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Early pregnancy diagnosis in Assam hill goat

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Abstract
A total of thirty healthy does maintained at Goat Research Station, Byrnihat, Assam Agricultural University, were used in the present study for early pregnancy diagnosis by Real time B-mode Ultrasonography. Observation were made for detection of various parameter like embryonic vesicle, embryo proper, foetal heartbeat, placentomes, twins, foetal movement, foetal head, foetal limb buds, vertebral column and rib cageby both the transrectal and transabdominal method. using transrectal methods 100% detection of embryonic vesicle, embryonic proper and foetal heartbeat were on day 27, 27, and 30 days after breeding which was 30, 30 and 35 days using transabdominal methods The diameter of embryonic vesicle, length of embryo proper and diameter of foetal head increased gradually with the advancement of pregnancy.

Keywords: Assam hill goat, real time b mode ultrasonography, early pregnancy diagnosis

1. Introduction

Early detection of pregnancy is extremely essential and prerequisite for success of animal husbandry enterprise. Early identification and diagnosis of pregnant animals helps in managing the animals in a planned and scientific way for improving reproductive efficiency and hence the economy [1]. Moreover, early identification of non-pregnant animals allows breeders to step up for bringing the animals to pregnant state by application of various techniques. Diagnosis of pregnancy at an early stage is therefore of great economic importance which helps in timely culling or rebreeding of barren animals. On the ground of traditional method like non return to estrus alone cannot be taken to consider an animal to be pregnant because non pregnant females may fail to show subsequent estrus behaviour due to prolonged estrus cycle, silent estrus and anestrus or due to some other pathological condition. Therefore, regular diagnosing of pregnancy at the very early stage seems to be essential. Some of the methods that have been used to diagnose pregnancy in goats and ewes over the last few decades include: Radiography, assessment of Estronesulphate, Progesterone assay, vaginal biopsy, Pregnancy Specific Antigen. But, the application of these methods on a large scale in veterinary practice has remained limited in field condition due to either non- availability of sophisticated laboratory facilities or longer time required for confirmation of pregnancy or the methods are insufficiently inaccurate. Rectal abdominal technique using Hulet's rod also proved hazardous with respect to rectal injury and abortion [2]. For the last three and eighties decades, Real time ultrasonography the noninvasive and painless on application offered a new tool for early pregnancy diagnosis in small ruminants [3]. Taking into account of all the above points a study was conducted on Assam Hill Goat using TR (transrectal) and TA (transabdominal) ultrasonography for pregnancy diagnosis by studying different parameters viz., embryonic vesicle, embryo proper, foetal heartbeat, placentomes, twins, foetal movement, foetal limb buds, vertebral column and rib cage. The objective of the study is to detect the pregnancy at an early stage and to find the accuracy of the methods used.

2. Materials and Methods

The present study was carried out on 30 does (Assam Hill Goat) maintained at the Goat Research Centre (Byrnihat). The does were maintained under uniform managemental system. All does undergoes mating were subjected to pregnancy diagnosis by Real time Ultrasonography. A Son sound ultrasonography machine with a linear rectal probe of 5-10 MHz frequency was used for the present study. The scanning of animals for detection of pregnancy was carried out in the morning half of the day before providing any feed to the animals. Transrectal and transabdominal methods were used for diagnosing the pregnancy by Ultrasonography.

2.1 Transrectal examination

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 materials were removed manually prior to ultrasonography. About 20 milliliter (2%) suspension of carboxy-methylcellulose in lukewarm water was infused into the rectum for lubrication and to avoid air gap between the probe and rectal wall. The animals were controlled manually in standing position. The probe was modified by fixing a 9inch long polyvinyl pipe (1/2inch diameter) with adhesive tape for required manipulation of the scan head inside the rectum. The probe was lubricated with commercially available ultrasound gel for easy passage. The probe was inserted into the rectum until the urinary bladder was displayed on the screen as black anechoic circumscribed area. The urinary bladder was considered as acoustic window for uterus. The probe was moved forward and backward and rotated clockwise and anticlockwise for complete survey of genitalia [4]. Accuracy of diagnosis was determined based on percentage of animal detected positive.

2.2 Transabdominal examination

The experimental 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 and thoroughly cleaned for better probe skin contact. The same rectal probe of 5 to 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. Observations were made for detection of different parameters using the following method as presented in the table 1.

