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Possibilities of Using Ultrasonography in Prenatal Diagnosis in Sheep

[1]Abaleke IO ; [2]Nwankwo CI ; [3]Amah Anthony Okechukwu

[1]West Pomeranian Univeristy of Technology in Szczecin Poland,
Department of Biotechnology in Animal Production and Environmental Protection
Faculty of Biotechnology
[2,3]Department of Biochemistry, College of Natural Sciences,
Michael Okpara University of Agriculture, Umudike
Correspondence Author: Nwankwo CI

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Abstract: The heart is the first organ to function in the embryo and blood begins to flow in early days of pregnancy. The heart is a large organ relative to body size. Examinations of fetal heart are a very essential factor in assessing fetal viability. Ultrasonographic evaluation of pregnant sheep can enable the diagnosis of fetal growth problems and develop prenatal support accountable for lowering morbidity and perinatal death. The aim of this study is to carryout ultrasound measurements of fetal heart in sheep between 75 and 80 days, 85 and 95 days, and between 120 and 130 days of gestation. Ultrasound examination was done transabdominally using an ultrasound scanner (EDAN U500) equipped with a sector probe with a frequency of up to 5 MHz (Model, C352UB). Before the examination, the sheep were placed in a standing position. The inguinal and caudal areas were shaved and the skin was cleaned with soap and water. A sufficient amount of transmission gel was applied prior to transabdominal examination. The measurements were made using B-Mode. The length and width of the heart, the height of the ventricles and atria were twice measured for each fetus. The obtained results were further submitted for a statistical analysis. The resulting data are presented as the mean ± SD, marked on the tabulated. The differences were analysed by analysis of variance and a posthoc test. Duncan multiple range test was used to verify the significance of differences at P<0.01 and P<0.05. The correlations between the studied parameters were calculated with the Pearson rank correlation coefficient (r; correlation coefficient). The result of this study showed that between days 75 and 80 of pregnancy, the fetal heart rate was significantly smaller than in the later stages of pregnancy at p<0.01. There was significant difference in the parameters of the ventricles and the artria measurement was done between 85th and 95th day of pregnancy, the size of artria was significantly smaller in the period between day 120 and 130 at p<0.01. It was also observed that most of the parameters measured were highly significantly and positively correlated with the day of pregnancy at p>0.01. The heart size parameters were postitively correlated with the size of both ventricles and right artrium at correlation coefficient (r) at p>0.01. In concusion, this study suggests that ultrasound measurements of the heart could be used to determine gestational days and study normal and abnormal cardiac growth in Sheep.

Keywords*: Heart; Ultrasound Examination; Pregnancy; Cardiac Growth; Gestational Age.*

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I. INTRODUCTION

The embryonic stage of development is exceedingly tiny and not observable in sheep. Due to the difficulty to estimate exactly the length of time which elapses between coitus and fertilization, there is substantial individual variation in the developmental stage of a conceptus at a given period after mating (Rowson and Moor, 1966). Shiota (2009) revealed that from known-age embryos show comparable variance. Variation in development of embryos of the same coital age, albeit large, is not particularly remarkable. The latest achievements in understanding myocardial shape and function have caused a paradigm shift in study and offered a promising platform for new integrated knowledge of heart anatomy and

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function (Torrent-Guasp *et al.*,, 2001). The heart is the first organ to form and function throughout development (Sakabe *et al.*,, 2005) and it undergoes considerable structural remodelling during development. For the human, the earliest phases are to be found in the Glaevecke embryo of von Spee and in the Sternberg and Payne embryos. B-mode ultrasonography is a rapid and reliable procedure used to screen for pregnancy in small ruminants. Transrectal ultrasonography is much superior to the transabdominal approach in getting more accurate findings during the early gestational phase (days 27–30) (Ishwar 1995; Doize *et al.*,,1997; Gürler and Kaymaz 2011; Karadaev *et al.*,,2016). In recent times, technological advancement has made it possible to determine gestational age in humans by measuring the biparietal diameter (BPD). Similarly, there are reported studies conducted in sheep and goats using BPD measurements (Haibel 1988; Haibel *et al.*,,1989; Reichle and Haibel 1991; Lee *et al.*,,2005). In addition to BPD, gestational age may also be determined by measuring the heart diameter (HD), placentome diameter (PD), crown-rump length (CRL), trunk diameter (TD), the aorta, or the umbilical cord (Martinez *et al.*,,1998; Lee *et al.*,,2005; Kuru *et al.*,, 2018; Kandiel *et al.*,,2015). To this, there is no study with a detailed ultrasound measurement of sheep fetal heart, its relationship with gestational age and normal cardiac growth in sheep. The present study will carry out ultrasound fetal heart measurement begining from 75th day to 130th days of gestation. Ultrasonography is a simple, reliable and non-invasive imaging technique without secondary effects. Application of ultrasonography in veterinary practice, and specifically in small ruminants, has developed to become the most efficient diagnostic tool for managing reproduction (Antonio Gonzalez-Bulnes et al, 2010). The aim of this study therefore is to assess the possibility of performing ultrasound measurements of the heart of sheep fetuses in different stages of pregnancy.

