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ALTERATION OF PHYSIOLOGICAL PARAMETERS IN REPEAT BREEDER COWS

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors JS and AHS planned the research design, authors MRP, MAI, FKN collected the samples and performed laboratory analysis authors SAK, JS, AS and AHS wrote the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Repeat breeding is a significant reproductive problem to develop in a cattle industry in developing country. In this study, we demonstrated physiological alterations in terms of hematological, biochemical and hormonal profile being developed in repeat breeder cows. However, the biochemical profiles of 30 repeat breeders and 30 fertile cyclic cows were investigated and compared in this study. Hematological parameters (Hb, ESR, PCV etc) were measured in both cohort of animals. Serum was tested for biochemical (glucose, total protein, cholesterol, low density lipoprotein, high density lipoprotein) and hormonal (follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, testosterone, thyroxine) assays using a commercially available ELISA kit after a routine blood examination. Paired T-test was used to check the significance of differences ($P< .05$) in blood profiles and hormonal profiles between repeat breeder and normal cycle cows. Repeat breeder cows had considerably lower total erythrocyte count, haemoglobin, platelets, blood glucose, luteinizing hormone, progesterone, and higher testosterone levels than typical cyclic cows. Overall, haemoglobin deficiency and low blood glucose concentrations possibly alters ovarian function along with lower concentrations of LH surge for ovulation. Correcting plasma glucose, Hb, LH, and testosterone levels may be an effective strategy for treating repeat breeding cows, based on the findings.

Keywords: Biochemical; hormonal; normal cyclic cow; repeat breeder cow.

1. INTRODUCTION

The cows that failed to conceive after three or more successive inseminations and lack of any detectable abnormalities are called repeat breeders [1]. Repeat breeding is one of the major causes of infertility; that leads to economic losses due to repeated inseminations, reduced milk yield, extended calving interval, increased culling rates and loss of genetic gain [2]. There are many potential risk factors involved with repeat breeding incidence including genetics, age, uterine infection, repeat oestrus cycles,

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anatomical defects of the genital tract, improper ovarian function, nutrition and artificial insemination (semen quality, inseminator or insemination techniques) [3,4]. Among these factors improper ovarian function is directly associated with reproductive and metabolic hormonal status of animals. However, pituitary hormones, Follicular stimulating hormone (FSH) and Luteinizing hormone (LH) regulate the production of estrogen and progesterone from the ovary and corpus luteum respectively. In this process, FSH stimulates the follicular growth, development, and function, while LH causes the follicle to rupture and the corpus luteum to develop. Several research articles have indicated endocrine impairment in estrogen (E_2), progesterone (P_4), or luteinizing hormone (LH) as the potential reasons for the repeat breeding problem [5-7]. In addition, usual levels of various biochemical constituents are indispensable for regular function of various systems of the body. These are closely associated with hormone bio synthesis in animal body from different precursors. However, Cholesterol is a derived lipid compound that is responsible for the synthesis of steroid hormones that include sex hormones like estrogen, and progesterone. Besides, protein hormones such as FSH, LH, thyroxine concentrations could be deteriorating due to the disturbances of the parent biomolecules like protein, and amino acids. Moreover, lipids are high energy generating compounds that required for oocyte maturation, and postpartum reproductive behavior by increasing the energy status of the animals and thus stimulates the ovarian follicular growth, oocyte maturation and luteal functions [8].

Therefore, the present study is aimed to investigate the serum biochemical profile (plasma protein, lipid profiles) and associated hormones related to reproduction and metabolism in cycling and repeat breeding cows as those parameters are related with endocrine and reproductive physiology of cattle.