3. Results and Discussion

The experimental does were examined with the help of real time transrectal and transabdominal ultrasonography for diagnosis of pregnancy using a rectal probe. The percentage of animals detected positive for pregnancy by both the methods for different parameter such as embryonic vesicle, embryo proper, foetal heartbeat, placentomes, twins, foetal movement, foetal head, foetal limb bud and vertebral column and rib cage are presented in table 2& 3.

In the present study embryonic vesicle was visible as an anechoic area with or without echogenic embryonic mass depending on the stage of pregnancy in both transrectal and transabdominal ultrasonography (Figure 1). Similar observations were also made by Hussein in the year 2010 (5). On perusal of the data recorded it was observed that embryonic vesicle was first detected on day 19 by transrectal and on day 25 after breeding by transabdominal method in 10.00 and 26.66 per cent of animals respectively which is in agreement with many researchers on day 19.5 ± 0.3 and 24.7 ± 0.4 after breeding in goats [3, 5]. According to Mohsin Ali Gazi in the year 2017 early diagnosis of pregnancy is possible by 26 days after breeding [6]. Karen et al. in the year (2009) reported that it was possible to detect the amniotic vesicle as early as 16 days after insemination in ewes and does by transrectal route [7] but according to the recent report, scanning at 30 days pregnancy is necessary to ensure fetus viability [6].

With the help of Transrectal and Transabdominal it was ultrasonography possible to detect embryonic vesicle with 100.00 per cent accuracy on day 27 and 30 after breeding in the present study (Table 3 & 4). Gonzalez and his coworkers in the year 2004detected the embryonic vesicle on day 26 after breeding with 100.00 per cent accuracy in goats which was comparable to the present observation [8]. The different in the finding may be due to breed difference, different climatic conditions or environmental factors. On the other hand, Yotov (2005) observed the amniotic vesicle transabdominally with 98.00 per cent accuracy on day 35 post AI in ewes which was later than the present study [9]. According, to the report of Amanda John and Sarah A Reedin the year 2017 pregnancy diagnosis is most sensitive after day 30 of gestation using transrectal and transabdominal ultrasonography [10]. The appropriate time for transabdominal ultrasonography is between post breeding days 40–45 and for transrectal examination is between post breeding days 25 and 30 [11]. Transrectal ultrasonography could be accurately used for early pregnancy diagnosis and fetal number estimation at days 24–29 and days 31–36, respectively, with accuracy similar to that of transabdominal ultrasonography at days 39–51 [12], which is in agreement with the present study.

Embryo proper was first detected in the present study in 33.30 and 10.00 per cent of animals on day 21 and 25 respectively after breeding by transrectal and transabdominal methods (Table 1&2; Figure 2). Early detection of embryo proper as observed in the present study was comparable to that reported by Karen and his coworkers in the year 2009 who could detect embryo proper on day 22 after breeding by transrectal ultrasonography [7]. However, the embryo proper could be detected later on day 35 and 30 after breeding by transabdominal method [13]. In the present study, the embryo proper was detected with an accuracy of 100.00 per cent on day 27 and 30 after breeding by transrectal and transabdominal methods respectively (Table 4&5; Figure 3).

In the present study heart beat was first visualize on day 25 and 27 after breeding by transrectal and transabdominal methods in 76.60 and 56.66 per cent of the does (Table 2& 3) which is in agreement with the findings in boer goats [14] and the foetal heart beat transabdominally on day 27 after breeding [5]. In the present study, it was possible to detect foetal heart beat by transrectal and transabdominal methods in all the animals (100 %) from day 30 and 35 onwards after breeding.(Table4 &5).The embryonic heart beat detected by transrectal route in 100.00 per cent of the animals on day 28 after breeding which was comparable to the present finding [13]. The earlier or later detection may be due to differences in skill, breed differences or climatic changes.

Circular or C- shaped placentomes were detected in the present study on day 30 (7.00 %) and 35 (16.66%) after breeding by transrectal and transabdominal methods respectively (Table2&3; Figure 4) which is in accordance with Suguna and his coworkers in the year 2008 [13].While, Medan et al., (2004) could detect placentomes later on day 35 after breeding in goats by transrectal method [14]. In the present study, Placentome was detected with 100.00 per cent accuracy from day 45 after breeding by both the methods (Table 4 & 5). Anwar et al., in the year 2008 could also detect placentomes on 45 days after breeding which is compared well with the present observation [15].

