ELSEVIER

DETERMINATION OF GESTATIONAL AGE IN SHEEP AND GOATS USING TRANSRECTAL ULTRASONOGRAPHIC MEASUREMENT OF PLACENTOMES

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ABSTRACT

Three experiments were conducted to determine gestational age in the ewe and doe by measuring placentomes with a B-mode ultrasonograph and a 5 MHz transducer. Transrectal measurements were obtained by placing the female over a bale of hay. In Experiment 1, ewes (n=12) and does (n=15) were examined by transrectal ultrasonography every week from breeding to parturition to determine the growth pattern of placentomes during pregnancy. In Experiment 2, placentomes from 132 ewes and 169 does were measured between 30 and 90 d of gestation. A linear regression relationship between fetal age in days and placentome size in mm was calculated and adjusted for does (gestational age = 28.74 + 1.80PL + e, r^2 = 70.34) and for ewes (age = 47.98 + 0.62PL + e, r^2 = 15.59). In Experiment 3, the placentomes of 63 does were measured to validate this relationship by using linear regression. Gestational age was determined correctly in 66% of the does, with a range of ± 7 d and in 96% with a margin of ± 14 d. In conclusion, transrectal ultrasonography allowed for the measurement of placentome size, which increased rapidly during the first 70 to 90 d of gestation in ewes and does. In ewes, however, there was a poor correlation of placentome size with gestational age, while in goats, measurement of placentomes could be used along with pregnancy diagnosis by transrectal ultrasonography as an indication of gestation age.

Keys words: ultrasonography, gestational age, placentome measurements, ewes, does

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INTRODUCTION

In most flocks of sheep and goats, natural service dates are generally unobserved or unrecorded, making fertile breeding impossible to determine. This is particularly so in the goat (13,21). Accurate pregnancy diagnosis would provide essential information for effective herd management practices such as the culling of nonpregnant females and the determination of the number of fetuses, the latter being especially important in sheep herds. Such information would allow producers to group animals based on their nutritional needs so that they are fed appropriate rations during the last trimester of pregnancy. Furthermore, accurate information on the stage of gestation would be useful to dry off lactating females at an adequate period of time and to monitor females near term.

B-mode ultrasonography is an accurate, rapid and safe method for diagnosing pregnancy in small ruminants. Transrectal or transabdominal approaches can be used with a nearly 100% accuracy rate (3,5,6,10,13,18). One main advantage of the transrectal approach in small ruminants is that it does not require a special transducer. A regular linear transducer can be used just as for examination of larger animal species. In our experience, this method quickly provides clear images of the uterus and it is well tolerated by the animals.

Determination of gestational age by ultrasonography has been described in the ewe and in the goat using a transabdominal approach (11,12,24,26). The size of the fetal head (biparietal diameter) and the thoracic depth between 40 and 100 d of gestation are reliable measures of gestational age. Using the transrectal approach and a 7.5 MHz human prostate transducer on ewes lying on their backs, embryo length can be accurately measured during the first 40 d of pregnancy (27). A good correlation was observed between embryo size and gestational age. However, in advanced gestation, the various positions of the fetus in utero make it impossible to measure it accurately (3,27).

The other possible measurable objective variables included those of the size of the fluid-filled uterus and size of placentomes. While the usefulness of fluid-filled uterus measurements is limited since the uterus rapidly becomes too large to be accurately measured, placentomes are readily observable by transrectal ultrasonography throughout pregnancy, as has been reported in the goat (3) and in other small ruminants along with pregnancy examination (5). However, the relationship between ultrasonographic measurement of placentomes and stage of pregnancy has not been determined in small ruminants.

According to anatomical study of the ewe, placentomes can be discerned as small nodules by Day 21 of gestation (9). At Day 30, the periphery of the nodule begins to rise to form a thin linear lip around the flat center. And by approximately Day 90 of gestation, the placentome reaches its maximum weight and diameter (1,9).

Placentomes can be detected by transrectal ultrasonography and a 5 MHz linear transducer at Days 28 to 30 of gestation. The placentomes appear as small echogenic areas on the surface of the endometrium (3,6); later, they are readily imaged in cross section as cup-shaped hyperechogenic structures with the concave surface directed toward the uterine lumen (3,5,13). The ultrasonographic measurement of placentomes during gestation offers an objective means of determining gestational age.

