Regression Models Based on Ultrasonography Fetal Ocular Measurements for Determining Gestational Age in Arab-Barb Mares

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RESEARCH ARTICLE

Abstract
The aim of the present study was to develop regression models based on ultrasoundographic measurements of fetal eyes (orbital diameter (OD) and eyes volume (EV)) to determine mare gestational age (fetal age) in Arab-Barb breed and to compare the obtained formula with similar formulas used in practice. Twenty-two Arab-Barb mares were included in this study. For each mare, an average of 6 ultrasound examinations per month of gestation, from day 90 of pregnancy to foaling were performed. The parameters examined, OD and EV, were used to establish regression models to determine gestational age of mares. The results led to establish linear regressions of the two parameters, EV and OD. The derived gestational age (GA) prediction formulas were: GA = 0.0097EV + 93.887; GA = 8.687OD + 0.6598 (GA in day; EV in mm3; OD in mm). The coefficients of correlation (R) between EV (R=0.96), OD (R=0.93) and mare gestational age were highly significant (P<0.001) indicating that the two eye measurements can be used to determine accurately mare gestational age after 90 days of pregnancy in Arab-Barb breed. In addition, the formula established in the present work from the OD is more accurate in determining the GA in the Arab-Barb compared to that predefined for English Thoroughbred. This comparison reveals that the growth of fetal orbit of Arab-Barb breed is slower than that of the English Thoroughbred.

Keywords: fetal age; fetal orbital diameter; fetal eye volume.

INTRODUCTION

Determination of gestational age of mares has a great importance on equine breeding management. Indeed, it can be used to ensure: the food need of mare according to the age of the fetus, reducing, therefore, embryonic mortalities and metabolic imbalances, an effective sanitary and vaccination program, a good quality of colostrum, a safe and successful foal delivery (Aoki et al., 2012; Morel et al., 2002; Samper, 2009). However, determination of pregnant mares and their gestational ages in equine farms is sometimes difficult and complicated by an unknown breeding or ovulation date.

In the early stage of pregnancy of mare, particularly before 70 days, the estimation of the size and the asymmetry of uterus by transrectal palpation (Ginther, 1983) and some ultrasound measurements on the fetus can be used for determining gestational age (Acker et al., 2001; Ginther, 2007). Between 70 and 90 days, the fetus sinks into the abdominal cavity and becomes no longer explorable (Bucca, 2005; Mebarki et al., 2019). After 90
days of gestation, ultrasound measurements of fetal ocular orbit are an easy technic, widely used to determine gestational age (Renaudin et al., 2000; Turner et al., 2006).

Kähn and Leidl (1987) developed a formula based on fetal ocular diameter, measured by ultrasonography, to determine gestational age in Thoroughbred horse (Kähn and Leidl, 1987). This formula was integrated in several ultrasound machines, as a reference formula. However, this formula could not provide the accurate gestation age for other breeds owing the genetic and morphological variations between breeds (Hevia et al., 1994; Mebarki et al., 2019; Panchal and Gujarati 1995). In this context, Hartwig et al. (2013) found a divergence in fetal orbit measurements between Crioulo horse and those of Thoroughbred of similar size (Hartwig et al., 2013). Moreover, Turner et al. (2006) reported that Pony fetal eye measures are smaller than those of light horse mares, reported by Kähn and Leidl (1987). From findings of those studies, the need to assess fetal development based on each horse breed is evident.

Arab-Barb is the dominant horse breed in Algeria, with approximately 90,000 of heads, representing more than 90% of the equine population (Rahal, 2005). Despite its recognized importance in the history, culture and agribusiness of the country, there is a lack of scientific research on this breed, particularly on reproductive management. In addition, implementation of natural covering, in Arab-Barb horse, renders very difficult the determination of the ovulation dates, the early detection of pregnant mares and gestation age.

To our knowledge, no study was performed on the determination of gestational age in Arab-Barb mares by ultrasound measurements on the fetus. Thus, the objective of this work was to use fetal eye measurements by ultrasonography to develop a formula to accurately estimate gestational age in Arab-Barb mares and to compare it with similar formulas used in practice.

MATERIALS AND METHODS

Study design

This study was carried out on 22 multiparous pregnant mares of Arab-Barb breed, aged between 6 and 15 years (9.09 ± 2.84 years). These mares had body condition scores greater than 5 (on a scale that ranged from 1 to 10). Before the experiment, the mares included had no abnormalities of the genitalia after breeding soundness examination. They were the subject of 1012 fetal orbit measurements between 2017 and 2019 at the Institute of Veterinary Sciences of Batna (Algeria) with an average of 46 measurements per mare. To determine ovulation date, ultrasonography of follicular growth was evaluated every 24 hours, starting from 15 days of the estrous cycle. Ultrasound gestation follow-up was performed every 15 days until 3 months of pregnancy. All experimental procedures were approved according to the guidelines established by the Institutional Animal Care Committee of the National Administration of Algerian Higher Education and Scientific Research. Ethical approval number: 98–11 law of August 22, 1998.

