Transabdominal ultrasound for detection of pregnancy, fetal and placental landmarks, and fetal age before day 45 of gestation in the sheep.

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Abstract

Detection of pregnancy during early gestation is advantageous for flock breeding management. Transabdominal ultrasound is a practical and efficient approach for monitoring pregnancy and fetal growth in small ruminants. However, there is limited information using the transabdominal technique before day (d) 45 of gestation in sheep. Therefore, our objective was to determine how accurately transabdominal ultrasound could be used to detect pregnancy, to identify pregnancy landmarks, and to quantify fetal length before d 45 in ewes. Multiparous Western White-faced ewes ($n = 99$) were estrus synchronized and exposed to one of four Dorset rams. The day a ewe was marked by a ram was considered d 0 of gestation. Ewes not re-marked by d 20 were separated for ultrasonography. To detect pregnancy and landmarks, ewes were scanned three times per week between $d 26.0 \pm 0.3$ (mean $\pm$ standard error) and $d 40.0 \pm 0.2$. A single technician performed all scans in the right non-haired abdominal pit using a real-time portable EasiScan machine and a 5 MHz linear rectal transducer. All data were analyzed using the MIXED procedure in SAS (with repeated measures where appropriate). Due to rebreeding activity, 113 ultrasound periods were initiated. The specificity and positive predictive value were 100% during the entire study. The accuracy, sensitivity, and negative predictive value of ultrasound scanning were greater than 90% beginning at $d 33 \pm 1$. On average, pregnancy ($n = 85$) was detected at $d 28.7 \pm 0.4$ and non-pregnancy ($n = 28$) at $d 25.5 \pm 0.6$. Three early fetal losses were identified at $d 39.7 \pm 0.7$. In pregnant ewes ($n = 82$) the overall accuracy of fetal counting was 78%. The first observance of an enlarged uterus ($P = 0.05$) and pregnancy ($P = 0.03$) were detected earlier when multiple fetuses were developing compared with singletons. Placentome evagination was first observed earlier in triplets compared with twins and singletons ($P = 0.02$). Fetal length increased with day of gestation ($P < 0.0001$), but not fetal number ($P =$

0.72). A fetal number by day of gestation interaction ($P = 0.01$) indicated differences in fetal length at $d\ 29 \pm 1$ and $d\ 32 \pm 1$. These data demonstrate that a portable ultrasound using the transabdominal technique can be used to accurately determine pregnancy, identify landmarks indicative of gestation, and estimate fetal age, before $d\ 45$ of gestation in sheep.

Key words: pregnancy detection, sheep, small ruminant, transabdominal ultrasound, uterus

1. Introduction

In 2015, the United States sheep and lamb industry registered its first population increase since 2006, which included an additional 35,000 breeding ewes and replacement lambs [1,2]. These data suggest a current industry focus on increasing flock breeding inventory; thereby generating new interest towards improved management of pregnant ewes. Pregnancy diagnosis is a vital aspect of flock management, and detection of pregnancy during early gestation provides a larger window for sheep producers to make important economic decisions [3]. This includes identifying non-pregnant ewes for culling and rebreeding, thereby maintaining reproductive efficiency within the flock and limiting investment into non-pregnant ewes [4–6]. Additionally, early identification of pregnant ewes ensures adequate nutrition to a gestating ewe and provides an opportunity to estimate litter size. Importantly, the nutritional plane of a ewe can be improved to optimize birth and weaning weights of offspring, prevent pregnancy toxemia, and increase milk production [3,5,7,8].

