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Some Biochemical and Immunogenetic Investigations on Buffaloes Suffering from Chronic Endometritis

Samy I.A. Shalaby, Wahid M. Ahmed and Magdy M. Zaabal

Department of Animal Reproduction and A.I.,
Veterinary Research Division, National Research Centre, Giza, Egypt

Abstract: Endometritis is among the main cause of failure of conception in buffaloes in many developing countries including Egypt, In this study the prevalence of the affection and blood changes in affected buffalo-cow were investigated as compared with normal fertile partners. A total number of 385 buffalo-cows was investigated. Blood samples were collected and plasma was analyzed for some biochemical and genetic components. Results indicated that 5.97% of examined buffalo-cows suffer from chronic endometritis. Affected animals revealed high cortisol ($p < 0.01$), A1P, LDH and AST activities ($P < 0.01$), iron ($p < 0.01$) and creatinine ($P < 0.01$) and low progesteronel ($P < 0.01$), total lipid ($P < 0.05$), zinc($P < 0.05$) and copper ($p < 0.05$) concentration in their blood as compared with normal fertile animal. Total proteins and urea values were not significantly changed in buffalo-cows suffering from endometritis, Electrphoretic pattern of plasmo protein in buffalo-cows with chromic endometritis was characterized by significant changes in proalbumin ($P < 0.05$), post albumin ($P < 0.05$) and transferrin ($P < 0.01$) values compared to normal cyclic buffaloes. Distribution of serum protein genotypes and estimation of their gene frequencies indicated that the most predominant genetic alleles in buffalo-cows with endometritis are Al^S (0.714) and Ptf^B(0.643) while in healthy animals, Al^F (0.684), Pal^A (0.833), $F\alpha_2^A$ (0.684) and Tf^D (0.763) alleles predominated It was concluded that chronic endometritis are usually associated with disturbed blood constituents such as steroids, total lipids, enzymes, trace elements and protein electrophoresis.

Key words: Buffalo · Endometritis · Biochemical parameters · Genotypes

INTRODUCTION

Buffalo has an important role in the agricultural economy of several developing countries, whereas, such animals significantly contribute to the total milk, meat and work production. Despite having several merits such as utilization of poor-quality roughage, adaptation on harsh environments and resistance to several bovine tropical diseases, the buffalo has relatively poor reproductive efficiency irrespective of their location throughout the world [1].

During peripartum period, animals undergo pronounced physiological changes that suppress both the cellular and humoral defense mechanisms of the hosts thus increasing their susceptibility to various uterine infections Further, the physiological barriers of reproductive tract are weakened at parturition and the commensals take upper hand in creating infection more so when the hygienic surroundings are not at their best [2].

A large pro portion of failure of conception in farm animals could be attributed to endometritis [1]. Such affection is the most common ailment under field/farm conditions in buffaloes causing decreased fertility resulting in high economic losses [3, 4].

In Egypt, endometritis is an important cause of reproductive failure among buffaloes. It represents 87.3-93.5% from infectious causes of infertility. The incidence was recorded as 4.5- 27% In abattoir surveys and 2.4-27% in living buffaloes [4]. Many etiological factors have been incriminated as a cause of endometritis among which, hereditary, nutritional, managemental, hormonal, disturbance, specific and nonspecific infectious factors [5].

Corresponding Author: Samy I.A. Shalaby, Department of. Animal Reproduction and A.I., Veterinary Research Division, National Research Centre, Giza, Egypt. E-mail: samyshalaby58@yahoo.com.

Several authors [4-9] indicated that endometritis induced biochemical alterations in the blood of affected farm animals.

Reproductive performance may be changed as a result of changes in frequency of different genotypes. An increase in proportion of homozygotic loci is typically associated with a decrease in performance and vise versa for heterozygosity [10]. Moreover, correlation between immunogenetics and liability for infection and /or resistance has been recorded [11-13]. There are many studies performed to identify genes coding for productive, reproductive and economically significant traits by analysis of the whole-genome sequence of swamp and river buffaloes [14-16].

The present study was carried out to build a composite diagnostic approach for endometritis in buffaloes at an early stage through investigating some biochemical and immunogebetic aspects in the blood buffaloes suffering from chronic endometritis as compared to normal fertile partners.

