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Transabdominal ultrasonographic measurement of placentome length to estimate and validate gestational age in Nellore brown ewes

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Abstract
Detection of gestational age helps in nutritional management of pregnant ewes and optimizes the lamb survival. Nellore Brown ewes (n=20) aged 1-4 years were subjected to short day estrus synchronization protocol by inserting vaginal sponges and received 300 IU of PMSG and 75 µg of $PGF_{2\alpha}$ intra muscularly at sponge withdrawl and matings were monitored. The last day of receiving mating mark was treated as day 0 start of pregnancy. Transabdominal ultrasonography weekly once starting from day 8 onwards until parturition. On day 29, in more than 50% of pregnant animals placentome were observed as raised, nodular, convex shaped that projected into the lumen of uterus. The cup shaped placentome were first observed on day 36 and by day 43 in all ewes cup shape or C shaped placentomes were evident. Up to day 85 the placentomes were uniformly hyperechoic in texture. By day 92 the borders of placentomes started becoming more hyperechoic and within uniformly hypoechoic. As the gestation advanced the placentome hollowed out. Just before lambing the placentome widened and were distorted in shape. A moderately positive correlation was established for placentome length and gestational age between days 43 and 78 ($r = 0.685$; $R^2 = 0.470$). Using the regression equation developed for up to 78 days of gestation $y = 11.87x + 34.13$, 62 and 100% of pregnant ewes delivered within ±7 and ±14 days respectively of expected parturition dates. From this study it can be concluded that the echogenicity of placentome gives the rough estimate and progression of the gestation and the placentome length can be used to estimate gestational age in early pregnancy up to first half of the gestation in Nellore Brown ewes.

Keywords: Nellore brown ewes, estrus synchronization, transabdominal ultrasonography, placentome, hyperechoic, correlation, regression equation

Introduction
Sheep breeders need accurate information about gestation age of ewes to maximize survival rates of lambs, to form appropriate rations based on their nutritional needs and to dry off the lactating ewes at appropriate time [1] as lamb survival is an important factor affecting profitability in sheep production. Accurate mating date is usually unknown in most of the sheep flocks. Ultrasonographic assessment of placental and fetal measurements during pregnancy is useful to estimate gestational age and to obtain fetal growth curve in order to predict parturition date [2]. The placentomes are the most available structure of the pregnant uterus for ultrasound examination [3, 4] throughout pregnancy in small ruminants [1, 5]. The study was conducted to document the normal placentome growth throughout the pregnancy in Nellore Brown ewes using real time B mode ultrasonography, to establish the correlation coefficient and to construct prediction equation for gestational age besides its validation in field conditions.

Materials and Methods
Nellore Brown ewes (n=20) aged 1-4 years reared under semi intensive conditions, with standard management conditions fed with greens, concentrates and adlib fresh water and salt licks were selected for the study.

The ewes were subjected to estrus synchronization protocol of seven days using vaginal sponges and 300 IU of PMSG (Folligon, Intervet International, Boxmeer, Netherlands) and 75 µg of $PGF_{2\alpha}$ (Cloprostenol; Pragma, Intas Pharmaceutica Limited, Matoda, Ahmedabad) intra

muscularly at sponge withdrawl. Upon sponge withdrawl the synchronized ewes were kept with a ram, color painted, and each day ram was replaced with a new one with changed color paint at brisket region. The last day of receiving mating mark was treated as day 0 start of pregnancy. Ultrasonography was conducted using a real time B mode scanning (ALOKA SSD 500, Aloka co Ltd, Japan) equipped with 5 MHz convex transducer on mated ewes. Serial transabdominal ultrasonographic examinations were carried out on weekly basis starting from day 8 of mating until parturition. For transabdominal examination, the animals were restrained on dorsal recumbency and the transducer was placed on the hairless area of the ventral abdominal wall caudal and lateral to the udder. Once embryonic vesicle was identified the pregnancy was confirmed and scanning continued weekly until end of parturition. During scanning placentomes were identified and images frozen, saved and measured with built in electronic callipers. The transducer was manipulated until the largest section of placentome was obtained and measurements were done. Measurement of 2– 4 big sized placentomes were done and mean diameter of placentomes were calculated [6].

