

Age Estimation of Arabian Mares by Incisors Morphometry and Dentition Changes

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Abstract

Accurate estimation of 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 genuine Arabian horses of Khuzestan (southern west of Iran). The purpose of study was to investigate the age-dependent changes in morphology and morphometry of the incisors in the Arabian mares of Khuzestan in order to age estimation and to compare the estimated dental age with ancestral age. In this study, eighty-four Arabian mares of Khuzestan were examined at a number of 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 lower incisors including appearance and disappearance of cup, enamel spot, dental star and appearance and changes of Galvayne's groove in upper corner incisor were investigated. Comparison of clinical crown length of incisors in each group showed that first, second and third incisors (I_1 , I_2 , and I_3) had the maximum to minimum crown length, respectively. The correlation between ancestral age and clinical crown length was strong in I_1 ($r = 0.73$, $p \leq 0.001$), I_2 ($r = 0.8$, $p \leq 0.001$) and I_3 ($r = 0.81$, $p \leq 0.001$) in the Arabian mares of Khuzestan. The correlation of estimated dental age with ancestral age in the Arabian mares of Khuzestan was very strong ($r = 0.992$, $p \leq 0.001$). Therefore, the dental age estimation in the Arabian mares of Khuzestan seems to be very close to the actual age of the animal.

Introduction

Horse is a beautiful and magical animal that has 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). The importance of precise estimate of a horse's age, largely based on teeth, is clear to everyone (Muylle et al. 1996). The ability to estimate age can be useful when buying a livestock, predicting useful sport 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. Both during the life of the horse and many years after their death, the appearance of the teeth may determine the age, sex and health status of the animal (Luszczynski and Pieszka 2011). The estimation of horse's age based on the appearance and morphology of incisors was first introduced 600 Before Christ (Navin 1882). Since then, this technique has been employing throughout history. This method is nowadays considered as one of the common practices of equine veterinarians. In estimating the age of a horse, it should be noted that there may be disagreements between several veterinarians (Richardson et al. 1994). Significantly, researchers know that age estimation based on tooth structure not only is a science but also is 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 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 so far in the genuine Arabian horses of Khuzestan (southern west of Iran), and also given the interest of horse owners in the accurate estimation of horse's age and its applications, the purpose of this study was to investigate the age-dependent changes in morphology and morphometry of the incisors in the Arabian mares of Khuzestan in order to prepare the age profile of incisors changes and to compare the estimated dental age with ancestral age.

Materials And Methods

In the present study, 84 Arabian mares of Khuzestan were examined at a number of equestrian clubs and horse care centers in the Khuzestan province, Iran. The horses were clinically healthy and had no significant dental problems in their primary examination. So, 6 mares were excluded because of 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 less than 2.5 years, 3) between 2.5 years and less than 5 years, 4) between 5 years and less than 10 years, and 5) older than 10 years.

In each case, the originality 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 also noted on the basis of these documents. After confirming the originality of the mares, the mouth was opened by tethering the animal, and first images were taken with a digital camera (Canon, 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 from the upper corner if there was Galvayne's groove.

If permanent lower incisors were present, their clinical crown length was measured in the left mandible by two persons using a digital caliper (Guanglu, China), and then the mean of the two numbers was recorded.

The images were analysed by computer. Parameters of deciduous and permanent teeth eruption and their number, occlusal surface changes in lower incisors including appearance and disappearance of cup, enamel spot, dental star and appearance (Dyce et al. 2018; Nicks et al. 2007) and changes of Galvayne's groove in 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 25–50% proximal, from 50–75% proximal, from 75% proximal to 100%, up to 75% distal, from 75–50% distal, from 50–25% distal and from 25% distal to disappear. The index of hook appearance in upper corner on the left side was also assessed.

Statistical analysis

The studies were performed by at least two blind researchers and finally the dental age estimation was presented based on the mean analysis and then statistically compared with ancestral age. When

analysing the images, the angle between the left upper and lower corner incisors in the vestibular view image was measured using AutoCAD 2016 software.

The collected data were analysed using descriptive and analytical methods in SPSS version 16 software. Data were analysed 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 as significant difference. Charts were plotted with Excel 2013 software.

Results

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

Table 1. Sample size of mares examined in each age group

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

Time of lower incisor eruption

This index was evaluated in the groups 1 to 3 (according to the time of teeth eruption), because all incisors were permanent in the horses of groups 4 and 5.

Deciduous teeth

Regarding the lower deciduous incisor eruption in the Arabian mares of Khuzestan, first deciduous incisor (dl_1) was present in the youngest horse examined in this study (15 days old) and also in other mares with deciduous tooth aged more than 15 days. The second deciduous incisor (dl_2) was seen from age 1 month onwards and the third deciduous incisor (dl_3) in spite of appearance in 2 horses aged 3 and 4 months was absent in horses at ages 6 and 6.5 months, and had begun to erupt among other mares over 10 months old (Fig. 1A).

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, though the permanent incisors were erupted in seven mares earlier than expected (Fig. 1B).

Clinical crown length of lower permanent incisors

In the studied Arabian mares, the clinical crown length of lower permanent incisors was increased with age (Table 2). Moreover, first incisor (I_1), second incisor (I_2) and third incisor (I_3) had maximum to minimum crown length, respectively.

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

clinical crown length (mM)			
Age groups	I_1	I_2	I_3
1	-	-	-
2	15.47 \pm 1.87 ^c	-	-
3	18.27 \pm 1.57 ^{Ac}	16.18 \pm 0.63 ^{Ac}	11.06 \pm 5.38 ^{Ac}
4	24.29 \pm 2.18 ^{Ab}	21.52 \pm 2.46 ^{Bb}	18.32 \pm 1.2 ^{Cb}
5	27.37 \pm 1.88 ^{Aa}	27.37 \pm 2.97 ^{Aa}	22.83 \pm 2.88 ^{Ba}

Different lowercase letters in each column indicates a significant difference.

Different uppercase letters in each row indicate a significant difference.

As shown in the Table 2, the crown length of tooth I_1 in the groups 2 and 3 was significantly different from groups 4 and 5 ($p \leq 0.001$), and increased with age. There was also a significant difference ($p \leq 0.01$) between the groups 4 and 5.

The crown length of I_2 and I_3 was also increased significantly with age, with a significant difference ($p \leq 0.001$) between all groups.

When comparing the crown length of the incisors in each group, the I_1 , I_2 and I_3 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 ($p \leq 0.001$) between teeth in the group 4. In the group 5, the crown length of I_1 and I_2 had no significant difference and was approximately equal, while the I_3 was significantly ($p \leq 0.01$) shorter than the others.

