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# Evaluation of Ultrasonography to Measure Fetal Size and Heart Rate as Predictors of Fetal Age in Hair Sheep

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## Summary

There is little information available on methods to estimate fetal age of hair sheep. The objective of this study was to determine whether crown-rump length (CRL) and/or fetal heart rate (FHR) could be used to predict fetal age in hair sheep using transrectal B-mode and Doppler ultrasonography. St Croix White, Barbados Blackbelly and Dorper X St. Croix White ewes (n = 54) were scanned weekly beginning 28 d after a successful mating. Linear array B-mode ultrasonography (5 MHz) was used to measure CRL and visual FHR, and Doppler ultrasonography was used to measure audible FHR. Due to the size of the fetus CRL was not measurable after 42 d of gestation, and visual FHR was not measurable after 70 d. Audible FHR was not consistently measurable before 35 d but could be measured through 140 d of gestation. Single fetuses had greater CRL ($P < 0.01$) than multiple fetuses at d 35 and d 42 of gestation. There was no difference ($P > 0.10$) in visual or audible FHR between single and multiple fetuses. Visual FHR was higher ($P < 0.0001$) than audible FHR at 49 d of gestation. Both CRL and audible FHR had a linear relationship with days of gestation for single and multiple fetuses. The relationship between days of gestation and visual FHR was best described by a cubic equation for single fetuses and a quadratic equation for multiple fetuses. Accuracy of the regression equations and the software in the ultrasound machine was evaluated by scanning a set of ewes (n = 51) also with known breeding dates. Both the equations and the software underestimated actual age of single fetuses by 2.5 d ($P < 0.01$). For multiple fetuses the equation overestimated the age by 1 day, and the software underestimated age by over 2 d ($P < 0.01$). Overall, the regression equations underestimated fetal age by 1 d and the software underestimated fetal age by more than 2 d ($P < 0.004$). Fetal age can be estimated with acceptable accuracy in hair sheep breeds, regardless of fetal number, using existing methods that were developed using other breeds of sheep.

**Key Words:** Sheep; Fetus; Ultrasonography; Fetal Heart Rate

## Introduction

Pregnancy diagnosis in sheep usually is done using some form of ultrasonography, since rectal palpation is impractical due to the size of the animals. Linear-array ultrasonography is a common method because it allows the operator to view the fetus, determine fetal number and collect measurements of the fetus to determine fetal age (Griffin and Ginther, 1992; Karen et al. 2004; Romano and Christians, 2008). One measurement that is commonly used to estimate fetal age is crown-rump length (CRL). In addition to CRL, other measurements used to estimate fetal age in sheep include head width and thoracic depth (Sergeev, et al., 1990) and biparietal skull and body trunk diameter (Aiumlamai, et al., 1992). Neither of these studies indicated that there was an effect of fetal number on the estimated age of the fetus based on the measurements collected. Audible Doppler ultrasonography also has been used for pregnancy detection in the ewe (Lindahl, 1971), sow (Too et al., 1974; Pierce et al., 1976), mare (Mitchell, 1973) and cow (Mitchell, 1973).

Because hair sheep breeds in the tropics are smaller than most wool breeds in temperate areas and lamb birth weights are smaller (Godfrey et al. 2004; Godfrey, 2005), it is unknown if CRL measurements can accurately predict fetal age in these breeds using existing criteria. In addition, there is little information available on the effect of multiple fetuses on the accuracy of fetal age predictions. The objectives of this study were to compare measurements of CRL and fetal heart rate (FHR) throughout gestation using transrectal B-mode and Doppler ultrasonography in hair sheep. The effect of multiple fetuses on the accuracy of the measurements was evaluated.

