

Assessment of fetal sex determined and eye diameter to detection of gestational age in mares by ultrasonography

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Abstract

The objective of the current study was to estimate the gestation age and measurement of the fetal eye and evaluate the accuracy of fetal sex identification using transrectal and transabdominal ultrasonographic approaches in mares. Eleven mares were employed in this study from day 60 to 125 of pregnancy. The ultrasound examination was performed a transrectal and subsequently used the transabdominal approach between 125-240 days of pregnancy. The results led to a found linear model of fetal eye diameter (FED), and the consequent gestation age (GA) prediction formula was $y = 0.1071x + 2.3143$ ($Y=GA$ in a day; $X=FED$ in mm). The determined coefficient (r^2) between FED ($r^2=0.91$) and gestation age in mares were highly significant ($P \leq 0.01$). Furthermore, the accuracy of the fetal sex determination was evaluated during 65-125 days of gestation using the transrectal ultrasonographic approach. The overall accuracy of fetal sex determination was 81.8%. Meanwhile, the accuracy increased from 72.7% on days 65-85 to 90.9% on days 105-125. The accuracy of male and female fetuses was 75% and 85.7%, respectively. This study suggests that the measurement of the fetal eye is applied to the farm procedures for estimating the period of pregnancy in mares, as well as an assessment of fetal sex with greater accuracy.

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Introduction

With the improvement and subsequent Advantest of B-mode compound ultrasonography, it is probable to achieve more comprehensive information about the different measurements of a fetus during gestation. Estimation of the gestational age using ultrasonography approach to the evaluation of fetal-metric parameters was also practical in several species of animals in the many studies, including camels (1), buffaloes (2), and equines (3-5), who indicative of that have some effects in the fetal orbit growth and then prediction foaling. These previous studies were also stated in the small ruminants: sheep (6) and goat (7). These studies determined that ultrasonography fetal parameters have been revealed to make available an accurate estimation of pregnancy and expectancy of parturition. The eye of the fetus is the organ that is furthest recurrently accessible for

feto-metry using trans-rectal ultrasonography, even during mid to late gestational age (4). The evaluation of fetal sex might be incredibly significant in equine horse owners as the pregnant mares carrying a female fetus have potential value, and fetal sex might play an essential role in selling pregnant mares with high genetic quality (8). The ultrasonic approach of genital tubercle has been used to evaluate fetal sex in cattle (9), buffaloes (10), sheep (11), and goats (12). The best windows for fetal sex determination are between 100 and 220 days in equines (13,14). The genital tubercle (GT) is a small bi-lobulated structure that is ventral and situated between the hind leg; it can be detected on days 52-53 of pregnancy; it signifies the forerunner of the male penis and clitoris in the female fetus (15), it is placed at an identical distance between the tail and umbilical cord. After 55 days, it starts to migrate in the cranial toward the umbilical cord in the male fetus and the caudal direction toward the bottom in

the female fetus. The most significant outcomes in gender identification can be accomplished between days 59 and 68 of gestation (13,16). Beyond day 70, the fetus develops too large and goes downhill over the pelvic brim to be dependably examined in its wholeness by the trans-rectal ultrasound approach (17). Therefore, the transabdominal ultrasound approach is the safest and minimally invasive technique for scanning the mare throughout the last two trimesters of pregnancy (18,14). Unfortunately, transabdominal ultrasound examination of the fetus is time-consuming. Furthermore, there is no danger of perforating the rectum, and pregnancy checking-ups via trans-abdominal ultrasound in non-lactating mare cause a lower stress reaction than the trans-rectal ultrasound approach (19).

The current study aimed to assess fetal eye diameter and fetal sex in mares via transrectal and transabdominal ultrasound approaches.

