Estimation of gestational age by ultrasonographic measurement of different fetal parameters in goats

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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Table of content

	Titles	Page No.
	Authorization	ii
	Acknowledgement	iv
	Content	v-vi
	List of Tables	vii
	List of Figures	viii
	List of Graphs	ix
	List of Abbreviations	x
	Abstract	xi
Chapter 1	Introduction	1-3
Chapter 2	Review of literature	4-14
	2.1. Historical background of ultrasound scanning in pregnancy diagnosis	4
	2.2. Principle of ultrasound scanning	5
	2.3. Factors affecting the accuracy rate of pregnancy diagnosis using ultrasound scanning in goat	6-7
	2.4. Gestational length in goat	7
	2.5. Method of pregnancy diagnosis in goat	7
	2.6. Ultrasonography and early pregnancy diagnosis	8-9
	2.7. Fetometry for assessing gestational age in goat	9-14
	2.7.1. Fetal Crown Rump Length (CRL) in goat	9-11
	2.7.2. Fetal Bi-Parietal Diameter (BPD) in goat	11-12
	2.7.3. Fetal Femur Length (FL) in goat	13
	2.7.4. Placentomes Diameter length in goat	14
Chapter 3	Materials and methods	15-22
	3.1. Location of study	15
	3.2. Experimental animal	16
	3.3. Materials	16
	3.4. Experimental design	17-18
	3.5. Ultrasonic assessment of fetal biometry in goat	19-22
	3.6. Statistical analysis of data	22
Chapter 4	Results	23-33
	4.1. Detection of pregnancy by ultrasonography in goat	23-24
	4.2. Ultrasonographic measurement of different fetal parameters and estimation of gestational age and comparison with actual gestational age	25-33

Chapter 5	Discussion	34-39
	5.1. Detection of pregnancy by ultrasonography in goat	34
	5.2. Estimation of gestational age by measuring CRL and comparison with actual gestational age in goats	35-36
	5.3. Estimation of gestational age by measuring BPD and comparison with actual gestational age in goat	36-37
	5.4. Estimation of gestational age by measuring FL and comparison with actual gestational age in goat	37-38
	5.5. Estimation of gestational age by measuring PD and comparison with actual gestational age in goat	38-39
Chapter 6	Conclusions	40
Chapter 7	References	41-47
	Appendix- I	48
	Appendix-II	49
	Biodata	50

LIST OF TABLES

Serial No.	Title	Page No.
Table 1	Detection of pregnancy by ultrasonography	23
Table 2	Estimation of gestational age by measuring CRL and comparison with actual gestational age	25
Table 3	Estimation of gestational age by measuring BPD and comparison with actual gestational age	27
Table 4	Estimation of gestational age by measuring FL and comparison with actual gestational age	29
Table 5	Estimation of gestational age by measuring PD and comparison with actual gestational age	31
Table 6	Regression equations for determination of gestational age in goats	33

LIST OF FIGURES

Serial No.	Title	Page No.
Figure 1	Geographical location of study area	15
Figure 2	Ultrasound Machine (EXAGO, transabdominal probe (3.5-5.0 MHz) and coupling reagent (Jelly)	16
Figure 3	Preparation of goat for ultrasonography (shaving on lower abdomen)	17
Figure 4	Transabdominal ultrasonography in goat	18
Figure 5	Illustration of reference points for CRL measurements using ultrasound	19
Figure 6	Ultrasound image showing the measurement of CRL at different gestational age	19
Figure 7	Illustration of reference points for BPD measurements using ultrasound	20
Figure 8	Ultrasound image showing the measurement of BPD at different gestational age	20
Figure 9	Illustration of reference points for FL measurements using ultrasound	21
Figure 10	Ultrasound image showing the measurement of FL at different gestational age	21
Figure 11	Illustration of reference points for PD measurements using ultrasound	22
Figure 12	Ultrasound image showing the measurement of PD at different gestational age	22
Figure 13	Ultrasound imaging showing gravid uterus (left) and non-gravid uterus (right)	24
Figure 14	Ultrasound image showing fetal heart (left) and ribs and internal organs (left)	24

LIST OF GRAPHS

Serial No	Titles	Page no.
Graph 1	Linear regression curve representing the relationship between fetal CRL and AGA	25
Graph 2	Linear regression curve representing the relationship between fetal BPD and AGA	28
Graph 3	Linear regression curve representing the relationship between FL and AGA	30
Graph 4	Linear regression curve representing the relationship between PD and AGA	32

LIST OF ABBREVIATIONS

%	Percent
mm	Millimeter
A-Mode	Amplitude Mode
B-Mode	Brightness Mode
AGA	Actual Gestational Age
cm	Centimeter
BPD	Biparietal Diameter
CRL	Crown Rump Length
CVASU	Chattogram Veterinary and Animal Sciences University
EDD	Estimated Date of Delivery
EGA	Estimated Gestational Age
G	Gestational Age
Mz	Mega hertz
FL	Femur Length
GA	Gestational Age
FAO	Food and Agricultural Organization
PD	Placentome Diameter
TA	Transabdominal probe
TR	Transrectal probe
SAQTVH	Shahedul Alam Quadary Teaching Veterinary Hospital
USG	Ultrasonography

Abstract

The study was designed to estimate the gestational age in goat by measuring different fetal parameters (CRL, BPD, FL) and PD by ultrasonography. The study was also aimed to compare the estimated gestational age with actual gestational age and design reference charts to calculate the gestational age in Bangladeshi goats for future use. The experiment was conducted at SAQ Teaching Veterinary Hospital, CVASU, Khulshi, Chattogram. All the registered outdoor female patient (does) which brought for pregnancy diagnosis in SAQ Teaching Veterinary Hospital CVASU were considered for the study. The does were examined transabdominally in standing position using 3-5MHz convex transducer by real time ultrasonography. Measurement of fetal parameters and placentomes were from ultrasongraphic images by electronic caliper by freezing the desired image on screen. Pregnancy detection was confirmed by amniotic fluid and fetal heartbeat in early stage and other fetal structures (skull, ribs, femur, placentomes, etc) in later stages of pregnancy. Fetal CRL, BPD, FL and PD were measured and gestational age of does were estimated. The fetal CRL, BPD, FL and PD were 58 ± 8.67, 28.17 ± 8.02, 47.88 ± 9.50 and 29.47 ± 2.46 mm, respectively. The estimated gestational age were 54.91 ± 7.33, 78.82 ± 17.11, 97.23 ± 11.95 and 96.94 ± 8.98 days, respectively. However, the recorded actual gestational age were 58.08 ± 8.57, 76.62 ± 16.31, 99.47 ± 13.39 and 93.38 ± 13.72 days, respectively. The estimated and actual GA in goats were significantly differed ($P<0.05$) with respect to CRL, BPD and FL. However, in case of PD estimated and actual GA were not statistically different. The formula generated from linear regression equation from actual age of Jamunapari cross to estimate the gestational age in Bangladeshi goats as GA=1.27+0.98CRL, GA= 22.99+1.94BPD, GA= 34.67+1.35FL and GA= 4.43PD-37.41 with Coefficient of determination (R^2 = 98, 96, 91 and 63) respectively. Transabdominal ultrasonography is a practical and reliable method for pregnancy detection in goat. From the present study it could be concluded that the CRL, BPD were highly reliable for the estimation of GA in 1st and 2nd trimester whereas during 3rd trimester FL is recommended.

Keywords: Ultrasonography, Goat, Pregnancy, Fetal parameters, Gestational age.

Chapter-1

Introduction

Goat industry plays a vital role with its multipurpose utility of meat, milk, wool, leather products and manure thereby contributing a great deal towards national economy. The dairy goat population in the world is around 218 million estimated in 2017 (FAO, 2019). About 26 million goats are being reared in Bangladesh, mainly by middle class people as means of their family income (FAO, 2016). It is major contributor of protein and fat and often the goat rearing can help farmers to overcome an unexpected crisis.

The profitability of goat farming could be improved if a simple and reliable technique is available for the detection of pregnancy. The accurate prediction of pregnancy in goat would greatly increase the efficiency of goat farming (Doize et al., 1997). Earlier, various techniques have been used to diagnose the early pregnancy in goat like laparoscopy, laparotomy, vaginal biopsy, abdominal inspection and palpation, hulet rod method, hormonal assay and radiography (Karadaev, 2015). Most of these techniques have been unsatisfactory due to factors such as expenses, low accuracy rates, impracticability and long delay in availability of result.

Ultrasonography is commonly used in the management of livestock operations to diagnose pregnancy because of its user-friendly technique, economic, fast scanning time and immediate results. Ultrasonography is simple, quick, non-invasive and no hazard to the operator or patient. Recent advances in ultrasound technology have improved both in image clarity and the portability of ultrasound machines, improving usefulness of this tool in the field and for research. Ultrasonography enable remating or culling of non-pregnant (Amer, 2010), information about conception rates after artificial insemination (Matsas, 2007), gestation stages (Amer, 2010), time for drying-off and parturition date (Doize et al., 1997; Gonzalez et al., 2004). The separation of pregnant and non-pregnant does in different groups reduces the losses from abortions, stillbirths and optimizes labor, feed and medication costs (Wani et al., 1998).

