

SHORT COMMUNICATION

## Serum glucose, urea nitrogen, cholesterol, and total proteins in crossbred repeat breeder and normally cyclic cows

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### ABSTRACT

**Objective:** This study was designed to determine and compare the serum glucose, urea nitrogen, cholesterol, and total protein (TP) level in crossbred repeat breeder (RB) and normally cyclic cows to find out the relationship of these metabolic factors with repeat breeding syndrome (RBS).

**Materials and Methods:** A total of 592 breedable cows from 34 farms were examined from Mymensingh and Chittagong districts. Seventy cows were identified as RB cows and another 10 cows were randomly selected as normally cyclic control cows for this study. Blood sample from each cow was collected and the serum was separated. The serum samples were analyzed by auto blood analyzer.

**Results:** Cows affected with RBS had significant variation in their glucose, urea, and cholesterol levels. Serum glucose (43.00 gm/dl) level was significantly lower than in normally cyclic cows. On the other hand, serum urea and cholesterol levels were significantly higher than in normally cyclic cows. However, the TP concentrations did not differ between RB and cyclic cows. RB cows had a lower trend (6.815 mg/dl) of serum TP than in normally cyclic cows.

**Conclusion:** This research might help scientists and veterinarians to understand that the high serum urea and cholesterol level along with low glucose and TP level could have some effect in the development of RBS in crossbred cows. It will potentially help veterinary practitioners and farmers to take preventive measures against RBS of crossbred cows.

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### Introduction

Productive and profitable dairy is crucially dependent on the sound reproductive health of the dairy animals. However, dairy farmers are increasingly concerned about the reproductive performance of cattle as reproductive performance is decreasing year to year among dairy farms around the world [1,2]. Repeat breeding syndrome (RBS) is one of the main reproductive diseases responsible for decreasing the reproductive performance of a cow. Repeat breeder (RB) cows are less than 10 years old, cycle in regular intervals, and are free from clinical abnormality; however, they fail to conceive after three or more consecutive inseminations [3]. A RB cow can bring a tremendous loss to the farmer because it increases the cost of insemination, decreases productivity, and increases the culling rates [4]. Nowadays, increasing numbers of RBS and reproductive

problems are found in small holding farms, animal welfare camps, and veterinary clinics [5]. An optimal nutritional state is vital for sound reproductive potential, which is reflected by the variations in biochemical or hematological factors in blood plasma or serum. Alternations in biochemical profile might result in many reproductive insufficiencies [6]. Both blood metabolic and mineral profiles are reflective of the health and nutritional status of dairy cows.

Biochemical component plays a significant role in the maintenance of normal physiology. Therefore, optimum blood biochemical components are essential for the normal function of the reproductive system, as well as other systems of the body. If any of those components such as glucose, urea, cholesterol, total protein (TP), and other metabolites of the blood have altered, many disorders

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may arise which might result in reproductive failure. Thus, it is important to elucidate the serum biochemical profile of dairy cows in different states of health and disease. Biochemical profiling will help to characterize metabolic disorders. Blood cholesterol, glucose, urea, and TP were found to affect fertility and cyclicity in farm animals [7]. Deficiency of one or more of these components could impair the reproductive function. A cow's good reproductive performance is characterized by the resumption of ovarian cyclicity within 30 days postpartum, first service conception is less than 90 days and has an average calving interval of 1 year. Adequate nutrient availability enhances postpartum reproductive performance by increasing the energy status of the animals followed by the stimulation of the ovarian follicular growth and luteal functions [8,9]. In limited studies, repeat breeding was associated with the alterations of cholesterol [10], glucose [11], protein, albumin, and globulin levels [12]. However, very limited studies on the biochemical profile of crossbred RB cows have been performed. Therefore, our main objective is to determine the differences in selected biochemical parameters of RB and normally cyclic crossbred cows. The studies will also identify the present prevalence of RBS in the study areas and any managemental factor might be the cause of RBS in crossbred dairy cows.

## Materials and Methods

### Study areas and ethical consideration

The study was conducted in different villages of Phulpur and Potiya *Upazila* (sub-district) under Mymensingh and Chittagong districts, Bangladesh. Community-based Dairy Veterinary Foundation (CDVF) provided veterinary services to the farms. The study was conducted in accordance with the Guidelines of the international animal ethical standard and was approved by the Animal Welfare and

Ethical Committee, Bangladesh Agricultural University, Mymensingh (No. 01/AWEC/2019).

### Data and sample collection

Data such as the farmer's name and address, animal identification number, breed, age, parity, body weight, date of service, Body condition score during artificial insemination, date of parturition, any medication given during pregnancy, any complication during parturition, and previous history of retention of fetal membrane were recorded. The local and Frisian cross cows were considered as *Bos taurus* subfamily, whereas local and Sahiwal or Shindhi cross animals were considered as *Bos indicus* subfamily.

Blood sample collected from cows was identified or complaint having RBS was considered as a positive sample. Correspondingly, blood samples from normal healthy cows were collected as normally cyclic control cows. The diagnosed RB animals were treated according to the normal treatment regime. Blood samples were collected from the jugular vein in vacutainer tube containing clot activator. The tubes were kept at 4°C immediately after collection and transported to the laboratory within 4 h of collection.

### Serum separation

The blood sample was kept at 4°C for 4 to 5 h in the laboratory to activate the clot. After complete clotting, serum was separated and collected in a separate tube. Then the serum sample was centrifuged for further removal of clotted blood.

### Serum analysis to determine cholesterol, glucose, urea, and TP

Blood serum was taken to the laboratory of CDVF at the Department of Surgery and Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh and analyzed by auto blood analyzer ERMA AE-600 (MedWOW, Nicosia, Cyprus).