Materials and methods

Eleven mares between 4 and 12 years old were used for the current study. Four were maiden, and seven were barren mares. The last day of breeding was considered day 0 of gestation. In a transrectal ultrasonography approach using a linear probe with 5-7.5MHz, the scanning was conducted from 60-125 days of pregnancy. In contrast, the transabdominal ultrasonography approach was performed from 125-240 days of gestation using a sector probe (3.5-5MHz, WellD ultrasonic, Shenzhen Well. D. Medical Electronics Co. Ltd China). Serial ultrasonic examinations were carried out monthly. Every mare was restrained in stock; sedation was used in three mares. To localize the eye diameter of the fetus, the transducer (probe) was moved from right to left over the uterus. The investigation was carried over the founded orbit; this image of the eye orbit was frozen, two fetal eye diameter measurements were made, and an average was calculated (Figure 1).

Fetal sex identification trans-rectal ultrasonographic scanning was carried out between 65 and 125 days of pregnancy at three different stages of pregnancy 65-85, 85-105 and 105-125 days. Each ultrasonic scanning was accomplished independently without references to its previously observed. During ultrasonic scanning, the part from the umbilical cord (UC) to the base of the fetal tail was checked to recognize the appearance of the genital tubercle (GT). The fetus was documented as female when the GT was located toward the base of the fetal tail (Figure 2) or behind the hind limbs. The fetus was considered male when the GT was situated closely caudal to the hind limbs (Figure 3). The accuracy of the ultrasonography for predicting fetal sex was calculated.

The transabdominal ultrasonographic approach was performed for mares on the left flank; clipping was unnecessary, the transducer was held with the left hand, and gradually moving along the abdomen was started around the

udder and ongoing in the cranial way (14). The fetus was observable, and the focus was on the beating heart, umbilical cord, stomach, and spine (Figure 4).

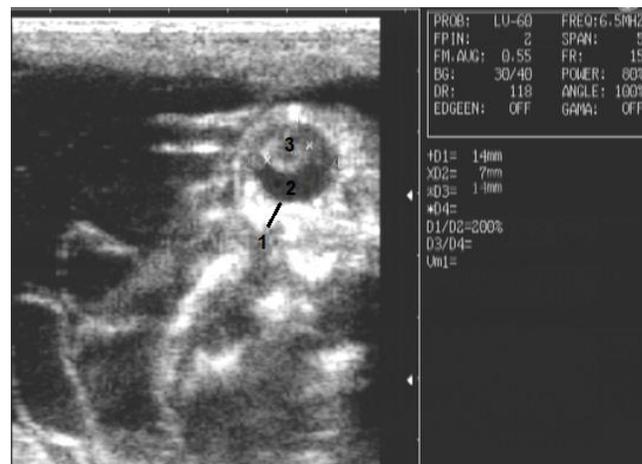


Figure 1: Transrectal ultrasonic image at the sixth month of pregnancy. Measurement of fetal eye orbital parameters (width and length) are shown and it's measured from the maximum length of the inner limits of the vitreous body

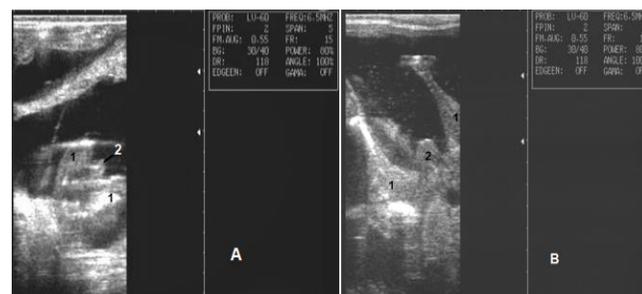


Figure 2: Trans-rectal ultrasonic image of female fetus at 75day (A) and 113 days of gestation. 1: Hindlimbs; 2A: Genital tubercle; 2B: mammary gland; 3A: tailhead; 3B: umbilical cord.

