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## Detection of pregnancy with the determination of foetal indices in Assam hill goat

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**Abstract**

The present study was conducted for pregnancy diagnosis and determination of gestational age by measuring the diameter of embryonic vesicle (mm), length of the embryo proper (CRL, crown rump length, mm) and diameter of the foetal head (BPD, Biparietal Diameter, mm) in 30 does using transrectal and transabdominal ultrasonography. The embryonic vesicle was possible to detect from day 19 after breeding onwards with a diameter of  $10.52 \pm 0.58$  mm and increased gradually till day 35 after breeding with the diameter of  $28.21 \pm 0.33$  mm using transrectal ultrasonography. But by transabdominal ultrasonography it was possible to detect the embryonic vesicle from day 25 after breeding onwards with a diameter of  $18.16 \pm 0.91$  mm and gradually increased to  $27.45 \pm 0.48$  mm till day 35 after breeding. The second parameter is the measurement of the length of the embryo proper. The embryo proper was first visible on 21 day after breeding with a diameter of  $6.99 \pm 0.36$  mm and gradually increased till day 50 after breeding with a diameter of  $42.09 \pm 0.87$  mm by transrectal method. But it was not possible to measure the fetus due to increase in size of the fetus. The embryo proper was visible on day 25 after breeding with a diameter of  $8.31 \pm 1.52$  mm and gradually increased till day 50 of pregnancy with a diameter of  $41.52 \pm 1.07$  mm by transabdominal method and also similarly it was difficult to measure the length of the embryo after day 50 after breeding. The third parameter was the measurement of the BPD. It was possible to detect the foetal head only after day 40 after breeding by both the methods with a diameter of  $23.79 \pm 0.29$  mm and  $23.49 \pm 0.42$  mm but was not possible after day 80 and day 90 after breeding by transrectal and transabdominal ultrasonography. The present study could be concluded that it was possible to detect pregnancy as well as the gestational age by measuring all the three parameters.

**Keywords:** Ultrasonography, embryonic vesicle, CRL, BPD, Assam hill goat

**Introduction**

Early detection of pregnancy is one of important aspects in reproductive management and should be performed after mating. Early detection of pregnancy can offer rapid information of mating success so that production efficiency due to infertility could be achieved and in turn would reduce the expense of breeding program and aid livestock management economically. Furthermore, faster evaluation and differentiation between pregnant and non-pregnant livestock could increase the efficiency of reproduction and reduce the production cost (Gonzalez *et al.*, 2004; Karen *et al.*, 2004) [8, 22]. The development of a method to accurately estimate the stage of pregnancy when precise mating dates are not available would assist management to maximize survival rates of off spring (Greenwood *et al.*, 2002) [9]. The use of ultrasonography could also be performed to observe the embryo and fetal development in order to estimate the gestational age of Garut lamb (Amrozi and Setiawan, 2011) [3]. Ultrasound image of certain organ from fetus could be utilized to estimate gestational age. The measurement of fetal body and head diameter obtain high accuracy in estimating gestational age and determining birth (Goddard, 1995) [6]. Since pregnant and non-pregnant animals require different care and feeding regimes, ultrasonographic methods have been used in the past to estimate gestational age (Haibel 1988; Haibel *et al.*, 1989 [15]; Doize *et al.*, 1997 [5]; Karen *et al.*, 2009) [23]. B-mode ultrasonography is a fast and reliable method used to check for pregnancy in small ruminants. Transrectal ultrasonography is significantly better than the transabdominal method in obtaining more accurate results during the early gestational period (days 27–30) (Ishwar 1995 [18]; Doize *et al.*, 1997 [5]; Gürlér and Kaymaz, 2011 [10]; Karadaev *et al.*, 2016) [21]. BPD with ultrasonography is commonly used to determine foetal age in humans (Kieler *et al.*, 1995 [24]; Sailaja *et al.* 1996) [32]. And these days it has been extended to sheep and goat. For determination of gestational age, measurement of the diameter of the foetal embryonic vesicle (González de Bulnes A *et al.* 1998 [7]; Martineze MF *et al.*, 1998 [26]; Suguna *et al.*, 2008) [37], length of the embryo proper, CRL (Santiago-Moreno J *et al.*, 2005 [33];

