

Use of Audible and Chart-Recorded Doppler Ultrasonography to Monitor Fetal Heart Rate and Uterine Blood Flow Parameters in Cattle

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Abstract: The objective of the present study was to evaluate the use of audible chart-recorded doppler ultrasonography (DUS) to monitor both uterine blood flow and fetal heart rate (FHR) during pregnancy in dairy cattle. Possible applications of DUS include the monitoring of fetal distress when a pregnancy becomes compromised, or as a method for estimating fetal age through changes in FHR during gestation. In this study, 30 pregnant Holstein heifers at varying stages of pregnancy were sampled repeatedly between 54 to 262 d post-breeding. On test days, transrectal DUS (Medata Systems) was performed to quantify FHR and dam uterine artery pulse rates (UAP). Following DUS, B-mode ultrasonography was performed to obtain placentome diameter measurements as an additional measure of pregnancy stage. For the 104 total individual tests, 48 FHR, 101 UAP and 78 placentome measurements were obtained. Doppler US was also linked to a PowerLab Chart Recorder (ADI Instruments) to record FHR and UAP wave-forms for analysis. Data were analyzed using correlation and regression analysis relative to stage of gestation. Fetal heart rate was negatively correlated ($R = -0.61$, $P < 0.01$) with d of gestation. Fetuses that were < 140 d of age had higher ($P < 0.01$) FHR (155.8 ± 3.2 beats per minute; BPM) than fetuses > 140 d of age (142.8 ± 2.4 BPM). As expected, average placentome size was positively correlated ($R = 0.83$; $P < 0.01$) with d of gestation. However, UAP of the dam did not change ($R = 0.16$; $P > 0.10$) relative to d of gestation (mean: 74.1 ± 1.0 pulses per minute). Of the 104 attempts at DUS, FHR were determined 46% of the time and UAP found 97.1% of the time. Using the PowerLab, FHR wave-forms were 808.0 ± 13.9 mV in amplitude from baseline (62.9 ± 5.9 mV). In contrast, UAP signatures were not different ($P > 0.10$) from background levels (81.2 ± 3.2 MAX mV; 44.8 ± 3.1 MIN mV). In summary, transrectal audible DUS is effective for estimating fetal age in the bovine and can be coupled to a low-cost chart recording system for monitoring FHR characteristics.

Key words: Doppler ultrasonography, fetal heart rate, bovine

Introduction

The Doppler principle was first described in 1842 by the Austrian physicist Christian Doppler, and describes the relationship between the velocity of objects and transmitted or received wave frequencies. When the object reflecting the transmitted sound waves moves towards or away from the source, the sound waves become either compressed or expanded, respectively. The change in frequency that occurs is referred to as the 'Doppler Shift', and is dependent on the rate of movement of the objects reflecting the sound waves. By filtering out reflected background signals detected from stationary objects and amplifying shifted signals indicative of moving objects, the operator can discriminate signals of physiological significance. For example blood flow, with moving circulatory cells acting as the reflecting objects, can be discriminated with respect to its origin in the pregnant animal as either fetal heart rate or maternal uterine artery pulse rate or other circulatory processes. Current technologies utilize the Doppler principle linking it with B-scan ultrasonography and pseudo-color to produce color Doppler ultrasonography to visualize (in real-time) blood flow to the fetus, various organs, extremities or other parts of the anatomy (Jaffe, 1995). Of note, Bollwein *et al.* (2002) have recently reported the use of transrectal color Doppler ultrasonography to monitor and quantitate uterine blood flow parameters in the bovine. One draw-back, however, is the cost of these systems and the need when using less sophisticated systems to obtain both qualitative and quantitative assessments of blood flow characteristics. As part of this study, we sought to link a hand-held, battery-operated audible Doppler ultrasonography system to a low-cost chart recorder to permit the collection of fetal heart rate and uterine blood flow parameters from pregnant dairy heifers throughout gestation.

