al. 1999). In addition, morphological analysis of teeth can also be used to identify the type of feed consumed or the type of environment in which horses live (Luszczyński and Pieszka 2011). During the life of the horse and many years after their death, the appearance of the teeth may allow to determine the age, sex, and health status of the animal (Luszczyński and Pieszka 2011). The estimation of a horse’s age based on the appearance and morphology of incisors was first introduced in 600 BC (Navin 1882). Since then, this technique has been employed throughout history. This method is nowadays considered one of the common practices of equine veterinarians. In estimating the age of a
horse, it should be noted that there might be disagreements between several veterinarians (Richardson et al. 1994). Significantly, researchers maintain that age estimation based on tooth structure is both a science and an art (Martin et al. 1999). The effect of age on horse dental morphology has been studied more on incisors. This is probably due to the ease of access to these teeth and their significant ontogenetic changes (Carmalt and Allen 2008).

Since it is necessary to accurately estimate the horse’s age based on the tooth status as a scientific and artistic technique, which has not been performed thus far in the genuine Arabian horses of Khuzestan (southern west of Iran) as well as given the interest of horse owners in the accurate estimation of horse’s age and its applications, the purpose of this study aimed to investigate the age-dependent changes in the morphology and morphometry of the incisors in the Arabian mares of Khuzestan to prepare the age profile of incisors changes and to compare the estimated dental age to actual age.

Materials and methods

In the present study, 84 Arabian mares of Khuzestan were examined in several equestrian clubs and horse care centers in the Khuzestan province, Iran. The horses were clinically examined and healthy animals without significant dental problems were selected for study. Therefore, 6 mares were excluded (84–6) owing to their dental problems.

According to morphological changes in teeth (Dyce et al. 2018), the mares were divided into five age groups, including 1) under 1 year, 2) between 1 year and under 2.5 years, 3) between 2.5 years and under 5 years, 4) between 5 years and under 10 years, and 5) older than 10 years.

In each case, the breed of the horse was confirmed according to the genealogical documentation recorded on the birth certificate, passport, or cauterized neck, and the age of the animal was recorded based on these documents. After confirming the breed of the mares, the mouth was opened by tethering the animal, and the first images were taken with a digital camera (Canon G9, Japan) from the left vestibular views of the overlapped incisors. The images were then taken from a close-up view from the occlusal surface of the lower incisors and the upper corner if Galvayne’s groove was present.

If permanent lower incisors were present, their clinical crown length was measured in the left mandible by two persons using a digital caliper (Guanglu 0–150 mm, China). This measurement was performed along the central portion of the tooth, and then the mean of the two numbers was recorded.

Two observers evaluated the photographs of the Arabian mares in this study. For every individual horse, each observer estimated the dental age of the horse based on dental traits observed in that individual. The mean dental age for that particular horse was calculated from the dental age estimates obtained by the two observers. Parameters of deciduous and permanent teeth eruption and their number, occlusal surface changes in the lower incisors including appearance and disappearance of the cup, enamel spot, dental star (Dyce et al. 2018) and appearance (Dyce et al. 2018; Nicks et al. 2007), and changes of Galvayne’s groove in the upper corner incisor were investigated (Dyce et al. 2018; Muylle et al. 2007). According to previous studies (Dyce et al. 2018; Muylle et al. 2007), the appearance of Galvayne’s groove was classified as up to 25% proximal, from 25 to 50% proximal, from 50 to 75% proximal, from 75% proximal to 100%, up to 75% distal, from 75 to 50% distal, from 50 to 25% distal and from 25% distal to disappearance. The index of hook appearance in the upper corner on the left side was also assessed.

Statistical analysis

Two blind researchers conducted the studies, and the dental age estimation was calculated based on the mean analysis, and then it was statistically compared to actual age. When analyzing the images, the angle between the left upper and the lower corner incisors in the vestibular view image was measured using the AutoCAD 2016 software.

