

13 **Diagnosis of pregnancy in dairy cows using Barium Chloride test**

14 **Abstract**

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

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

32 **INTRODUCTION**

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

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

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

61 chemicals to screen for conception. A recent study has observed a significant increase in the
62 use of a specific blood test for pregnancy (Stevenson, 2008). However, it is neither easier nor
63 more cost-effective for a farmer at the field level. The farming industry is increasingly adopting
64 alternative pregnancy detection methods that do not necessitate specialist equipment or
65 extensively trained workers (Lucy *et al.*, 2011). The barium chloride test is an inexpensive and
66 time saving alternative compared to ELISA in pregnancy diagnosis when the insemination date
67 is unknown (Dana *et al.*, 2020). In the 1st trimester, pregnancy diagnosis using the barium
68 chloride test was performed better, respectively, in terms of accuracy and specificity when
69 compared to progesterone-ELISA (P4-ELISA) (Shaikat *et al.*, 2024). Ultrasonography provides
70 accurate diagnosis from 28 days, but its use is limited in low-resource settings due to
71 equipment costs and the need for technical expertise (Silva *et al.*, 2007)
72 In testing using the BaCl₂ method (Lalrintluanga and Dutta, 2009) the urine sample was diluted
73 1:1 with 1% BaCl₂ solution. Samples from pregnant animals will result in a clear solution, and
74 those from non-pregnant animals will result in precipitation and a turbid solution (Azmi *et al.*,
75 2020)

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

84 Early Pregnancy Detection (EPD) is crucial for minimizing the interval between calvings.
85 Early pregnancy diagnosis is crucial to shortening the calving interval by enabling the farmer to
86 identify open animals so that they can be treated and/or rebred at the earliest opportunity
87 (Azmi *et al.*, 2020). It is essential for effective management of pregnant animals and early

88 submission of non-pregnant animals for subsequent breeding to reduce calving to conception

89 interval (Abdullah *et al.*, 2014).

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

93 **MATERIALS AND METHODS**

94 **Selection of the study area and duration**

95 This research was conducted inside the Department of Anatomy, Histology and Physiology
96 (ANHP) at Sher-e-Bangla Agricultural University, Dhaka, for a period of 12 months, from July
97 2021 to June 2022, to diagnose pregnancy in dairy cows during the first trimester. The study
98 areas included four upazilas of Dhaka division: Savar (Dhaka District), Shivalaya (Manikganj
99 District), Sreenagar, and Serajdikhan (Munshiganj District).

100 **Ethical statement**

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

103 **Study animals and reproductively sound cow selection**

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

114 Their Body Condition Score (BCS) was assessed appropriately.

115 **Animal preparation, application of Ovosynch program and insemination**

116 Throughout the study, the selected cows were provided with a balanced diet and allowed to
117 graze freely. Before Artificial Insemination (AI), each cow undergoing therapy was
118 incorporated into an own Ovosynch program (Figure 1). The single Ovosynch program and the
119 exact day of ovulation were implemented with slight adjustments, as previously stated
120 (Shirasuna *et al.*, 2015). The cows exhibiting large follicles were administered an injection of
121 PGF2 α seven days after a single intramuscular dose of GnRH. Another dose of GnRH was
122 administered 48 to 56 hours after the initial dose. Timed Artificial Insemination (TAI) was
123 conducted 16 (8-18) hours after the second GnRH dose. The entire animal population was
124 inseminated appropriately.

125 **Preparation of solution**

126 1% barium chloride (BaCl₂) solution was produced by combining refined water and the
127 chemical agent, barium chloride. Initially, 1 gm of barium chloride was meticulously measured
128 and combined with 100 ml of distilled water. Subsequently, the vessel containing the solution
129 was shaken for a few minutes using a Vortex machine (Figure 2).