Karen *et al.*, 2009<sup>[23]</sup>; Hussein AA, 2010<sup>[17]</sup> Abdelghafar RM *et al.*, 2011<sup>[1]</sup>; Mohamed Kandiel MM *et al.*, 2015<sup>[27]</sup>; Karadaev M *et al.*, 2016<sup>[21]</sup>; Juli Melia *et al.*, 2018<sup>[20]</sup>; Mushap Kuru *et al.*, 2018)<sup>[28]</sup>, measurement of the foetal head diameter, BPD (G.K. Haibel *et al.*, 1989<sup>[15]</sup>; G.K. Haibel, 1991<sup>[19]</sup>; Santiago-Moreno J *et al.*, 2005<sup>[33]</sup>; Lee Y *et al.*, 2005<sup>[25]</sup>; Suguna *et al.*, 2008<sup>[37]</sup>; Karen *et al.*, 2009<sup>[23]</sup>; Hussein AA, 2010<sup>[17]</sup>; Abdelghafar RM *et al.*, 2011<sup>[1]</sup>; J.K. Reichle and G.K. Haibel, 1991<sup>[19]</sup>; Mohamed Kandiel MM *et al.*, 2015<sup>[27]</sup>; Karadaev M *et al.*, 2016<sup>[21]</sup>; Juli Melia *et al.*, 2018<sup>[20]</sup>; Mushap Kuru *et al.*, 2018)<sup>[28]</sup> and placentome size (F Doizé *et al.*, 1997)<sup>[5]</sup> were the parameters mainly used. In goats, measurement of placentomes could be used along with pregnancy diagnosis by transrectal ultrasonography as an indication of gestation age but in ewes there was a poor correlation of placentome size with gestational age (F Doizé *et al.*, 1997)<sup>[5]</sup>. Transcutaneous ultrasonography may prove a useful method of estimating gestational age in the absence of accurate breeding records (JC Hunnam *et al.*, 2009)<sup>[16]</sup>. The Estimation of pregnancy diagnosis and gestational age is also important to achieve maximum reproductive efficiency (Heibel and Perkins, 1989)<sup>[14, 15]</sup>.

### Materials and Methods

A total of thirty healthy does with recent mating history maintained at Goat Research Station, Assam Agricultural University, Burynihat were taken for the present study using real time B mode ultrasonography for early pregnancy diagnosis and to determine the gestational age by measuring the diameter of the embryonic vesicle, length of the embryo proper (Crown rump length) and foetal head diameter (BPD).

A son sound ultrasonography machine with a linear rectal probe of 5-10 MHz frequency was used for the present study. The scanning of the animals for detection of pregnancy was carried out in the morning half of the day before providing any feeds to the animals. Ultrasonography was done by transrectal and transabdominal methods.

Transrectal examination was carried out from day 17 onwards at alternate day upto 27 day and on 30, 35, 40, 45, 50, 60, 70 and 80 days after mating. The faecal material were removed manually prior to ultrasonography. The animal were controlled manually in standing position. The probe was modified by fixing a 9 inch long polyvinyl pipe (1/2 inch diameter) with adhesive tape for required manipulation of the scan head inside the rectum. About twenty milliliter (2%) suspension of carboxymethylcellulose in lukewarm water was infused into the rectum for lubrication and to avoid air gap between the probe and rectal wall. The probe was inserted into the rectum until the urinary bladder was displayed on the screen as black anechoic circumscribed area. The probe was moved forward and backward and rotated clockwise and anticlockwise for complete survey of genitalia as per the method described by (Martinez *et al.*, 1998)<sup>[26]</sup>.

In Transabdominal method animals were scanned transabdominally from day 25 to 35 at alternate day, then at 40, 45, 50, 60, 70, and 80 days after mating. The right inguinal area upto the ventral midline was shaved thoroughly cleaned for better probe skin contact. The same rectal probe of 5-10 MHz frequency was used. The animals were controlled in standing position. Ultrasonic gel was put liberally on the scan head and skin. Scanning was started from the uppermost part of the inguinal region placing the probe longitudinally to the body of the doe. The probe was slowly moved towards the ventral abdomen for complete scanning of

genitalia.

