Chapter 1: Introduction

Goats play an important socio-economic role in Bangladesh. Many stakeholders consider to increase the goat population due to their versatility in producing meat, milk, leather products, and manure and raising goats frequently enables farmers to overcome an economic crisis. According to estimates from 2017 (FAO, 2019), there were around 218 million dairy goats worldwide. Bangladesh has the second-highest population of goats among the Asiatic countries with around 26.77 million goats being raised primarily by middle-class individuals (DLS, 2021–2022). The increase in livestock productivity has recently become the main demand concomitant with the plan to declare self-sufficiency in meats. The productivity of livestock mammals depends on the success of reproduction and goat farming is becoming a profitable source in the livestock sector.

The precise prediction of pregnancy might boost goat farming's productivity. It is crucial for improving the effectiveness and management of reproduction by giving data on the gestational course among other things (Amer *et al.,* 2010) and the rates of conception following artificial insemination (Matsas *et al.,* 2007). It also aids in the appropriate handling of management practices and the dietary plan to be established in connection to the nutrients needed for optimal fetal growth in addition to the maternal requirements according to the stage of pregnancy and fetal numbers. Early pregnancy detection can provide quick feedback on mating performance, allowing for the achievement of production efficiency. This would lower the cost of the breeding program and help livestock management economically by reducing economic losses in milk and kid production. It is also helpful to increase the herd's reproductive efficiency by early eliminating females with reproductive issues early, interrupting pregnancies (Al-Merestani *et al.,* 2003).

However, there are still issues with pregnancy detection. It is hard to determine pregnancy as the majority of goats are bred naturally and are usually not documented (Doize *et al.,* 1997). Numerous methods have been used to diagnose pregnancy. Abdominal ballotment and monitoring udder development are the traditional ways to diagnose pregnancy in ruminants, although they are only reliable in late pregnancy. A number of methods including laparoscopy, uterine palpation by laparotomy (Smith, 1980), abdominal examination and palpation, hormonal assays such as progesterone (Murray and Newstead, 1988), radiography and ultrasonography (Karadaev *et al.,* 2016) have been used early to identify pregnancy in goats. The majority of these methods have not been successful because of issues including costs, poor accuracy rates, a high chance of gestational failure, impracticality, and lengthy delays in the availability of results. Ultrasonography is the most reliable, rapid and cheap method for pregnancy detection among these techniques.

Ultrasound is frequently utilized in the management of livestock operations to determine pregnancy because of its user-friendly approach, affordability, quick scanning time, and instant results (Haibel, 1990). The first reported pregnancy diagnosis in goats by using ultrasonography was in 1983. Since then, the examination of small ruminants using real-time ultrasound gives an unmatched variety of information about pregnancy status, the number of fetuses, gestational age, fetal sex, abnormalities of reproductive tract etc. (Scott, 2012). Portable ultrasonography is commonly used to detect the physiological and pathological condition of goats due to its transport friendly approach during handling. The use of trans-rectal and transabdominal ultrasonic probes allowed for the repeated, direct, noninvasive monitoring and measurement of the fetus inside the uterus as well as the opportunity to observe the fetus and uterine structures for studying the dynamics of fetal growth. These procedures can be carried out with the doe standing, sitting, or in a dorsal recumbent position without the use of sedatives (Vinoles *et al.,* 2010). Due to the tiny rectal anatomy in goats, trans-rectal scanning is more difficult but much more effective than transabdominal scanning in terms of producing more precise findings during the early stages of pregnancy (days 27–30) (Doize *et al.,* 1997; Karadaev *et al.,* 2016). In order to accurately diagnose pregnancy and calculate the number of fetuses in goats, the trans-abdominal ultrasonography technique has been adopted widely nowadays (Gonzalez *et al.,* 2004).

By measuring the fetus using ultrasound, it is possible to determine the stage of the pregnancy and the age of the fetus based on the conceptus's developmental state, which aids in optimal gestational management and lowers producer input costs (Kahn, 1994). It also measures a number of organs' average growth rates during pregnancy, including PD, BPD, TD, tibia length, etc. (Lee *et al.,* 2005). Numerous goat breeds, including Saanen goats (Abdelghafar *et al.,* 2011), Asam hill goats (Devi *et al.,* 2019), Jamunapari goats (Abubakar *et al.,* 2016) etc. have already been reported to use these techniques. However, there is limited study (Ali *et al.,* 2020) in Bangladeshi Jamunapari crossbreed goats in terms of the association between goat pregnancy age and structures relevant to pregnancy. The ability to do ultrasound exams on various fetal parts relied on the day of gestation such as the gestational sac first appeared on day 19 when the long bones appeared in the mid-gestation (Hayder and Ali, 2007). Predictive equations may be used to estimate GA and parturition dates (EPD) when breeding dates are unknown, which can then lead to the monitoring of fetal growth curves and the identification of abnormal fetal development. Variations in fetal growth allow the breeder to provide adequate nutritional management at the end of pregnancy (Santos *et al.,* 2007).

Only a small number of studies have been conducted in Bangladesh on the use of ultrasonography to detect pregnancy and estimate gestational age in goats, despite the fact that early knowledge of pregnancy status could be very advantageous to the management and breeding of goats. The application of ultrasonography is restricted to academic and research institutions as owners are open to learning about the early pregnancy diagnosis as well as the fetal number, health, and expected date of delivery date nowadays. As a result, we are driven to do a study that there is a substantial relationship between the eco-biometric characteristics of the embryo, the fetus and the goats' gestational age. This correlation will allow the development of accurate mathematical formulas for predicting gestational age. Thus, the present work was performed to estimate the gestational age and EDD using trans-abdominal USG in Bangladeshi Jamunapari crossbreed goats based on fetal growth imaging over the pregnancy period.

The study was conducted with the following objectives-

1. To estimate gestational age by measuring six different gestational parameters (GSD, GSL, PD, CRL, BPD and TD) and detect the appropriate parameter for measuring gestational age at different stages of gestation of goats.

2. To obtain eco-biometric variables, and assess mathematical equations for the prediction of gestational age by measuring six different studied gestational parameters in pregnant goats.

3. To estimate the expected days from delivery (EDD) in Bangladeshi Jamunapari crossbreed goats.

Chapter 2: Review of literature

2.1. Pregnancy diagnosis in goat

The timely repeated insemination, breeding, or culling of non-pregnant and barren does is made possible by the use of an accurate and simple approach for pregnancy testing (Amer, 2010). It is a useful tool for controlled breeding programs. The inability to identify pregnancy might lead to financial losses in milk and kid production due to extended kidding intervals. For the diagnosis of pregnancy in goats, a number of techniques are available, including non-return to estrus, recto-abdominal palpation, vaginal biopsy, abdominal palpation (Pratt and Hopkins, 1975), estimation of progesterone (Medan *et al.,* 2004), estimation of oestrone-sulfate (Refstal *et al.,* 1991), bimanual palpation technique (Kutty, 1999), radiography (Bar Barker and Cawley,1967), ultrasonography like A-mode ultrasound (Watt *et al.,* 1984), Real-time B-mode ultrasonography (Wani *et al.,* 1998) and Doppler (Ott *et al.,* 1981) etc. Most of these procedures have not proven effective due to problems including high prices, low accuracy rates, a high risk of gestational failure, impracticality, and protracted delays in the release of data. But in recent years, ultrasonography has emerged as one of the most crucial imaging methods for controlling the reproduction of small ruminants. It is the recommended diagnostic tool because it's reasonably straightforward and non-invasive procedure (Erdogan, 2012). The two primary practical uses of ultrasonography in the breeding of small ruminants are to verify that mating has successfully resulted in pregnancy and to perform accurate biometric measures known as embryo-fetal foetometry (Karen *et al.,* 2009). Available reports describe this diagnostic imaging as one of the most important elements of monitoring pregnancy and performing fetal biometric measurements to assess gestational age and thus determine the date of parturition in goats (Abdelghafar *et al.,* 2011, Amer, 2010, Padilla-Rivas *et al.,* 2005; Raja-Ili Airina *et al.,* 2011).

2.2. Development of ultrasound scanning

Ian Donald established the use of ultrasound in human obstetrics in the 1950s (Donald *et al.,* 1958). When utilized for tissue evaluation, one of the most crucial aspects of ultrasonography is operator and patient safety. Although Donald's work in the 1950s marked the beginning of the use of ultrasonography in human medicine, it was not used in the management of cattle obstetrics until the 1980s. Early in the 1980s, ultrasound was utilized for the first time to diagnose pregnancies in small ruminants (Buckrell, 1988). However, worldwide use has been delayed due to elaborate management techniques. Ultrasonography is becoming more useful for managing small ruminant's reproduction because of advancements in technology. This includes counting the number of fetuses and measuring fetal characteristics at various stages of pregnancy to determine the gestational age.

All three methods of ultrasonography—amplitude depth (A-scan), Doppler, and real-time B-scan—can be used to identify pregnancy in does. Ian Donald and Tom Brown created a 2D contact scanning machine that produced static, bistable (pure black and white) images because A-mode ultrasound only produced a one-dimensional image of the uterus and its interpretation was challenging and resulted in image artifacts (Donald *et al.,* 1958). A-scan ultrasonography applied to the flank area in goat has shown to be largely reliable from 50 to 120 days of gestation (Watt *et al.,* 1984). False positive results might result from an expanded urine bladder, hydrometra, or pyometra. This approach cannot identify prenatal viability or fetal numbers. Doppler-based ultrasound techniques (Lindahl, 1971) are able to identify multiple pregnancies with a respectable level of accuracy, although they are not always able to tell whether a doe is carrying two, three, or more fetuses. Real-time, B-scope ultrasound scanning seems to provide a reliable, quick, safe, and useful way to diagnose pregnancy and figure out how many fetuses there are. It generates a two-dimensional image on a screen that a Polaroid camera may capture. The uterus, fetal fluids, fetus, fetal heartbeat, and placentomes are all captured in a moving image. In the last 25 years, B-mode real-time ultrasonography has been effectively incorporated into the control of goat reproduction (Quintela *et al.,* 2012). For researchers, doctors, and producers, ultrasound has developed into a very important tool for obtaining crucial information during gestation, improving management choices, and providing prenatal fetal development assessments using a non-invasive technique in small ruminants.

2.3. The Principle of Ultrasound Scanning

Ultrasound is defined as any sound having a frequency above the normal human hearing range greater than 20,000 Hz. Ultrasonography uses 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 transducer is placed on the lower right flank in front of the udder of the standing ewe or doe. Hair or wool in this area should be clipped to facilitate optimal contact. A coupling agent such as commercial ultrasonic gel or vegetable oil should be applied to the transducers to eliminate air spaces between the skin and the transducer head. B-scan units emit ultrasonic waves from a hand-held transducer placed externally against the skin of the abdomen and directed toward the uterus. The ultrasound beam is produced 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 to 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 to as hyperechogenic or hyperechoic and appear white on the screen. The ultrasound waves travel with a speed of approximately 1540 m/sec through the body until they reach the tissue reflector. In these, some of the waves are reflected and returned to the piezoelectric 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 the transducer and the reception of returning echoes. There is an 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.

2.4. Factors and recommendations associated with the accuracy rate of ultrasound scanning

The accuracy rate using ultrasound scanners may vary 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). In order to acquire the highest accuracy rate, many factors are considerable like-

Skilled person: The operator's ability to interpret desired images is highly important, as expected outcomes on images taken at a certain stage of pregnancy exclusively depend on the operator's knowledge and abilities. Although specialized knowledge and abilities may be acquired quickly, it takes consistent and regular practice to become proficient with the approach and confident in the outcomes (King, 2006). To prevent any misinterpretation, operators must be able to distinguish between normal and abnormal anatomical structures related to pregnancy.

Scanning area: The positioning of the animals is crucial for obtaining great precision. Karen *et al.* (2004) performed ultrasonography in Awassi x Merino ewes, which provided a substantial difference in accuracy rate since the pregnant uterus tips over the pelvic brim and descends into the abdominal cavity at an early stage of pregnancy, especially by pluriparous ewes.

Age, breed, and status of the doe may play a little role because uniparous does gave higher accuracy than pluriparous does (Karen *et al.,* 2004).

Some recommendations (Buckrell, 1988) have been made for having the highest accuracy rate of pregnancy as- (1) Feed should be withheld for 12 hours prior to diagnosis. Fasting reduces intestinal gases, which might obstruct views of the reproductive system and, as a result, improves diagnosis. (2) Rectal scanning should be avoided unless an early diagnosis is absolutely necessary, it is preferable to utilize a 5-7.5 MHz head starting on Day 25. (3) For transabdominal scanning, the 3.5- 5 MHz head should be put high in the fleece-less groin, near to the udder, and simple to identify at 25-30 days using the 5 MHz head. (4) Any doe determined to be non-pregnant by rectal scan should also be examined high in the groin to prevent false negatives. (5) Fetal counts are performed on standing ewes or does 20 cm above the udder using a 5 MHz head from Day 40 to Day 50 and a 3 MHz head from Day 50 to Day 100. (6) A 3.5 MHz head is advised for late-term pregnancy diagnosis and fetal monitoring on the clipped ventral abdomen. (7) For the best image visibility, examinations should be conducted away from direct sunlight in soft lighting.

2.5. Application of Ultrasonography in Theriogenology

Ultrasonography has developed over the past 25 years into an important diagnostic technique for small ruminant reproductive management and research. However, due to its ease of use, reliable, and non-invasiveness, ultrasonography is still the favored diagnostic method (Gonzale *et al.,* 2010). There are three ways to use ultrasonography on goats: trans-rectal, transvaginal, and transabdominal. These procedures can be carried out with the doe standing, sitting, or in a dorsal recumbent position without the use of sedatives (Vinoles-Gil *et al.,* 2010). These approaches differ significantly in terms of the precision of diagnosis, timeliness of the test, fetal numbers, fetal age, and fetal viability. Ultrasound provides a number of advantages compared to other techniques used for reproductive issues, including pregnancy diagnosis. These include an earlier time of pregnancy diagnosis, earlier determination of conceptus number, embryo or fetus viability, the estimation of embryo or fetus age, reduction of misdiagnosis, sexing, and the diagnoses of abnormal pregnancies (Bretzlaff *et al.,* 1993; Coubrough and Castell, 1998).

Pregnancy is confirmed by imaging fluid in the uterine lumen, evidence of placentomes, or identifying one or more fetuses (White and Russell, 1984). An experienced examiner can anticipate correctly diagnosing pregnancy at 91–100% (White *et al.,* 1984, Buckrell, 1988). False positives are uncommon. This could be the result of early fetal death, an unnoticed abortion, or occasionally mistaking the bladder for the uterus. False negatives may be caused by operator inexperience or by failing to scan the tract early in gestation (White *et al.,* 1984; Haibel, 1990). Ultrasonography can also be used to determine early pregnancy (up to two months of gestation) (Kahn.1992).

Fetal movement or the beating of the fetal heart can be seen via real-time imaging to determine the viability of the fetus, and this is typically the case after Day 25. The fetal heart has an anechoic structure with echogenic walls serving as chamber division and heart valves, making it simpler to view (Raja-Ili Airina *et al.,* 2011).

Real-time ultrasound has an advantage over previous ultrasound procedures in that it is more accurate when counting the number of fetuses. Between 45 and 90 days of gestation are the best times to count the number of fetuses (White and Russell, 1984; Haibel, 1990). Fetuses are too big to distinguish one from another consistently after 90–100 days of gestation. Diagnoses for twins are more reliable than for triplets.

