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Research Article
Association of Some Hematological Parameters, Blood Glucose, Thyroid Hormones and Repeat Breeding Syndrome: A Case-Control Study

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Abstract

Background and Objective: A repeat breeder (RB) cow is not conceived after three consecutive inseminations. This study examined the association of Haemoglobin (Hb), packed cell volume (PCV), total erythrocytes count (TEC), total leukocytes count (TLC), plasma glucose and thyroid hormones (T3 and T4) with repeat breeding syndrome in cross-bred dairy cows reared under the semi-intensive system. **Materials and Methods:** Twenty crossbred multiparous, apparently healthy dairy cows with the age of 4-8 years and a body condition score of 2.5-3 were used. The animals were divided into two equal groups. Group A (n = 20) were healthy normal cyclic cows used as control. Group B (n = 20) were RB cows. Blood samples were collected once from all animals for measuring the Hb, PCV, TEC, TLC, glucose, T3 and T4. **Results:** When compared to the normal cyclic cows, no significant differences were detected ($p>0.05$) in the Hb, PCV, TEC, serum T3 and T4 in RB cows, but the TLC was significantly higher (8.2 ± 0.5 vs 5.5 ± 0.12 103 mL^{-1}, $p<0.05$) and the blood glucose level were significantly lower in the RB (44.9 ± 5.0 vs. 54.6 ± 6.5 mg dL^{-1}, $p<0.05$). **Conclusion:** The hypoglycemia and leukocytosis were associated with repeat breeding syndrome in cross-dairy cows under the study.

Key words: Repeat breeder, animal reproduction, cow fertility, blood biochemistry, packed cell volume, hypoglycemia, hyperthyroidism

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

A repeat breeder (RB) cow is unable to get pregnant after more than three successive natural services with the well-known fertile bull or artificially with fertile semen without the presence of abnormalities in the genital tract. The RB syndrome is considered to be an important cause of economic loss and bad reproductive performances in the dairy industry[1]. The potential causes of RB among dairy cows mainly include: Sub-clinical endometritis, nutritional deficiency especially trace minerals, hypoglycemia and vitamin A, age and improper detection of estrous and endocrine dysfunction[2,3]. Sub-clinical endometritis is reported to be an important factor that causes RB syndrome in dairy cattle[4]. In Sudan, there is a very high incidence of RB syndrome in dairy cows (35%)[5]. In RB syndrome, the costs of herd management and rearing are increased by an increment of expenses of unsuccessful frequent artificial insemination (AI), extended length of days open (DO) as well as culling and replacement of infertile cows[6]. Moreover, RB syndrome influences the haematological parameters of the affected cows. It has been reported that there are significantly reduced Haemoglobin (Hb) and packed cell volume (PCV) values in RB dairy cows compared to normal cycling animals. The White Blood Cell (WBC) value was significantly elevated in RB dairy cows in comparison to normal cycling ones[7,8]. Glucose is crucial for dairy cattle since it is highly required for the production of milk reproduction and regulation of the endocrine function[9]. Normal blood glucose in dairy cows varies within breeds, countries and geographical locations[10]. The plasma level of glucose was reported to be reduced in RB cows than that of normal cycling ones[3,11]. The thyroid hormones have vital roles in metabolism and affect reproduction, growth and development[12]. They are crucial for the growth and normal functioning of the female genital system. Hypo and hyperthyroidism have been reported to cause subfertility or infertility in women and animals[12]. It has been reported that adequate thyroid hormone concentration is required for the resumption of postpartum (PP) ovarian cyclicity in dairy cattle[13].

In this study, the complete blood count (CBC), plasma glucose level and thyroid hormones were examined in crossbred RB cows reared under a semi-intensive system.

MATERIALS AND METHODS

Study area: This study was performed during September 1st, 2017. Healthy twenty multiparous cross-bred (Kenana × Holstein Friesian) dairy cattle of 4-8 years old were included. These cows were from the dairy farm of the University of Khartoum, Sudan. The animals were maintained on a similar feeding program, that composed of two types of feeds: Roughage in Table 1 which included alfalfa (*Medicago sativa*) and Abu 70 (Sorghum) and concentrate which consisted of 37% sorghum seeds, 21% cottonseed cake, 40% wheat brand and 2% NaCl[14]. The roughage was provided every day in the morning and the evening. The concentrate was provided to individual cows every day during the milking time (2.00 am and 2.00 pm). The cows graze every day for 3 hrs in the morning.

Experimental plan: Twenty multiparous crossbred dairy cows 4-8 years old and body condition scores (BCS) of 2.50-3.00 were included in this study. The body condition score measured as suggested in other study[15,16], were 1.00: Emaciated, 2.00: Thin, 3.00: Fair, 4.00: Fat and 5.00: Obese cow. Cows were assigned into two groups (each contained 10 cows). Group A contained the normally cyclic cows (healthy control). Group B contained the RB cows.