Real-time ultrasonography is routinely used for pregnancy detection in small ruminants and provides critical information during early gestation, such as estimations of fetal number and gestational age [7,9,10]. Pregnancy detection via transrectal ultrasound has been reported to be

77 to 100% accurate as early as day (d) 19 to 29 of gestation in sheep [7,11–13]. Although this level of accuracy has been achieved using a transabdominal approach it has not been reported until later in gestation, between d 40 to 80 in sheep [8,14–16] and d 39 to 51 in goats [17]. Positive indicators of pregnancy, such as placental and fetal landmarks, are detectable by transrectal ultrasound in sheep. This includes identification of a fetal heartbeat as early as d 20, and fetal elongation and placental developments before d 45 of gestation [11,13,18,19]. However, there is limited information on the use of transabdominal ultrasound for identifying such landmarks or fetal elongation [16,20], and therefore transrectal ultrasound has been favored for early pregnancy detection in sheep [10,18].

Although transrectal ultrasound has been reported to be safe in ewes [21], the application of this approach under field conditions has limitations, requiring a dorsal recumbent position of the ewe for optimal probe contact and ultrasound imaging [13,21]. This positioning is laborious and stressful to both the ewe and technician. Alternatively, the transabdominal approach, applied with ewes in a standing position, may be more adaptable to field conditions and less invasive to the ewe [6]. However, transabdominal ultrasound is used less frequently during early gestation because the accuracy of detecting pregnancy status and identifying fetal development using this approach has not been well described. This information is critical as producers increase their focus on breeding management and researchers continue to utilize the pregnant sheep as a biomedical model. Fortunately, advancements in ultrasound technology provide opportunity for more detailed imaging when scanning transabdominally, and therefore the potential of using this technique accurately and efficiently for flocks during early gestation is thoroughly addressed in the current study. We hypothesized that transabdominal ultrasound, using a portable ultrasound machine, would accurately detect pregnancy before d 45 of gestation. Therefore, the objectives

of this study were to use transabdominal ultrasound during early gestation to 1) detect pregnancy status and litter sizes before d 45, 2) establish a timeline of identifiable early placental and fetal landmarks indicative of pregnancy and fetal length measurement, and 3) determine the relationship of this information to gestational age.

2. Materials and Methods

The University of Connecticut's Institutional Animal Care and Use Committee approved all animal procedures.

2.1 Animals and Breeding

Multiparous Western White-faced ewes ($n = 99$) were estrus synchronized in groups of 24 with progesterone via vaginal insertion of a controlled intravaginal drug releasing device (Easi-Breed CIDR Sheep Insert, Zoetis, Florham, NJ). After 12 d, CIDRs were removed and ewes received 2 mL prostaglandin im (Lutalyse, 5 mg/mL; Zoetis; Florham, NJ) [22,23]. Ewes were housed with 1 of 4 genetically related Dorset rams for breeding. Each ram was fitted with a marking harness for the entirety of the breeding period. Marking activity was observed and recorded twice daily. The day a ewe received a rump mark was considered d 0 for calculating gestational age. If a ewe was remarked within 20 d, the ewe remained with the ram and calculation of gestational age was restarted. If a ewe was not remarked within 20 d, the ewe was removed from the ram, individually housed and scanned for pregnancy via transabdominal ultrasound. If pregnancy was not confirmed by d 40, the ewe was returned to a ram for rebreeding.

Due to a large collaboration, the pregnant ewes involved in this study were randomly assigned into a 3 x 4 factorial treatment structure, involving three dietary regimens and four necropsy time points. The dietary regimen had no effect on the fetal variables reported herein ($P \geq 0.27$, see supplementary material); however relevant data obtained from necropsy are included.

2.2 Transabdominal Ultrasound

Real-time ultrasound was performed transabdominally using a portable EasiScan machine (BCF Technologies, Rochester, MN) with ewes in the standing position. A 5 MHz rectal probe was positioned in the right non-haired abdominal pit with 70% isopropyl alcohol applied topically for optimal contact. A single qualified technician (author R.E.G., DVM) performed all scans ($n = 514$). Each ultrasound period consisted of scanning each ewe three times/wk beginning at $d 26.0 \pm 0.3$ and continuing through $d 40 \pm 0.2$ of gestation. Due to rebreeding activity, 113 different ultrasound periods were initiated from the 99 ewes.

