The Haryana Veterinarian (September, 2023) **62(SI-2),** 38-41 ISSN 0033-4359 (P) **Research Article**

TRANS ABDOMINAL ULTRASONOGRAPHIC MEASUREMENT OF EMBRYONIC VESICLE DIAMETER AND CROWN RUMP LENGTH AIDS IN GESTATIONAL AGE DETERMINATION IN NELLORE BROWN EWES IN EARLY PREGNANCY

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ABSTRACT

Gestational age estimation in small ruminants helps in nutritional management of pregnant animals and optimizes the young one survival. In this study, Nellore Brown ewes (n=20) were subjected to short day estrus synchronization protocol and trans abdominal ultrasonography once weekly from day 8 of last mating until parturition. The embryonic vesicle diameter (EVD) and crown rump length (CRL) of fetus measured from day 22 and 29, respectively until day 64 of gestation. A positive correlation was obtained between gestational age and EVD (r=0.902) and CRL (r=0.916) and regression equations were generated as y=5.319x+18.09 and y=3.808x+27.23, respectively for EVD and CRL where y is gestational age (GA) and X is respective embryonic measurements and these prediction equations were validated under the field conditions. It was concluded that the ultrasonographic measurements of embryonic measurements were well correlated with gestational age and can be used for estimation of pregnancy duration; however, to define the significance of equations in the field a large data is needed.

Keywords: Estrus synchronization, Ewe, Nellore, Transabdominal ultrasonography

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Gestational age estimation in sheep provides their owners valuable information that aids in the late gestation management as lamb survival is an important factor affecting the sheep profitability (Ekiz *et al.*, 2005). Ultrasonographic fetometry allows not only estimation of fetal age but also determination of sex and measurement of number of foetuses carried, assessment of vitality of fetus and diagnosis of pregnancy disorders which would be useful for specific clinical management or research applications. Numerous researchers used transrectal or transabdominal B mode ultrasonography to predict gestational age by means of fetal measurements in small ruminants (Frank *et al.*, 1982; Ali and Hayder, 2007). Various embryonic/fetal parameters have been studied so far for estimating gestational age. However, the most practical measurements of fetal structures are embryonic vesicle, crown rump length, fetal head diameters, placentome size (Karen *et al.*, 2001). One of the important limitation of estimation of gestational age using fetal parameters is their accessibility throughout the gestation (Airina *et al.*, 2011). The crown rump length (CRL) and embryonic vesicle diameter (EVD) were accessible only during the first trimester of gestation, however regarded as an important conformational parameters and can be done in relatively early stage of gestation than the other parameters (Petrujkic *et al.*, 2016). Hence the present study was undertaken to establish correlation and construct prediction equations for embryonic parameters in Nellore brown ewes.

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MATERIALS AND METHODS

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

Estrus Synchronization protocol: The ewes were subjected to short term estrus synchronization protocol of seven days using indigenous vaginal progesterone sponges (Avikesil-S® ICAR-CSWRI, Avikanagar, Rajasthan) containing 350 mg of natural progesterone and 300 IU of PMSG (Folligon®, Intervet International, Boxmeer, Netherlands) and 75 µg of $PGF_{2\alpha}$ (Cloprostenol; Pragma®,

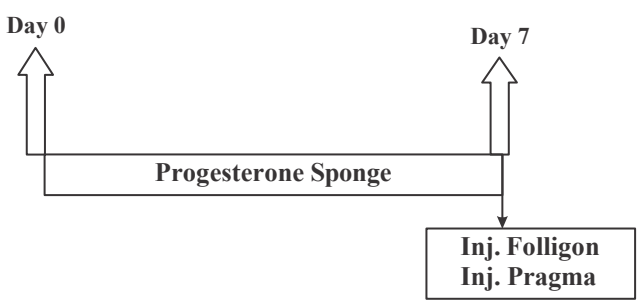

Table 1. Range and Mean (±SE) of EVD and CRL of fetus during pregnancy in Nellore Brown ewes