The collected data were analyzed using descriptive and analytical methods in the SPSS version 16 software. Data were analyzed by repeated-measures ANOVA, one-way ANOVA, Tukey’s post-hoc test, independent T-test, correlation analysis, and regression. P-value of 0.05 was considered a significant difference. Charts were plotted using Excel 2013 software.

Results

In this study, 78 Arabian mares of Khuzestan with age ranging from 15 days to 24 years were evaluated. Table 1 shows the number of mares examined in each group.

Time of lower incisor eruption

This index was evaluated in the Groups 1 to 3 (according to the time of teeth eruption), since all incisors were permanent in the horses of Groups 4 and 5. The results of this index for deciduous and permanent teeth are as follows:

1. Deciduous teeth

Regarding the lower deciduous incisor eruption in the Arabian mares of Khuzestan, the first deciduous incisor
(dI₁) was present in the youngest horse examined in this study (15 days old) and also in other mares with deciduous teeth over 15 days of age. The second deciduous incisor (dI₂) was seen from age 1 month onward, and the third deciduous incisor (dI₃), despite its appearance in 2 horses aged 3 and 4 months was absent in horses aged 6 and 6.5 months and had begun to erupt among other mares over 10 months old (Fig. 1A).

2. Permanent teeth

The time of permanent incisor eruption in the Arabian mares of Khuzestan investigated in this study was approximately similar to the standard eruption time of these teeth in horses; however, the permanent incisors erupted in seven mares earlier than expected (Fig. 1B).

The morphological changes in occlusal surface of lower incisors

The cup

In the Arabian mares of Khuzestan, the cup of deciduous incisors appeared at ages 1 to 3 months, and then the enamel spot appeared at ages 10 to 26 months (Figs. 2A-D). In the permanent teeth, there was a cup on all lower incisors up to 4.5 years old, but the disappearance of the cups included a wide age range. Thus, the cup disappeared in I₁ and I₂ usually after 8 years old and in I₃ after 10 years old (Fig. 3A-F). However, occasionally, the cup was also observed on all three teeth up to 13 years old.

The dental star

The dental star appeared on the dI₂, dI₁, and dI₃ at the ages of 10 months, 1 year and 3 months, as well as 2 years and 5 months, respectively (Fig. 4A-C). The appearance of a dental star on the lower permanent incisors in the Arabian mares of Khuzestan investigated in this study was first observed on the I₁ at the ages of 5 years and 5 months and on the I₂ and I₃ at the ages of 7 years and 10 months (Fig. 4D, E). As the age of the animal increased, the dental star shifted to the center of the occlusal surface and gradually became rounder, with the rounding of the dental star on the I₁ and I₂ at the age of 15 years and on the I₃ at the age of 19 years (Fig. 4F).

The enamel spots

The enamel spot in the permanent teeth of Arabian mares of Khuzestan was first observed on the tooth I₁ at the age of 7 years and 10 months old, and on the I₂ and I₃ at the ages of 12 years old (Fig. 5A, B). This trait was seen on the occlusal surface of the lower incisors up to 16 years old, while 19-year-old mares had no enamel spot and none of the older ones had an enamel spot (Fig. 4F).

The morphology of the occlusal surface

The occlusal surface of the lower incisors in the Arabian mares of Khuzestan was initially transverse oval. With increasing age, rounding of the occlusal surface occurred in I₁ at the age of 7 years and 10 months old and in the I₂ and I₃

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Table 1 The sample size of mares examined in each age group

| Age groups                  | Number | Mean age ± Standard deviation (months) |
|-----------------------------|--------|---------------------------------------|
| 1) under 1 year             | 14     | 6.25 ± 4.00                           |
| 2) between 1 year and less  | 15     | 19.13 ± 6.45                          |
| 3) between 2.5 years and    | 15     | 45.00 ± 6.88                          |
| 4) between 5 years and      | 21     | 84.19 ± 13.59                         |
| 5) older than 10 years      | 13     | 199.23 ± 53.51                        |
| Total                       | 78     |                                       |