## Materials and Methods

The Institutional Animal Care and Use Committee of the Agricultural Experiment Station of the University of the Virgin Islands approved all procedures prior to commencement of this study (FASS, 1999). Hair sheep ewes (n = 54) were bred during a 35-d period in October 2003, and the number of ewes of each breed type that was carrying sin-

**Table 1. Number of ewes of each breed type with either single, twin or triplet fetuses.**

| | Breed type | | | |
|---|---|---|---|---|
| Sire breed | Barbados Blackbelly | Dorper | Dorper x St. Croix White | St. Croix White |
| Dam breed | Barbados Blackbelly | St. Croix White | Dorper x St. Croix White | St. Croix White |
| Fetal number | Number of ewes | | | |
| 1 | 12 | 2 | 5 | 1 |
| 2 | 10 | 4 | 4 | 10 |
| 3 | 0 | 6 | 0 | 0 |
| Number of fetuses | 32 | 28 | 13 | 21 |

**Figure 1. Crown-rump length (CRL) of single and twin hair sheep fetuses measured between d 28 and 42 of gestation.**

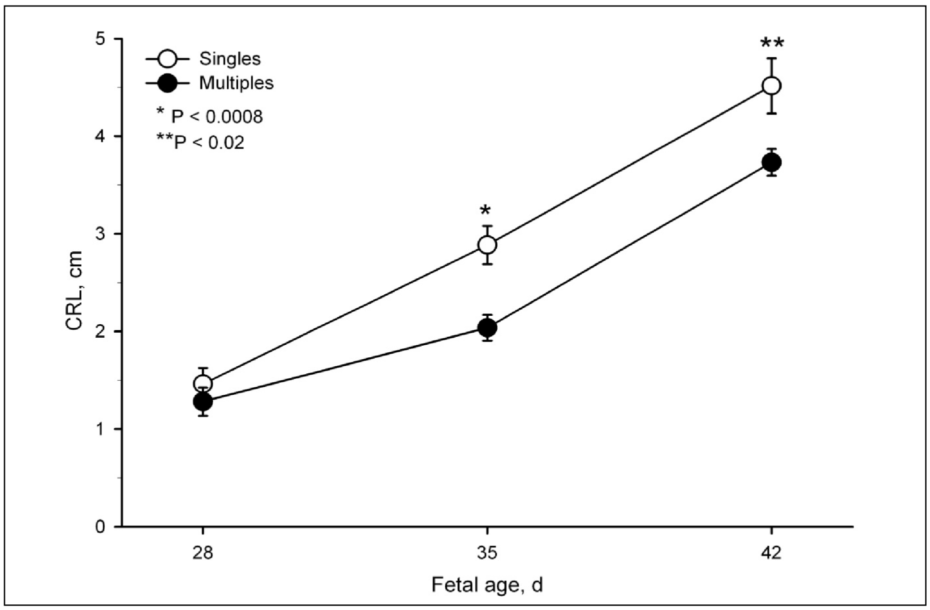
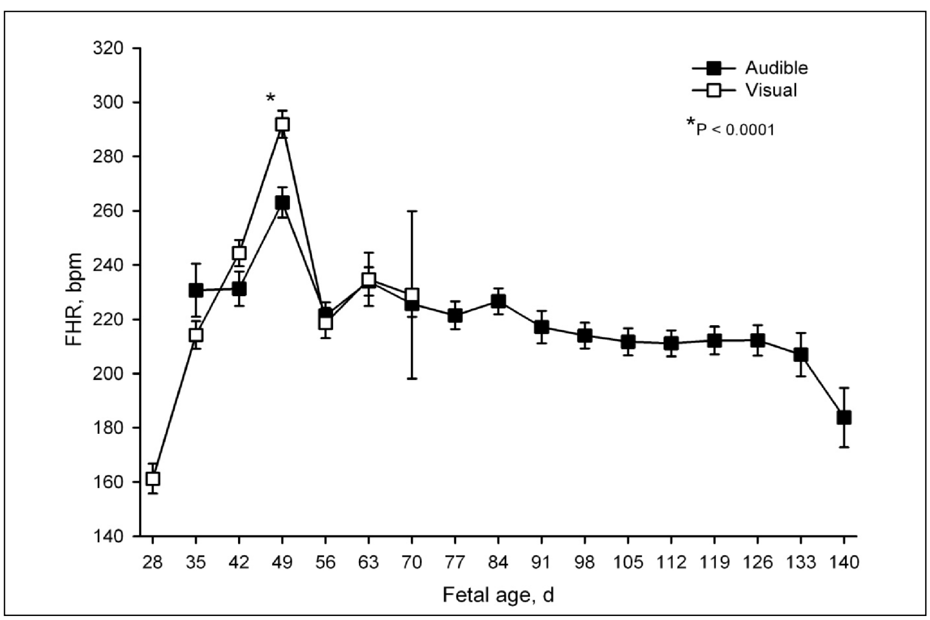



