

UNDER PEER REVIEW

Abstract

The objective of this study was to evaluate the accuracy of pregnancy diagnosis in dairy cows by using barium chloride in urine samples. The investigation was conducted from July 2021 to June 2022. Urine samples were collected from four distinct Upazillas in three districts: Savar in Dhaka, Shivalaya in Manikganj, and Sreenagar and Serajdikhan in Munshiganj, Bangladesh. At three-week intervals, urine samples were collected non-invasively into a plastic test tube that was tightly sealed with a plastic cork. In total, urine samples were obtained from 50 inseminated dairy heifers, with 20 of them located in Sreenagar & Serajdikhan, 15 in Savar, and the remaining 15 in Shivalaya Upazila. The urine samples were transported to the Physiology Laboratory at the Department of Anatomy, Histology, and Physiology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. 5 ml of 1% Barium chloride solution was mixed with 5 ml of urine to conduct the barium chloride test. The reproductive status of dairy cows significantly affected the results of the tests, resulting in a success rate of nearly 80% during the first trimester of pregnancy. It was concluded that the Barium chloride test is suitable for pregnancy diagnosis in dairy cows. In brief, the Barium chloride test has the potential to become a standard diagnostic method for pregnancy in cows during the first trimester.

Keywords: Barium chloride, Dairy cows, Diagnosis, Pregnancy, Urine.

INTRODUCTION

A profitable dairy farming system largely depends upon the production level of the farm. Reproduction with a high fertility rate plays an important role in this case (Plaizier *et al.*, 1997 and Meadows *et al.*, 2005). It is imperative for a dairy farm to be sustainable and to ensure that

cows conceive at the appropriate time. A reproductive frequency of 60 days postpartum dryness is more suitable for a dairy heifer. Dairy farmers must determine non-pregnancy as soon as feasible in order to rebreed the cows (Balhara *et al.*, 2013). Despite the pervasive use of artificial insemination (AI), extensive cross-breeding programs, and an increase in the total number of days open, the fertility of dairy cows in lactation has been declining globally in recent years, with a reduction in conception rate (Smith, 2010). This decline in reproductive performance in dairy cows is closely associated with disrupted ovary function as a result of repeat breeding (RB) or other factors. Ovarian dysfunction induces abnormal endocrine surges at various phases of the estrus cycle. During the estrus cycle, elevated progesterone (P4) levels are associated with diminished estradiol levels, a delayed surge of luteinizing hormone (LH), and suboptimal fertilization, leading to the aging of formed follicles due to these causes. An earlier study indicated that during the initial luteal phase, suboptimal conception rates correlated with a delayed increase in progesterone levels (Bage *et al.*, 2002; Canu *et al.*, 2010). Furthermore, it is recognized that cows designated as repeat breeders exhibit qualitative changes in their oocytes and follicular fluids. It may result in fertilization failure or premature embryonic mortality (Kurykin *et al.*, 2011; Kafi *et al.*, 2017; Sood *et al.*, 2017). The ovary's mature and persistent follicles maintain a higher estradiol level, which results in recurrent estrus. Extended periods of anestrus with a non-pregnant state are the consequence of a persistent corpus luteum in the ovary. Determining the ovary status is the critical regulator for achieving optimal performance in dairy heifers.

Ultrasonography and manual palpation are among the conventional methods employed to determine the status of the ovary. It necessitates professional personnel and a skilled hand. In order to expedite the pregnancy examinations following insemination, it may be advantageous to identify non-pregnant heifers at an early stage and reschedule them for a subsequent artificial insemination. The most significant aspect of these elements is the potential for the use of

chemicals to screen for conception. A recent study has observed a significant increase in the use of a specific blood test for pregnancy (Stevenson, 2008). However, it is neither easier nor more cost-effective for a farmer at the field level. The farming industry is increasingly adopting alternative pregnancy detection methods that do not necessitate specialist equipment or extensively trained workers (Lucy *et al.*, 2011).