Repeat breeding (RB) and normal cyclic cows: A cow that showed regular estrous cycles and no clinical endometritis or other abnormalities and served with a fertile bull more than three times but failed to conceive was considered an RB cow[17]. The normal cyclic cow was the cow that showed a regular estrous cycle and had less than three services per conception when inseminated naturally with a fertile bull was considered to be a normal cyclic cow[17].

Blood samples collection: Ten milliliters of blood was collected aseptically by jugular venipuncture. Two milliliters of blood were kept in a tube containing sodium fluoride for glucose determination. Two milliliters of the blood sample were allowed to clot at room temperature and centrifuged at 3000 rpm for 15 min. The serum samples were transferred to fresh plastic vials and immediately frozen at -20°C for measurements T3 and T4. The rest of the blood sample was kept in EDTA tubes for the determination of HB, PCV, TEC and total leukocytes count (TLC).

Table 1: Approximate analysis of the roughage provided to the Dairy Cattle

Ingredients (%)	Alfalfa (*Medicago sativa*)	Abu 70 (*Sorghum bicolor*)
Dry matter	23.00	28.00
Crude protein	3.60	3.10
Crude fibre	6.70	8.70
Fat	1.75	1.33
Ash	2.60	4.10

Haematological parameters: The haematological analyses were conducted with standard protocols. The PCV was assayed in the plain capillary tube using a microhematocrit centrifuge (Hettich-Germany), Hb concentration was measured with Drab kin's solution by the Cyanmethemoglobin Method[18]. The TLC was done in an improved Neubauer hemocytometer using dilution fluid of Turk's solution. The TEC was done using an improved Neubauer hemocytometer with Hyme's solution.

Measurement of blood glucose: The blood glucose concentration was measured with the enzymatic method using a kit from Biosystem, Spain.

Measurement of T3 and T4 concentrations: Serum levels of T3 and T4 were determined using ST AIA-PACK TT3 (Cat. No. 025282, Japan) and ST AIA-PACK T4 (Cat. No. 025258, Japan), respectively on TOSOH AIA System Analyzers (Tokyo, Japan).

Statistical analyses: Statistical Package for Social Science (SPSS) was employed to analyze the data and a T-test was used to compare the means between the two groups.

RESULTS

Haematological parameters: The mean values of Hb concentration for the control and RB cows were 10.2±0.7 and 9.6±1.7 g dL^{-1}, respectively, there was no significant difference in HB ($p>0.05$) between the control and RB cows. Moreover, no significant difference ($p>0.05$) in PCV 30.9±1.4 of controls and PCV of 29.2±5.0 RB cows was observed. The TEC of the control and RB cows were 6.9±1.4 vs 7.1±1.6, respectively without a significant difference in Table 2. A significant difference ($p>0.05$) in the TLC between the normal cyclic cows (5.5±0.5) and the RB cows was seen (Table 2). The blood glucose of the control and RB cows were 54.6±6.5 and 44.9±5.00 mg dL^{-1}, respectively, which was significantly different ($p<0.05$) between the two groups in Table 3.

Thyroid hormones: The blood levels of T3 of the control and RB cows were 0.9±0.2 and 0.9±0.1 μg dL^{-1}, respectively. No significant difference ($p>0.05$) was found (Table 3). The blood levels of Thyroxin (T4) of the control and RB cows were 4.50±0.60 and 5.00±1.00 μg dL^{-1}, respectively, which were not significantly different ($p>0.05$) (Table 3).

DISCUSSION

A repeat breeder cow shows normal cycling and has no clinical abnormality but is unable to conceive after two or three successive inseminations[1,19]. The RB syndrome is a major economic problem in dairy farms. Nutrition and postpartum uterine diseases can significantly influence the cow's reproductive performance[20-22]. The results of the present indicated that Hb concentrations of RB cows were not significantly different from the normal cyclic ones (9.6±1.7 vs 10.2±0.7, respectively, $p>0.05$, Table 2). This indicates the ratio was rich in trace elements such as iron and cobalt, that the RB syndrome is not affected by Hb concentration in this study and there is adequate oxygenation of the reproductive tissue of the animals. This result is inconsistent with the result of a previous study that showed lowered Hb levels in RB buffaloes[8]. The current results showed that the PCV levels were not different between RB cows and normal cyclic cows (29.2±5 vs 30.9±1.4, $p>0.05$, Table 2). This result is inconsistent with previous studies that reported reduced PCV in RB syndrome[8,23]. The inconsistency between our results in this studies[8,23] regarding Hb and PCV may be due to the different breeds, different environmental conditions or different sample sizes.

Results also indicated that there was no significant difference in the TEC between the RB and normal cyclic cows (6.9±1.4 vs 7.1±1.6, $p>0.05$, Table 2). These results indicate that the RBCs of the RB cows were normal[24].

