Clinico-pathological profiles of sub-fertility in Zebu Cows

K. F. Pariza*, A. S. M. Bari, F. Y. Bari[1], M. G. S. Alam[2] and M. Noor
Department of Pathology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

Blood samples from cows with reproductive problems (anoestrus, repeat breeder, uterine infection) were collected for haematological, biochemical and humoral immune response analyses. The erythrocyte sedimentation rate (ESR) was higher and haemoglobin (Hb) lowers in subfertile cows than innormal cycling cows. The packed cell volume (PCV) was lower in anoestrus and repeat breeding cows than in control. Total erythrocyte count (TEC) was not significantly different, whereas total leukocyte count (TLC) significantly higher (p<0.01) in anoestrus and repeat breeder cows. The bilirubin concentration was significantly higher (p<0.005) in anoestrus and uterine infected cows compared with control. The mean serum IgG was lower in all subfertile cows than control. It is suggested that there is no specific clinico-pathological marker for reproductive disorders in cows. (*Bangl. vet.* 2009. Vol. 26, No. 1, 1-7)

Introduction

Sub-fertility in cows is an important hindrance to dairy farmers (Serur *et al.*, 1982; Albin *et al.*, 1989; Jainuddin and Hafez, 1993). The causes are many, including management, type of service, uterine infection, oestrus detection, nutrition, and immune status. Defective management can increase sub-fertility (Shamsuddin *et al.*, 1988). Important reproductive disorders encountered in Bangladesh are anoestrus, repeat breeder, endometritis, and repeated conception failure (Samad *et al.*, 1978, Vale, 1997) causing considerable economic loss (Jainuddin and Hafez, 1993). Several reports have implicated various blood chemical constituents in sub-fertile cows (Serur *et al.*, 1982; Albin *et al.*, 1989; Dhaliwal *et al.*, 1996; Hoeben *et al.*, 2000), but no such reports are available in Bangladesh.

The present investigation was undertaken to evaluate the haematological and biochemical profiles of cows with reproductive problems in Bangladesh.

Materials and Methods

Animals selected
A total of 30 lactating multiparous mixed breed cows weighing 220 - 355 kg were divided into three groups, Group A (n = 7): were suffering from postpartum anoestrus of more than 90 days (Chowdhury *et al.*, 1998); Group B (n = 10): were

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[1&2] Department of Surgery & Obstetrics, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh
*Correspondence: *E-mail*:- bari.bau.bd@gmail.com

repeat breeders (Zemjanis, 1980) and Group C (n = 13) : had suspected uterine infection (Sheldon *et al.*, 2003). All were outpatients at the Veterinary Clinic. For control (Group D), cycling lactating multiparous cows (n = 30) of mixed breeds were selected from the university dairy farm. Cows belonged to small-holders where only one to 15 cows were kept. They normally maintained their animals on rice straw cut-and-carry grass and limited grazing on roadside and community land as forage; and milling by-product as concentrate (rice polish, wheat bran, mustard and til oil cakes fed at 1-2 kg per 1.5 - 2.0 litres of milk). There was no system for oestrus detection, but farmers relied on visible signs of oestrus.

Blood sampling: Five-ml blood was collected from each cow by jugular vein puncture using 10ml plastic syringe on the first day after examination and every week for three weeks. Half the blood sample was kept with heparinized saline for routine haematological examination; rest of the sample was taken in vials for collection of serum. Serum samples were kept at -20°C until analyzed.

Haematological studies: Erythrocyte sedimentation rate (ESR), Packed cell volume (PCV), Haemoglobin percentage (Hb%), Total erythrocyte count (TEC), Total leukocyte count (TLC) and Differential leukocyte count (DLC) were carried out as described by Coffin (1953).

Total serum protein assay: Total blood protein was measured using Folin-phenol method (Lowry *et al.*, 1951). Briefly, a standard curve was made with equilibrated (200 μl distilled water) bovine serum albumin solution (1 mg/ml) ranging from 0 to 60 μl. Unknown serum samples (10 μl) were taken in separate tubes after similar equilibration. After addition of prepared solution (one ml), each tube was incubated in a dark place for 30 minutes at room temperature. The absorbance of standard proteins at 660 nmol using spectrophotometer was plotted against concentration of BSA and the concentrations of proteins were extrapolated by putting the value of optical density (OD) in the standard curve.