130 **Urine collection**

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

140 **Urine analysis using BaCl₂ solution**

141 Barium chloride tests were carried out in the laboratory of Physiology, Department of Anatomy,
142 Histology and Physiology, Faculty of Animal Science and Veterinary Medicine of Sher-e-
143 Bangla Agricultural University, Dhaka, Bangladesh, within one week after collection. In a test
144 tube, 5 ml of urine was mixed with 5 ml of a 1% BaCl₂ solution (Krishna and Veena 2009,
145 Lalrintluanga and Dutta 2009). Then, the solution was agitated and left to stand for 5 min (Ndu
146 *et al.*, 2021); after this, the results were evaluated. Samples that showed any degree of
147 cloudiness or turbidity after 5 min were regarded as having shown precipitation with the
148 reagent, and the test was concluded as negative (animal should be non-pregnant). When no
149 reaction occurred in 5 min, the test was concluded as positive (animal should be pregnant) (Ndu
150 *et al.*, 2021) (Figure 3). Finally, the result was observed carefully and kept for further analysis
151 of the ovary status of the cow.

152 **Rectal palpation**

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

156 **Statistical analysis**

157 The precision of Barium Chloride (BaCl₂) tests was assessed by comparing the results with the
158 actual reproductive state of females, categorized as either pregnant or non-pregnant. The test
159 results, aligned with the reproductive status of females, were evaluated as accurate. The
160 reliability of individual tests in pregnant females was assessed solely during the first trimester
161 of pregnancy. The analysis in dairy cows was categorized into three distinct periods: the initial
162 42 days of gestation, the subsequent 63 days, and the last 84 days of pregnancy. The data
163 utilized in this study are computed and analyzed using Microsoft Excel software. The analysis
164 of this data is considered statistically significant. This analysis will assist us in creating a more
165 effective results sheet.

166 **RESULTS**

167 The total number of study animals, specifically dairy cows, is 50, of whom 44 are pregnant.
168 Confirmation is subsequently achieved via rectal palpation and later through parturition. The
169 other six dairy cows are non-pregnant, constituting 12% of the total, while the forty-four
170 pregnant dairy cows represent 88%. These data represent the current reproductive state of the
171 dairy cows (Table 1 and Figure 4).

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

175 The Barium Chloride (BaCl_2) test is conducted on 44 pregnant dairy cows using urine analysis
176 utilizing a 1% Barium Chloride (BaCl_2) solution on days 42, 63, and 84 post artificial
177 insemination (AI) (Table 2). The colorless translucent solution in the BaCl_2 test indicated a
178 positive pregnancy result. Conversely, the white precipitate in the BaCl_2 test cocktail indicated
179 a negative pregnancy result. The highest accuracy was observed on the 84th day, validated by
180 rectal palpation and parturition records. The least accurate result was observed on the 42nd day
181 post-AI. On day 42, the count of identified pregnant cows is 31, constituting 70.45%, whereas
182 the count of non-pregnant cows is 13, representing 29.55% (Table 2). On day 63, the count of
183 identified pregnant cows is 35, constituting 79.55%, while the count of non-pregnant cows is
184 9, representing 20.45%. On day 84, the count of identified pregnant cows is 37, representing
185 84.09%, while the count of non-pregnant cows is 7, accounting for 15.91% of the total (Figure.
186 5).

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

190 **DISCUSSION**

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

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

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

200 The study was conducted meticulously. Urine samples were obtained from several locations
201 within the Dhaka division, including Dhaka, Manikganj, and Munshiganj districts. Subsequent
202 to the collection of these samples, they were stored in a refrigerator at a temperature of 5-7 °C
203 until testing. A 1% Barium Chloride (BaCl₂) solution was utilized for this study. Barium
204 Chloride (BaCl₂) solution has been demonstrated to be effective in identifying pregnancy in
205 multiple animal species.

206 This study revealed an accuracy rate of 70.45% (31 cows) on day 42, 79.55% (35 cows) on day
207 63, and 84.09% (37 cows) on day 84 using the barium chloride (BaCl₂) test for genuine
208 pregnant cows. The mean of these three outcomes is 78.0%, indicating approximately an 80%
209 success rate throughout the first trimester of pregnancy.

