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ORIGINAL ARTICLE

Evaluation of the performance of three tests for the early pregnancy diagnosis in ewes and does

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Abstract

Background: The aim of this study was to evaluate the performance of three tests for the early pregnancy diagnosis in ewes and does.

Methods: A total of 50 ewes and 50 does urine samples were collected in which 25 ewes and 25 does were pregnant, and 25 ewes and 25 does were non-pregnant from different farmers in Babugonj upazila of Barishal district and Sadar upazila of Bhola district. The pregnant and non-pregnant ewes and does were primarily selected from farmer's record of management history. The pregnancy of ewes and does were confirmed through scanning by B-mode ultrasound machine. Three pregnancy diagnostic tests like barium chloride, copper sulfate and ballottement were evaluated considering B-mode ultrasound technique as gold standard.

Results: The sensitivity of manual ballottement technique (MBT) was highest among the three tests: 96% in ewes and 92% in does. On the other hand, the specificity (96%) of the MBT was higher in ewes and that of the copper sulfate test (CST) (91.7%) was higher in does. The highest positive predictive value (PPV) of MBT (95.8%) was in ewes but the PPV of CST was highest (91.7%) in does. The negative predictive value of the MBT was highest both in ewes (92.3%) and does (95.7%).

Conclusions: MBT can be considered as a screening test for the early diagnosis of pregnancy in ewes and does. MBT positive cases can be confirmed by ultrasonograpy whenever needed.

Key words: Ultrasonography, Barium chloride, copper sulfate, ballottement

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Introduction

The people of Bangladesh are loving animals from very old years for domestic as well as companion animals. The productivity of farm animals is essential for profitable farming. The early pregnancy diagnosis is one of the keys for animals' management. In human, there is a lot of early pregnancy diagnostic kits, available in the market. Peoples are also habituated to use that kit as well. It is also well known that the laboratory test is needed for confirmative diagnosis. However, the scenario in case of animals is quite reverse from human. People used to practice rectal palpation in case of large animals i.e. cows, buffaloes, mares etc. In case of sheep and goat, the assumptive absence of next cyclicity is only tool for pregnancy diagnosis. Most of the time, it proofs false pregnancy diagnosis. The ewe may not show cyclicity due to nutritional deficiency, hormonal imbalance, and season etc. The rectal palpation in cows is the method for pregnancy diagnosis on 60-90 days of pregnancy (Cowie, 1948). The ultra-sonogram may use in animals at early days of pregnancy but the ultrasound machine is very unavailable and expensive. Though per-rectal palpation is the cheapest pregnancy diagnosis method, it is suggested that examining pregnant cows early in gestation by trans-rectal palpation increases the risk of iatrogenic embryonic mortality (Franco *et al*., 1987) Therefore a newer technology may replace the trans-rectal method of pregnancy diagnosis in the dairy industry as well as for others domestic animals. For diagnosing pregnancy, intra-rectal Doppler technique is superior to the external technique during early second trimester (Ott *et al*., 1981). Purohit (2010) reviewed that pregnancy diagnosis might be done by observing signs of pregnancy exhibited by female, examination of ovary, uterus and vagina, laboratory tests and use of rapid milk progesterone test kits. Analysis of progesterone level in blood and urine has been studied by different researcher. Laboratory methods like copper sulfate test, Sodium hydroxide test, Barium chloride test, sodium benzoate test are used in different farm animals infrequently. But these methods are not reliable. Recently, determination of progesterone in blood and milk was based on radio immune assay (RIA) and enzyme-linked immunasorbent assay (ELISA) test (Pieterse *et al*., 1990). Plasma progesterone concentration is determined 18 days post breeding in ewes (Dobeli and Schwander, 1985) and does may be tested on 19-23 days after breeding with high accuracy. (Murray and Newstead, 1988). Hulet (1972) was first to describe rectal abdominal palpation technique for diagnosing pregnancy in the ewe, which has also been used in the doe (Ott *et al*., 1981). However there are very few studies for early diagnosis of pregnancy in ewes and does (Lone *et al*., 2016; Thakchos, 2011; Mayura *et al*., 2009). In Bangladesh, there is no specific study for small ruminant (sheep and goat) pregnancy diagnosis method. Only rectal palpation is practicing in case of cows but the pregnancy diagnosis of small ruminants is very difficult. Therefore, the aim of present study is to evaluate the performance three tests for the early pregnancy diagnosis in pregnant ewes and does.

Materials and methods

Study location and animals

The urine samples were collected from different farmers in Babugonj upazila of Barishal district and sadar upazila of Bhola district. The laboratory test was conducted in the Theriogenology and Animal Reproductive Biotechnology laboratory, Department of Medicine, Surgery and Obstetrics, Faculty of Animal science and Veterinary Medicine, Patuakhali Science and Technology University, Barishal Campus, Barishal. A total of 50 ewes and 50 does samples were collected in which 25 ewes and 25 does were pregnant, and 25 ewes and 25 does were non-pregnant. The pregnant and non-pregnant ewes and does were primarily selected from farmer's record of management history. The pregnancy of ewes and does were confirmed through scanning by B-mode ultrasound machine.