Chemical examinations have been conducted for the diagnosis of pregnancy based on the hormone secreted from the ovary. However, with the ovarian state, it is not optimized. At the field level, sufficient information is lacking for detecting ovarian state through chemical analysis of urine samples. The current study posits that the ovary secretes several hormones that are excreted in urine, and the condition of the ovary is reflected by chemical analysis. It is essential to optimize and standardize the chemical analysis of urine concerning ovarian conditions in field settings, without requiring specialist equipment or skilled workers, to enhance the performance of dairy cows.

Early Pregnancy Detection (EPD) is crucial for minimizing the interval between calvings. It enhances the productive lifespan of cows and enhances daily milk yield throughout the herd's lifetime.

This study intended to develop a novel, economical, and early approach for detecting pregnancy in dairy cows and to evaluate the accuracy of this diagnostic compared to other techniques.

MATERIALS AND METHODS

Selection of the study area and duration

This research was conducted inside the Department of Anatomy, Histology and Physiology (ANHP) at Sher-e-Bangla Agricultural University, Dhaka, for a period of 12 months, from July 2021 to June 2022, to diagnose pregnancy in dairy cows during the first trimester. The study

areas included four upazilas of Dhaka division: Savar (Dhaka District), Shivalaya (Manikganj District), Sreenagar, and Serajdikhan (Munshiganj District).

Ethical statement

The experimental protocols of this work received approval from the Local Animal Care and Ethics Committee of Sher-e-Bangla Agricultural University (ASVM/SAU/07/2023).

Study animals and reproductively sound cow selection

Fifty reproductively sound cows were utilized for the experiment. The experimental cows were chosen from various farms in certain regions. Urine samples were obtained from 50 dairy cows across four distinct upazilas: Savar in Dhaka, Shivalaya in Manikganj, and Sreenagar and Serajdikhan in Munshiganj. Of them, 20 are from Sreenagar and Serajdikhan, 15 from Savar, and the remaining 15 cows are from Shivalaya Upazila. All cows were in their estrous cycle. The date of parturition was documented for each pregnancy based on the date of Artificial Insemination (AI), and the sampling period in cows was quantified as the number of days preceding parturition. Furthermore, the date of the last successful mating, regarded as the probable conception date, was acquired from the breeders. The selection of reproductively viable cows is contingent upon the outcomes of a physical examination of the female genitalia. Their Body Condition Score (BCS) was assessed appropriately.

Animal preparation, application of Ovsynch program and insemination

Throughout the study, the selected cows were provided with a balanced diet and allowed to graze freely. Before Artificial Insemination (AI), each cow undergoing therapy was incorporated into an own Ovsynch program (Figure 1). The single Ovsynch program and the exact day of ovulation were implemented with slight adjustments, as previously stated (Shirasuna *et al.*, 2015). The cows exhibiting large follicles were administered an injection of PGF2 α seven days after a single intramuscular dose of GnRH. Another dose of GnRH was

administered 48 to 56 hours after the initial dose. Timed Artificial Insemination (TAI) was conducted 16 (8-18) hours after the second GnRH dose. The entire animal population was inseminated appropriately.

Preparation of solution

1% barium chloride (BaCl_2) solution was produced by combining refined water and the chemical agent, barium chloride. Initially, 1 gm of barium chloride was meticulously measured and combined with 100 ml of distilled water. Subsequently, the vessel containing the solution was shaken for a few minutes using a Vortex machine (Figure 2).

Urine collection

Urine samples were obtained from each individual animal at three distinct intervals. The initial sampling occurred on day 42, the second on day 63, and the last one on day 84 of pregnancy, spanning from July 2021 to June 2022, with a total of three samplings conducted for each female. The sample interval for routine chemical analysis of the Barium Chloride test spanned from day 42 to day 84 post-conception. Urine was caught during spontaneous urination of the animals in 250 ml plastic test tube that were fastened onto a plastic cord (Fedorova *et al.*, 2015). Collected urine was placed into plastic test tube holders for the purpose of transport and storage. Samples were refrigerated (Fedorova *et al.*, 2015) at 5-7 °C until testing. Before testing, urine was kept outside the refrigerator to reach room temperature and was then homogenized.