The first reported pregnancy diagnosis in goats by using ultrasonography was in 1983. Since then, pregnancy diagnosis in small ruminants by transabdominal real time ultrasonography became rampant. In goats, ultrasonography can be used via transrectal and transabdominal. These techniques can be performed with the doe standing, sitting

or in dorsal recumbency without using sedative drugs (Vinoles et al., 2010). Transrectal scanning is more challenging in small ruminants, due to the small rectal anatomy. B-mode real time ultrasonography is a reliable and most convenient means of detecting early pregnancy in goats from day 19 onwards. This is the only accurate method for prediction of gestational age, fetal numbers and approximate date of kidding under field conditions (Amer, 2010). Nowadays, transrectal ultrasonography is widely used to study early stages of pregnancy and transabdominal ultrasonography for the later stage of pregnancy studies. Ultrasound examination can be expanded through the application of fetometry and determination of gestational age of the fetus. Unlike other pregnancy detection methods, ultrasound scanning gives the exact image of fetus, confirming the pregnancy status of the animal.

Prior to the widespread use of ultrasound scanning, the conventional technique used to study the fetal development was slaughter house specimens and its measurements, histological evaluation of fetal structures at various stages of pregnancy. In contrast, with the development of transrectal and transabdominal ultrasonographic probes opportunities were opened up to visualize fetus as well as uterine structures for examining the dynamics of fetal growth and provided a means for repeated, direct, non-invasive monitoring and measuring of fetus within the uterus. The stage of the pregnancy and age of the fetus could be estimated based on the developmental status of the conceptus by taking fetal measurements (Kahn, 1994). Ultrasonographic estimation of gestational age was practiced in various domestic animals based on the change in relation of parameters like crown rump length, biparietal diameter, trunk diameter and femur length in cattle (White et al., 1985), sheep (Metodiev et al., 2012) and goats (Haibel and Perkins, 1989; Haibel 1990; Suguna et al., 2008; Karen et al., 2009; Gonsalez et al., 2010; Amer, 2010; Abdelghafar et al., 2011; Kandiel et al., 2015).

Ultrasonographic fetal biometry constitutes an efficient tool to estimate the stage of pregnancy and the crown rump length, biparietal diameter, femur length and trunk diameter were correlated with the gestational age in Saanen goats (Abdelghaffar et al., 2011), Japanese miniature Shiba goats (Kandiel et al., 2015), and Jamnapari goats (Abubakar et al., 2016).

Ultrasonography during an optimal timeframe in the doe can improve the gestational management, and therefore the health of the fetus. Scanning throughout mid and late

gestation allows clinicians to monitor growth patterns of the offspring, identifying abnormalities in growth before parturition. Additionally, the gestational age of the fetus can be determined by the measurements of different fetal parameters. Ultrasound allowing for the estimation of parturition dates and improved late gestational management even when the mating dates are unknown. These all factors contribute to proper gestational management and reduce input costs for the producer.

Studies on pregnancy detection and estimation of gestational age in small ruminant are vital, however only few researches are conducted related to the fetal parameter measurement and estimation of fetal age in goats by using ultrasonography in Bangladesh. Thus, this study is conducted with followings objectives.

1. To determine the efficacy of ultrasonography in pregnancy detection of goats.
2. To estimate the gestational age by using ultrasonographic measurement of different fetal parameters and placentomes diameters in goats.
3. To compare the estimated gestational age with actual gestational age and make reference charts for future application in estimation of gestational age in goats.

Chapter 2

Review of literature

2.1. Historical background of ultrasound scanning in pregnancy diagnosis

Ultrasonography in human obstetrics was pioneered by Ian Donald in the 1950s (Donald et al., 1958). A-mode ultrasound provided a one-dimensional image of the uterus. Interpretation of A-mode for pregnancy detection was difficult and resulted in image artifacts, leading to the development of a 2D contact scanning machine by Ian Donald and Tom Brown that designed static, bistable (purely black and white) images (Donald et al., 1958). Although the use of ultrasonography in human medicine began with Donald's work in the 1950s, its application in livestock obstetrical management did not begin until the 1980s, with the advent of real-time B-mode (brightness mode) imaging. Since that time, the use of ultrasound has become well integrated into reproductive management of the horse (Ginther, 2014) and the cow (Quintela et al., 2012).

Ultrasound was first used for pregnancy diagnosis in sheep and goat as early 1980s (Buckrell, 1988); however, widespread uses in small ruminants has been slower due to extensive management practices. As technologies have improved, ultrasonography has become more applicable for sheep and goat management. This includes detection of fetal number and estimating of gestational age by measurement of fetal parameters during mid and late gestation. Additionally, goat have become a popular research model for pregnancy, and therefore application of ultrasound to monitor fetal growth during gestation in multiple experimental conditions is also relevant. Ultrasound has become an extremely useful tool for researchers, clinicians and producers to obtain valuable information during gestation, improve management decisions, and provide prenatal fetal growth measurements using a non-invasive approach in small ruminants.

2.2. The Principle of Ultrasound Scanning

Ultrasound is defined as any sound having frequency above the normal human hearing range greater than 20,000 Hz. Ultrasonography uses the high-frequency sound waves to produce an image. Because ultrasonography is relatively safe and noninvasive, it has become a useful diagnostic tool in veterinary medicine (Laurel, 1993). Ultrasound frequencies range from 2 to 15 MHz, although even higher frequencies may be used in some situations. The ultrasound beam produces from mechanical oscillations of numerous crystals in a transducer, which is excited by electrical pulses (piezoelectric effect). The transducer converts electrical energy to sound. The ultrasound waves are sent from the transducer, propagate through different tissues, and then return to the transducer as reflected echoes. The returned echoes are converted back into electrical impulses by the transducer crystals and are further processed to form the ultrasound image on the monitor. In ultrasound images, the reflected portion of ultrasound waves will be represented by different shades of grey extending from black to white. Body fluids such as follicular fluid and fetal fluids do not reflect the ultrasound waves and hence they are referred as non-echogenic or anechoic which appear black on screen. However, the dense and hard tissues like bone, cervix, ligaments etc., reflect most of the sound waves and hence, they are referred as hyper echogenic or hyperechoic and appear white on screen. The ultrasound waves travel with a speed of approximately 1540 m/sec through the body until it reaches the tissue reflector. In these, some of the waves are reflected and returned to the piezo electric crystals and other waves continue to interact with deeper structures of the body. The distance from the crystals to the tissue reflector is calculated by using the time delay between the propagation of waves from transducer and reception of returning echoes. There is inverse relationship between the frequency and the wavelength of a sound: the higher the frequency, the shorter the wavelength. Higher-frequency ultrasound waves create higher-resolution images, but their shorter wavelength makes them unable to penetrate deeper tissues and organs (Jainudeen and Hafez, 2000). While lower-frequency waves have better penetrating power, because of their longer wavelengths the resolution is low. When selecting a transducer frequency need for higher resolution versus more penetrating power should always be considered.

2.3. Factors affecting the accuracy rate of pregnancy diagnosis using ultrasound scanning

Accuracy rate using ultrasound scanners may varies with different materials and methods used. The age and breed of the animals and the experience of the operators are among the main factors responsible for these variable results (Buckrell, 1988; Bretzlaff and Romano, 2001).

In order to acquire the highest accuracy rate, operator's competency in interpretation of desired images is highly important. Meaningful information on image indicators during pregnancy may be difficult to obtain and scarcely reported in the literature. Thus, predicted results on images during specific time of pregnancy solely depend on operator's experiences and skills. Although specific skills and information may be rapidly acquired, it requires a constant and regular practice in order to achieve a broad-base competency with the technique and confidence in the results (King, 2006). Operators are required to have competency in differentiating the normal and abnormal anatomical structures associated with pregnancy in order to avoid any misdiagnosis.

Equipment's availability holds an important role as well. Ultrasonography have several advantages over other methods used for pregnancy diagnosis. These include an earlier time of pregnancy diagnosis, earlier determination of conceptus number, embryo or foetus viability, the estimation of embryo or foetus age, reduction of misdiagnosis, sexing and the diagnoses of abnormal pregnancies (Bretzlaff et al., 1993; Coubrough and Castell, 1998).

Kahn (1992) reported there are three periods or lengths of time for sonographic diagnosis on early pregnancies (the first 2 months of gestation). First, detection can be carried out the first 14 days of pregnancy whereby, early detection of pregnancy may be visible but diagnostic accuracy was low. This diagnosis is normally used for research purposes. Second, the succeeding period (days 14-28 of gestation) gives reliable diagnosis but needs favorable conditions. Normally, accuracy of diagnosis is low under field condition. Third, days 29-56 of pregnancy gives reliable accurate results of diagnosis even under practical field conditions and on large-scale basis. Romano and Christians (2008) prioritize on obtaining the earliest period to detect pregnant ewe and reported day 20 of gestation gave 100% accuracy.

Position of animals plays important role in acquiring high accuracy. Karen et al. (2004) performed ultrasonography examinations in Awassi x Merino ewes, gave a significant different in accuracy rate since the pregnant uterus tops over the pelvic brim and descends into the abdominal cavity at an early stage of pregnancy especially by pluriparous ewes (Buckrell, 1988; Bretzlaff et al., 1993). Thus, took the initiative to lift the abdomen wall of the ewe.

Age, breed and status of does may be a slight factor, as nulliparous gave higher accuracy than pluriparous ewes (Karen et al., 2004). Some does were deprived from feed or water before the examination (Buckrell, 1988). Fasting minimizes the presence of intestinal gases, which might obscure images of reproductive tract and consequently, increases in correct diagnosis.

2.4. Gestational length in goat

In goats, the normal length of gestation is approximately 150 days, even though it varied between the individuals and breeds. In most of the goat breeds, the gestation length ranged within 2 to 3 days on average of the species, however in Black Bengal goats the average gestational length reported was 144 days (Jainudeen and Hafez, 2000).