2.3 Ultrasound Data Collection

During each scan, the ultrasound technician reported a ewe as either pregnant or non-pregnant. Pregnancy was defined by the presence of a fetus(es) with a heartbeat. In pregnant ewes, the first observances of placental or fetal landmarks were recorded (Figure 1). This included placentome evagination from the uterine wall, leg bud separation from the fetal body, a fetal genital spot, an umbilical cord, placentome maturation into a hollow circle or c-shape, and visualization of fetal ribs. Images of enlarged fluid-filled uterine cross-sections, distinct from the bladder, were supportive of pregnancy throughout the entire scanning period. Prediction of litter size was performed for each ewe. Twin and triplet pregnancies were predicted only when two or three fetuses, respectively, could be observed in the same ultrasound image to prevent recounting

the same fetus. Fetal elongation was quantified by measuring fetal length along the longitudinal axis of the fetus using a 1 cm grid super-imposed on the ultrasound screen (Figure 2). The fetus was aligned cranio-caudally along the parallel lines of the grid, and the portion of 1 cm squares covered by the fetus was reported in millimeters. Fetal length measurements were obtained from 16 singleton, 46 twin and 15 triplet pregnancies. Obtaining measurements of fetal length was limited due to improper positioning of the fetus in utero for a longitudinal measurement, or the fetal length extending longer than the ultrasound screen.

2.4 Necropsy Data Collection

At one of three gestational time points, d 45 (n = 21), d 90 (n = 20), or d 135 (n = 20), pregnant ewes were euthanized with sodium pentobarbital iv (0.22mL/kg; Beuthanasia-D Special; Merek Animal Health; Summit, NJ), and a hysterotomy was performed. The uterine horn(s) of fetal implantation were recorded and fetuses were subsequently excised. A fourth group of ewes was allowed to undergo parturition (n = 21). The number of offspring each ewe carried was recorded at necropsy or following parturition. At the d 45 necropsy, fetal length (n = 36 fetuses) was recorded by measuring craniocaudally along the longitudinal axis of each fetus with a metric digital caliper.

2.5 Data Analysis

To assess the validity of transabdominal ultrasound to discriminate between pregnancy and non-pregnancy outcomes, each ultrasound result was categorized into a 2 x 2 contingency table as follows [28,29]:

True Positive (TP) = pregnant ewe correctly identified as pregnant on ultrasound

False Positive (FP) = non-pregnant ewe incorrectly identified as pregnant on ultrasound

False Negative (FN) = pregnant ewe incorrectly identified as non-pregnant on ultrasound

True Negative (TN) = non-pregnant ewe correctly identified as non-pregnant on ultrasound

Accuracy (Acc) calculated the proportion of pregnant and non-pregnant ultrasound predictions that were correct pregnant or non-pregnant outcomes [$Acc = \frac{TP+TN}{TP+FP+FN+TN}$]. Sensitivity (Sen) calculated the proportion of pregnant ewes that were correctly identified as pregnant by ultrasound [$Sen = \frac{TP}{TP+FN}$]. Specificity (Sp) calculated the proportion of non-pregnant ewes that were correctly identified as non-pregnant by ultrasound [$Sp = \frac{TN}{TN+FP}$]. Positive predictive value (PPV) calculated the portion of ewes with pregnant predictions on ultrasound that were actually pregnant [$PPV = \frac{TP}{TP+FP}$]. Negative predictive value (NPV) calculated the portion of ewes with non-pregnant predictions on ultrasound that were actually non-pregnant [$NPV = \frac{TN}{TN+FN}$]. Each variable was calculated for every three days of scanning, inclusive of all scans performed during each three-day timeframe.

In pregnant ewes (n = 82), accuracy of fetal counting was calculated as the proportion of litter size predictions made on ultrasound that were correct litter size outcomes at necropsy or parturition. False positive predictions were considered when a larger litter size prediction was made on ultrasound than was present at necropsy or parturition. False negative predictions were considered when a smaller litter size prediction was made on ultrasound than was present at necropsy or parturition.