During the length of pregnancy, fetal orbit measurements were taken 6 times/month from the 3rd month (day 0 = day of ovulation determined by ultrasound machine) to foaling (328 ± 12.41 days).

Fetal orbit measurements were taken using a portable ultrasound, DRAMINSKI model 4 Vet mini, equipped with a linear probe from 5 to 7.5 MHz. A transrectal palpation was performed to empty the rectum and examine the reproductive tract. Then, a scan of the uterus by inserting the ultrasound probe into the mare’s rectum was performed to locate the fetal skull and identify the orbital cavities.

Once identified, the ultrasound image of the orbital cavity was frozen when the maximum transverse diameter of the vitreous was obtained (Figure 1).

![Figure 1. Ultrasound image of measurement of fetal orbital parameters (width and length (OD))]
Two measurements on fetal orbital were taken on the frozen image: the width and length of the eye (Figure 1). The width represents the distance between the middle of the edge of the anterior lens capsule and the middle of the inner edge of the optic disc. The length or orbital diameter (OD) represents the greatest distance between the inner edges of the vitreous. The averages of the width and the length of the eye were obtained from 3 repeated measurements. The two measurements, the width and the length of the eye, were used to estimate the eye volume (EV) according to the formula developed by Renaudin et al. (2000) (Renaudin et al., 2000): \[\text{volume} = \text{length} \times \text{length} \times \text{width}\]

Statistical analyses

Descriptive statistics, particularly, calculations of means, standard deviations and coefficients of variation of fetal orbit diameters (OD) were performed to estimate the homogeneity of mares group divided by OD with respect to gestational age. A polynomial regression analysis was used to predict the effect OD and EV on gestational age, and models were chosen based on the significance of the regression coefficients (P <0.05) and the determination coefficient (r²). The fetal orbit diameter of Arab-Barb mares was compared with the data that Kahn and Leidl (Kähn and Leidl, 1987) obtained for Thoroughbred horse using an analysis of variance. All statistical analyses were performed using SPSS statistical analysis software version 26.0 (SPSS Inc. USA).

RESULTS AND DISCUSSIONS

Figure 2 represents the means± SD of OD according to gestation age. The small values of SD and CV revealed a high degree of between-group homogeneity. The largest value of CV 15.93 was observed for orbits 23 mm in diameter.

The growth curves of OD and EV relative to gestational ages of Arab-Barb mares showed that the best suitable curves to obtained data were linear. The obtained linear regression models were \[y = 0.0097x + 93.887\] (Figure 3) and \[y = 8.687x_1 + 0.6598\] (Figure 4), where y represents gestational age of mares in days, x represents EV in mm³ and \(x_1\) represents OD in mm. These 2 formulas had \(r^2\) of 0.96 (P <0.001) and 0.93 (P <0.001), respectively.

Figure 2. Graphical representation of evaluation of the homogeneity of the different groups of mares by gestational age, showing the mean and standard deviation of gestational age in relation to fetal ocular orbit measurement

Figure 3. Linear regression model showing the relationship between the EV (eye volume in mm³) and gestational age (days) in Arab-Barb mares
As reported by Kahn and Leidl (1987), the OD development of Thoroughbred horses occurs linearly following the model $y = 0.14x + 0.77$, where "y" represents the orbit diameter in mm, and "x" represents the gestational age in days. The comparison between the linear regression model of OD of the present study and that of Kähn and Leidl (1987) revealed that gestational age provided by Kähn and Leidl model was significantly ($P < 0.05$) lower than actual age obtained from our model (Figure 4). In similar studies, in the English thoroughbred (Ginther, 1992; Mckinnon et al., 1988), pony (Turner et al., 2006), Crioulo (Hartwig et al., 2013) and Standardbred mares (Lanci et al., 2019), the regression models were also linear. However, the formulas obtained are different from each other and from those obtained in our study indicating that each equine breed needs an assessment of fetal development. This divergence between breeds could be due to genetic variation between breeds expressed by differences in fetal development. In this context, Turner et al. (2005) reported that fetal eye measures are not affected by mare's BCS, mare's weight and foal weight. They concluded that the ratio of eye size to skull width may vary as a characteristic of breed, and may confound application of prediction models among breeds, even among breeds of similar size.

In addition, the comparison between linear growth curve of OD of the current study and that of Kähn and Leidl (1987) showed that Arab-Barb fetuses have a slower growth rate than that of the English thoroughbred. Therefore, the models established in the current study are strongly recommended to more accurately assess the fetal age in Arab-Barb breed.

**CONCLUSIONS**

Based on the results of the fetal eye measurements, particularly EV and OD relative to gestational age in Arab-barb mares, two linear regression models were obtained. These models led to establish 2 reliable formulas ($GA = 0.0097EV + 93.887$; $GA = 8.687OD + 0.6598$). The formula of OD in the present work is more precise in determining the GA in the Arab-Barb compared to that predefined for English Thoroughbred (Kähn and Leidl, 1987). Moreover, this comparison reveals that the growth of fetal orbit of Arab-Barb breed is slower than that of the English Thoroughbred.

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Conflicts of Interest
The authors declare that they do not have any conflict of interest.

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