2.5. Method of pregnancy diagnosis goat

There are several methods available for the diagnosis of pregnancy in goats, which includes non-return to estrus, recto-abdominal palpation, vaginal biopsy, abdominal palpation (Pratt and Hopkins, 1975), estimation of Progesterone (Medan et al., 2004) , oestrone sulphate estimation (Refstal et al., 1991), Bimanual palpation technique (Kutty, 1999), radiography (Barker and Cawley,1967; West, 1986), A-mode ultrasound (Watt et al., 1984), Real time B-mode ultrasonography (Wani et al., 1998; Langfort, 2003; Kähn, 2004; Sousa et al., 2004; Johnson, 2005) and Doppler (Ott et al., 1981; Wani et al., 2003).

2.6. Ultrasonography and early pregnancy diagnosis in goat

Several authors had been reported the higher accuracy rate of ultrasound scanning for early pregnancy diagnosis in small ruminants (Martinez et al., 1998; Amer, 2010). Since past two decades mainly three types of ultrasound systems (A-mode, Real-time B-mode and Doppler) have been commonly in use for pregnancy diagnosis in sheep and goat. However, Real-time B-mode ultrasound is found to be an accurate, rapid, practical and safe tool for pregnancy diagnosis, determination of fetal numbers, estimation of gestational age and detection of fetal sex in small ruminants (Karen et al., 2001).

Martinez et al. (1998) scanned the does daily by transrectal ultrasonography using 5 MHz transducer, the average day of first detection of embryo proper with a beating heart was Day 20. But using a transrectal ultrasonography (7.5 MHz), embryonic vesicle was identified on Day 12 after mating, although the first visualization of the embryo was on day 19 (Gonzalez et al., 1988). Gonzalez et al. (1988) reported embryonic vesicles diameter were detected with 7.5 MHz linear array transrectal probe were highly correlated with gestational age from day 12 to 19. From day 19 onwards, crown–rump measurements, occipital-snout lengths, thoracic, biparietal and orbit diameters gave the highest correlation in determining ages of ewes. Medan et al. (2004) reported an accuracy of 100 percent in diagnosis of pregnancy in goats using transrectal ultrasonography (7.5 MHz transducer) and detected gestational sac, embryonic heartbeat and placentomes on 20.20 ± 0.60, 24.30 ± 0.70 and 35.40 ± 1.0 days of gestation, respectively. Padilla-Rivas et al. (2005) recommended transrectal ultrasonography for maximum reliability and considered the observation of fetal heartbeat from Day 22 onwards as the evidence of live fetus. They detected the allantoic fluid earlier from 19.5 ± 0.3 days after insemination in Boer goats using transrectal ultrasonography. Suguna et al. (2008) diagnosed early pregnancy and monitored the fetal development using transrectal (6.0 MHz transducer) ultrasound scanning. She concluded that the earliest identification of an embryonic vesicle on Day 21 by transrectal scanning and confirmation of its viability by fetal heartbeat from Day 28 onwards were the most reliable ultrasonographic finding for early pregnancy diagnosis in goats. Placentomes were detected from Day 42 onwards and skull, rib cage and vertebral column were detected from Day 56 of gestation. Karen et al. (2009) reported the average days of first detection of allantois fluid is 16.98 days but the embryo along

with fetal heartbeat were first detected on day 22.87 ± 2.66 by transrectal ultrasonography (7.0 MHz) in Egyptian native goats. Sreejith (2009) detected the embryonic vesicle on Day 19 by transrectal scanning and on Day 26 of gestation by using transabdominal scanning. He found embryo on Day 22 and fetal heartbeat on Day 24 by transrectal scanning. But on transabdominal scanning embryo was observed on Day 28 and heartbeat on Day 34 of gestation.

According to Amer (2010), the first thing that could be visualized by the transrectal probe was a small non-echogenic vesicle, about 1 cm in diameter, in the uterine lumen which could be reliably detected from Day 19.5 ± 0.3 onwards and fetal heartbeat was recognizable three and half days later, on Day 22.9 ± 0.7 after mating. He concluded that real-time ultrasonography is a convenient, reliable and only accurate method for determination of fetal numbers, sex and prediction of gestational age and kidding date under field conditions. Abubakar et al. (2016) concluded that the transrectal real-time ultrasound scanner can be considered a reliable and convenient tool for the early pregnancy detection in Jamunapari goat from Day 28-100.

2.7. Fetometry for assessing gestational age in goat

Fetal biometry means the measurement of the fetal anatomic segments by ultrasound. The following measurements are the most common: Fetal Crown Rump length (CRL), Biparietal Diameter (BPD), Femur Length (FL) and Placentome Diameter (PD). The age of the pregnancy could be estimated, and the developmental status of the conceptus could be assessed by taking the fetal measurements (Kahn, 1994). Ultrasonographic estimation of gestational age was applied to various species of animals based on the fetal development in relation to CRL, BPD, FL and PD

2.7.1. Fetal Crown Rump Length (CRL) in goat

Crown rump length is commonly used as a reliable parameter in fetometry. The measurement should be taken from the crown (most upper part of the skull) to the buttock (end of sacrum) when the fetus is fully extended (Amer 2008). In early years, the CRL had been used frequently in post-mortem ageing of fetuses in different species and hence, it was considered as one of the representative measures in relation to the day of gestation (Evans and Sack, 1973). Ultrasonographic measurement of fetal CRL was highly correlated with that of measured after dissection for prediction of gestational age

of goat fetuses (Singh et al., 2004). During early gestation, the entire fetus can be visualized on the same ultrasound screen, aiding in whole body measurements as crown rump length (CRL).

The fetal CRL measurement in goats beyond 70 days of gestation was difficult because of the length of foetus (>10 cm) which exceeded the viewing screen and penetration properties of the transducer (Karen et al., 2009; Kandiel et al., 2015). Martinez et al. (1998) measured the CRL of Anglo-Nubian goat embryos by transrectal ultrasonography (5.0 MHz) and concluded that ultrasonic measurement of fetal CRL was useful in predicting the gestational age from Days 19 to 38 post mating and was highly correlated (R^2 = 0.94) with the gestational age. Azevedo et al. (2007) reported a high correlation (R^2 =0.96) between fetal measurements and gestational age in different breeds of sheep using transrectal ultrasonography (6.0-8.0 MHz) from Day 30 to 45 of gestation. They found a higher coefficient of determination of gestational age for foetus than embryo vesicle especially regarding the fetal CRL. They also concluded that fetometry by using ultrasound and calculation with specific linear regression equations could be the most effective method for gestational aging. Karen et al. (2009) concluded that fetal CRL of Egyptian native breeds of goats was significantly and highly correlated (R^2 = 0.94) with the gestational age from Day 25 to 70. Similarly, Amer (2010) measured the CRL of dairy goat embryo using transrectal ultrasonography (6.0 MHz) from Day 40 to 89 at weekly interval and established a gestational equation viz, CRL = 0.464x−17.767 where, x is the gestational age in days. The correlations between the gestational age and CRL were found highly significant and CRL was progressively increased with the days of gestation proportional to the fetal growth in dairy goats. After Day 89 of gestation, they also reported difficulties to measure the CRL because of larger fetal size.

In Saanen goats, the measurement of CRL between 5 and 10 weeks of gestation provided a lower correlation (R^2 = 0.90) with its gestational age (Abdelghafar et al., 2011). They remarked that the fast fetal movements during ultrasound examination might be an obstacle for the accurate measurement of fetal CRL which questions the feasibility of this method in goats at mid-gestation. In Miniature Shiba goats, Kandiel et al. (2015) also reported a high (R^2= 0.9848) correlation between fetal CRL and the gestational age from first to third month of gestation and the measurements were recorded till 90 days of gestation. Karadev et al. (2015) measured uterine lumen

diameter and embryo length of local goat breeds of Bulgaria on Day 21 of gestation which were 10.3 ± 2.7 and 5.2 ± 0.9 mm, respectively. The mean fetal CRL gradually increased as pregnancy progressed, and they opined that the ultrasound measurement of fetal CRL was much easier and advantageous as compared to the measurement of other parameters for gestational age determination in local goats between 28 and 49 days of pregnancy. In Jamunapari goats, Abubakar et al. (2016) measured the fetal CRL using transrectal ultrasound probe (7.5MHz) from Day 37 to day 72 of gestation and reported that the relationship between fetal CRL and gestational age was highly correlated (R^2=0.969). Rasheed (2017) established gestational equation as follows: GA=4.712 + 0.445 CRL, Where the estimated gestational age (EGA) in weeks and CRL was in centimeter. The measurements of CRL were obtained from the greatest upper part of fetal skull to the base of tail when the fetus completely expanded. The Crown-Rump length was strongly positively correlated with gestational age (R^2=0.99).

2.7.2. Fetal Biparietal Diameter (BPD) in goat

The criteria for measuring the BPD were oval shape as possible, closed contour of the skull table, flax cerebri midline dividing hemispheres into 2 equidistant parts and measurements were taken from outer surface of the proximal calvarium to the inner surface of the distal calvarium (Amer 2008). The BPD has been widely used in the human medicine and also measured in domestic animals for the estimation of gestational age. The ultrasonic measurement of BPD was performed during the prenatal examination and estimation of gestational age in various breeds of goats. The BPD is difficult to measure after day 95 of gestation because of the increase in fetal size and the head of the fetus compressed by other fetal parts (Abdelgafar et al. 2007) and also the variability of fetal location and posture. Haibel and Perkins (1989) measured the Biparietal Diameter (BPD) on Day 46 to estimate the fetal age from the second trimester of pregnancy. They concluded that the BPD was a well-suited parameter for fetal biometry and it increased linearly during the course of gestation in ovine and caprine fetuses and also opined that there was a marked variation in fetal BPD among different goat breeds.