Detection of twins was first observed on day 35 by transrectal method in 7.00 per cent animals and on 40 after breeding in 10 per cent of animals using transabdominal method (Table 2 & 3; Figure 5). Detection of twins was possible on day 45 and

50 after breeding in 100.00 per cent of the animals in the present study by transrectal and transabdominal methods respectively (Table 4 & 5). Medan *et al.,* (2004) obtained an accuracy of 83.30 and 91.7 per cent on day 50 and 60 after breeding respectively using real time ultrasonography [14]. Suguna*et al.,* (2008) obtained 100.00 per cent accuracy on day 49 after breeding transabdominally and found similar to the present finding [13].

Foetal movement could be detected in the present study on day 40 after breeding in 40 and 16.66 per cent of the animals by transrectal and transabdominal methods by which 100.00 per cent accuracy could be obtained on day 45 after breeding by both the methods (Table 2, 3, 4 & 5). Perusal of available literature reveals no study on foetal movement for comparison with the results obtained in the present study. However it could be detected the foetal movement on day 50.70 ± 1.00 and 49.40 ± 2.31 after breeding in cattle [16, 17].

In the present study, the foetal head was first detected on day 40 after breeding in 66.60 per cent animals using transrectal and in 16.66 per cent of the animals using transabdominal methods and 100% accuracy was obtained on 70 and 80 days after breeding (Table 2, 3, 4 & 5; Figure 6). The foetal head could also be detected on day 40 after breeding [5] as in the present study.

Foetal limb buds could be detected in the present study on day 40 after breeding in 50 per cent of the animals by transrectal as well as by transabdominal method and with 100.00 per cent accuracy on day 45 after breeding (Table 2, 3, 4 & 5). The present finding was similar to the finding of Ali and Hayder (2007) who could detect the limb buds on day 40 after breeding by both the methods [18]. Anwar *et al.,* in the year 2008 also could detect foetal limb buds on day 41-45 and 45-50 after breeding with an accuracy of 66.70 and 100.00 per cent respectively by both the methods [15].

Present study, was possible to detect vertebral column and rib cage on day 50 after breeding in 50.00 per cent of the animals

and 100.00 per cent accuracy could be obtained on day 60 in both methods (Table 2, 3, 4 & 5; figure 7). The present finding was comparable to the observation of Anwar *et al.,* (2008) who could detect the vertebral column and rib cage on day 45-50 and 51-55 after breeding in 50.00 and 100.00 per cent of the animals respectively [15]. The vertebral column and ribs could also detect on day 46-55 after breeding similar to that observed in the present study [18].

Embryonic vesicle was detected earliest by transrectal ultrasonography compared to transabdominal ultrasonography, embryonic vesicle was detected on day 19 in 10% of animals by transrectal method compared to transabdominal which was on 25th day after breeding (26.66%). Similarly, using transrectal methods 100% detection of embryonic vesicle, embryonic proper and foetal heartbeat were on day 27, 27, and 30 days after breeding which was 30, 30 and 35 days using transabdominal methods. Earlier detection of all the above parameters using TR ultrasonography might be closeness of the transducer to the reproductive tract as compared to TA ultrasonography where the efficiency of ultrasonography might be inferred by presence of various abdominal structure and the distance of transducer from the reproductive tract (Table 6). Placentomes was detected first time using transrectal ultrasonography on day 30 and in 100% of animals on day 45, while it was day 35 and 45 using transabdominal ultrasonography, similarly in case of foetal movement it was on day 40 and 45 using transrectal and transabdominal ultrasonography on first and 100% of animals. Similar observation was recorded in foetal skeletal structure where it was first detected using transrectal ultrasonography (Table 6). Hence it can be concluded that since transrectal ultrasonography method detected all the stages of foetal development to the earliest and hence it can be employed for study of foetal development as it provides better accuracy compared to transabdominal ultrasonography.

Table 1: Various parameters observed by both the methods are:

Sl no.	Parameter	Observation
1.	Embryonic Vesicle	Recognized as no echogenic black area of spherical shape inside the uterus with or without echogenic embryonic mass Hussein (2010).
2.	Embryo Proper	Identified as a defined echogenic structure located eccentrically within the embryonic vesicle. (*Karen et al.,* 2009).
3.	Foetal heartbeat	Presence of rhythmic pulsation in a defined echogenic embryonic mass (*Martinez et al.,* 1998).
4.	Placentomes	Detected as C-shaped or O-shaped gray images against the uterine fluid (*Suguna et al.,* 2008).
5.	Foetal movement	Observed as moving echoes within the anechoiec vesicle (Pegu2011).
6.	Skeletal structure	Recognised as highly echogenic structure of definite shape inside the uterus (Pegu2011).