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Fig. 1 A Occlusal surface view of deciduous incisors in a 10-month-old Arabian mare of Khuzestan (Arrow shows the newly erupted I₃). B Vestibular surface view of permanent incisors in an Arabian mare of Khuzestan aged 4.5 years (No eruption of the permanent corner)
at the age of 12 and 13 years old. Triangular occlusal surface was first observed at the age of 15 years and then all lower incisors were triangular at the age of 20 years old (Fig. 6). Fig. 7A-C shows the percentage of the morphological changes in the occlusal surface of the lower incisors with age.

The hook

The appearance of a hook in the upper corner incisor was observed only in six Arabian mares of Khuzestan, 4 mares were 7 to 9 years old and 2 mares were 15 years old (Fig. 5C, D).
Galvayne’s groove

In the studied mares, the Galvayne’s groove was observed after 11 years and 10 months old, and was present up to 24 years old (the oldest studied mare) (Fig. 8).

Correlation of estimated dental age based on lower incisor status with actual age

Fig. 9 shows the correlation of the estimated dental age based on lower incisor status with actual age in the Arabian mares of Khuzestan.

The correlation of the estimated dental age with actual age in the Arabian mares of Khuzestan was very strong ($r=0.992$, $p \leq 0.001$).

According to Fig. 9, the equation between dental age and actual age in the Arabian mares of Khuzestan is as follows:

$$\text{actual age} = (\text{dental age} \times 0.983) + 2.701, R^2 = 0.985$$

where, $R^2$ is the coefficient of determination which was 0.985 in this study, so that dental age could justify the actual age up to 98.5%.

The morphometric changes

Clinical crown length of lower permanent incisors

In the studied Arabian mares, the clinical crown length of lower permanent incisors increased with age (Table 2).
Moreover, the first incisor (I₁), second incisor (I₂), and third incisor (I₃) had the maximum to minimum crown length, respectively.

As Table 2 shows, the crown length of tooth I₁ in the Groups 2 and 3 was significantly different from that in Groups 4 and 5 (p ≤ 0.001) and it increased with age. There was also a significant difference (p ≤ 0.01) between the Groups 4 and 5.

The crown length of I₂ and I₃ significantly increased with age, with a significant difference (p ≤ 0.001) between all groups.

When comparing the crown length of the incisors in each group, the I₁, I₂, and I₃ had the maximum to minimum crown length, respectively. There was no significant difference in the crown length of teeth in Group 3, but there was a significant difference (p ≤ 0.001) between teeth in the group 4. In group 5, the crown length of I₁ and I₂ had no significant difference and was approximately equal, while the I₃ was significantly (p ≤ 0.01) shorter than the others.

The correlation of actual age with the clinical crown length of lower incisors The correlation coefficient between the actual age and the clinical crown length index in the I₁, I₂, and I₃ was strong (r = 0.73, p ≤ 0.001), (r = 0.8, p ≤ 0.001), and (r = 0.81, p ≤ 0.001), respectively.

The equation for age estimation based on the clinical crown length of lower incisors Age (month) = 40.89 — [Length of I₃ (mM) × 7.78] + [Length of I₂ (mM) × 24.47] + [Length of I₁ (mM) × 24.78].

These three teeth explained 78.3% of the age-dependent changes.

The angle between the lower and upper corner incisors

Fig. 10 presents the measurement of the angle between lower and upper corner incisors in the Arabian mares of Khuzestan. This angle decreased with age and this difference was considered statistically significant between the Groups 4 and 5 (p ≤ 0.001). The correlation of this index with actual age was strong and accordingly an equation was presented for age estimation.

The correlation coefficient in this index was strong (r = 0.66).

The equation for actual age estimation corresponding to the angle index is as follows:

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\text{Age (month)} = 509 - (\text{angle between lower and upper corner incisors} \times 3.24).
\]
Fig. 11 displays the sharpening of the angle between upper and lower corners with increasing age of Arabian mares in Groups 4 and 5.

