Original Article

Pregnancy diagnosis in goat by using vaginal cytology and trans-abdominal ultrasonography

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ABSTRACT The present study was conducted in a goat farm for pregnancy diagnosis by using vaginal cytology and B-mode real time ultrasound using 5 MHz probe by trans-abdominal method. Seventeen pregnant does were used for this study. The objective of the study was to determine the earliest day of pregnancy and describe the chronological characteristics of pregnancy from day 22 to day 40 for vaginal cytology and day 25 to day 60 for ultrasonography of gestation. The differences among the average percentage of cell value in different age of pregnancy were significant ($p < 0.05$). The average percentage of intermediate cells (81.12%) was also significantly ($p < 0.05$) higher than superficial (9.41%), keratinized (7.10%) and neutrophil (2.61%) on 22-40th days of pregnancy. In case of real time B-mode ultrasonography, the gestational sac was observed only in three does out of seventeen (17.6 %) at 25-30 days whereas the placentomes and heart beat of the foetus were first detected at 31-35 days in six does (35.3%). The foetal leg buds were first visualized at 36-40 days in four does (23.5%) whereas the foetal vertebral column was first observed at 36-40 days of gestation in only three does (17.6%). In conclusion, vaginal cytology and trans-abdominal ultrasonography can be used for detection of early pregnancy in does.

Keywords: does, pregnancy diagnosis, trans-abdominal ultrasonography, vaginal cytology

INTRODUCTION

Bangladesh has a subtropical monsoon climate characterized by wide seasonal variations in rainfall, moderately warm temperatures, and high humidity. Bangladesh lies in the North-Eastern part of South Asia between 20.02° and 26.038° north latitude and 88.001° and 92.041° east longitude. The goats of Bangladesh usually show estrus across the year with no anovulatory period. Bangladesh has the second highest population of goat among the Asiatic countries which accounts for about 60.60 million heads representing 57% of total ruminant livestock (Fao-stat et al., 2009). The intensive goat production and the

wide spread application of the controlled breeding techniques, such as artificial insemination increased the need for an accurate early pregnancy diagnosis. The pregnancy diagnosis in goats is essential for better efficacy and management of reproduction (Doize et al., 1997), providing information about conception rates after artificial insemination (Matsas et al., 2007), gestation course (Ameret al., 2010), time for drying-off and parturition date (Doize et al., 1997; González et al., 2004). The utilization of an accurate and easily applicable method for pregnancy diagnosis allows the timely repeated insemination, breeding or culling of non-pregnant animals (Ameret al., 2010). A number of techniques for pregnancy diagnosis have been

implemented; some of them are efficient and applicable under field condition, but not enough accurate. Several investigation were carried out during early pregnancy in the Black Bengal breed of goat (Talukder et al., 2020). Trans-abdominal ultrasonography (Gonzalez et al., 2004) and vaginal cytology are a simple technique that can be used by practitioners for pregnancy diagnosis in goat (Sharma and Sharman et al., 2016).

In Bangladesh, most of the goats are bred by natural service and are generally unobserved or unrecorded making fertile breeding impossible to determine early detection of pregnancy. Pregnancy diagnosis is depending mainly on the non-return to estrous after service. However, recently some researchers have been taken initiative to diagnosis the pregnancy in large and small ruminants by using ultrasonography. Thus, the present work was performed to determine the pregnancy by using vaginal cytology, to determine the earliest stage of pregnancy using trans-abdominal ultrasonography and to assess the chronological changes of the foetal development in the pregnant does at different gestation period that not being studied before in does in Bangladesh.

MATERIALS AND METHODS

Study area and period

The study was carried out on a goat farm located at Paharpur in Dinajpur under the supervision of Department of Medicine, Surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The research units are located on 25.38°N latitude and 88.41°E longitude, respectively and elevated 37.5 m above sea level. The area receives on average 170 mm of rainfall. Mean annual minimum and maximum temperatures experienced at the site are 33.5 and 10.5°C, respectively. The study was conducted between December' 2017 to April' 2018.