Thus, the objectives of the present study were to use transrectal ultrasonography 1) to determine placentome development in ewes and goats throughout pregnancy, 2) to establish the relationship between gestational age and placentome measurement and 3) to present a formula for the accurate prediction of parturition at the time of pregnancy diagnosis.

MATERIALS AND METHODS

A real-time ultrasonograph scanner (Aloka SSD-210 DX) fitted with a 5 MHz linear-array transducer (model UST-58101-5) was used in 3 experiments. The transducer (90 x 19 x 29 mm) had a scanning width of 56 mm and was attached to a semi-rigid wire. Various methods of transrectal ultrasonography have been described in the ewe (6,25) and goat (3,5). The procedure used in the present study required that the animal be placed over a bale of hay in order to compress the abdomen and push the uterus up closer to the rectum (3). The lubricated transducer was slowly pushed transrectally without the use of any other rigid device. The transducer was moved forward until the bladder was visible, and the area scanned was essentially just cranial to the bladder. The transducer was then rotated 45^{o} to the right and to the left to scan the greater area of either the gravid or nongravid uterus. Thus, manipulation of the linear transducer was limited to cranio-caudal movement and to its rotation by the wire outside the animal (3). Examinations were recorded on videotape for subsequent analysis.

Experiment 1

Fifteen Alpine and mixed-breed goats and 12 mixed-breed ewes were used at the animal husbandry facility of the school of veterinary medicine. The animals were fed twice a day a ration of hay with some grains, and were kept outside in large parks with free access to a shelter. Experiment 1 commenced at the beginning of a normal breeding season (September), while data for the other 2 experiments were collected mostly during the fall and winter seasons. Estrus synchronization was accomplished by progesterone treatment with vaginal sponges (medroxyprogesterone acetate, Veramix[a]) for 7 d, followed by 2.5 mg, im $PGF_2\alpha$ (Lutalyse[a]). Thereafter, the animals were placed with a fertile male fitted with a marking harness, and the date of marking was recorded daily. This procedure allows for the establishment of the exact breeding and parturition dates. Transrectal ultrasonography was initiated on Day 7 after breeding (or estrus) for the does and Day 11 for the ewes. Animals were examined weekly until the end of gestation. Two to five placentomes were measured using electronic calipers. Measured placentomes had a circular shape when viewed in a longitudinal section or a regular C-shape in a cross-section.

Experiment 2

In Experiment 2, data were collected from goats and ewes at the time of pregnancy diagnosis at the ambulatory clinic of the veterinary faculty. A total of 169 goats, mostly Alpine

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breed (≈70%), but also Saanen, Toggenburg and LaMancha breeds as well as mixed breeds, were examined. The 132 experimental ewes were from Dorset, Suffolk, Dorset-Romanov crosses and mixed breeds. Several placentomes were usually imaged on the screen, and a representative size placentome was measured. Because the mating dates were unknown, we estimated the gestational age at the time of ultrasonographic examination by subtracting the length of time between examination and parturition from 150 d, which is the average gestation length for goats and ewes. A linear regression model was fitted to evaluate the relationship between gestational age and placentome size. A 5% significance level was used (α=0.05). The accuracy of the fitted line was tested by an analysis of the residual terms (19). Detection of extreme and influence values was assessed by the Cook's distances (19).

Experiment 3

Because of the poor results in sheep in Experiment 2, validation was only obtained for goats. Placentomes were measured in 63 goats (mostly Alpine breed) during pregnancy diagnosis at the ambulatory university veterinary clinic. Using the linear regression coefficients estimated in Experiment 2, gestational age was predicted with the mean squared predicted errors method (MSPE; 19). The value obtained was compared with the mean square of error (MSE) of the first fitted linear regression (from Experiment 2). This was done to compare the sum of variation of each observation around the fitted time in the 2 experiments. An MSPE value close to the MSE indicates that the line fits both data sets similarly.