Reichle and Haibel (1991) measured the BPD of pygmy goat fetuses between Day 36 to 102 of gestation at two to three days interval. They reported a significantly high correlation (R^2= 0.97) between BPD and gestational age of fetuses. They concluded

that BPD was an accurate parameter for the determination of gestational age from Day 40 to 100. Similarly, Gonzalez et al. (1988) also observed a very high correlation of head diameter (HD) with gestational age (R^2= 0.96) and they opined that the HD and lengths of fetal head could be a good index of fetal development because it showed high correlations with gestational age, enabled long periods of observation from 36–38 days of gestation onwards.

There are some reports stating difficulty in measuring BPD after Day 95 of gestation because of the increase in size of fetus and compression of the head by other fetal parts and also due to the variability of fetal location and posture (Haibel et al., 1989; Abdelgafar et al., 2007). Hence a lower correlation coefficient (R^2=0.80) has been reported from Korean Black goats between Days 60 and 135 of gestation using (4.0-9.0 MHz) convex transducer (Lee et al., 2005). Suguna et al. (2008) reported that the fetal HD increased significantly between Days 56 to 130 during gestation which was highly correlated (r = 0.99) with the gestational age and concluded that fetal growth could be assessed by the measurement of HD. Karen et al. (2009) stated that fetal BPD showed high significant correlation ($P \leq 0.0001$; R^2=0.956) with the gestational age by using (3.5-5.0 MHz) transabdominal ultrasonography between Days 30 and 105 of gestation in Egyptian native breed of goat. Amer (2010) observed highly significant correlations between the gestational age and BPD in dairy goats, where it was increased with the progression of gestation. Abdelghafar et al. (2011) determined the BPD in Saanen goats from sixth week of gestation until full term pregnancy and proved that there was a strong correlation with gestational age (R^2=0.91). Metodiev et al. (2012) recorded a positive, highly significant coefficients of determination (R^2= 0.93) between BPD and gestation length in Bulgarian ewes with (5.0 MHz) transabdominal sector probe. Kandiel et al. (2015) reported that braincase diameter showed a high positive correlation (R^2= 0.98) with gestational age and it increased substantially from 10.50 ± 0.17 mm at first month to 64.00 ± 0.91 mm at the fourth month of gestation in Miniature Shiba goat fetuses.

2.7.3. Fetal Femur Length (FL) in goat

The femur length has been widely in human pregnancy to predict gestational age (Bulnadra et al., 2004; Dare et al., 2004; Isobe, 2004). However, the measurement in veterinary medicine is limited. The observation of femur became possible upon ossification which will start at the end of fourth week of gestation. Initially, femur appeared cylindrical, with spherical shaped centers of ossification at both ends in early gestation within the first 4 weeks. Gradually, the centers broaden out and brings characteristic shape of adult bone. Femur length measurement showed increase in size as gestation advances.

Diana et al. (2007) reported that the femur length showed highly significant correlation (R^2= 0.95) with gestational age in Serrana goats between 47–82 days of gestation. Karen et al. (2009) reported that Femur length showed a correlation coefficient of R^2= 0.90 between 55–125 days of gestation by using (3.5-5.0 MHz) transabdominal ultrasonography in Egyptian Balady goats which is slightly lesser than in Saanen goats (R^2=0.95) reported by Abdelghafar et al. (2011). Kandiel et al. (2015) obtained a regression line is fitted to the scatter plot from measurements of femur length schemed against gestational stage and the equation for the regression line fit for femur length growth with fetal age with a correlation factor of R^2=0.9278 in miniature Shiba goats from second to fourth month of gestation. Zongo et al. (2018) monitor the fetal growth using ultrasonic assessment of tibia (TL) and femur (FL) with known gestational stage (GS) were obtained from Twenty-one (21) gravid Sahelian goat, performed twice-weekly using ultrasound machine having 5 MHz linear transducer. The fetal ages ranged between day 30 and day120. The threshold of accurate ultrasonic femur and tibia measurement in Sahelian goat is approximately days 43, with respectively 10.83 mm and 6.2mm. The derived gestational stage prediction equations were GS = 0.66TL – 21.09, (r=0.93) and GS = 0.59FL – 21.59, (r=0.92), where GS is in day, FL and TL are in mm.

2.7.4. Placentomes Diameter (PD) length in goat

Kahn (1994) observed that placentomes are the cardinal signs of pregnancy. In caprine pregnancies, placentomes are seen as a concave circular shape, which results in C-shaped or O-shaped grey images, depending on the plane of the sections, against the anechoic fetal fluid. To obtain an accurate measurement, the mean of the diameters of the placentomes (2–5) should be used (Doize et al. 1997; Lee et al. 2005).

A period of clear visualization of placentomes was reported to be from the middle of the first trimester to the end of the second trimester (Doize et al. 1997; Suguna et al. 2008). The diameter of placentomes was also evaluated for estimating GA; however, it had a low correlation coefficient (r = 0.45, r = 0.57, r = 0.70) with GA (Doize et al. 1997; Lee et al. 2005; Nwaogu et al. 2010). Buckrell et al. (1988) also reported that placentomes could be detected by transrectally ultrasonography with a 5 MHz linear transducer at Days 28 to 30 of gestation. Haibel (1990) imaged the placentomes in cross view as cup shaped hyperechoic structures, the concave surface directed towards the uterine lumen. Doize et al. (1997) concluded that placentome size increased rapidly during the first 70 to 90 days of gestation in ewes and does. But they found that in ewes there was a poor correlation of placentome size with gestational age. They used P-mode ultrasonography with 5 MHz transducer and did transrectal procedure.

Placentomes are difficult to visualize after approximately 100 days of gestation, and the resultant measurements are not reliable for determining the stage of pregnancy at this time (Doize et al. 1997). Karen et al. (2009) reported that the measurement of only the largest placentomes in the scanning field may result in higher coefficients of determination (r = 0.86). Kandiel et al. (2015) reported a high significant correlation (R^2 = 0.8999) was found between changes in placentomes diameter and the gestation period (Table 2) that fitted a polynomial regression line demonstrated with the equation $y = -0.3835x2 + 11.799x - 47.402$

Chapter 3

Materials and Methods

This study was carried out to diagnose pregnancy and to estimate the gestational age by measuring different fetal parameters and placentomes by ultrasonography in goats. The study was also designed to compare the estimated gestational age with actual gestational age and to make reference chart to use in future to calculate gestational age by ultrasonography in Bangladeshi goats. The study was performed from July 2019 to June 2020.

3.1. Location of study

The experiments were carried out at SAQ Teaching Veterinary Hospital, Chattogram Veterinary and Animal Sciences University, Khulshi, Chattogram.

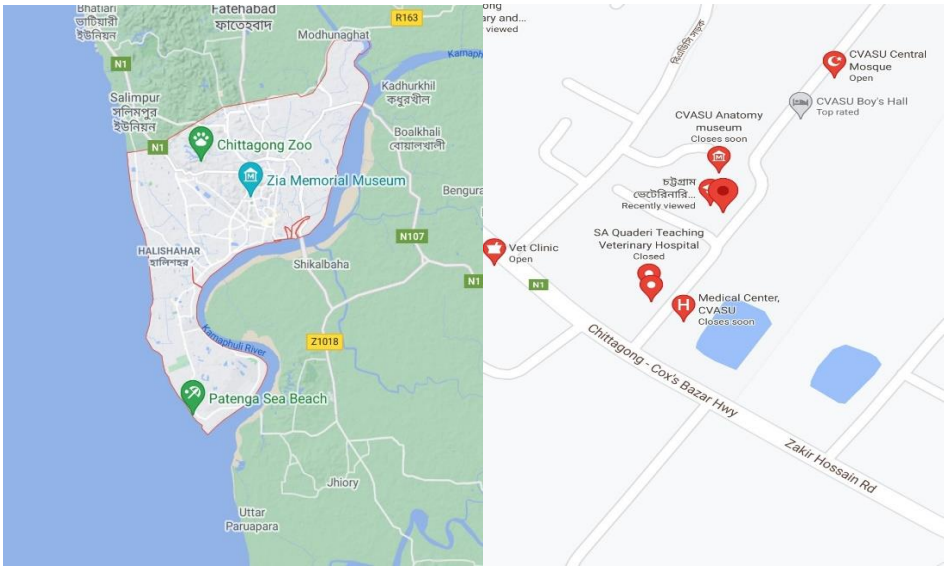


Fig. 1: Geographical location of study area

3.2. Experimental animal

All the registered outdoor female patient (does) which brought for pregnancy diagnosis in SAQ Teaching Veterinary Hospital, CVASU were considered for the study. The goats were ultrasound and only pregnancy positive animal were considered for this experiment.

3.3. Materials

Ultrasound equipment

Pregnancy detection and measurement of fetal parameters like Crown Rump Length (CRL), Biparietal Diameter (BPD), Femur Length (FL) and Placentome Diameter (PD) were conducted using real-time ultrasound scanner, equipped with a convex 3.5 to 5.0 MHz transabdominal scanner. (ExaGo Veterinary ultrasound scanner; France)

Fig. 2: Ultrasound Machine (EXAGO, transabdominal probe (3.5-5.0 MHz) and coupling reagent (Jelly)

Disposables/Miscellaneous

Contact fluid (carboxymethylcellulose gel) that reacts as a coupling agent for ultrasound transmission was used in transabdominal approach. Shaving blade was used to shave lower abdominal site before ultrasound examination (Fig. 2). Sometimes scissor was used to clip the lower abdominal hair before shaving. Shaved area was cleaned with gauge. After ultrasonography, scanned area was cleaned with tissue paper.