The embryonic vesicle was recognized as no nechogenic black area of spherical shape in the uterus with or without echogenic embryonic mass. Embryo proper was identified as a defined echogenic structure located eccentrically within the embryonic vesicle. Diameter of embryonic vesicle was measured as the ultrasonic cross sectional images of the embryonic vesicle at their maximal diameter following the method of (Martinez MF *et al.*, 1998)<sup>[26]</sup> and the length of the embryo proper (CRL) was measured as the maximum length of the echogenic embryo within the anechoic vesicle in millimeter using inbuilt caliper of the machine. The diameter of the fetal head (millimeter) (BPD) was measured from images of which the axis of the head symmetry was perpendicular to the ultrasound beam following the method of (Lee *et al.*, 2005)<sup>[25]</sup>.

### Results and Discussion

In the present study we could detect the embryonic vesicle as early as 19 day after breeding by transrectal method and on day 25 after breeding by transabdominal method which is in agreement with other researchers which could be detected on day  $19.5 \pm 0.3$  and  $24.7 \pm 0.4$  after breeding (Padilla-Rivas *et al.*, 2005<sup>[29]</sup>; Martinez MF *et al.*, 1998)<sup>[26]</sup>. In perusal to (Schrack and Inskeep, 1993)<sup>[35]</sup> and Ishwar, 1995)<sup>[18]</sup> it was more accurate to detect on day 25 after breeding and on day 26 after breeding by (Buckrell *et al.*, 1986)<sup>[4]</sup> transabdominally which is similar with the present study and for determination of the gestational age in the present study, the mean diameter of the embryonic vesicle was measured and was found to be  $10.52 \pm 0.58$  mm (Figure 1) on day 19 after breeding by transrectal and  $18.16 \pm 0.91$ mm on day 25 after breeding by transabdominal methods respectively (Table 1 and Table 2). (Padilla *et al.*, 2005 and Hussein AA, 2010)<sup>[29, 17]</sup> also reported that the diameter of embryonic vesicle on day 19 after insemination by transrectal ultrasonography was 1cm which was similar to the present finding. The diameter of embryonic vesicle as observed in the present study increased with the advancement of pregnancy and was measured as  $28.21 \pm 0.33$  mm on day 35 after breeding (Table 1 and Figure 2) which was in agreement with the observation of Martinez *et al.*, (1998)<sup>[26]</sup> who 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. Pegu (2011)<sup>[30]</sup> also recorded similar observation in cow.

The length of embryo proper was measured as  $6.66 \pm 0.36$  and  $8.31 \pm 1.52$  mm on day 21 and 25 after breeding in does under the present study (Table 1 and 2). (Martinez MF *et al.*, 1998)<sup>[26]</sup> recorded the length of embryo proper as 7 mm on day 20 after breeding which was comparable to the present study by transrectal method. According to the report by (Juli Melia *et al.*, 2018)<sup>[20]</sup> on Etawa crossbred goat, using transcutaneous ultrasonography, the mean diameter of the embryo was found to be  $2.65 \pm 0.75$  mm with is echogenic appearance and gestational sac with hypoechogenic appearance which could be detected quiet earlier than the present study. But according to (Rivas *et al.*, 2005 and Sayuti *et al.*, 2016)<sup>[31, 34]</sup> embryo images in Kacang goat and Boer goat could be detected on day 24 and day 26 after breeding with a size of 6.77 mm which was compared well with present study. The length of the embryo proper was recorded as  $15.85 \pm 0.48$  and  $17.54 \pm 0.39$  mm on day 30 and 35 respectively after breeding, in the present study by transrectal ultrasonography (Table 1). It was  $14.04 \pm 0.21$  and  $16.25 \pm 0.14$  mm by transabdominal method