The correlation of ancestral age with clinical crown length of lower incisors

The correlation coefficient between ancestral age and clinical crown length index in the I_1 , I_2 and I_3 were strong ($r = 0.73$, $p \leq 0.001$), ($r = 0.8$, $p \leq 0.001$) and ($r = 0.81$, $p \leq 0.001$), respectively.

The equation for age estimation based on clinical crown length of lower incisors

Age (month) = $40.89 - [\text{Length of } I_3 \text{ (mM)} \times 7.78] + [\text{Length of } I_2 \text{ (mM)} \times 24.47] + [\text{Length of } I_1 \text{ (mM)} \times 24.78]$

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

The angle between the lower and upper corner incisors

The measurement of angle between lower and upper corner incisors in the Arabian mares of Khuzestan showed in Fig. 2, that this angle was decreased with age and this difference was considered statistically significant between the groups 4 and 5 ($p \leq 0.001$). The correlation of this index with ancestral 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 ancestral age estimation corresponding to the angle index is as follows:

Ancestral age of mares (month) = $509 - (\text{angle between lower and upper corner incisors} \times 3.24)$.

Figure 3 shows the sharpening of the angle between upper and lower corners with increasing age of Arabian mares in the groups 4 and 5.

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 disappeared at ages 10 to 26 months (Fig. 4A-D). In the permanent teeth, there was a cup on all lower incisors up to 4.5 years old, but disappearance of the cups included a wide age range. Thus, the cup was disappeared in I_1 and I_2 usually after 8 years old and in I_3 after 10 years old (Fig. 5A-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 dl_2 , dl_1 and dl_3 at the ages of 10 months, 1 year and 3 months, as well as 2 years and 5 months, respectively (Fig. 6A-C). The appearance of dental star on the lower permanent

incisors in the Arabian mares of Khuzestan investigated in this study was first observed on the I_1 at the age of 5 years and 5 months and on the I_2 and I_3 at the age of 7 years and 10 months (Fig. 6D,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 I_1 and I_2 at the age of 15 years and on the I_3 at the age of 19 years (Fig. 6F).

The enamel spots

The enamel spot in the Arabian mares of Khuzestan was first observed on the tooth I_1 at 7 years and 10 months old, and on the I_2 and I_3 at 12 years old (Fig. 7A,B). This index was seen on the occlusal surface of lower incisors up to 16 years old, while 19-years-old mares had no enamel spot and none of the older ones had enamel spot (Fig. 6F).

The morphology of occlusal surface

The occlusal surface of lower incisors in the Arabian mares of Khuzestan was initially transverse oval. With age increasing, rounding of the occlusal surface occurred in I_1 at 7 years and 10 months old and in the I_2 and I_3 at 12 and 13 years old. Triangular occlusal surface was first observed at 15 years old and then all lower incisors were triangular at 20 years old (Fig. 8).

Figure 9A-C shows percentage of the morphological changes in the occlusal surface of lower incisors with age.

The hook

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

The Galvayne's groove

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

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

Figure 11 shows the correlation of estimated dental age based on lower incisor status with ancestral age in the Arabian mares of Khuzestan.

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

According to Fig. 19, the equation between dental age and ancestral age in the Arabian mares of Khuzestan is as follows:

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

Where, R^2 is the coefficient of determination which was 0.985 in this study, meaning that dental age could justify the ancestral age up to 98.5%.

Discussion

The time of lower incisor eruption

The deciduous teeth

References of veterinary anatomy have described the eruption time of the dl_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 dl_2 eruption in the horses, including 3–4 weeks and rarely up to 8 weeks (Schummer and Nickel 1979), 4–6 weeks (Getty 1975), 1 month (De Lahunta and Habel 1986) and 6 weeks (Dyce et al. 2018; König and Liebich 2020). The largest difference with respect to deciduous incisor eruption in available references is related to the tooth dl_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 dl_3 in the Arabian mares of Khuzestan had a different eruption time, while the eruption time of the dl_1 and dl_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, this time is mentioned in some references up to 6-month delay for 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 l_2 and l_3 , consistent with the present study in some cases²¹). However, the teeth have been 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. Obviously, the reported differences may be due to racial and species differences between the different horses, in addition to species differences.

The clinical crown length of lower permanent incisors

Muyllé et al. (1999), evaluated the crown length of permanent incisors in horses slaughtered in a Belgian slaughterhouse, and reported that the I_1 to I_3 had the highest to shortest lengths, respectively, similar to the results of the present study. In their study, the length of the teeth was longer up to the age of 10 years, in line with the results of the present study. In a three-dimensional study of horse incisors after euthanize by Schrok et al. (2013), it was found that incisors reach a maximum length at 2 to 4 years after eruption, and this length is 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 present study are somewhat different from the results of their study because the horses of the Group 5 (over 10 years of age) in the present study had the highest clinical crown length. In explaining of this difference, first, the method and tools of measurement in the present study were different from those of of Schrok 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 less than 15 years old, thus 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, because the lack of horses older than 20 years, this was impossible.

According to the literature review, the present study seems to be unique study evaluating the clinical crown length of incisors in the live horses. Thus, the correlation between ancestral age and clinical crown length (which was particularly very strong for I_2 and I_3) and the stated equation for estimating age 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 the lower and upper corner incisors of horses with aging (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 years old horses, and 90 degrees in 15 years old horses. According to the mentioned study, the angle was sharper in horses over than 16 years old. Muyllé et al. (1996), stated that the angle between the lower and upper corner incisors was perpendicular in the 10 years old horses, and this angle increased in the older animals, with the angle being approximately 100 degrees in the 17 years old horses, but the angle again decreased after that age, and it was 90 degrees and less in the 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 theory, Muyllé 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 seems to have been able to answer this challenge. Richardson et al. (1995), used the scoring method to evaluate the angle between the lower and upper corner incisors in thoroughbred horses

and reported a strong correlation between angle and ancestral age, consistent with the present study. It seems for the first time in the present study, the angle between the 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.

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 ²⁶⁾ and 10–24 months ⁸⁾.

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 the 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 on 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 the I_1 , 7 to 11 years old in the I_2 and 9 to 15 years old in the I_3 (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 due to 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 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 horse's age. If it is possible to measure the depth of the cups, it seems likely that this challenge will be largely resolved, though it is not easy to do in live horses. In spite of what has been said, in the present study similar to study by Luszczynski et al. (2015), disappearance of the cup was found to be useful indicator for dental age estimation and also they, introduced this index as reliable for estimating the age of Hucul pony (Luszczynski and Pieszka 2011).