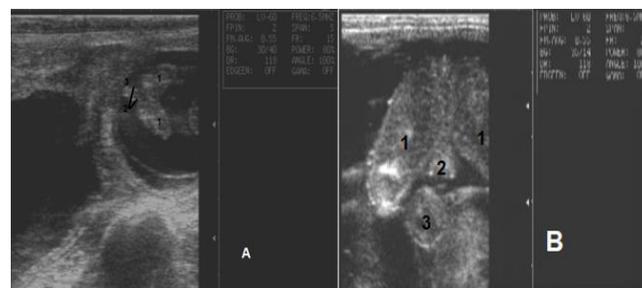


Figure 3: Trans-rectal ultrasonic image of male fetus at 80day (A) and 120 days of gestation. 1: Hindlimbs; 2: Genital tubercle.

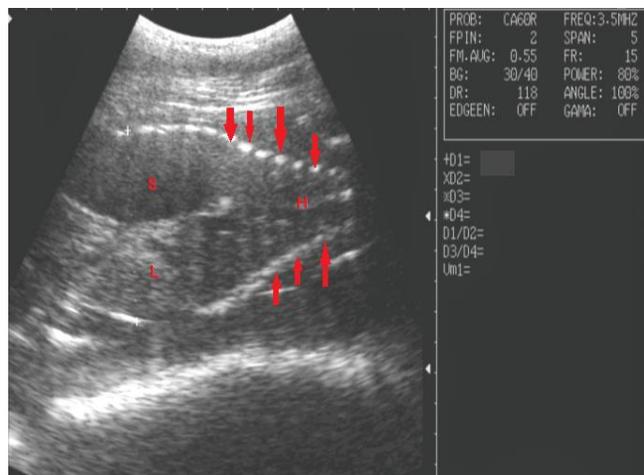


Figure 4: Trans-abdominal ultrasonic image of Mare at the seven month of gestation, red arrows: Ribs; H= heart; S= stomach; L= liver.

Ethical approval

The Scientific Ethical Committee of the College of Veterinary Medicine, University of Diyala, Iraq, was approved that the research proposal submitted, the approval no: Vet Medicine 312; June 2022.

Statistical analysis

The regression and coefficient correlation (r^2) models were fitted to estimate the relationship between fetal eye diameter and gestational age. A multiple-range test (Analysis of Variation-ANOVA) was performed to compare between incomes significantly. All analyses were conducted using the Statistical Analysis System program; the significance level was established at $P \leq 0.01$ (20).

Results

Eighty-seven ultrasonic scans were attained from the eleven mares between 60 and 240 days of pregnancy. The fetal eye diameter (FED) increased linearly from 7.0 ± 0.32 mm at 60 days of gestation to 27.7 ± 1.33 mm at 240 of gestation (Table 1 and Figure 5). The table denotes the most present data for linking the gestation age of a mare to the fetal eye diameter (FED). Using the trans-rectal ultrasound approach, we accomplished measurement via linear probe until 125 days and were afterward able to use the sector probe between 125-240 days of pregnancy. There was a higher significant and substantial determination coefficient (r^2) between fetal eye diameter (FED) and gestational age ($P \leq 0.01$, $r^2 = 0.91$). The consistent equation derived was $y = 0.1071x + 2.3143$; in the present equation, Y represents the current gestation period in days while X is the FED in millimeters. These parameters are determined to be the high coefficient determination with fetal age.

Table 1: Relationship between gestation period and fetal eye diameter (FED)

Gestation period (days)	FED (mm, Mean \pm SE)
60	7.0 ± 0.32^c
90	14.5 ± 0.78^b
120	16.8 ± 0.73^b
150	16.5 ± 0.86^b
180	18.7 ± 0.91^b
210	27.5 ± 1.26^a
240	27.7 ± 1.33^a

Level of Significant

**

This means having the different letters in similar columns differed significantly. ** significant at $P \leq 0.01$.