(Table 2). (Singh *et al.*, 2004) [36] reported the length of embryo as  $17.5 \pm 0.13$  and  $25.9 \pm 0.08$  mm on day 30 and 34 after breeding in does by transrectal method which was slightly higher than the present study. (Juli Melia *et al.*, 2018) [20] reported the size of the embryo proper was recorded as  $8.31 \pm 0.42$  mm on day 30 after breeding and the embryo size reached to  $10.44 \pm 0.51$  mm on day 35 after breeding on Etawa crossbred goat transabdominally in which the size is lower than the present finding. The measurement of the embryo proper in the present study was  $42.09 \pm 0.87$  and  $41.52 \pm 1.07$  mm on day 50 after breeding by transrectal and transabdominal methods (Table 1 and 2). It was difficult to measure the length of the embryo proper from day 50 after breeding as the fetus was extended beyond the focused area. (Habil, 2004) [11] stated that measurement of goat fetus was not possible after day 60 day with 5 MHz probe. (Hussein AA, 2010) [17] also reported that such measurement was not possible after day 89 of gestation. (Ali and Hayder, 2007) [2] also reported that length measurement of embryo and amnion sac on Ossimi lamb could only be detected at first trimester of pregnancy. The variation in the difference observed might be due to the breed of the animals, skill of the operator and quality of the ultrasound machine. Measurement of the foetal head observed in the present study was possible from day 40 to 70 (Figure 3 and 4) and 40 to 80 after breeding by transrectal and transabdominal methods (Table 1 and 2) which was comparable to (Gonzalez *et al.*,

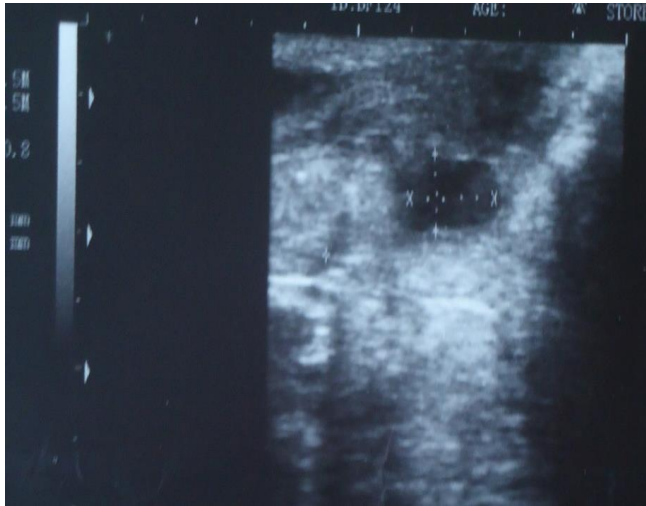
1998) [7] (day 32 to 90) and (Hussein AA, 2010) [17] (day 40 to 109) transrectally and Heibal and Parkins (1989) (day 40 to 95) transabdominally. The mean diameter of foetal head on day 40 after breeding was recorded as  $9.45 \pm 0.38$  mm by transrectal and  $9.50 \pm 0.48$  mm by transabdominal method (Table 1 and 2). The measurements on day 45, 50, 60 and 70 after breeding were  $13.24 \pm 0.20$ ,  $15.08 \pm 0.27$ ,  $20.48 \pm 0.26$  and  $23.79 \pm 0.29$  mm, respectively as recorded by transrectal Ultrasonography (Table 1). In transabdominal method these were  $12.57 \pm 0.40$ ,  $14.52 \pm 0.27$ ,  $20.39 \pm 0.20$ ,  $23.25 \pm 0.24$  and  $25.49 \pm 0.42$  mm on day 45, 50, 60, 70 and 80 after breeding respectively (Table 2), after which the measurement was not possible as observed in the present study. (Heibal and Fung, 1991 and Hussein AA, 2010) [13, 17] reported that the measurement of foetal head was difficult to measure after day 109 after breeding by real time ultrasonography due to the increase in foetal size and compression of the head by other foetal parts. The mean diameter of foetal head as observed in the present study increased gradually with the advancement of pregnancy. (Suguna *et al.*, 2008) [37] also reported that diameter of the head increased significantly from day 56 ( $1.97 \pm 0.00$  cm) today 98 ( $6.57 \pm 0.40$  cm) after breeding. (Juli Melia *et al.*, 2018) [20] also recorded with a mean diameter of  $25.5 \pm 0.1$  mm on day 60 after breeding and  $33.03 \pm 0.20$  mm on day 75 after breeding which is slightly higher than the present study.