**Figure 2. Audible and visual fetal heart rate (FHR) of hair sheep fetuses measured using Doppler or B-mode ultrasonography, respectively.**



gle, twin or triplet fetuses is shown in Table 1. Rams were equipped with marking harnesses during the breeding period, and ewes were checked twice daily for crayon marks to determine actual breeding dates of the ewes.

Fetal measurements were collected weekly beginning on d 28 after a successful mating (d 0), based on crayon marks and non-return to estrus. Ewes were placed in dorsal recumbency in a flip cradle for the transrectal ultrasound examinations. Linear-array, B-mode ultrasonography was used to measure CRL and visually measure FHR using a Pie Medical Scanner 480 with a 5/7.5 MHz linear-array, switchable transducer set to 5 MHz for all examinations or a 5 MHz linear-array intracavity probe (Classic Medical, Tequesta, Fla., USA). Doppler ultrasound coupled to a battery-operated headset was used to measure audible FHR using a rectal probe (Medata Systems Ltd., West Sussex, UK). Two trained technicians conducted all ultrasound exams distributed uniformly across days and breeds.

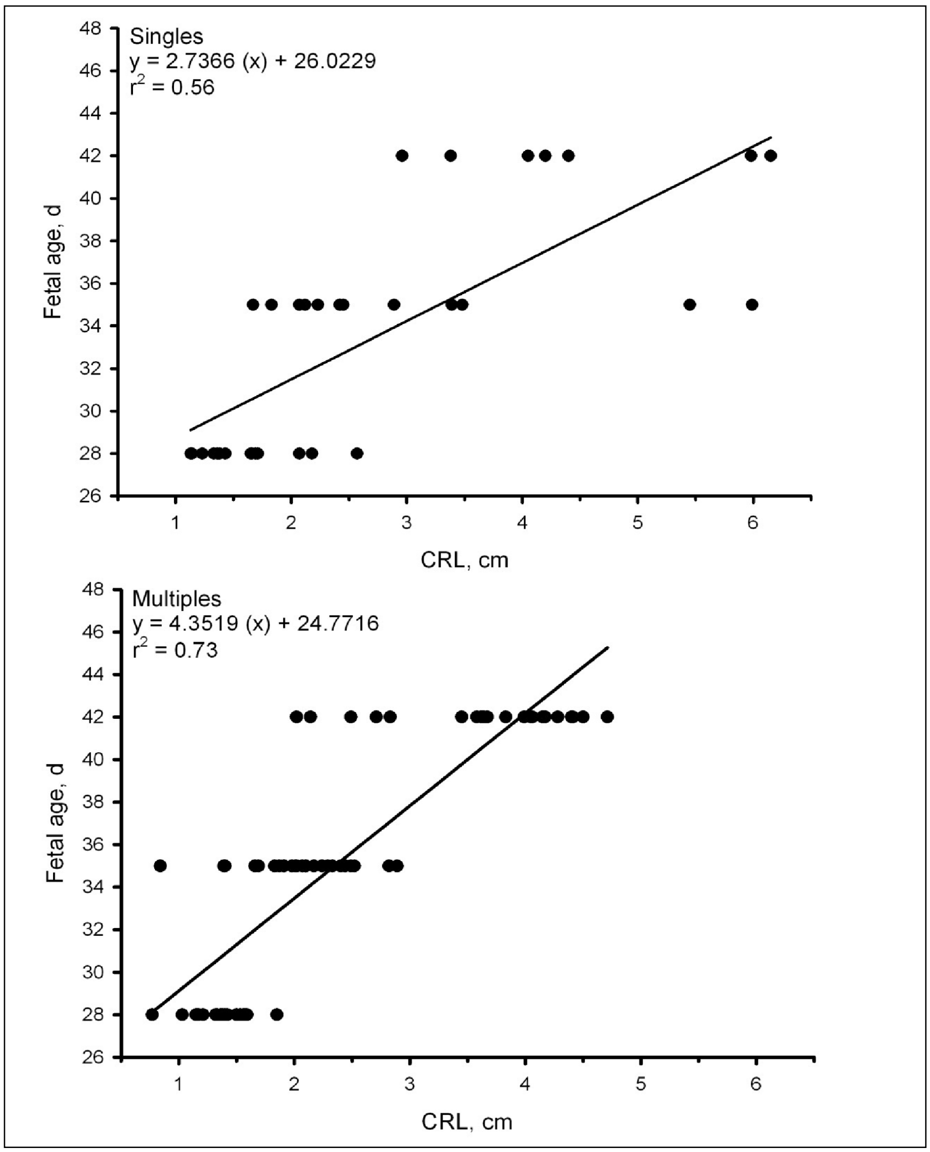
Visual FHR was measured on the image of the fetus using the linear-array ultrasound by counting the heartbeats on the screen for 10 s. The CRL was measured on a still image of the fetus on the screen using electronic calipers provided through the software on the machine. The cursor was placed on the top of the head and the second point was placed at the most distant point over the rump of the fetus.