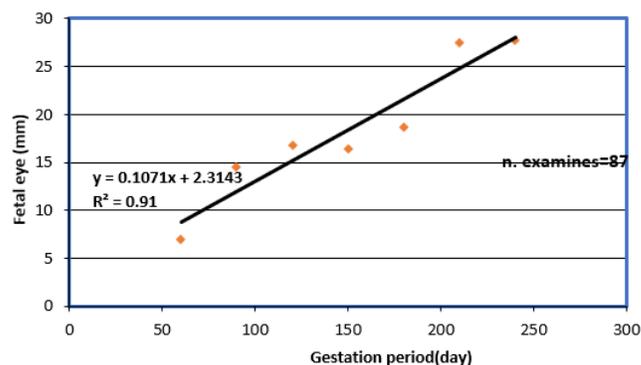


Figure 5: Relationship between Gestation period and Fetal eye

The results of fetal sex determination were reported in table 2. The possibility of fetal sex could be determined was increased during the first trimester of gestation. Afterward, identification of the fetal sex was intolerable due to the far location of the fetus. The fetal sex was diagnosed correctly in 27 of the 33 assigned occasions (overall accuracy of 81.8%), and the prenatal detection of the fetal sex was compared with that of foaling. From the table, the accuracy of female and male fetuses was 72.7% and 90.9%, respectively, during 65-125 days of gestation.

Discussion

This study used ultrasound to designate the fetal eye diameter growth pattern and assess the fetal sex between 65 and 125 days of pregnancy. In this study, the exactness of the ultrasonic measurement was adjudicated by the correlation coefficient between fetal eye diameter (FED) and gestational periods. A high correlation originated in the present data. Our results displayed that FED might be measured using a transrectal approach until 125 days and later using a transabdominal approach because the fetal location moved ventrally and cranially. The present result was supported by preceding reports (18,21), which observed that a linear probe

was only probable between 15 and 17 weeks of gestation. In contrast, observation of the fetal eye required the sector transducer during another gestation period. According to Toenissen *et al.* (14) and Quaresma *et al.* (22), transabdominal ultrasonography is the least invasive

approach to performing pregnancy determination if the mare agrees the transducer close to her udder; pregnancy scanning can be accomplished transabdominal after 90 days of gestation (13).

Table 2: Accuracy of transrectal ultrasonic prenatal fetal sex at different stages of pregnancy

Mares	Gestation period (day)			At foaling	Accuracy
	65-85	85-105	105-125		
Mare 1	Male	Female	Male	Male	2/3 (66.7%)
Mare 2	Male	Male	Female	Male	2/3 (66.7%)
Mare 3	Female	Female	Female	Female	3/3 (100%)
Mare 4	Male	Male	Female	Female	1/3 (33.4%)
Mare 5	Male	Male	Male	Male	3/3 (100%)
Mare 6	Male	Male	Male	Male	3/3 (100%)
Mare 7	Male	Female	Female	Female	2/3 (66.7%)
Mare 8	Female	Female	Female	Female	3/3 (100%)
Mare 9	Female	Male	Male	Male	2/3 (66.7%)
Mare 10	Male	Male	Male	Male	3/3 (100%)
Mare 11	Male	Male	Male	Male	3/3 (100%)
Accuracy	72.7%	81.8%	90.9%	Total Accuracy	27/33 (81.8%)

Accuracy: number of correct diagnoses/total number of diagnosed cases.

Our data yielded a linear growth model for the diameter of the fetal eye. This observation was in similarity to a linear model found in horse's fetuses (23,24) and in other species (1,25). According to the findings obtained in the current study, the FED was more significant than the researchers of the previous study (26), which measured the fetal eye diameter in Crioulo-breed and thoroughbred mares. These observations demonstrated variances in growth forms across breeds at the same gestation period (27).

Also, multiparous mares generally yield larger foals than primiparous mares (28). Table 1 observed the mean and prediction intervals for days of pregnancy based on fetal eye diameter up to 240 days of gestation (26,29). Hartwig *et al.* (26) and Quaresma *et al.* (22) also observed a significantly high determination coefficient between fetal eye diameter and gestation period 0.98 and 0.95, respectively. While according to the present data, the r^2 was 0.91. In previous studies, this might have contributed to measuring the fetal eye up to 340 days of gestation.