Field test: The ewes were randomly subjected to ultrasound scanning in farmers flocks in villages. Mean placentome length was calculated using 2 - 4 large placentomes. The lambing dates were obtained from farmers and calculated gestational age (GA) retrospectively assuming the average gestation length as 148 days.

Data analysis

The relationship of gestational age with placentome length was plotted as linear regression and expressed as quadratic line equation, the GA (days) was the independent variable (y) and the placentome length (cm) being the dependent variable (x), a 5% level of significance was used. Regression equation and correlation were established using statistical packages for social sciences (SPSS) version 20.

Results

Documentation of normal development of placentomes in Nellore Brown ewes using transabdominal ultrasonography:

The first placentome was observed on day 22 of scanning as echogenic dense line (Fig 1). By day 29 more than 50% of pregnant animals exhibited placentomes as raised nodular convex shaped structures that projected into the lumen of uterus making the gestational sac irregular in shape (Fig 2). First time cup shaped placentome were observed on day 36 and by day 43 in all ewes cup or C shaped placentomes (Fig 3) were evident. Up to day 85 the placentomes were uniformly hyperechoic in texture. By day 92 the borders of placentomes started becoming more hyperechoic with uniformly hypoechoic inner content. As the gestational age advanced the border of placentome exhibited intensified hyper echogenecity with anechoic inner content (Fig 4). As the lambing approached the cup shape of placentome widened straightened and became distorted in shape (Fig 5).

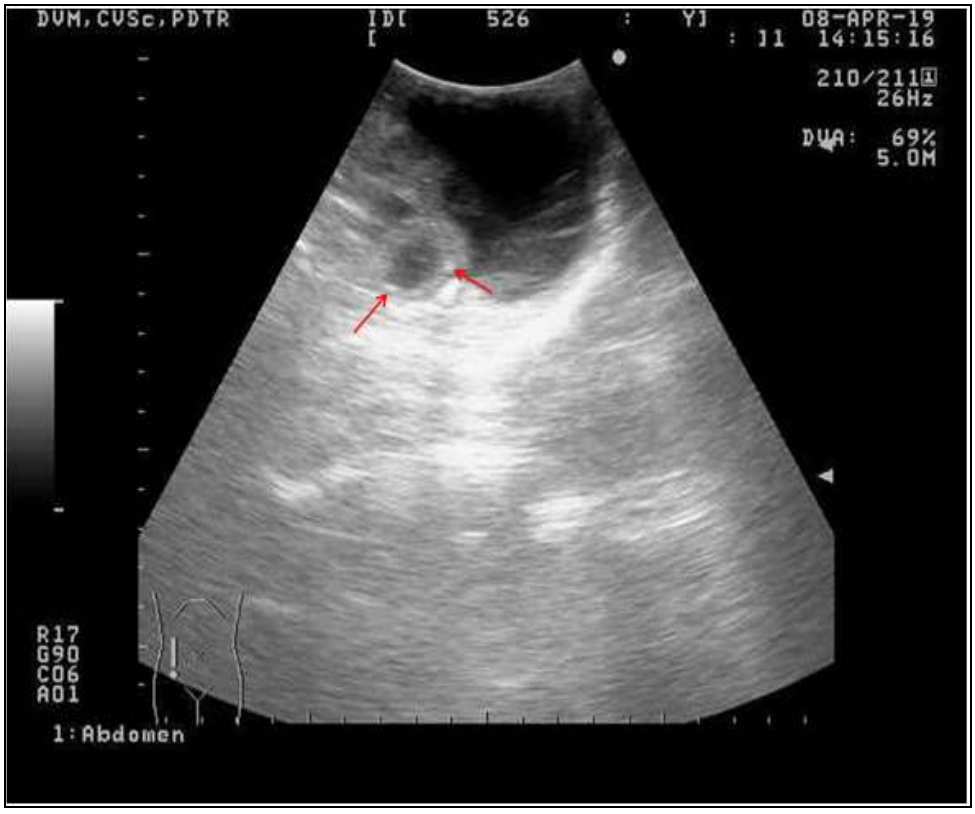

Fig 1: A 22 day gestational sac with echogenic densities (arrows) on endometrial surface