Pregnancy diagnosis by manual ballottement

The pregnancy was diagnosed by manual ballottement as described earlier (Cowie, 1948; Pratt and Hopkins, 1975). It is easier in thin ewes and does than does in fat animals. It was conducted as described by. Briefly by placing a hand on either side of the abdomen and squeezing or lifting upwards, the gravid uterus or fetus can sometimes be palpated through the relaxed abdominal wall. A fetus can sometimes be balloted low in the right flank during the last month of gestation. Examination becomes easy when feed and water are withheld for at least 12h before.

Diagnostic procedure

Collection of samples

Urine was collected from pregnant and pregnant ewes and does and brought to laboratory within icebox and stored in the refrigerator (4 to 8°C).

Copper Sulfate test

It was performed, in briefly, 0.25 ml cervical mucus was placed in CuSO4 solution. Mucus sinks indicate pregnancy positive case and mucus float pregnancy negative case.

Barium chloride test

It was conducted as described by Maslov and Smirnov (1965). Briefly, 5ml of urine will be added into 1% Barium chloride solution. Turbidity due to precipitation was indicated not pregnant animal whereas clear solution will be indicated pregnant animal.

Data analysis

The collected data was recorded and coded in Microsoft excel sheet. The sensitivity and specificity of the tests were calculated by dividing the true positive and true negative test results with the total number of pregnant and non-pregnant animals, respectively. The positive and negative predicative values of different tests were estimated by dividing the true positive and true negative test results with the total number of positive and negative results, respectively. The percent difference among sensitivities and specificities were assessed by the chi-square test using SPSS statistical Software. Differences were considered significant at a level of $P < 0.05$.

Results

Evaluation of different pregnancy diagnostic technique in ewes and does

The sensitivity of manual ballottement technique (MBT) was among the three tests: 96% in ewes and 92% in does. On the other hand specificity (96%) the MBT was higher in ewes and that of the copper sulfate test (CST) (91.7%) was higher in does. The highest positive predictive value (PPV) of MBT was highest (95.8%) in ewes but the PPV of CST was highest (91.7%) in does. The negative predictive value of the MBT was highest both in ewes (92.3%) and does (95.7%) (Tables 1 & 2).

Our findings have the similarity with the studies of Rao and Veena (2009) who found that the percentages of true positive reaction for pregnancy and non-pregnancy status in cows by urine barium chloride test were 80 and 10. Though the efficacy for true positive reaction for pregnancy is comparable with the reports of Kavani (1983), Vohra and Kaikini (1992), it is higher than those reported by Verma and Mishra (1981) and lower than those reported by Moslov and Smirnov (1965) and Temblador and Landa (1971). The efficacy of this test for detecting non-pregnancy is less than those reported by Elpakov and Cyganok (1966), Akhmadeev and Vasilev (1967), Temblador and Landa (1971), Kavani (1983), and Vhora and Kaikini (1992). While in this study false negative reaction for pregnancy was only 24%, false negative for non-pregnancy was 84%. Rao and Veena (2009) found that the false negative reaction for pregnancy was only 20%, false negative for non-pregnancy was 90%.

Table 1: Evaluation of different pregnancy diagnostic techniques in ewes considering ultrasonography as a gold standard

Tests	Pregnant ewes (25)		Non-pregnant ewes (25)			
	True Positive (Sensitivity: %)	False Negative	False Positive	True Negative (Specificity: %)	PPV (%)	NPV (%)
Barium Chloride test	18 (72.0)[a]	7	3	22 (88.0)	85.9	75.9
Copper sulfate test	20 (80.0)[a]	5	4	21 (84.0)	83.3	80.8
Manual ballottement	23 (92.0)[b]	2	1	24 (96.0)	95.8	92.3

[a, b] within the column indicates the significance difference ($p<0.05$), PPV=Positive Predictive Value, NPV=Negative Predictive Value

Table 2: Evaluation of different pregnancy diagnostic techniques in does considering ultrasonography as a gold standard

Test	Pregnant does (25)		Non-pregnant does (25)			
	True Positive (Sensitivity: %)	False Negative	False Positive	True Negative (Specificity: %)	PPV (%)	NPV (%)
Barium chloride test	19 (76.0)[a]	6	4	21 (84.0)	82.6	77.8
Copper sulfate test	22 (88.0)[b]	3	2	23 (92.0)	91.7	88.5
Manual ballottement	24 (96.0)[b]	1	3	22 (88.0)	88.9	95.7

[a, b,c] within the column indicates the significance difference ($p<0.05$)

Conclusion

The MBT can be considered as a screening test for the early diagnosis of pregnancy in ewes and does. MBT positive cases can be confirmed by ultrasonography whenever needed.

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Competing of interest

None to declare.

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