Audible FHR was measured using the Doppler ultrasound probe by counting the heartbeats through the earphones for 10 s. All FHR were adjusted to beats/min (BPM). In ewes with multiple fetuses, measurements were collected on each fetus at each time point. This was accomplished by rotating the head of the transducer and monitoring the sound as one fetal heartbeat faded out and the transducer picked up the next fetal heartbeat. Because there was no way to determine which fetal heartbeat was being measured using the Doppler ultrasound, the results were coded by fetus number (1, 2 or 3). Repeatability of fetal-heart-rate measurements was not determined in this study, so the error associated with making the FHR measurements cannot be partitioned from the variance associated with the trait.

Data were analyzed using GLM procedures for repeated measures (SAS, 1999). Fetal breed type, number of fetuses and day of gestation were the main effects used in the model for CRL and audible and visual FHR. Because there were only two sets of triplets, fetuses were classified as either singles or multiples for the final analysis. There was no effect ($P > 0.10$) of fetal breed type on CRL or FHR (audible and visual), so all data were pooled across breed types for subsequent analysis. Relationships between fetal age and CRL and FHR measurements were determined using stepwise regression analysis with linear, quadratic and cubic terms included in the models within litter size.

To compare fetal ages produced by the machine software and the generated regression equations for CRL and fetal age, a second set of St. Croix White, Barbados Blackbelly and Dorper x St. Croix White ewes (n = 51) with known breeding dates, carrying single (n = 21) or multiple (n=30) fetuses were scanned for CRL between 26 d and 45 d of gestation (validation group). The age of the fetus determined by the software in the ultrasound machine and the regression equations developed for single or multiple fetuses were compared to the actual fetal age based on breeding dates. The

**Figure 3. Regression of crown-rump length (CRL) on days of gestation in single or multiple hair sheep fetuses. The dots represent a measurement of CRL on day 28, 35 or 42 of gestation.**



