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A REGRESSION MODEL INCLUDING FETAL ORBIT MEASUREMENTS TO PREDICT PARTURITION IN STANDARD BRED MARES WITH NORMAL PREGNANCY

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Abstract

In the mare, foaling is a critical unpredictable event due to a wide range of gestational length and the absence of clear signs of impending parturition. To predict foaling, pH, inversion sodium potassium and increase of calcium concentration in mammary secretions are used. The aim of this study was to find how many days are left until parturition knowing mare's age (A) and parity (P) combined with ultrasonographic measurements of the fetal orbit in Standardbred mares with normal pregnancy. Eighty healthy Standardbred mares with normal pregnancy were hospitalized for attended delivery. Information about mare's age, parity and breeding date were recorded at admission. Transrectal ultrasonography were routinely performed at admission and every 10 days until parturition using a B-mode real time portable unit equipped with a 5-7.5 MHz linear transducer. The images of the fetal orbit were acquired when cornea, anterior and posterior chamber, vitreous body, lens and optic nerve were visible. Longitudinal diameter (LD) was considered as the distance between the two ocular poles, within the vitreous body; transverse diameter (TD), perpendicular to LD and bisecting the lens, was measured as the distance between cornea and retina. At delivery, length of pregnancy and gestational age at each exam were registered. For each ultrasound examination, days before parturition (DBP) were calculated. Seventy-eight Standardbred mares with normal pregnancies were included in the study. Mares' mean age was 9 ± 5 years old (range 4-20 years) and mean gestation length was 341 ± 7 days (range 327-366 days). Thirty-three mares were primiparous and 45 mares were multiparous. Data were analyzed using a regression tree: P, A, LD and TD were used as covariates. DBP was used as the variable of interest. Nine terminal nodes were identified based on the selected covariates. The first split is produced by the TD: fetuses with TD greater or equal than 2.97 cm are further split according to LD, with a threshold of 3.28 cm. The next split is dictated by A; after a further split on LD, the first terminal node is built, containing 34 fetuses with average DBP equal to 10 days. If the A is ≥ 9.5 years a further split is on P: when mares are multiparous, the TD built two different nodes. Since prediction of mare's foaling date is an important factor in stud farm management, the regression model developed may help the veterinarian to estimate the DBP in Standardbred mares with normal pregnancy.

Keywords: fetal eye, Standardbred mares, transrectal ultrasonography, predicting parturition, regression tree.

1. INTRODUCTION

In the mare, foaling is an unpredictable and critical event due to a wide range of gestational length and the absence of clear and constant signs of impending parturition [1]. Moreover, most mares foaled late at night or early in the morning. Signs of impending parturition include the development of the udder, waxing of the teat ends, relaxation of the vulvar lips, change in consistency and appearance of milk and sinking of the tail head on either side as the ligaments of the pelvis relax [2,3]. These signs could be indicative of foaling, but there is considerable variation among mares, since none of them is an accurate predictor of impending parturition.

The only method to predict the day of foaling is to identify the increase of calcium concentration in mammary secretions, which occurs 24-12 hours before foaling [4-7]. More recently, some studies showed a decline of pH in mammary secretion of normal pregnant mares [8-10].

Estimate the date of foaling could be further complicated by an unknown breeding date.

In equine reproduction, transrectal ultrasonography is the most important technique to evaluate the pregnancy; it is a quick and easy on-farm procedure that is widespread among reproduction specialists. With transrectal ultrasonography the fetal orbit is easily accessible in the last month of pregnancy, and its measurement has been widely used to estimate both fetal growth [11-14] and gestational age [15-16]. Turner et al. [15] and Hartwig et al. [16] developed a formula for predicting parturition or gestational age in ponies and Crioulo mares, using ocular longitudinal diameter.

In the mare, due to the fetus' size at term, only aortic diameter and ocular measurements are easily observable during the entire pregnancy [12,17-19]. At term, transrectal ultrasonographic measurement of the fetal eye is an easy on-farm procedure, also used for the evaluation of the combined thickness of utero-placental unit at the ventral aspect of the cervical pole [11]. The fetal aortic diameter can be visualized only with transabdominal ultrasonography, which requires more time, clipping of the abdomen and a 2.5 MHz transducer with a depth setting of 30 cm [20].

We hypothesized that ultrasonographic measurement of the fetal orbit in Standardbred could help the practitioners in predicting days before parturition. The purpose of this study was to find how many days are left until parturition using data information known by the breeder, such as mare's age and parity, combined with ultrasonographic measurements of the fetal orbit in Standardbred mares with normal pregnancy.