Experimental animals and management

A total of 17 clinically healthy does of 2.5-3 years old and 22-25 kg body weight were used for this study. The does were maintained on natural grazing along with concentrate mixture (25% crushed maize, 50% wheat bran, 20% soybean meal, 1% fish meal, 2% DCP powder, 1.5% salt and 0.5% vitamin-mineral premix) during the experimental period. Water was supplied ad libitum.

Oestrous detection

Estrous detection in does was carried out twice a day for 1 h period by observing clinical signs. All goats were naturally mated during heat according to the routine program adapted in the farm. Expectation of early pregnancy was done by non-return to oestrus (>17-21 days).

Vaginal cytology of pregnant does

For determining the early pregnancy stage vaginal smear was collected from the pregnant does with the aid of vaginal swabs. At the anterior vagina, the swab was gently and briskly rolled against the vaginal mucosa and carefully withdrawn. The swab was immediately smeared on a warm glass slide, air dried and immediately fixed with 100% ethanol. The smears were stained with Giemsa stain (Zohara et al., 2014). The cells encountered in the vaginal smear were categorized as percentage of superficial, parabasal, and intermediate cells, and leucocytes. Identification of vaginal epithelial cells was performed by microscopic observation ($\times 100$). The percentage of vaginal cells was calculated as the number of each type of cell divided by the total number of cells seen within 3 microscopic fields.

Real-time, B-mode ultrasonography

A real time B-mode ultrasound scanner equipped with a 5 MHz sector probe (DRAMINSKI ANIMAL proti portable ultrasound scanner, Poland) was used for scanning. Scanning was performed after shaving the inguinal region, in front and on either side of udder of the animal. The animal was lightly restrained by one person in standing position and also in dorsal recumbent position. One of the hind legs of the does was folded up at the time of scanning for proper placement of the probe. An ultrasound coupling gel was applied in each time to the probe to develop good contact and to remove air between probe and animal skin. Each animal was subjected to scanning for at least five minutes at different days (days 25-30, 31-35, 36-40, 41-45, 46-50, 51-55 and 56-60).

Statistical analysis

The data were recorded and analyzed statistically by using software SPSS (Version 20.0). Chi-square test was performed and the results were expressed in percentage with p -value and significance was determined ($p < 0.05$).

RESULTS

The study was conducted in a small scale goat farm located at Paharpur in Dinajpur. The purpose of this study was to diagnose the pregnancy by using vaginal cytology and real time B-mode ultrasonography (5 MHz).

Vaginal cytology

During the study, a total of 5 types of vaginal cells were identified and illustrated in figures (Fig. 1A-D). Parabasal (p) cells were small, round or nearly round and had a high nuclear to cytoplasm ratio, oval shape with large, prominent nuclei or polygonal shape with a small nuclear/cytoplasmic ratio were intermediate (i) cells. The Neutrophils (n) were the little ones with segmented nuclei. Polygonal

in shape and distinctly flat, with pyknotic nuclei (very small and dark) were superficial (s) cells. Normal bacterial flora was present and organisms often were attached to the superficial cells.

The percentages of vaginal epithelial cells according to different days of gestation length are presented in Table 1. Cell counts increased sharply after 21 days of gestation and clumping of the intermediate and superficial cells increased in the following days (Fig. 1E). The average percentages of intermediate cells during early stage of pregnancy were 78.84% at 22-25 days, 84.15% at 26-30 days, 81.73% at 31-35 days and 79.74% at 36-40 days. The average percentage of superficial cells and the keratinized cells were 9.61%, 7.76%, 9.90%, 10.35% and 6.87%, 5.64%, 7.34%, 8.54% respectively on the similar days. The para-

Fig. 1. Photomicrographs of exfoliative vagina cells during various days of the gestation period of does (A: smear of day 24, B: smear of day 29, C: smear of day 33, D: smear of day 39, E: clumping of the cells). Name of the vaginal epithelial cells shown as, intermediate (i), superficial (s), keratinized (k) and neutrophil (n).

