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Gestational Age Estimation and Validation using Ultrasonic Measurements of Fetal Biparietal Diameter and Occipito Nasal Length in Nellore Brown Ewes

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ABSTRACT

Gestational age estimation in small ruminants helps in nutritional management of pregnant ewes and optimizes the lamb survival. Nellore Brown ewes (n = 20) were subjected to short day estrus synchronization protocol and weekly once transabdominal ultrasonography from day 8 onwards until parturition. The biparietal diameter and occipito nasal length of fetal head measured from day 43 until day 113 of gestation. A positive correlation was obtained between gestational age and BPD (r = 0.9687) and ONL (r = 0.9873) and regression equations generated as $y = 15.94x + 28.43$ and $y = 9.471x + 25.66$ respectively for BPD and ONL where y is GA and X is respective head measurements. For validation of equations, BPD and ONL were measured in 21 and 12 pregnant ewes respectively, in field test. For linear relationship 61.9 and 71.4% of pregnant ewes delivered within ±7 and ±14 days of expected parturition dates for BPD and 58.3, and 100% of pregnant ewes delivered within ±3 and ±11 days of expected parturition dates for ONL. It was concluded that the ultrasonic measurements of fetal head diameters were well correlated with gestational age and can be used for estimation of pregnancy duration, however, ONL was precise over BPD in predicting parturition dates in Nellore Brown ewes.

HIGHLIGHTS

- The fetal head diameters were used to estimate and validate gestational age in Nellore ewes
- The fetal occipito nasal length was proved better in correlation and prediction of lambing dates.

Keywords: Estrus Synchronization, Transabdominal ultrasonography, Correlation, Regression equation

Gestational age estimation in sheep provides their owners valuable information that aids in the late gestation management. Real time ultrasonography is useful in determining the duration of pregnancy. In small ruminants the most practical measurement of fetal structures are

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fetal head diameters (Karen *et al.,* 2009). These can be observed from middle of first trimester to the end of second trimester of pregnancy and found to have high correlation with gestational age (Lee *et al.,* 2005, Suguna *et al.,* 2008). The data for the domestic breeds are scanty and the current paper presents analysis of data derived by transabdominal sequential ultrasonic measurements of bipariertal diameter (head width) and occipito nasal length (head length) in Nellore breed and validation of regression equations generated.

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 next day of receiving last mating mark was treated as day 1, start of pregnancy. Transabdominal 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 ultrasonographic examinations were carried out on weekly basis starting from day 8 of mating. Once embryonic vesicle was identified the pregnancy was confirmed and scanning was performed weekly till end of gestation. During scanning biparietal diameter and occipito nasal length were identified and images frozen, saved and measured with built in electronic callipers.

Biparietal Diameter (BPD)

It is the maximum diameter of the head width between two parietal bones and measured between day 43 to 113 (Fig. 1).

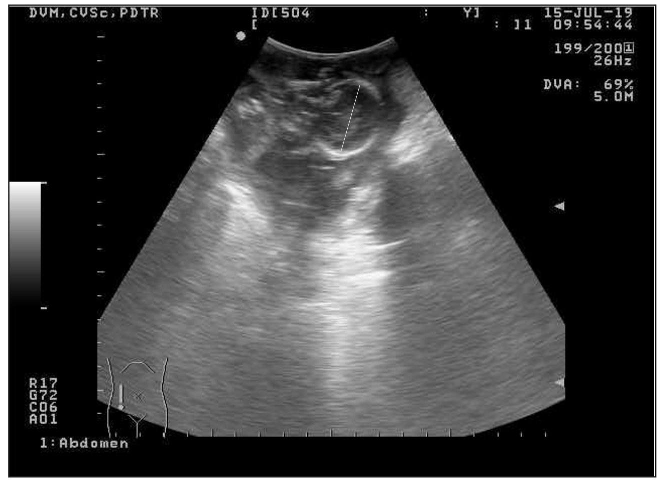

Fig. 1: Biparietal diameter measured in Nellore Brown ewes

Occipito nasal length (ONL)

The head length measured from the top of Os nasale to the end of Os occipital and measured between day 43 to 113 (Fig. 2).