3.4. Experimental Design

3.4.1. Preparation of animal

All the registered does for pregnancy diagnosis with the mating date known or unknown in SAQTVH, CVASU were selected for ultrasonography scanning. A well prepared questionnaire was filled after interviewing the goat owners (Annex I). The hairs at lower abdominal wall were clipped and shaved at outdoor of SAQTVH, CVASU.

Fig.3: Preparation of goat for ultrasonography (shaving on lower abdomen)

3.4.2. Ultrasonography of goat

After clipping, shaving and cleaning the lower abdomen, patients were brought in the ultrasonography room. Does were restrained in a standing position with the help of animal attendants for scanning. Ultrasound machine and probes were properly set up. Ultrasound scanning examinations were executed using transabdominal probes. (3.5-5.0 MHz frequency). Adequate quantity of ultrasound gel was applied over the probes to eliminate the air spaces to obtain better contact of skin with the probe surface which could enhance the image quality. The transducer was moved perpendicularly towards the ventral abdominal wall until the urinary bladder was becomes visible on the monitor. Parameters on early pregnancy such as sac, non-echogenic (NE) area, amniotic fluid and fetal heartbeat were indicators of early pregnancy. The fetal structural images and other related images, such as presence of placentomes, ribs, spinal cord, limbs, head and other skeletal structures were the indicators during later stages of pregnancy.

Pregnancy positive does were scanned for measuring fetal parameters. The measurements of fetal Crown Rump Length (CRL), Biparietal Diameter (BPD), Femur Length (FL) and Placentome Diameter (PD) were measured by electronic caliper after freezing the desired image to estimate the gestational age in goat. Gestational age of pregnant does were estimated by regression formula (Annex-II). All the pregnant cases were recorded in record book with the individual owner's mobile number. The owner were requested to inform when their does delivered. In addition, all the recorded pregnant does were followed frequently to get the actual date of delivery. The study was carried on those goats whose actual date of delivery were known. Actual gestational age was calculated retrospectively by subtracting the number of days elapsing between scanning and kidding. The actual gestational age and fetal parameters (CRL, BPD, and FL) and PD were plotted as linear regression and expressed as straight line equation. The gestation age (days) was the independent (Y) and the fetal parameters being the dependent variable (X). Data of this study fitted to their optimal regression line and described by the equation as well as the coefficient of determination and correlation were calculated. Thus the formula derived from above linear regression could be use in future for estimation of fetal age.

Fig.4: Transabdominal ultrasonography in goat

3.5. Ultrasonic assessment of fetal biometry in goat

The following parameters of fetuses were imaged and recorded as fixed images using ultrasound electronic calipers (Kahn, 2004). In each animal, biometric parameters of fetus were measured three times in every ultrasound scanning for minimizing the errors.

Crown-rump-length (CRL)

The measurement was taken from the crown (most upper part of the skull) to the buttocks (end of sacrum) when the fetus was fully extended (Amer, 2008); (Fig. 5 and 6). Initially, the embryo proper was detected within the apparent conceptus (fluid filled; anechoic elongated structure observed in the uterine lumen).

Figure 5. Illustration of reference points for CRL measurement using ultrasound

Figure 6. Ultrasound image showing the measurement of CRL at different gestational age

Biparital diameter (BPD)

To visualize and measure BPD, transabdominal Ultrasonography (USG) was carried. The fetal skull was visualized in its transverse axial plane and the axis of the head symmetry was perpendicular to the ultrasound beam. The largest distance between the outer limit of two parietal bones of fetal skull is measured (Amer, 2010). The cross-sectional images obtained were used to measure BPD after freezing it on the monitor. (Fig. 7 and 8)

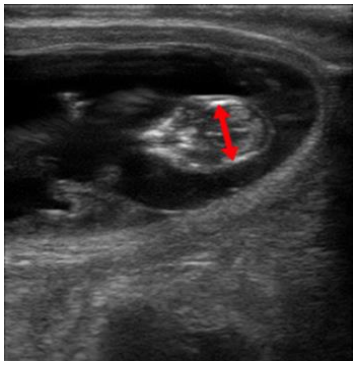


Figure 7. Illustration of reference points for BPD measurement using ultrasound

Figure 8. Ultrasound image showing the measurement of BPD at different gestational age

Femur length (FL)

The FL was measured in its longitudinal section by placing the caliper at the end of the diaphysis on both (Fig. 9 and 10) sides after visualizing hyper-echogenic femur with zones of intensive calcification which appeared near urinary bladder of the foetus (Kahn, 1992).

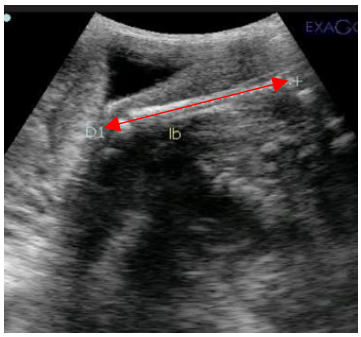


Figure 9. Illustration of reference points for FL measurement using ultrasound

Figure 10. Ultrasound image showing the measurement of FL at different gestational age

Placentomes diameter

Placentomes were observed as a concave circular shape, which results in C-shaped or O-shaped grey images, depending on the plane of the sections, against the anechoic fetal fluid. To obtain an accurate measurement, the mean diameters of 2-3 placentomes were measured as shown in Fig. 11 and 12 (Doize et al., 1997; Lee et al., 2005).

Figure 11. Illustration of reference points for PD measurements using ultrasound

Figure 12. Ultrasound image showing the measurement of PD at different gestational age

3.6. Statistical analysis of data

Independent variables	**Dependent variables**
Actual Gestational age	% pregnancy
CRL,BPD,PL and FL	Estimated Gestational Age

The data was expressed as the Mean ± SD. A paired t-test was performed to compare the estimated and actual gestational age of goat using MS excel 2010. P value was calculated at the significance level 0.05. The actual gestational age relationship with ultrasonic fetal parameters (CRL, BPD, FL and PD) were plotted as linear regression and expressed as straight line equation using MS Excel. The gestation age (days) was the independent (Y) and the fetal parameters being the dependent variable (X). Data of this study fitted to their optimal regression line and described by the equation as well as the coefficient of determination and correlation were calculated.

Chapter 4

Results

The study was conducted in goats with the objectives to diagnose pregnancy by ultrasonography and to estimate the gestational age by measuring different fetal parts (CRL, BPD and FL) and placentomes. This study was also conducted to compare the estimated gestational age with actual gestational age and to make reference chart to use in future to calculate gestational age by ultrasonography in Bangladeshi goats. The results are presented in the tables from 1 to 5 and graph 1 to 4.

4.1. Detection of pregnancy by ultrasonography in goat

Total 517 goats were scanned during study period. Out of them 170 (32.88%) were pregnant and 347(67.11%) non pregnant animals. Among 170 pregnancy positive does, 4 does were in early pregnancy, 140 were in mid gestation and 26 were in late gestation (Table 1). Accuracy of pregnancy detection was 100%.

Table 1: Detection of pregnancy by ultrasonography

<table>
<tr><td rowspan="2">No. of goats scanned by USG</td><td colspan="3">Pregnancy positive</td><td rowspan="2">Pregnancy negative</td><td rowspan="2">Accuracy</td></tr>
<tr><td colspan="3">170 (32.88%)</td></tr>
<tr><td rowspan="3">517</td><td>Early (≤50 days)</td><td>Mid (50-100 days)</td><td>Late (>100 days)</td><td rowspan="3">347(67.11%)</td><td rowspan="3">100%</td></tr>
<tr><td>4</td><td>140</td><td>26</td></tr>
</table>

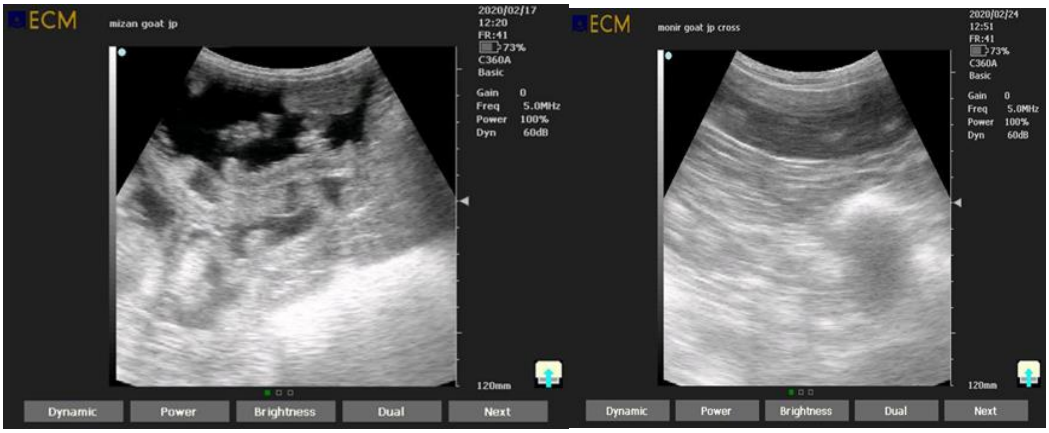


Figure 13. Ultrasound imaging showing gravid uterus (left) and non-gravid uterus (right)