Real-time ultrasonics may also be used to estimate the age of a doe's fetus by monitoring several fetal metrics, such as the fetal skull's width and the gestational sac's diameter, at various gestational stages (Haibel, 1988; Juli Melia *et al.,* 2018). The sonographic appearance of fetal organs can be used to estimate gestational age. For instance, Kahn (2004) reported that the early signs of pregnancy in goats could be seen on the 12th day with the appearance of anechoic (black) embryonic fluid from the embryonic vesicle using the trans-rectal ultrasonography method. When the actual day of breeding is unknown, this method might be useful for estimating parturition date.

In addition to assessing fetal viability, numbers, and age, real-time ultrasonography can also be helpful in diagnosing diseases of the reproductive tract (Buckrell, 1988). Another advantage of real-time ultrasonic is that it can distinguish a pregnancy from hydrometra, pyometra, and fetal mummification or maceration (Haibel, 1990). The uterus appears distended with anechoic fluid in hydrometra, with hypoechoic fluid in pyometra. Also, there is no appearance of placentomes or fetus. Fetal mummification is characterized by the presence of a dense, hyperechoic image with no fluid whereas disintegrated fetal parts with fluid found in the macerated fetus.

2.6. Gestational length in goat

The normal length of gestation is approximately 150 days in Bangladeshi goats, 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).

2.7. Fetal biometry for assessing gestational age in goats

Fetal biometry means the measurement of the fetal anatomic segments by ultrasound. The age of the pregnancy could be estimated, and the developmental status of the conceptus could be assessed by fetometry (Kahn, 1994). Ultrasonic estimation of gestational age was applied to various species of animals based on the fetal development in relation to different parameters like Fetal crown-rump length (CRL), bi-parietal diameter (BPD), trunk diameter (TD), gestational sac diameter (GSD), placentome diameter (PD), the long and short axis of the heart, chest diameter, femur and tibia length etc. (Haibel *et al.,* 1989; Martinez *et al.,* 1998; Lee *et al.,* 2005; Amer, 2010; Kandiel *et al.,* 2015). These parameters are fast becoming indispensable and reliable tools in estimating the age of fetuses in exotic breeds of animals. The information regarding the estimation of gestational stage by measuring fetal parts through ultrasonography has already been reported in different goat breeds, including Toggenbubrg, Nubian and Angora (Haibel *et al.,* 1989), Egyptian native goats (Karen *et al.,* 2009), Egyptian baladi goats (Amer,2008), Saanen goats (Abdelghafar *et al.,* 2012) etc. with a very significant correlation between the parameters.

2.7.1. Gestational sac diameter (GSD) and Gestational sac length (GSL)

The presence of the gestational sac is a reliable indicator of early pregnancy. Amrozi and Setiawan (2011) claim that embryonic fluids in the uterus are an early sign of pregnancy. The gestational sac or embryonic vesicle was visible as round or elongated anechoic regions in the uterine lumen in front of the urinary bladder (Ali *et al.,* 2020). The ultrasonic cross-sectional images of the embryonic vesicle at its maximum diameter or length are used to estimate the embryonic vesicle's diameter (Martinez *et al.,* 1998). The visualizing possibility of the gestational sac depends on the position and placement of the probe. Thus it must be placed precisely where the exact location is being found. The gestational sac's extremely tiny dimensions may make it challenging to see and identify. Kahn (2004) found that the early indication of pregnancy in goats could be observed on the 12th day with the appearance of anechoic (black) embryonic fluid from the embryonic vesicle using the trans-rectal ultrasonography method in contrast to other researchers (Buckrell *et al.,* 1986, Karen *et al.,* 2009; Ali *et al.,* 2020) reported at 25 to 30 days after mating through trans-abdominal ultrasonography. According to Sayuti *et al.* (2016), the embryonic vesicle continued to grow from the 14th to the 19th day. False positive tests are uncommon and are often caused by misreading the urine bladder for the uterus, early embryonic demise, and undetected abortion (White *et al.,* 1984; Haibel, 1990). The diameter of the embryonic vesicle increased with the advancement of pregnancy and was measured as 28.21 ± 0.33 mm on day 35 after breeding (Devi *et al.,* 2019). Martinez *et al.* (1998) observed that the diameter of the embryonic vesicle increased from 4 mm on day 18 to 7 mm on day 20 after breeding in goats.

2.7.2. Placentome diameter (PD)

The measurement of placentome diameter is a key parameter for determining gestational age. According to Kahn (1994), placentomes are the telltale indicators of pregnancy. In caprine pregnancies, placentomes are seen as a concave circular shape that, hyperechoic circular shape when viewed in a longitudinal section and the regular C-shaped shape when viewed in cross-section against the anechoic fetal fluid, with the concave surface directed towards the uterine lumen (Haibel, 1990). Doize *et al.* (1997) maintain that the average value of 2 to 5 placentomes diameter is the most consistent value while Karen *et al.* (2009) contend that only the dimensions of the largest placentomes closely connected with gestational age. Placentomes are often identified by Days 26–28 (Buckrell *et al.,* 1986) and Day 42 (Karadev *et al.,* 2016) of post-breeding and appear like echogenic densities in the uterine wall by trans-rectal ultrasonography in goats. With increasing gestational age, placentomes were found more often, and between 36 and 50 days after mating, 41 to 45 days, and 46 to 50 days after mating, respectively, the percentage accuracy of pregnancy detection was 52.9, 82.4, and 100% (Ali *et al.,* 2020). The placentom displayed a development pattern that continuously increased in significance pattern from the 56th day until the placentom's growth became negligible (essentially steady) (up to 90 days of gestation) (Doize *et al.,* 1997) in does. Additionally, according to Matsas (2007), the placentome diameter in dairy goat breeds was connected to gestational stage up to day 90 of gestation. After around 100 days of gestation, placentomes become difficult to see, and the measures that follow are not accurate indicators of the stage of pregnancy at this time (Hesselink and Taverne, 1994; Doize *et al.,* 1997).

In order to calculate the gestational age of goats, the diameter of the placentomes was also assessed. Studies (Leigh & Fayemi, 2010; Roukbi, 2013; Igwebuike & Ezeasor, 2013; Kandiel *et al.,* 2015) demonstrated a high correlation (R^2 = 0.89, R^2= 0.83, R^2 = 0.97, R^2=0.94, respectively) between changes in placentomes diameter and gestation period in various goat breeds between 30-78 days of gestation. According to Karen *et al.* (2009), the coefficients of determination may be greater (R^2= 0.86) if just the biggest placentomes are measured in the scanning field. In contrast, some authors found a low correlation coefficient (R^2 = 0.45 in red Sokoto goats, R^2= 0.57 in Korean black goats, R^2 = 0.70) between 30 and 90 days of gestation (Doize *et al.,* 1997; Lee *et al.,* 2005; Nwaogu *et al.*, 2010). Kandiel *et al.* (2015) found a higher correlation with gestational age and fitted a polynomial regression line demonstrated with the equation $y = -0.3835x2 + 11.799x - 47.402$.

This implies that the placentome diameter is not a reliable indication of gestational age during the third trimester of pregnancy (Waziri *et al.,* 2017). The existence of several placentomes of varied range, independent of gestational stage, at the same ultrasound image, particularly between closer sections with umbilical connection in the uterus and uterine horn tip, is likely the cause of the change in the correlation coefficient. In addition, breeds could be an influence. Additionally, it's possible that using the biggest placentomes on the display improved the association between gestational age and placentome size.

2.7.3. Crown-rump length (CRL)

Crown-rump length (CRL) is a dependable metric that is frequently used in fetometry. It could be measured when the top of the cranium and the rump could be differentiated (Revol and Wilson, 1991). The measurement should be made from the crown (the top of the skull) to the buttocks (the end of the sacrum) when the fetus is completely expanded (Amer, 2008), or from the peak of the head to the heart, then from the heart to the base of the tail if the screen is not changed (Abdelghafar *et al.,* 2011). It is helpful for figuring out the fetal growth rate and gestational stage since it measures the fetal length while it is in a curved position that resembles the letter C. The CRL has been utilized extensively in the post-mortem aging of fetuses in different species in the early years and hence, it was considered one of the representative measures in relation to the day of gestation (Evans and Sack, 1973).

Crown-rump measurements can be performed easily at 20–50 days of gestation in small ruminants (Schrick and Inskeep 1993; Karadev, 2015; Sayuti *et al.,* 2016). However, the Anglo–Nubian goat embryo was measured by trans–rectal ultrasonography (5 MHz) at 19–38 days after mating (Martinez *et al.,* 1998). They believed that fetal CRL measurements by ultrasonography were significantly simpler and more beneficial than measurements of other parameters for determining gestational age in local goats between 28 and 49 days of pregnancy. In contrast to others, Julli Melia *et al.* (2018) demonstrated that the embryo of Etawa crossbred goat could be detected using transcutaneous ultrasonography at 20th day where the embryo size was 2.65±0.75 mm with hyperechogenic appearance, others measured 5.2±0.9 mm on day 20 of the Bulgarian local goat breed (Karadev, 2015), 6.77 mm on day 24 of the Kachang goat (Sayuti *et al.,* 2016), 6.66±0.36 mm and 8.31±1.52 mm on day 21 and 25 after breeding, respectively (Abdelghafar *et al.* (2011). According to fetal development in dairy goats, the mean fetal CRL gradually increases during the course of gestation (Karadev *et al.,* 2016). On the 35th day of pregnancy, an Etawa crossbred goat's embryo measured 10.44 ± 0.51 mm by transabdominal method (Juli Melia *et al.,* 2018).

The ultrasound measurement of fetal CRL was significantly associated with that determined after dissection for the purpose of predicting the gestational age of goat fetuses (Singh *et al.,* 2004). In the first and second trimesters of various breeds, several researches found a strong link between GA and CRL (Martinez *et al.,* 1998; Singh *et al.,* 2004; Amer, 2008). Additionally, Singh *et al.* (2004) revealed that CRL was the metric with the highest reliability ($r = 0.99$) for predicting GA in early pregnancy. Other studies (Martinez *et al.,*1998; Karen *et al.,* 2009; Kandiel *et al.,* 2015; Abubakar *et al.,*2016) also discovered a strong association between CRL and gestational age in different goat breeds ($R^2= 0.98$ in Miniature Shiba goats from the first to third month of gestation, $R^2 = 0.94$ in Anglo-Nubian goats between the ages of 19-38 days, $R^2= 0.96$ in Jamunapari goats between the ages of 37 and 72, $R^2 = 0.94$ in Egyptian native goat breeds from Day 25 to 70, respectively). The variation in the observed difference might be due to the breed of the animals, the operator's skill, and the ultrasound machine's quality.

Gestational age can be measured by applying different established formulas during fetometry. Amer (2010) measured the CRL of dairy goat embryos using trans-rectal ultrasonography (6.0 MHz) from Day 40 to 89 at weekly intervals and established a gestational equation, $CRL = 0.464x - 17.767$ where x is the gestational age in days. Rasheed (2017) also established the gestational equation as follows: $GA = 4.712 + 0.445$ CRL, Where the estimated gestational age (EGA) in weeks and CRL was in centimeters. The correlation coefficient for Alpine goat fetuses between days 33 and 146 of gestation was found to be decreased, indicating that this measurement cannot be made with accuracy as the pregnancy advances (Gall *et al.,* 1994). Due to the fetus's larger-than-10 cm size, which is incompatible with the transducer's size and penetration capabilities, it became inaccessible after the tenth week (Karen *et al.,* 2009). A further challenge to the viability of CRL measurement in goats is the rapid fetal mobility during ultrasound evaluation (Abdelghafar *et al.,* 2007).

2.7.4. Bi-parietal diameter (BPD)

Bi-parietal diameter (BPD) measurements with ultrasonography has been widely used in the human medicine and also measured in domestic animals for the estimation of gestational age (Kieler *et al.,* 1995). The ultrasonic measurement of BPD is routinely performed during the prenatal examination and estimation of GA in various breeds of goats (Haibel, 1988; Haibel *et al.,* 1989; Haibel and Perkins, 1990; Reichle and Haibel, 1991). The criteria for measuring the BPD are as follows: as oval shape as possible, closed contour of skull table, falx cerebri mid-line dividing the hemispheres into two equidistant parts and measurements taken from the outer surface of the proximal calvarium to the inner surface of the distal calvarium, is the maximum diameter of head width at the plan of the orbit (Haibel, 1988, Amer, 2008). Scanning of the head should be conducted on the transverse axial plane. Image symmetry is essential for an accurate measurement (Lee *et al.,* 2005).

Haibel and Perkins (1989) stated that BPD was a well-suited parameter for fetal biometry and it increased linearly during the course of gestation in caprine fetuses and also opined that there was a marked variation in fetal BPD among different goat breeds. The visualization and measurement of the bi-parietal diameter in local goats was possible by the day 32 to 90 (Gonzalez *et al.,* 1998) and day 40 to 109 (Hussein AA, 2010) trans-rectally and day 40 to 95 (Heibal and Parkins, 1989) trans-abdominally and considered as an accurate parameter for determination of gestational age up to 100 days (Haibel, 1988; Reichle and Haibel, 1991). However, marked variations in BPD were shown among goat's breeds from the second trimester of pregnancy (Haibel *et al.,* 1989). The mean diameter of fetal head is increased gradually with the advancement of pregnancy. Kandiel *et al.* (2015) reported that braincase diameter 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. Suguna *et al.* (2008) also reported that diameter of the head increased significantly from day 56 (1.97 ± 0.00 cm) to 98th day (6.57 ± 0.40 cm) after breeding whereas Juli Melia *et al.* (2018) found the mean diameter of 25.5±0.1 mm and 33.03±0.20 mm on day 60 and 75 after breeding.

Comparably, a number of researchers established a very strong correlation between gestational age and bi-parietal diameter during the second trimester of pregnancy. In studies conducted with different breeds of goats (Haibel *et al.,* 1989; Reichle and Haibel, 1991; Suguna *et al.,* 2008; Karen *et al.,* 2009; Kandiel *et al.,* 2015), the higher coefficient of determination found (R^2= 0.97, R^2= 0.99 , R^2=0.95, R^2 = 0.99, R^2 = 0.98, respectively) between BPD measurements at 32–90 days of gestation and determination of gestational age and they opined that the BPD 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. Therefore, Korean black goats have reported a lower coefficient (R^2=0.80) between days 60 and 135 of gestation (Lee *et al.,* 2005). Abdelghafar *et al.* (2007) affirmed that bi-parietal diameter was difficult for measuring after the 95th day of pregnancy by real time ultrasonography because of the marked growth of the fetus and the compression of the head by other fetal body parts. Also, the non-static position and posture of the fetus impede the proper scanning of the bi-parietal diameter measurement observed in different goat breeds (Toggenburg, Nubian, Angora) during the 2nd trimester of pregnancy (Haibel *et al.,* 1989).

2.7.5. Trunk diameter (TD)

TD measurements were performed by detecting the diaphragm limit (Karen *et al.,* 2009). It is the maximum diameter of the body of the fetus measured from the ventral aspect of the spinal column through the abomasum to the ventral aspect of the abdomen (Aiumlamai *et al.,* 1992). Measurement of the fetal trunk by ultrasound had an advantage through its feasibility to be measured than other fetal parameters along gestation period demonstrating that it could be the preferred measurement for determining gestational age in goats (Kuru *et al.,* 2018). In goats, gestational age reportedly could be determined by measuring embryonic and fetal TD using ultrasonography at 30–120 days (Karen *et al.,* 2009; Kandiel *et al.,* 2015) or 60–135 days (Lee *et al.,* 2005) of gestation. Karadev *et al.* (2016) attained 18.8±1.1 mm by the end of the first trimester of pregnancy.