Urine analysis using BaCl_2 solution

Barium chloride tests were carried out in the laboratory of Physiology, Department of Anatomy, Histology and Physiology, Faculty of Animal Science and Veterinary Medicine of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh, within one week after collection. In a test tube, 5 ml of urine was mixed with 5 ml of a 1% BaCl_2 solution (Krishna and Veena 2009, Lalrintluanga and Dutta 2009). Then, the solution was agitated and left to stand for 5 min (Ndu

et al., 2021); after this, the results were evaluated. Samples that showed any degree of cloudiness or turbidity after 5 min were regarded as having shown precipitation with the reagent, and the test was concluded as negative (animal should be non-pregnant). When no reaction occurred in 5 min, the test was concluded as positive (animal should be pregnant) (Ndu *et al.*, 2021) (Figure 3). Finally, the result was observed carefully and kept for further analysis of the ovary status of the cow.

Rectal palpation

The selected cows had manual rectal palpation at a specific cycle point, which was associated with standard chemical analysis of barium chloride. Additional assessment has been conducted by examining the parturition history.

Statistical analysis

The precision of Barium Chloride (BaCl_2) tests was assessed by comparing the results with the actual reproductive state of females, categorized as either pregnant or non-pregnant. The test results, aligned with the reproductive status of females, were evaluated as accurate. The reliability of individual tests in pregnant females was assessed solely during the first trimester of pregnancy. The analysis in dairy cows was categorized into three distinct periods: the initial 42 days of gestation, the subsequent 63 days, and the last 84 days of pregnancy. The data utilized in this study are computed and analyzed using Microsoft Excel software. The analysis of this data is considered statistically significant. This analysis will assist us in creating a more effective results sheet.

RESULTS

The total number of study animals, specifically dairy cows, is 50, of whom 44 are pregnant. Confirmation is subsequently achieved via rectal palpation and later through parturition. The

other six dairy cows are non-pregnant, constituting 12% of the total, while the forty-four pregnant dairy cows represent 88%. These data represent the current reproductive state of the dairy cows (Table 1 and Figure 4).

During the estrus period, urine analysis utilizing the barium chloride (BaCl_2) test revealed white precipitation indicative of mature follicles in the ovary, while during the diestrus period, colorless precipitation was associated with the corpus luteum.

The Barium Chloride (BaCl_2) test is conducted on 44 pregnant dairy cows using urine analysis utilizing a 1% Barium Chloride (BaCl_2) solution on days 42, 63, and 84 post artificial insemination (AI) (Table 2). The colorless translucent solution in the BaCl_2 test indicated a positive pregnancy result. Conversely, the white precipitate in the BaCl_2 test cocktail indicated a negative pregnancy result. The highest accuracy was observed on the 84th day, validated by rectal palpation and parturition records. The least accurate result was observed on the 42nd day post-AI. On day 42, the count of identified pregnant cows is 31, constituting 70.45%, whereas the count of non-pregnant cows is 13, representing 29.55% (Table 2). On day 63, the count of identified pregnant cows is 35, constituting 79.55%, while the count of non-pregnant cows is 9, representing 20.45%. On day 84, the count of identified pregnant cows is 37, representing 84.09%, while the count of non-pregnant cows is 7, accounting for 15.91% of the total (Figure 5).

The study's findings at the field level provide a straightforward manual tool for employing chemical analysis to figure out an ovary's condition. As a result, the farmers will get greater benefits at the initial stage.

DISCUSSION

Identifying non-pregnant cows is essential to reduce the calving interval, hence facilitating subsequent pregnancies. Furthermore, milk production will be augmented, rendering dairy

farming more lucrative. Ultimately, producers will achieve increased earnings and exhibit greater interest in dairy production. Ciplak (2024) emphasized that early pregnancy identification on cattle farms is crucial for the reproductive management of dairy operations.

Out of the total of 50 heifers, 44 were actually pregnant, which accounted for 88% of the study. The remaining six heifers were not pregnant. It was a reference to 12% of the total animal population. All of the calves were initially examined for physical health. The research animals that were chosen did not have a history of any significant genital diseases.