Discussion

The time of lower incisor eruption

The deciduous teeth

References of veterinary anatomy have described the eruption time of the $dI_1$ in horses from birth to one week of age (De Lahunta and Habel 1986; Dyce et al. 2018; Getty 1975; König and Liebich 2020; Schummer and Nickel 1979). References cited different times for the $dI_2$ eruption in the horses, including 3–4 weeks and rarely up to 8 weeks (Schummer and Nickel 1979), 4–6 weeks (Getty 1975), and 1 month (De Lahunta and Habel 1986), and 6 weeks (Dyce et al. 2018; König and Liebich 2020). The largest difference concerning deciduous incisor eruption in available references is related to the tooth $dI_3$, as reported from 5 to 9 months (Schummer and Nickel 1979), 6 to 9 months (Getty 1975), 6 months (Dyce et al. 2018; König and Liebich 2020) and 8 months (De Lahunta and Habel 1986). This difference in time was also observed in the present study, and the $dI_3$ in the Arabian mares of Khuzestan had a different eruption time, while the eruption time of the $dI_1$ and $dI_2$ was approximately similar to the other horses.

The permanent teeth

Similar to the results of the present study, most of the available references reported that the time of lower incisors eruption in the horses was 2.5, 3.5, and 4.5 years old from center to corner, respectively (De Lahunta and Habel 1986; Dyce
et al. 2018; Getty 1975; König and Liebich 2020; Lowder and Mueller 1998; Martin et al. 1999). However, some references mention a delay of up to 6 months for the eruption of any mentioned tooth (Schummer and Nickel 1979; Silver 1963). Richardson et al. (1994), investigated the eruption time of the lower permanent incisors in thoroughbred horses and reported earlier eruption for the $I_2$ and $I_3$, being consistent with the present study results in some cases (Richardson et al. 1994). However, the teeth have erupted at the determined time in most cases. Muylle et al. (1997), reported that the eruption time of permanent incisors in the Belgian draft horses was up to 7 months later than usual. The reported

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**Fig. 8** Galvayne’s groove status in the upper left corner in some Arabian mares of Khuzestan. **A)** Absence of Galvayne’s groove in a 10 years old mare, **B)** Appearance of Galvayne’s groove up to 25% proximal in 11 years and 10 months old mare, **C)** Appearance of Galvayne’s groove up to 50% proximal in a 16 years old mare, **D)** Appearance of Galvayne’s groove on the all of the vestibular surface in a mare with 20 years old, **E)** Appearance of Galvayne’s groove up to 75% distal in a 23 years old mare. The arrow shows the size of Galvayne’s groove on the vestibular surface of the upper corner

**Fig. 9** Correlation of estimated dental age based on lower incisor status with actual age

$y = 2.701 + 0.983x$

$R^2 = 0.985$
differences, in addition to species differences, may be due to racial and species differences between the different horses.

**The morphological changes on the occlusal surface of lower incisors**

The cup

Available references have reported the time of disappearance of the cup in deciduous incisors approximately similar to that of the Arabian horses examined in the present study; for example, 12–24 months (Silver 1963) and 10–24 months (König and Liebich 2020).

Similar to the present study, a wide range of ages have been reported in various references regarding the disappearance of the cup in permanent incisors. Most references of veterinary anatomy have stated that ages 6 to 8 years are the time of disappearance of the cup in lower incisors (De Lahunta and Habel 1986; Dyce et al. 2018; König and Liebich 2020), while the time of disappearance of the cup in permanent incisors of horses has been reported from 7 to 9 years in a study of different methods of age estimation in domestic animals (Silver 1963). Richardson et al. (1995), reported that the time of disappearance of cups in permanent incisors in thoroughbred horses was 3 to 17 years. Muylle et al. (1996), reported the disappearance of the cup in permanent incisors in trotter horses at the age of 7 to 14 years. The disappearance of the cup has been reported in 5 to 10 years old Belgian draft horses, so that the horses older than 11 years had no cups in the lower incisors (Muylle et al. 1997). In the study of Arabian horses in Belgium, the disappearance of the cup was reported at 6 to 7 years old in I1, 7 to 11 years old in I2, and 9 to 15 years old in I3 (Muylle et al. 1998).