Gestational age	EVD (Mean± SE)	Range (Min-Max)	CRL (Mean± SE)	Range (Min-Max)
22	1.48±0.14[a]	0.9-2.3	-	
29	2.22 ±0.16[a]	1.2-3.6	1.26±0.13[a]	0.5-1.9
36	3.49 ±0.17[b]	2.4-4.9	2.19 ±0.20[a]	1.0-3.6
43	4.67 ±0.15[c]	3.6-5.9	4.03 ±0.18[b]	2.7-5
50	5.79 ±0.17[d]	4.3-6.6	5.93 ±0.36[c]	4.5-7.7
57	6.77 ±0.19[d]	4.6-8.0	7.70 ±0.25[d]	5.2-8.6
64	8.73 ±0.27[e]	7.6-10.6	9.28 ±0.27[e]	8.2-10.3

*Means with different superscripts differ significantly (P<0.05) within columns

Intas Pharmaceutica Limited, Matoda, Ahmedabad) intra muscularly at sponge withdrawal.

Breeding of the synchronized ewes: Upon sponge withdrawal the synchronized ewes were kept with a ram, color painted (mixture of glycerin and dye) and mating marks were recorded both morning and evening for three days. 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 or start of pregnancy.

Procedure followed for ultrasonography: 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 the pregnancy was confirmed, the scanning was continued weekly and embryonic vesicle diameter and crown rump lengths were identified and images were frozen, saved and measured with built in electronic calipers.

Embryonic vesicle diameter (EVD): The largest diameter of the gestational sac was chosen as the measure of EVD between gestational days 22 to 64 (Fig. 1).

Crown rump length(CRL): The CRL was measured as a straight line between fetal crown and origin of the tail when the fetus was fully extended between gestational days 29 to 64 (Fig. 2).

Field test: The ewes were randomly subjected to ultrasound scanning in farmers' flocks in villages and EVD and CRL were measured in ten and nine 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 EVD and CRL was plotted as linear regression and the gestational age (GA; days) was the independent variable (y) and EVD and CRL (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

The EVD was detectable from day 22 till the end of parturition, however measurable from day 22 until day 64. The EVD was measured between days 12 to 65 in ewes and does by various researchers (Airina *et al.*, 2011; Petrujkic *et al.*, 2016; Santos *et al.*, 2018, Devi *et al.*, 2019; Haq *et al.*, 2020). Haq *et al.* (2020) reported non-detectability of amniotic vesicle diameter 65 days post mating. In contrast, Santos *et al.* (2018) stated that at 7th week of gestation itself gestational vesicle could no longer be measured due to increase in fetal size. These variations of duration of measurements might be due to method of ultrasonography used to measure gestational vesicle and rapid accumulations of fetal fluids making uterus too large to be accurately measured.

On day 22 in 40 per cent of animals (8/20) EVD could be measured and in others could not identified until Day 29. On day 64 in 55 per cent of animals (11/20) EVD could be measured and in rest exceeded the ultra sound screen. High positive correlation (r=0.9497) (p<0.05) was obtained between EVD and GA and regression equation was generated (Fig. 3). These findings were in agreement with Petrujkic *et al.* (2016) and Haq *et al.* (2020), however, Santos *et al.* (2018) reported lower correlation R^2 = 0.689 and r= 0.76, respectively. This could be due to the difficulties to visualize the entire transverse or longitudinal images, accessibility of the sac, number of days in gestation measured, method of ultrasound used and breed of ewe studied. Santos *et al.* (2018) generated prediction equation as GW=3.1+0.1×GV where GW is gestational week and GV is gestational vesicle in Santa Inn sheep. Haq *et al.* (2020) had given regression equation as y=1.7383x-27.79 where y is EVD in mm and x is gestational age in days in Beetal goats.