The dental star

The time of appearance of 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), although 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, roughly similar to the present study (De Lahunta and Habel 1986; Dyce et al. 2018). Richardson et al. (1995), reported the appearance of dental star in thoroughbred horses at 3 years and 9 months old. Also, observed the dental star in the I_1 to I_3 in trotter horses at 5 years old, over than 6 years and 7 to 8 years old, respectively (Muyllé 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 (Muyllé et al. 1997), while the same researchers reported the appearance of dental star in the lower incisors at the ages of 5 to 7 years in the Belgian Arabian horses, that is 2 to 3 years earlier (Muyllé et al. 1998), approximately similar to the results of the present study. As previously mentioned, the appearance of 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. On the other hand, the appearance of dental star due to large age scattering in the present study was not considered as a reliable indicator, while Muyllé 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 present study.

It seems that since the appearance of dental star is heavily influenced by the type of nutrition, the arrangement of teeth and the amount of tooth wear, it has a wide range of ages, and the racial and individual differences that affect the dentition and jaw shape of horses contribute to the reasons. Therefore, with respect to 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 references of veterinary anatomy.

The enamel spots

The review of the references of veterinary anatomy showed that the enamel spot appears on the lower incisors in horses between 9 to 11 years old, and disappears between 13 to 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 I_1 , seems to appear about one year earlier than usual, while being observed one to two years later in the I_2 and I_3 , but the time of disappearance of this index in present study, is almost similar to that of other horses and ages recorded in the references.

The morphology of 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 present study, but 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), 9–12 years (Martin 2002; Schummer and Nickel 1979), 12–17 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) point out 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 appropriate index for age estimation due to high data scattering. Muylle et al. (1997), reported that the morphological changes in occlusal surface of incisors in the draft horses are 1 year earlier than in the trotter horses. According to the researchers, the morphological changes occur in the Arabian horses later than 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 do not follow the expected pattern of morphological changes (De Lahunta and Habel 1986; Dyce et al. 2018).

By reviewing various references and studies, it seems that the morphological changes on occlusal surface in the Arabian mares of Khuzestan are largely consistent with the model mentioned in American Association of Equine Practitioners (AAEP) (Martin, 2002), though due to the large age scattering, this index in the present study was not considered 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 believe that this index disappears at 9 years old and reappears between 11 to 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 were between 5 to 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 on one hand and the possibility of reappearance at different ages make the hook not a reliable and suitable parameter for dental age estimation for the horses.

The Galvayne's groove

According to the references of veterinary anatomy, 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; Muylle et al. 1997; Muylle et al. 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 identified as suitable for age estimation in some studies due to variation in groove length (Muylle et al. 1996; Muylle et al. 1997; Muylle et al. 1998; Muylle et al. 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 (the pattern mentioned in the references of veterinary anatomy).

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), thus providing the possibility of age estimation with the approximate of 1 to 2 years. To confirm these 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 seems to be able to respond to the challenge of age estimation with a few years.

Correlation of estimated dental age with ancestral age

The correlation between estimated dental age and ancestral 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 the Anglo-Arabian mares in Poland (Muylle et al. 1996). Nicks et al. (2007) showed that the difference between estimated dental age and ancestral age in the horses older than 8 years was greater than in the younger horses. Also, noted a significant correlation between estimated dental age and ancestral age in the horses less than 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 the significant probability of age estimation error in 15 to 20 years old horses. Muylle et al. (1996), reported that the accuracy of dental age estimation in the horses was 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 the various reported differences, can be considered as an auxiliary approach. It should be borne in mind that racial differences are of the factors influencing the estimation of horse's age (Gaspard et al. 2009). Muylle et al. (1998), observed these differences between Arabian, trotter and Belgian draft horses, and reported that tooth wear in Arabian horses was slower than

the other two breeds ¹⁴). However, the very strong correlation between estimated dental age and ancestral age in the present study made it possible to design an equation for the age estimation.

Conclusions

In the examined mares from 15 days to 24 years old, 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 valid than the dental star, enamel spot and hook. Comparison of clinical crown length of the incisors in each group showed that the I_1 , I_2 and I_3 had the highest to the lowest crown length, respectively. The correlation of estimated dental age with ancestral 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 ancestral age, though 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 ancestral age.

Declarations

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Conflicts of interest/Competing interests

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

All authors contributed to the study conception and design. Sample preparation and data collection were performed by [Hamid Pesarakli] and [Alireza Ghadrddan Mashhadi]. Data analysis were performed by [Kaveh Khazaeel] and [Mahdi Pourmahdi Borujeni]. The first draft of the manuscript was written by [Kaveh Khazaeel] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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).

Consent to participate

Not applicable

Consent for publication

Not applicable

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Figures



Figure 1

A: Occlusal surface view of 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 permanent corner).