II. MATERIALS AND METHODS

➢ *Animals*

Twelve Pomeranian sheep, all with a single pregnancy, were used in the study. The sheep were housed in pastures and indoor systems at the Experimental Station of the National Research Institute of Animal Production in Kołbacz. The animals were fed according to the standards adopted for this species, which were based on pasture green fodder and other roughage and concentrated feed, depending on the season. The animals had constant access to water and salt licks for the duration of the study.

➢ *Ultrasound Examination*

The sheep were placed in a standing position before the ultrasound examination, and the inguinal and caudal areas were shaved and the skin was cleaned with soap and water. A sufficient amount of transmission gel was applied before the transabdominal examination, and the measurements were made using B-Mode. The measurements were taken twice for each fetus, and the length and width of the heart, the height of the ventricles, and the atria were measured. The ultrasound was conducted using an EDAN U500 equipped with a sector probe with a frequency of up to 5 MHz (Model, C352UB).

➢ *Statistical Analysis*

The obtained results were then submitted for statistical analysis, and the results are shown as the mean ± SD, indicated on the tabulated data. Analysis of variance and a posthoc test were used to analyze the differences, and the Duncan multiple range test was used to confirm that the differences were significant at $P<0.01$ and $P<0.05$. The Pearson rank correlation coefficient (r; correlation coefficient) was used to calculate the correlations between the parameters under study. STATISTICA version 13.1, Stat Soft, Poland, was used for the statistical analyses.

III. RESULTS

Table 1 presents the results of ultrasound measurements of the heart of sheep fetuses in the period from 75 to 130 days of pregnancy. Between days 75 and 80 of pregnancy, the fetal heart rate was significantly smaller than in the later stages of pregnancy.

In the present study, significant differences were also noted in the parameters of the ventricles. Due to the too small size of the atria of the heart, the first measurements of these anatomical structures were made only between the 85th and 95th days of pregnancy. During this period, the size of atria was significantly smaller than in the period between day 120 and 130 of pregnancy.

Table 1. Mean (± SD) Value of Heart Ultrasound Measurements in Sheep Fetuses (n = 12)

Ultrasound parameters of the heart	Days of gestation		
	75-80	85-95	120-130
The length of the heart (mm)	$18{,}18\pm0{,}96^{A}$	$33{,}95\pm2{,}32^{B}$	$38{,}93\pm1{,}62^{C}$
The width of the heart (mm)	$15{,}33\pm1{,}71^{A}$	$19{,}58\pm1{,}82^{B}$	$31{,}25\pm0{,}87^{C}$
Left chamber - height (mm)	$13{,}48\pm1{,}42^{A}$	$14{,}62\pm0{,}50^{A}$	$17{,}64\pm1{,}31^{B}$
Right chamber - height (mm)	$13{,}57\pm1{,}32^{A}$	$14{,}90\pm0{,}79^{A}$	$20{,}20\pm1{,}98^{B}$
Left atrium - height (mm)	-	8,73±0,84	9,01±0,92
Right atrium - height (mm)	-	$5{,}40\pm0{,}63^{A}$	$8{,}58\pm0{,}52^{B}$

A, B - Different letters indicate significant difference at $p<0.01$, within the same rows

