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ULTRASONOGRAPHIC DIAGNOSIS OF EARLY PREGNANCY IN BLACK BENGAL GOAT

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ABSTRACT

The study was aimed to diagnose the pregnancy at the earliest possible time by USG and, to assess the fetal growth and development (FGD), and fetal number in Black Bengal (BB) goats. Ten cyclic BB does were used and does in estrus were allowed to be mated by fertile BB bucks. Ultrasonographic examinations for pregnancy diagnosis (PD) were performed trans-abdominally using real-time B-mode scanner equipped with a 5 MHz transducer. The results demonstrated early pregnancy in BB does as early as 21 to 25 days of gestation with an accuracy of 20% (only in two does out of ten; 2/10). However, the accuracy of PD increased to 80% (8/10) at 31 to 35 days and up to 100% (10/10) at 36 to 40 days (onwards) of pregnancy. The gestational sac (GS) was observed in the form of circular and elongated fluid filled structure in only 5 does (5/10) with 50% accuracy at 26 to 30 days after mating. The GS became clearer and more prominent with gestation advances, and accuracy rate was 100% within 36 to 40 days. Placentomes, fetal leg buds and vertebral column were visualized as early as 30 to 40 days with accuracy of 50% (5/10), 40% (4/10) and 30% (3/10), respectively. Additionally, the visualization accuracy of placentomes, fetal leg buds and vertebral column was increased to 100% (10/10) between 51 to 60 days of gestation in BB does. The overall accuracy in fetus number detection was 86.67% on 31 to 35 days of gestation.

Keywords: Black Bengal does, early pregnancy, diagnosis, transabdominal ultrasonography

Introduction

Black Bengal (BB) goat is the only recognized goat breed in Bangladesh and famous for its high fertility, prolificacy, superior chevon quality, best quality skin, early sexual maturity, disease resistance, low kidding interval and adaptability (Talukder *et al.* 2020). The early and accurate pregnancy diagnosis (PD) in goats is very essential for the maintenance of high levels of reproductive efficiency (Kumar *et al.* 2015). Early PD also tends to improve the life time productivity of the goat (Medan and Abdelaty 2010, Talukder *et al.* 2020). The intensive goat production and wide spread application of the controlled breeding techniques, such as artificial insemination (AI) and out of seasonal breeding, increase the need for an accurate early PD (Ali *et al.* 2020). The early PD in goats is also very essential for better efficacy and management of reproduction (Doize *et al.* 1997, Omontese *et al.* 2012), providing information about conception rates after AI and gestation course (Amer *et al.* 2010), time for drying-off and parturition date (González *et al.* 2004).

The reliable technique for early PD would allow early culling or rebreeding of non-pregnant or barren does (Gonzales-Bulnes *et al.* 2010). Methods of PD depending on visualization of the conceptus or determination of its secretory products in the maternal blood or in the milk are the most accurate and specific methods for pregnancy (Doize *et al.* 1997, Padilla-Rivas *et al.* 2005). The traditional methods of PD in small ruminants like goats and sheep such as non-return to estrus, palpation through external abdomen, abdominal ballottement and noting udder enlargement (Goel and Agrawal 1992). However, all these methods are applicable only in late pregnancy and are not satisfactory (Islam *et al.* 2014). Furthermore, a variety of techniques have evolved in goat PD over the years such as laparotomy, laparoscopy, rosette inhibition test and vaginal biopsy, though, these methods are

impractical under farm conditions and have limited utility (Gordon 1999, Medan *et al.* 2004). Ultrasonography (Haibel 1990), progesterone assay (González *et al.* 2004 and Islam *et al.* 2014) and radiography (Ruder *et al.* 1988) have emerged as the most useful methods utilized today. Among the three methods described, ultrasonography provides a simple, rapid, accurate and non-invasive means for PD and counting fetal numbers, as well as, considered a good alternative method to check embryonic mortality in small ruminants on the farm (Buckrell 1988). The technique of ultrasonographic diagnosis has been used with great accuracy as a means for PD and estimation of fetal numbers in sheep (Garcia *et al.* 1993) and goat (Martínez *et al.* 1998). The optimum time for using trans-abdominal or trans-rectal ultrasonography in sheep and goats ranges from 19 to 100 days of gestation (Medan and Abdelaty 2010).