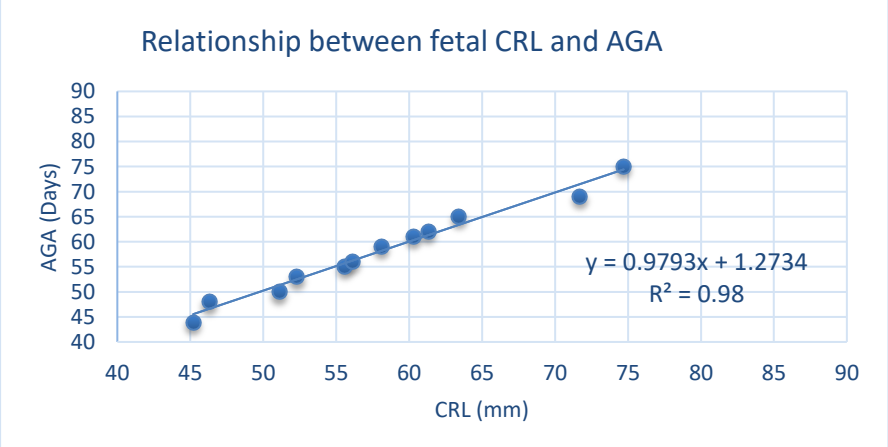
Figure 14. Ultrasound image showing fetal heart (left) and ribs and internal organs (left)

4.2. Ultrasonographic measurement of different fetal parameters and estimation of gestational age and comparison with actual gestational age

Table 2. Estimation of gestational age by measuring CRL and comparison with actual gestational age

No. of observation	CRL(mm)	EGA(Days)	AGA (Days)	P value
1	45.2	46	44	0.002
2	46.3	47	48	
3	51.1	50	50	
4	52.3	50	53	
5	55.6	52	55	
6	56.1	53	56	
7	58.1	54	59	
8	60.3	56	61	
9	61.3	56	62	
10	63.4	58	65	
11	71.7	63	69	
12	74.7	74	75	
Mean ± SD	58 ±8.67	54.91±7.33	58.08 ± 8.57	

Relationship between fetal CRL and actual gestational age in goats



Graph 1. Linear regression curve representing the relationship between fetal CRL and AGA

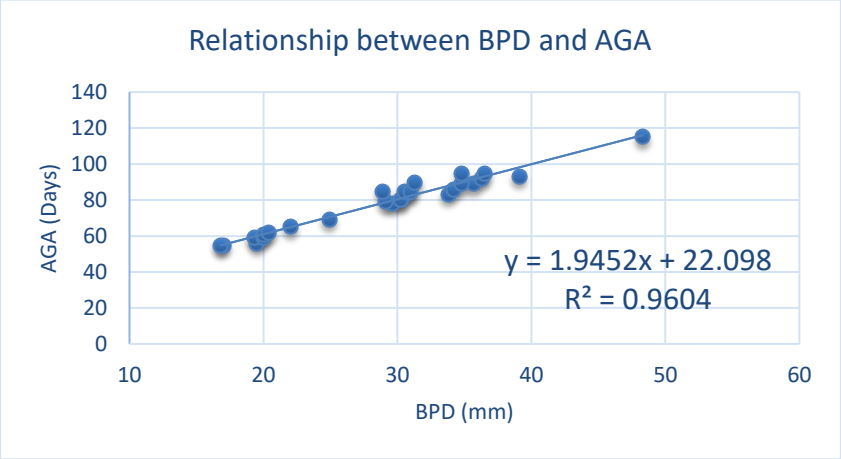
12 pregnant does were scanned and gestational age of pregnant goats were estimated by measuring CRL and results are shown in table 2. In respect to CRL of fetus the EGA and AGA was 54.91 ± 7.33 and 58.08 ± 8.57, respectively in goats. The lowest CRL of the fetus measured as 45.2mm, at that time estimated gestation age was 45 days but, the recorded actual gestational age was 44 days (Table. 2). The highest CRL of fetus was measured 74.7mm, which estimated gestation age was 74 days. According to the recorded information of delivery the actual gestational age was 75 days when the CRL was 74.7mm in goats. The result showed that it was possible to measure fetal CRL (mm) from 46 to 74 days in pregnant goats. The estimated gestational age and actual gestational age was significantly different ($P<0.05$) (table 2). There was significant correlation between the estimated and actual gestational age ($R=0.956$) with a mean difference of 3.16 days (SD 2.64, range -7 to +2 days).

The relationship between the crown rump length (x) and the gestational stage (y) was $y = 0.98x+1.27$ described by fitting linear regression equation with measured CRL and actual gestational age (Graph 1). Coefficient of determination (R^2) is found to be 0.98.

Table 3. Estimation of gestational age by measuring BPD and comparison with actual gestational age

No. of observation	BPD (mm)	EGA (Days)	AGA (Days)	P value
1	15	51	48	0.0009
2	16.8	54	50	
3	17	55	55	
4	16.8	55	55	
5	19.5	60	56	
6	19.3	61	59	
7	20	60	59	
8	20	60	61	
9	20.4	63	62	
10	22	66	65	
11	24.9	72	69	
12	29.6	82	78	
13	29.1	81	79	
14	30.3	83	80	
15	30.2	83	80	
16	33.8	91	83	
17	30.9	85	84	
18	30.5	84	85	
19	31	85	85	
20	28.9	81	85	
21	34.2	92	86	
22	35.7	95	89	
23	34.8	93	89	
24	31.3	85	90	
25	36.3	96	92	
26	39.1	102	93	
27	34.8	93	95	
28	36.5	97	95	
29	48.3	121	115	
Mean ± SD	28.17±8.02	78.82 ±17.11	76.63±16.31	

Relationship between fetal BPD and actual gestational age in goats



Graph 2: Linear regression curve representing the relationship between fetal BPD and AGA

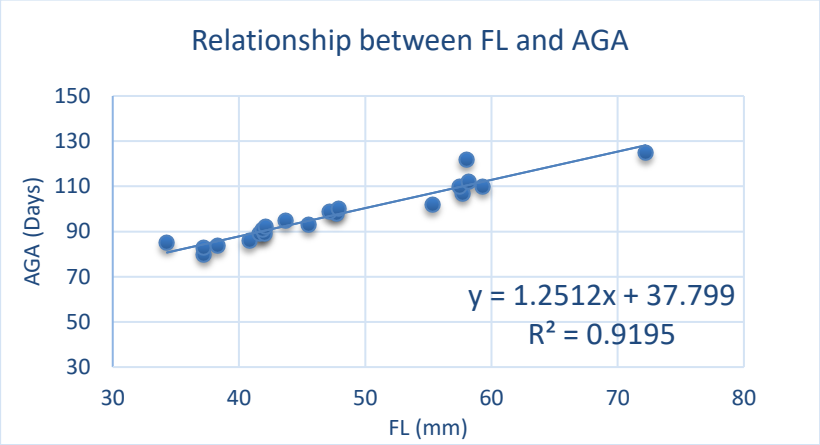
A total of 29 pregnant does were scanned and gestational age was estimated by measuring BPD of goat's fetus and results are shown in table 3. Among 29 observations, the lowest and the highest measured BPD were 15mm and 48.3mm, respectively. Estimated gestational age and actual gestational age of goats were recorded as 78.82 ± 17.11 and 76.63 ± 16.31 days. The BPD of fetus in different gestation period was calculated as 28.17±8.02 mm. The estimated gestational age and actual gestational age in goats were significantly differed ($P<0.05$). The results of this study showed that BPD (mm) of the fetus could be measured from 51 to 121 days. There was significant correlation between the estimated and actual gestational age (R= 98) with a mean difference of 2.20 days (SD 3.16, range -1 to +9 days).

The relationship between BPD (mm) and actual gestational stage in goats had a high Coefficient of determination ($R^2 = 0.96$) and plotted in a regression equation which was expressed by fitting linear regression equation $y = 1.94x + 22.10$ (Graph 2).

Table 4. Estimation of gestational age by measuring FL and comparison with actual gestational age

No. of observation	FL (mm)	EGA(Days)	AGA(Days)	P value
1	37.2	84	80	0.04
2	37.2	84	89	
3	38.3	83	88	
4	34.3	80	85	
5	40.8	88	86	
6	41.6	89	89	
7	42	89	95	
8	41.9	89	91	
9	42.1	90	97	
10	45.5	102	93	
11	43.7	92	95	
12	47.7	97	98	
13	47.2	96	93	
14	47.9	97	100	
15	55.3	106	102	
16	57.7	108	115	
17	59.3	110	119	
18	57.5	109	115	
19	58.2	110	108	
20	58	122	117	
21	72.2	127	134	
Mean ± SD	47.88 ± 9.50	97.23 ± 11.95	99.47 ± 13.39	

Relationship between fetal FL and actual gestational age in goats



Graph 3: Linear regression curve representing the relationship between FL and AGA