The differences observed in the time of disappearance of the cup in the lower incisors, in addition to differences in species, race, and breed may be due to differences in the cup depth as well as different tooth wear rates in various odd-toed ungulates (Perissodactyla). Another important factor that may have caused this age range is the fact that the indicator is evaluated by different people using visual perception. Therefore, a tooth detected with no cup by one person may be classified as a superficial and shallow cup by another. This may be why some researchers (Muylle et al. 1997) have not considered the value of cup disappearance in estimating a horse's age. If it is possible to measure the depth of the cups, it seems likely that this issue will be largely resolved, however, it is not easy to do in live horses. In spite of what is stated, in the present study similar to the study by Luszczyński et al. (2015), the disappearance of the cup was found to be a useful indicator for dental age estimation. They also regarded this index as reliable to estimate the age of Hucul pony (Luszczyński and Pieszka 2011).

**The dental star**

The time of appearance of the dental star in references of veterinary anatomy is stated to be 8 years old (De Lahunta and Habel 1986; Dyce et al. 2018; Getty 1975), however, this index was first observed in this study at about 5.5 years old. The time of dental star rounding has also been reported in references at the age of 15 to 16 years, being roughly similar to the present study results (De Lahunta and Habel 1986; Dyce et al. 2018). Richardson et al. (1995) reported the appearance of the dental star in thoroughbred horses at 45 months old. In another study, the dental star was observed in I1 to I3 in trotter horses at 5, over 6, and 7 to 8 years old, respectively (Muylle et al. 1996). The dental star in the lower incisors of the Belgian draft horses was seen, respectively, at 4, 5, and 6 to 7 years old, respectively (Muylle et al. 1997), whereas the same researchers reported the appearance of

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**Table 2** Mean ± standard deviation of clinical crown length of lower permanent incisors in Arabian mares of Khuzestan

| Age groups | I₁ | I₂ | I₃ |
|------------|----|----|----|
| 1          | -  | -  | -  |
| 2          | 15.47±1.87<sup>c</sup> | -  | -  |
| 3          | 18.27±1.57<sup>Ac</sup> | 16.18±0.63<sup>Ac</sup> | 11.06±5.38<sup>Ac</sup> |
| 4          | 24.29±2.18<sup>Ab</sup> | 21.52±2.46<sup>Bb</sup> | 18.52±1.2<sup>Cb</sup> |
| 5          | 27.37±1.88<sup>As</sup> | 27.37±2.97<sup>As</sup> | 22.83±2.88<sup>As</sup> |

Different lowercase letters in each column indicate a significant difference

Different uppercase letters in each row indicate a significant difference.

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**Fig. 10** The angle measured between lower and upper corner incisors in Arabian mares of Khuzestan. A significant difference was observed between groups 4 and 5.
of the dental star in the lower incisors at the ages of 5 to 7 years in the Belgian Arabian horses, which is 2 to 3 years earlier (Muylle et al. 1998), almost similar to the present study results. As previously mentioned, the appearance of the dental star in the Arabian mares of Khuzestan occurred before 8 years old (time cited in the references of veterinary anatomy), similar to that of Arabian and non-Arabian horses examined by other researchers. Moreover, the appearance of the dental star due to large age scattering in the present study was not considered as a reliable indicator, while Muylle et al. (1997), introduced this parameter as one of the most reliable dental features in the Belgian draft horses. Contrary to their view, Richardson et al. (1995), stated a large age scattering in this index, consistent with the results of the present study. It appears that since type of nutrition, arrangement of teeth, and amount of tooth wear heavily influence the appearance of a dental star, it has a wide range of ages, and the racial and individual differences affecting the dentition and jaw shape of horses contribute to the reasons. Therefore, concerning the differences observed with references of veterinary anatomy, this index should be judged and used with greater caution. However, broader studies of different horse breeds in the future may be able to alter the age ranges associated with this index in veterinary anatomy references.