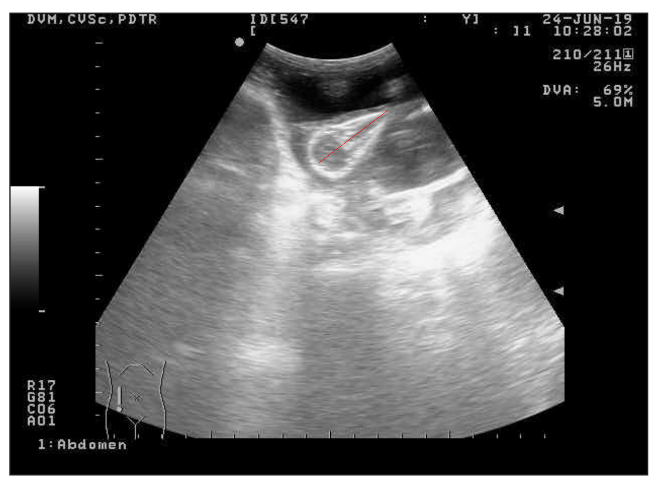

Fig. 2: Occipito nasal length measured in Nellore Brown ewes

Field test

The ewes were randomly subjected to ultrasound scanning in farmers' flocks in villages. BPD and ONL were measured in 21 and 12 pregnant ewes, respectively. The gestational age was estimated using the regression equations generated in experimental animals. The lambing dates were obtained from farmers and gestational age was calculated retrospectively taking the average gestation length as 148 days.

Data analysis

The relationship of gestational age with BPD and ONL was plotted as linear regression and the GA (days) was the independent variable (y) and the head diameters (cm) being the dependent variable (x), a 5% level of significance was used using statistical packages for social sciences (SPSS) version 20. In the field test, Mean square errors method used to compare fetal parameters.

RESULTS AND DISCUSSION

By day 36, head and trunk of the embryo could be visualized, by day 43, a clear organization of the fetus into head, neck and body was observed in this study and these findings were in agreement with Ali and Hayder (2007) and Valasi *et al.* (2017) who reported differentiation of embryo into head, body and limbs on 38 ± 3.2 and 35 days respectively. On the contrary, Kumar *et al.* (2015) reported organization of fetal body on day 42, later than recorded in this study while Santos *et al.* (2018) reported in 4th week, earlier than observed in this study. In 1939, Cloette grossly observed these structures between days 28 and 35 after conception.

The Orbits of the eye observed as anechoic pockets, mandible, nasal bone ossification was noticed in few by day 43. Ossification of the head bones was observed by Day 50. Present findings were in corroboration with Ali and Hayder (2007) and Kumar *et al.* (2015) who reported skull ossification between 42 - 48 days of gestation and differed from Valasi *et al.* (2017), who reported mandible and orbit walls ossification on day 36 and head bones ossification on day 56. These differences could be attributed to breed variation (Kumar *et al.,* 2015), individual differences and inability to establish precise timing of ovulation as well as fertilization (Valasi *et al.,* 2017).

The BPD was detected from day 36 until end of pregnancy. However, measured between day 43 to 113, after that the BPD exceeded the ultrasound screen length and obtaining images became difficult. Even though, head was detected from day 36, BPD could be measured in 50% (10/20) of ewes only on day 43. However, by day 50 BPD was measurable in all ewes. Many authors (Sergeev *et al.,* 1990; De Bulnes *et al.,* 1998; Airina *et al.,* 2011; Santos *et al.,* 2018 and Haq *et al.,* 2020) measured BPD between day 32 and 125 in ewes and goats. Haibal and Perkins (1989) stated that it was not possible to obtain symmetrical images of skull before 40-50 days of pregnancy. Sergeev *et al.* (1990) opined that fetal skull was clear and defined between 49 – 84 days and was easy to measure, however fetal positioning influenced the ease of obtaining an image suitable for measurement.