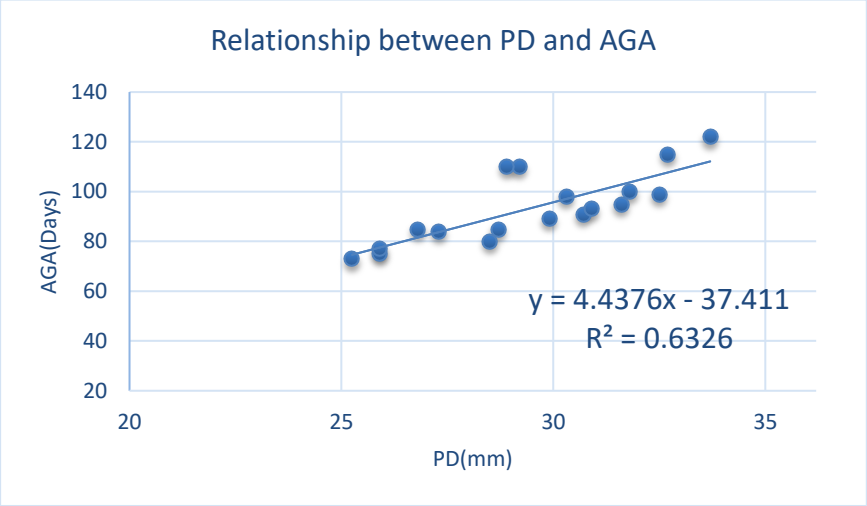
The fetal FL was measured from 21 pregnant does for the estimation of gestational age in goats and results are presented in table 4. The EGA and AGA were recorded as 97.23 ± 11.95 and 99.47 ± 13.39, respectively (Table 4). Result from above data showed that when FL was 37.2 mm in goats, the estimated gestational age was 84 days while actual gestational age was recorded 80 days. Similarly, when the highest FL 72.2 mm was measured, gestational age was estimated 127 days while actual gestational age was recorded 134 days. The actual gestational age and estimated gestational age were significantly differed with respect to FL ($P<0.05$) as shown in table 4. The FL of the fetus can be measured from days 84 to 127 of gestation in goats. There was significant correlation between the estimated and actual gestational age ($R= 0.93$) with a mean difference of 2.23 days (SD 4.61, range -9 to +9 days).

The relationship between the FL (mm) and actual gestational age (days) were expressed by fitting linear regression equation, $y=1.25x + 37.80$ with measured femur length and actual gestational age (Graph 3). Coefficient of determination R^2 is found to be 0.92.

Table 5. Estimation of gestational age by measuring PD and comparison with actual gestational age

No. of observation	PD (mm)	EGA(Days)	AGA(days)	P value
1	25.25	84	73	0.10
2	25.9	86	75	
3	25.9	85	77	
4	28.5	94	80	
5	27.3	90	84	
6	28.7	94	85	
7	26.8	88	85	
8	29.9	98	89	
9	30.7	100	91	
10	30.9	101	93	
11	31.6	102	95	
12	30.3	98	98	
13	32.5	106	99	
14	31.8	103	100	
15	29.2	95	110	
16	28.9	94	110	
17	32.7	105	115	
18	33.7	122	122	
Mean ± SD	29.47±2.46	96.94± 8.89	93.39 ± 13.72	

Relationship between PD and actual gestational age in goats



Graph 4: Linear regression curve representing the relationship between PD and AGA

Total 18 pregnant does were scanned and gestational age of pregnant goats was estimated by measuring placentome diameter (PD) and results are presented in table 5. The estimated gestational age and actual gestational age were found 96.94 ± 8.89 and 93.39 ± 13.72 days, respectively. The actual gestational age and estimated gestational age were not significantly different with respect to PD (P>0.05) as shown in table 5. There was significant correlation between the estimated and actual gestational age (R= 0.79) with a mean difference of 3.55 days (SD 8.54, range -14 to +15 days).

The relationship between PD (mm) and actual gestational age (days) was fitted to a regression line, gestational equation y=4.4329x-37.41 with measured PD and actual gestational age (Graph 4). Coefficient of determination R^2 is found to be 0.63 (Graph 4), with a coefficient of determination R^2= 0.63.

Table 6. Regression equations for determination of gestational age in goats

Fetometry parameters	Regression equations	Gestational age prediction equations	Coefficient of determination (R^2)
CRL	y= 0.98x+1.27	GA= 1.27+0.98CRL	98
BPD	y=1.94x+22.10	GA= 22.10+1.94BPD	96
FL	y=1.35x+34.67	GA= 34.67+1.35FL	92
PD	y=4.43x-37.41	GA= 4.43PD-37.41	63

y = gestation age in days, x = fetal parameters (CRL, BPD, FL) and PD in mm.

The table 6 represent that the regression equations obtained from the linear regression curve with respect to different fetal parameters and actual gestational age (recorded). The formula derived from linear regression can be used in future for estimating the gestational age in Bangladeshi goats. The actual gestational age relationship with ultrasonic fetal parameters (CRL, BPD, FL and PD) were plotted as linear regression and expressed as straight line equation. The actual gestation age (days) was the independent (Y) and the fetal parameters being the dependent variable (X). Further the regression equations were converted into the formula for the estimation of gestational age in goats.

Chapter 5

Discussion

5.1. Detection of pregnancy by ultrasonography in goat

The study, found amniotic fluid, heartbeat, different fetal organs and placentomes in pregnant positive goats during the transabdominal ultrasonography scanning. Total 517 goats were scanned and 170 were pregnant positive cases. The non-pregnant uterus of goat was found inside the pelvis in the vicinity of the apex of the urinary bladder (Fig 13). No amniotic fluid accumulation was seen inside the uterus. In the early gestational period amniotic fluid and/or fetal heart beats were observed on the monitor during ultra-scanning (<50 days). From the mid gestation (50-100 days) to advanced gestation period (>100 days) different fetal parts like rib cage, spinal cord, skull, bones were visualized. Kumar et al. (2015) explained that in goats the umbilicus can be visualized beginning on day 39 with the limbs extending from the abdomen at day 42 and skeletal structures including the rib cage, spinal cord and skull visualized from day 48 of gestation onward.

B-mode real-time ultrasonography is highly, quick and easy imaging technique used for the detection of pregnancy in goats. The accuracy of pregnancy detection in the present study was found 100%. Buckrel (l988) also described that transrectal or transabdominal ultrasonography applications can be used with a nearly 100% accuracy which supports this study.

In the present study, the CRL was able to measure from 46 to 74 days, while BPD could be measured from 51 days to 121days. Santiago-Moreno et al. (2005) also measured BPD throughout mid-gestation, as late as day 115 of gestation in small ruminants. Femur length could be measured for estimating the fetal age up to the 4^{th} month of gestation, when measurement of CRL and BPD cannot be measured easily. In both the ewe and doe, accurate measurement of placentomes using ultrasound was difficult after approximately day 90 due to the increased distention of the uterus (Doize et al., 1997). But in the present study PD was measured up to 122 days.

5.2. Estimation of gestational age by measuring CRL and comparison with actual gestational age in goat

A strong positive correlation (R= 0.956) existed between estimated gestational age and actual gestational age. The estimated and actual gestational age of pregnant goat were 54.91±7.33 and 58.08 ± 8.57 days respectively in average with respect to CRL. In the present study gestational age in goat was estimated by using equation, GA=8.776+0.606CRL that was previously published (Abubakar et al., 2016) for Jamunapari breed. The variation between estimated and actual gestational age might be due to breed specific. Faulty measurement, technician skill and inaccurate details of parturition date provided by owners might be other causes for variation between estimated and actual kidding dates. The mean difference between the estimated and actual gestational age was less than 3.16 days. Habiel and Pekins (1989) also reported that there was a range in variation of 6 days for gestational age in goats based on fetal parameters measurement by ultrasonography. It was shown that the delivery date may vary ±5days from the actual date in goats. So the equation which was used to predict gestational age can be used with sufficient accuracy to estimated GA in goats. The estimated gestational age was proved with actual (recorded) gestational age of goats.

From this study, conducted on local Bangladeshi goats in Chattogram a new regression formula was generated to estimate gestational age with higher accuracy than previous one. The relationship between the fetal CRL (x) and the actual gestational age (y) was described by linear regression equation, y= 0.978x+1.27 and the coefficient of determination (R^2) is found to be 0.98. In future, the above derived equation from this study can be used more precisely in Bangladeshi goats for estimating the gestational age with the accuracy of 98%

The results of the study showed that CRL could be considered as a good predictor of gestational age from 44- 74days. After 75 days the reliability of CRL measurement has reduced due to larger fetal size which couldn't be measured in a single focus in the monitor. Karen et al. (2009) and Kandiel et al. (2015) terminated the measurement on 70th day when the length of the fetus exceeded 10 cm due to the lower frequency transducer used by them which is in close agreement with present study.

CRL is one of the important parameters used for estimating the gestational age of ruminants and other mammals (Karen et al., 2009). Karen et al. (2009) and Abubakar

et al. (2016) obtained correlation coefficients (R^2= 0.94 and 0.969) from 25-70 days of gestation in Egyptian native goats and 37-65 days of gestation in Jamunapari goats, respectively. However, Kandiel et al. (2015) reported higher correlation (R^2=0.985) with gestational age from third to tenth week of gestation in Miniature Shiba goats which support this study. In contrast, a lower correlation (R^2=0.90) of fetal CRL with gestational age was reported by Abdelghafar et al. (2011) in Saanen goats between 5th and 10th week of gestation which might be due to breed differences in which the studies were conducted.

The regression equation GA =1.27+0.978CRL derived in the study could be used for predicting the gestational age in Bangladeshi goat. Whereas, the regression equations reported by other authors were y= 30.15+2.74x in Alpine goats (Gall et al., 1994) and y=4.712+0.445x in Saanen goats (Abdelghafar et al., 2011).