Lee *et al.* (2005) found that the trunk diameter (TD) was more correlated (R^2 = 0.88) with the gestational age than other fetal parts such as orbit, aorta, placentome, umbilical cord and umbilical vein from Days 60 to 135 in Korean black goats. Correlation found in Saanen goats by Léga *et al.* (2007) was R^2= 0.91 whereas the correlation found in Egyptian native goats by Karen *et al.* (2009) was R^2 = 0.96, showed that the trunk was correlated well with gestational stage. Kuru *et al.* (2018) found that there was a high correlation at days 30–90 of gestation between TD and gestational age for Abaza (R^2 = 0.94) and Gurcu goats (R^2 = 0.92). Kandiel *et al.* (2015) showed the changes in the trunk diameter with the progress of gestation showed a considerable correlation with the age of goat fetuses (R^2=0.89). Clinicians can use the TD in the third trimester when CRL and BPD cannot be measured.

Chapter 3: Materials and methods

3.1. Study area and period: The study was carried out at SAQ Teaching Veterinary Hospital, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh. The study was conducted from February'21 to January'23.

3.2. Experimental animal: All of the registered female goats (N=917) that came to be checked for pregnancy were taken into consideration for the study. Only pregnancy positive Jamunapari cross breed goats (N=150) were included in this study characterized with large sized, tall, leggy with large folded pendulous ears and prominent Roman nose with different coat color. The studied does were of various ages (from 8 months to 6 years where most of them, N=104 were within 1-3 years) and of different body weights (19-60 kg of weight). Uniparous or multiparous does with BCS 2-4 (most of the does, N=110 were of BCS 3) were included in the study. All of the does were inseminated naturally. All the studied goats were reared by goat owners at their house. Some of them were reared intensively, semi-intensively where some of them were exposing extensive management system.

Fig 1: Jamunapari Cross breed goat

3.3. Materials

Ultrasound equipment: A real-time B-mode ultrasound equipped with a convex 3.5 to 5.0 MHz transabdominal transducer (ExaGo Veterinary ultrasound scanner; France) was used. A coupling reagent (ultrasound gel) was used over the probe's head during scanning to reduce the air space between the animal's skin and the transducer probe. Six parameters like crown rump length (CRL), bi-parietal diameter (BPD), trunk diameter (TD), placentome diameter (PD), gestational sac diameter (GSD) and gestational sac length (GSL) were measured to estimate gestational age in studied animals.

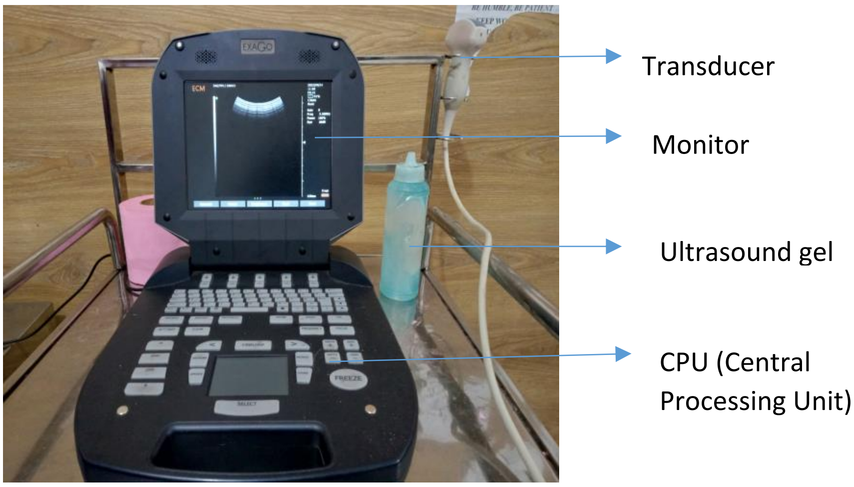


Fig 2: Ultrasound Machine (EXAGO, transabdominal probe (2.5-5.0 MHz) and coupling reagent (ultrasound gel)

Disposables/Miscellaneous

Prior to the ultrasound examination, the lower abdominal area was shaved using a shaving blade or hair was clipped where required using scissors before shaving. The shaving area was cleaned using the gauge. After completing the ultrasonography, tissue paper was used to clean the scanned area and transducer scanner.

3.4. Preparation of animal

All the registered does for pregnancy diagnosis with the known or unknown mating date at SAQTVH, CVASU were selected for ultrasonography scanning. A well-prepared questionnaire entitled with ‘Gestational age and EDD determination in goats by ultrasonic assessment’, including feeding history, BCS, insemination type and time, etc. was filled out by interviewing the goat owners (Appendix). Animals were restrained in right lateral recumbency for clipping or shaving the scanned area. The hairs of an area of 100–200 cm^2 of the coat in the inguinal region, in front and on either side of the udder up to the ventral midline of the animal were clipped with scissors and shaved with a shaving blade at the outdoor of hospital **(Fig 3)**. An antiseptic solution like savlon or alcohol using gauge was used for cleaning the shaving area and then ready for ultrasound scanning.

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

3.5. Ultrasonography of goat

Does were restrained in a standing position without sedation with the assistance of animal attendants after preparation of animal. The probes and ultrasound machine were set up properly. Transabdominal probes with a frequency of 2.5 to 5.0 MHz were used for ultrasound scanning. In order to get better skin contact with the probe surface and improve image quality, a suitable amount of ultrasonic gel was put over the probe's head to close any air pockets (Haibel, 1990). The probe was positioned longitudinally to the doe's body as scanning began from the top portion of the inguinal area and then moved forward to the abdomen **(Fig 4)**. To make the urinary bladder visible on the monitor, the transducer probe was pushed perpendicularly towards the ventral abdominal wall and softly pressed on the abdomen in the direction of the bladder. The transducer was rotated 45° to 90° in a clockwise direction and 180° in a counterclockwise direction to image the whole reproductive system and the fetus if present. When the does were being scanned, one of its hind legs may also be held up for optimal probe placement. A minimum of five minutes of scanning were spent on each animal. Images were frozen when it was shown a satisfactory level for measuring with higher resolution. During each assessment, a single or multiple reading for each biometric parameter was taken. In addition to taking measures after the examination, videos were also filmed throughout it. Real-time interpretation of shape, size changes, and organ detection was done.

Fig 4: Transabdominal scanning of goat by ultrasound machine

Sonography image consists of three parts, that is; white (hyper-echogenic), grey (hypo-echogenic), and black (an-echogenic). A doe was considered to be pregnant if there was evidence of definite, intrauterine, clearly defined anechoic round, or oval structure (Gestational sac) with hypoechoic C-shaped or round-shaped structure (cotyledon) or embryo proper (hyperechoic, elongated structure) present in the uterine lumen (Medan et al., 2004). Pregnancy-positive does were scanned for measuring fetal parameters. In order to estimate the gestational age in goats, ultrasound measurements of the following biometric parameters were made by electronic calipers: gestational sac diameter (GSD), gestational sac length (GSL) crown-rump length (CRL), trunk diameter (TD), bi-parietal diameter (BPD), placentome diameter (PD). The established regression formulae were used to estimate the gestational age of the pregnant doe. All of the pregnant does with all measurements of parameters were recorded in a book along with the owner's mobile number. All of the reported pregnant does were followed frequently over the phone to determine the precise delivery date. The research was conducted on does whose actual delivery date was known. On the basis of a 150-day gestation, the actual gestational age was determined retroactively by deducting the number of days that passed between scanning and kidding. EDD and actual delivery date, which was acquired from the owners, were compared.

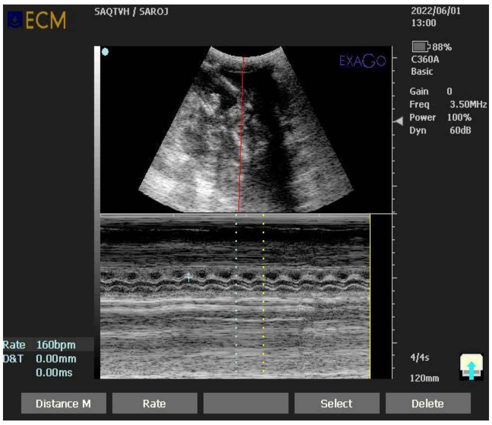
3.6. Pregnancy diagnosis of goat

During ultrasonography of goats, different structures like gestational sac, placentomes, fetal bones, fetal skulls etc. were found in the uterus that indicate pregnancy. Fluid filled anechoic gestational sac was the indicator of early pregnancy (Fig 5,b). Embryo with placentomes were observed gradually along with the gestational age. Different fetal parts (skull, bone, ribs etc.) were observed thereafter that helped to diagnose pregnancy and to estimate the gestational age (Fig 5,c). Fetal movements and heart rate were measured to identify the viability of fetus (Fig 5,d).

(a)

(b)

(c)



(d)

Fig 5: Ultrasound images of non-pregnant (a), embryonic and fetal structures (b), (c) and (d) at different gestational age

3.7. Ultrasonic assessment of fetal biometry in goat

Six parameters (GSD, GSL, PD, CRL, BPD and PD) were measured during ultrasonography in 150 pregnant Jamunapari crossbreed does for estimation of gestational age at three different gestational age (early, mid and late). Every stage of gestation was divided into different sub groups at 10 days interval. The first measurements (gestational sac) was possible to take after 20 days of gestation. The study showed that most of the measurements of gestational sac (77.77%) and CRL (58.06%) were possible to take during early gestation where placentome (81.58%), bi-parietal (97.87%) and trunk diameter (74.73%) measurements were mostly possible to take from a significant number of animals in mid gestation (Table 1). In late stage of pregnancy, TD was possible to measure from higher animals among other measured parameters. In each animal, the biometric parameters of the fetus were measured three times in every ultrasound scanning to minimize errors.

Table 1: Number of animals measuring six different parameters at three different gestation stages by ultrasound-scanning

Stage of gestation		**No of scanned animals**	**Parameters n**					
			GSD	**GSL**	**PD**	**CRL**	**BPD**	**TD**
Early	1-10 days	-	-	-	-	-	-	-
	11-20 days	-	-	-	-	-	-	-
	21-30 days	1	1	1	-	-	-	-
	31-40 days	10	10	10	-	5	-	-
	41-50 days	13	10	10	4	13	-	4
Mid	51-60 days	11	5	5	8	8	4	6
	61-70 days	19	1	1	19	2	8	12
	71-80 days	25	-	-	24	1	17	17
	81-90 days	20	-	-	19	-	9	15
	91-100 days	25	-	-	23	1	8	18
Late	101-110 days	8	-	-	7	-	1	6
	111-120 days	7	-	-	3	-	-	5
	121-130 days	6	-	-	6	-	-	4
	131-140 days	3	-	-	-	-	-	3
	141-150 days	2	-	-	1	-	-	2
Total		**150**	**27**	**27**	**114**	**31**	**47**	**91**

3.7.1. Gestational sac length (GSL) and Gestational sac diameter (GSD)

The embryonic vesicle or gestational sac was recognized as an indication of early pregnancy. It is an anechoic black area of spherical shape in the uterus with or without echogenic embryonic mass **(Fig 6)**. The length and diameter of the gestational sac was measured as the ultrasonic cross-sectional images of the embryonic vesicle at their maximal length or diameter (Martinez MF *et al.,* 1998).

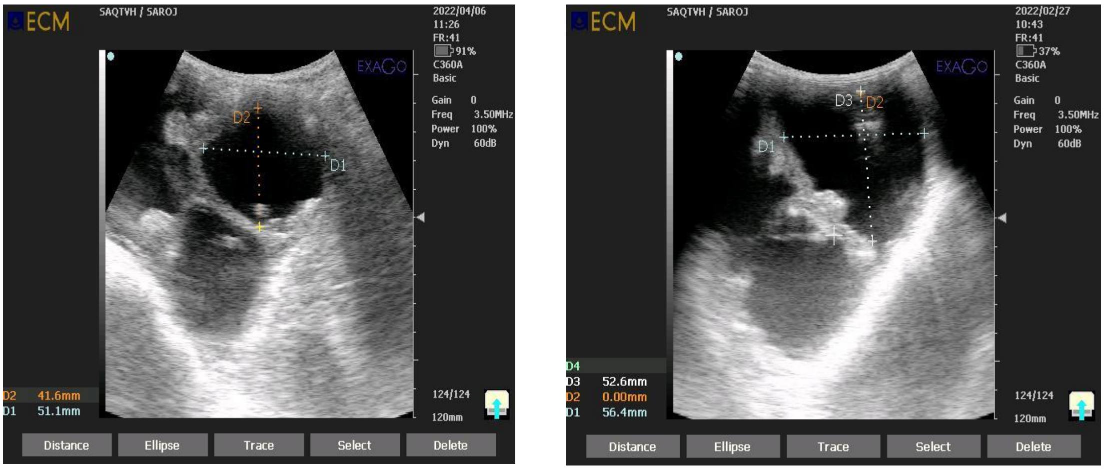


Fig 6: Ultrasound image showing the measurement of GSL and GSD at different gestational age

3.7.2. Placentome diameter (PD)

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 like longitudinal and cross-section, respectively against the anechoic fetal fluid. The broadest part of placentome is measured for obtaining the measurement **(Fig 7)**. To obtain an accurate measurement, the mean diameters of 2-3 placentomes were measured by electronic caliper (Doize *et al.,* 1997).

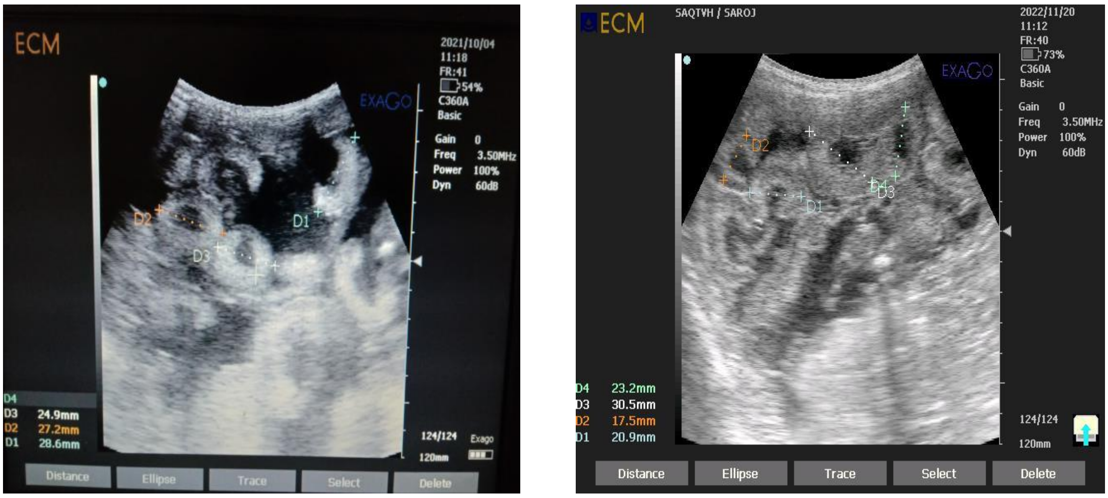


Fig 7: Ultrasound image showing the measurement of PD at different gestational age

3.7.3. Crown-rump-length (CRL)

The embryo proper was diagnosed as the hyper-echogenic elongated structure within the anechoic vesicle in millimeters using the inbuilt caliper of the machine. The crown-rump-length (CRL) measurement was taken from the crown (most upper part of the skull) to the buttocks (end of the sacrum) when the fetus was fully extended (**Fig 8**) (Amer, 2008). When the fetus was larger than the probe head length, the measurements were taken in two phases; firstly from the occipital bone to the heart area, and secondly from the heart area to the first vertebrae of the tail (Abdelghafar *et al.,* 2011).