In present study, BPD could be measured in all ewes until day 99, however on day 106 and day 113, head width could not be measured in 20% and 45% ewes respectively either due to fetal positioning or fetal size. This was in agreement with findings of Haibel (1988) who stated that the BPD measurement becomes difficult after day 105 of gestation due to variability of fetal head location and posture which may be anywhere in the abdomen from the inguinal region to the xiphoid (Jones and Reed, 2017) and diminished uterine fluids as proportional to uterine contents often defies measurements of head in transabdominal position. Kelly and Newnham (1989) also experienced constraints in measurement of head width from about day 100-110 of gestation. It could be due to increasing calcification of bones and shadowing effects. Sergeev *et al.* (1990) were unable to measure head width in the later stages of their study between day 106 to 119, due to inadequate depth of penetration of the 5MHz transducer employed. Haq *et al.* (2020) detected head occasionally after day 125, in the last month of pregnancy. This difficulty was attributed to the increase in fetal size and compression of the head by other fetal parts (Amer, 2010).

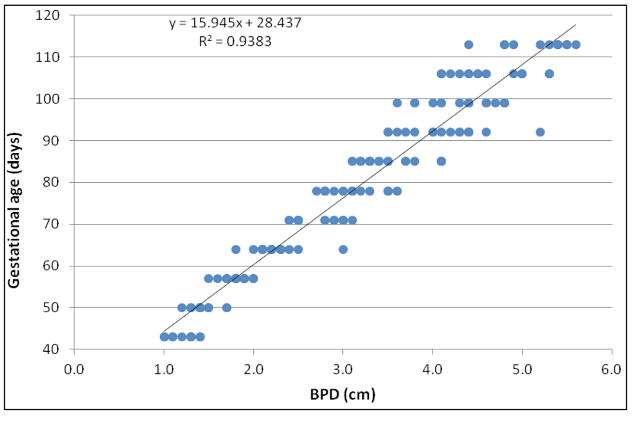

Fig. 3: Scatter plot and linear regression line of BPD and GA in Nellore Brown ewes

A high positive correlation (r = 0.96867; R^2 = 0.938) was derived between GA and BPD in the present investigation. These results were in consistent with findings of Sergeev *et al.* (1990), De Bulnes *et al.* (1998) and Gunduz *et al.* (2010) who reported correlation as r = 0.961, 0.96 and 0.977 respectively between GA and BPD in sheep and goats. Kuru *et al.* (2018) also reported similar results in Abaza (R^2 = 0.925) and Gurku (R^2 = 0.928) goats.

However, higher correlation (0.97-0.99) was reported by Haibel (1988), Haibel and Perkins (1989), Raichle and Haibel (1991), Suguna *et al.* (2008) and Santos *et al.* (2018) between GA and BPD in sheep and goats than the present study. Lower correlation in the present study might be attributed to the fact that the scanning was extended to third trimester, the intervals between consecutive scanning and breed differences. BPD measurements at early gestational ages (day 40 -50) had moderate variability due to depth of the uterus in the abdomen (Haibel, 1988). In contrast some authors like Abdelghafar *et al.* (2011), Venkato *et al.* (2013) Petrujkic *et al.* (2016) and Haq *et al.* (2020) reported lower coefficient of determination (0.75 – 0.91) between GA and BPD in sheep and goats.

In the present investigation, ONL was detected from day 36 until end of parturition however measurements could be recorded between days 43 and 113. This was in consistent with Santos *et al.* (2018) who measured ONL from 6th week of gestation, while De Bulnes *et al.* (1998) and Yazici *et al.* (2018) recorded ONL earlier than this study. However, Nwaogu *et al.* (2010) and Petrujkic *et al.* (2016) obtained measurements in later gestational days (from day 57 and day 46 respectively) than current study.