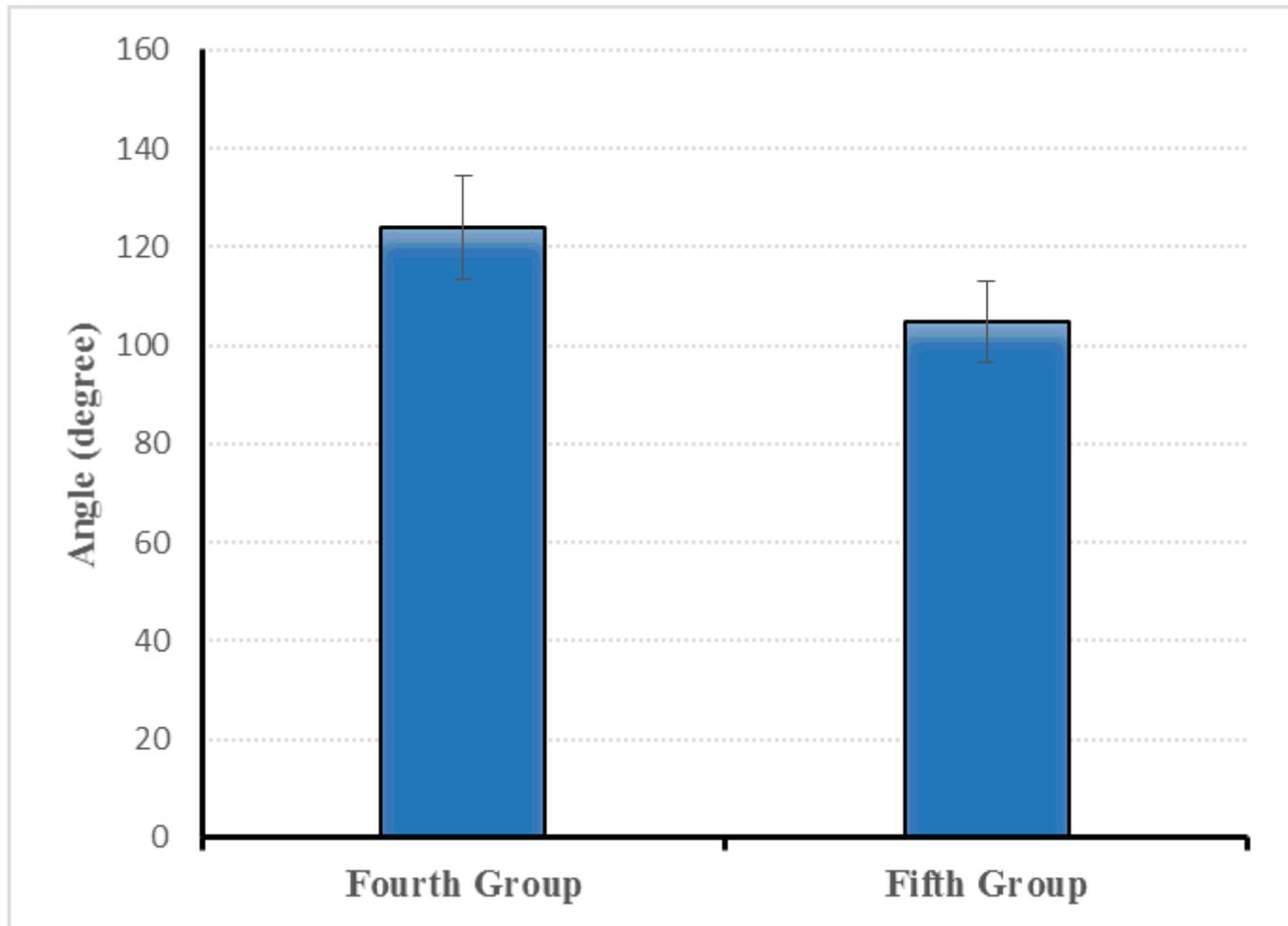


Figure 2

Angle measured between lower and upper corner incisors in Arabian mares of Khuzestan. A significant difference observed between the groups 4 and 5.

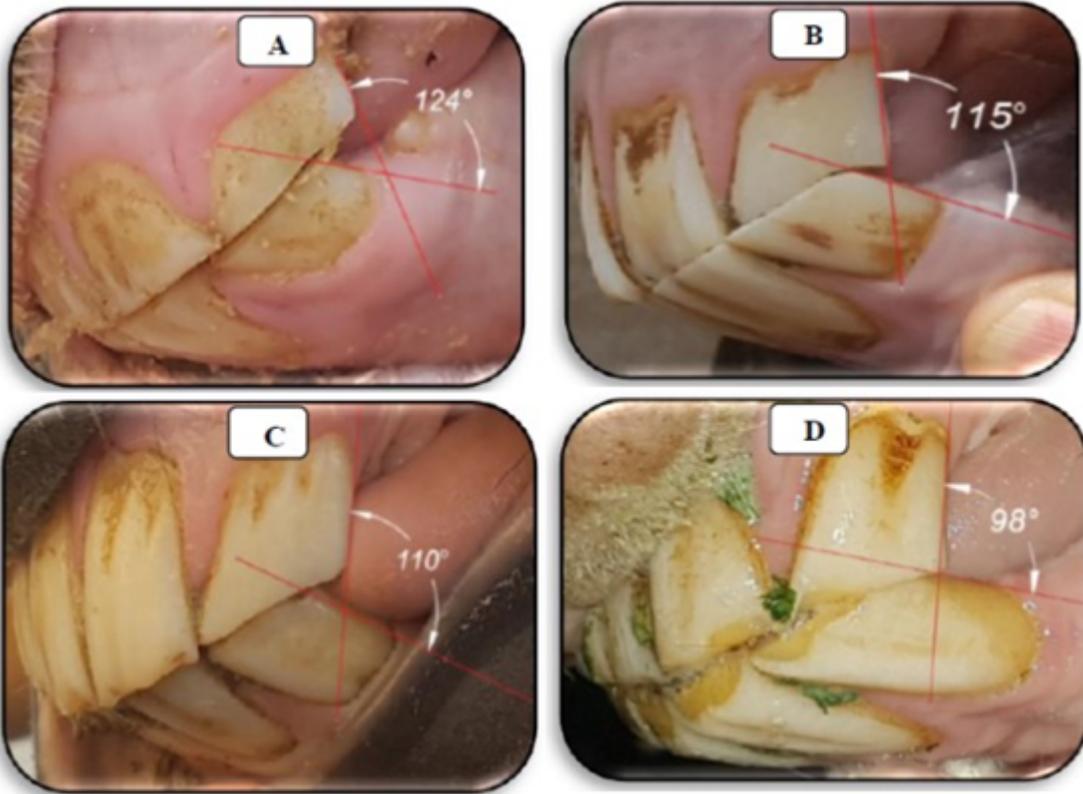


Figure 3

Vestibular view of incisors in Arabian mares of Khuzestan. A) 5 years and 7 months; B) 8 years and 4 months; C) 12 years; D) 23 years. The angle between lower and upper corners has sharpened with age.