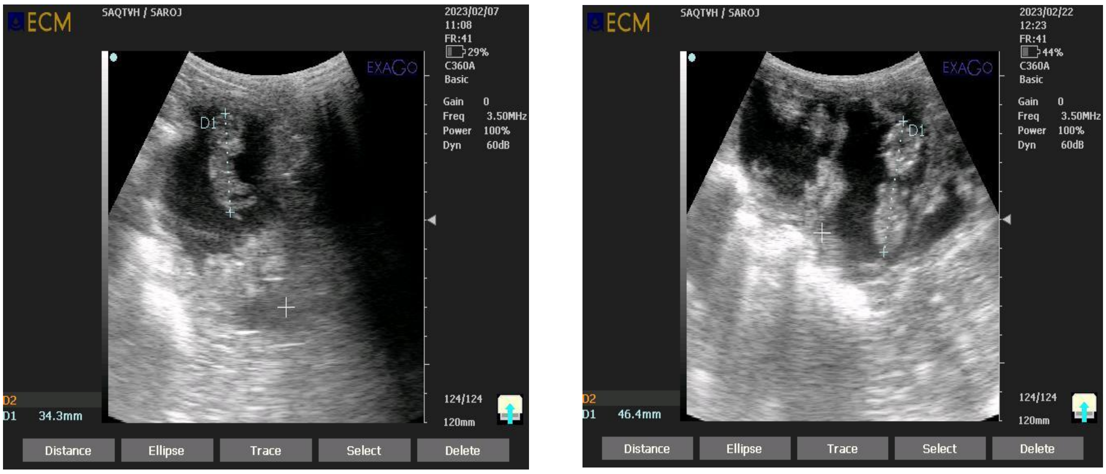


Fig 8: Ultrasound image showing the measurement of CRL at different gestational age

3.7.4. Biparietal diameter (BPD)

The diameter of the fetal head (BPD) was measured from images in which the fetal skull was visualized in its transverse axial plane and the axis of the head symmetry was perpendicular to the ultrasound beam (Lee et al., 2005). To measure BPD, the largest distance between the outer limit of two parietal bones of the fetal skull is measured (**Fig 9**) (Amer, 2010).

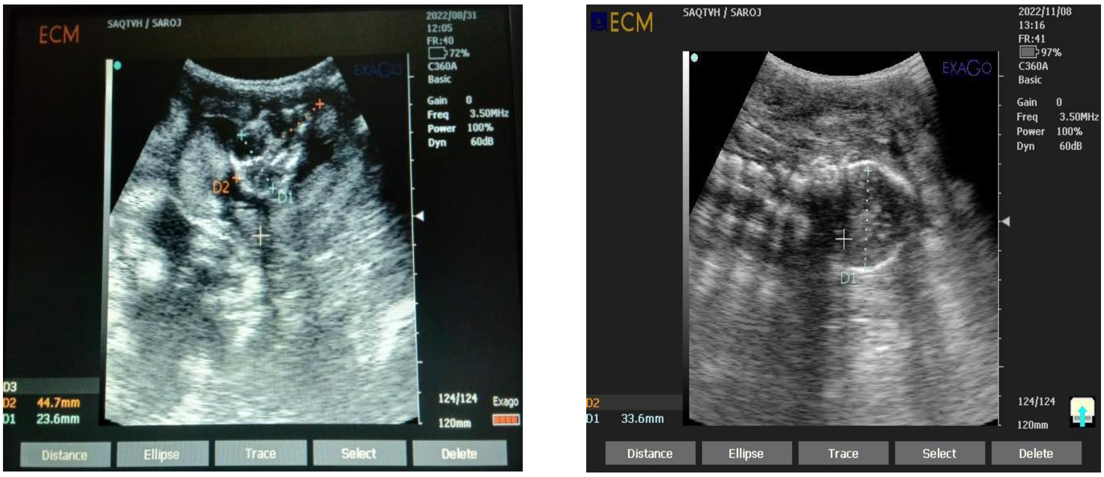


Fig 9: Ultrasound image showing the measurement of BPD at different gestational age

3.7.5. Trunk Diameter (TD)

Trunk Diameter (TD) measurements were performed by detecting the diaphragm limit. It is the maximum diameter of the body of the fetus measured from the ventral aspect of the spinal column through the abomasum to the ventral aspect of the abdomen (**Fig 10**) (Aiumlamai et al., 1992).

Fig 10: Ultrasound image showing the measurement of TD at different gestational age

3.8. Statistical analysis of data

The observation data of ultrasound of the embryo and fetal growth related to the gestational age of Bangladeshi cross breed goats were analyzed descriptively. The data were recorded, compiled and analyzed statistically in Microsoft Excel spreadsheet 16 (Microsoft office 2016). The result was expressed as the Mean ± SD (Appendix). The mean value of measurements was calculated and the gestational age based on measurements was estimated by following the findings of different authors (Appendix). The actual gestational age was calculated by subtracting the expected days from 150 (days), the gestation length of goat. The mean of estimated and actual gestational age was calculated in goats by measuring six different parameters. A paired t-test was performed to compare the estimated and actual gestational age of goats using MS excel 2016. P value was calculated at the significance level 0.05 ($p < 0.05$ was accepted as significant).

Regression co-efficient models were fitted to evaluate the relationship between the parameters (X-axis) and GA (Y-axis) when determinations were made using ultrasound scanning. The actual gestational age and fetal parameters (CRL, BPD, and TD), GSD, GSL and PD were plotted as linear regression and expressed as a straight-line equation using the gestational age formula '$y = ax + b$', where y = gestational age, a = predictors (constant), x = measured value, and b = dependent variable. The gestation age (days) was the dependent (Y) and the fetal parameters being the independent 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 the above linear regression could be used in future to estimate fetal age in Bangladeshi Jamunapari crossbreed goats.

Chapter 4: Results

4.1. Inventory of animals by ultrasound scanning

A total 917 does were scanned during the study period who came for pregnancy diagnosis at SAQTVH, CVASU. Out of them, 348 (37.95%) does were pregnant and 569 (62.05%) does were non-pregnant. Among 348 pregnancy-positive does, 150 animals were included in this study as measurements were not possible to take appropriately from all pregnancy positive animals due to stage of gestation, image clearance etc. Animals were divided into three different gestational ages, early gestation (<50 days of gestation), mid gestation (51-100 days of gestation) and late gestation (101-150 days of gestation). Among the studied animals, 24 (16%) does were in early gestation, 100 (66.67%) does were in mid-gestation and 26 (17.33%) does were in late gestation **(Table 2)**.

Table 2: Assign the animals using ultrasonic scanning

No. of goats scanned by USG	Pregnancy positive (%)			Pregnancy negative (%)	Accuracy (%)
917	348 (37.95%)			569 (62.05%)	100
	150 animals were selected based on study objectives				
	Early (<50 days)	Mid (51-100 days)	Late (101-150 days)		
	24 (16%)	100 (66.67%)	26(17.33%)		

4.2. Estimation of gestational age by ultrasound scanning and comparing the resultant total to the actual gestational age

Different parameters (GSD, GSL, PD, CRL, BPD and TD) related to pregnancy were measured by ultrasound scanning for estimation of the gestational age in studied goats. Among 150 animals, PD was possible to measure from the highest number of animals (76%) whereas gestational sac measurements were possible to take from the lowest number (18%) of animals. A significant difference ($P<0.05$) was found between the estimated and actual gestational age in most of the parameters (GSD, GSL, PD, and CRL) except BPD and TD which showed no significant variation ($P>0.05$) in studied population **(Table 3)**.

Table 3: Estimation and comparison of gestational age by measuring different parameters in goats-

Parameters	Number of scanned does	Measurements (mm) (Mean ± SD)	EGA (days) (Mean ± SD)	AGA (days) (Mean ± SD)	P-value
GSD	27	35.43 ± 9.76	38.96 ± 5.68	44 ± 9.16	0.0003
GSL	27	48.16 ± 18.34	38.96 ± 5.68	44 ± 9.16	0.0003
PD	114	23.71 ± 4.90	79.18 ± 14.67	83.04 ±18.71	0.02
CRL	31	44.86 ± 19.87	47.55 ± 9.73	51.16 ±11.87	0.02
BPD	47	27.18 ± 6.19	75.28 ± 12.18	78.32 ±12.15	**0.22**
TD	91	41.57 ± 15.58	88.60± 21.38	87.05 ± 22	**0.63**

EGA= Estimated gestational age, AGA= Actual gestational age

4.3. Comparative evaluation of estimated and actual gestational age among three different gestational stage

The estimated and actual gestational age was compared at three different gestational stages (early, mid and late) based on the measurements of six parameters to detect the appropriate parameters for measuring gestational age in three different stages of gestation **(Table 4)**. The study showed that BPD at early stage and GSD, GSL, CRL at late stage of pregnancy was unable to measure where all parameters was possible to measure at mid stage of gestation. The findings of the study showed no significant variation (P>0.05) of GSD, GSL and CRL measurements at early stage, PD and BPD at mid gestational stage and TD at late gestational stage of pregnancy while other studied parameters were significantly varied (P<0.05) at different gestational stage.

Table 4: Comparative study of gestational age between estimated and actual gestational age in goats among three different gestational stages using ultrasonography scanning

Stages of gestation	**Gestational age (days) (Mean± SD)**	**GSD**	**GSL**	**PD**	**CRL**	**BPD**	**TD**
Early	EGA	37.57±5.35	37.57±5.35	55.5±4.79	42.88±7.19	-	51.50±3.11
	AGA	41.375±7.01					
	P-value	**0.057**	**0.057**	0.004	**0.499**	-	0.001
Mid	EGA	43.83±4.07	43.83±4.07	79.37±13.60	54±9.26	75.04±12.20	83.25±15.01
	AGA	78.39±12.83					
	P-value	-	-	**0.605**	-	**0.133**	0.03
Late	EGA	-	-	83.70±17.03	-	86	113.95±17.70
	AGA	118.07±12.10					
	P-value	-	-	-	-	-	**0.378**

EGA= estimated gestational age, AGA=actual gestational age

4.4. Relationship of different gestational parameters with actual gestational age

4.4.1. Relationship between GSD and AGA

27 does of all gestational age were considered for estimation of gestational age by measuring GSD. The lowest and highest measurement of GSD was 14.6 mm and 54.4 mm at day 22 and day 55 of gestation, respectively. The measurements of GSD (mm) and actual gestational age (AGA) were fitted into linear regression and found a correlation between the gestational sac diameter (x) and the gestational age (y) was **$y = 0.7157x + 18.534$ (Fig 11)**. The coefficient of determination was found **$R^2 = 0.75$** in the present study indicates that the acquired formula can be applied accurately in 75% cases.

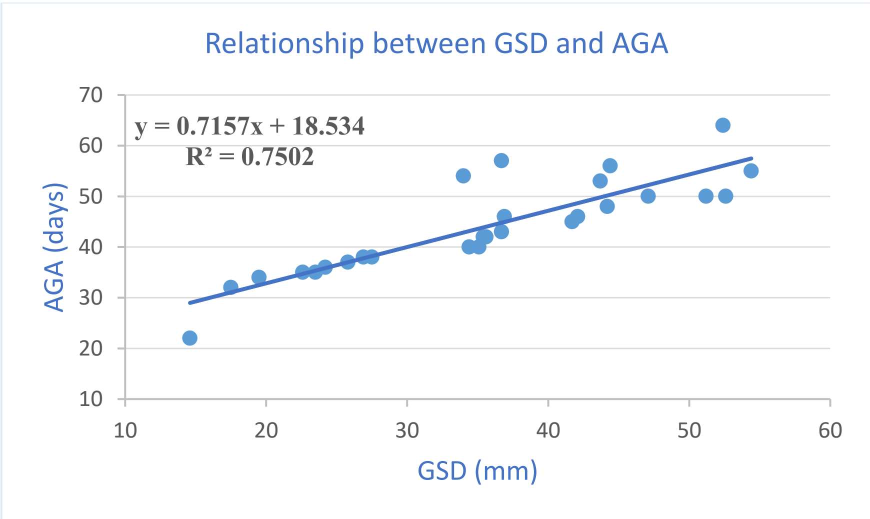


Fig 11: Linear regression curve representing the relationship between GSD and AGA

The estimated and actual gestational age based on GSD measurements were not significantly varied (P>0.05) at early stage of gestation while it was significantly differed at the total studied population. A total of 21 animals were included for estimation of gestational age by measuring GSD at early stage of gestation. A correlation of **$y = 0.6053x + 20.234$** was discovered between the actual gestational age (y) and the gestational sac diameter (x) when put into linear regression with a high coefficient of determination (**$R^2 = 0.92$**) **(Fig 12)**. There was also found a strong correlation (**$r=0.96$**) between the estimated and actual gestational age at early gestational age (<50 days of gestation).

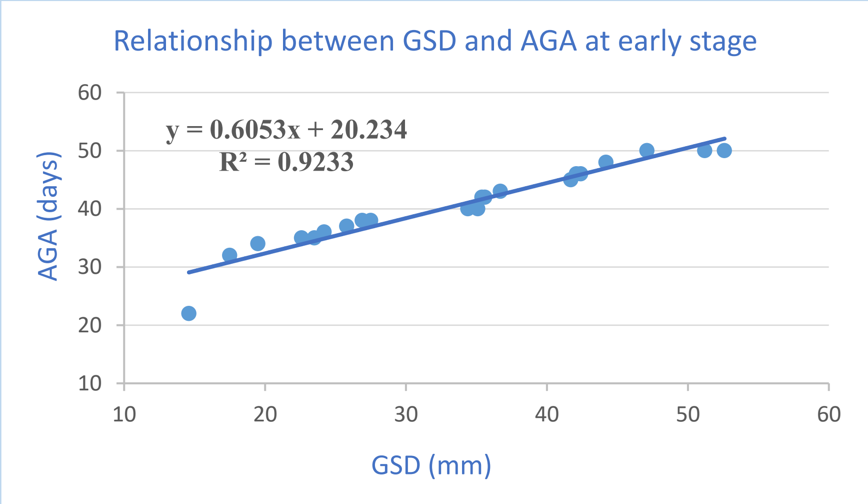


Fig 12: Linear regression curve representing the relationship between GSD and AGA at early stage of gestation

4.4.2. Relationship between GSL and AGA

Among the studied animals, GSL measurements were taken from 27 pregnant does for estimation of gestational age. The lowest and highest measurement of GSL was 23.4 mm and 95.3 mm at 22 days and 64 days respectively. The measurements of GSL at different stages of gestation and AGA were fitted into linear regression and found an equation to measure the gestational age in goats. The relationship between the gestational sac length (x) and the gestational stage (y) was described **y = 0.45x + 25.885**. The coefficient of determination was found to be **R^2=0.72 (Fig 13)**.

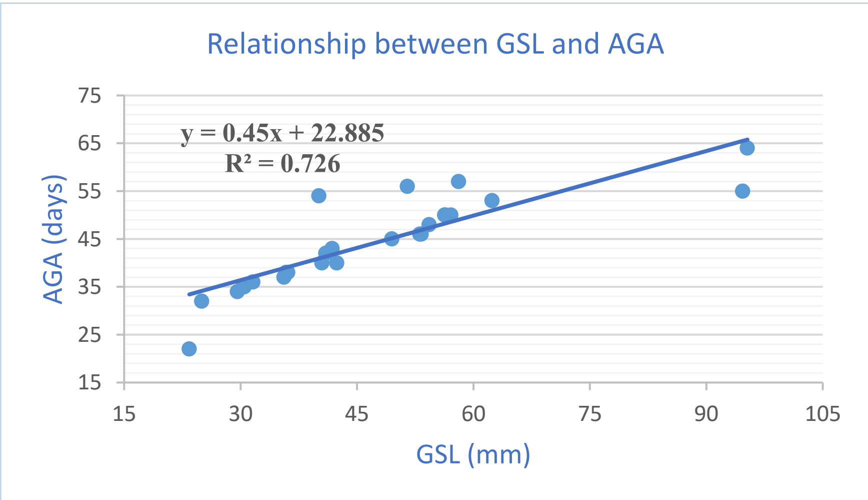


Fig 13: Linear regression curve representing the relationship between GSL and AGA

In early gestational period, there was no significant difference (P>0.05) between the estimated and actual gestational ages based on measures of the gestational sac length. 21 animals were enrolled in order to estimate gestational age by assessing GSL at early stage of gestation. When the measures of actual gestational age (AGA) and GSL (mm) were placed into linear regression, a correlation of **y = 0.6161x + 15.118** was found at early sage of gestation with a high coefficient of determination **(R^2 =0.91) (Fig 14)**. Additionally, at early gestational age (<50 days of gestation), there was a significant correlation (**r=0.95**) between the estimated and actual gestational age.