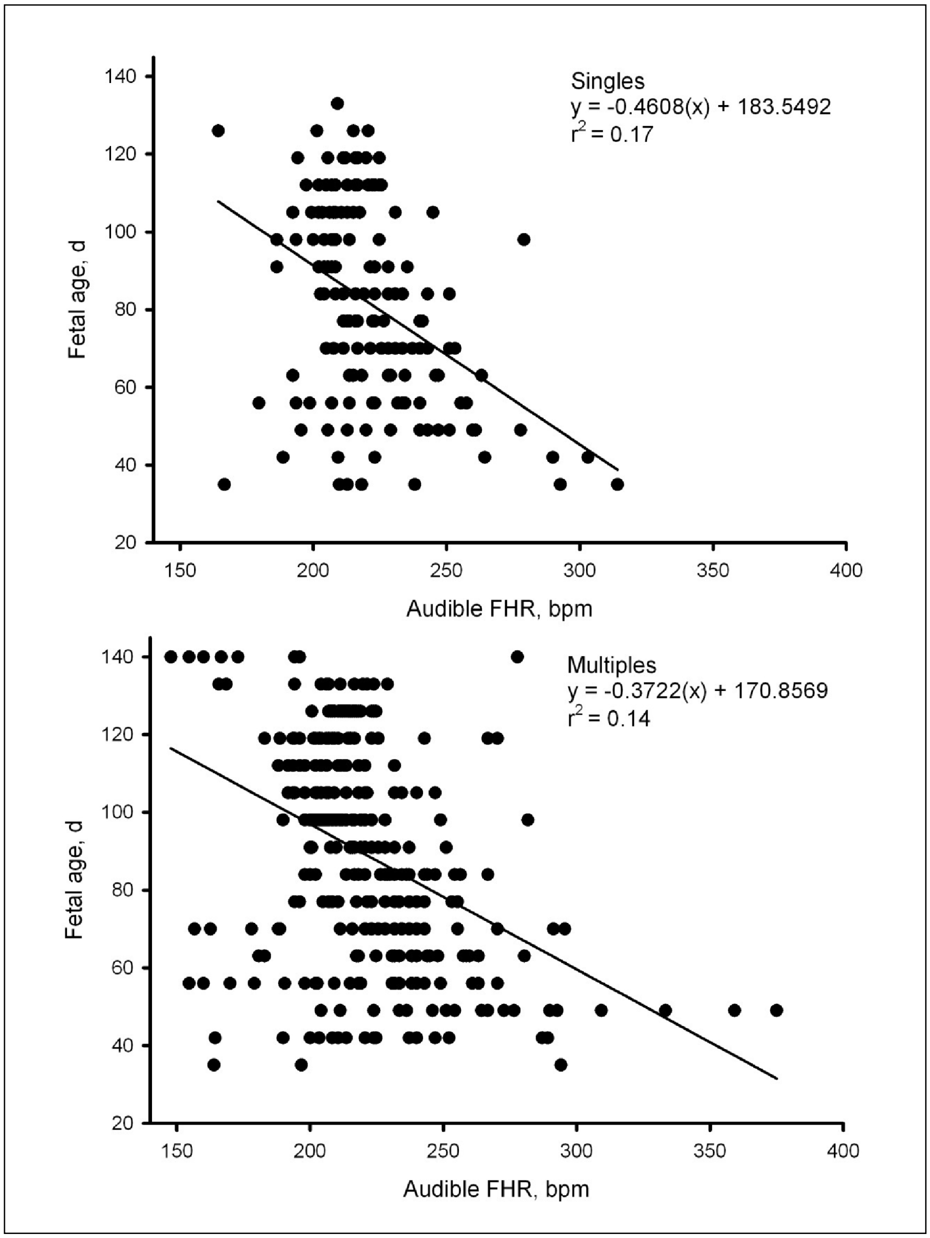
specific algorithm used in the ultrasound machine to estimate fetal age was not specified in the operators' manual. The differences were calculated as (actual age – estimated age), where estimated age was produced either by the software in the ultrasound machine or by the regression equations generated in this study. General Linear Models procedures of SAS were used to compare the differences using number of fetuses (single or multiple) in the model as the main effect. The Student's t-test was used to determine if the differences were equal to zero within fetal number groups.