2. MATERIAL AND METHODS

2.1. Animals

Eighty healthy Standardbred mares with normal pregnancy and healthy foals were enrolled in the study. All the mares were housed for attended delivery at the Equine Perinatology and Reproduction Unit - Department of Veterinary Medical Sciences - University of Bologna, during four breeding seasons (2014-2017).

The mares were hospitalized at about 310 days of pregnancy, and remained under observation for at least 7 days postpartum. They were housed in separate 4*4m straw-bedded boxes, fed hay ad libitum and concentrates twice a day and were allowed to go to pasture during the day. Information about mare's age, parity and breeding date were recorded at admission.

Transrectal ultrasonography were routinely performed at admission and every 10 days until parturition using a B-mode real time portable unit (Aloka Prosound 2; Hitachi Aloka Medical Ltd., Tokyo, Japan) equipped with a 5-7.5 MHz linear transducer. The mares were restrained in steel stock, with both sides opened and sedation was always avoided. The duration of transrectal ultrasound examinations varied from 5 to 15 minutes.

2.2. Data collection

The images of the fetal orbit were acquired when cornea, anterior and posterior chamber, vitreous body, lens and optic nerve were visible (Fig. 1a). Images were analyzed using the software ImageJ (<http://imagej.nih.gov/ij/>). Longitudinal diameter (LD) and transverse diameter (TD) were measured. LD was considered as the distance between the two ocular poles, within the vitreous body; TD, perpendicular to LD and bisecting the lens, was measured as the distance between cornea and retina (Fig. 1b). All measurements were repeated 3 times and the average was calculated.

At delivery, length of pregnancy and days of pregnancy at each exam were registered. For each ultrasound examination, days before parturition (DBP) were calculated with this formula:

$$\text{DBP} = \text{gestation length} - \text{days of pregnancy at each ultrasound examination.}$$

The foals were classified as healthy when they had an APGAR score ≥ 8 [21], a normal clinical evaluation during the course of hospitalization, including a complete blood count and serum biochemistry at birth and an IgG serum concentration ≥ 800 mg/dL at 18-24 hours of life.

2.3. Statistical analysis

Descriptive statistics of mare's age, parity, DBP, LD and TD, including mean \pm SD and range (min/max values) were calculated.

Data were analyzed using a regression tree. Regression tree is a recursive algorithm where the full sample is split into smaller groups called 'nodes' [22]. In every step of the procedure, nodes are split according to covariates values, following the criteria of homogeneity: units put together in the same node have similar values for the variable of interest. At the end of the procedure, all units are allocated into terminal nodes. Covariates determine the path to the terminal node. Each node can be characterized by computing averages and variabilities for the variable of interest.

Parity, mare's age, longitudinal diameter and transverse diameter, were used as covariates for the regression tree. Days before the parturition (DBP) was used as the variable of interest.

3. RESULTS

Seventy-eight Standardbred mares with normal pregnancies were included in the study, since the ultrasound studies of two mares were not suitable for the ocular image inclusion criteria. Mares' mean age was 9 ± 5 years old (range 4-20 years). Thirty-three mares were primiparous and 45 mares were multiparous, the mean parity was 5 ± 3 pregnancies (range 1-12). Mean gestation length was 341 ± 7 days (range 327-366 days).

One hundred seventy-eight ultrasound examinations were performed, but 132 ultrasound evaluations were included; forty-six images were excluded because the ocular images were not appropriate for inclusion criteria. Two examinations were excluded from the statistical analysis because there were two values of DBP which may be considered outliers. The included ultrasonographic evaluation were performed between 293 and 357 days of gestation.

Results of the regression tree algorithm are visualized in Fig. 2. The following covariates were used to determine the terminal nodes: transverse diameter (TD), longitudinal diameter (LD), mare's age (A) and parity (P). Nine terminal nodes were identified based on the selected covariates; for each terminal node, boxplots of DBP are shown. Averages and standard deviations of each terminal node are reported in Table 1. Covariates splitting the nodes in the top part of the tree can be regarded as more important in discriminating between DBP. Different paths lead to different nodes. The first split is produced by the TD, with a threshold of 2.97 cm: fetuses with TD greater or equal than 2.97 are further split according to LD, with a threshold of 3.28 cm. Following the path, next split is dictated by mare's age (A): for mares younger than 9.5 years, after a further split on LD the first terminal node (node 5) is built, containing 34 fetuses with average equal to 10 days. If the mare's age is ≥ 9.5 years a further split is on parity: when mares are multiparous the TD built two different nodes (node 9 and 10). For instance, in Node 14 there are 9 foals with average DPB equal to 18 days, all having transverse diameter greater or than 2.97 cm, longitudinal diameter smaller than 3.28 cm, and further classified by having transverse diameter smaller than 3.09 cm.