Audible Doppler ultrasonography has been used in livestock since the late 1960s for pregnancy detection in the ewe (Lindahl, 1971), sow (Too *et al.*, 1974 and Pierce *et al.*, 1976), mare (Mitchell, 1973) and cow (Mitchell, 1973). Since the work of Mitchell (1973) in the bovine, little additional research in cattle using audible Doppler ultrasonography has been conducted to date. The use of palpation for pregnancy in cattle and the advent of B-mode ultrasonography have largely made the use of traditional audible Doppler ultrasonography obsolete for routine detection of pregnancy. However for the analysis of blood flow characteristics of the uterus, umbilical vessels or fetus during gestation, use of the Doppler principle is an important application.

With the exception of recent advances in color Doppler ultrasonography technologies (Bollwein *et al.*, 2002), or

the use of sophisticated invasive probes in chronically instrumented animals (Ferrell and Ford, 1980), radioisotope labeling or steady state diffusion procedures (Reynolds *et al.*, 1985) for quantifying actual uterine blood flow parameters, other studies have relied upon simple measurements of fetal heart or uterine artery pulse rates without qualitative or quantitative assessments of blood flow parameters. Moreover, at least in cattle, little additional data since Mitchell (1973) has been collected documenting the changes that occur during pregnancy in fetal heart rate and uterine blood flow characteristics with respect to the application of audible Doppler ultrasonography. Therefore, the objectives of this study were to couple a low-cost audible Doppler ultrasonography system with chart-recording technologies to quantify the changes in fetal heart rate and uterine artery pulse rate with respect to day of gestation in pregnant dairy heifers, and to permit an assessment of fetal heart rate and uterine blood flow characteristics during pregnancy.

Materials and Methods

Animals: Thirty pregnant dairy heifers were randomly selected from the breeding herd at the Bearden Dairy Research Heifer Unit at Mississippi State University for use on this trial. Heifers had been bred previously by artificial insemination following detected estrus, and were confirmed pregnant by rectal palpation and B-mode transrectal ultrasonography at approximately 45 days of gestation. Animals were restrained for ultrasound examinations in a standard cattle squeeze chute. This study followed the FASS Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, and was approved by the MSU Institutional Animal Care and Use Committee (protocol approval number #01-019).

Doppler and B-Mode Ultrasonographic Measurements and Chart Recording: Transrectal Doppler and B-mode ultrasonography were performed repeatedly between 54 and 262 days of gestation among a group of 30 pregnant heifers. Not all 30 heifers were sampled each session, however among the nine ultrasound sessions conducted over four months a total of 104 individual ultrasound (Doppler followed by B-mode) examinations were performed. The heifers were not bred by fix-time artificial insemination, therefore heifers were at varying stages of known gestation at ultrasound examination periods. The operator was not informed at the time of each exam as to the stage of gestation of each heifer until after the examinations were concluded. This prevented any possible biases in the data collection process by the operator in knowing which heifers were in early versus later stages of gestation. The Doppler ultrasound unit was manufactured by Medata Systems Ltd. (Series 5; West Sussex, England). It consisted of a battery-operated headset coupled with a rectal Doppler probe. This Medata Doppler system was interfaced with a real-time chart recorder using a Gold series 3.6 m video cable (#15-1517) with a female indoor "F" connector to an 0.32 cm plug (1/8" in. gold plated; #278-277) and a female indoor "F" connector to a BNC (gold plated #278-267; Radio Shack, Fort Worth, TX) to connect the Doppler headset to a PowerLab 400 receiver (ADI Instruments, Castle Hill, NSW, Australia). The receiver was connected to a desktop computer and the data collected and integrated using Chart for Windows v4.0 software (ADI Instruments, Castle Hill, NSW, Australia). Data was streamed from the Doppler headset by plugging in the video cable into the headset once the fetal heart or uterine pulse was identified. If the animal moved or the operator lost the signal, the process was completed until at least two series of at least three or more wave-forms were obtained. Each animal was attempted for at least 5 min, after which if no identifiable audible Doppler signals were obtained, a new animal was examined. Of the 104 individual Doppler ultrasound exams, 48 fetal heart rates and 101 uterine artery pulse rates were obtained. B-mode ultrasonography was performed following Doppler ultrasound using an Aloka 500V ultrasound unit with a 7.5 MHz transrectal probe (Aloka Co. Ltd., Wallingford, CT). This was conducted to verify stage of pregnancy at each examination period, regardless of whether a Doppler profile was obtained, and to obtain placentome cross-sectional diameters for correlations to Doppler fetal heart rate and stage of gestation.