## Results and Discussion

Due to the size of the fetus and 5 MHz transducer depth of penetration, CRL was not consistently measurable before 28 d and after 42 d of age, and visual FHR was not consistently detectable before 28 d and after 70 d of age. Audible FHR was not consistently detectable before 35 d of age but could be measured through 140 d of age using the Doppler ultrasound. Romano and Christians (2008) reported that pregnancy could be detected in ewes as early as d 16 using a 7.5 MHz transducer, but the authors did not conduct any measurements of the fetus in the study. Other studies have shown that fetal measurements, such as biparietal-skull diameter and thoracic diameter, can be measured between 49 d and 140 d of gestation (Sergeev et al., 1990, Aiumlamai et al., 1992). In both of these studies the ultrasound was conducted transabdominally using either a 7.5, 5 or 3.5 MHz transducer, as opposed to transrectally using a 5 MHz transducer as in the present study.

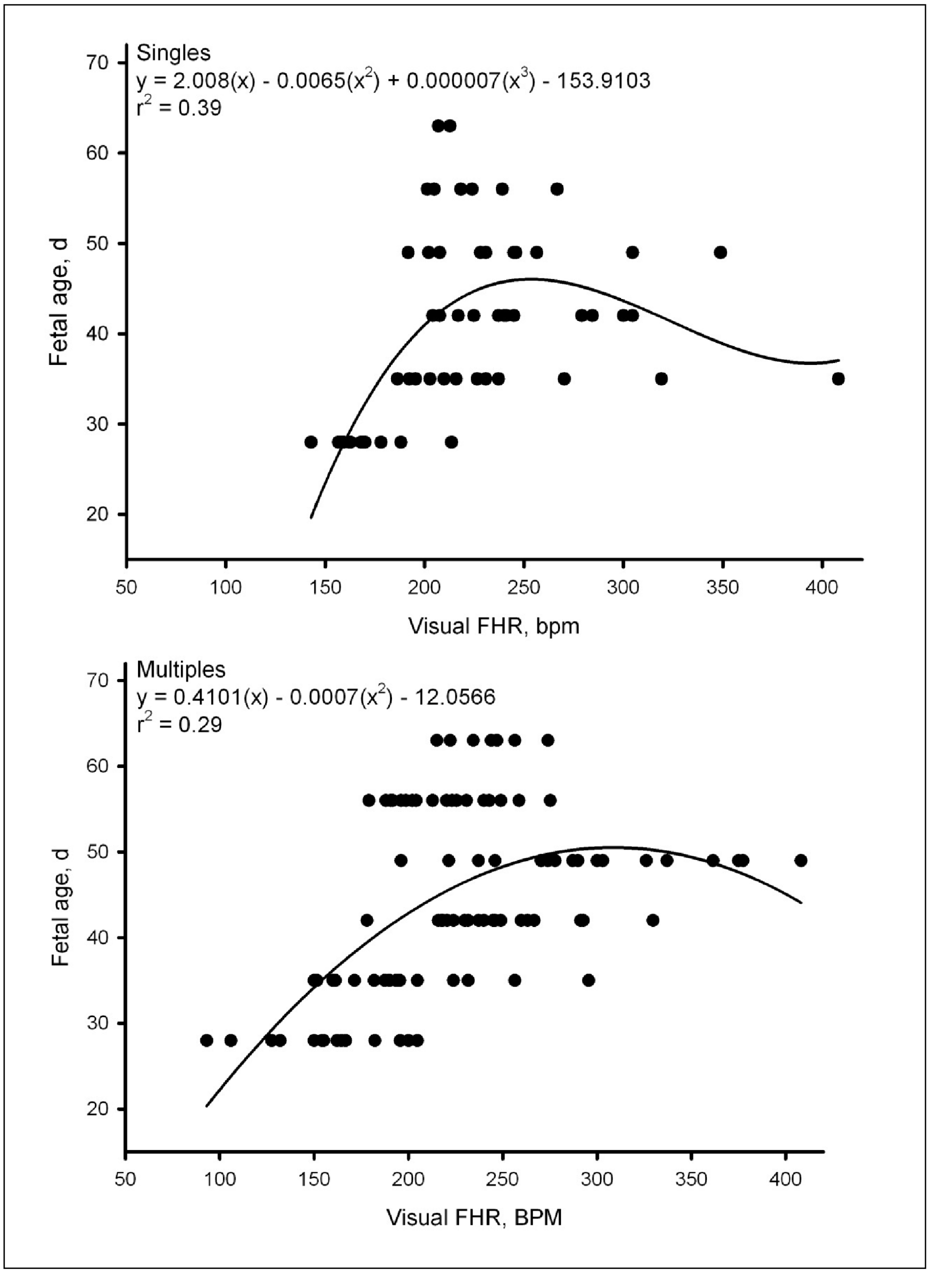
There was no difference ($P > 0.10$) in CRL between single and multiple fetuses at 28 d of age, but single fetuses had a greater CRL than multiple fetuses at 35 d ($P < 0.0008$) and 42 d ($P < 0.02$) (Figure 1). In contrast, Sergeev et al. (1990) reported that there were no differences in head length among litter sizes of 1, 2 or 3 lambs. The difference in results between the two studies may be due to the type of fetal measurements being collected. This highlights the need to validate whatever method is chosen to estimate fetal age in sheep, whether it is CRL or head length or diameter. Visual FHR was similar to audible FHR at all times except for 49 d of age ($P < 0.0001$, Figure 2). There was an increase in FHR using both methods on d 49 but it was unclear what might have caused this. The values reported for FHR in the current study were higher than those reported by Aiumlamai et al. (1992) during the entire gestation. The decrease in FHR as fetal age increases was consistent with reports in sheep (Aiumlamai et al., 1992) and cattle (Breukelman et al., 2004).