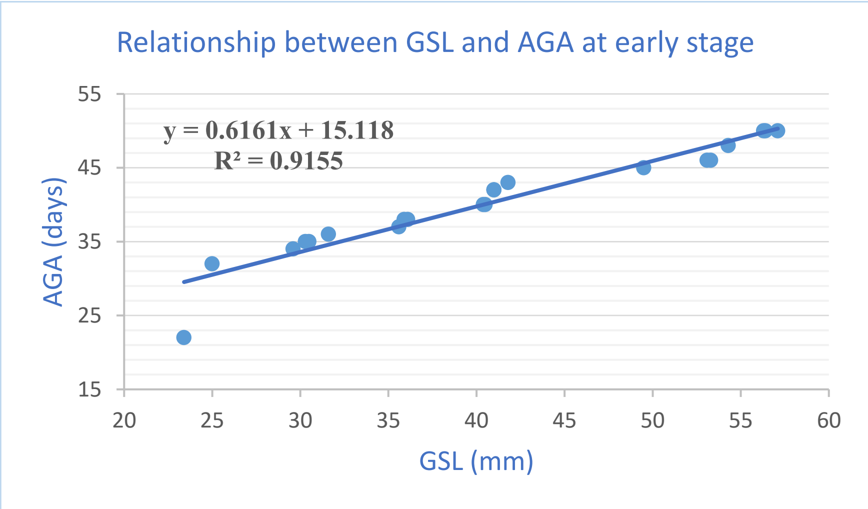


Fig 14: Linear regression curve representing the relationship between GSL and AGA at early gestational stage

4.4.3. Relationship between PD and AGA

A total of 114 pregnant does were scanned among the studied goats and gestational age was estimated by measuring placentome diameter (PD). The first detectable measurements of placentome was 13.45 mm at 48 days and the highest measurement was 38 mm at 122 days where at 19.3 mm found at 145 days in a doe in the present study. Most of the measurements were taken from 45-75 days of pregnancy as a growing pattern but measurements were decreased gradually after that period. There was a low correlation between the estimated and actual gestational age at total population **(r= 0.44)**. The relationship between PD (mm) and actual gestational age (days) was fitted to a regression line, gestational equation **y=1.6991x + 42.743** with measured PD(mm) in horizontal axis and actual gestational age(AGA) in vertical axis with a low coefficient of determination **R^2= 0.19** (**Fig 15**).

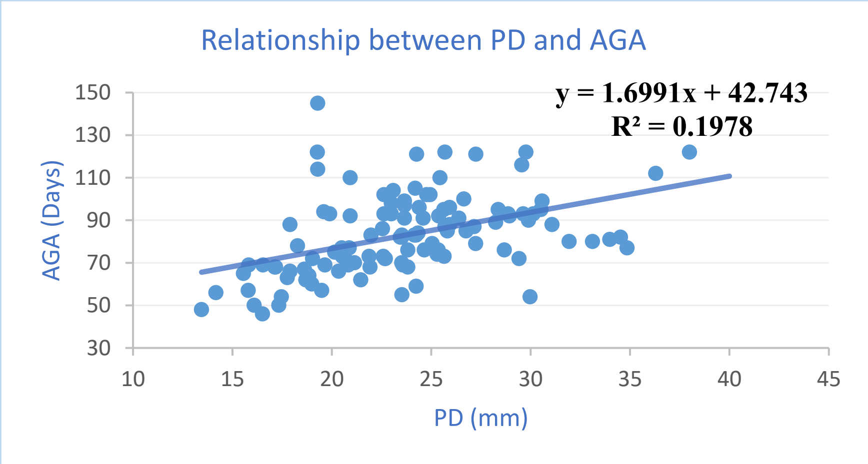


Fig 15: Linear regression curve representing the relationship between PD and AGA

The estimated and actual gestational age based on placentome diameter measurements were not significantly varied (P>0.05) at mid stage of gestation where it was significantly differed at the studied population. A correlation of **y = 2.1104x + 27.613** was discovered for mid stage of gestation between the gestational age (y) and the placentome diameter (x) when put into linear regression with a coefficient of determination **(R^2 =0.80)** (**Fig 16**). There was also found a strong correlation **(r=0.89)** between the estimated and actual gestational age at mid gestational age (<50 days of gestation).

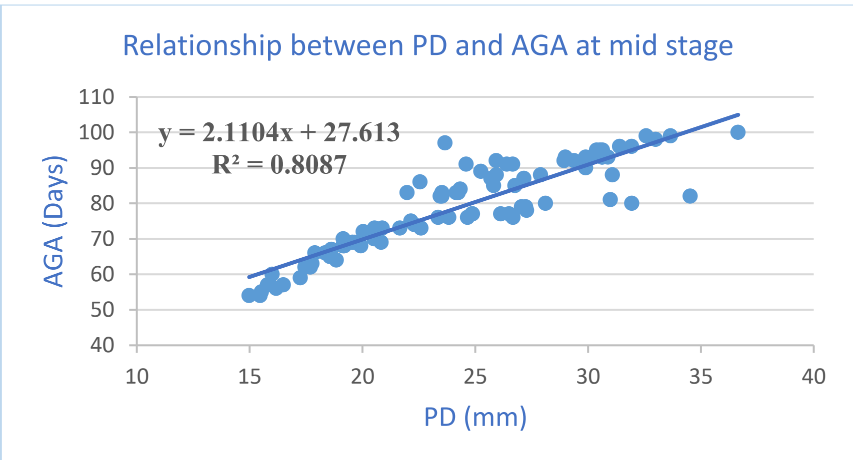


Fig 16: Linear regression curve representing the relationship between PD and AGA at mid stage of gestation

4.4.4. Relationship between CRL and AGA

Among the pregnant does, 31 does were scanned for crown rump length measurements for estimation of gestational age. CRL was first measured at 34 days (20.1 mm) and highest measurement was taken at 93 days (65 mm) although most of the measurements were taken within 30-60 days of pregnancy. There was a great correlation between the estimated and actual gestational age (**r=0.72**). The relationship between the crown rump length (x) and the gestational stage (y) was **y = 0.4976x + 28.106** described by fitting linear regression equation with measured CRL (mm) and actual gestational age where coefficient of determination (**R^2 =0.57**) was found in the present study (**Fig 17**).

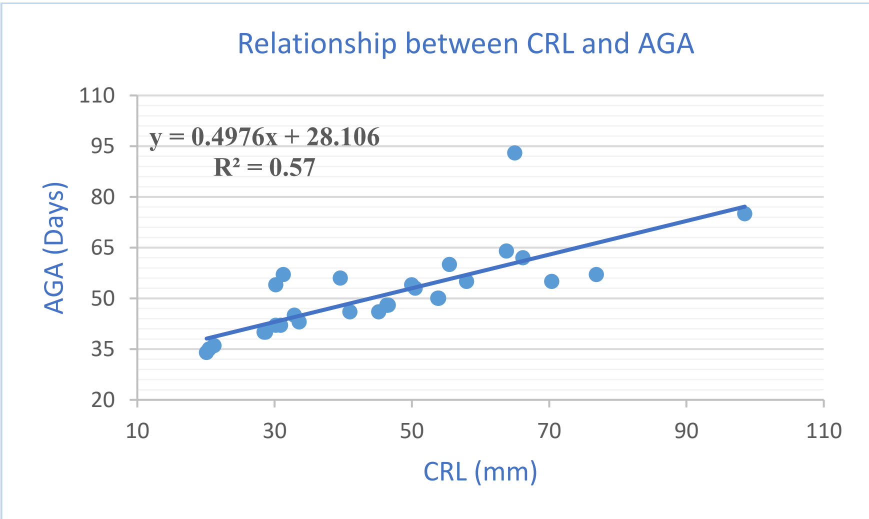


Fig 17: Linear regression curve representing the relationship between CRL and AGA

In early gestational period (<50 days of gestation), there was no significant difference (P>0.05) between the estimated and actual gestational ages based on measures of the crown rump length measurements although there was a significant difference at whole studied animals. 12 animals were considered in order to estimate gestational age by assessing CRL at early gestation. When the measures of actual gestational age (AGA) and CRL (mm) were placed into linear regression, a correlation of **y = 0.4335x + 27.559** was found between the gestational age (y) and the gestational sac diameter (x) with a high coefficient of determination (**R2 =0.94**) (**Fig 18**). Additionally, there was a significant correlation (**r=0.97**) between the estimated and actual gestational age at early gestational age.

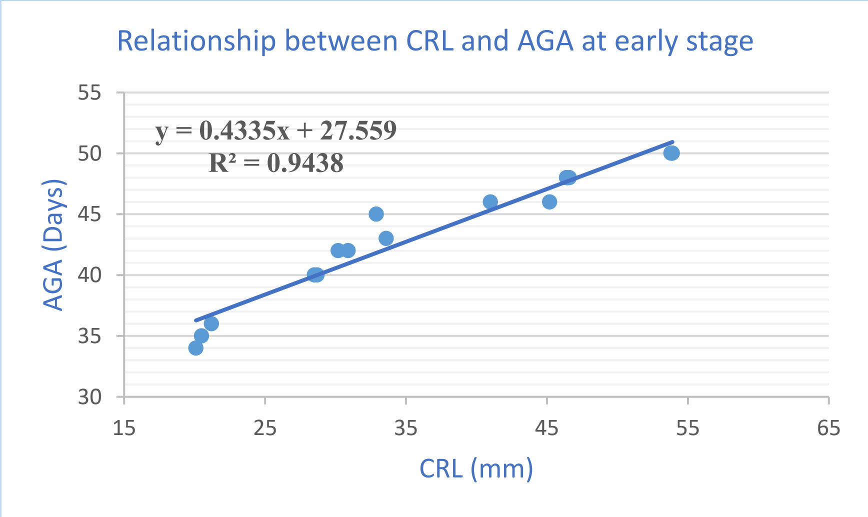


Fig 18: Linear regression curve representing the relationship between CRL and AGA at early stage of gestation

4.4.5. Relationship between BPD and AGA

47 pregnant does were scanned and gestational age was estimated by measuring BPD of goat's fetus at mid stage of gestation as 46 animals are included in this stage. The BPD was first measured at 55 days (21.1 mm) and lately measured at 109 days (32.6 mm). The results of this study showed that BPD (mm) of the fetus could be measured from 55 to 109 days. The estimated gestational age and actual gestational age in goats were not significantly differed (P>0.05) (**Table 4**). There was a high correlation found between the estimated and actual gestational age (**r= 0.72**).

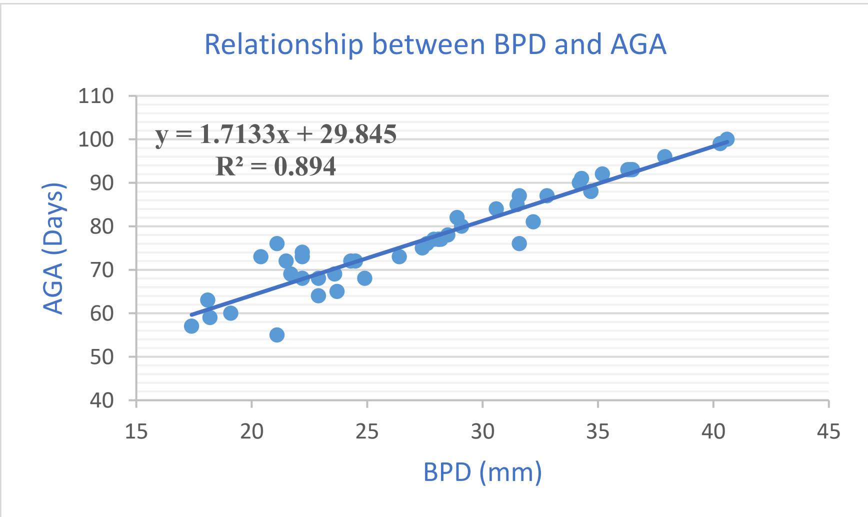


Fig 19: Linear regression curve representing the relationship between BPD and AGA

The relationship between BPD (mm) and actual gestational stage in all stages of gestation of goats were plotted in a linear regression which was expressed by an equation **y = 1.7133x + 29.845** with measured BPD(mm) in horizontal axis and actual gestational age(AGA) in vertical axis with a coefficient of determination **R^2= 0.89** (**Fig 19**).

4.4.6. Relationship between TD and AGA

Among the studied goats, 91 pregnant does were considered for measuring TD for estimation of gestational age. Among observations, the lowest and the highest measured TD were 11.4 mm at 46 days and 93.9 mm at 145 days, respectively. The results of this study showed that BPD (mm) of the fetus could be measured mainly from 45 to 120 days of pregnancy. It was difficult to measure after 4 month of pregnancy in this study as fetus was not covered in the monitor of ultrasonography machine. There was no significant differentiation (P>0.05) between the estimated and actual gestational age in goats (Table 4). There was also found a significant correlation between the estimated and actual gestational age (**r= 0.92**). The relationship between TD (mm) at different gestational age and actual gestational age in goats plotted in a linear regression which was expressed by an equation **y = 1.3062x + 32.756** with a high coefficient of determination **R^2= 0.85** (**Fig 20**).

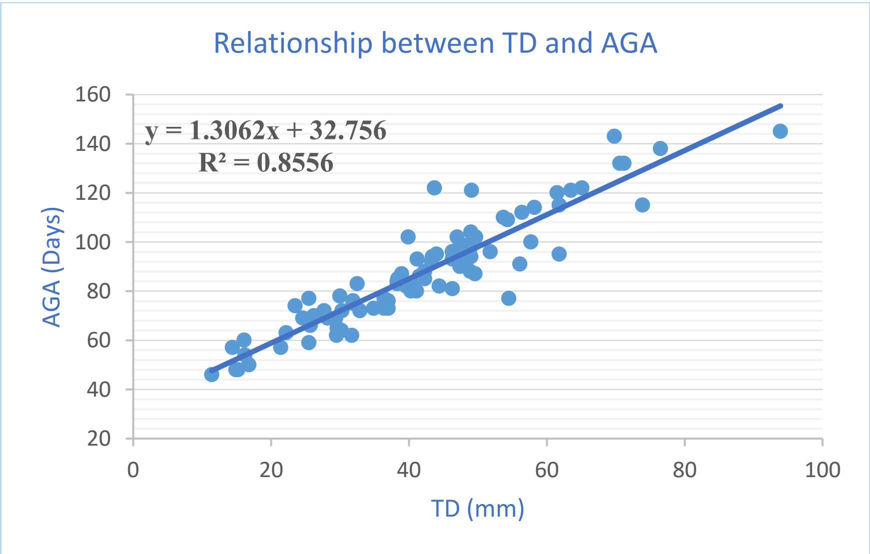


Fig 20: Linear regression curve representing the relationship between TD and AGA

The estimated and actual gestational age based on trunk diameter measurements were not also significantly varied (P>0.05) at late stage among three stage of gestation. A correlation of **y = 1.0401x + 54.161** was discovered for last stage of pregnancy between the gestational age (y) and the trunk diameter (x) when the measurements of PD (mm) and actual gestational age (AGA) were put into linear regression with a strong coefficient of determination (**R^2 =0.96**) (**Fig 21**). There was also found a strong correlation (**r=0.98**) between the estimated and actual gestational age.