210 The non-pregnancy rates were 29.55% (13 cows) at day 42, 20.45% (9 cows) at day 63, and
211 15.91% (7 cows) at day 84. These pregnancy diagnosis results correlate with similar research
212 published in reputable international publications.

213 Samsonova *et al.* (2014) found that the accuracy of milk progesterone ELISA for pregnancy
214 identification in cows was 87.0%. However, it is not particularly economical. Ghaidan *et al.*
215 (2019) noted that the accuracy of rectal palpation was 87.2%, whereas the accuracy of the
216 progesterone assay was 84.8%. The conventional rectal palpation method poses certain health

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

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

226 **CONCLUSION**

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

235 **ACKNOWLEDGMENTS**

236 The authors extend their appreciation to the Ministry of Science and Technology, the People's
237 Republic of Bangladesh, and the Sher-e-Bangla Agricultural University Research System.

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237 **AUTHOR'S CONTRIBUTION**

238 Md. Rakib Khan, Md. Mosharaf Hossain, Jahagir Alam, Md. Abdur Raihan Ratul

239 **Md. Rakib Khan:** Methodology, data collection, writing, and editing manuscript;

240 **Md. Mosharaf Hossain:** Conceptualization, supervision, reviewing, and editing manuscript;

241 **Jahagir Alam:** Investigation, data analysis, writing, and reviewing of manuscript;

242 **Md. Abdur Raihan Ratul:** Data analysis, writing, reviewing, and editing of manuscript

243 **CONFLICTS OF INTEREST**

244 The authors declare that there are no conflicts of interest in this publication.

245 **FUNDING INFORMATION**

246 This work was funded by the Ministry of Science and Technology, the People's Republic of
247 Bangladesh, and the Sher-e-Bangla Agricultural University Research System.

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335 Table 1. Actual Reproductive Status of the Dairy Cows

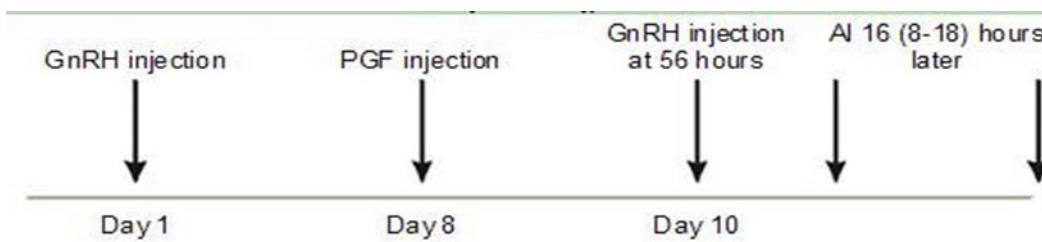
Total Number of Dairy Cows	Actual Reproductive Status of Dairy Cows		Percentage (%)	
	Pregnant	Non-pregnant	Pregnant	Non-pregnant
n = 50	44	06	88	12

336

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

Time of pregnant dairy cows	Total number of pregnant dairy cows	Barium Chloride (BaCl2) 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

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339

340 **Figure 1.** Ovosynch Program.

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342

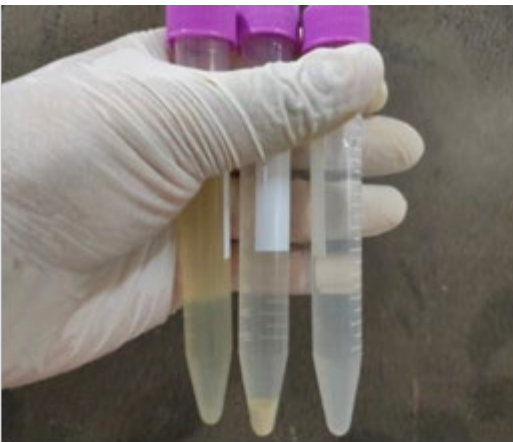


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Figure 2. Measuring Barium Chloride (A), Shaking the mixer with vortex machine (B), Final solution (C).