**Figure 4. Regression of audible fetal heart rate (FHR) on days of gestation in single or multiple hair sheep fetuses. The dots represent a measurement of audible FHR between days 35 and 140 of gestation.**




Crown-rump length had a linear relationship ($P < 0.0001$) with age (Figure 3) in single and multiple fetuses. The brief time period (14 d) when CRL could be measured in the current study limits the amount of information that can be obtained about fetal age using the methods described. The use of other fetal measurements, such as skull or thoracic diameter, may expand the portion of gestation during which fetal age can be estimated in hair sheep (Sergeev et al., 1990; Aiumlamai et al., 1992). In cattle biparietal diameter of the cranium was measureable between d 35 and d 100 of gestation, and the relationship was linear (Breukelman et al., 2004). In rein-

**Figure 5. Regression of visual fetal heart rate (FHR) on days of gestation in single or multiple hair sheep fetuses. The dots represent a measurement of visual FHR between days 28 and 70 of gestation.**




deer CRL was measurable in week 3 through week 17 of gestation, and chest width and depth was measurable through week 19 of gestation, after which the fetus became too large to measure (Vahtiala et al., 2004). The authors also reported that CRL and chest width and depth had quadratic relationships with gestational age in reindeer.

Audible FHR had a linear relationship ($P < 0.0001$) with day of age (Figure 4) in single and multiple fetuses. Visual FHR had a curvilinear relationship ($P < 0.0001$) with day of age, that was best described by a cubic equation in single fetuses and a quadratic equation in multiple fetuses (Figure 5). In contrast, Aiumlamai et al. (1992) reported that visual FHR had a linear relationship with fetal age during the second half of gestation, but they did not distinguish between single or twin fetuses. In the current study the visual FHR is lowest at d 28, before audible FHR was consistently measureable, and this may contribute to the curvilinear relationship being reported.