All statistical analyses were performed using Statistical Analysis Software version 9.4 (SAS Institute Inc., Cary, NC). Data were analyzed using the MIXED procedure. Fetal length

was analyzed using repeated measures, with ewe as the experimental unit. Fetal length measurements obtained from multiple fetuses in a single ewe during a scan were averaged within ewe. Compound symmetry covariance structure was chosen based on the lowest Akaike information criterion (AIC) values. When appropriate, mean comparisons were made using a least square means (LSMEANS) statement and PDIFF option. Data are reported as mean $\pm$ SE. Significance was considered at $P \leq 0.05$.

3. Results

3.1 Identification of Pregnancy Status

Accuracy of detecting pregnancy status increased from 68.8% at d 21 ± 1 to 100% at d 39 ± 1 of gestation (Table 1). Sensitivity of detecting pregnant ewes was 44.4% at d 21 ± 1 and reached 100% at d 39 ± 1 of gestation. Specificity of detecting non-pregnant ewes and the positive predictive value were 100% during the entire scanning period. The negative predictive value increased from 58.3% at d 21 ± 1 to 100% at d 39 ± 1 of gestation. Greater than 90% accuracy, sensitivity and negative predictive value were achieved between d 33 ± 1 and d 42 ± 1 of gestation.

In total, 85 pregnancies were detected at d 28.7 ± 0.4 and 28 non-pregnancies were detected at d 25.5 ± 0.6 . Of the 85 pregnancies, three early embryonic losses were identified between d 39 and d 41 (mean: d 39.7 ± 0.7) of gestation, generating a flock embryonic loss rate of 3.5%. The remaining data are presented for the 82 sustained pregnancies, of which pregnancy was detected at d 28.5 ± 0.4 of gestation.

3.2 Identification of Multiple Offspring

Twenty singleton, 46 twin, and 16 triplet litters resulted from the pregnant ewes. Twin fetuses could be observed on ultrasound between d 23 and d 44 (mean: d 34.5 ± 0.5), whereas triplet fetuses were observed between d 25 and d 34 (mean: d 31.0 ± 1.3). Accuracy of predicting fetal number decreased with increasing number of fetuses, with 100, 87 and 31% accuracy for singleton, twin, and triplet pregnancies, respectively. The overall accuracy of predicting fetal number was 78%. No false positive predictions were reported. All inaccuracies were a result of false negative predictions (predicting fewer fetuses than actually present), which included 8 twins and 2 triplets predicted as singletons, and 9 triplets predicted as twins.

3.3 Observance of Placental and Fetal Landmarks

In pregnant ewes, placental and fetal landmarks indicative of fetal development were observed throughout early gestation (Table 2). Fluid-filled uterine cross-sections were observed beginning at d 27.9 ± 0.4 and persisted in pregnant ewes throughout the remainder of the scanning period. Immature placentomes emerged from the uterine wall as button-like structures beginning at d 33.8 ± 0.4 . Fetal landmarks, such as limb bud separation from the body, a fetal genital spot, and the umbilical cord were first observed at d 35.2 ± 0.7 , d 37.9 ± 0.7 , and d 38.4 ± 0.7 , respectively. Placentome maturation into a hollow circle or c-shape was observed starting at d 40.6 ± 0.4 and visualization of ribs began at d 42.2 ± 0.7 .

Multiple offspring had a significant effect on pregnancy detection and landmarks related to placental development (Table 2). An enlarged uterus ($P = 0.05$) and pregnancy (fetal heartbeat; $P = 0.03$) were detected earlier when multiple offspring were developing compared with singletons (enlarged uterus: d 25.5 ± 1.1 , d 26.5 ± 0.8 , d 28.6 ± 1.0 ; pregnancy: d 26.8 ± 1.0 , d 27.3 ± 0.7 , d 29.7 ± 0.7 ; triplet, twin, singleton, respectively). Similarly, placentome evagination

was observed earlier in triplet than in twin and singleton pregnancies ($P = 0.02$; d 31.8 ± 0.8 , d 33.9 ± 0.5 , d 34.8 ± 0.7 ; triplet, twin, singleton, respectively).