Table 1. Percentage of different exfoliative cells of vaginal smears in early stages of pregnancy in does

Gestation length (days)	Average percentage of vaginal cells				
	Parabasal (%)	Intermediate (%)	Superficial (%)	Keratinized (%)	Neutrophils (%)
22-25	0	78.84 ^{Aa}	9.61 ^{Bb}	6.87 ^{Bc}	4.68 ^{Cd}
26-30	0	84.15 ^{Aa}	7.76 ^{Bb}	5.64 ^{Bc}	2.45 ^{Cd}
31-35	0	81.73 ^{Aa}	9.90 ^{Bb}	7.34 ^{Bc}	1.95 ^{Cd}
36-40	0	79.74 ^{Aa}	10.35 ^{Bb}	8.54 ^{Bc}	1.37 ^{Cd}
Average (22-40)	0	81.12 ^A	9.41 ^{BC}	7.10 ^{BC}	2.61 ^{BC}

^{A-C} superscripts in a row indicate statistically significant difference ($p < 0.05$).

^{a-c} superscripts in a column indicate statistically insignificant difference ($p > 0.05$).

basal cells were absent during the early stages of pregnancy. The differences among the average percentage of cell value in different age of pregnancy were significant ($p < 0.05$). The average percentage of intermediate cells (81.12%) was also significantly ($p < 0.05$) higher than superficial (9.41%), keratinized (7.10%) and neutrophil (2.61%) on 22–40 days of pregnancy.

Findings of real-time, B-mode ultrasonography in pregnant does

The present study was conducted to diagnose pregnancy in does using the real time B-mode ultrasonography by taking the following criteria or chronological changes.

Gestational sac

In the present study, gestational sac was detected be-

tween 25–30 days in 3 out of 17 does with the accuracy of 17.6%. The presence of anechoic areas cranial to the bladder was considered as positive for pregnancy (Table 2 and Fig. 2).

Heart beat

In the present investigation the heart beat was detected for the first time at 31–35 days with accuracy of 35.3%. The heart beat became more prominent with the advancement of gestation and the rate of accuracy was 58.8, 88.2 and 100% at 36–40, 41–45 and 46–50 days after mating, respectively (Table 3).

Placentomes

No placentome was detected at 25–30 days after the mating. But, it was detected only in six does (35.3%) as small circular, concave structures between 31–35 days of

Table 2. Percentage of pregnant does based on observation of gestational sac

Days after mating	Gestational sac observed	Gestational sac not observed	Total
25–30	17.6% (3)	82.4% (14)	17
31–35	100% (17)	0% (0)	17
36–40	100% (17)	0% (0)	17
41–45	100% (17)	0% (0)	17
46–50	100% (17)	0% (0)	17
51–55	100% (17)	0% (0)	17
56–60	100% (17)	0% (0)	17

Table 3. Percentage of pregnant does based on foetal heart beat

Days after mating	Heart beat observed	Heart beat not observed	Total
25–30	0% (0)	100% (17)	17
31–35	35.3% (6)	64.7% (11)	17
36–40	58.8% (10)	41.2% (7)	17
41–45	88.2% (15)	11.8% (2)	17
46–50	100% (17)	0% (0)	17
51–55	100% (17)	0% (0)	17
56–60	100% (17)	0% (0)	17

Fig. 2. Gestational sac at different days of gestation (A: Gestational sac below the bladder at 25 days after mating; B: Gestational sac with embryonic vesicles at 26 days after mating; C: Gestational sac at 28 days of gestation; D: Gestational sac with embryonic vesicle at 30 days after the mating).

pregnancy. The frequency of detection of placentomes increased with the increase of gestation age and the percentage accuracy of detecting pregnancy was 52.9, 82.4 and 100% between 36-40 days, 41-45 days and 46-50 days after mating respectively (Table 4 and Fig. 3).

Leg buds

Leg buds could not be detected till 36-40 days following mating. However, the detection of leg buds was first observed at 36-40 days in four does (23.5%). The accuracy of pregnancy by detection of leg buds were 52.9, 76.5 and 100% between 41-45, 46-50 and 51-55 days respectively (Table 5 and Fig. 4).