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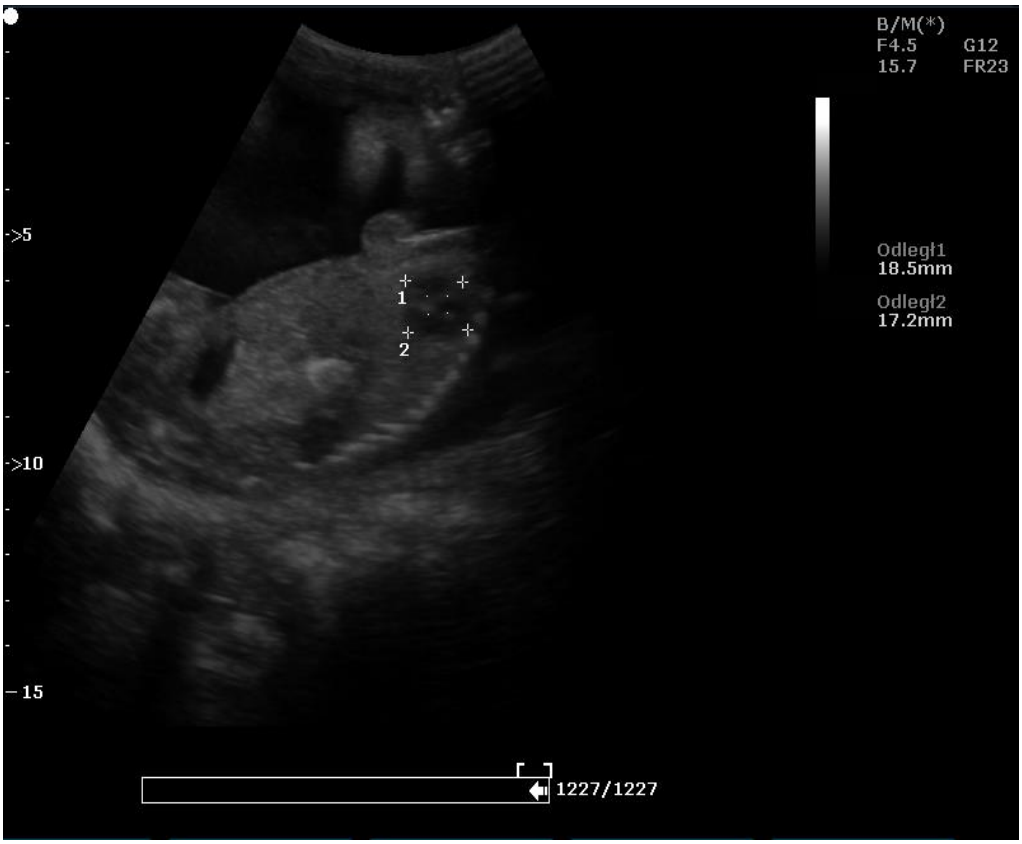

Fig 1 Shows the Ultrasound Image of Theheart of Fetus in 75 Day Gestation in Sheep.

Fig 2 Shows the Ultrasound Image of the Heart of Fetus in 94 Days Gestation

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Fig 3 Shows the Ultrasound Image of the Heart of Fetus in 125 Day Gestation in Sheep

Table 2 presents the values of the correlation coefficients between the ultrasound measurements of the heart and the day of pregnancy. Most of the parameters were highly significantly and positively correlated with the day of pregnancy.

Table 2. Correlations (r) Between Ultrasound Measurements of the Heart of Sheep Fetuses and the Day of Pregnancy (n = 12)

Ultrasound Parameter	Day of Gestation
The length of the heart	0,74**
The width of the heart	0,98**
Left chamber - height	0,88**
Right chamber - height	0,96**
Left atrium - height	0,20
Right atrium - height	0,92**

** - Correlation significant at p <0.01

Table 3 presents the values of the correlation coefficients between the examined ultrasound measurements of the heart. Heart size parameters were positively correlated with the size of both ventricles and the right atrium.

Table 3. Correlations (r) between Ultrasound Measurements of the Heart of Sheep Fetuses.