RESULTS

Experiment 1

The first signs of pregnancy, in the form of circular and elongated anechogenic images located in utero cranial to the bladder, were observed on Day 21 in does and on Day 18 in ewes. In the following days the fetus and the fetal heart could be seen in every animal. On Day 32 for the ewes and Day 35 for the does placentomes were visible as small nodules. On Day 42, the placentomes of all does were cup-shaped, whereas only 9 of the 12 ewes presented cup-shaped placentomes on Day 39 of gestation (Figure 1).

The relationship between days of gestation and diameter of placentomes in both ewes and goats is shown in Figure 2. In ewes, placentome development was rapid, and maximal size was reached by Day 74 of gestation. In contrast, placentomes in does exhibited a slower growth, with the largest diameter being reached by Day 91 (Figure 1). In both species, images of the uterus were from the portion close to the rectum, which is limited by the dorsocaudal aspect of the uterus after about 90 d of pregnancy. Afterwards, accurate measurements of placentomes were difficult to obtain due to the increased distension of the uterus.

Experiment 2

Scatter plots and the fitted regression lines between placentome measurements and age of the fetus in sheep and goats are shown in Figure 3. The linear model fitted between fetal age (age) and placentome diameter (PL) was of the following form in does: age (days) = 28.74 +

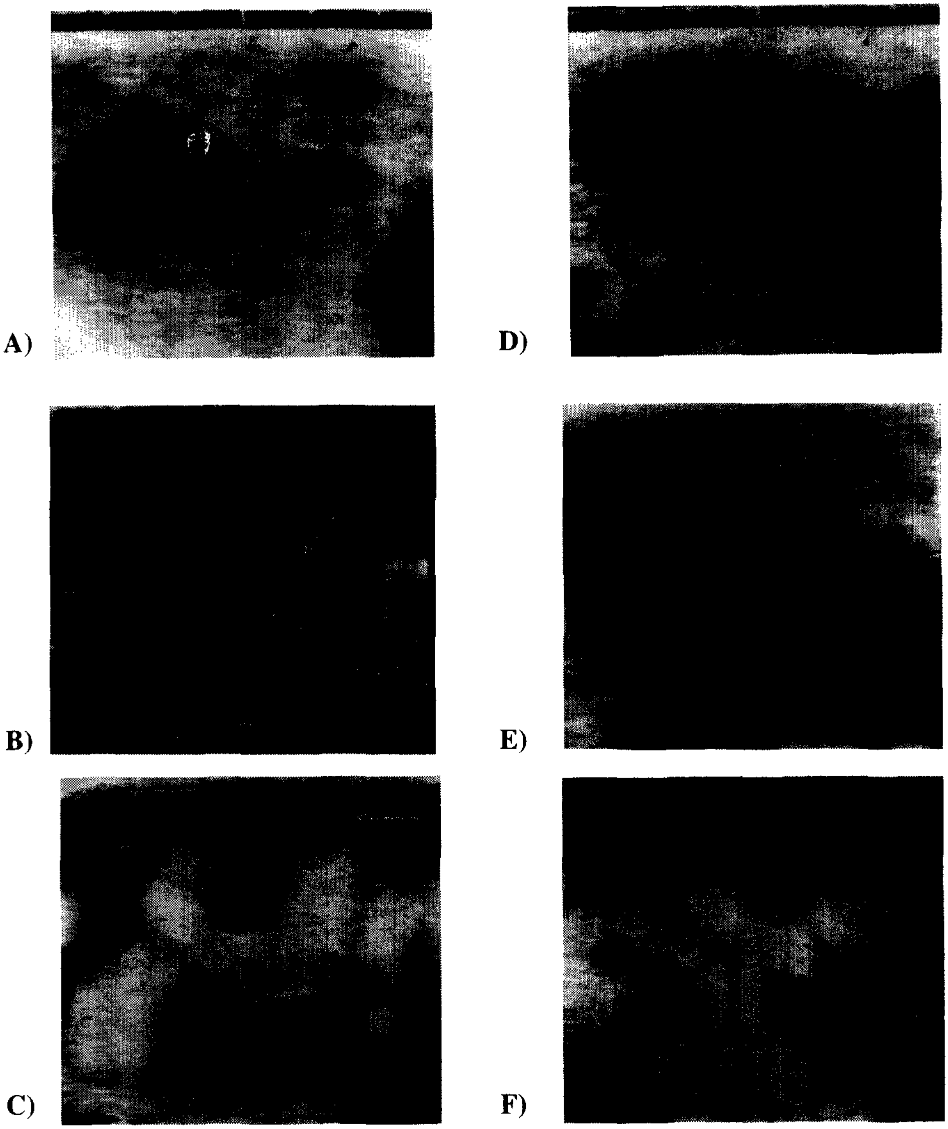

Figure 1. Transrectal ultrasonographic images of placentomes (←) in cm at day 46 (A), 67 (C), 86 (E) of pregnancy in ewe and day 42 (B), 63 (D), 84 (F) in goat.

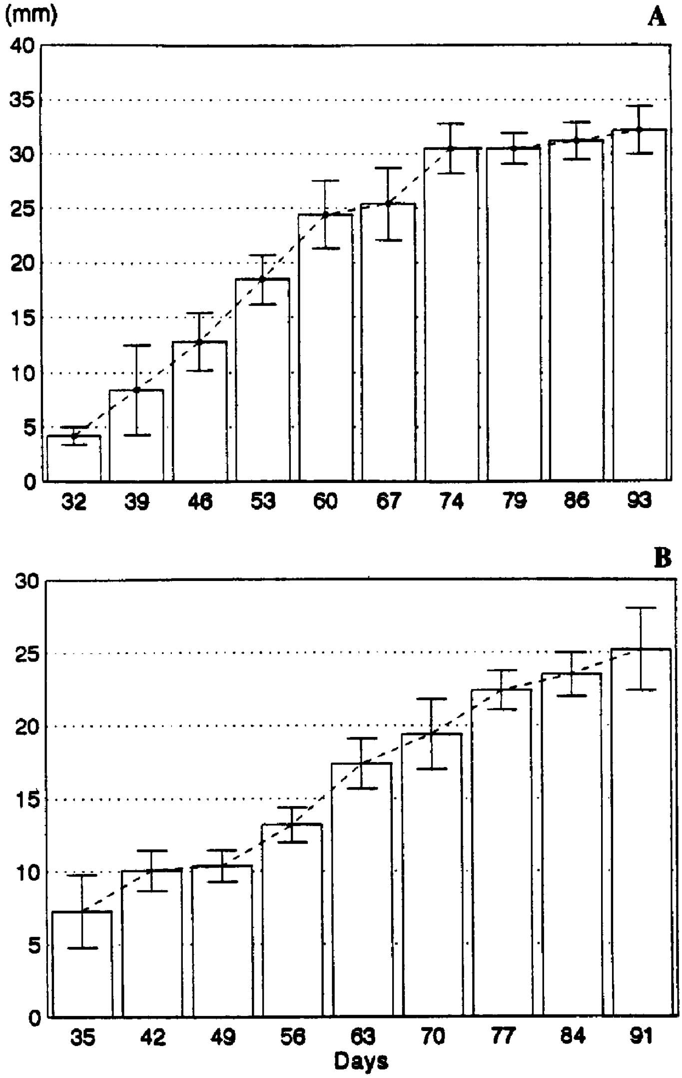

Figure 2. Relationship between days of gestation and placentome (mm) diameter in ewes (A) and goats (B). The bar represent the 95% confidence interval with 12 ewes and 15 goats in Experiment 1.