5.3. Estimation of gestational age by measuring BPD and comparison with actual gestational age

In this study, fetal BPD measured was 28.17±8.02 mm, while estimated gestational age and actual gestational age were 78.82±17.11 and 76.63±16.31days, respectively. A strong positive correlation (R= 0.98) existed between estimated gestational age and actual gestational age with respect to BPD. In the present study gestational age in goats was estimated by using equation, GA= 19.638 + 2.1BPD (Abubakar et al., 2016) for Jamunapari breed. This breed specific variation, fetal movement during ultrasonography leading to faulty measurement and inaccurate kidding dates might be reasons for the differences in two assessed gestational ages. The estimated gestational age was 2.2 days more than the actual gestational age. It was shown that the delivery date may vary ± 5days in goats, therefore above formula for estimation of GA in Jamunapari can be applied to the goats of Bangladesh. However, for higher accuracy a new formula was derived from this study by plotting the regression equation between actual gestational age (recorded) and BPD from local goats of Chattogram. The regression equation GA=22.098+1.94BPD derived in the study could be used for predicting the gestational age in goats with 96% efficacy in future.

Karen et al. (2009) reported highly significant correlation (R^2=0.956) between fetal BPD and gestational age between Days 30 and 105 of gestation which is in favors of this study. Haibel (1990) obtained a high correlation (R^2 =0.98) between fetal BPD and

gestational age from Days 39 to100 after mating in dairy goats. Similarly, Reichle and Haibel (1991) reported a high coefficient of determination (R^2=0.97) in pygmy goats between Days 36 and 102 of gestation. Haibel and Perkins (1989) reported a high correlation between fetal BPD and gestational age in Purebred Toggenburg goats (R^2=0.9949), Purebred Nubian goats (R^2=0.9875) and Angora goats (R^2=0.9775) between Day 40 and 100 of gestation. Similarly, Suguna et al. (2008) and Kandiel et al. (2015) also reported much higher correlation (R^2=0.9831) between fetal BPD and gestational age between Day 56 and 130 gestation in Indian crossbred goats and from first to fourth month of gestation in Miniature Shiba goats, respectively. In Sannen goats, Abdelghafar et al. (2011) found significant correlation (R^2=0.91) between gestational age and fetal BPD from Days 46 to 95 of gestation which was found comparably lower than the present study. In contrast, Lee et al. (2005) reported much lower correlation ($P<0.05$, R^2=0.65) between gestational age and fetal BPD in Korean black goats between Days 60 and 135 of gestation. The variation in the correlation between these studies might be due to the breed differences, time interval between scanning, extended period of scanning beyond 120 days and positioning of the transducer for measurement as well as posture of fetus.

5.4. Estimation of gestational age by measuring FL and comparison with actual gestational age

There is a high correlation (R= 0.93) existed between estimated gestational age and actual gestational age. EGA and AGA were calculated as 97.23±11.95 days and 99.47±13.39 days, respectively. In the present study gestational age in goats was estimated by using the previous published formula 0.59FL – 21.59 (Zongo et al., 2018).

This variation in between estimated and actual gestational age may be due to breed specific formula, error in measurement, size of fetus, USG machine and transducer used. The mean difference between the estimated and actual gestational age was less than 2 days. It was shown that the delivery date may vary ± 5days in goats, so the equation which was used to predict gestational age can be used with sufficient accuracy.

The regression formula GA=1.35FL+34.67 derived in the study could be used for predicting the gestational age in goat after 80 days onward. The advantage of this measurement is that clinicians can use the FL measurement in the third trimester of

pregnancy when CRL and BPD cannot be measured easily. Present study implies that FL can be used for predicting the gestational age of a goat with 91 percent reliability

In the present study, a significant and high correlation (R^2= 0.92) was observed between the fetal FL and gestational age which is almost similar to finding of Kandiel et al. (2015) who observed correlations in Serrana Egyptian native goats (R^2= 0.91) and in Miniature Shiba goats (R^2=0.9278), respectively. Abdelghafar et al. (2011) reported a highly significant correlation (R^2= 0.95; P < 0.0001) between the femur length and gestational age from Day 56 to 146 in Saanen goats. The variations in results between present and previous studies may be due to the differences in the time intervals between consecutive ultrasonographic examinations and its schedules, frequency of the transducers used in previous studies, as well as the breed differences.

5.5. Estimation of gestational age by measuring PD and comparison with actual gestational age

The fetal PD was measured 29.47±2.46 mm, while the estimated gestational and actual gestational age were recorded as 96.94 ± 8.89 and 93.39 ± 13.72 days, respectively. The difference between average EGA and AGA was 3.55 days. The mean difference between estimated and actual gestational age was 3.5 days. This variation might be explained by factors such as variations in the shape and size of the placentomes.

The relationship between PD (mm) and gestational age (days) was fitted to a regression line with a gestational equation, y= 4.4329x-37.41, with a coefficient of determination R^2= 0.63 (Table 3.). It indicates that about 63 percent of the variability in gestational age is predicted by the fetal PD up to fourth month of pregnancy. Despite of some limitations, placentome measurement remains a valuable method for estimating fetal age for goat owners. In conclusion, placentome diameter measurement could not useful in determining gestational age. Thus, it is suggested that for accurate determination of gestational age other available fetal parameters along with PD should be used.

Lee et al. 2005 and Nwaogu et al. 2010 reported correlation R^2 ranging from 0.45 and 0.57 which is closer of this findings. Doize et al. (1997) reported the correlation between gestational age and placentome diameter which was moderate in the doe (R^2 = 0.70) and it is higher than current study. In contrast to present study, placentome size was strongly correlated with gestational age between Days 25 and 130 of gestation (R^2=

0.91) (Karen et al., 2009) in Egyptian goats. The smallest placentomes were found at the tips of the horns and the largest near the junction of both horns.

From the present study it could be concluded that the CRL, BPD were highly reliable for the estimation of GA in 1st and 2nd trimester whereas during 3rd trimester FL is recommended. The gestational equations designed from actual gestational age in this study can be used to estimate the age of the fetus with high accuracy especially in Bangladeshi cross breed goat. Since in most of the cases the mating date is unknown, gestational age estimation by ultrasonography helps in drying off lactating does, and to take care of the pregnant doe by planned nutrition and management. Thus, it is suggested that accurate determination of gestational age could be increased by measurement of other available fetal parameters.

Chapter 6

Conclusions

The current study was conducted with the objectives to diagnose pregnancy and measure the fetal parameters like CRL, BPD, FL and PD by ultrasonography to estimate gestational age and comparison with actual gestational age after kidding. The study was also aimed to compare the estimated gestational age with actual gestational age and to design reference charts to calculate the gestational age in Bangladeshi goats for future use.

Pregnancy detection was confirmed by amniotic fluid and fetal heartbeat in early stage and other fetal structures (skull, ribs, femur, placentomes, etc) in later stages of pregnancy.

Fetal CRL, BPD, FL and PD were measured and gestational age of does were estimated. The fetal CRL, BPD, FL and PD were 58 ± 8.67, 28.17 ± 8.02, 47.88 ± 9.50 and 29.47 ± 2.46 mm, respectively. The estimated gestational age were 54.91 ± 7.33, 78.82 ± 17.11, 97.23 ± 11.95 and 96.94 ± 8.98 days, respectively. However, the recorded actual gestational age were 58.08 ± 8.57, 76.62 ± 16.31, 99.47 ± 13.39 and 93.38 ± 13.72 days, respectively.

The estimated and actual GA in goats were significantly differed ($P<0.05$) with respect to CRL, BPD and FL. However, in case of PD estimated and actual GA were not statistically different.

The formula generated from linear regression equation to calculate the gestational age in goats as GA=1.27+0.98CRL, GA=22.99+1.94BPD, GA= 34.67+1.35FL and GA= 4.43PD-37.41 with Coefficient of determination (R^2 = 98, 96, 91 and 63), respectively.

Chapter 7

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Appendix-I

Estimation of gestational age by ultrasonographic measurement of different fetal parameters in goats

Questionnaire Date:

Name and address of the owner.....
Patient name: ..... Phone no: .....
Reg. no: ..... Age: .....
Species..... Beed..... Body weight.....
Housing system intensive/ semi intensive / free Range feeding habits.....
Owner complains.....
History Related to Pregnancy.....
Date of last natural service/ AI .....Did you detect your goat is pregnant? Yes/ No
If yes, how? History/Non return to estrus/ Examined by doctor/ other
Method of pregnancy diagnosis
Abdominal palpation: Positive / Negative Ultrasound: Positive / Negative / Abnormalities
If abnormal, what was it: pyometra/hydrometra/mucometra/mummified fetus/others?
Gestation period during ultrasonography: Early/mid/later
No of fetus detected: ..... Status of fetus: live/dead
Structures found during scanning: sac`/heartbeat/fetus/ head/ limbs/ abdomen/ fluid/ cotyledons/ femurs/ spinal code/ ribs/ others
Parameter measured to detect EDD
GSD..... CRL.....
BPD..... PL.....
FL..... Other.....
Fetal age in days: ..... EDD in days.....
Follow up.....

Appendix-II

Formula used to calculate GA by measuring different fetal parameters

Fetal Parameter	Regression equation or Formula for gestational age (GA)	Author	Correlation Coefficient
CRL	18.776 + 0.606CRL	Abubakar et al., (2016)	r^2= 0.96
BPD	19.638 + 2.1BPD	Abubakar et al.,(2016)	r^2=0.98
PL	2.930PL+9.611	Waziri et al., (2017)	r^2= 0.90
FL	0.59FL – 21.59	Zongo et al.,(2018)	r^2= 0.92

Gestational age is in days and measurements are in mm

Biodata

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