3.4 Uterine Horn Distribution

In singletons, fetuses were distributed 56% and 39% in the right and left horns, respectively. One uterine abnormality accounted for the remaining 5% of singletons, where a ewe carrying a singleton had only one uterine horn. A similar uterine distribution was observed in twins, with 53.3% in the right horn and 46.7% in the left horn. For twins, one fetus was implanted in each horn per pregnancy in all but two ewes, which both had two fetuses implanted in the right horn. In triplets, 36.4% of fetuses were implanted in the right horn and 63.6% in the left horn. In all but two ewes, two fetuses were implanted in the left horn and one in the right horn. No cases were observed in which three offspring were implanted in one horn.

Since scanning was performed only in the right abdominal pit, the uterine horn of implantation was analyzed for an effect on pregnancy detection. Only singleton pregnancies were analyzed since those are the only litter size in which the fetus visualized on ultrasound corresponds exactly with fetal information at necropsy. In singletons, uterine horn had no effect on when pregnancy could be detected ($P = 0.29$; right horn d 31.7 ± 1.6 ; left horn d 29.2 ± 1.3).

3.5 Fetal Length

Fetal length increased with day of gestation ($P < 0.0001$) but fetal number had no effect on fetal length ($P = 0.72$). A fetal number by day of gestation interaction ($P = 0.01$) was observed for fetal elongation (Figure 3). At d 29 ± 1 , singletons were longer than twins and triplets ($P \leq 0.01$; 20.0 ± 4.9 mm, 12.8 ± 0.5 mm, 13.1 ± 0.8 mm; singleton, twin, triplet,

respectively). At d 32 ± 1 , singletons were shorter than twins ($P = 0.05$; 13.8 ± 1.8 mm, 18.6 ± 0.7 mm; singleton, twin, respectively). At necropsy at d 45 of gestation, fetuses were 59.7 ± 0.8 mm long, with no effect of fetal number on fetal length ($P = 0.72$).

4. Discussion

In the current study, the overall ability to discriminate pregnancy versus non-pregnancy using transabdominal ultrasound steadily improved from d 21 to d 31 of gestation, with greater than 90% accuracy and sensitivity consistently achieved after d 31 of gestation. Increased accuracy and sensitivity correspond with the ability to detect pregnancy landmarks beginning at approximately d 30 of gestation. This included recognizing enlarging cross-sections of uterine fluid that were supportive of pregnancy, and placentome evagination and the fetal heart beat which were positive indicators of pregnancy, between d 28 and d 34. The timing of recognition may be a result of the uterus expanding from its intra-pelvic location towards the right abdominal wall [30], thereafter positioning the uterus and fetus(es) within the depth that the ultrasound probe could penetrate [3,16]. The reduced NPV between d 21 and d 31 indicates that a larger number of non-pregnancies were predicted during this timeframe on ultrasound, yet fewer ewes were actually non-pregnant, which suggests that technicians should use caution when scanning ewes transabdominally before d 31 of gestation. Additionally, the low accuracy and sensitivity before d 31 indicate difficulty recognizing positive indicators of pregnancy during this time of gestation. However, when pregnancy and fetal landmarks can be identified, technicians can expect confidence in pregnant diagnoses made using transabdominal ultrasound, regardless of the time of gestation, as supported by the PPV of 100% reported during the entire scanning period of this study.

The timeline reported in the current study for observing early placental and fetal landmarks is novel using the transabdominal approach, and consistent with transrectal reports [18]. Using the transrectal technique, the fetus, limb bud separation and rib structures have been observed at d 25, 38 and 50, respectively using a 6/8 MHz probe [18]. Transabdominally, using a 3.5 MHz probe, Anwar et al. (2008) observed limb buds and mature placentomes in all ewes between d 51 and 55, when scanning was initiated at d 26 of gestation. The earlier observance in the current transabdominal study (limb buds at d 35.2 and mature placentomes at d 40.0) may be attributed to improved ultrasound technology and use of a 5 MHz transducer, which lacks the penetration of a 3.5 MHz transducer, but provides a more detailed image [3].