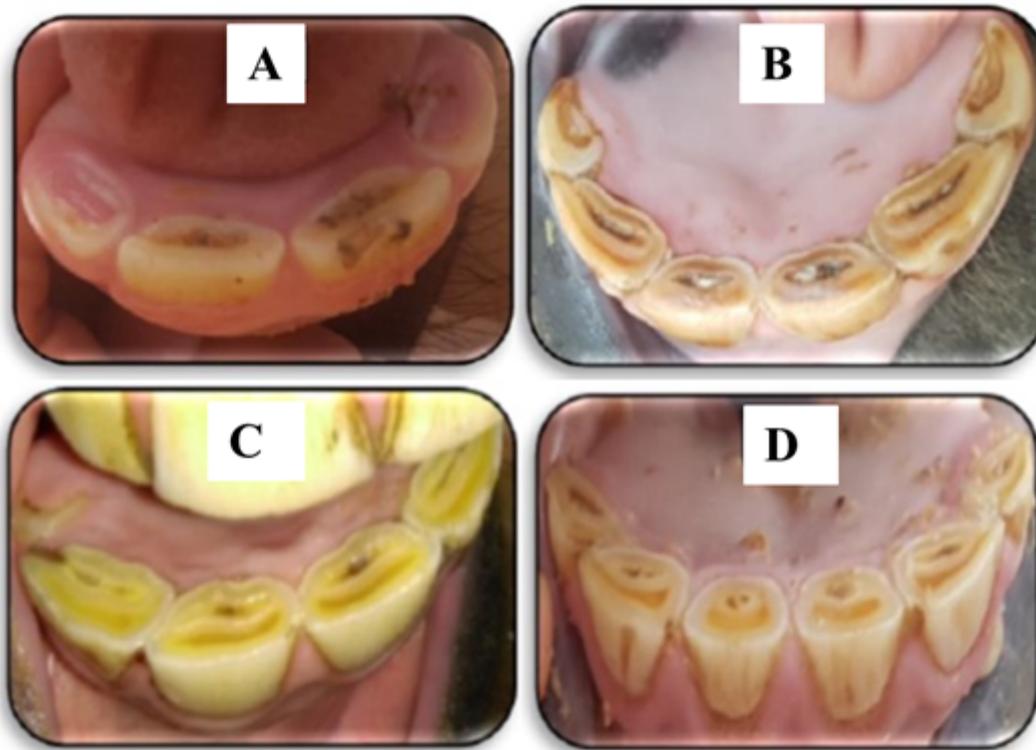


Figure 4

Occlusal view of lower incisors in Arabian mares of Khuzestan. A) Appearance of cup in dl1 at 1 month old, B) All lower deciduous incisors at 3 months old have cup. C) Disappearance of cups in dl1 and dl2 at 10 months old, D) Disappearance of cup in dl3 at 2 years and 2 months old.

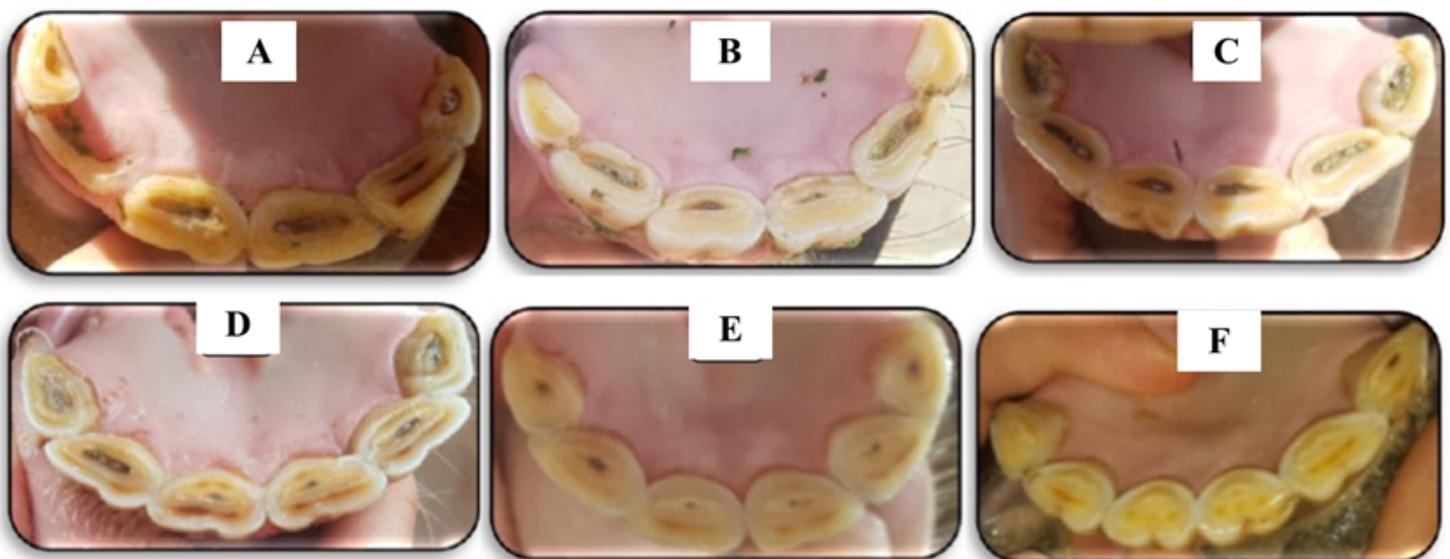


Figure 5

Occlusal view of lower incisors in Arabian mares of Khuzestan. A) Appearance of the cup in I1 at 2 years and 5 months old, B) Appearance of the cups in I1 and I2 at 3 years and 3 months old, C) Appearance of

the cups in I1, I2 and I3 at 4.5 years old, D) Disappearance of the cup in I1 at 5 years and 5 months old, E) Disappearance of the cups in I1 and I2 at 6 years and 3 months old, F) Disappearance of the cups in I1, I2 and I3 at 7 years and 10 months old.