MATERIALS AND METHODS

Animals: A total number of 385 buffalo-cows (at least 6 months after last calving0 was investigated at Veterinary Clinics and small private farms nearby great Cairo, Egypt. Owner complains, case history and general health status were recorded. Gynaecological examination was perfumed by rectal palpation aided by ultrasonography with an endorectal array (8.6 M Hertz; Pia Medical Flacse Saote, Netherland) at least twice for two successive weeks and the reproductive status and/or disorders were recorded. Cases revealing chronic endometritis were registered taking in consideration the criteria of reported by Robert [3] such as exudation, congestion, erosion of lining epithelium and enlargement.

Samples: Blood samples on EDTA were collected from buffalo-cows (normal cyclic at luteal phase and those revealing picture of chronic endometritis). Plasma was separated by centrifugation at 3000 r.p.m. for 15 minutes and kept at -20°C until biochemical analysis.

Hormonal Analysis: Plasma progesterone and cortisol levels were assayed using ELIZA micro wells technique, kits from Novotec, Germany [17] and ELIZA reader (Anthos Zenyth 200rt).

Some Other Biochemical Parameters: Glucose, cholesterol, total lipids, triglycerides, total protein, urea, creatinine, calcium, inorganic phosphorus, magnesium, alkaline phosphatase (AlP), lactate dehydrogenase (LDH) and transaminase (AST, ALT) were calorimetrically determined [18]. Proteins were electrophoretically analyzed using polyacrylamide gel [19].

Immunogenetic Investigation: Gene distribution frequency was determined [20] using the following formula: $P^2+2pq+q^2=1$ where p and q are the homozygotic genotypes (AA) and (BB) respectively. In the present study 6 blood protein loci were analyzed by electrophoresis, Pre-albumin (Pr) – Albumin (Alb)-post-albumin (Pal)- α-globulin ($F\alpha_2$) -transferrin (Tf) and post-transferrin (Ptf).

Statistical Analysis: Means were calculated for buffalo-cows suffering from endometritis and normal cyclic ones at the luteal phase. Student (t) test and x^2 were used for studying the differences between the above mentioned groups [21].

RESULTS

Examinations of 385 buffalo cows revealed that 23 animals suffer from chronic endometritis with an incidence of 5.97%.

Progesterone level was low ($P<0.01$) while, cortisol level was high ($P<0.01$) in affected animals as compared with normal animals (Table 1).

Energy metabolies and cholesterol revealed little changes in buffalo-cows suffering from endometritis except for total lipids which was significantly decreased ($P<0.05$) compared to normal (Table 1).

Narrow C/P ratio ($P<0.05$) with high iron ($P<0.01$) and low zinc ($P<0.05$) and copper ($P<0.05$) were the main characteristic alterations in mineral profiles due to chronic endometritis in buffalo-cows (Table 1).

Total protein and urea values were not significantly changed due to endometritis (Table 1) While, creatinine is high ($P<0.01$) in affected animals (Table 1). On the other hand, electrophretic analysis indicated that low albumin ($P<0.05$), postalbumin ($P<0.05$) and post transferrin ($P<0.01$) values were obvious in the plasma of buffalo-cows suffered from chronic endometritis compared to normal cyclic one (Table 1).

Distribution of serum protein genotypes and estimation of their gene frequencies indicated that the most predominant genetic alleles in buffalo-cows with endometritis are Al^S (0.714) and Ptf^B (0.643) while in healthy animals, Al^F (0.684), Pal^A (0.833), $F\alpha_2{}^A$ (0.684) and Tf^D (0.763) alleles predominated (Table 2).

Table 1: Some Biochemical paramters in buffalo-cows suffering from chronic endomteritis (Mean ± S.E.)