2. MATERIALS AND METHODS

This study was conducted in five commercial dairy farms in Chattogram district, Bangladesh because of available number of same cross breed, body condition score and management practiced. A total of 30 repeat breed and 30 normal cyclic cattle were included in this study which were clinically sound. The selected repeat breeder cows were failed to conceive after 3 or more successive insemination whereas the normal cyclic cows were conceived normally after inseminations and all the animals were kept in good managemental condition with balanced ration. A pre-structured questionnaire was prepared to record required information along with demographic data like age, parity, insemination and, disease history. Blood samples were collected from tail vein in two separate vacutainers, one having anticoagulant (Na_2-EDTA, BD Vacutainer®, BD Franklin lakes, NU, USA.) and other without anticoagulant. Serum was separated immediately from blood without anticoagulant using centrifuage machine at 3000 RPM for 15 mins and transfer to 1.5 ml Eppendorf tube (Axyen, USA) and preserved at −20°C until analysis. Hematological indices viz. Red Blood Cell (RBC), Haemoglobin (Hb), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV), Mean Corpuscular haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Total Leukocyte Count (TLC), Platelet (PLT), Lymphocyte (Lym), Monocyte (Mon), Eosinophil (Eos) were determined by the Nihon Kohdens hematology analyzer Celltac Alpha VET MEK-6550. Biochemical parameters viz glucose, Triglyceride (TG), Cholesterol, Total protein (TP), HDL, and LDL were measured spectrophotometrically (Human analyzer 3000®) and kits from Randox (Ireland). Plasma testosterone, progesterone, estradiol, Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Thyroxine were estimated by ELISA kits (Testosterone- BIOS Microwell diagnostic system), LH -Atlas Medical, UK and estrogen progesterone-Nova Tec Immunodiagnostica GmbH, Germany). Obtained laboratory data were analyzed by Paired T-Test to determine the significance of difference between normal cyclic (NC) and repeat breeder (RB) cows. The analysis was performed using Microsoft Excel 2007 and the level of significance was determined at $p<0.05$.

3. RESULTS

Haematological parameters of NC and RB are presented in Table 1. Mean blood TEC, Hb, PCV, MCH, lymphocytes, monocytes, platelets and eosinophil were higher in normal cyclic than repeat breeder cows. Among these parameters TEC, PCV and platelets are significantly ($P< .05$) higher in NC cows.

Serum biochemical parameters in the two groups are depicted in Table 2. Glucose level of NC cows was higher (74.73 vs 65.68 gm/dl, $P< .05$) than RB cows.

Serum hormone concentrations were found to be different in RB and NC cows. Our study found that progesterone and LH levels were significantly higher in NC cows than RB cows (25.40 vs 15.49 and 1.23 vs 0.63; $P< .05$) while testosterone was higher in RB than NC cows (0.73 vs 0.24).

Table 1. Haematological parameters of NC and RB cows

Parameters	Normal cyclic cows (n=30)	Repeat breeder cows (n=30)	*P* value
TEC(x 10^6/cumm)	6.71 ± 0.33	5.61 ± 0.39	*
WBC (x 10^3/cumm)	7.99 ± 0.56	8.84 ± 0.83	ns
Hb (g/dl)	9.9 ± 0.39	8.47 ± 0.43	*
PCV (%)	27.39 ± 1.24	24.04 ± 1.23	ns
MCV(µm^3)	41.40 ± 1.29	43.58 ± 0.96	ns
MCH ((pg)	15.96 ± 1.03	15.89 ± 0.71	ns
MCHC (g/dl)	35.29 ± 0.14	35.76 ± 0.54	ns
Platelets (x10^3/cumm)	280.05 ± 29.59	187.85 ± 28.97	*
Lymphocyte(%)	43.56 ± 3.85	39.23 ± 2.64	ns
Monocyte (%)	0.55 ± 0.14	0.29 ± 0.09	ns
Eosinophil (%)	7.93 ± 1.29	5.07 ± 0.82	ns
Granulocyte (%)	48.73 ± 3.79	54.8 ± 2.88	ns

**means significantly different (P< .05)*

Table 2. Biochemical parameters of NC and RB cows

Parameters	Normal cyclic cows (n=30)	Repeat breeder cows (n=30)	*P* value
Glucose (mg/dl)	74.73 ± 2.65	65.68 ± 2.04	*
Total protein (g/l)	56.46 ± 1.92	54.13 ± 0.67	ns
Triglyceride (mg/dl)	44.7 ± 3.22	48.72 ± 6.20	ns
Cholesterol (mg/dl)	168.41 ± 8.59	151.93 ± 12.36	ns
HDL (mg/dl)	120.16 ± 7.48	96.19 ± 6.19	ns
LDL (mg/dl)	43.72 ± 5.55	53.18 ± 7.05	ns

**means significantly different (P< .05)*

Table 3. Hormonal indices of NC and RB cows

Parameters	Normal cyclic cows (n=30)	Repeat breeder cows (n=30)	*P* value
Estradiol (ng/ml)	55.15 ± 3.41	48.84 ± 5.55	ns
Progesterone (pg/ml)	25.40 ± 4.07	15.49 ± 2.45	*
Testosterone (ng/ml)	0.24 ± 0.06	0.73 ± 0.13	*
LH (ng/ml)	1.23 ± 0.26	0.63 ± 0.09	*
FSH (pg/ml)	0.50 ± 0.2	0.63 ± 0.35	ns
Thyroxine (nmol/l)	40.3 ± 1.18	40.36 ± 1.06	ns