In the current study, the accuracy of the fetal sexing presented a significant difference within gestation of 65 to 125 days, and we observed that the accuracy of fetal sex was 72.7%, 81.8%, and 90.9% during days 65-85, 85-105 and 105-125, respectively. The low accuracies in the current study during the first and second periods of gestation might be due to difficulty in identifying genital tubercles because they are not well developed (14). Similar accuracy was stated by Merkt *et al.* (30) and approvingly by that recorded by Curren and Ginther (31), which were 85% and 89%, respectively. According to the present study, the overall accuracy of ultrasonographic scanning was 81.8%, slightly

higher than in Mari *et al.* (32), which was 74.3%. In our estimation, the most common causes for a divergence with our data could be attributed to a single ultrasound examination done in a previous study. Furthermore, in the current data, the low level of accuracy compared to that recorded in camels 91.7% (33) and cattle 92% (31), due to misdiagnosed of sex-determining was more common in males than in females, this observed approves result previously followed by Ahmed *et al.* (33), as well as due to deference specious of animals.

In contrast, Mari *et al.* (32) suggested that male sex determination was easier than female fetuses due to the more prominent genital tubercle. According to earlier and current studies, the supposition is to discharge misdiagnosis, for example, diagnosing the pelvic bones as the female genital tubercle (34,35). In our estimation, the severity of detection in an individual parameter depends on conditions of examination, the examiner's patience and experience, the cooperativeness of the mare, and fetal positioning (14,31,36).

Furthermore, Bollwein *et al.* (36) described that the accuracy of fetal sex determination reached 100% in mares. The most common reasons for disagreeing with the present data might be the use of 3D ultrasound imaging and the working conditions (37).

Conclusions

This study provided critical data about fetal eye growth in mares. A high correlation was provided between the gestational period and FED. The best window for prenatal

fetal gender determination was found during 105-125 days of pregnancy.

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Conflict of interest

None.

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تحديد جنس الجنين وقياس قطر العين لتقدير عمر الحمل في الأفراس باستخدام الموجات فوت الصوتية

ياسين محمود رشيد، فلاح حسن خلف و صلاح نوري محمد

فرع الجراحة والتوليد، كلية الطب البيطري، جامعة ديالى، ديالى، العراق

الخلاصة

هدفت الدراسة الحالية الى تقدير مدة الحمل في الأفراس باستخدام الموجات فوت الصوتية في قياس قطر العين للجنين ولتحديد جنس الجنين عن طريق إجراء الفحص عبر المستقيم وجدار البطن. تضمنت الدراسة أحد عشر فرس فحصت عبر المستقيم باستخدام المسبار الخطي 6,5-7,5 ميغاهيرتز وخلال الفترة 60-120 يوم من الحمل، أعقبه الفحص عبر البطن باستخدام المسبار المنحني 3,5-5 ميغاهيرتز لغاية الفترة 240 يوم من الحمل. أظهرت نتائج الدراسة وبوضوح وجود فارق إحصائي بين تحديد مدة الحمل وقياسات قطر العين للجنين خلال فترات الحمل المختلفة حيث بلغت قيمة معامل التحديد 0,91. كما بينت الدراسة الحالية أن دقة تحديد جنس الجنين خلال الأيام 65-125 من الحمل قد بلغت 81,8%، كما لوحظ أن دقة تحديد جنس الجنين خلال فترات الحمل المختلفة 65-85 و 85-105 و 105-125 يوماً بلغت 72,7، 81,8، و 90,9% وعلى التوالي. في حين كانت دقة جنس الجنين الذكر اقل مقارنة بالجنين الأنثى 75 و 85,7% وعلى التوالي. نستنتج من الدراسة الحالية أن استخدام تقنية الموجات فوت الصوتية لقياس عين الجنين لتحديد مدة الحمل وفي تحديد جنس الجنين يعد طريقة عملية وموثوقة وبدقة عالية كما يمكن تطبيق المعادلة المستنبطة بشكل عملي في الظروف الحقلية.