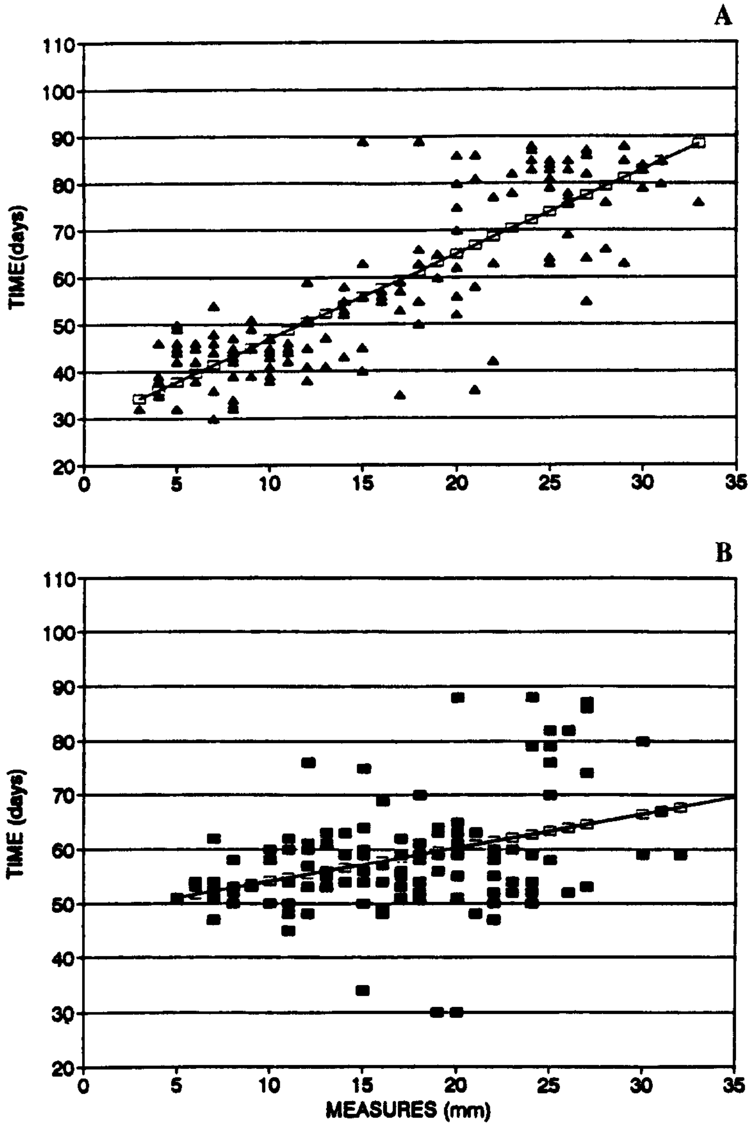

Figure 3. Scatter plots and regression line of placentome diameter (mm) and gestational age (days) in ewes (A, n=132) and goats (B, n=169) in Experiment 2.

1.80 PL (mm) + e (residual). This regression was statistically significant (F=339.20; P=0.0001). The coefficient of determination (r^2) was 70. The analysis of the residuals showed that the linear model adjusted was appropriate. No extreme or influent variables were detected using the Cook's distances. In ewes, the linear association was less accurate but still significant (F=63.74; P=0.0001): age = 47.98 + 0.62 PL + e, with a coefficient of determination of 16. Thus, placentome diameter explained only 16% of the variation in age.

Experiment 3

The results obtained during Experiment 3 are shown in Figure 4. A difference of ±7 d between the expected and the real fetal age was found in 41 goats (65%) and ±14 d in another 20 goats (31%). The difference between the MSPE and MSE was not significant, indicating that linear regression is sufficiently accurate for predicting new data (17).