Parameter (mg/dL)	Normal cycilc (luteal)	Endometritis
Progesterone (ng/ml)	4.21±0.52	1.87±0.17**
Cortisol (µg/dL)	1.31±0.23	3.11±0.19**
Glucose(mg/dL)	67.55±3.08	71.28±4.54
Cholesterol(mg/dL)	201.65±4.58	183.78±9.90
Total lipids(mg/dL)	416.80±13.26	367.73±19.36*
Triglycerides(mg/dL)	81.20±1.84	81.85±2.27
A1P U/L)	12.99±0.39	21.62±1.84**
LDH U/L)	45.63±3.12	56.80±2.26**
AST U/L)	31.30±2.84	51.14±2.42**
ALT U/L)	12.70±0.11	12.51±0.10**
Calcium (mg/dL)	10.54±0.34	9.93±0.24
Inorg.phosphorous	5.08±0.26	5.24±0.92
Calcium: phosphorous	2.08±030	1.21±0.19*
Mg (mg/dL)	3.54±0.04	3.54±0.20
Iron(µg/dL)	217.15±5.63	250.14±11.73**
Zinc (µg/dL)	108.75±4.16	86.57±8.24*
Copper (µg/dL)	90.63±5.57	70.86±8.24*
Total proteins	6.52±0.13	6.35±0.20
Urea (mg/dL)	33.80±1.56	35.45±2.70
Creatinine (mg/dL)	2.00±0.80	2.35±0.03**
Electrophoresis (%)		
Prelabumin	8.55±1.89	6.96±0.33
Albumin	18.34±1.41	13.46±1.71*
Postalbumin	5.82±0.36	3.42±0.68**
A-globulin	6.03±1.02	4.87±1.82
Transferrin	16.33±1.02	23.78±6.33
Posttransferrin	12.28±1.47	5.83±0.84**

*p<0.05 **p<0.01

Table 2: Distribution of plasma protein genotype in buffalo-cows suffering from endometritis (Mean ± S.E.)

Protein		Normal cyclic (luteal)	Endometritis
Pre-albumin	AA	7 (5.200)	2 (1.750)
	AB	6(9.500)	3 (3.500)
	BB	6 (4.300)	2 (1.750)
	Gene frequency	Pr^A =0.526	Pr^A =0.500
		Pr^B =0.474	Pr^B =0.500
	X^2	2.600	0.130
Albumin	FF	7 (8.900)	3 (3.500)
	FS	12 (8.200)	4 (4.900)
	SS	- (1.900)	- (0.600)
	Gene frequency	Alb^F =0.684	Alb^F =0.714
		Alb^S =0.316	Alb^S=0.286
	X^2	4.000*	1.400
Post-albumin	AA	11 (10.400)	4 (3.500)
	AB	3 (4.200)	2 (2.900)
	BB	1 (0.400)	1 (0.600)
	Gene frequency	Pal^A=0.833	Pal^A = 0.714
		Pal^B=0.167	Pal^B =0.286
	X^2	1.200	0.640
A-globlin	AA	4 (1.900)	2 (0.900)
	AB	4 (8.200)	1 (3.200)
	BB	11 (8.900)	4 (2.900)
	Gene frequency	$F\alpha_2A$ =0.316	$F\alpha_2A$ = 0.357
		$F\alpha_2B$ =0.684	$F\alpha_2B$ =0.643
	X^2	4.900*	3.22**
Transferrin	DD	12 (11.000)	2 (2.900)
	DE	5 (6.900)	3 (3.200)
	EE	2 (1.100)	2 (0.900)
	Gene frequency	Tf^D =0.763	Tf^D =0.643
		Tf^E =0.237	Tf^E =0.357
	X^2	1.300	1.600
Postransderrin	AA	7 (5.800)	2 (0.900)
	AB	7 (9.400)	1 (3.200)
	BB	5 (3.800)	4 (2.9000)
	Gene frequency	Ptf^A = 0.553	Ptf^A = 0.357
		Ptf^B = 0.447	Ptf^B = 0.643
	X^2	1.200	3.200**

*p > 0.05 **p > 0.01

DISCUSION

Endometritis is among main causes of low reproductive potentials in buffaloes and causes great financial losses represented by the coast of therapy, failure of conception and repeat breeding [1, 22]. Since the endometrium is central to the control of reproductive events such as luteolysis, maternal recognition of pregnancy, early embryo development and placentation in the ruminants, it is logical to assume that its inflammation would cause infertility in the ruminants [22]. In fact, postpartum (PP) endometritis which occurs during the first three to six weeks of calving adversely affects the fertility indices in cattle. The condition not only affect the uterus but also the ovary [23] and may be due to various factors viz. dystocia, retained foetal membranes (RFM), caesarean section, contamination of uterus at the time of artificial insemination or natural insemination, induced parturition, stillbirth, twins, ovarian inactivity, cystic ovaries, unhygienic calving environment and metabolic disorder associated with parturient metabolic disorders [24].