Ultrasonography permits a close relationship between research and practice, as findings in research can be directly translated to the field (Islam *et al.* 2014). Increased portability also makes ultrasound possible and convenient on the farm. Fetal number can be determined during early- to mid-gestation, allowing prediction of litter size. As gestation advances, fetal features can be used to estimate gestational age and track growth over time. Thus, ultrasound is useful for producers, aiding with efficient culling and rebreeding decisions, fetal counting and sexing, and feeding the dam appropriately throughout the majority of gestation (Medan and Abdelaty 2010). Furthermore, ultrasound also allows producers and clinicians to calculate parturition dates, monitor does susceptible to pregnancy toxemia during late gestation, supervise parturition, and monitor the fetus for abnormal growth in a non-invasive manner (González *et al.* 2004, Islam *et al.* 2014). These factors all contribute to proper gestational management in goats and sheep that can ultimately improve the health and operation efficiency and profitability (Amer *et al.* 2010). Considering the above facts and to establish a reliable field technique for PD in BB goats, the study considered the use of real-time B-mode trans-abdominal ultrasonography as means of PD at the earliest possible time and, to assess the fetal growth and development (FGD) and fetal number following breeding in BB goats.

Materials and Methods

Study area and period: The study was carried out on a goat shed of Livestock and Poultry Farm (LPF) (Figure 1A, 1B and 1C), Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during the period from July 2019 to June 2020 with a view to diagnose the pregnancy as the earliest possible time by real-time B-mode ultrasonography and, to assess the fetal growth and development (FGD), and fetal number in Black Bengal (BB) goats.

Materials and equipment: Ten cyclic and healthy BB does (female goats) were used in the study (Figure 1C and 1D). Two Ultrasound Machines (DIGI 600 MW VET, portable ultrasound machine, India; DRAMINSKI-4 VET SLIM, portable ultrasound machine, Poland) and ultrasound probes of 5 MHz frequency were used in the current study to diagnosis pregnancy in BB does (Figure 1E-G). Ultrasound coupling gel (KONIX ECG gel-250ml, ARC Medicine Suppliers INC.) was used for clear and accurate visualization of the objects to be scanned. Marking tags were also used to identify the individual doe.

Experimental does and their management: A total of ten clinically healthy and cyclic BB does of 2 to 2.5 years old, 10 to 15 kg body weight and kidding once before were used in the study. The does were maintained on natural grazing inside BSMRAU campus area along with concentrate mixture (25% crushed maize, 50% wheat bran, 20% soybean meal, 1.0% fish meal, 2.0% DCP powder, 1.0% salt and 1.0% vitamin-mineral premix) during the experimental period. Water was supplied ad libitum.

Ultrasonographic examination: Ultrasonographic examination of BB does were performed as describe elsewhere (Medan *et al.* 2004) with partial modification. Ultrasound examinations was performed periodically at days 20 to 30, 31 to 40, 41 to 50, 51 to 60 of gestation using a real-time B-mode scanner (DRAMINSKI-4 VET SLIM, portable ultrasound machine, Poland) equipped with 5 MHz transducer after properly clipping and shaving of both side of lower abdomen of BB does. Experimental BB doe was lightly restrained by one person in standing position and also in dorsal recumbent position (Figure 1D). Ultrasound coupling gel was applied in each time



Figure 1. Ultrasonographic diagnosis of the BB does. **A**. Goat shed of Livestock and Poultry Farm, BSMRAU where the does were kept. **B.** Interior wooden platform on which does is kept. **C**. Experimental does grazing on field. **D**. Shaving of does before ultrasonographic examination. **E.** Ultrasound scanner and gel used in ultrasound examination. **F**. A single healthy doe with a healthy kid.

to the probe with a view to develop good contract and to remove air between probe and skin of the doe. Each doe was subjected to scanning for at least 4-5 minutes at each time. All the ultrasonographic records were kept in the data record sheet.

Experiment outline: The experimental methodology is presented schematically in the Figure 2.

Data analysis: The data generated from this experiment were entered in Microsoft Excel worksheet, organize and process for further analysis. Descriptive statistics were performed to calculate mean, and percentages. Accuracy rate was calculated as the total percentage of does correctly diagnose pregnant during USG examination periodically.

Results and Discussion

The early PD in BB does is a primary factor for improving the reproductive performance in herds and achieving high economic efficiency. The study demonstrated to diagnose the pregnancy of does at the earliest possible time after breeding by using real-time B-mode ultrasonography equipped with a 5 MHz transducer and, to assess the FGD and fetal number.