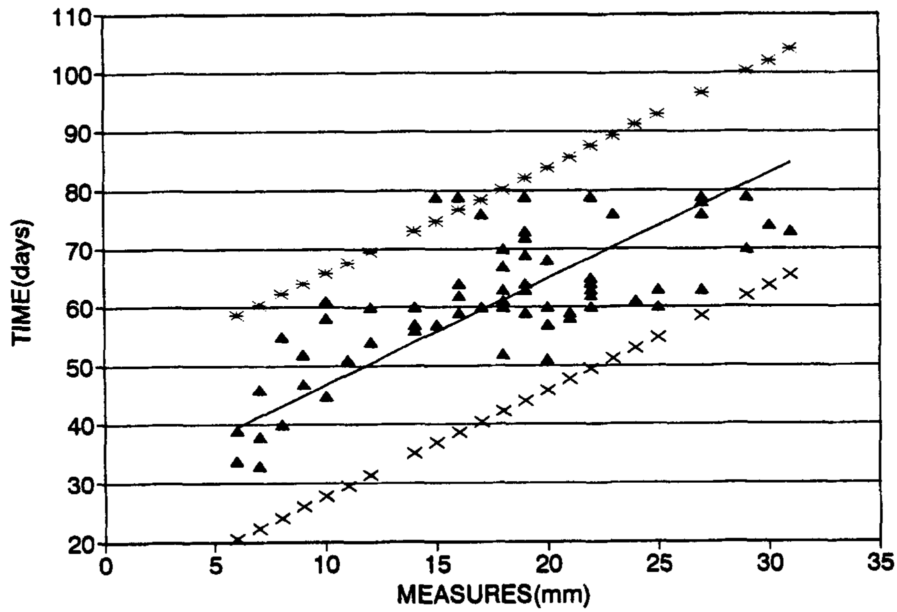

Figure 4. Predicted placentome diameter (mm) in relation to the time of gestation in goats using the linear regression calculated in Experiment 2. The X represent the calculated mean square error and the ▲ are the placentome measures obtained in Experiment 3.

DISCUSSION

Placentomes were noticeable around 30 d of gestation in the ewe and goat using our transrectal method of ultrasonography. This finding is similar to that of previous reports (3,6), and is an improvement over the transabdominal approach, in which placentomes can be seen only at 40 d of gestation (13). The maximal size of placentomes, as measured by ultrasonography, was reached at 74 and 91 d of gestation in sheep and in goats, respectively. Again, this was similar to earlier anatomical studies (1,2,9) reporting that the weight of placentomes peaks before the third month of gestation in the 2 species. The gradual increase in the weight of placentomes was correlated with a gradual increase in size and, as previously reported (2). Furthermore, the largest cotyledons measured either after necropsy at different stages of gestation (9) or after expulsion of the fetal membranes (4) in the previous studies had a diameter of about 3 cm, which is the same as in our study.

The development of placentomes as observed by ultrasonography was also similar to that of previous observations (2,9,15). According to these studies, caruncles are noticeable on the endometrial surface by 21 to 26 d of gestation. However, they were readily detectable by transrectal ultrasonography and a 5 MHz transducer by 32 to 35 d of gestation. The placentomes were imaged as small nodules on the surface of the endometrium, the periphery of which became elevated to form a circular lip around a flat center within a few days.

The anatomical shape of the placentome is different between the goat and sheep species. In ewes, the placentomes are nearly hemispherical when seen in transverse section, while in goats the placentomes have a saucer-like shape (2). This difference is also readily observable in ultrasonographic images (Figure 1A and B). In ewes, as the placentomes reached their maximal size, the center became partly filled, and the tissues were characterized by thick echogenic walls with a small anechoic center. In contrast, the central cavity of the goat placentome increased in size, with the borders remaining thin. These produced images of ovoid-shaped structures with a thin hyperechoic wall and a large anechoic center.

Placentome diameter and weight progressively decreased during the second half of the pregnancy in the monotocous ewe (1,2). This aspect of placentome development may be associated with increased placental perfusion and tissue permeability during the second half of gestation (17). Furthermore, the determination of gestational age is not possible after 70 and 90 d of gestation using this criteria in the ewe and the goat, respectively. In the present study, placentome measurements were not possible beyond 90 d of gestation due to variability and difficulty in imaging the distended uterus, as was also observed in the first experiment. A similar limitation has been reported for fetal measurement by transabdominal approach (13).

The poor correlation between gestational age and size of placentomes in ewes might be explained by factors such as variations of the number and size of the placentomes within the uterus as well as among females. The number and weight of the placentomes were found to be quite variable between the 2 uterine horns in the ewe (1). The smallest placentomes were found at the tips of the horns and the largest near the junction of both horns. The number of functional caruncles varied between uterine horns, and the weight of placentomes for a given day of pregnancy varied greatly between females and breeds of ewes (1,9). The results of our second experiment confirm these earlier observations (Figure 3a). Variation of placental development in relationship to season has also been reported (14). The number of developed placentomes and their total weight was much greater in females bred during the normal breeding season than those bred during the anestrus season. In the present study, examinations were done on females bred during the normal breeding season.