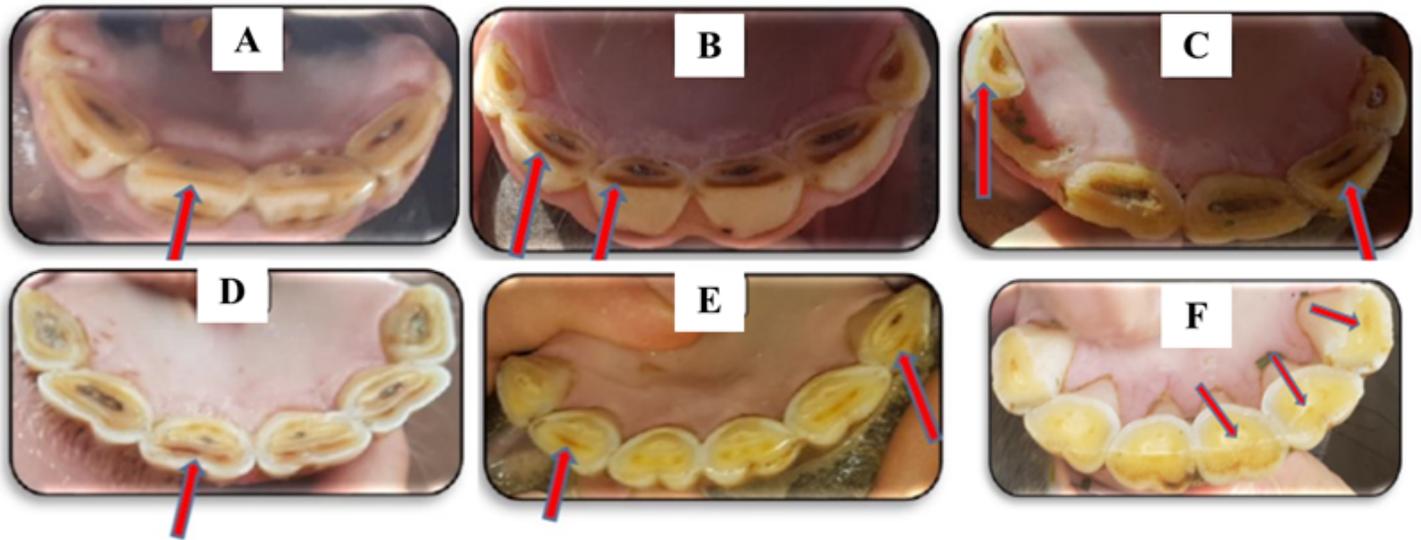


Figure 6

Occlusal view of lower incisors in Arabian mares of Khuzestan. A) Disappearance of the cup and the appearance of the dental star on the I1, the appearance of cup on the I2 at 10 months old, B) All lower deciduous incisors have cups, the I1 and I2 have dental star at the age of 1 year and 3 months, C) The I1 with cup, the I2 with eruption on the right and the I2 with no cup on the left, the I3 with cup and dental star at the age of 2 years and 5 months, D) Appearance of dental star on the I1 at the age of 5 years and 5 months, E) Appearance of dental star on the I2 and I3 at the age of 7 years and 10 months, F) the round dental star in all lower incisors in the center of the occlusal surface (Arrows show the dental star).



Figure 7

A & B: Occlusal view of lower incisors in Arabian mares of Khuzestan. A) I1 with dental star and enamel spot, I2 and I3 with dental star at 7 years and 10 months old, B) I1, I2 and I3 with dental star and enamel

spot at 12 years old (Arrows show the enamel spot), C & D: The appearance of the hook in the left upper corner of the Arabian mares of Khuzestan. C) 7 years and 5 months old (the youngest), D) 15 years old (the oldest) (Circles show the hook).

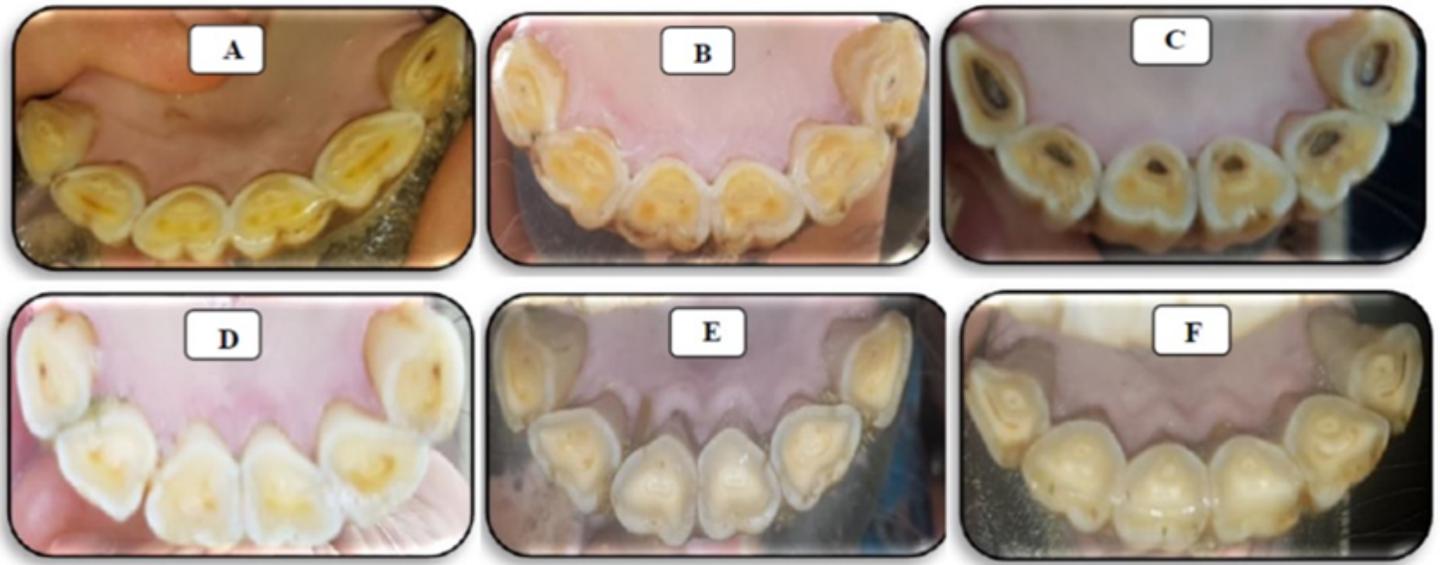


Figure 8

Morphological changes in the occlusal surface of lower permanent incisors in Arabian mares of Khuzestan. A) At the age of 7 years and 10 months, the occlusal surface was rounding in the I1 and transverse oval in the I2 and I3, B) At the age of 12 years, the occlusal surface was rounded in the I1, rounding in the I2 and transverse oval in the I3, C) At the age of 13, all three lower incisors were rounding, D) At the age of 15, all three lower incisors were rounded, E) At the age of 15 years, the occlusal surface was triangular in the I1 and rounding in the I2 and I3, F) At the age of 20, the occlusal surface of all three incisors was triangular.