Early pregnancy diagnosis: In the present study, the pregnancy in BB does was diagnosed as early as 21 to 25 days of gestation with an accuracy of 20% (only in two does out of ten; 2/10) by using real-time B-mode ultrasonography equipped with 5 MHz probe. However, the accuracy of PD increased to 50% (5/10) at 26 to 30 days, and 80% (8/10) at 31 to 35 days and up to 100% (10/10) at 36 to 40 days (onwards) of pregnancy with the same equipment. The presence of anechoic areas was considered as positive for pregnancy (Table 2 and Figure 3). The results obtained in the present study were consistent with a previous study, where Ali *et al.* (2020)

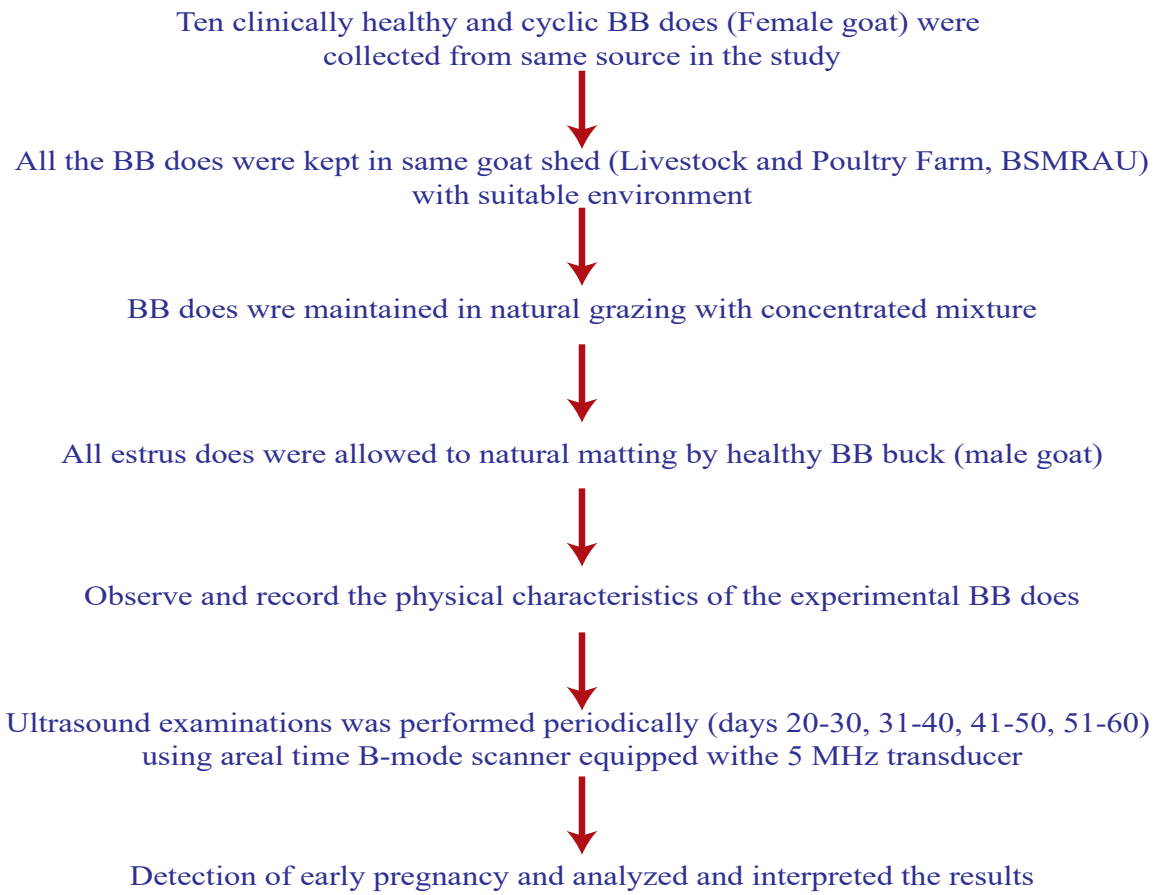


Figure 2. Experimental outline used in the present study

Table 1. Identification of gestational sac (GS) as a means of early pregnancy in BB does by ultrasonography

Gestational age (days)	No. of pregnant does	Accuracy of pregnancy diagnosis (%)
15-20	0	0%
21-25	2	20%
26-30	5	50%
31-35	8	80%
36-40	10	100%
41-45	10	100%
46`-50	10	100%
51-55	10	100%
56-60	10	100%
61-65	10	100%

reported the 100% pregnancy in does at days 31 to 35 days post mating. No false positive cases were recorded in the present study. False positive results are attributed to the early embryonic death, unobserved abortion and sometimes misinterpreting the urinary bladder as the uterus (Fowler and Wilkins 1984, Haibel *et al.* 1990).