Table 2: Mean (±SD) Hb, PCV, TEC and TLC of crossbreed RB cows and normal cyclic cows

Estimation	Control	RBCS	p-values
Hb (g L^{-1})	10.2±0.7[a]	9.6±1.7[a]	0.34
PCV (%)	30.9±1.4[a]	29.2±5[a]	0.34
TEC×10^6 mL^{-1}	6.9±1.4[a]	7.1±1.6[a]	0.1
TLC×10^3 mL^{-1}	5.5±0.5[b]	8.2±0.12[a]	0.002

[a,b]Superscripted alphabets describe significant differences ($p>0.05$) while the same alphabets in both columns indicate no significant difference, RBCS: Repeat breading syndrome, Hb (g dL^{-1}): Hemoglobin, PCV (%): Packed cell volume, TEC: Total erythrocytes count (cell×10^6 mL^{-1}) and TLC: Total leukocytes count (cell×10^3 mL^{-1})

Table 3: Biochemical parameters of plasma glucose, serum T3 and T4 in crossbred RB cows and normal cyclic cows

Estimation	Control	RBCS	p-values
Glucose (mg dL^{-1})	54.6±6.5[b]	44.9±5[a]	0.002
T3 (μg dL^{-1})	0.9±0.2[a]	0.9±0.1[a]	0.84
T4 (μg dL^{-1})	4.5±0.6[a]	5±1[a]	0.16

[a,b]Superscripted alphabets describe significant differences ($p<0.05$) while the same alphabets in both columns indicate no significant difference ($p>0.05$)

The present results showed that the total leukocytes count (TLC) was elevated in repeat breeder cows compared to the normal cyclic cows (8.2±0.12 vs 5.5±0.5, p<0.05, Table 2). This indicates that there is an infection, which is probably due to subclinical endometritis and intrauterine infections in RB cows. This result is consistent with some studies[25,26]. It has been reported that the prevalence of subclinical endometritis in RB cows was 12.7%[25]. The uterus is commonly infected with *Escherichia coli*, *Trueperella pyogenes* and alpha-hemolytic streptococci[24]. It was reported that the RB syndrome can be treated successfully with the intra-uterine infusion of 1% Lugol's iodine[5]. The infection has been reported as a minor cause of RB syndrome and there have been other causes of RB syndrome such as ovarian hormone profile alterations, which cause prolongation of the estrus duration and delayed peak of the luteinizing hormones[25]. Moreover, an insufficient postovulatory progesterone concentration has been also suggested as a cause of RB syndrome[25,27].

Results showed that there were lower glucose levels in RB cows compared to normal cyclic cows (44.9±5 vs 54.6±6.5, p<0.05, Table 2). This result is in agreement with the previous reports[3,8,11,26]. Glucose is the primary source of energy in animals. Low levels of blood sugar may cause reduced levels of energy and infertility. Reduced availability of energy in cattle results in a negative energy balance. This would convert the cow to the catabolic condition[28]. This condition elevates the plasma growth hormone and the unesterified fatty acid levels and decreases insulin levels and blood glucose[28]. The negative energy balance is accompanied by many metabolic changes that influence the resumption and normality of estrous cyclicity and fertility[29]. It has been reported that cows with a blood glucose of 60 mg/100 had a bigger chance of getting conceived from the first insemination than cows with a blood glucose of 45 mg/100[30]. Blood glucose is very important for cow fertility[29]. Current results indicated that there were no significant differences in the thyroid hormone levels between RB and normal cyclic cows (Table 2). This result is consistent with a study that suggested that the levels of T3 and T4 did not differ between the cyclic and acyclic Nili-Ravi buffaloes[31]. However, it has been reported that cows with cystic ovarian follicles had lower levels of T3 and T4 than normal cyclic cows[32]. The current results showed that the levels of T3 and T4 were within normal in the RB cows (Table 3).

CONCLUSION

In summary, the data indicate the need for blood biochemical metabolites and haematological parameters monitoring in dairy cattle concerning RB syndrome. A reasonable nutritional and treatment strategy should be adopted to mitigate the negative effects of hypoglycemia and leukocytosis on the reproductive efficiency of dairy cows.

SIGNIFICANCE STATEMENT

The repeat breeder is a cow with a regular estrous cycle and with no illnesses but fails to get pregnant after at least three repetitive services. The repeat breeding syndrome causes subfertility and reduces profitability and frustration for the owners. This study reported that low blood glucose may reduce the conception rate (CR) and consequently increases the number of services preconception (NSPC). Moreover, elevated WBCs count is associated with subclinical endometritis perhaps one of the factors that lead to repeat breeding syndrome. The result of the present study indicates that repeat breeding syndrome may be avoided by correction of blood glucose via improvement of nutrition and by treatment of subclinical endometritis.

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