The study was conducted meticulously. Urine samples were obtained from several locations within the Dhaka division, including Dhaka, Manikganj, and Munshiganj districts. Subsequent to the collection of these samples, they were stored in a refrigerator at a temperature of 5-7 °C until testing. A 1% Barium Chloride (BaCl_2) solution was utilized for this study. Barium Chloride (BaCl_2) solution has been demonstrated to be effective in identifying pregnancy in multiple animal species.

This study revealed an accuracy rate of 70.45% (31 cows) on day 42, 79.55% (35 cows) on day 63, and 84.09% (37 cows) on day 84 using the barium chloride (BaCl_2) test for genuine pregnant cows. The mean of these three outcomes is 78.0%, indicating approximately an 80% success rate throughout the first trimester of pregnancy.

The non-pregnancy rates were 29.55% (13 cows) at day 42, 20.45% (9 cows) at day 63, and 15.91% (7 cows) at day 84. These pregnancy diagnosis results correlate with similar research published in reputable international publications.

Samsonova *et al.* (2014) found that the accuracy of milk progesterone ELISA for pregnancy identification in cows was 87.0%. However, it is not particularly economical. Ghaidan *et al.* (2019) noted that the accuracy of rectal palpation was 87.2%, whereas the accuracy of the progesterone assay was 84.8%. The conventional rectal palpation method poses certain health

risks to the animal being examined. Ongoing hand examinations may lead to infections in the cow's reproductive organs. It also elevates the risk of infertility. Conversely, the progesterone assay is not well recognized at the farm level. Pennington *et al.* (1985) observed that accuracy rates linked with this study increased from 70.45% to 84.09% between day 42 and day 84.

The Barium Chloride (BaCl_2) test demonstrates a notable accuracy rate in diagnosing pregnancy in cows at the foundational level. It is also economically efficient. The accuracy of the research results was verified by examining the parturition history of the experimental animals. Ultimately, the findings of this study will be beneficial for further research that may assist dairy farmers.

CONCLUSION

The optimal dairy yield significantly relies on the timely identification of early pregnancy. However, regarding accuracy, the currently employed procedures are not flawless diagnostic methods due to their inherent limits. Their subsequent phases of application may be questionable. Additionally, intricate apparatus and a scientific environment are also necessary. Cows in the first trimester of gestation can safely utilize the barium chloride test as a diagnostic method for pregnancy. This study technique may enable farmers to readily identify the early pregnancy of dairy cows. Additional study is necessary to guarantee the enhancement of this approach.

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Table 1. Actual Reproductive Status of the Dairy Cows

Total Number of Dairy Cows	Actual Reproductive Status of Dairy Cows		Percentage (%)	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
n = 50	44	06	88	12

Table 2. Result of Barium Chloride (BaCl₂) Test of the Pregnant Dairy Cows

Time	Total number of pregnant dairy cows	Barium Chloride (BaCl ₂) Test Result		Percentage (%)	
		Pregnant	Non-pregnant	Pregnant	Non-pregnant
At day 42	n = 44	31	13	70.45	29.55
At day 63		35	09	79.55	20.45
At day 84		37	07	84.09	15.91

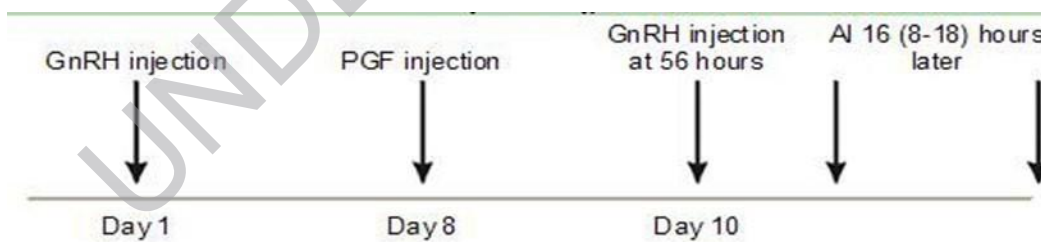


Figure 1. Ovosynch Program.