**Table 1:** Measurement of embryonic vesicle, embryo proper and foetal head diameter by transrectal real time ultrasonography in does at different days of pregnancy

Days of pregnancy	Diameter of embryonic vesicle (mm)	Length of embryo proper (mm)	Diameter of foetal head (mm)
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
17	0.00	0.00	0.00
19	$10.52 \pm 0.58$	0.00	0.00
21	$14.55 \pm 0.25$	$6.99 \pm 0.36$	0.00
23	$17.11 \pm 0.52$	$8.67 \pm 0.33$	0.00
25	$19.53 \pm 0.87$	$10.41 \pm 0.03$	0.00
27	$23.33 \pm 0.08$	$12.45 \pm 0.02$	0.00
30	$25.63 \pm 0.36$	$15.85 \pm 0.48$	0.00
35	$28.21 \pm 0.33$	$17.54 \pm 0.39$	0.00
40	---	$26.01 \pm 1.08$	$9.45 \pm 0.38$
45	---	$31.21 \pm 1.15$	$13.24 \pm 0.20$
50	---	$42.09 \pm 0.87$	$15.08 \pm 0.27$
60	---	---	$20.48 \pm 0.26$
70	---	---	$23.79 \pm 0.29$
80	---	---	---

**Table 2:** Measurement of embryonic vesicle, embryo proper and foetal head diameter by transabdominal real time ultrasonography in does at different days of pregnancy.

Days of pregnancy	Diameter of embryonic vesicle (mm)	Length of embryo proper (mm)	Diameter of foetal head (mm)
	Mean $\pm$ SE	Mean $\pm$ SE	Mean $\pm$ SE
25	$18.16 \pm 0.91$	$8.31 \pm 1.52$	0.00
27	$19.52 \pm 0.54$	$10.19 \pm 0.41$	0.00
30	$22.18 \pm 0.45$	$14.04 \pm 0.21$	0.00
35	$27.45 \pm 0.48$	$16.25 \pm 0.14$	0.00
40	---	$26.52 \pm 0.88$	$9.50 \pm 0.48$
45	---	$33.17 \pm 1.35$	$12.57 \pm 0.40$
50	---	$41.52 \pm 1.07$	$14.52 \pm 0.27$
60	---	---	$20.39 \pm 0.20$
70	---	---	$23.25 \pm 0.24$
80	---	---	$25.49 \pm 0.42$



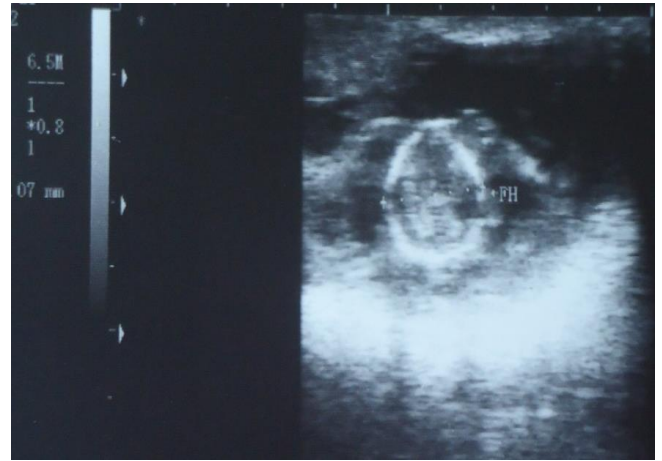
**Fig 1:** Ultrasonic image showing measurement of the diameter of anechoic embryonic vesicle on day 19 after breeding in goats.



**Fig 2:** Ultrasonic image showing measurement of the length of the echogenic embryo proper on day 35 of pregnancy in goats



**Fig 3:** Ultrasonic image showing the embryo proper with visible head on day 40 of pregnancy in goats.



**Fig 4:** Ultrasonic image showing the measurement of the foetal head in the anechoic uterine fluid on day 70 of pregnancy in goats.

### Conclusion

By using ultrasonography, detection of early pregnancy is possible and apart from this by measuring the diameter of the embryonic vesicle, length of the embryonic proper and foetal head diameter it was possible to find the gestational age.

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