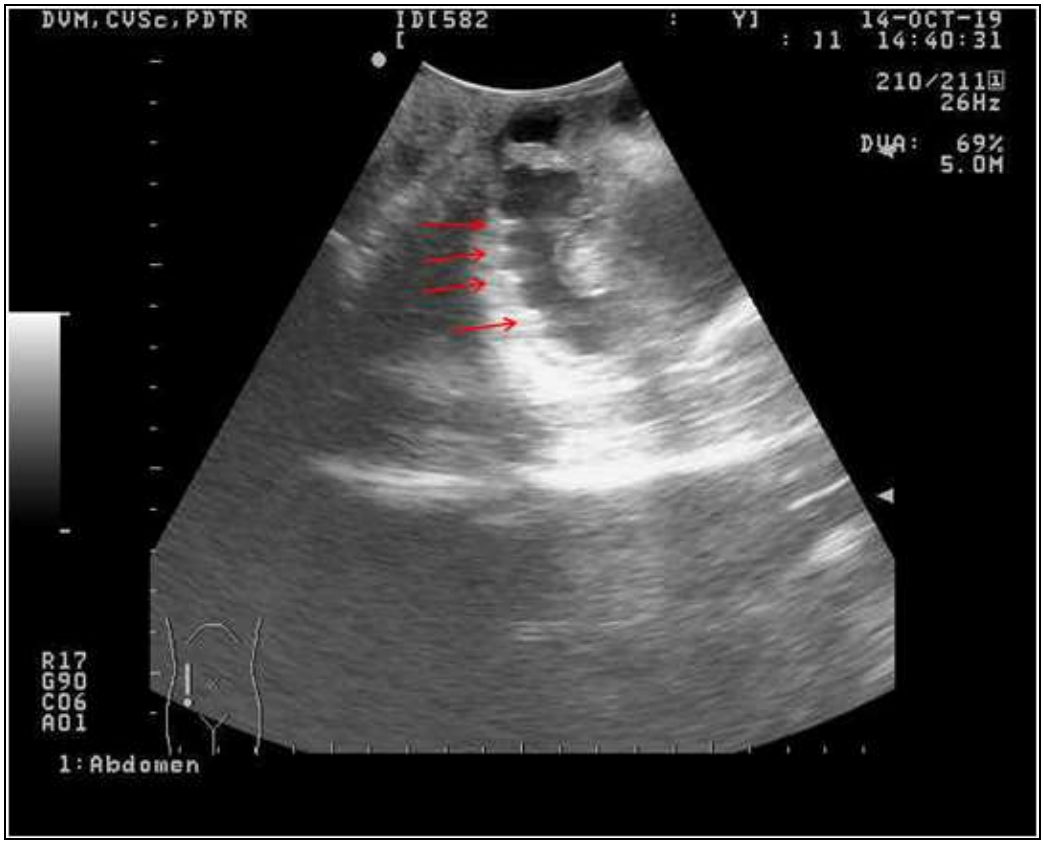

Fig 2: A 29 day gestational sac with raised, nodular placentomes (yellow arrows) making the lumen irregular in shape

Fig 3: Uniformly hyperechoic C shaped placentome (arrow) on day 64 of gestation in ewes