**means significantly different (P< .05)*

4. DISCUSSION

Notable variations were found in several hematological and hormonal values of repeat breeder and normal cyclic cows in this study. The amount of oxygen in the reproductive tract's cells, as well as normal cyclicity, are determined by the amount of haemoglobin in the blood. The appropriate transfer of oxygen and nutrients to the critical organs, as well as the metabolic processes of the gonadal cells, require an adequate concentration of haemoglobin in the blood during oestrus. As a result, a drop in haemoglobin levels in cross-bred cows in the current study may be a factor for repeat breeding.

Additionally, reduced Hb levels in the blood are indicated by decreased RBC and PCV. Increased enzymatic activity in the body or intrauterine infection in repeat breeder cows could account for a non-significant increase in WBC count in animals. Sabasthin et al. [9] discovered elevated TLC in RB cows, which corroborated this finding. There was no significant difference in lymphocytes, monocytes, or eosinophils between the repeat breeding and regularly cycling cows in this investigation. The findings showed that lymphocytes, monocytes, and eosinophils numbers could not be employed as a predictor of recurrent breeding in a herd. The number of platelets differs dramatically between NC and RB cows; in normal cycle cows, this value is larger than

in RB cows. Platelets are responsible for the generation of thrombocytokinin, which leads to the development of additional prostaglandins (PGF2 and PGE2), which aid in ovulation and subsequent reproductive tract contraction and fertilisation [10].

Blood glucose levels in normal cyclic cows were substantially higher (P<0.01) than in repeat breeder cows, according to our research**.** Repeat breeder cows had significantly lower glucose levels than normal cyclic cows, and that low glucose levels had adverse effects on reproduction, such as repeat breeding [11-14]. Low blood glucose levels indicate a lack of energy and, as a result, infertility. Contrary to present study, Awasthi and Kharche [15] and Sulieman et al. [16] discovered that RB cows had considerably greater blood glucose than NC cows. The occurrence of a condition such as endometritis can explain the elevated level of glucose in repeat breeding cows reported in those investigations. Endometritic cross-bred cows have higher blood glucose levels [16, 17]. Between the fertile and repeat breeder cows, there were no significant differences in total cholesterol, HDL, or LDL levels (Table 2). This is in line with the findings Guzel and Tanriverdi [11] found no significant difference in blood cholesterol levels between RB and NC cows. It has been discovered that RB buffaloes and cows have lower plasma cholesterol than normal cyclic (NC) buffaloes and cows [18, 19].

Plasma progesterone, and LH hormone levels in normal cyclic and repeat breeder cows were found to be significantly different *P*< .05 in this study. Previous studies showed decreased progesterone levels in RB cows [20,21]. Theca cells produce testosterone from healthy ovarian follicles, and granulosa cells convert testosterone to oestrogen. Testosterone levels in plasma rose prior to estrus and then fell during the period [22,23]. In addition, the degree of luteinization appears to be linked to testosterone synthesis. As a result, our findings are consistent with those of the writers previously cited.

5. CONCLUSION

This study found that LH and progesterone hormone is significantly lower and testosterone is higher in RB cows than NC cows. Moreover, glucose, TEC, PCV and Hb level also significantly low in repeat breeder cow. So, regular monitoring and management of these parameters by proper nutrition and treatment might be possible solution to overcome repeat breeding problem.

ETHICAL APPROVAL

The study was approved by the Animal Experimentation Ethics Committee (AEEC), Chattogram Veterinary and Animal Sciences University, Bangladesh.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Gustafsson H, Emanuelson U. Characterisation of the repeat breeding syndrome in Swedish dairy cattle. Acta Veterinaria Scandinavica. 2002;43:115-125.
2. Bartlett PC, Kirk JH, Mather EC. Repeated insemination in Michigan Holstein-Friesian cattle: Incidence, descriptive epidemiology and estimated economic impact. Theriogenology. 1986;26(3):309-322.
3. El-Khadrawy HH, Ahmed WM, Hanafi M. Observations on repeat breeding in farm animals with emphasis on its control. Journal of Reproduction and Infertility. 2011;2(1):1-7.
4. Walsh SW, William EJ, Evans ACO. A review of the causes of poor fertility in high milk producing dairy cows. Animal Reproduction Science. 2001;123(3-4):127-138.
5. Båge R., Gustafsson H, Larsson B, Forsberg M, Rodrıguez-Martınez H. Repeat breeding in dairy heifers: follicular dynamics and estrous cycle characteristics in relation to sexual hormone patterns. Theriogenology. 2002;57(9):2257-2269.
6. Saumande J, Humblot P. The variability in the interval between estrus and ovulation in cattle and its determinants. Animal Reproduction Science. 2005;85(3-4):171-182.
7. Bloch A, Folman Y, Kaim M, Roth Z, Braw-Tal R, Wolfenson D. Endocrine alterations associated with extended time interval between estrus and ovulation in high-yield dairy cows. Journal of Dairy Science. 2006;89(12):4694-4702.
8. Hightshoe RB, Cochran RC, Corah LR, Kiracofe GH, Harmon DL, Perry RC. Effects of calcium soaps of fatty acids on postpartum