Serum bilirubin was measured using the standard technique of Harrison and Barlow (1989). Briefly, five-ml methanol, one-ml diazo reagent and four-ml diazo blank were taken as blank sample. For unknown serum sample, five-ml methanol, one-ml diazo reagent and four-ml 1: 10 diluted serum were mixed thoroughly and incubated for 30 minutes at room temperature. The blank was set at 100% at 540 nm and the % of transmission (T) was read in the unknown sample. The result of % T was then converted to mg/dl.

Serum immunoglobulin-G (IgG): IgG was estimated by the single radial immune-diffusion technique (Mancini *et al.,* 1965). The polyclonal antibody IgG was raised by immunizing New Zealand White Rabbit with purified 0.5 mg/ml Bovine IgG (Sigma, USA). Blood (Serum) was collected from ear vein every week after the first booster

dose. Sera were used to check the antibody response and quantify the antigen by double immuno-diffusion test and single radial immuno-diffusion technique, respectively, following the procedures described by Bari (1989). The antibovine IgG was titrated using ELISA technique (Bari 1989) in 5 fold dilutions starting from 1 in 50. All the data were analyzed by comparison between groups using students "t" test (Bailey, 1981).

Results and Discussion

Haematological examination: The results are shown in Table 1. The mean values of ESR in mm/24 hour were 9.3 ± 2.6; 11.1 ± 5.6; 15.5 ± 7.3 and 7.5 ± 1.2 in anoestrus (Group A), repeat breeder (Group B), uterine infection (group C) and control (Group D), respectively. The mean values of ESR were significantly higher in problem groups than in control group (A: p<0.05; B: p<0.001 and C: p<0.001). The mean Hb% in Groups A, B, C and D were 10.2 ± 1.5, 9.6 ± 1.1, 9.7 ± 7.3 and 13.1±2.6, respectively. The Hb concentration was significantly lower in problem groups than in control: (A: p<0.05; B: p<0.01 and C: p<0.01). The mean PCV% in groups A, B, C and D were 31.3 ± 5.2, 28.7 ± 7.0, 38.1 ± 12.8 and 43.2 ± 7.9, respectively. PCV in Groups A and B were significantly lower than Group D, The TEC of Groups A, B, C and D were 5.5 ± 1.2; 5.1 ± 1.0; 5.9 ± 1.8; 4.5 ± 1.5 million/µl, respectively. The differences were not significant. The mean TLC in groups A, B, C and D were 9.3±5.6, 10.0±5.7, 5.0 ± 3.5 and 6.5 ± 1.5, respectively. The TLC in groups A and B were significantly higher than the control.

Total serum protein (mg/dl): The mean total serum protein in Group A, B, C and D was 3.4 ± 0.8, 3.7 ± 1.1, 3.7 ± 1.6, and 5.2 ± 0.8) mg/dl, respectively. In problem groups it was significantly (p<0.001) lower than Group D (Table 2).

Table 1 Haematological values

Parameters	Group A (n=7)	Group B (n=10)	Group C (n=13)	Group D (n=13)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
ESR mm/24hr	9.3 ± 2.6*	11.1 ± 5.6***	15.5 ± 7.3***	7.5 ± 1.2
Hb gm%	10.2 ± 1.5*	9.6 ± 1.1**	9.7 ± 0.6**	13.1 ± 2.6
PCV%	31.3 ± 5.2***	28.7 ± 7.0***	38.1 ± 12.8	43.2 ± 7.9
TEC million/µl	5.5 ± 1.2	5.10 ± 1.0	5.9 ± 1.8	4.5 ± 1.5
TLC thousand/µl	9.3 ± 5.6**	10.0 ± 5.7**	5.0 ± 3.5	6.5 ± 1.5

*p<0.05 **p<0.01 ***p<0.001

Serum bilirubin: The mean concentration of serum bilirubin in group A, B, C and D were 0.6 ± 0.2, 0.3 ± 0.2, 0.4 ± 0.4 and 0.3 ± 0.3, respectively (Table 2). The mean serum bilirubin in Groups A and C was significantly (p<0.005) higher than Group D (0.3 ± 0.3).