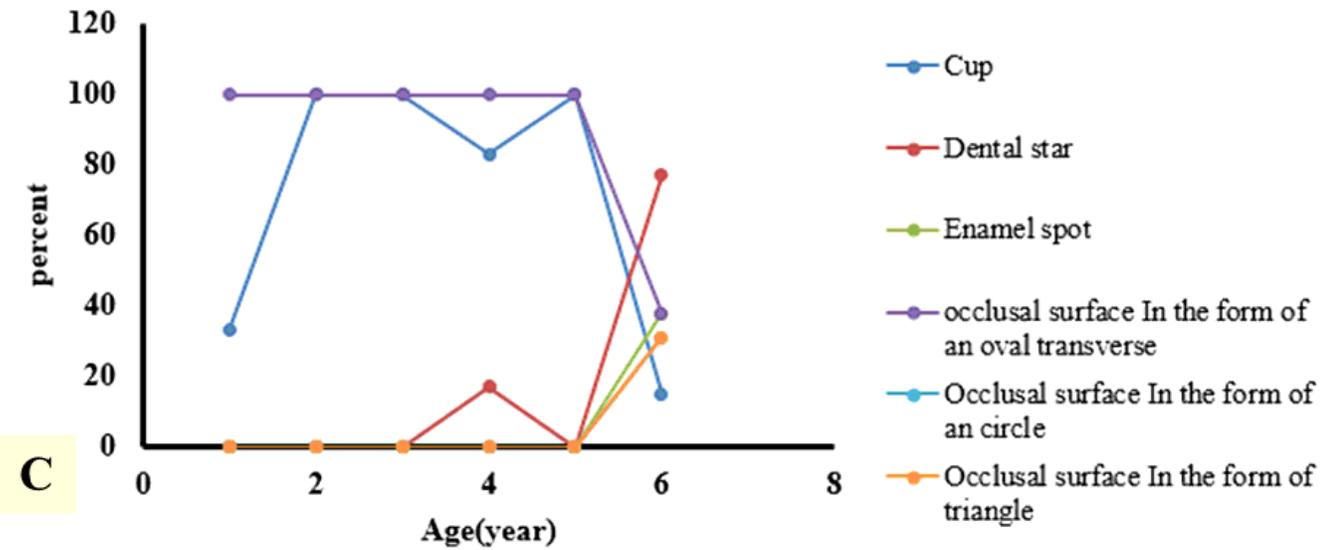
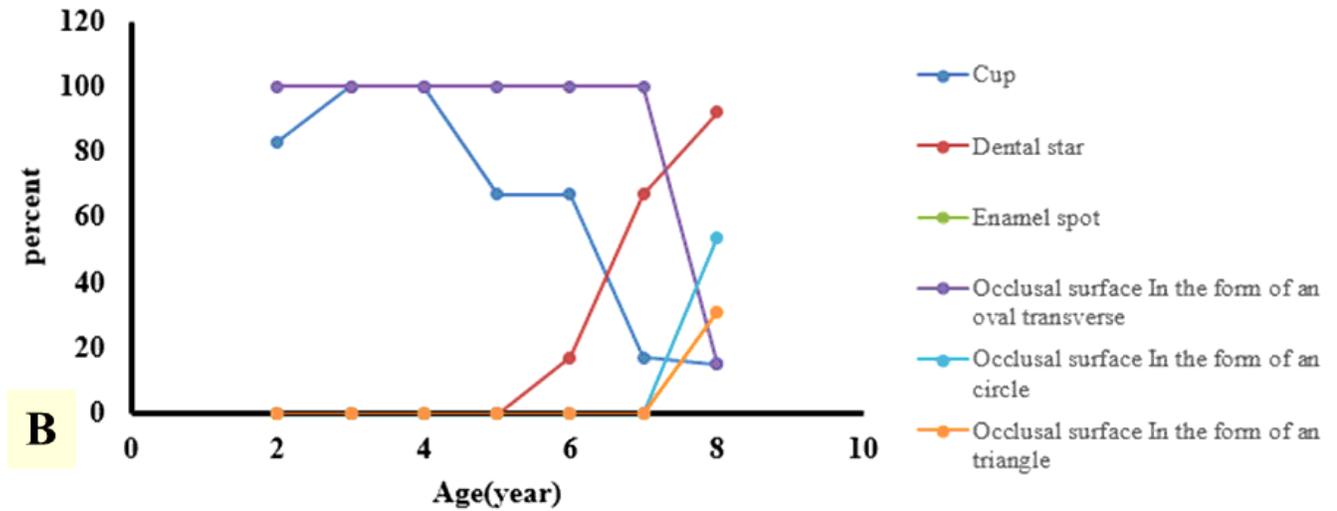
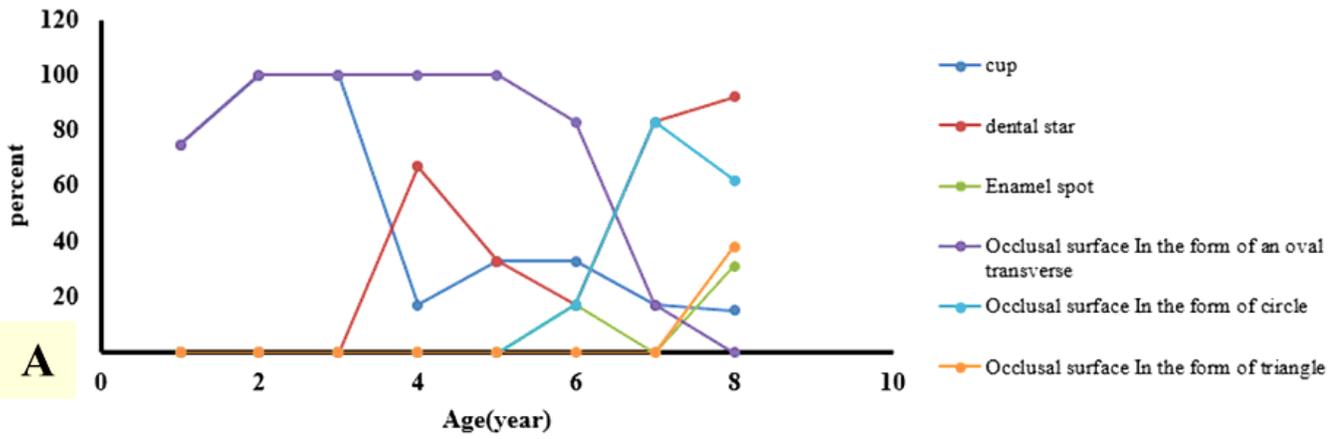


Figure 9

Morphological changes in occlusal surface of the I1 (A), I2 (B) and I3 (C) with age.