The enamel spots

The review of the references of veterinary anatomy revealed that the enamel spot appeared in lower incisors in horses between 9 and 11 years old, and disappeared between 13 and 16 years old (De Lahunta and Habel 1986; Dyce et al. 2018; Martin 2002). Thus, the enamel spot on incisors in the Arabian mares of Khuzestan, especially on the I₁, seems to appear about one year earlier than usual, while it is observed one to two years later in the I₂ and I₃. Nevertheless, the time of disappearance of this index in the present study, is almost similar to that of other horses and ages recorded in the references (De Lahunta and Habel 1986; Dyce et al. 2018).

The morphology of the occlusal surface

The morphological changes in the occlusal surface were reported to be the most diverse and most variable parameter in studies on horse incisors. The pattern of morphological change from transverse oval to round and then triangular form has been reported in most references, similar to the results of the present study results; however, the age of occurrence of these changes was different in references, so that rounding time varied from 9 - 10 years old (De Lahunta and Habel 1986; Dyce et al. 2018), 9 to 12 years (Martin 2002; Schummer and Nickel 1979), 12 and 17 years (König and Liebich 2018) and 15 years old (Getty 1975). The formation of triangular occlusal surfaces in the incisors has been reported at 14 - 17 years old (Schummer and Nickel 1979), 15 - 17 years (Martin 2002), 16 - 17 years (De Lahunta and Habel 1986), and 16 - 18 years old (Dyce et al. 2018). However, some references (De Lahunta and Habel 1986; Dyce et al. 2018) reveal that changes of the corner do not follow the aforementioned pattern.
Various researchers have reported a large age scattering for this index. Richardson et al. (1995), noted that despite the significant correlation between the age and the morphology of incisors occlusal surface in thoroughbred horses, this parameter was not an inappropriate index for age estimation due to high data scattering. Muylle et al. (1997), reported that the morphological changes in the occlusal surface of incisors in the draft horses occurred 1 year earlier than those in the trotter horses. According to the researchers, morphological changes occur in the Arabian horses later than those in the draft and trotter horses (Muylle et al. 1998). Along with some references of veterinary anatomy, these researchers reported that the occlusal surface of the corners did not follow the expected pattern of morphological changes (De Lahunta and Habel 1986; Dyce et al. 2018).

By reviewing various references and studies, it appears that the morphological changes on the occlusal surface in the Arabian mares of Khuzestan are largely consistent with the model mentioned in the American Association of Equine Practitioners (AAEP) (Martin 2002), however, due to the large age scattering, this index in the present study was not regarded as a suitable parameter for the age estimation. To support these results, Luszczynski and Pieszka (2011), did not consider occlusal surface morphology to be reliable for age estimation in the Hucul pony.

The hook

According to most references of veterinary anatomy, the hook appears at 7 years old. They maintain that this index disappears at 9 years old and reappears between 11 and 13 years old (De Lahunta and Habel 1986; Dyce et al. 2018; König and Liebich 2020; Martin 2002). It should be noted that this index has been observed up to 20 years old (Getty 1975). Muylle et al. (2007), examined Standardbred, Belgian draft, and Arabian horses as well as Shetland pony, and reported that the age of hook appearance was 5 to 19 years. This index was observed in 16% of the horses examined by them. In other studies, these researchers also pointed to the invalidity of the hook index in age estimation in the Arabian (Muylle et al. 1998), draft (Muylle et al. 1997), and trotter (Muylle et al. 1996) horses. Richardson et al. (1995) stated that the appearance of the hook on the upper corner in the thoroughbred horses was between 5 and 18 years old.