Additionally, the day of gestation that pregnancy landmarks and the fetus were identified was indicative of whether multiple or single fetuses were implanted. This is consistent with the biological phenomena of pregnancy, since placental growth is exponential during early gestation, preceding fetal development, and placentome number increases with more fetuses [30–32].

When using ultrasound to estimate litter size in sheep, false positive predictions are reported more commonly than false negative predictions [33]. False positives, or overestimations, are caused by one of two possibilities; unaccounted early fetal loss or recounting of the same fetus [3,33]. False positive predictions were prevented in the present study by 1) accounting for three early fetal losses by scanning ewes repeatedly before d 45, and 2) differentiating fetuses in a single ultrasound image. Reports of early fetal loss rates in flocks are limited; however, Diskin and Morris, (2008) reported that embryo survival decreased with increasing ovulatory rates in sheep. Mono-ovular sheep had a fetal loss rate of 12%, much less than other mono-ovular species such as bovines (40 to 60%; [34]), suggesting that early

embryonic loss is a small contributor to overestimations of litter size. This is in agreement with the 3.5% fetal loss rate identified in the current study.

Although false positive predictions were eliminated in this study, 10 false negative predictions of singletons on ultrasound resulting in either twins or triplets at necropsy or parturition did occur. While only a small portion of the flock, such error could result in restricted feeding of a pregnant ewe and compromising fetal development. Importantly, error in predicting fetal number can result in under-feeding (resulting from false negative prediction) or over-feeding (resulting from false positive prediction) pregnant ewes, and therefore these consequences must be considered when using ultrasound to predict litter size.

Measurement of fetal length was dependent on fetal positioning in utero and limited by equipment capacity. With the EasiScan, fetal length was measurable until d 42 of gestation, at which point fetal length reached greater than 40 mm, exceeding the ultrasound image limits. Differences in singleton versus twin and/or triplet length were observed at d 29 and d 32, but not at other time points. These differences may have resulted from a smaller number of singleton observations obtained at these specific time points, or the limited accuracy of estimating fetal length on a 1 cm grid. Despite these limitations, a significant relationship was observed between fetal length and day of gestation between d 23 and d 41. Similarly, positive correlation between fetal length and age has been established between d 25 and 46 in sheep using the transabdominal technique [35]. Further, Metodiev et al. (2012) reported a similar fetal length on ultrasound at d 46 (51.8 ± 1.7 mm) to that obtained at necropsy in our study at d 45 (59.7 ± 0.8 mm), suggesting agreement between ultrasound estimations and the actual size of the fetus during early gestation.

5. Conclusion

Transabdominal ultrasound was used to accurately discriminate pregnancy and non-pregnancy before d 45 in the sheep, with greater than 90% accuracy, sensitivity and negative predictive value achieved at d 33 onward. By detecting enlarged uterine cross-sections, technicians can suspect pregnancy even if a fetus is not readily identified. When a fetus and heartbeat are observed, structural developments and fetal length are important indicators of gestational age and breeding date. Furthermore, detection of multiple offspring can be improved by accounting for early placental development and fetal identification. Thus, the use of transabdominal ultrasound before d 45 of gestation in sheep can result in improved flock management by early detection of pregnancy, estimation of fetal number and measurement of fetal length.

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Table 1: Accuracy (Acc), sensitivity (Sen), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of transabdominal ultrasound for pregnancy status prediction.