Data Analysis: Statistical analysis was performed using StatView (SAS Institute Inc., Cary, NC). The relationship between placentome diameters, fetal heart rate, uterine artery pulse rate and day of gestation were measured using regression analysis and Pearson's correlations, with the significance of correlations established using Fisher's *r* to *z* transformations. One-way ANOVA followed by unpaired comparisons for the contrasts of means for placentome diameters, fetal heart rates, uterine artery pulse rates and blood flow parameters relative to day of gestation were also conducted.

Results and Discussion

Fetal heart rate was negatively correlated with day of gestation ($R = -0.61$; $P < 0.001$), decreasing ($P < 0.01$) from around 190 to 160 beats per minute between 60 to 100 days of gestation to around 140 to 120 beats per minute between 180 to 220 days of gestation (Figure 1). These data mirror those of Mitchell (1973) who also documented a significant negative correlation with respect to bovine fetal pulse rate and day of gestation. Predictive regression equations were similar between our study ($y = 188.3 - 0.279x$) and the study of Mitchell

(1973; $y = 188.4 - 0.1903x$). However, Mitchell (1973) noted a "gradual but not consistent decline in fetal pulse rate with gestational age" and noted little confidence in precise aging with audible Doppler ultrasonography. Nevertheless this study coupled with our current results would suggest that a reasonable estimate of fetal age could be obtained with audible Doppler ultrasonography for animals with unknown breeding dates. As expected, placentome diameter was positively correlated with day of gestation ($R=0.83$; $P<0.001$), increasing from 10 to 20 mm between 50 to 100 days of gestation to 40 to 80 mm after 180 days of gestation (Fig. 2). As such, fetal heart rate and placentome diameter were also negatively correlated ($R=-0.52$; $P<0.001$). However, no relationship ($P>0.10$) was observed between uterine blood flow pulse rate and fetal heart rate ($R=-0.12$), placentome size ($R=0.09$) or day of gestation ($R=0.16$). This lack of a relationship to day of gestation is depicted in Fig. 3. Heart rate in the mature bovine is reported to be between 60 to 70 beats per min (Merck Veterinary Manual, 1986), which is consistent with the uterine artery pulse rate recorded for pregnant heifers in this study (Fig. 3). As partitioned relative to early versus late pregnancy, with 140 days of gestation selected as a mid-point, these relationships are further reflected in Table 1 with differences ($P<0.01$) in fetal heart rates and a notable lack of change in uterine artery pulse rates relative to time-points < 140 versus > 140 days of gestation. Representative profiles of Doppler fetal heart rate and dam uterine artery pulse rate using the PowerLab 400 chart recorder interfaced with the Doppler headset are depicted in Fig. 4 and 5, respectively. Note the different time-series (x-axis; i.e., frequency of beats or pulses) and mV readings between the two Doppler profiles captured. While frequency of fetal heart rates differed with respect to day of gestation (depicted in Fig. 1 and Table 1), variability among fetal heart rate wave-forms remained constant and did not differ ($P>0.10$) during gestation in amplitude using the chart recorder, with fetal heart wave-forms of 808.0 ± 13.9 mV in amplitude relative to baseline (62.9 ± 5.9 mV). Calculations of fetal heart blood flow resistance indices (RI; Pourcelot, 1974), pulsatility indices (PI; Gosling and King, 1975), and systolic to diastolic ratios (S/D; Stuart *et al.*, 1980) were 0.91 ± 0.01 , 1.65 ± 0.04 and 12.7 ± 2.1 for RI, PI and S/D, respectively. Nevertheless, the extent to which these indices may be of use in the bovine for monitoring fetal heart rate characteristics in response to physiological and environmental challenges have yet to be determined with this audible Doppler ultrasound and chart recording system. Unlike fetal heart rate characteristics, uterine artery pulse signatures, when converted to mV readings,