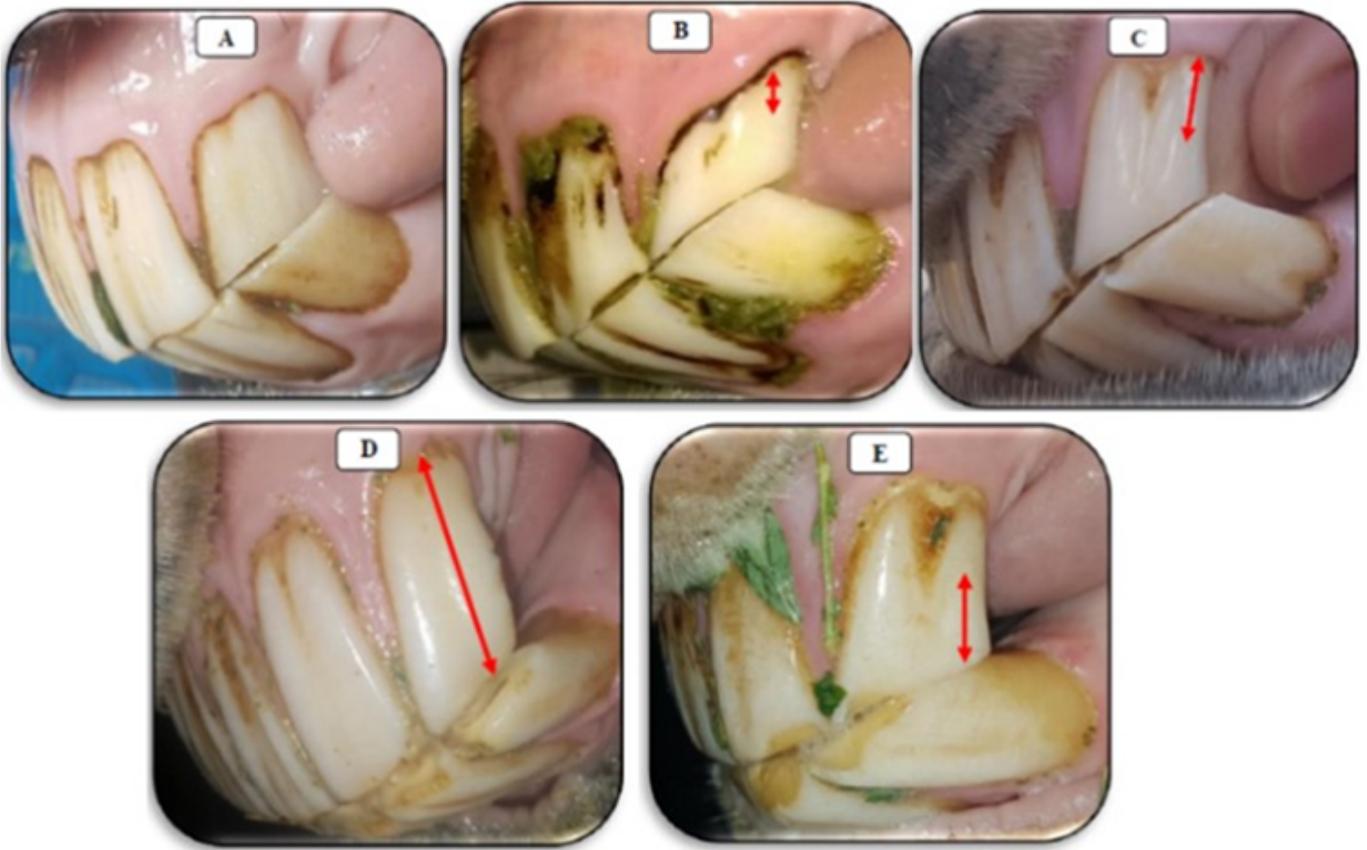


Figure 10

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 a 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 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. Arrow shows the size of Galvayne's groove on the vestibular surface of the upper corner.

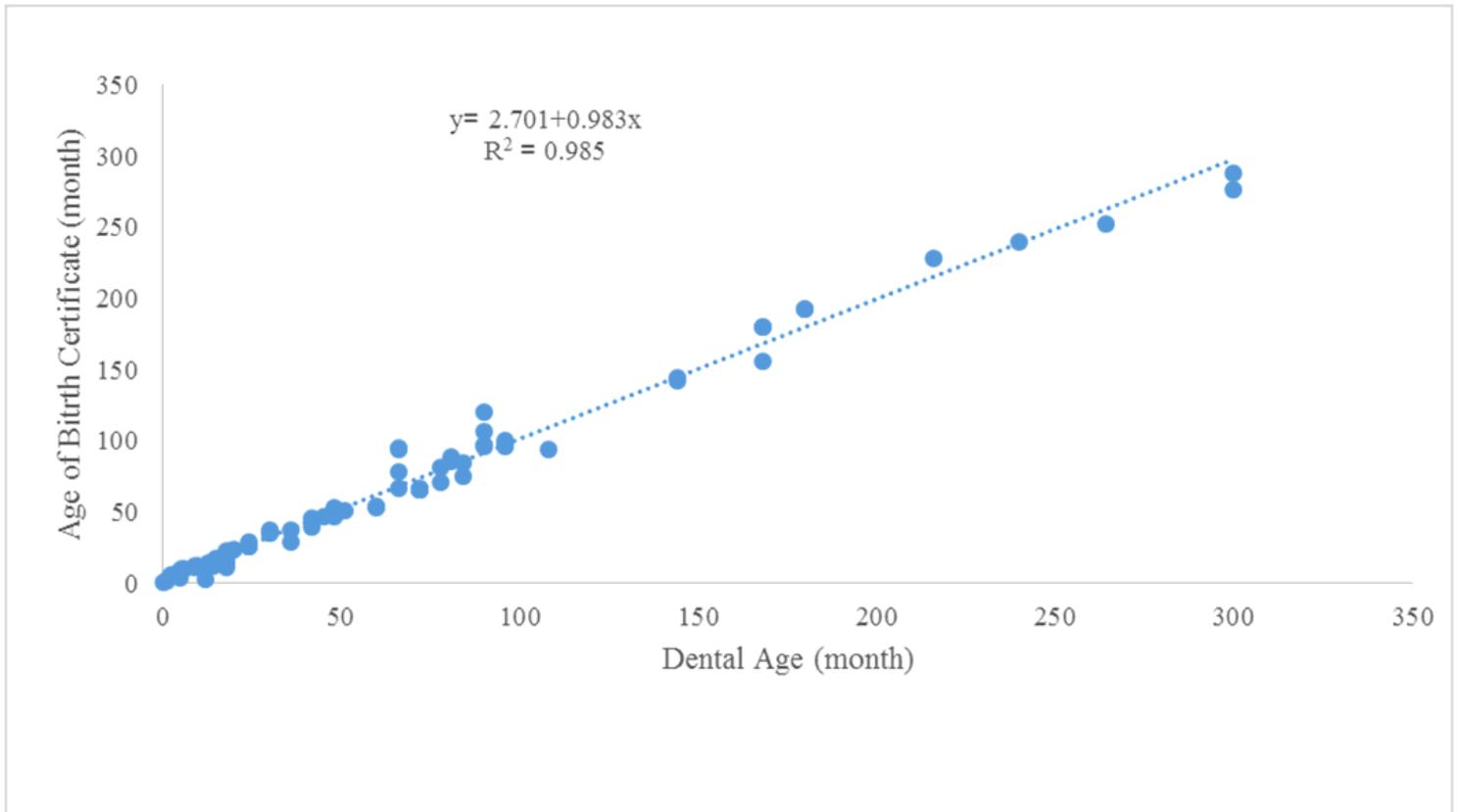


Figure 11

Correlation of estimated dental age based on lower incisor status with ancestral age.