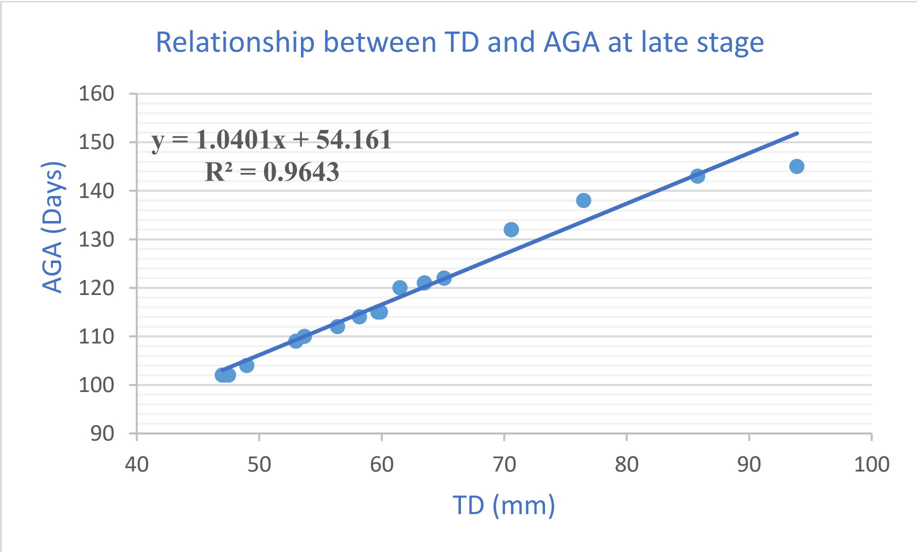


Fig 21: Linear regression curve representing the relationship between TD and AGA at late stage of gestation

Table 5. Gestational age prediction equation in Jamunapari crossbreed goats in Bangladesh

Parameters	Gestational age prediction equation	Coefficient of determination (R^2)
GSD	GA= 0.7157 × GSD (mm) + 18.534 (any stage)	0.75
	GA= 0.6053 × GSD (mm) + 20.234 (early stage)	**0.92**
GSL	GA= 0.45 × GSL (mm) + 22.885 (any stage)	0.72
	GA= 0.6161 × GSL (mm) + 15.118 (early stage)	**0.91**
PD	GA= 1.699 × PD (mm) + 42.743 (any stage)	0.19
	GA= 2.1104 × PD (mm) + 27.613 (mid stage)	**0.80**
CRL	GA= 0.4976 × CRL (mm) + 28.106 (any stage)	0.57
	GA= 0.4335 × CRL (mm) + 27.559 (early stage)	**0.94**
BPD	GA= 1.713 × BPD (mm) + 29.845 (any stage)	0.89
TD	GA= 1.3062 × TD (mm) + 32.756 (any stage)	0.85
	GA= 1.0401 × TD (mm) + 54.161 (late stage)	**0.96**

GA= gestational age

The actual gestational age relationship with ultrasonic fetal parameters (CRL, BPD, TD, GSD, GSL, and PD) was plotted as linear regression and expressed as a straight-line equation. GSD, GSL, CRL at early stage, PD at mid stage and TD at late stage of gestation had a higher correlation with actual gestational age which expressed as different equations where BPD expressed as a same equation at all stages of pregnancy. **Table 5** represents the gestational age prediction equations with coefficient of determination (R^2) obtained from the linear regression curve with respect to different fetal parameters and actual gestational age (recorded).

4.5. Determination of expected days from delivery (EDD)

Expected days from delivery (EDD) was estimated by measuring different parameters through ultrasonography at three different gestational age in goats. EDD (days) was determined by subtracting the EGA from 150 as the gestation period of goat is considered 150 days. The expected days from delivery (EDD) based on finally estimated gestational age and actual days from delivery (ADD) was found 72.22 ± 24.15 and 70.71 ± 25.14 days, respectively (**Table 6**). The study showed that there was no significant difference (P>0.05) between the EDD and ADD of the studied does, means the EDD was statistically same as the ADD of does. There was also found a significant relationship (**r= 0.92**) between estimated and actual days from delivery in the present study (Table 6).

Table 6: Comparison of expected days from delivery (EDD) and actual days from delivery (ADD) in goats:

No of observation	**EDD (days) Mean ± SD**	**ADD (days) Mean ± SD**	**P value**	**Correlation coefficient (r)**
150	72.22 ± 24.15	70.71 ± 25.14	0.06	0.92

EDD= expected days from delivery, ADD= actual days from delivery

As the gestation period of goat is considered 145-155 days in normal ranges, the EGA ± 1-5 days was also calculated in this study to evaluate the significant relationship between estimated and actual gestational age. The present study showed that estimated days from delivery can be varied from ±1-5 days based on the different measured parameters. There was no significant different (P>0.05) between the estimated and actual gestational age when added 1-5 days with EGA in case of all parameters. Contrariwise, GSD GSL, CRL, PD and BPD significantly varied (P<0.05) but TD showed no significant difference (P>0.05) between the estimated and actual gestational age while subtracted 1-5 days from EGA in goats (**Table 7**). So it was found that EDD can be varied ±4 days in case of EGA based on TD measurements, +1-5 days varied in EGA based on GSD, GSL, PD, CRL and BPD.

Table 7: Comparative evaluation of estimated (EGA ± 5 days) and actual gestation age in goats

EGA ± 5 (days)	GSD/GSL	PD	CRL	BPD	TD
EGA	38.96 ± 5.68	79.18 ± 14.67	47.54 ± 9.73	75.28 ± 12.18	88.6 ± 21.38
AGA	44 ± 9.16	83.04 ± 18.71	51.16 ± 11.86	78.32 ±12.15	87.05 ± 22
EGA + 1	39.96 ± 5.68	80.18 ± 14.67	48.54 ± 9.73	76.28 ± 12.18	89.6 ± 21.38
P-value	0.002	**0.20**	**0.09**	**0.41**	**0.49**
EGA+2	40.96 ± 5.68	81.18 ± 14.67	49.54 ± 9.73	77.28 ± 12.18	90.6 ± 21.38
P-value	0.01	**0.40**	**0.28**	**0.67**	**0.27**
EGA+3	41.96 ± 5.68	82.18 ± 14.67	50.54 ± 9.73	78.28 ± 12.18	91.6 ± 21.38
P-value	**0.33**	**0.70**	**0.68**	**0.98**	**0.15**
EGA+4	42.96 ± 5.68	83.18 ± 14.67	51.54 ± 9.73	79.28 ± 12.18	92.6 ± 21.38
P-value	**0.62**	**0.94**	**0.79**	**0.70**	**0.08**
EGA+5	43.96 ± 5.68	84.18 ± 14.67	52.54 ± 9.73	80.28 ± 12.18	93.6 ± 21.38
P-value	**0.98**	**0.60**	**0.62**	**0.43**	0.04
EGA-1	37.96 ± 5.68	78.18 ± 14.67	46.54 ± 9.73	74.28 ± 12.18	87.6 ± 21.38
P-value	0.005	0.03	0.004	**0.11**	**0.86**
EGA-2	36.96 ± 5.68	77.18 ± 14.67	45.54 ± 9.73	73.28 ± 12.18	86.6 ± 21.38
P-value	0.001	0.005	0.0007	0.04	**0.88**
EGA-3	35.96 ± 5.68	76.18 ± 14.67	44.54 ± 9.73	72.28 ± 12.18	85.6 ± 21.38
P-value	0.0003	**-**	**-**	0.01	**0.65**
EGA-4	34.96 ± 5.68	75.18 ± 14.67	43.54 ± 9.73	71.28 ± 12.18	84.6 ± 21.38
P-value	**-**	**-**	**-**	0.02	**0.09**
EGA-5	33.96 ± 5.68	74.18 ± 14.67	42.54 ± 9.73	70.28 ± 12.18	83.6 ± 21.38
P-value	**-**	**-**	**-**	0.002	0.04

EGA=estimated gestational age, AGA=actual gestational age

Expected days from delivery (EDD) = 150 – estimated gestational age (EGA based on TD) ± 4 days

Expected days from delivery (EDD) = 150 – estimated gestational age (EGA based on GSD, GSL, CRL, PD and BPD) + 5 days

Chapter 5: Discussion

5.1. Pregnancy detection by ultrasound scanning

In gestational ages of does, amniotic fluid and/or fetal heart beats were observed on the monitor during ultra-scanning in the early gestational period (<50 days) (Kahn, 2004). From the mid gestation (50-100 days) to advanced gestation period (>100 days) placentomes and different fetal parts like rib cage, spinal cord, skull, bones were visualized (Hayder and Ali, 2007). 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. The accuracy of pregnancy detection in the present study was found 100%. Buckrel (1988) also described that trans-rectal or transabdominal ultrasonography applications can be used with a nearly 100% accuracy which supports this study.

5.2. Estimation of gestational age by measuring different parameters through ultrasonography and comparison with actual gestational age in goat

Different gestational parts like GSD, GSL, PD, CRL, BPD, and TD was measured for estimation of gestational age in pregnant does form a different number of animals.

GSD and GSL was able to measure from 22 to 64 days of pregnancy (mainly at the 1st trimester of pregnancy) from most of the does which is similar to the findings of Ali *et al.* (2020) when other structures are difficult to measure. The value of EGA and AGA was found 38.96 ± 5.68 and 44 ± 9.16 days, respectively. The mean difference between the estimated and actual gestational age was less than 5.04 days which was almost close to the findings of Karadev *et al.* (2016) who reported a range in variation of 4.9 days for gestational age in goats. The variation between estimated and actual gestational age might be due to body size of does, faulty measurement, and inaccurate details of parturition date provided by owners etc.

In case of PD measurements, the value of EGA and AGA was found 79.18 ± 14.67 and 83.04 ± 18.71 days, respectively which showed a significant variation ($P<0.05$). This variation might be explained by factors such as variations in the shape and size of the placentomes as the smallest placentomes were found at the tips of the horns and the largest near the junction of both horns. It may also be the cause of considering the placentomes measurements during the whole gestation period. Because the placentomes displayed a development pattern that continuously increased in significance pattern from the 56th day until the placentom's growth became negligible (essentially steady) (up to 90 days of gestation) (Doize *et al.,* 1997; Matsas, 2007)) in does. After around 100 days of gestation, placentomes become difficult to see, and the measures that follow are not accurate indicators of the stage of pregnancy at this time due to its reduced size (Hesselink and Taverne,1994; Doize *et al.,* 1997). In the present study, there was no significant difference found (P= 0.486 > 0.05) between the estimated and actual gestational age up to 2nd trimester (up to 100 days) of pregnancy. This implies that the placentome diameter is not a reliable indication of gestational age during the third trimester of pregnancy which is in agreement with other findings (Waziri *et al.,* 2017).

The CRL was able to measure from 34 to 75 days in this study. The average value of EGA and AGA was found 47.55 ± 9.73 and 51.16 ± 11.87 days, respectively and the difference was less than 3.62 days which was lower than the findings of Habiel and Pekins (1989) who reported a range in variation of 6 days for gestational age in goats. It may be due to the fast fetal movement during ultrasound examination represented as an obstacle for the feasibility of CRL measuring in goats (Abdelghafar *et al.,* 2007).

BPD was possible to take from 51 days to 121days in this study. Santiago-Moreno *et al*. (2005) also measured BPD throughout mid-gestation, as late as day 115 of gestation in small ruminants. The mean diameter of fetal head was increased gradually with the advancement of pregnancy which was in the agreement of Suguna *et al.* (2008). Trunk diameter was measured for estimating the fetal age mainly at 2nd trimester of gestation, when measurement of CRL and BPD cannot be measured easily (Kuru *et al.,* 2018).

5.3. Comparative evaluation of estimated and actual gestational age among three different gestational stages

During evaluation of gestational stages of pregnancy, all the parameters (GSD, GSL, PD, CRL, and TD) except BPD were able to measure at early stage of gestation where showed no significant difference (P>0.05) between the estimated and actual gestational age with respect to GSD, GSL and CRL. Researchers (Karadev *et al.,*2016; Devi *et al.,*2019) also found GSD and GSL as reliable parameter in early stage of pregnancy which is similar to the findings of present study. Singh *et al.* (2004) also revealed that CRL was the metric with the highest reliability (**r = 0.99**) than other parameters for predicting GA in early stage of pregnancy. In mid stage of gestation, all parameters were able to measure but PD and BPD were measured from the highest number of animals and showed no significant difference (P>0.05) between the estimated and actual gestational age. The finding was in agreement with the statement of Matsas (2007) who stated that the placentome diameter in does was significantly connected to gestational stage but in contrast to Lee *et al.* (2005) who found low correlation in second stage of gestation. Some researchers (Haibel, 1988; Reichle and Haibel, 1991) also considered BPD as a reliable parameter for determination of gestational age at mid stage of gestation which is similar to the findings of this study. PD and TD were able to measure only among six studied parameters at late stage gestation where TD can be considered as a reliable parameter in third stage of pregnancy as showed no significant variation (P>0.05) between the estimated and actual gestation age. Lee *et al.* (2005) also found a significant relationship which had an agreement with the present study which in contrast to Kuru *et al.* (2018) who stated a great relationship of TD at mid stage of gestation.

5.4. Relationship of different measurable parameters with actual gestational age

5.4.1. Gestational sac (GSD and GSL)

The presence of anechoic areas cranial to the bladder was considered as positive for pregnancy that was also described by Ali *et al.* (2020). In the present study, GSD and GSL were detected as early as 22th day (14.6 mm) after breeding by transabdominal method which is in agreement with other researchers (Padilla-Rivas *et al.,* 2005) who found at 24^{th} day after mating where Kahn (2004) found at 12^{th} day after mating. The diameter and length of embryonic vesicle as observed in the present study increased with the advancement of pregnancy and was measured as 52.4 mm and 95.3 mm on day 64 after breeding (Graph 1,3) which was in agreement with the observation of Martinez *et al.* (1998) who observed that the diameter of the embryonic vesicle increased along with gestation. From this study, conducted on Bangladeshi jamunapari crossbreed goats, new regression formulae (**y = 0.708x + 18.916** and **y = 0.3803x + 25.684** with respect to GSD and GSL, where x was the measurements of GSD and GSL, y was the actual gestational age) were generated to estimate the gestational age. The coefficient of determination (R^2) was found to be **0.75** and **0.72,** respectively. There was a great relationship found in this study between the estimated and actual gestational age in respect to GSD and GSL at early stage of gestation (Table 4). So new regression formulae (**y y= 0.6053x + 20.234 and y= 0.6161x + 15.118** were generated to estimate the gestational age at early gestational stage with a high coefficient **R^2 = 0.92 and R^2 = 0.91** respectively. In future, the above derived equations from this study can be used more precisely in Bangladeshi goats for estimating the gestational age.

5.4.2. Placentome diameter (PD)

The placentome was first able to measure at day 46 after breeding in the present study which is almost similar to the findings of Karadev *et al.* (2016) who reported day 42 of post breeding and Ali *et al.* (2020) found 100% accuracy at 46-50 days of post breeding but identified as early as day 26 by Buckrell *et al.* (1986). In the current study, the placentomes progressively increased in diameter until the 3rd month of gestation and slowly grew thereafter which was similar to the statements of Kandiel *et al.* (2015). The relationship between PD (mm) and gestational age (days) was fitted to a regression line with a gestational equation, **y = 1.6991x + 42.743**, with a low coefficient of determination R^2= 0.19 which was close to the findings of Lee *et al.* (2005) (R^2= 0.45). Also found R^2= 0.30 during consideration of the value up to the 2nd trimester (up to 100 days) of pregnancy where found a higher degree of determination **R^2= 0.80** at mid stage of gestation with a regression equation **y = 2.1104x + 27.613** having almost similar to the statements of Doize *et al.* (1997) and Karen *et al.* (2009), reported higher correlation between gestational age and placentome diameter (R^2 = 0.70 and R^2= 0.91, respectively). In conclusion, placentome diameter measurement could not useful in determining gestational age throughout the whole gestational period but it could be a reliable parameter up to or the 2nd trimester of pregnancy to estimate the gestational age.