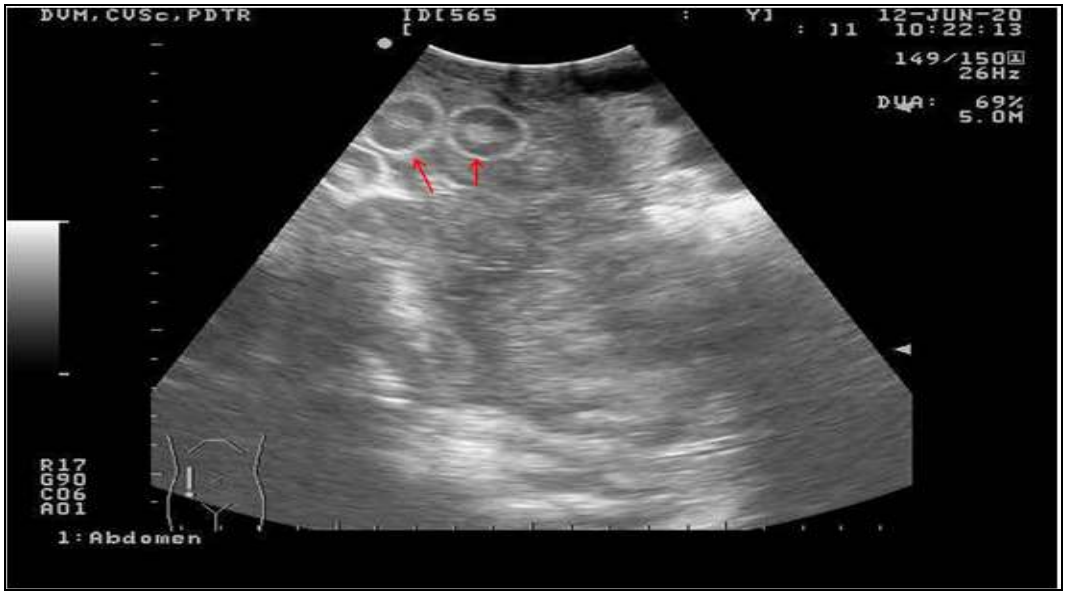

Fig 4: 'O' shaped placentomes with borders hyperechoic (arrow) and within hypoechoic on day 113 of gestation

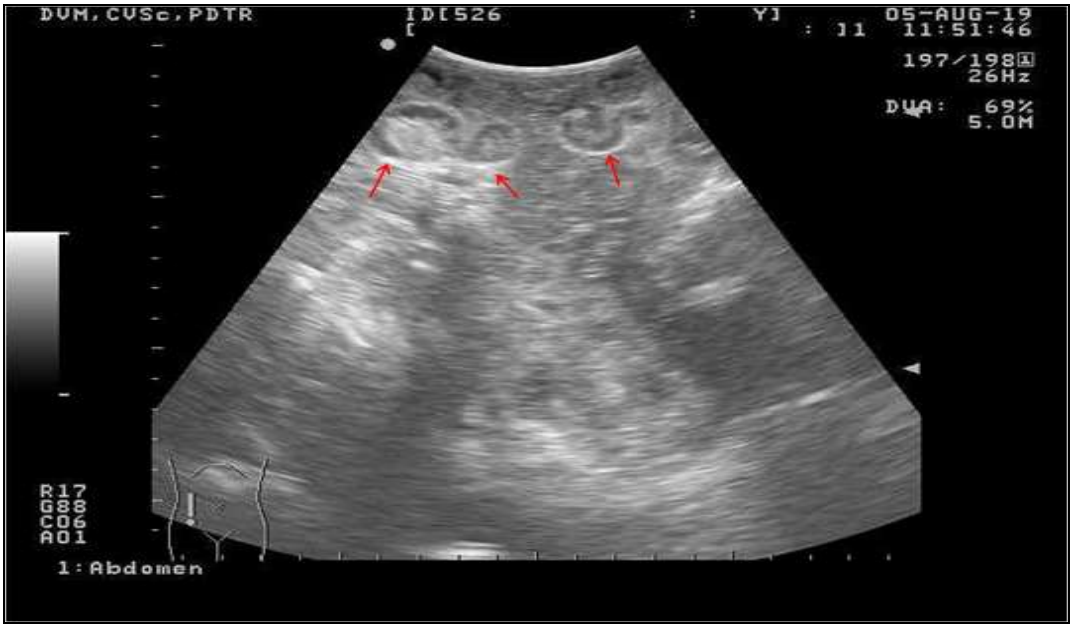

Fig 5: Distorted placentomes (arrows) on day 148 of gestation in Nellore Brown ewes showing indications of degeneration

The relationship between gestational age and placentome length:

The length of placentome was measured from day 43 to day 141of gestation. The mean placentome length measured as 1.25±0.13 on day 43, and showed a substantial increase in length until day 57 and to attain maximum mean length by day 78 (Table 1).

Low correlation was observed for placentome length and GA between days 43 and 141 (r = 0.05875; R^2=0.003) (Fig 6). However a moderately positive correlation was established for placentome length and GA between days 43 and 78 (r = 0.685; R^2 =0.470) (Fig 7).