### Statistical analysis

All the data were entered in the Microsoft Excel Spread Sheet and analyzed by GraphPad Prism 5 (San Diego, CA) and SPSS 11.5 statistical software (IBM, New York). Differences were considered significant when the *p*-value was less than 0.05.

**Table 1.** Age, parity, and body weight wise distribution of repeat breeding cow.

Indices	Groups	Number of RB cows
Age	2–5 years	46
	6–8 years	22
	9–12 years	2
Parity	1	4
	2	5
	3	24
	4	30
	5	5
	6	2
Body weight	120–350 kg	44
	351–500 kg	26

**Table 2.** Percentage of repeat breeding cows in a different subfamily of crossbred cows.

Breed	Number of RB cows	Percent
<i>Bos taurus</i>	45	64.28
<i>Bos indicus</i>	25	35.71
Total	70	100

**Table 3.** Blood metabolite (Glucose, urea, cholesterol, and TP) levels in healthy and RB cows.

Group	Glucose gm/dl (Mean ± SEM)	Urea, nitrogen mg/dl (Mean ± SEM)	Cholesterol mg/dl (Mean ± SEM)	Total Protein mg/dl (Mean ± SEM)
RB cow	43.00 ± 1.420	25.05 ± 0.3401	144.5 ± 0.7400	6.815 ± 0.1485
Healthy cows	53.98 ± 4.089	22.71 ± 0.8485	135.8 ± 1.362	7.102 ± 0.4388
P values	0.0296	0.0250	0.0003	0.5510
Level of significance	*	*	***	NS

NS = not significant; SEM = standard error of the mean. Asterisks indicate the significant differences within columns.

\*Indicates  $p = 0.01-0.05$ , \*\*Indicates  $p = 0.001-0.01$ , \*\*\*Indicates  $p = 0.0001-0.001$ .

## Results

The age, numbers of calving, and body-weight based distribution of repeat breeding cows are presented in Table 1. The cows' age ranged from 2 to 12 years. The greater numbers of repeat breeding cows were between 2 and 5 years old and the majority were at fourth parity. Most of the repeat breeding cows' body weight ranged from 120 to 350 kg. The percentages of repeat breeding cows in each subfamily are presented in Table 2. The greatest number of repeat breeding cows were crossbred *B. taurus* (64%) compared to *B. indicus* (35%).

In total, 80 crossbred cows' blood urea, cholesterol, TP, and glucose level were included for analysis. Among the 80 cows studied, 70 were RB and 10 were normally cyclic cows. There were significant differences in serum glucose ( $43.00 \pm 1.420$  gm/dl versus  $53.98 \pm 4.089$  gm/dl,  $p = 0.0296$ ), urea ( $25.05 \pm 0.3401$  mg/dl versus  $22.71 \pm 0.8485$  mg/dl,  $p = 0.0250$ ), and cholesterol ( $144.5 \pm 0.740$  mg/dl versus  $135.8 \pm 1.362$  mg/dl,  $p = 0.0003$ ) levels between RBS affected cows and normally cyclic cows (Table 3).

## Discussion

Serum glucose (43.00 gm/dl) level was significantly ( $p < 0.05$ ) lower in RB cows than in normal healthy cows. It is in agreement with previous studies [13–15]. There might be three causes of hypoglycemia in RB cow. These are increased peripheral glucose uptake; failure of gluconeogenesis or glycogenolysis and endogenous hyperinsulinemia [16]. The serum glucose is an important factor which modulates reproduction, and the lower glucose level is responsible for decreased fertility rate [17].

The serum cholesterol level was significantly ( $p < 0.05$ ) higher than in the normal healthy cow which is in agreement with Dutta et al. [18] who observed higher cholesterol levels in RBs than in normally cycling cows. Whereas Ceylan et al. [19], Ramakrishma [20], and Singh and Pant [21] reported lower cholesterol levels in RBs than in normally cycling cows. The level of cholesterol may depend upon various physiological factors of a cow. Importantly,

cholesterol levels in cows vary on various physiological states, such as pregnancy and lactation, and low cholesterol levels may affect reproduction potential [22].

We found serum urea level (25.05 mg/dl) was significantly ( $p < 0.05$ ) higher in RB cows than in normally cyclic cows. Higher concentration of urea is related to fertility disorder, it also decreases the energy level causing economic loss [23].

The TP concentration had a lower trend (6.815 mg/dl) than in normally cyclic cows. Unlikely, Ramakrishma [20] and Gandotra et al. [24] did not find significant variation in blood protein content of normally cycling and repeat breeding cows. Deficiency of certain amino acid might result in the reduction of plasma protein, which ultimately hampers gonadotropins and gonadal hormones biosynthesis. This results in the reproductive hormonal disturbances, leading to inactive ovaries [12,25]. Moreover, the higher dietary protein could decrease the uterine pH, interfering embryonic development [26].

## Conclusions

A lower level of glucose, TP and the higher level of urea, cholesterol in crossbred RB cows suggest that the alteration of some metabolites might be the key to the development of RBS. The study might help to make a proper treatment regimen for RB cows, as well as preventive measures for RB cows to improve fertility.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## Authors' contributions

Jayonta Bhattacharjee was responsible for the conceptualization, designing, and supervision of the research. Mohammad Musharraf Uddin Bhuiyan was co-supervising the research and helped in manuscript preparation. Rashed Khan Barson, Shasthi Padder, Abu Sadath Md. Sayam and Mohammad Moshir Rahman performed most of the experiments. Jayonta Bhattacharjee and Rashed Khan Barson analyzed the data and wrote the manuscript.

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