Table 2: Per cent does detected positive by transrectal real time ultrasonography for embryonic vesicle, embryo proper, foetal heartbeat, placentomes, twins, foetal movement, foetal head, limb buds and vertebral column and rib cage

Days of pregnancy	Per cent does detected positive								
	Embryonic vesicle	Embryo proper	Foetal heart beat	Placentome	Twins	Foetal movement	Foetal head	Limb bud	Vertebral column and rib cage
17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
19	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0. 00	0.00
	(3)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
21	40.00	33.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(12)	(10)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
23	83.30	70.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(25)	(21)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
25	86.60	83.3	76.60	0.00	0.00	0.00	0.00	0.00	0.00
	(26)	(25)	(23)	(0)	(0)	(0)	(0)	(0)	(0)
27	100.00	100	93.30	0.00	0.00	0.00	0.00	0.00	0.00

	(30)	(30)	(28)	(0)	(0)	(0)	(0)	(0)	(0)
30	100.00	100	100.00	7.00	0.00	0.00	0.00	0.00	0.00
	(30)	(30)	(30)	(2)	(0)	(0)	(0)	(0)	(0)
35	100.00	100	100.00	16.60	7.00	0.00	0.00	0.00	0.00
	(30)	(30)	(30)	(5)	(2)	(0)	(0)	(0)	(0)
40		100	100.00	83.30	57.00	40.00	66.60	50.00	0.00
	---	(30)	(30)	(25)	(17)	(12)	(20)	(15)	(0)
45		100	100.00	100.00	100.00	100.00	93.3	100.00	0.00
	---	(30)	(30)	(30)	(30)	(30)	(28)	(30)	(0)
50	---	100	100.00	100.00	100.00	100.00	100.00	100.00	50.00
		(30)	(30)	(30)	(30)	(30)	(30)	(30)	(15)
60			100.00	100.00	100.00	100.00	100.00	100.00	100.00
	---	---	(30)	(30)	(30)	(30)	(30)	(30)	(30)
70		---	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	---		(30)	(30)	(30)	(30)	(30)	(30)	(30)
80		---	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	---		(30)	(30)	(30)	(30)	(30)	(30)	(30)

Figures within the parentheses are numbers of doe detected positive.

Table 3: per cent does detected positive by transabdominal real time ultrasonography for embryonic vesicle, embryo proper, foetalheartbeat, place tomes, twins, foetal movement, foetal head, limb buds and vertebral column and rib cage.

Days of pregnancy	Per cent does detected positive								
	Embryonic vesicle	Embryo proper	Foetal heart beat	Placentome	Twins	Foetal movement	Foetal head	Limb bud	Vertebral column and rib cage
25	26.66	10.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(8)	(3)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
27	56.66	56.66	56.66	0.00	0.00	0.00	0.00	0.00	0.00
	(17)	(17)	(17)	(0)	(0)	(0)	(0)	(0)	(0)
30	100.00	100.00	83.33	0.00	0.00	0.00	0.00	0.00	0.00
	(30)	(30)	(25)	(0)	(0)	(0)	(0)	(0)	(0)
35	100.00	100.00	100.00	16.66	0.00	0.00	0.00	0.00	0.00
	(30)	(30)	(30)	(5)	(0)	(0)	(0)	(0)	(0)
40	---	100.00	100.00	76.66	10.00	16.66	16.66	50.00	0.00
		(30)	(30)	(23)	(3)	(5)	(5)	(15)	(0)
45	---	100.00	100.00	100.00	50.00	100.00	43.33	100.00	0.00
		(30)	(30)	(30)	(15)	(30)	(13)	(30)	(0)
50	---	100.00	100.00	100.00	100.00	100.00	73.33	100.00	50.00
		(30)	(30)	(30)	(30)	(30)	(22)	(30)	(15)
60	---	---	100.00	100.00	100.00	100.00	100.00	100.00	100.00
			(30)	(30)	(30)	(30)	(30)	(30)	(30)
70	---	---	100.00	100.00	100.00	100.00	100.00	100.00	100.00
			(30)	(30)	(30)	(30)	(30)	(30)	(30)
80	---	---	100.00	100.00	100.00	100.00	100.00	100.00	100.00
			(30)	(30)	(30)	(30)	(30)	(30)	(30)

Figures within the parentheses are numbers of doe detected positive.