Figure 2. Measuring Barium Chloride (A), Shaking the mixer with vortex machine (B), Final solution (C).



Figure 3. Chemical analysis of urine using Barium Chloride (BaCl_2) solution.

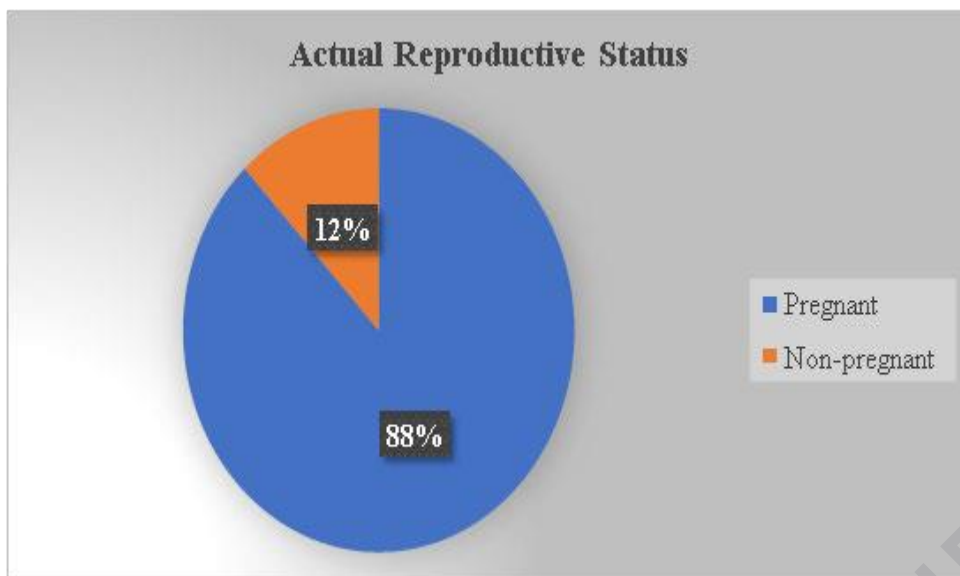


Figure 4. Actual reproductive status of the cows.

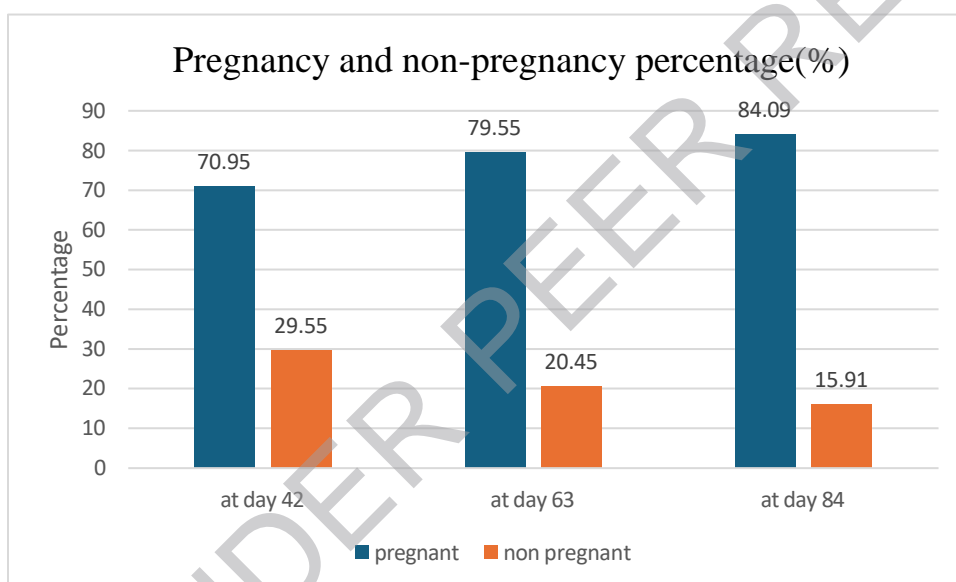


Figure 5. Pregnancy percentage of the cows at different times using Barium Chloride Test.