reproductive function in beef cows. Journal of Animal Science. 1991;69(10): 4097-4103.
9. Sabasthin A, Kumar VG, Nandi S, Murthy VC. Blood haematological and biochemical parameters in normal cycling, pregnant and repeat breeding buffaloes (*Bubalus bubalis*) maintained in isothermic and isonutritional conditions. Asian Pacific Journal of Reproduction. 2012;1(2):117-119.
10. Donna SW, Joanne KL, Shelley A, Lubica R, Kowalska MA, Magnus AB, Gunnar P, James GW, Barbara PS. Serglycin proteoglycan deletion induces defects in platelet aggregation and thrombus formation in mice. Blood. 2008;111(7):3458-3467.
11. Guzel S, Tanriverdi M. Comparison of serum leptin, glucose, total cholesterol and total protein levels in fertile and repeat breeder cows. Revista Brasileira Zootecnia. 2014;43:643-647.
12. Khan S, Thangavel A, Selvasubramaniyan S. Blood biochemical profile in repeat breeding cows. Tamilnadu Journal of Veterinary and Animal Sciences. 2010;6:75-80.
13. Kumar AS. Profile in repeat breeding crossbred dairy cows. International Journal of Veterinary Sciences. 2014;3:172-173.
14. Selvaraju S, Agarwal SK, Karche SD, Srivastava SK, Majumdar AC, Shanker U. Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. Animal Reproduction Science. 2002;73: 141-149.
15. Awasthi MK, Kharche KG. Studies on some constituents in normal cycling, fertile and infertile repeat breeder crossbred cows. Indian Journal of Animal Reproduction. 1987;8:95-97.
16. Sulieman MS, Makawi SEA, Ibrahim KEE. Association between postpartum blood levels of glucose and urea and fertility of cross-bred dairy cows in Sudan. South African Journal of Animal Science. 2017; 47(5):595-605.
17. Ijaz A, Lodhi LA, Gureshi ZI, Younis M. Studies on blood glucose, total protein, urea and cholesterol levels in cycling, nonocycling and endometritic crossbred cows. Pakistan Veterinary Journal. 2004;24:92-94.
18. Amle M, Patodkar V, Shelar R, Birade H. Serum biochemical levels of repeat breeder cross bred cows under rural condition of Satara District of Maharashtra. International Journal of Advanced Veterinary Science and Technology. 2014;3(1):109-113.
19. Ceylan A, Serin İ, Akşit H, Seyrek K, Gökbulut C. Döl tutmayan veanöstruslu süt ineklerinde vitamin A, E, Beta-karoten, kolesterol ve trigliserid düzeylerinin araştırılması. Kafkas Üniversitesi Veteriner Fakültesi Dergisi. 2007;13(2):143-147.
20. Akhtar MS, Farooq AA, Mushtaq M. Biochemical and hormonal profile in anoestrus Nili-Ravi buffaloes. Indian Veterinary Journal. 2010;87(6):603-604.
21. Wm A, Ek H, Hanafi EM, Ali AH, Sa S. Clinical perspective of repeat breeding syndrome in buffaloes. Journal of American Science. 2010;6(11):661-666.
22. Shemesh M, Hansel W. Measurement of bovine plasma testosterone by radioimmunoassay (RIA) and by a rapid competitive protein binding (CPB) assay. Journal of Animal Science. 1974;39(4):720-724.
23. Kanchev LN, Dobson H, Ward WR, Fitzpatrick, RJ. Concentration of steroids in bovine peripheral plasma during the estrous cycle and the effect of betamethasone treatment. Journal of Reproduction and Fertility. 1976;48:341.