Characteristics of pregnancy by Real-time B-mode ultrasonography: The chronological sequence of ultrasonographic observations of gestational sac, placentomes, fetal leg buds, vertebral column were identified.

Gestational sac identification: The presence of a gestational sac (GS) is the positive sign of pregnancy. In the current study, the GS was observed in the form of circular and elongated fluid filled structure in only 5 does (5/10) with 50% accuracy at 26 to 30 days after mating. The GS became clearer and more prominent with the advancement of gestation period and accuracy rate was 100% within 36 to 40 days of gestation (Figure 3). The findings of the current study are in accordance with the

several previous observations (Ali *et al.* 2020, Bretzlaff *et al.* 1993, Mialot *et al.* 1991, Picazo *et al.* 1991), where authors reported that pregnancy could be first verified at 25 to 30 days after the breeding.

Identification of placentomes: In the present study, placentomes were visualized as early as 30 to 40 days of gestation with 50% accuracy (in 5 does out of 10; 5/10) (Table 2, Figure 4). Additionally, the visualization accuracy of placentomes, was increased to 80% (8/10) between 41 to 50 days and to 100% (10/10) between 51 to 60 days of gestation in BB does. Studies by Russel (1989) revealed that placentomes were identified from about day 40 as echoic circular structures in sheep. On day 42, the ovine placentomes appears to be cup-shaped nd reaching the maximum size by day 74 (Doize *et al.* 1997). Placentomes were detected with 100% at 45-50 days after the mating (Anwar *et al.* 2008). However, a poor correlation between placentomes size and ovine gestational age has been reported which was ascribed to great variation in the size of placentomes in different breeds (Doize 1997, González *et al.* 1998). Hence, the finding of present study is in agreement with the previous reports that have also employed trans-abdominal ultrasonography using a 3.5 MHz probe (Wani *et al.*