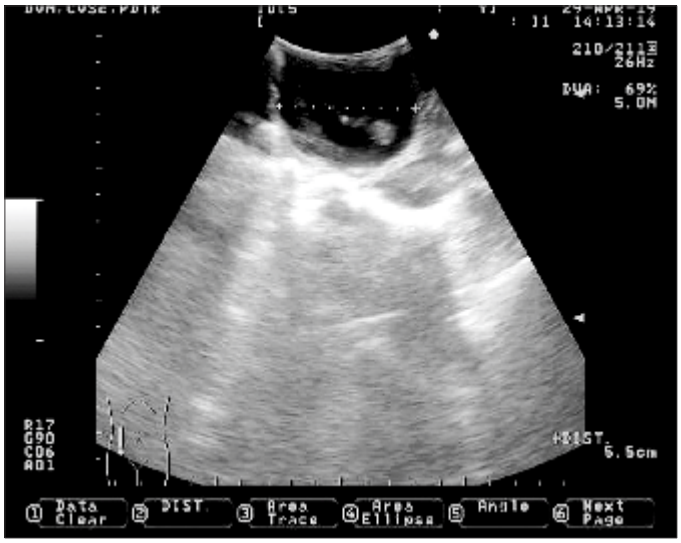

Fig. 1. EVD measured in Nellore Brown ewes

Fig. 2. Crown rump length measured in Nellore Brown ewes

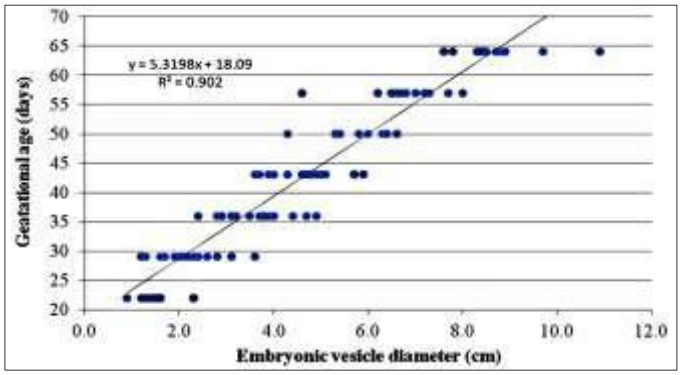

Fig. 3. Scatter plot and linear regression line of EVD and GA in Nellore Brown ewes

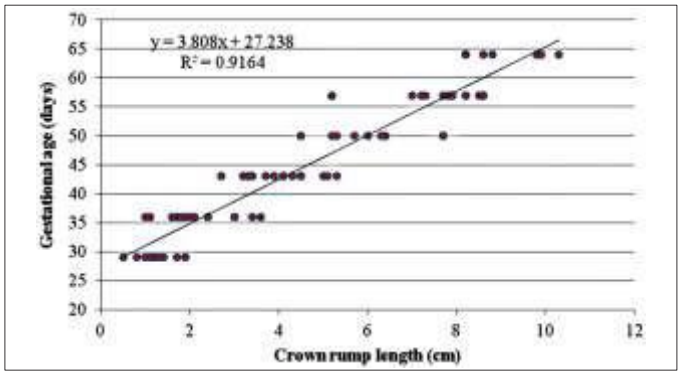

Fig. 4. Scatter plot and linear regression line of Crown Rump Length and gestational age in Nellore Brown ewes