Vertebral column

The vertebral column was detected in three does out of

seventeen with accuracy of 17.6% at 36-40 days after the mating. The percentage of pregnancy was increased with the advancement of gestation and was 41.2, 70.6, 82.4 and 100% at 41-45, 46-50, 51-55 and 56-60 days of pregnancy, respectively (Table 6 and Fig. 5).

DISCUSSION

The pregnancy diagnosis in goats is a primary factor for improving the reproductive performance in herds and achieving high economic efficiency. During the years, multiple techniques for pregnancy detection in small ruminants have been developed.

Vaginal cytology

During the study a total of 5 types of vaginal cells were identified. The typifying of morphologic and morphometric characteristics of the epithelial cells from the vagina was accordance with the study of other researchers (Grant, 1933) that the vaginal epithelial cells thrown into four types such as parabasal, intermediate, superficial and superficial keratinized cells. The average percentage of intermediate, superficial, keratinized and neutrophils cells during early stage of pregnancy were 81.12%, 9.41%, 7.10% and 2.61% respectively on 22-40 days. This results recorded in this study are similar to the study of Ghannam

Table 4. Percentage of pregnant does based on placentomes

Days after mating	Placentomes observed	Placentomes not observed	Total
25-30	0% (0)	100% (17)	17
31-35	35.3% (6)	64.7% (11)	17
36-40	52.9% (9)	47.1% (8)	17
41-45	82.4% (14)	17.6% (3)	17
46-50	100% (17)	0% (0)	17
51-55	100% (17)	0% (0)	17
56-60	100% (17)	0% (0)	17

Fig. 3. Placentomes at different stages of gestation (Placentomes-A: at 31-35 days of gestation; B: at 36-40 days of gestation; C: at 41-45 days of gestation; D: at 46-50 days of gestation).

Table 5. Percentage of pregnant does based on observation of leg buds

Days after mating	Leg bud observed	Leg bud not observed	Total
25-30	0% (0)	100% (17)	17
31-35	0% (0)	100% (17)	17
36-40	23.5% (4)	76.5% (13)	17
41-45	52.9% (9)	47.1% (8)	17
46-50	76.5% (13)	23.5% (4)	17
51-55	100% (17)	0% (0)	17
56-60	100% (17)	0% (0)	17

Table 6. Percentage of pregnant does based on observation of vertebral column

Days after mating	Vertebral column observed	Vertebral column not observed	Total
25-30	0% (0)	100% (17)	17
31-35	0% (0)	100% (17)	17
36-40	17.6% (3)	82.4% (14)	17
41-45	41.2% (7)	58.8% (10)	17
46-50	70.6% (12)	29.4% (5)	17
51-55	82.4% (14)	17.6% (3)	17
56-60	100% (17)	0% (0)	17

Fig. 4. Leg buds at different stages of gestation (A: at 31-35 days of gestation; B: 36-40 days of gestation; C: 41-45 days after mating). **Fig. 5.** Vertebral column at different stages of gestation (A: at 36-40 days of gestation; B: Vertebral Column and head of foetus at 41-45 days of gestation; C: at 51-55 days of gestation; D: Vertebral Column and heart of foetus at 56-60 days of gestation).

et al. (1972) observed that during the early stage of pregnancy of gestation length in does; there is an increase in intermediary cell numbers. Grant (1933) was also reported similar histological changes in vaginal mucosa during an-

oestrus and pregnancy in goat.

Leigh et al. (2010) confirmed 83.3% pregnancy in does where there was sharp increase in superficial cells and clumping of epithelial cells. Only one doe (17.7%) was

not pregnant and this was the doe whose vaginal smear though showed similar pattern of exfoliated cells with others, was negative for sperm cells. Sharma (2016) reported various vaginal epithelial cells during early, mid and advanced stage of pregnancy in cows as 81.63, 85.9 and 88.23% of intermediate, 13.83, 5.97 and 4.53% of parabasal cells, 2.97, 5.5, 4.64% of superficial squamous and 1.57, 2.55 and 2.60% of cornified cells respectively which was more or less similar to our study except present of a few percentages of parabasal cells.