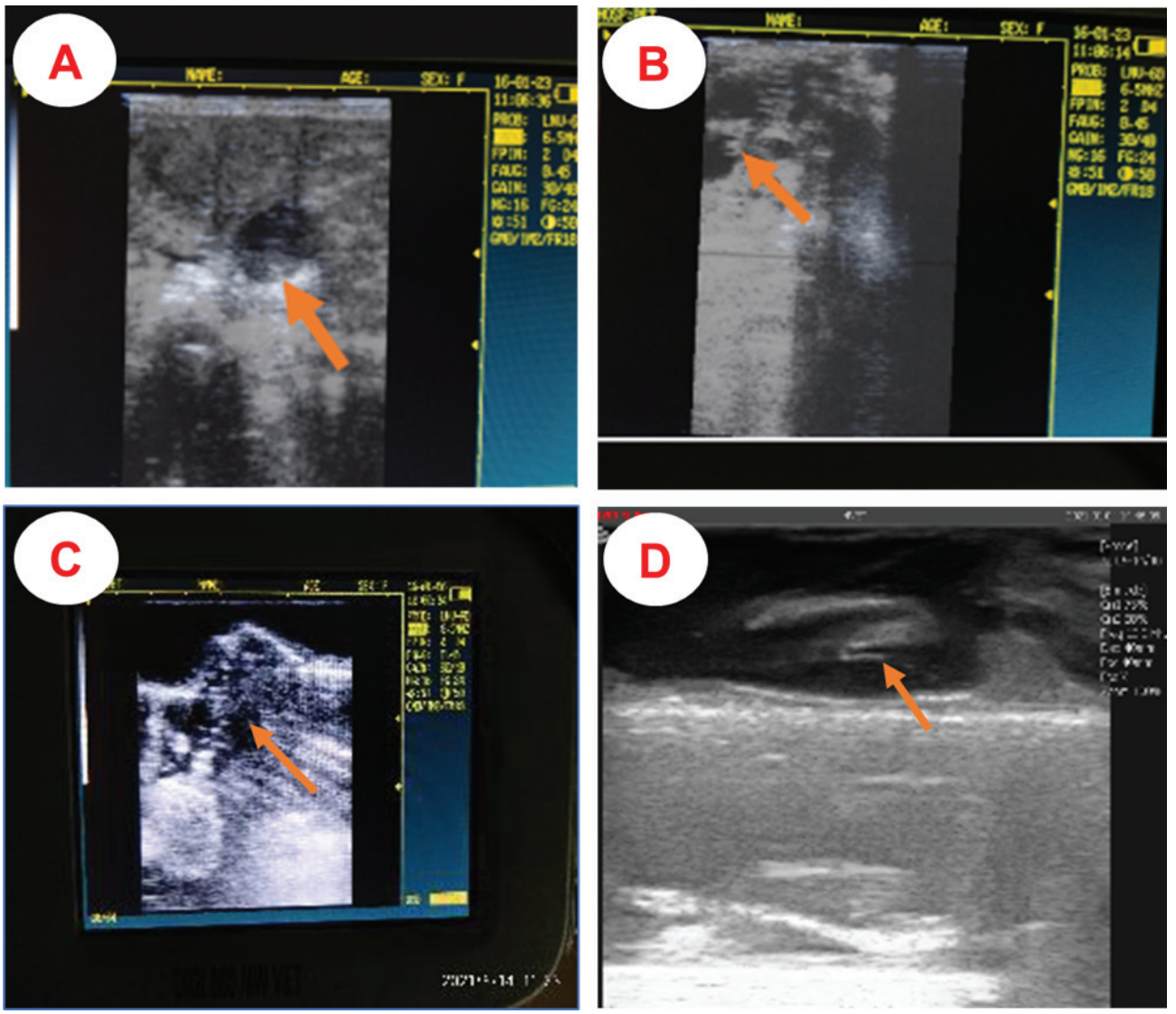


Figure 3. Gestational Sac (GS) at different age of gestation. **A**. GS at 26 days after mating. **B**. GS with embryonic vesicle at 31 days of gestation. **C**. GS with embryonic vesicle at 36 days of gestation. **D.** GS with embryonic vesicle at 55 days of gestation.

Table 2. Developmental characteristics of fetuses during pregnancy in BB does observe ultrasonography

Gestational age (days)	Parameters (Total number of does=10)		
	No. of goats with placentomes detected	No. of goats with leg buds detected	No. of goats with vertebral column detected
30-40	5 (50%)	4 (40%)	3 (30%)
41-50	8 (80%)	7 (70%)	7 (70%)
51-60	10 (100%)	10 (100%)	10 (100%)

2003, Anwar *et al.* 2008).

Identifications of fetal leg buds: In the present study, fetal leg buds were visualized as early as 30 to 40 days of gestation with accuracy rate 40% (in 4 does out of 10; 4/10) (Table 2, Figure 5). Additionally, the visualization accuracy of fetal leg buds was increased to 70% (7/10) by 41 to 50 days and to 100% (10/10) between 51 to 60 days of gestation in BB does. No fetal leg buds were detected before 35 days of gestation in the current study. Similar observations have also been reported previously by others (Anwar *et al.* 2008, Ali *et al.* 2020). The results indicated in the study that, PD by transabdominal ultrasound based on fetal leg buds could be detected