The fetal age of the validation group that was scanned to evaluate the accuracy of the machine software and the generated regression equations for CRL ranged from 26 d to 45 d of age, based on breeding dates. On d 26, d 29 and d 38 fetal ages estimated by measuring CRL and using the regression equations developed in the current study (Figure 3) were greater ($P < 0.05$) than those estimated using CRL and the software in the ultrasound machine (Figure 6). For single fetuses, both the equation and the software underestimated actual age by 2 d to 3 d based on CRL measurements (Table 2). For multiple fetuses, the equation overestimated the age by 1 d and the software underestimated age by more than 2 d ($P < 0.0001$). Overall, the equations generated in the current study underestimated fetal age by 1 d, and the software underestimated fetal age by more than 2 d ($P < 0.04$). This small discrepancy between actual age and estimated age may not be important enough to impact decisions regarding management or predicting lambing time of ewes carrying single or multiple fetuses.

## Conclusions

Fetal age can be estimated with acceptable accuracy in hair sheep breeds, regardless of fetal number, using existing methods that were developed using other breeds of sheep. It may be beneficial to develop systems that utilize measurements besides crown-rump length, such as cranial or thoracic diameter, to expand the number of days during gestation during which fetal age can be estimated. Because audible and visible fetal heart rate can be monitored for more days during gestation, it has the potential to be developed more fully as a tool for estimating fetal age.

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**Figure 6. Relationship between actual fetal age and fetal age estimated by software included with the ultrasound equipment and the equations generated in this study (Figure 3).**

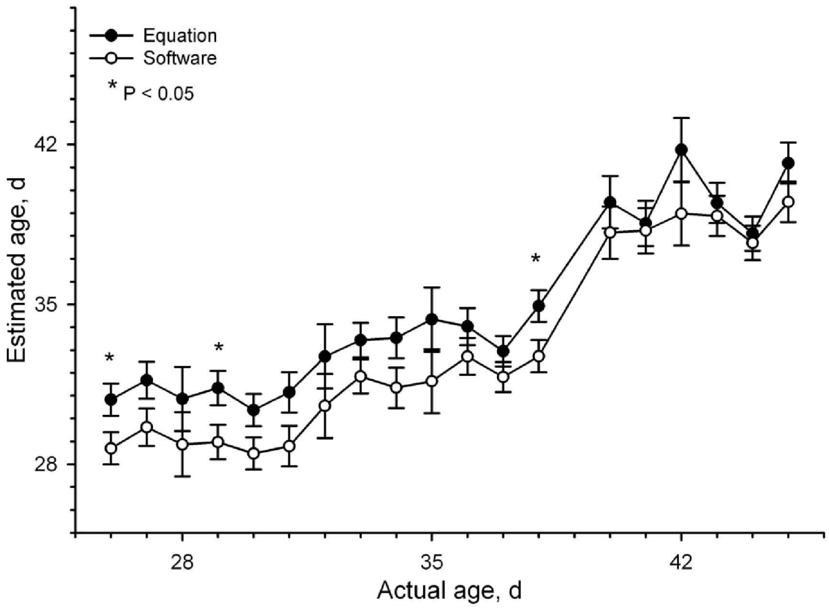


**Table 2. Differences between actual and estimated fetal ages using CRL measurements based on fetal number in the validation group of ewes.**

| Number of fetuses | Comparison | Difference[a] |
|---|---|---|
| Single | Actual - Equation | 2.6 ± 0.5[b] |
| | Actual - Software | 2.4 ± 0.5[b] |
| Multiple | Actual - Equation | -0.4 ± 0.5[c] |
| | Actual - Software | 2.4 ± 0.5[b] |
| Pooled | Actual - Equation | 1.1 ± 0.4[d] |
| | Actual - Software | 2.4 ± 0.4[e] |

[a] Difference was calculated by subtracting fetal age estimated by equations generated in this study or fetal age estimated by ultrasound machine software from the actual fetal age, based on day of breeding. The equation used for a single fetus was $y = 2.7366(x) + 26.0229$ and for multiple fetuses was $y = 4.3519(x) + 24.7716$ (Fig. 3).

[b,c] Values within fetal number group or within comparison are different (*P < 0.0001*).

[d,e] Pooled values are different (*P < 0.02*).

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