Nwaogu *et al.* (2010) measured ONL up to day 124 which is later than this study, While Kelly and Newnham, (1989), De Bulnes *et al.* (1998), Petrujkic *et al.* (2016), Santos *et al.* (2018) and Yazici *et al.* (2018) took measurements up to day 95, 91, 63, 98 and 74 respectively in sheep and goats. The limited observations made for ONL was due to specific fetal posture (Khan *et al.,* 2015) or fetal dimensions exceeding the width of transducer in many ewes (Kelly and Newnham, 1989). From 13 week onward it was difficult to measure nasal to snout length due to fetal size, ultrasonographic characteristics, positioning difficulty and fetal movement (Santos *et al.,* 2018).

A high positive correlation (r = 0.9873 and R^2 = 0.974) was established between ONL and gestational age in the present study. The correlation developed between ONL and GA was in accordance with the findings of Petrujkic *et al.* (2016) and Yazici *et al.* (2018). In contrast, De Bulnes *et al.* (1998), Nwaogu *et al.* (2010), Khan *et al.* (2015) and Santos *et al.* (2018) reported lower correlation (r=0.7 to 0.97).

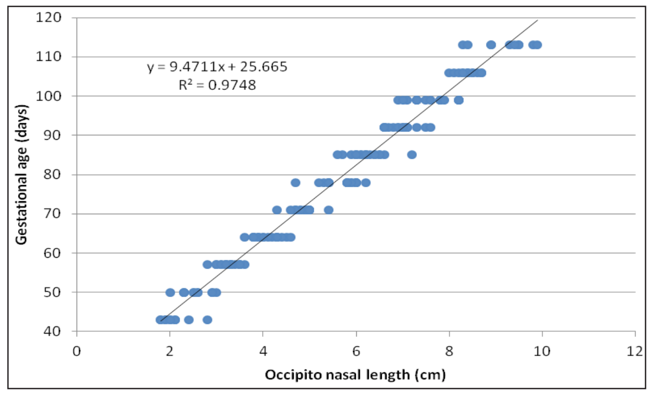

Fig. 4: Scatter plot and linear regression line of ONL and GA in Nellore Brown ewes

The mean BPD and ONL (Table 1) showed significant increase ($p<0.05$) during the measurement period.

Table 1: Range and Mean (±SE) of BPD and ONL of fetus during pregnancy in Nellore Brown ewes

GA	BPD		ONL	
	Mean±SE	Range	Mean±SE	Range
43	1.19 ± 0.05^{a}	1.0-1.4	2.08 ± 0.10^{a}	1.8-2.8
50	1.43 ± 0.04^{ab}	1.2-1.7	2.54 ± 0.09^{a}	2.0-3.0
57	1.80 ± 0.03^{bc}	1.5-2.0	3.25 ± 0.05^{b}	2.8-3.6
64	2.22 ± 0.06^{c}	1.8-3.0	4.08 ± 0.06^{c}	3.6-4.6
71	2.71 ± 0.06^{d}	2.4-3.1	4.85 ± 0.06^{d}	4.3-5.4
78	3.13 ± 0.07^{de}	2.7-3.6	5.67 ± 0.09^{e}	4.7-6.2
85	3.49 ± 0.08^{e}	3.1-4.1	6.22 ± 0.09^{e}	5.6-7.2
92	4.14 ± 0.11^{f}	3.5-5.2	6.86 ± 0.15^{f}	4.6-7.6
99	4.33 ± 0.09^{fg}	3.6-4.8	7.58 ± 0.12^{g}	6.9-8.2
106	$4.64\ ^{G}\pm0.11^{g}$	4.1-5.3	8.39 ± 0.06^{h}	8.0-8.7
113	5.18 ± 0.11^{h}	4.4-5.6	9.19 ± 0.17^{i}	8.3-9.9

*Means with different superscripts differ significantly ($p<0.05$) within columns.