In the present study, the percentages of vaginal cells during early stages of pregnancy easily differentiated with percentages of vaginal cells during the stages of oestrous cycle of goats (Ola et al., 2006). Higher percentages of intermediate cells were found at early stages of pregnancy but in case of oestrous cycle few percentages were found.

Real-time, B-mode ultrasonography

1) Gestational sac

In the present investigation, the gestational sac, in the form of circular and elongated fluid filled anechogenic images located cranial to bladder was seen in only three does with 17.6% accuracy at 25 to 30 days after mating. The findings of current study are in accordance with Gearhart et al. (1988), Taverne et al. (1985) and Bretzlaff et al. (1993) who also reported that pregnancy could be first verified at 25 to 30 days after the breeding. No false positive cases were recorded in the present study. False positives results are rare and are attributed to the early embryonic death, unobserved abortion and sometimes misinterpreting the urinary bladder as the uterus (Fowler and Wilkins, 1984; White, 1984; Haibel et al., 1990).

2) Foetal heart beat

In the present study, heart beat was observed for the first time at 31-35 days of gestation in six does out of seventeen with 35.3% of accuracy. The heart beat became more prominent with the advancement of gestation and the rate of accuracy detected was 58.8, 88.2 and 100% at 36-40, 41-45 and 46-50 days of pregnancy, respectively.

The fetal heartbeat is one of the preferred indicators of pregnancy in does and ewes because it provides conclusive evidence of the presence of a live foetus (Aiumlamai, 1992; Amer et al., 2007). Similar observation have been reported where in fetal heart beats was determined clearly as early as 25 to 30 day of pregnancy and the foetus visu-

alized by transrectal ultrasound (Oral et al., 2007). However, Aiumlamai et al. (1992) reported that fetal hearts in ewes were clearly seen at day 44 to 63 days of gestation and heart chambers and valves are distinguishable from day 97 to 103 of gestation by trans-abdominal ultrasonography. Further, the author also reported that the foetal heart rate reached a plateau by 7 weeks before lambing (167 ± 1.5 beats per minute) and decreased to 139.0 ± 15.7 and 117.0 ± 9.2 bpm at 3 weeks before and at lambing respectively.

3) Placentomes

In the present study, placentomes were detected with accuracy of 35.3, 52.9, 82.4 and 100% at 31-35, 36-40, 41-45 and 46-50 days of pregnancy, respectively. Studies by Russel (1989) revealed that placentomes were identified from about day 40 as echoic circular structures in sheep. On day 42, the ovine placentomes appears to be cup-shaped form and reaching the maximum size by day 74 (Doize et al., 1997). Placentomes were detected with 100% at 45-50 days after the mating (Anwar et al., 2008). However, a poor correlation between placentomes size and ovine gestational age has been reported which was ascribed to great variation in the size of placentomes in different breeds (Doize, 1997; González et al., 1998). Hence, the finding of present study is in agreement with the previous reports that have also employed trans-abdominal ultrasonography using a 3.5 MHz probe (Russel, 1989; Anwar et al., 2008).

4) Foetal leg buds

In the present study, the leg buds were first observed at 36-40 days of gestation only in four does with accuracy of 23.5% and was seen in all the seventeen does by 51-55 days after the mating. Similar observations have also been reported previously by Anwar et al. (2008). It can be opined that the pregnancy diagnosis by trans-abdominal ultrasound based on foetal leg buds could be detected with 100% accuracy between 51 to 55 days of gestation.

5) Vertebral column

The vertebral column was first seen at 36-40 days of gestation and the percentage of accuracy of detecting foetal vertebral column increased with the advancement of gestation period. The pregnancy was detected in all the does by 55-60 days of gestation which is similar to the

findings of Anwar et al. (2008). He confirmed that pregnancy diagnosis by trans-abdominal ultrasound based on vertebral column was done by 55–60 days of pregnancy with 100% accuracy.

CONCLUSION

The study concluded that vaginal cytology may be used for detection of pregnancy in doe. The real time transabdominal ultrasonography can be easily utilized for early pregnancy diagnosis in does at field level.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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