Table 1: Mean placentome size, fetal heart rate (FHR) and uterine artery pulse rate (UAP) at < 140 and > 140 days of gestation

Measurement	< 140 Days	> 140 Days	P-value
Placentome Size (mm) *	27.8 ± 9.3	43.7 ± 1.6	< 0.0001
FHR (beats / min)	155.8 ± 3.2	142.8 ± 2.4	0.002
UAP (pulses / min)	72.8 ± 1.4	75.0 ± 1.4	0.339

*Placentome size measured as cross-sectional diameter

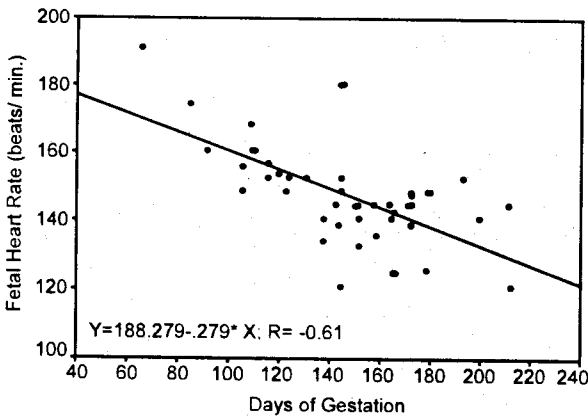


Fig. 1: Changes in fetal heart rate during gestation in the bovine. Fetal heart rate decreased ($P<0.01$) from around 190 to 160 beats per minute between 60 to 100 days of gestation to around 140 to 120 beats per minute between 180 to 220 days of gestation. To this end, fetal heart rate was negatively correlated ($P<0.001$) with day of gestation (See also Table 1).

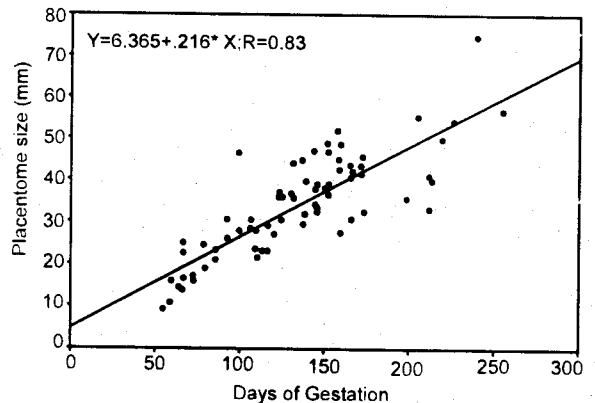


Fig. 2: Changes in placentome diameter during gestation in the bovine. Placentome diameter was positively correlated with day of gestation ($R=0.83$; $P<0.001$), increasing from 10 to 20 mm between 50 to 100 days of gestation to 40 to 80 mm after 180 days of gestation (See also Table 1).

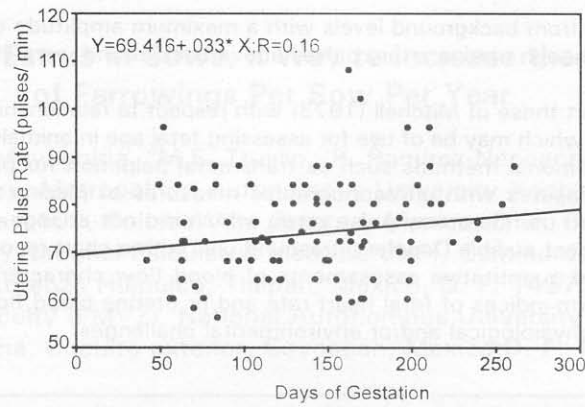


Fig. 3 Dam uterine artery pulse rate during gestation in the bovine. Dam uterine artery pulse rate did not change ($P>0.10$) during gestation, and was not correlated with day of gestation or fetal heart rate (See also Table 1).