Validation of prediction equations in the field

In the farmers flocks in 21 pregnant ewes BPD measurements were taken for validation and it was found that 61.9 (13/21) and 71.4 (15/21) % of deliveries occurred within ±7, and ±14 days of expected lambing dates. Amle *et al.* (2014) evaluated the equation developed by Abdelghafar *et al.* (2007) for BPD in Sangamneri and Osmanabadi goats (n=10 each) and found GA as 85.99 ±1.29 and 81.03 ± 2.16 days when observed GA was 88 and 81 days respectively. However, Haq *et al.* (2020) validated regression equation for BPD and recorded difference between actual and estimated day of gestational stage was 6.8± 1.0 days in Beetal goats ($n = 20$). In human fetuses the measurement of BPD before gestational week 20 predict the gestational age with an accuracy of ±7-11 days and the precision tolerance of BPD dimension decreased during the third trimester of pregnancy (Hadlock *et al.,* 1991).

The regression equation for BPD used in this study was applicable between day 43 – 113 of gestation only. Further, the BPD measurements taken in the third trimester may not be true to its actual value due to fetal head positions which might lead to incorrect estimation of gestational age. Gestational age is best estimated by measurements in early pregnancy since at later gestational ages these measurements reflects the growth characteristics of the fetus rather than its age (Kurtz and Needleman, 1988). Prediction of GA would be increasingly unreliable from day 80 onwards due to differtial growth rates of skull even if accurate measurements were made as opined by Kelly and Newnham (1989).

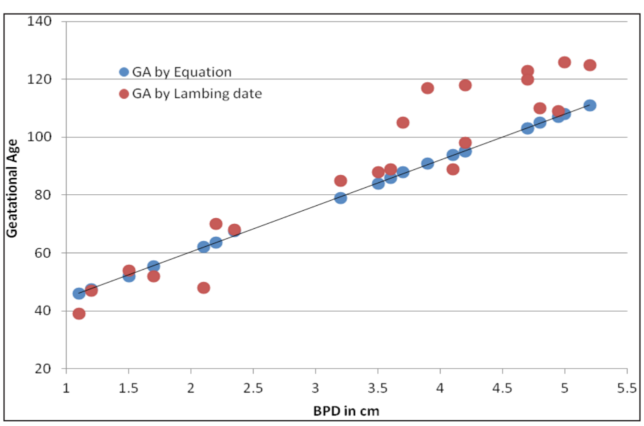

Fig. 7: Validation of Biparietal diameter measurements under field conditions

In farmers flocks in villages in 12 pregnant ewes ONL measurements were obtained and validated. 58.3 (7/12) and 100 (12/12) per cent of ewes lambed within ±3 and ±11 days of expected lambing dates, respectively, for ONL The highest correlation $r = 0.9873$ obtained for ONL in the present study justified more than 50% of deliveries occurring within ±3 days of expected dates. No previous literature for validation of ONL in field condition obtained.

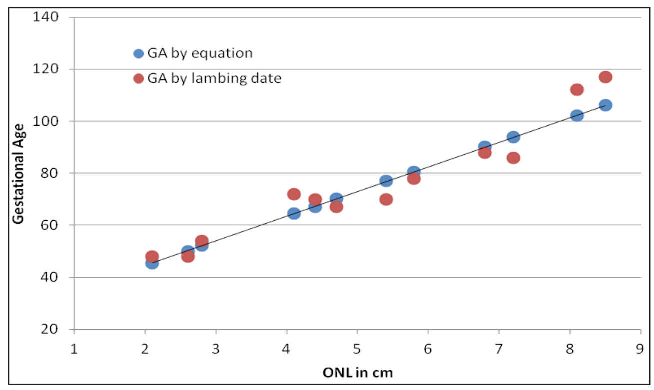

Fig. 7: Validation of occipito nasal length measurements under field conditions

CONCLUSION

It was concluded that the ultrasonic measurements of BPD and ONL of the fetus could be valuable in the estimation of gestational age and for the use of the regression formulae to predict the parturition under field conditions, however ONL measurements gave better prediction than BPD and it is recommended to conduct the study on large number of ewes.

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