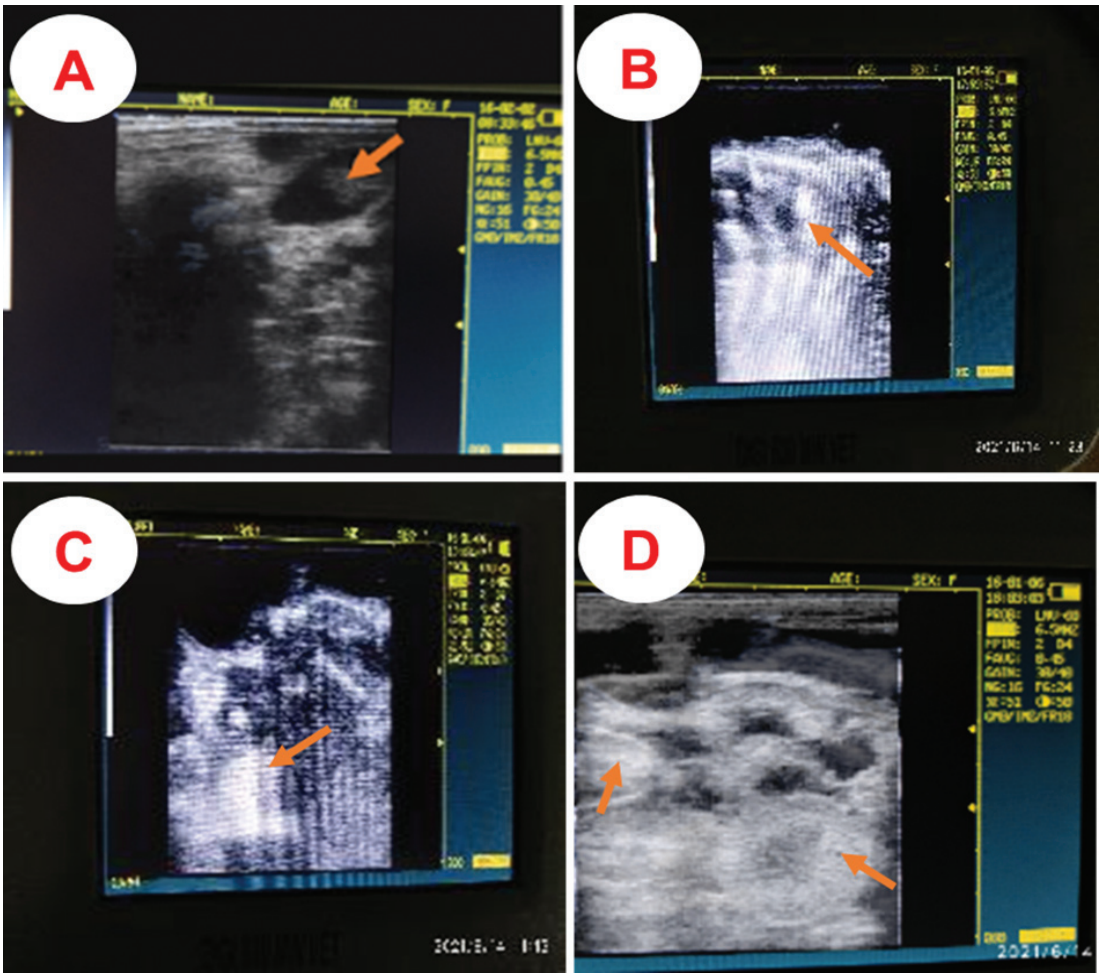


Figure 4. Identification of placentomes at different gestation stage. **A.** Placentomes as a distinct echodense structure on day 30 **B.** Placentomes at day 35 **C.** Prominent Placentomes at 41-45 days of gestation; **D.** C- or O-shaped gray images of placentome on day 70.