4. DISCUSSION

In veterinary medicine, fetal morphometry is used to evaluate fetal development, to estimate gestational age and could be useful for predicting parturition. Normal gestation length in Standardbred mares ranges between 322 and 389 days [23-25] and it could be influenced by many factors such as season, sex [26], daylight [27,28], geographical region, latitude [25,29,30], sire [23], mare's age and parity [31-37]. The selected covariates used for the regression tree (the two ocular diameters, mare's age and parity) were the ones that from a practical point of view are available for the veterinary practitioner. Foal's sex and daylight were not used as covariates because breeders and practitioners usually do not know them. The stallion effect was found to contribute only 1%-3% to the variation of gestational length [35-37] and for this reason, it was not considered as a covariate.

About mare's age and parity, they are controversial. Davies Morel et al. [26] did not find any relationship between mare's age and gestational length, but other studies conducted in different breeds found it. Satué et al. [34] found that gestational length was influenced by mare's age and parity: older mares and primiparous mares had a longer gestational length. The authors hypothesized that with increasing age there is a progressive degenerative change in the endometrium that might potentially reduce its nutritive capacity for

the development of the fetus [31,37-38]. Recent studies confirmed that primiparous mares produce smaller foals compared to multiparous [39,40].

The equine fetal ocular measurements were investigated in several studies: longitudinal diameter [15,16], both longitudinal and transverse diameter [12], circumference and area of vitreous body [13]. In the previous studies [12,14-16,19], the two calipers were positioned excluding the anterior chamber to measure the transverse diameter. In this study, the transverse diameter was measured including the anterior chamber from the cornea to the retina as described by other studies about the ultrasonographic ocular measures of the growing foal [41,42] and adult horse [43]. The TD, which is axial globe length (AGL), was measured as the distance from the central cornea to the retina and the LD, the longitudinal globe length (LGL), was measured as the distance from the lateral globe to the medial globe through the long axis of the lens [41,42].

The regression tree obtained showed that both the diameters might help in predicting parturition. What is interesting to note is the influence of mare's age and parity in the regression results, suggesting that they have some influences in the growth of the fetal eyes and in predicting parturition.

In this study, a group of mares of the same breed and with known breeding dates were included. Ultrasound examinations were performed between 293 and 357 days of pregnancy depending on the hospitalization dates. Differently, Turner et al. [15] included pony mares of different breeds and body weight and with unknown breeding or ovulation dates. Hartwig et al. [16] included Crioulo mares to develop a linear regression model to determine the gestational age based on longitudinal ocular diameter measured parallel to the lens. The fetus orbit diameter of Crioulo mares was also compared with the data obtained in light horses [44] and resulted significantly larger than in light horses. As also suggested by Hartwig et al. [16], this kind of study must be conducted in every single breed because ocular sizes could be different and are not related with the sizes of the horse. The ocular shape is an intrinsic characteristic of the breed as also reported by biometric ultrasonographic measurements of the equine eye [43,45].

The previous formula to calculate DBP [15] and estimate the gestational age [16] refer to a (mixed-effect) linear regression model. By using the regression tree model, which is a non-parametric procedure, some strict assumptions of the other formula, such as normality of the response variable and independent observations, were avoided.

The regression model obtained in this study does not predict exactly the date of parturition, but it could help to estimate how many days are missing in foaling, mostly when the breeding date is not known. From a

practical point of view, practitioners can follow the structure of the regression tree to determine, based on the splits and the covariate values, which node the new observations refer to. Once the node is determined according to the tree, one has a visualized distribution of the previous observations inside that node, and the average value of the node can be used as a proxy for the predicted DBP. Also, each node as an associated measure of uncertainty, represented by the standard deviation, gives an idea of the variability associated to the predicted DBP.

Further studies are needed to evaluate if the model could be useful during high-risk pregnancy, particularly with intrauterine growth retardation and/or placental problems that influence both the fetal growth and the gestational length.

4.1 Conclusions

Since prediction of mare's foaling date is an important factor in stud farm management, the regression model developed, combined with other strategies (e.g. changes in mammary secretions), may help the veterinarian to estimate the days before parturition in Standardbred mares with normal pregnancy.

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