Gestation, d $\pm$ 1	TP ¹	FN	FP	TN	Acc ² , %	Sen, %	Sp, %	PPV, %	NPV, %
21	4	5	0	7	68.8	44.4	100.0	100.0	58.3
24	18	9	0	14	78.0	66.7	100.0	100.0	60.9
27	40	14	0	17	80.3	74.1	100.0	100.0	54.8
30	44	9	0	19	87.5	83.0	100.0	100.0	67.9
33	55	3	0	32	96.7	94.8	100.0	100.0	91.4
36	71	1	0	20	98.9	98.6	100.0	100.0	95.2
39	70	0	0	24	100.0	100.0	100.0	100.0	100.0
42	26	0	0	12	100.0	100.0	100.0	100.0	100.0

¹ TP = correct pregnant prediction; FN = incorrect non-pregnant prediction; FP = incorrect pregnant prediction; TN = correct non-pregnant prediction

² $Acc = TP+TN/(TP+FP+FN+TN)$; $Sen = TP/(TP+FN)$; $Sp = TN/(TN+FP)$; $PPV = TP/(TP+FP)$; $NPV = TN/(TN+FN)$

Table 2: Fetal number affects first observance of pregnancy detection and landmarks.

Landmark	First Observance, d ¹			
	Overall	Singleton	Twin	Triplet
Enlarged uterus	27.9 ± 0.4	28.6 ± 1.0 ^a	26.5 ± 0.8 ^b	25.5 ± 1.1 ^b
Pregnancy detection	28.5 ± 0.4	29.7 ± 0.7 ^a	27.3 ± 0.7 ^b	26.8 ± 1.0 ^b
Placentome evagination	33.8 ± 0.4	34.8 ± 0.7 ^a	33.9 ± 0.5 ^a	31.8 ± 0.8 ^b
Limb buds	35.2 ± 0.7	38.0 ± 1.6	34.7 ± 0.8	35.4 ± 1.5
Fetal genital spot	37.9 ± 0.7	39.4 ± 1.2	37.9 ± 0.8	35.8 ± 1.4
Umbilical cord	38.4 ± 0.7	40.3 ± 1.5	38.0 ± 0.9	37.6 ± 1.5
Mature placentome	40.6 ± 0.4	41.5 ± 0.7	40.3 ± 0.5	40.8 ± 0.9
Ribs	42.2 ± 0.7	43.0 ± 1.4	42.4 ± 1.1	38.7 ± 1.7

¹ Day of gestation; Mean ± SE^{a-b} Means within a row with different superscripts differ ($P \leq 0.05$)

Figure 1. Representative images of ovine placental and fetal landmarks, as viewed transabdominally between d 21 and 45 of gestation. Panel A: cross sections of an enlarged, fluid-filled uterus (arrowhead) accompany pregnancy and the presence of a fetus with a heartbeat (arrow). Panel B: the hind limb buds (arrows) separate from the fetal body, and female genitalia (arrowhead) can be identified between the hind legs and tail. Panel C: the male genitalia (arrow) can be identified in the abdomen of the fetus. Panel D: the umbilical cord (arrow) extends from the fetal abdomen, with the rib cage (arrowhead) is also visible. Panel E: an immature placentome (arrow) begins to evaginate from the uterine wall. Panel F: a hollow, mature placentome unit (arrow) has developed along the uterine wall.

Figure 2. Representative images of fetal length when scanning transabdominally between d 21 and 45 of gestation in the ovine. Fetal length was measured cranio-caudally using a 1 cm grid superimposed on the ultrasound screen. When the longitudinal plane of the fetus could be visualized, the fetus was aligned with the parallel lines of the grid, and the portion of 1 cm squares that a fetus covered was reported in millimeters. Panel A: a fetus at d 25. Panel B: a fetus at d 32. Panel C: a fetus at d 37.

Figure 3. Fetal length increases with gestational age. Fetal length was measured along the longitudinal axis of the fetus using a 1 cm grid superimposed on the ultrasound screen. Fetal length measurements were obtained from 16 singleton, 46 twin, and 15 triplet pregnancies. Fetal length increased with day of gestation ($P < 0.0001$) but not fetal number ($P = 0.72$). A fetal number by day of gestation interaction was observed ($P = 0.01$). * $P \leq 0.01$, singleton versus twin and triplet; † $P = 0.05$, singleton versus twin.