The present procedure of transrectal ultrasonography is also suspected as a cause of variation of measurements. As previously discussed, placentome size varies in relation to its location in the uterine horns (1). In our study, the bladder was systematically used as visual landmark for every examination. Thus, the area of the uterus scanned was the same for every animal, since the bladder was kept on or close to the screen. Usually, the entire uterus is visible on the screen in the nongravid female and during the first month of gestation in the pregnant female (3,6). Afterwards, the region of the body of the uterus and of the junction of the gravid uterine horns were readily observed in proximity to the bladder.

In the present study, the gestational age was calculated from the day of examination by ultrasonography to the day of parturition, assuming an average of gestation length of 150 d. The normal variation of pregnancy length among breeds of ewes might be another reason for the reduced level of correlation with placentome measurements (28). The range of duration of gestation was 8 d for different breeds of ewes. The sex of the fetus and ewe age were not related to the duration of gestation. Several factors should not have been a source of variation of the placental development. The weight of placentomes was not affected by the sex of the fetus, the age and the nutrition of the ewes, or litter size (1). The number of fetuses per litter caused little variation ($\approx$1 d) in pregnancy duration in the goat (22), and was associated with a small reduction of duration in the ewe (28). Furthermore, the number and the size of the placentomes increased only a little in the ditocous compared with the monotocous ewe (1). Thus, these differences should not be discernable by ultrasonographic examination. These findings support the concept that fetal growth is not limited by the placental development early in pregnancy (2); moreover, they might explain the similar growth rate of single and twin fetuses during the first 3 mo of pregnancy (23, 29).

In the goat, the correlation between gestational age and placentome size (r^2=70.34) correctly predicted age in 66% of the examinations with a margin of ± 7 d, and in 96% with a margin of ± 14 d. Although there is no detailed study on placentome development in the goat like in the ewe, placentome size should be more uniform in the goat than ewe at the junction of the uterine horns, as viewed by transrectal ultrasonography (Figure 2B). This is supported by our present findings of a higher correlation between placentome diameter and gestational age than that obtained in the ewe. Furthermore, the results of Experiment 3 show that the model in Experiment 2 can adequately predict the gestational age from a new set of samples.

The normal range of gestation duration is 142 to 162 d in dairy breeds of goats (22,25); this variation might explain the reduced predictability on an individual basis. The occurrence of short estrous cycles is another important source of variation. The latter is of concern, especially at the beginning of the breeding season when up to 44% of the goats have a short estrous cycle (5 to 7 d), which may occur several times in the same individual early in the breeding season (7,21). The resultant delay in conception is impossible to determine on the basis of placentome size, and might account for the reduced level of correlation between placentome size and determination of gestational age in goats. The results of Experiment 1 showed only a small variation in placentome size between females, possibly due to the reduced incidence of abnormal estrous cycles after progesterone treatment (8,20).

Finally, fetal dimensions (the head or the thorax) were accurate predictors of gestational age due to precise measurements by transabdominal approach, with smaller variations at 40 to 100 d of gestation in both goats and sheep (11,12,26). A range of variation of 6 d for parturition was observed in goats based on fetal measurements. This variation was explained by breed differences and age of the fetus at the time of examination (12). The length of the embryo as measured using the transrectal method correlated with gestational age only during the first 40 d of pregnancy in ewes (27), possibly due to inaccurate measures of the entire fetus after that date.

Despite some limitations, placentome measurement remains a valuable method for estimating gestational age for goat producers. Our transrectal approach of ultrasonography is quickly performed in the goat, and determination of gestational age is done earlier than 90 d after breeding. Precise measurement of fetal segments requires experience and time (13,18), and some methods are not practical under field conditions because of the required dorsal recumbency (26,27). Using the present transrectal approach, it is nearly impossible to image a fetus adequately beyond 35 to 40 d of gestation. Likewise, placentomes were difficult to visualize after about 100 d of gestation and the resultant measurements are not reliable for determining the stage of pregnancy after this time. The correlation between size of placentome and gestational age is validated in our study for the Alpine, Saanen and Toogenburn dairy breeds of goats.

Determination of the gestational age in goats using placentome measurements should provide valuable information for producers to more accurately assess the best time to end lactation in the goat and thus to improve herd productivity (18). Additional study is indicated in the ewe, due to the poor correlation between placentome size and gestational age, where breed would be considered as a critical variable.

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