According to the references of veterinary anatomy, the time of appearance of the hook in the Arabian mares of Khuzestan is the same as other horses. However, the observational ratio of this index in the present study was only 24% (according to the number of horses older than 7 years). However, the relatively low occurrence of this index and the possibility of reappearance at different ages cause the hook to be an unreliable and unsuitable parameter for dental age estimation for the horses.

The Galvayne’s groove

According to veterinary anatomy references, the time of the appearance of Galvayne’s groove is mostly at 9 to 10 years old; almost all of them pointed to the progression of Galvayne’s groove up to 20 years old and then disappearance up to 30 years old (De Lahunta and Habel 1986; Dyce et al. 2018; Getty 1975; Martin 2002). Most available reports have also indicated that the time of appearance of Galvayne’s groove in the horses is after 9 years old (Muylle et al. 1996, 1997, 1998). There is a case report of the appearance of Galvayne’s groove in a thoroughbred horse at 4 years and 11 months old (Richardson et al. 1995). Given the time of the appearance of Galvayne’s groove in the present study (11 years and 10 months old), this finding is similar to that reported in the Arabian horses, as they also referred to 11 years and 6 months old (Muylle et al. 1998). Despite the emphasis on the almost permanent appearance of Galvayne’s groove in middle-aged and older horses, this feature has not been regarded as suitable for age estimation in some studies due to variation in groove length (Muylle et al. 1996, 1997, 1998, 1999). This is likely due to the neglect of the forward and backward groove to the middle of the tooth, respectively at 15 to 25 years old, respectively (the pattern mentioned in veterinary anatomy references).

In the present study, which assessed Galvayne’s groove forward and backward based on references of veterinary anatomy, this index was found to be a useful index for the age estimation (in Group 5), thereby providing the possibility of age estimation with approximately 1 to 2 years. To confirm these, the results of other studies (Richardson et al. 1994) also introduced the Galvayne’s groove as a useful feature for horse’s age estimation. Given that the morphological changes in the occlusal surface in older horses are not very reliable, the Galvayne’s groove appears to be able to respond to the challenge of age estimation with a few years.

The clinical crown length of lower permanent incisors

Muylle et al. (1999), evaluated the crown length of permanent incisors in horses slaughtered in a Belgian slaughterhouse, and reported that I1 to I3 had the highest to shortest lengths, respectively, similar to the present study results. In their study, the length of the teeth was longer up to the age of 10 years, in line with the present study results. In a three-dimensional study of horse incisors after euthanizing by Schrock et al. (2013), it was found that incisors reached a maximum length at 2 to 4 years after the eruption, and this length was almost stable for 10 years. Thus, in horses over the age of 13 to 15 years, the length of incisors was significantly reduced. The results of the present study are somewhat different from the results of their study, since the horses of Group 5 (over 10 years of age) in the present
study had the highest clinical crown length. In explaining this difference, first, the method and tools of measurement in the present study were different from those in the study conducted by Schrock et al. (2013). Second, the mentioned study found a significant reduction in tooth length after 15 years old, while nearly half of the Group 5 horses in the present study were under 15 years old; therefore, a reduction in clinical crown length would be indicated if other groups were defined for horses older than 15 years. However, in the present study, due to the lack of horses older than 20 years, this was impossible.

According to the literature review, the present study appears to be the first to evaluate the clinical crown length of incisors in live horses. Thus, the correlation between actual age and clinical crown length (which was particularly very strong for I2 and I3) and the stated equation for estimation based on clinical crown length in this study, is unique. However, a larger sample size could be associated with a higher percentage of justification for age-dependent changes in the teeth.