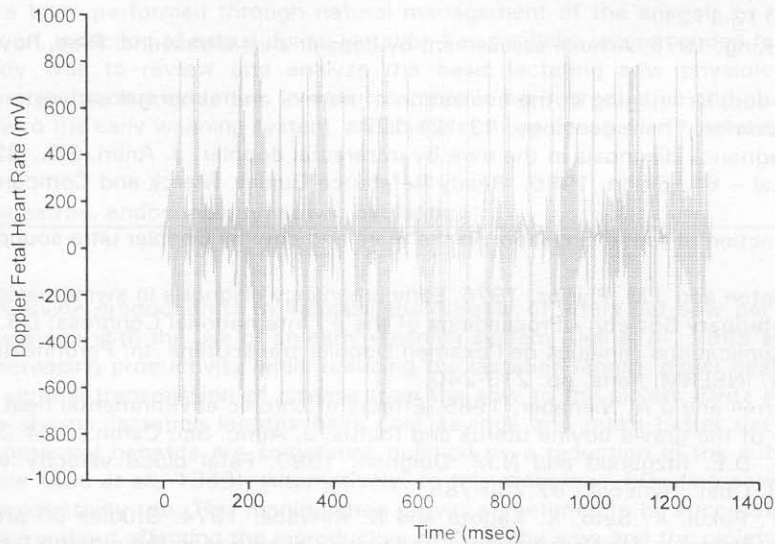


Fig. 4: Representative chart-recorded wave-forms of fetal heart beats as measured by Doppler ultrasonography. This representative profile of fetal heart wave-forms was recorded at 145 days of gestation, with a fetal heart rate of 150 beats / min.

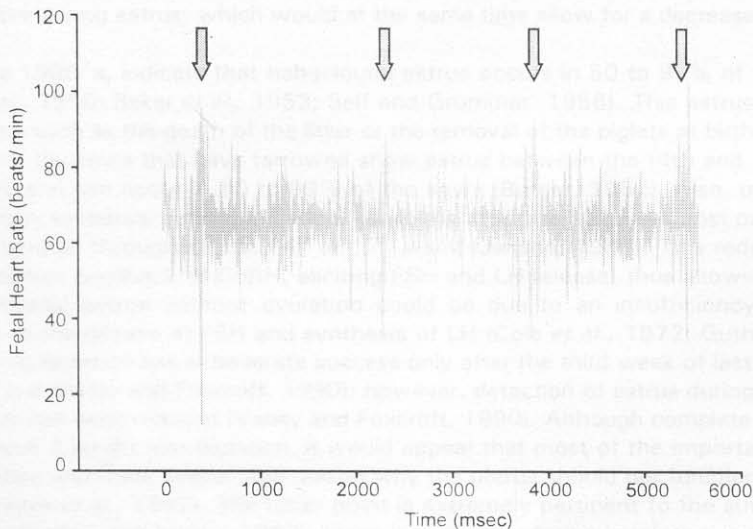


Fig. 5: Representative chart-recorded wave-forms of dam uterine artery pulses (arrows) as measured by Doppler ultrasonography during gestation in the bovine

were not appreciably different from background levels with a maximum amplitude of 81.2 ± 3.2 mV and minimum amplitude of 44.8 ± 3.1 mV; though uterine artery pulses above background were clearly evident in many instances (Fig. 5).

In summary, these data support those of Mitchell (1973) with respect to relationships between fetal heart rate and day of gestation in the bovine which may be of use for assessing fetal age in animals of unknown breeding; in place of or in conjunction with traditional methods such as transrectal palpation for pregnancy. This study has also paralleled fetal heart rate measures with ultrasonographic measures of placentome development, which were negatively correlated, and dam uterine artery pulse rates, which did not change during gestation, in this study. Finally, we have linked a low-cost audible Doppler ultrasound unit with a chart recording system that may be used to obtain both qualitative and quantitative assessments of blood flow characteristics. This may be of use for calculating functional wave-form indices of fetal heart rate and/or uterine blood flow parameters during gestation in the bovine in response to physiological and/or environmental challenges.

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