Table 4: Earliest day of pregnancy for accurate detection of embryonic vesicle, embryo proper, foetalheartbeat, placentomes, twins, foetal movement, foetal head, limb buds and vertebeal column and rib cage by transrectal ultrasonography.

Parameters	Days of pregnancy
Embryonic vesicle	27
Embryo proper	27
Foetal heart beat	30
Placentomes	45
Twins	45
Foetal movement	45
Foetal head	50
Limb bud	45
Vertebral column and rib cage	60

Table 5: Earliest day of pregnancy for accurate detection of embryonic vesicle, embryo proper, foetal heartbeat, place tomes, twins, foetal movement, foetal head, limb buds and vertebral column and rib cage by transabdominal ultrasonography.

Parameters	Days of pregnancy
Embryonic vesicle	30
Embryo proper	30
Foetal heart beat	35
Placentomes	45
Twins	50
Foetal movement	45
Foetal head	60
Limb buds	45
Vertebral column and rib cage	60

Table 6: Comparison of accuracy of detection (%) of embryonic vesicle, embryo proper and foetal heart beat between transrectal and transabdominal ultrasonography technique at different days for early pregnancy.

Days of pregnancy	Embryonic vesicle		Embryo proper		Foetal heartbeat	
	Transrectal	Transabdominal	Transrectal	Transabdominal	Transrectal	Transabdominal
17	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
19	10.00 (3)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)	0.00 (0)
21	40.00 (12)	0.00 (0)	33.30 (10)	0.00 (0)	0.00 (0)	0.00 (0)
23	83.30 (25)	0.00 (0)	70.00 (21)	0.00 (0)	0.00 (0)	0.00 (0)
25	86.60 (26)	26.66 (8)	83.30 (25)	10.00 (3)	76.60 (23)	0.00 (0)
27	100.00 (30)	56.66 (17)	100.00 (30)	56.66 (17)	93.30 (28)	56.66 (17)
30	100.00 (30)	100.00 (30)	100.00 (30)	100.00 (30)	100.00 (30)	83.33 (25)
35	100.00 (30)	100.00 (30)	100.00 (30)	100.00 (30)	100.00 (30)	100.00 (30)

Fig 1: Ultrasonic image showing anechoic embryonic vesicle on day 19 after breeding in goats.

Fig 2: Ultrasonic image showing echogenic embryonic mass within the anechoic embryonic vesicle on day 21 of pregnancy in goats.
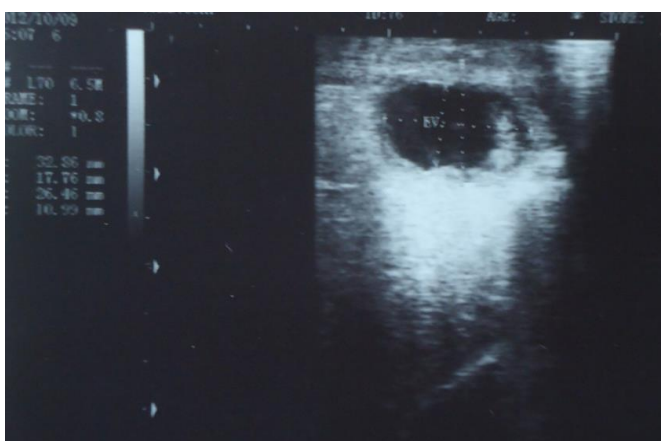

Fig 3: Ultrasonic image showing echogenic embryonic mass within the anechoic embryonic vesicle on day 27 of pregnancy in goats.

Fig 4: Ultrasonic image showing placentomes on day 45 of pregnancy in goats.

Fig 5: Ultrasonic image showing twins within the anechoic embryonic vesicle on day 35 of pregnancy in goats.

Fig 6: Ultrasonic image showing the foetal head in the anechoic uterine fluid on day 70 of pregnancy in goats.

Fig 7: Ultrasonic image showing the vertebral column and rib cage on day 50 of pregnancy in goats

4. Conclusion

Real time B mode ultrasonography is a painless, noninvasive and reliable method for early pregnancy diagnosis in goat. Both transrectal and transabdominal ultrasonography is convenient forearly pregnancy diagnosis in goats.

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