Table 1: Ultrasonic measurements of placentome length (Mean±SEM) and gestational age in Nellore Brown ewes

Day of Gestation	Mean Placentome length (cm) (Mean±SE)
43	1.25 ± 0.13^{A}
50	1.85 ± 0.05^{B}
57	2.36 ± 0.09^{CD}
64	2.62 ± 0.13^{D}
71	2.66 ± 0.09^{D}
78	2.72 ± 0.12^{D}
85	2.51 ± 0.09^{CD}
92	2.36 ± 0.09^{CD}
99	2.42 ± 0.07^{CD}
106	2.24 ± 0.07^{BCD}
113	2.33 ± 0.09^{BCD}

120	2.26 ± 0.08^{BCD}
127	2.32 ± 0.13^{BCD}
134	2.12 ± 0.10^{BC}
141	2.05 ± 0.08^{BC}
ANOVA	F = 12.200; p = 0.000

Mean with different alphabet as superscript differ significantly ($p<0.05$)

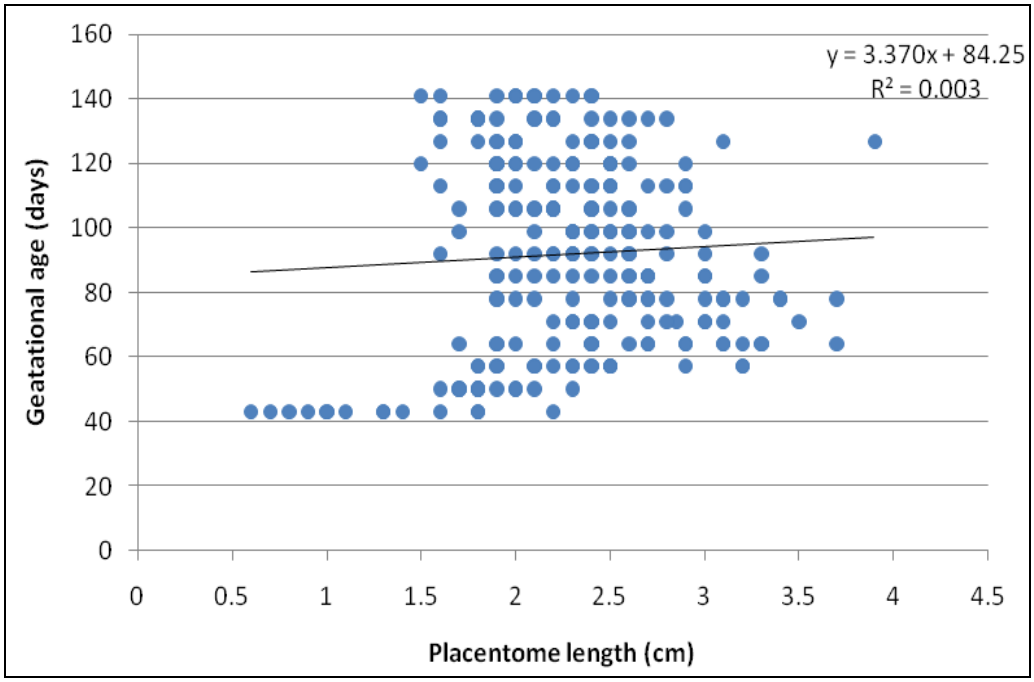

Fig 6: Scatter plot and linear regression line of placentome length and gestational age (days 43 to 141) in Nellore Brown ewes