5.4.3. Crown rump length (CRL)

CRL is one of the important parameters used for estimating the gestational age of goats (Karen *et al.,* 2009). In the present study, CRL was able to measure from day 34 to onward of pregnancy (up-to 75 days) when Karen *et al.* (2009) reported the first measured at day 22 of post breeding. After 75 days the reliability of CRL measurement had reduced due to larger fetal size which couldn't be measured in a single focus in the monitor in this study which was in close agreement Karen *et al.* (2009) and Kandiel *et al.* (2015) who terminated the measurement on 70th day when the length of the fetus exceeded 10 cm due to the lower frequency transducer used by them. From this study, a new regression formula was generated to estimate gestational age conducted on the relationship between the fetal CRL (x) and the actual gestational age (y) was described by linear regression equation, **y = 0.4976x + 28.106** with coefficient of determination R^2= 0.57 where found an equation **y = 0.4335x + 27.559** with a high value of **R^2= 0.94** at early stage of gestation as it showed no significant variation (P>0.05) between the estimated and actual gestational age at this stage. The finding was similar to the findings of Karen *et al.* (2009) and Abubakar *et al.* (2016) who obtained a greater correlation (R^2= 0.94 and 0.96) from 25-70 days of gestation in Egyptian native goats and 37-65 days of gestation in Jamunapari goats, respectively. In future, the above derived equation from this study can be used more precisely in Bangladeshi goats for estimating the gestational age at early gestational stage.

5.4.4. Bi-parietal diameter (BPD)

The BPD was first possible to measure at the day 55 of post breeding with a diameter of 21.1 mm along with gradual increasement in the current study where Heibal and Parkins (1989) and Karadev *et al.* (2016) measured at day 40 and day 42 respectively. The results showed that it was unable to measure BPD in the first trimester of pregnancy which was in agreement with Karadev *et al.* (2016), who found hardly measured of BPD in 1st trimester in Bulgarian local goats. The measurements were able to take only at 2nd stage of pregnancy in this study like Abdelgafar *et al.* (2007) and Haibel (1988), who found that the BPD was difficult to measure after day 105 of gestation because of the increase in fetal size and compression of the head by other fetal parts and also the variability of fetal location and posture. However, for higher accuracy a new formula (**y = 1.7133x + 29.845)** was derived from this study by plotting the regression equation between actual gestational age (y) and BPD (x) with 89% efficacy in future where Karen *et al.* (2009) and Haibel (1990) also reported highly significant correlation (R^2=0.95, R^2=0.98, respectively) between fetal BPD and gestational age between Days 30 and 105 of gestation.

5.4.5. Trunk diameter (TD)

TD was considered as a preferred measurement for estimating the gestational age from 2nd trimester of pregnancy to the end of gestation (Lee *et al.,* 2005). TD was able to measure from 46-145 days of gestation in this study which was almost close to 60–135 days, findings by Lee *et al.* (2005) where Kandiel *et al.* (2015) measured the parameter during 30-120 days of pregnancy. The relationship between TD (mm) and actual gestational age (days) was fitted to a regression line with a gestational equation, **y = 1.3062x + 32.756**, with a high correlation (R^2= 0.85) which was similar to the findings of Kandiel *et al.* (2015) (R2 = 0.86). The present study showed a significant relationship of TD at late stage of gestation with a new regression equation **y = 1.0401x + 54.161**, having a strong coefficient of determination (R^2 = 0.96) similar to Kuru *et al.* (2018) (R^2 = 0.96).

5.5. Expected days from delivery (EDD) in Bangladeshi crossbreed goats

In order to predict the expected days from delivery (EDD), six different parameters were measured using ultrasonography at three different gestational stages of goats. The study found no significant difference (P>0.05) and a strong relationship (**r=0.92**) between the expected and actual dates from delivery of the studied does. This study additionally estimated the EGA ± 5 days based on six parameters to assess any significant correlation with the actual gestational age as the usual range for a goat's gestation time is thought to be 145–155 days (Kahn, 1994).

The results revealed no significant difference (P>0.05) between the estimated and actual gestational ages when added 1–5 days using EGA for all parameters. In contrast, TD revealed no significant variation (P>0.05) when subtracting 1–5 days from EGA between the estimated and actual gestational age in goats when other parameters differed considerably (P<0.05). So It was discovered from the study that, delivery dates can be changed by ±1-4 days based on TD measurements where by +1–5 days depending on GSD, GSL, PD, CRL, and BPD. There was no study found related with the study of gestation length in goats.

Chapter 6: Conclusions

Pregnancy detection was confirmed by amniotic fluid and fetal heartbeat in early stage and other fetal structures (skull, ribs, femur, placentomes, etc.) in mid and late stages of pregnancy by ultrasonography. The estimated and actual GA in goats based on six different gestational parameters were significantly differed ($P<0.05$) with respect to GSD, GSL, PD and CRL where BPD and TD showed no significant variation($P>0.05$). But PD showed no significant variation up to the 2nd trimester of pregnancy as placentome size decreased gradually along with the gestation after 100 days of pregnancy. Among the stages of gestation, the GSD, GSL and CRL at early stage, PD and BPD at mid stage, and TD at later stage of pregnancy was identified as a reliable parameter for estimation of gestational age and new formulae was found with a high accuracy (R^2=0.92, 0.91, 0.94, 0.80, 0.89 and 0.96, respectively). The delivery date may also vary based on the measurements of different parameters as EGA ±1-5 days where found that EDD can be varied for ±1-4 days based on TD measurements where other parameters showed variation of + 1-5 days from EDD. The findings will help to estimate gestational age and expected days from delivery in future in Bangladeshi Jamunapari cross breed goats.

Chapter 7: Recommendations and future perspectives

7.1. Recommendations

While doing the research, a significant amount of issues was encountered. These could be successfully used for further research. As goats are increasing rapidly in our country, accurate diagnosis of pregnancy must be needed for improving the reproduction. To estimate the age and stage of gestation of a pregnant animal, appropriate measurements of different fetal parameters should be taken by freezing the proper and clear images. By improving the ultrasonic image quality, fetal sex and fetal numbers can also be detected easily. The study will be significant if it can be done in a development pattern of measurements from a group of studied animals. The communication with the owners should maintain properly to collect the data about reproduction of animals.

7.2. Future perspectives

Researchers can do further study to detect the gestational age of goats by using ultrasonography.

- Need to study with breed specific in goats to increase the accuracy in estimation of gestational age.
- Study all the gestational parameters of pregnant goats to detect the appropriate parameter for estimation of gestational stage of pregnancy.
- Should focus on determining the fetal sex and fetal numbers of pregnant animals.

References

Abdelghafar RM, Ahmed BH, Abdelrahim MS, Ibrahim MT. 2012. The accuracy of gestational age predicted from femur and humerus length in Saanen goat using ultrasonography. Acta Veterinaria Brno. 81: 295–299.

Abdelghafar RM, Ahmed BH, Bakhiet AO. 2007. Ultrasonic measurements of crown-rump length and bi parietal diameter to predict gestational age in Saanen goats. Journal of Animal and Veterinary Advances. 6(3): 454-457.

Abdelghafar RM, Ahmed BH, Ibrahim MT, Mantis P.2011. Prediction of gestational age by transabdominal real-time ultrasonographic measurements in Saanen goats (Capra hircus). Global Veterinaria. 6: 346–351.

Abubakar F, Kari A, Ismail Z, Rashid BA, Haruna UT. 2016. Accuracy of transrectal ultrasonography: in estimating the gestational age of Jamnapari goats. Malaysian Applied Biology Journal. 45: 49–54.

Aiumlamai SF, Nilsfors GL. 1992. Real-time ultrasonography for determining the gestational age of ewes. Veterinary Record. 131: 560–562.

Ali A, Hayder M. 2007. Ultrasonographic assessment of embryonic, fetal and placental development in ossimi sheep. Small Ruminant Research. 73: 227-282.

Ali AM, Islam FA, Rahman LMS, Zohara FB. 2020. Pregnancy diagnosis in goat by using vaginal cytology and trans-abdominal ultrasonography. Journal of Animal Reproduction & Biotechnology. 35: 338-346.

Al-Merestani MR, Zarkawi M, Wardeh MF. 2003. Improving the reproductive efficiency, pregnancy diagnosis and monitoring the resumption of luteal activity in indigenous Damascus goats. Reproduction in Domestic Animals. 38: 36-40. Available from: https:// doi.org/10.1046/j.1439-0531.2003.00394.

Amer HA. 2008. Determination of first pregnancy and fetal measurements in Egyptia Baladi goats (Capra hircus). Veterinaria Italiana. 44(2): 429-437.

Amer HA. 2010. Ultrasonographic assessment of early pregnancy diagnosis, fetometry and sex determination in goats. Animal Reproduction Science. 117: 226-231.

Amrozi, Setiawan B. 2011. Sinkronisasi estrus dan pengamatan ultrasonografi pemeriksaan kebuntingan dini pada domba garut (Ovis aries) sebagai standar penentuan umur kebuntingan. Journal Kedokteran Hewan. 2(5): 73-77.

Barker CA, Cawley AJ. 1967. Radiographic detection of fetal numbers in goats. The Canadian Veterinary Journal. 8(3): 59.

Bretzlaff K, Edwards J, Forrest D, Nuti L. 1993. Ultrasonographic determination of pregnancy in small ruminants. Veterinary Medicine. 88: 12-24.

Buckrell BC. 1988. Applications of ultrasonography in reproduction in sheep and goats. Theriogenology. 29(1): 71-84.

Buckrell, BC, Bonnett BN, Johnson WH. 1986. The use of real-time ultrasound rectall for early pregnancy diagnosis in sheep. Theriogenology. 25: 665-673.

Coubrough CA, Castell MC. 1998. Fetal sex determination by ultrasonically locating the genital tubercle in ewes. Theriogenology. 50(2): 263-267.

Department of Livestock Services. 2021-2022. Ministry of Fisheries and Livestock, Government of the Peoples Republic of Bangladesh, Khamar Bari, Farmgate, Dhaka.

Devi BN, Bhuyan D, Das A, Kalita D, Ozukum S. 2019. Detection of pregnancy with the determination of foetal indices in Assam hill goat. The Pharma Innovation Journal. 8(7): 221-225

Doize F, Vaillancourt D, Carabin H, Belanger D. 1997. Determination of gestational age in sheep and goats trans-rectal ultrasonographic measurement of plancentome. Theriogenology. 48:449-460.

Donald I, Macvicar J, Brown TG. 1958. Investigation of abdominal masses by pulsed ultrasound. The Lancet. 271(7032): 1188-1195.

Erdogan G. 2012. Ultrasonic assessment during pregnancy in goats - a review. Journal in domestic animals. 47:157e63. Available from: https://doi.org/10.1111/j.1439-0531.2011.01873.x.

Evans HE, Sack WO. 1973. Prenatal development of domestic and laboratory mammals: growth curves, external features and selected references. Anatomia, Histologia, Embryologia. 2(1): 11-45.

Ford EJH. 1983. Pregnancy toxaemia. In: Martin, W.B.: Diseases of sheep. Blackwell Scientific Publications, London, pp. 147-151.

Gall CF, Stier CH, Frahm K. 1994. Age estimation of goat fetus. Small Ruminant Research. 14(1): 91-94.

Gonzale BA, Pallares P, Vazquez MI. 2010. Ultrasonographic imaging in small ruminant reproduction. Reproduction in Domestic Animal. 45(2): 9–20.

Gonzale De BA, Moreno JS, Sebastián AL. 1988. Estimation of fetal development in Manchega dairy ewes by transrectal ultrasonographic measurements. Small Ruminant Research. 27(3):243-250.

Gonzales F, Cabrera F, Batista M, Rodriguez N, Alamo D, Sulon J, Beckers JF, Gracia A. 2004. A comparison of diagnosis of pregnancy in the goat via transrectal ultrasound scanning, progesterone and pregnancy-associated glycoprotein assays. Theriogenology. 62: 1108– 1115.

Haibel GK. 1988. Real-time ultrasonic fetal head measurement and gestation age in dairy goats. Theriogenology. 30: 1053-1057.

Haibel GK. 1990: Use of ultrasonography in reproductive management of sheep and goat fetuses. Veterinary Clinics of North America. 6: 597– 613.

Heibel GK, Perkins NR. 1989. Real-time ultrasonic biparietal diameter of second trimester Suffolk and Finn sheep fetuses and prediction of gestational age. Theriogenology. 32:863-869.

Haibel GK, Perkins NR. 1990. Real time ultrasonic measurements of foetal biparietal diameter for the prediction of gestational age in small domestic ungulates. Society of Theriogenology. Newsletter. 13.

Haibel GK, Perkins NR, Lidl GM. 1989: Breed differences in biparietal diameters of second trimester Toggenburg, Nubian and Angora goat fetuses. Theriogenology. 32: 827-834

Hesselink JW, Taverne MA. 1994. Ultrasonography of the uterus of the goat. Veterinary Quaterly. 16(1): 41-45.

Hussein A. 2010. Ultrasonographic assessment of early pregnancy diagnosis, fetometry and sex determination in goats. Animal Reproduction Science. 117(3- 4):226-231.

Igwebuike UM, Ezeasor DN. 2013. The morphology of placentomes and formation of chorionic villous trees in West African Dwarf goats (Capra hircus). Veterinarski Arhiv. 83(3): 313- 321.

Jainudeen MR, Hafez ESE. 2000. Pregnancy diagnosis. Reproduction in Farm Animals. B. HAFEZ and ESE HAFEZ (6th Ed). Lippincott Williams & Wilkins. Philadelphia, USA. Pp: 395-404.

Juli M, Fauziah, Hamdan, Panjaitan B, Syafruddin, Armansyah T, Siregar NT. 2018.The image of embryo and fetal of etawa crossbred goats using ultrasonography at different gestational ages. Jurnal Kedokteran Hewan. 12(2): 47-52. ISSN: 1978-225X; E-ISSN: 2502-5600

Kandiel MM, Watanabe G, Taya K. 2015. Ultrasonographic assessment of fetal growth in miniature "Shiba" goats (Capra hircus). Animal Reproduction Science. 162: 210. Available from; https://doi.org/10.1016/j.anireprosci.2015.08.007.

Karadaev M. 2015. Pregnancy diagnosis techniques in goats – a review. Bulgaria Journal of Veterinary Medicine. 18 (3): 183–193.

Karadaev M, Fasulkov I, Vassilev N, Petrova Y, Tumbev A, Petelov Y. 2016. Ultrasound monitoring of the first trimester of pregnancy in local goats through visualization and measurements of some biometric parameters. Bulgarian Journal Veterinary Medicine. 19: 209-217.

Karen A, Szabados K, Reiczigel J, Beckers JF, Szenci O. 2004. Accuracy of transrectal ultrasonography for determination of pregnancy in sheep: effect of fasting and handling of the animals. Theriogenology. 61(7-8): 1291-1298.

Karen AM, Fattouh ESM, Abu-Zeid SS. 2009. Estimation of gestational age in Egyptian native goats by ultrasonographic fetometry. Animal Reproduction Science. 114: 167–174.