Parameter Ultrasonography	The Length of the Heart	The Width of the Heart	Left Chamber - Height	Right Chamber - Height	Left Atrium - Height
The width of the heart	0,72**				
Left chamber – height	0,69**	0,89**			
Right chamber – height	0,83**	0,95**	0,89**		
Left atrium - height	-0,06	0,19	0,02	0,08	
Right atrium – height	0,70**	0,88**	0,82**	0,88**	0,08

** - Correlation significant at p <0.01

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IV. DISCUSSION

This current study largely aims to conduct a fetal cardiac measures from 75 prenatal day to 130 gestational dayby use of ultrasonography. The embryonic heart rate can be identified at 19–20 days post-mating (Medan *et al.*,,2004; Padilla-Rivas *et al.*,,2005) and monitored at 22–36 days post-mating (Karen *et al.*,,2004). It has been reported that the heart diammeter of sheep and goats may be identified at 30–40 days of gestation however the ultrasonography fetal heart measurements in this study were collected from day 75 of gestation to 130 gestational day. Visual fatal heart rate was not consistently seen before 28 days of age and beyond 70 days, as demonstrated by Godfrey *et al.,* (2010).

According to the current study, even though the fetal heart rate was measured on day 75 of pregnancy, the visual fetal heart rate is lowest at day 28 of pregnancy, aside from later stages. The fetal heart rate was also significantly lower than in the later stages of pregnancy. The fetal heart rate was not consistently detectable before 35 days of pregnancy, but it could be measured through 140 days of age using Doppler ultrasound. Additionally, the results of the previous study were contradicted by Frasch *et al.,* (1995), who demonstrated that FHR decreases in the latter stages of pregnancy. The reason for this disparity is that the FHR was assessed in fetuses with low body weight. The current study found that the ventricles' characteristics varied significantly in the later stages of pregnancy, indicating that the fetal heart's growth was normal throughout the gestation. Due to its small size, the artria were only measured anatomically between the 85th and 95th days of pregnancy, not at 70 or 80. Additionally, it was noted that the atria were noticeably larger between days 120 and 130 of pregnancy than they were between days 85 and 95. According to Pillai *et al.,* (2017), depending on the stage of gestation, inadequate maternal nutrition affects the body size and organ growth of the offspring differently. In Manchega sheep, Gonzalez *et al.,* (1998) found a positive connection (R = 0.72) between the diameter of the umbilical chord and the age of the fetus. According to Doize *et al.,* (1997), in sheep and goats, gestational age rose quickly as placentome size increased. In goats, placentome size can be utilized (R2 = 70.32%), however in ewes, there was a little association between placentome size and gestational age (R2 = 15.59%). This is also consistent with the results of Aiumlamai *et al.,* (1992), who found that visual FHR during the second half of pregnancy showed a linear relationship with fetal age; however, they did not differentiate between single and twin. 56 to 135 days of pregnancy, according to Hulet (1969), the distinctive sound of the fetus's heartbeat, arterial pulse, or movement was interpreted as a sign of pregnancy. Aiumlamai *et al.,* found that the heart rate, trunk diameter, and skull biparietal diameter were all correlated with fetal age between 56 and 135 days of pregnancy. They concluded that these measurements could be used to estimate fetal age. The current study found that the majority of fetal heart measurements were highly significantly and positively correlated with the day of pregnancy. Fetometry, on the other hand, is better suitable for estimating gestational age and forecasting paturition than fetal heart rate measurement, according to Chavez *et al.,* (1996).

In the current study, heart measurements showed a positive correlation with gestational age ($P<0.01$), while Branko et al. (2016) proposed that occipital nasal diameter correlated with gestational age with both transabdominal ($P<0.05$) and transrectal ultrasound ($P<0.01$), and that the biparietal diameter of the fetal head measured by transabdominal ultrasound during the period from the 46th to 63rd day of gestation correlated with gestational age ($P<0.05$).

Giannico et al. (2015) reported that the heart width and heart diameter were used as surrogates of cardiac development because these measurements showed the best statistical correlation and quantitative evaluation of fetal cardiac structures can be used to monitor normal and abnormal cardiac development in canine fetuses. These findings are consistent with the present study's findings that the heart size parameters were positively correlated with the size of both ventricles and the right atrium, indicating normal fetal heart growth.

V. CONCLUSION

In conclusion, a strong and positive correlation between the studied ultrasonography parameters of the heart and gestational ages was discovered. The size of the right atrium and both ventricles as determined by B-Mode ultrasonography showed a favorable correlation with the heart size metrics. Additionally, there were substantial differences in the fetal heart rate, heart length and breadth, right and left ventricle length and width, and right and left atrium length and width throughout the various gestational days.

Accordingly, the study proposes that ultrasound measurements of the heart could be used to examine normal and aberrant cardiac development in sheep as well as to identify gestational days.

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