Table 2. Serum protein bilirubin and Bovine serum

Parameter	Group A (n=7)	Group B (n=10)	Group C (n=13)	Group D (n=13)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Serum protein (mg/dl),	3.4 ± 0.8***	3.7 ± 1.1***	3.7 ± 1.6***	5.2 ± 0.8
Serum bilirubin (mg/dl)	0.5 ± 0.2*	0.3 ± 0.2	0.4 ± 0.4*	0.3 ± 0.3
IgG IgG (µg/dl)	807.7 ± 170.0*	869.6 ± 109.4*	857.9 ± 120.0*	1222.2 ± 178.9

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Serum IgG The mean concentration of IgG in groups A, B, C, D was 807.7 ± 170.0; 869.6 ± 109.4, 857.9 ± 120.0 and 1222.2 ± 178.9, respectively (Table 2). The concentration in all problem groups were significantly ($p < 0.001$) lower than Group D. The result of 3 different bleedings (Table 3) shows that the 3rd bleeding before 3rd booster had highest concentration of antibody.

Table 3. Titration of anti-bovine IgG by ELISA (OD at 405 ηm and serum dilution as five fold)

Serum dilution	A (Mean ± SD)	B (Mean ± SD)	C (Mean ± SD)	D (Mean ± SD)
Blank	0.16 ± 0.00	0.19 ± 0.00	0.21 ± 0.00	0.25 ± 0.00
0.00000128	0.24 ± 0.00	0.43 ± 0.03	0.48 ± 0.01	0.55 ± 0.01
0.0000064	0.38 ± 0.00	0.50 ± 0.03	0.65 ± 0.03	0.89 ± 0.03
0.000032	0.45 ± 0.01	0.73 ± 0.02	0.89 ± 0.01	1.23 ± 0.03
0.00016	0.57 ± 0.02	1.39 ± 0.01	1.51 ± 0.06	2.10 ± 0.01
0.008	0.78 ± 0.01	2.10 ± 0.02	2.74 ± 0.01	3.02 ± 0.02
0.004	0.92 ± 0.00	2.83 ± 0.04	3.09 ± 0.05	3.60 ± 0.03
0.02	1.06 ± 0.01	3.313 ± 0.01	3.28 ± 0.03	4.10 ± 0.01

A = Control; B = First boostered bleeding; C = Second boostered bleeding; D = Third boostered bleeding

ESR is generally high in chronic infection and malnutrition (Samad *et al.,* 1978; Dutta *et al.,* 1991; Islam *et al.,* 1999). Low Hb indicates anaemia and its values are significantly low in all problem groups compared to control. Baqi and Rahman (1981) showed low Hb in anoestrus cows. The animals in the present study were outpatients at the Veterinary clinic, and had been suffering from gastrointestinal parasites causing anaemia and hyproteinaemia (Murthy *et al.,* 1975). The PCV % was significantly decreased only in the anoestrus (A) and repeat breeder group (B), but not in the uterine infection group (C) compared to control. PCV is another index of anaemia (Samad *et al.,* 1978; Baqi and Rahman 1981 and Islam *et al.,* 1999). It is difficult to

interpret the TEC as these were not significantly different from the control. TLC in anoestrus (A) and repeat breeding (B) cows was significant higher than in control group, but the uterine infected animals were not significantly different from the control. Possibly bacterial infection was present in these two groups. Indeed, chronic microbial infection may cause endometritis that may be responsible for repeat breeding and anoestrus (Jahan and Myenuddin, 1996). Total serum protein was low in all subfertile groups compared to control which may indicate malnutrition. Safaa-Yessein *et al.* (1995) found no difference in total protein between cows with active ovaries and those with inactivate ovaries group.

In the present investigation, total serum bilirubin was higher in anoestrus and uterine infected animals than in control: this may have been due to liver fluke infestation.

The serum IgG in all subfertile groups was significantly (p<0.001) lower than control. This could indicate that lower humoral immunity is a factor in subfertility in zebu cows. Indeed, humoral immunity is low in cows with reproductive disease (Dhaliwal *et al.*, 1996; Sanin *et al.*, 1999). The results showed that the 3rd bleeding before 3rd booster dose had highest concentration of antibody against bovine IgG. In one hand, the mean value of anti-bovine IgG were highly significant (P<0.01). The total anti-bovine IgG was higher probably due to immuno-potentiating action against bovine IgG treated rabbit (Bari *et al.*, 1997).

It is concluded that there is no specific haematological, biochemical or immunological marker for specific reproductive problems in cows. Non-specifically ESR values were high in all reproductive disorders in the cows; however the Hb and PCV values were low. Humoral immune responses are non-specifically lower in all subfertile cows than the control group.

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