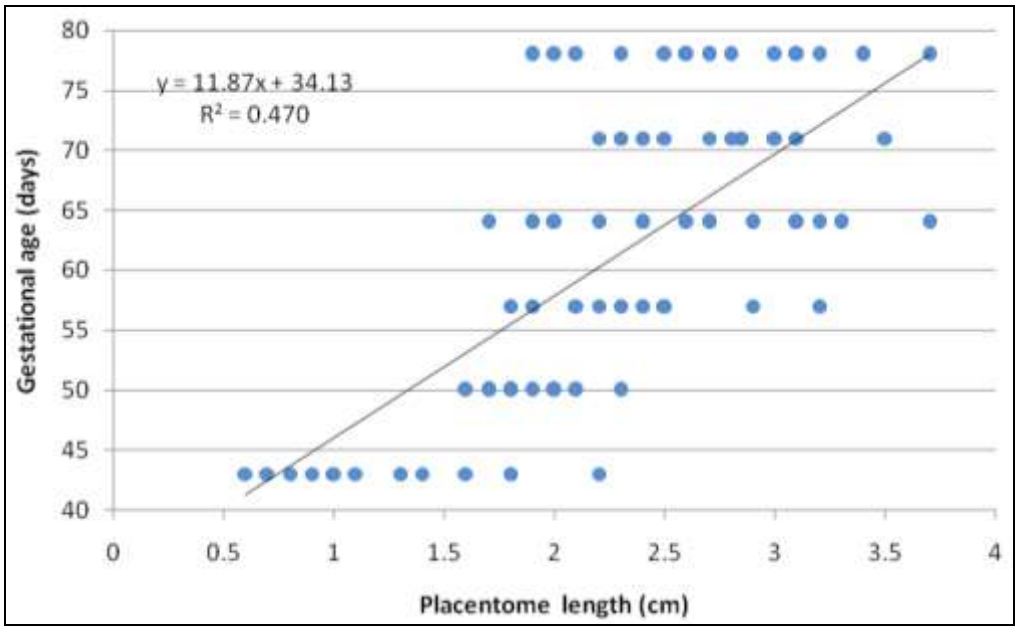

Fig 7: Scatter plot and linear regression line of placentome length and gestational age (days 43 to 78) in NB ewes

Regression equation for GA and placentome length and its validation in the field

A field test was conducted on ewes randomly, in villages and placentome length was measured when placentomes were in cup shape. The placentomes can be visualized and measured in 33 Nellore Brown ewes. However only eight ewes were in first half of gestation as per the lambing dates. Using the regression equation developed for up to 78 days of gestation y = 11.87x + 34.13, (where y is GA in days and x is placentome length in cm) 62 and 100% of pregnant ewes delivered within ±7 and ±14 days of expected parturition dates (Table 2)

Table 2: Validation of regression equation developed for up to 78 days of pregnancy using placentome length for the estimation of gestational age in the field

Fetal parameter measured	Total No of ewes	No of ewes in first half of gestation according to lambing date	Lambed ± 7 days	Lambed ± 14 days
Placentome	33	08	62% (5/8)	38% (3/8)

Discussion

In the present study placentomes were first observed on day 29 and 'C' like on day 36. These findings were in convergence with reports of Valasi [7]. The first detection of placentomes between day 27 – 32 has been reported by many authors [1, 8-10]. However Kasiksi [11] and Santos [12] observed placentome earlier than present study, on day 25 and 21 in sheep respectively. In 1939, Cloate [13] discerned placentome on day 21 of gestation and cup shape on day 30 in an anatomical study of ewe.

Ali and Heyder [14] first observed placentome on day 37.29±5.1 (range 30-42) in Ossimi sheep. Suguna [15], Kumar [16], Rasheed [17] and Haq [18] reported observation of placentomes on day 42, 35, 35 and 41 post breeding in goats respectively. These reports were in divergence with the present study findings. Doize [1] expressed difficulty in finding placentome in the first month of gestation since placentome lied very close to uterine wall, until it projects from endometrium into anecoic fluid filled uterine horns. The difference among the reports could be attributed to species difference, equipment quality, transducer frequency, examination position in addition to experience of operator [2].

The echogenecity of placentomes recorded in this study throughout pregnancy were similar to that of Ali and Heyder [14] in Ossimi sheep. These changes in the placentome may be attributed to increased placentome perfusion and permeability of tissues as the gestation advanced [19]. Near lambing collapsing of placentome might be an indication of degenerative process [20, 21].

The placentome length was measured from day 43 until day 141 in this study. However Suguna [15], Rasheed [17], Nwaogu [22] Airina [23], and Yaziki [24] measured placentome length between gestational days 50-130, 35- 135, 57-124, 42 to parturition, and 30-121 respectively in does. However Doize [1] stated that after day 90 it was difficult to measure length of placentome using transrectal ultrasonography due to increased distension of uterus.