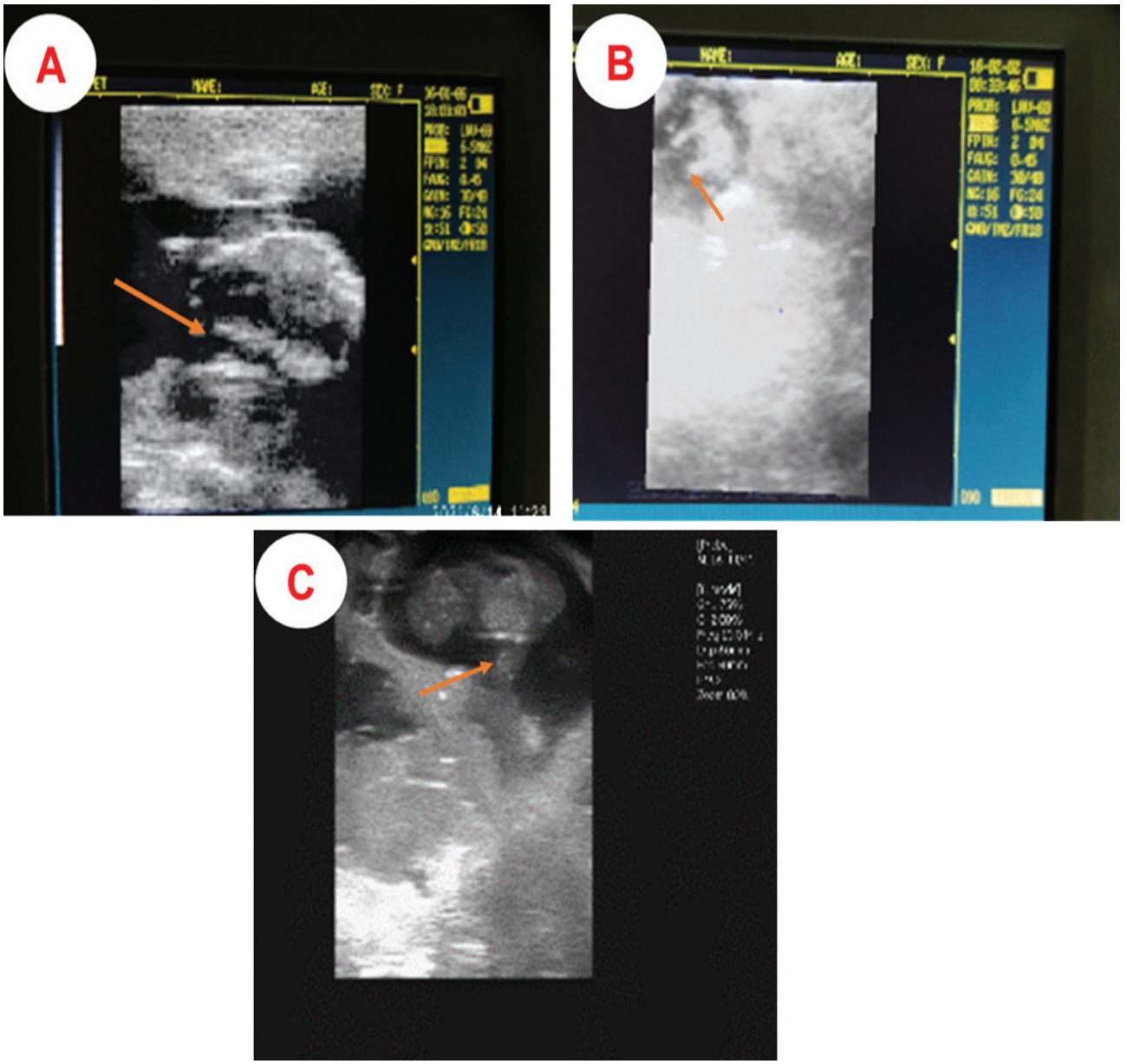


Figure 5. Ultrasonographic identification of fetal leg buds at different gestational age; **A**. Leg buds at day 34 of gestation **B**. Legs buds at day 42 days of gestation. **C**. A prominent fetal structure with leg buds at day 55-60 days of gestation.

with 100% accuracy between 51-55 days of gestation in does.

Identification of fetal vertebral column: Fetal vertebral column identification is one of the key factors for pregnancy diagnosis via trans-abdominal ultrasonography. As found in Table 2, Figure 6 fetal vertebral column was visualized as early as 30 to 40 days of gestation with accuracy of 30% (only in 3 does out of 10; 3/10). Additionally, the visualization accuracy of fetal vertebral column was increased to 70% (7/10) by 41 to 50 days and to 100% (10/10) between 51 to 60 days of gestation in BB does. The pregnancy was detected in all the does by 51 to 60 days of gestation which is similar to the findings of others (Ali *et al.* 2020, Anwar *et al.* 2008). The results obtained in the current study confirmed that PD by trans-abdominal ultrasound based on vertebral column could be done by 51-60 days of pregnancy with 100% accuracy.