**The angle between the lower and upper corner incisors**

Many references have pointed to the reduction of the angle between lower and upper corner incisors of horses with increased age (De Lahunta and Habel 1986; Dyce et al. 2018; Schummer and Nickel 1979; Silver 1963). Some references cited numbers for this angle; for example, Habermehl (1981), reported the angle was 180 degrees in 8-year-old horses, and 90 degrees in 15-year-old horses. According to the mentioned study, the angle was sharper in horses over 16 years old. Muylle et al. (1996), stated that the angle between lower and upper corner incisors was perpendicular in the 10-year-old horses, and this angle increased in older animals, with the angle being approximately 100 degrees in the 17-year-old horses. However, the angle again decreased after that age, and it was 90 degrees and less in horses older than 19 years. This is inconsistent with the results of the present study and the numerous references cited above. This is probably due to the differences in measurement methods and tools, since the numbers presented in the mentioned study are neither qualitatively as anatomical references nor methodological and quantitative as this study. In support of this interpretation, Muylle et al. (1999), pointed out that because the exact size of angle between incisors is unavailable, the evaluation of this parameter does not provide appropriate age estimation. Therefore, software measurement of this parameter in the present study appears to have been able to answer this challenge. Richardson et al. (1995), used the scoring method to evaluate the angle between lower and upper corner incisors in thoroughbred horses and reported a strong correlation between angle and actual age, consistent with the present study results. For the first time in the present study, the angle between lower and upper corners was quantitatively measured using the software, and repeating this method in other studies with a larger sample size may provide a new age estimation approach.

**Correlation of estimated dental age with ancestral age**

The correlation between estimated dental age and actual age was very strong in the Arabian mares of Khuzestan, while a study found that the percentage of error in the estimated dental age was significantly higher in the Arabian mares of Khuzestan than in Anglo-Arabian mares in Poland (Muylle et al. 1996). Nicks et al. (2007) showed that the difference between estimated dental age and actual age in horses over 5 years old (Richardson et al. 1994). The difference in the age estimation of horses studied by these researchers was greater after 11 years old. Garrott (1991), found a significant probability of age estimation error in 15 to 20-year-old horses. Other researchers have also noted a significant correlation between estimated dental age and actual age in horses under 5 years old (Richardson et al. 1994). The difference in the age estimation of horses studied by these researchers was greater after 11 years old. Garrott (1991), found a significant probability of age estimation error in 15 to 20-year-old horses. Muylle et al. (1996), reported that the accuracy of dental age estimation in horses significantly decreased with age. Errors in dental age estimation are inevitable, as the best way to estimate an animal's age is to know the time of birth (De Lahunta and Habel 1986). Nevertheless, the horse's dental age estimation technique, despite its various reported differences, can be considered an auxiliary approach. It should be noted that racial differences are among the factors influencing the estimation of a horse's age (Gáspárdy et al. 2009). Muylle et al. (1998), observed these differences between Arabian, trotter, and Belgian draft horses, and reported that tooth wear was slower in Arabian horses than in the other two breeds (Muylle et al. 1999). However, the very strong correlation between estimated dental age and actual age in the present study made it possible to design an equation for age estimation.

**Conclusions**

In the examined mares from 15 days to 24 years, the lower incisor eruption and some related morphological changes were observed earlier than usual in some cases, probably due to faster physical maturity in the Arabian mares of Khuzestan. Among the indicators of dental age estimation in the Arabian mares of Khuzestan, the incisor eruption, cup, and Galvayne’s groove were rated more reliable than the dental star, enamel spot and hook. Comparison of the clinical crown length of incisors in each group revealed that I1, I2, and I3 had the highest to the lowest crown length, respectively. The correlation of estimated dental age with actual age in the Arabian mares of Khuzestan was estimated to
be very strong. Therefore, the dental age estimation in the Arabian mares of Khuzestan appears to be largely close to the animal’s actual age, however, the presence of error in the dental age estimation is unavoidable. However, it could be proposed that dental age estimation using a combination of several indicators is closer to the animal’s actual age.

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Code availability Not applicable.

Declarations

Ethics approval Approval was obtained from the ethics committee of Shahid Chamran University of Ahvaz (Ethics approval number: EE/97.24.3. 49903/scu.ac.ir).

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