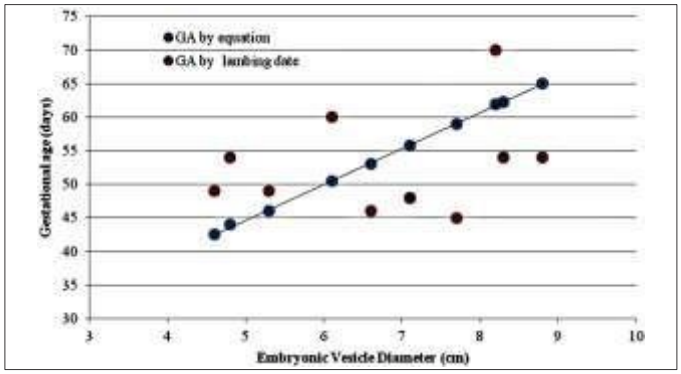

Fig. 5. Validation of EVD measurements under field conditions

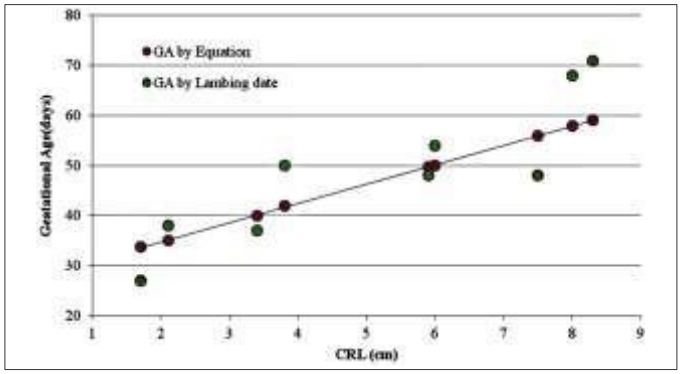

Fig. 6. Validation of CRL measurements under field conditions

The CRL was detectable from day 22 till day 71 however, was measurable between days 29 to days 64 and exceeded the ultrasound screen limits after that.

In the present study, the chance to access CRL during the gestational period between 29 and 64 was 62% (74/120) and 38% of CRL observations were inaccessible either due to immeasurable embryo (day 29) or fetus exceeding of screen of ultrasound (day 64). The accessibility of various fetal parts for scanning depends on the method of scanning. Transabdominal scanning was much inferior to that of the transrectal in the early pregnancy.

A high positive correlation was established (r=0.9573) between CRL and GA and regression equation was generated (Fig. 4). These results were in accordance with Abdelghafar *et al.* (2011), Kuru *et al.* (2018) and Haq *et al.* (2020), however, Metodiev *et al.* (2012) and Santos *et al.* (2018) reported lower correlation and Abubakar *et al.* (2016) reported a slightly higher correlation than this study. These discrepancies could be explained by

difference in breeds of sheep, intervals between consecutive scanning between studies difficulty in acquiring high quality images due to fetal size, position and movements and inaccurate measurement of the CRL (Abubakar *et al.*, 2016). Abubakar *et al.* (2016) developed regression equation in Jamnapari goats between day 37 -72 gestational age as y=18.776+0.606x where y is gestational age and x is CRL. Santos *et al.* (2018) expressed the relationship as GW = 2.65+0.11×CR where GW is gestational week and CR is CRL in Santa Inn ewes. Abubakar *et al.* (2016) opined that CRL is the best predictor of GA however Santos *et al.* (2018) considered CRL as less accurate to estimate GA.

Validation of prediction equations in the field: A field test was conducted on ewes in surrounding villages for GA estimation and EVD was measured in 10 pregnant ewes and CRL was measured in nine pregnant ewes. The regression equation established for EVD and CRL was used to predict the GA. For linear relationship 30 (3/10) and 100 (10/10) per cent of pregnant ewes delivered within ±7 and ±14 days of expected parturition dates, respectively (Fig. 5) for EVD and 55.5 (5/9) and 100 (9/9) per cent of pregnant ewes delivered within ±7 and ±12 days of expected lambing dates, respectively (Fig. 6). The high correlation established in this study for EVD was justified in the field study by recording 100 per cent of parturitions within ±14 days of expected parturition dates. Estimation of GA was more accurate during early pregnancy since the values obtained in latter gestation were also affected by the individual characteristics of the fetus. Godfrey *et al.* (2010) validated the accuracy of regression equation developed for CRL in ewes by scanning a set of ewes (n=51) with known breeding dates. There is much scope to extend the study to more number of ewes in field to solidify the relationship between the GA and embryonic parameters.

CONCLUSION

It could be concluded that the embryonic parameters studies in the present study were well correlated and validated with gestational age in early pregnancy. Further research is required to define the significance of these equations in the field conditions.

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