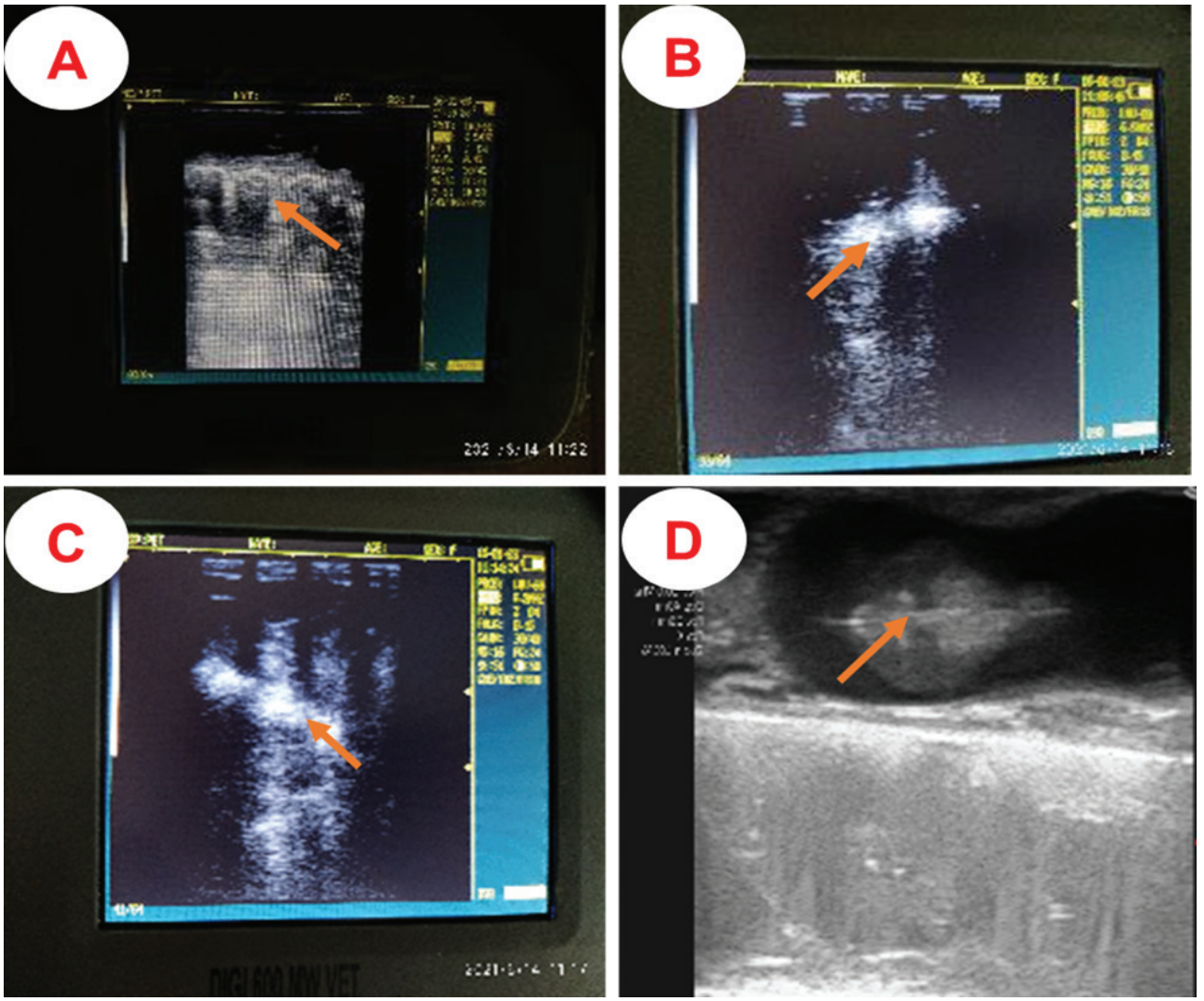


Figure 6. Ultrasonographic identification of fetal vertebral column at different gestational age. **A.** Vertebral column at 36 days of gestation, **B.** Vertebral column at 39 days of gestation. **C.** A prominent structure of fetus with vertebral column at day 45-50 days of gestation. **D.** Fetal thorax at 60-65 days of gestation.

Table 3. Identification of fetal number in does by ultrasonography

Does ID	Fetal number identification in different gestational age (days)						Number of kids born at parturition	Accuracy of fetal number detection (%)
	31-35	36-40	41-45	46-50	51-55	56-60		
1	2	2	2	2	2	2	3	66.7
2	1	1	1	1	1	1	1	100
3	2	2	2	2	2	2	2	100
4	1	1	1	1	1	1	1	100
5	2	2	2	2	2	2	2	100
6	2	2	2	2	2	2	4	50
7	2	2	2	2	2	2	4	50
8	2	2	2	2	2	2	2	100
9	2	2	2	2	2	2	2	100
10	1	1	1	1	1	1	1	100
							Mean	86.7

Determination of fetal number: Determination of fetal number is also one of the key factors while diagnosing early pregnancy. In the present study, we observed that, the optimal period for determining single and multiple pregnancies by trans-abdominal ultrasounds starts around Day 30-35 of gestation, as the efficiency for identifying single embryos reaches 100% and accuracy is more than 66% for triplets and >50% quadruplets. The overall accuracy in the detection of fetus number was 86.7% on 31 to 35 days of gestation in BB does (Table 3). Observations have been reported similarly by Goel and Agrawal (1992) that it is difficult to differentiate between twins and triplets or quadruplets at any stage of gestation while Karen *et al*. (2014) reported that the accuracy of ultrasound in detecting ewes carrying 2 fetuses or more was disappointing.

Conclusion

The present study revealed that the optimal period for diagnosis of single and multiple pregnancies in BB does at the earliest possible time by trans-abdominal real-time B-mode ultrasonography was at 31 to 35 days of gestation. The study also evaluated both FGD and fetal number in all the experimental BB does successfully during the study period. Therefore, the real-time B-mode trans-abdominal ultrasonography can be used as a simple, non-invasive PD technique in BB does in field condition. Further studies may direct to in-depth assessment of FGD characteristics as well as determination of fetal sex during pregnancy in small ruminants like sheep and goats.

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