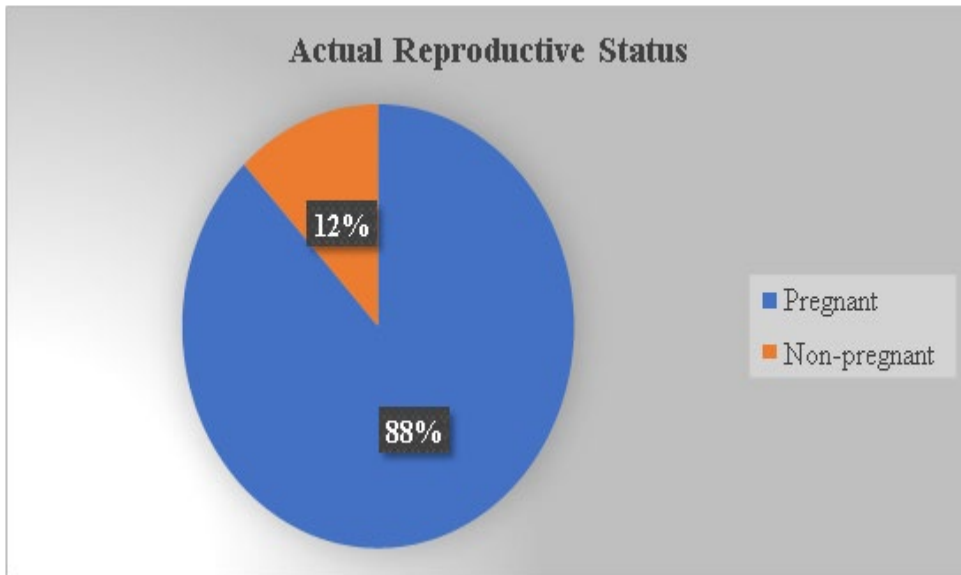


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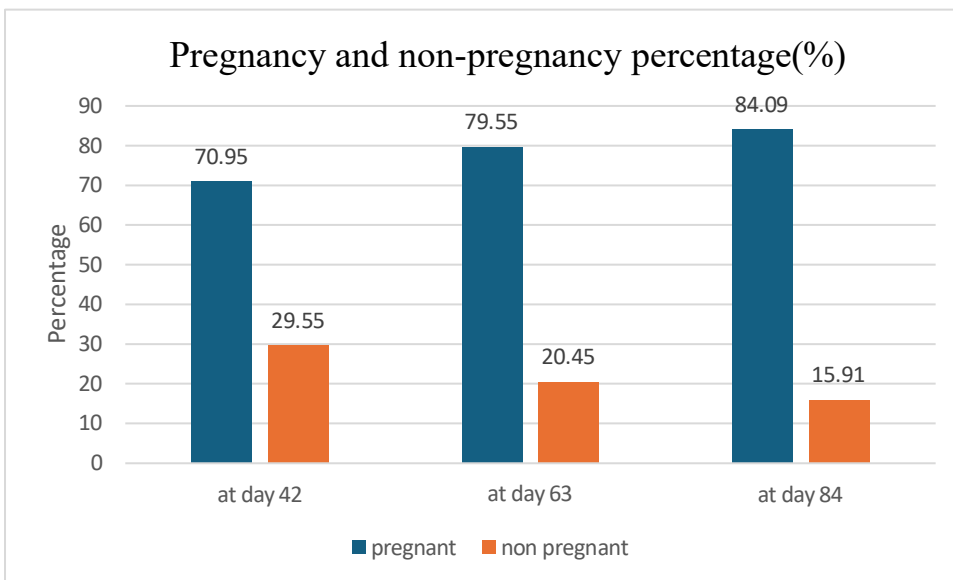
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Figure 3. Chemical analysis of urine using Barium Chloride (BaCl_2) solution.



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350 **Figure 4.** Actual reproductive status of the cows.



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

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