The mean placentome length as measured on day 43 was 1.25±0.13 cm. This was in comparison with Rasheed [17] who reported placentome length as 12.35± 0.17mm on day 46 in does. The maximum length reported in our study was 2.72±0.12 cm. In contrast Kasiksi [11] and Rasheed [17] obtained placentome length as 34.02±0.05mm in ewes and 39.6±2.37mm in goats respectively. This variance might be attributed to species, breed difference, transducer frequency [17], number of placentomes measured and season [25].

Mean placentome length increased rapidly until day 57 and reached maximum by day 78 of gestation in Nellore Brown ewes. This was in agreement with findings of Doize [1] who reported the rapid development of placentome in ewes and maximum size attainment by day 74 in ewes. However in does they reported largest diameter attainment by day 91. Kasikci [11] also found rapid placentome development between 5th and 9th week of pregnancy and no significant change in the placentome size between the 10th and 20th weeks. Placentome reached maximum size in does by Day 126 as said by Rasheed [17].

Metodiev [26] who established moderate correlation and coefficients of determination (r = 0.68 and R^2=0.47) for placentomes in ewes during the first half of pregnancy were in accordance with our findings. They stated that Placentome size measurement was not a good indicator for prognosis of GA and could be used as auxiliary parameter for prognosis. Santos [12] opied that placentome was moderately reliable (R^2=71.6) to estimate GA between third and eighth weeks in sheep. After this period its growth not correlated with GA. Some researchers like Doize [1] (R^2= 15.59), Ali and Heyder [14] (R^2= 0.38) also reported weak positive correlation in ewes. Doize [1] considered breed of ewe as critical variable and recommended additional studies in ewes. The variation in the duration of gestation was 8 days for different breeds of ewes and this might be another reason for the poor correlation with placentome measurements [27].

However in goats some authors like Kuru [10] Suguna [15], Haq [18] Karen [28], Leigh and Fayemi [29], and Kandiel [30] reported high significant correlations and coefficient of determination, R^2 =0.908, r=0.99, R^2 =0.84, r=0.86, R^2 = 0.94 and R^2 =0.899 respectively for placentome length and GA. However low correlation were reported by Doize [1] (R^2= 70), Lee [6] (r=0.5740), Nwaogu [22] (r = 0.45). Nwaogu [22] opied that the placentome diameter was not an useful indicator for GA prediction in Red Sokoto goats. The difference in correlation between placentome length and GA may have resulted from the varience in placentome size and goat breeds [10].

With the advancement of gestation visualizing larger placentome was increasingly difficult further more consistent evaluation of the placentome throughout pregnancy not possible [12]. Haibel and Perkins [31] considered placentome a poor parameter for estimating GA as placentome size differed between tip of uterine horn and middle of uterus. Jenkinson [25] reported a variation of placentome development in relationship to season. Accuracy of GA for placentomes diameter might be reduced as smaller size placentomes might accidentally be taken for GA estimation. Even though to minimize the error 2-3 larger placentomes of representative size measured there still will be high chance for error when it practiced at farm level.

A field test was conducted using the regression equation developed for up to 78 days of gestation y = 11.87x + 34.13, and found that 62 and 100% of pregnant ewes delivered within ±7 and ±14 days of expected parturition dates which were in first half of gestation. However Doize [1] reported a difference of ±7 days between the expected and the real fetal age was found in 65% and ±14 days in 86%. Amle [32] also validated GA in Sangemneri and Osmanabadi goats using regression equation developed by Doize [1] for placentomes and found 87±0.94 and 78.08±1.6 days when observed GA was 88 and 81 days respectively.

In our study the regression equation developed for placentome length up to day 78 of gestation in ewes was used for GA estimation in the field. The discrepancy between real and expected gestational age could be attributed to broad range of duration of gestation in ewes, breed of ewe, occurrence of short estrous cycles [1] and time of ovulation. In the present field validation test, lambing dates recorded revealed that 24% of ewes were in first half of gestation. The echogenicity

of placentome provides rough estimate of gestational age. The small discrepancy between the actual age and estimated age may not be important enough to impact decisions regarding management or predicting lambing time of ewes carrying single or multiple fetuses.

Conclusion

From this study it can be concluded that the echotextural changes of placentome gives the rough estimate and progression of the gestation and the placentome length can be used to estimate gestational age in early pregnancy up to first half of the gestation in Nellore Brown ewes.

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