Kahn W. 1992. Ultrasonography as a diagnostic tool in female animal reproduction. Animal Reproduction Science. 28(1-4):1-10.

Kahn W. 1994. Veterinary Reproductive Ultrasonography. In: Revised Edition. Mosby-Wolfe, Germany. Pp: 256-275

Kahn W. 2004. Ultrasonography in sheep and goats. Veterinary reproductive ultrasonography. 187-210.

Kieler H, Axelsson O, Nilsson S, Waldenström U. 1995. The length of human pregnancy as calculated by ultrasonographic measurement of the fetal biparietal diameter. Ultrasound in Obstetrics and Gynecology. 6: 353-357.

King AM. 2006. Development, advances and applications of diagnostic ultrasound in animals. The Veterinary Journal. 171(3): 408-420.

Kumar K, Chandolia RK, Kumar S, Pal M, Sandeep K. 2015. Two-dimensional and Three dimensional ultrasonography for pregnancy diagnosis and antenatal fetal development in Beetal goats. Veterinary World. 8(7): 835.

Kuru M, Oral H, Kulaksiz R. 2018. Determination of gestational age by measuring defined embryonic and foetal indices with ultrasonography in Abaza and Gurcu goats. Acta Veterinaria Brno. 87:357e62. Available from: https://doi.org/10.2754/avb201887040357.

Kutty CI. 1999. Gynecological examination and pregnancy diagnosis in small ruminants using bimanual palpation technique: A review. Theriogenology. 51(8): 1555-1564.

Laurel MD. 1993. Bioeffects and Safety of Diagnostic Ultrasound. American Institute of Ultrasound in Medicine.

Lee Y, Lee O, Cho J, Shin H, Choi Y, Shim Y, Choi W, Shin H, Lee D, Lee G, Shin S. 2005. Ultrasonic measurement of fetal parameters for estimation of gestational age in Korean black goats. Theriogenology. 67: 497–502.

Léga E, Toniollo GH, Oliveira JA, Resende KT, Rodrigues V. 2007. Determinac¸ ão da idade fetal por meio da técnica ultrasonográfica de etometria e de morfologia fetal em cabras. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 59: 851–856.

Leigh OO, Fayemi OE. 2010. Estimation of gestational age using ultrasonic measurement of fetal parameters in West African Dwarf goat. Tropical Veterinarian. 28(2): 43-49.

Lindahl IL. 1971. Pregnancy diagnosis in ewe by intrarectal Doppler. Journal of Animal Science. 32: 92.

Luvoni GC, Beccaglia M. 2006. The prediction of parturition date in canine pregnancy. Reproduction of Domestic Animals. 41 (1), 27–32. Available from: https://doi.org/10.1111/j.1439- 0531.2006.00641.x.

McDonald B, O'Rourke P, Connell J, Hoey W. 1988. Prenatal growth and estimation of fetal age in the Australian feral goat. Australian Journal of Agricultural Research. 39: 729–734.

Martinez MF, Bosch P, Bosch RA. 1998. Determination of early pregnancy and embryonic growth in goats by transrectal ultrasound scanning. Theriogenology. 49(8): 1555-1565.

Matsas D. 2007. Pregnancy diagnosis in goats. In: Youngquist RS, Threlfall WR (Eds.), Current Therapy in Large Animal Theriogenology. 2nd edition, Saunders, Philadelphia. Pp. 547- 554.

Medan M, Watanabe G, Absy G, Sasaki K, Sharawy S, Taya K. 2004. Early pregnancy diagnosis by means of ultrasonography as a method of improving reproductive efficiency in goats. Journal of Reproductive Development. 50(4): 391-397.

Murray RD, Newstead R. 1988. Determination of steroid hormones in goat milk and plasma as an aid to pregnancy diagnosis using an ELISA. Veterinary Record. 122: 158-161.

Naznina F, Hasana M, Mahmudb K, Sarkara KA, Hasana IMM, Bhuiyana UMM , Baria YF, Juyenaa SN. 2020. Consideration of breed specific fetal trunk diameter for the estimation of gestation age using trans-abdominal ultrasonography in sheep. Animal Reproduction Science. 212: 106237

Nwaogu IC, Anya KO, Agada PC. 2010. Estimation of foetal age using ultrasonic measurements of different foetal parameters in red Sokoto gaots (*Capra hircus*). Veterinarski Arhiv. 80: 225–233

Ott RS, Braun WF, Lock TF, Memon MA, Stowater JL. 1981. A comparison of intrarectal Doppler and rectal abdominal palpation for pregnancy testing in goats. Journal of the American Veterinary Medical Association. 178(7): 730-731.

Padilla-Rivas GR, Sohnrey B, Holtz W. 2005. Early pregnancy detection by real-time ultrasonography in Boer goats. Small Ruminant Research. 58(1): 87-92.

Pratt MS, Hopkins PS. 1975. The diagnosis of pregnancy in sheep by abdominal palpation. Australian Veterinary Journal. 51(8): 378-380.

Quintela LA, Barrio M, Peña AI, Becerra JJ, Cainzos J, Herradón PG, Díaz C. 2012. Use of ultrasound in the reproductive management of dairy cattle. Reproduction in domestic animals. 47(3): 34-44.

Raja-Ili Airina RK, Mohad Nizam AR, Abdullah RB, Wan-Khadijah W.E. 2011. Using fetal heart measured from ultrasound scanner images to estimate age of gestation in goat. Journal of Animal and Veterinary Advances. 10: 2528–2540.

Rasheed YM. 2017. Assessment of gestational age in goats by Real-Time Ultrasound measuring the fetal crown rump length, and bi-parietal diameter. The Iraqi Journal of Veterinary Medicine. 41(2): 106-112.

Refstal KR, Marteniuk JV, Williams CS, Nachreiner RF. 1991. Concentrations of estrone sulfate in peripheral serum of pregnant goats: relationships with gestation length, fetal number and the occurrence of fetal death in utero. Theriogenology. 36(3): 449-461.

Reichle JK, Haibel GK. 1991. Ultrasonic biparietal diameter of second trimester Pygmy goat fetuses. Theriogenology. 35(4): 689-694.

Revol B, Wilson PR. 1991. Ultrasonography of the reproductive tract and early pregnancy in red deer. Veterinary Record. 128: 229–233

Roukbi M. 2013. The use of ultrasonography for early detection of pregnancy and measurement of some foetal pattern in Damascus goats. Iraq Journal of Vetereinary Sciences. 27: 35–44.

Santiago-Moreno J, González-Bulnes A, Gómez-Brunet A, Toledano-Díaz A, López-Sebastián A. 2005. Prediction of gestational age by transrectal ultrasonographic measurements in the mouflon (Ovis gmelini musimon). Journal of Zoo and Wildlife Medicine. 36(3):457-462.

Santos MHB, Rabelo MC, Filho CRA, Dezzoti CH, Reichenbach HD, Neves JP, Lima PF, Oliveira MAL. 2007. Accuracy of early fetal sex determination by ultrasonic assessment in goats. Research in Veterinary Science. 83: 251–255.

Sayuti A, Melia J, Marpaung IK, Siregar TN, Panjaitan B. 2016. Diagnosis kebuntingan dini pada kambing kacang (Capra sp.) menggunakan ultrasonografi transkutaneus. Journal Kedokteran Hewan. 10(1): 63-67.

Schrick FN, Inskeep EK. 1993. Determination of early pregnancy in ewes utilizing trans-rectal ultrasonography. Theriogenology. 40: 295–306.

Scott PR. 2012. Applications of diagnostic ultrasonography in small ruminant reproductive management. Animal Reproduction Science. 130 (3-4): 184–186. Available from: https://doi.org/10. 1016/j.anireprosci.2012.01.013.

Singh NS, Gawande PG, Mishra OP, Nema RK, Mishra UK, Singh M. 2004. Accuracy of ultrasonography in early pregnancy diagnosis in doe. Asian-Australasian Journal of Animal Sciences. 17(6): 760-768.

Smith MC. 1980. Caprine reproduction. In: (Ed. D. A. Morrow), Current therapy in Theriogenology. 1st edition, W.B. Saunders, Philadelphia, P.A. Pp: 975-977.

Suguna K, Mehrotra S, Agarwal SK, Hoque M, Singh SK, Shanker U, Saratha T. 2008. Early pregnancy diagnosis and embryonic and fetal development using real time B mode ultrasound in goats. Small Ruminant Research. 80(1-3): 80-86.

Vinoles-Gil C, Gonzalez-Bulnes A, Martin GB, Zlatar FS, Sale S. 2010. Sheep and Goats. In: DesCoteaux L., Colloton J, Gnemmi G. (edition), Ruminant and Camelid Reproductive Ultrasonography. Willey-Blackwell, Hong Kong. Pp: 181–210.

Wani NA, Wani GM, Mufti AM, Khan MZ. 1998. Ultrasonic pregnancy diagnosis in gaddi goats. Small Ruminant Research. 29(2): 239-420.

Watt BR, Anderson GA, Campbell IP. 1984. A comparison of six methods used for detecting pregnancy in sheep. Australian Veterinary Journal. 61(12): 377-382.

Waziri MA, Ikpe AB, Bukar MM, Ribadu AY. 2017. Determination of gestational age through trans-abdominal scan of placentome diameter in Nigerian breed of sheep and goats. Sokoto Journal of Veterinary Sciences. 15(2): 49-53.

White IR, Russel AJF. 1984. Determination of fetal numbers in sheep by relative ultrasonic scanning. The Veterinary Record. 1: 200-203.

White IR, Russel AJF, Fowler DG. 1984. Real time ultrasonic scanning in the diagnosis of pregnancy and the determination of foetal numbers in sheep. Veterinary Record. 115: 140-143.

APPENDIX

Questionnaire

Gestational age and EDD determination in small ruminants by ultrasonic assessment

Reg no:	Date:	
Owner's name & address:		
Owner's contact num:		
Animal's breed:	Age:	Body weight:
Feeding history:		
Deworming: Y/N	Last date of deworming:	
Vaccination: Y/N	Type & date of vaccination:	
Physical status:	BCS: 1/2/3/4/5	Parity:
Last heat showing date:		
Last insemination type: Natural/ AI	Date:	
Gestation period (owner's statement):		
Cotyledon size (PD):	GP:	
GSD and GSL measurement:	GP:	
Head measurement (BPD):	GP:	
TD measurement:	GP:	
CRL measurement:	GP:	
USG results (EGA):	EDD:	
Parturition date:		

Table 8: Estimation of gestational age by measuring GSD and comparison with actual gestational age:

No of observation	GSD (mm)	EGA (Days)	AGA (Days)	P-value
1	36.7	41	57	**0.0003**
2	27.5	35	38	
3	17.5	28	32	
4	41	42	46	
5	52.6	49	50	
6	47.1	42	50	
7	52.4	49	64	
8	43.7	42	53	
9	54.4	49	55	
10	32.3	35	22	
11	34	40	54	
12	35.1	40	40	
13	33.6	40	50	
14	30.2	35	37	
15	41.7	42	45	
16	22.6	32	35	
17	36.7	41	43	
18	44.4	42	56	
19	33.1	35	35	
20	34.2	40	48	
21	19.5	27	34	
22	35.6	40	42	
23	21.6	30	38	
24	33.6	40	42	
25	24.2	35	36	
26	36.9	41	46	
27	34.4	40	40	
Mean ± SD	**35.43 ± 9.76**	**38.96 ± 5.68**	**44 ± 9.16**	

Table 9: Estimation of gestational age by measuring GSL and comparison with actual gestational age:

No of observation	GSL (mm)	EGA (days)	AGA (days)	P-value
1	58.1	41	57	
2	32.2	35	38	
3	53.1	42	46	
4	56.4	49	50	
5	82.3	42	50	
6	95.3	49	64	
7	62.4	42	53	
8	94.7	49	55	
9	35.3	35	22	
10	40.1	40	54	
11	35.7	40	40	
12	48.3	40	50	
13	35.6	35	37	
14	59.5	42	45	**0.0036**
15	27	32	35	
16	57.1	41	43	
17	51.5	42	56	
18	34.5	35	35	
19	41.3	40	48	
20	33.6	27	34	
21	32.2	30	38	
22	41	40	42	
23	31.6	35	36	
24	53.3	41	46	
25	42.4	40	40	
Mean ± SD	**48.16 ± 18.34**	**38.96 ± 5.68**	**44 ± 9.16**	

Table 10: Estimation of gestational age by measuring PD and comparison with actual gestational age:

No of observation	PD(mm) (Mean± SD)	EGA (days) (Mean± SD)	AGA (days) (Mean± SD)	P-value
114	23.71 ± 4.9	79.18 ± 14.67	83.04 ± 18.71	0.025

Table 11: 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	31.3	41	57	0.02
2	98.5	74	75	
3	46.4	48	46	
4	53.9	52	50	
5	66.2	58	62	
6	72	61	50	
7	63.8	57	64	
8	50.5	50	53	
9	70.4	60	55	
10	30.2	40	54	
11	18.1	34	40	
12	45	48	50	
13	65	57	93	
14	32.9	42	45	
15	33.6	42	43	
16	24.9	38	48	
17	39.6	45	56	
18	50	50	54	
19	19	35	35	
20	24.2	38	48	

21	20.1	35	34	
22	30.2	40	42	
23	30.9	40	42	
24	15.6	34	36	
25	58	54	55	
26	45.8	48	48	
27	55.5	53	60	
28	41	45	46	
29	34.7	43	40	
30	46.4	49	48	
31	76.9	63	57	
Mean ± SD	**44.86 ± 19.87**	**47.55 ± 9.73**	**51.16 ± 11.87**	

Table 12: Estimation of gestational age by measuring BPD and comparison with actual gestational age:

No of observation	**BPD(mm) (Mean**± SD)	**EGA (days) (Mean**± SD)	**AGA (days) (Mean**± SD)	**P-value**
47	27.18 ± 6.19	75.28 ± 12.18	78.32 ± 12.15	0.22

Table 13: Estimation of gestational age by measuring TD and comparison with actual gestational age:

No of observation	**TD(mm) (Mean**± SD)	**EGA (days) (Mean**± SD)	**AGA (days) (Mean**± SD)	**P-value**
91	41.57 ± 15.58	88.6 ± 21.38	87.05 ± 22	0.63

Table 14: Comparison of estimated gestational age with actual gestational age:

No of observation	EGA (days) Mean ± SD	AGA (days) Mean ± SD	P-value	Correlation coefficient (r)
150	77.67 ± 24.35	79.35 ± 25.22	0.05	0.92

Table 15: Regression equation that are used for estimation of gestational age:

Fetal Parameter	Regression equation or Formula for gestational age (GA)	Author	Correlation Coefficient
GSD, GSL	Different range of measurements	Singh *et al.* (2016)	-
PD	2.930PL+9.611	Waziri *et al.* (2017)	r^2= 0.90
CRL	0.49CRL + 25.454	Kuru *et al.* (2018)	r^2= 0.94
BPD	1.95 BPD+21.936	Kuru *et al.* (2018)	r^2=0.95
TD	1.3565TD +32.065	Naznin *et al.* (2020)	r^2= 0.92

BIOGRAPHY

I am Khadija Begum, daughter of Nurul Hoque and Sajeda Begum. I am from Kalipur, Banskhali, Chattogram, Bangladesh. I passed my Secondary School Certificate (SSC) examination from Kalipur Nasera Khatun R.K. Girls High School in 2011 and Higher Secondary Certificate (HSC) examination from Chittagong Govt. Women college in 2013. I have completed my graduation on Doctor of Veterinary Medicine (DVM) from Chattogram Veterinary and Animal Sciences University (CVASU) in 2020. Currently I am a Master of Science (MS) fellow in Theriogenology under the Department of Medicine and Surgery, Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University (CVASU). I intend to pursue my future research on the reproduction of livestock.