In the present study approximately 6% of the examined buffalo-cows suffered from endometritis. This incidence lies within ranges given by Vale *et al*. [25] and Yessein *et al*. [26] as 4.5-7.5%. However, high incidence (11.9-14.6%) was given by El- Hariri *et al.* [27]. Variations in the reported incidence was related to milk production, mangemental system and endocrine dysfunction [3, 28].

Progesterone level was lower while, cortisol level was higher in the affected animal compared to values of normal luteal phase. In this respect, Ismail *et al.* [29] concluded that uterine disorder disturbed steroidogenesis. The condition is mainly due to replacement of luteal cells with fibroblast in pathologically persisted corpora lutea [5] as well as the efficiency of the affected endometrium to produce prostaglandin $F\alpha_2$ [30]. Moreover, the type of microorganism could not be denied [7]. On the other hand, the high cortisol level agree with Zraly *et al.* [31] and Ezzo *et al.* [32] who related the condition to the stressful circumstances of the affected animals as the high cortisol level minimize the inflammatory reaction and suppresses the allergic response to various antigens.

Buffalo-cows suffering from chronic endometritis had low plasma level of energy metabolites and cholesterol, especially total lipids. Majeed *et al.* [33] found that buffaloes with metritis showed increased serum glucose level. Ash-turker *et al.* [34] revealed low total cholesterol level in infertile buffalo, while Salem *et al.* [35] and Yassein *et al.* [36] reported high cholesterol and total lipid in repeat breeder bovine, respectively. These conflicting results were mainly related to differences in the managerial systems of the affected animals.

Concerning A1P, AST and LDH higher activity in affected buffalo-cows, many authors [9, 24, 37] indicated that the higher enzymatic activity in infertile compared to fertile animals mainly reflected the hypermetabolic rate and stress in the affected animals [9]. Moreover, it was recorded that increased enzymatic activity in the uterine flushing could be diagnostic criteria in the assessment of the intensity of endometritis [24].

Changed C/P ratio with high iron and low zinc and copper concentrations were evident in the plasma of the affected buffalo-cows. However, balanced C/P ratio is essential for animal pituitary function and fertility [8]. Concerning iron, similar results were reported by Hahn *et al.* [37] who found high iron concentration, while Moustafa *et al.*[38] found low concentration in repeat breeder bovines. On the other hand, Hahn *et al.* [37] and Moustafa *et al.* [38] reported low copper concentration, while El-Taweel [39] found high value and Nada *et al*. [5] found no significant changes in plasma copper concentration in buffalo-cows with endometritis. Zinc concentration was low in repeat breeder [33] and did not changed in buffalo-cows with endometritis [7]. However, Ahmed *et al.* [8] concluded that mineral homeostasis is essential for normal fertility in farm animals. No significant changes could be detected in total plasma proteins in buffalo-cows with endometritis. However, creatinine value was high, while albumin and α-globulin values were low with high transferrin. Similar results were previously reported [34-37].

In the present study, the distribution of genotypes and gene frequencies of studied loci indicated that the albumin genotype SS (Alb^S=0.684), globulin genotype BB ($F\alpha_2^B$ =0.643) and post-transferrin genotype BB (Ptf^B = 0.643) were mostly associated with endometritis. and this finding agree with data obtained by Osman [40] who reported association of homozygote genotypes with endometritis in Egyptian buffaloes. On the other hand, the current results revealed more predominance of genetic alleles in were Alb^F (0.684), Pal^A (0.833) and Tf^D (0.763) in normal cyclic buffalo-cows and this result is similar to those previously recorded by Zheng *et al.* [41], Shalaby *et al*. [42] and Elkhadrawy *et al*. [43]. The possible correlation between some genetic markers and animal resistance could be attributed to the amplifying synthesis of TNF-α and IL-Iβ genes that contribute to protective immune response and exert anti-microbial action. Moreover, Yi *et al*. [12] recorded a significant association between CYP19 polymorphism and endometritis. Also, Anurag *et al.* [13] found a polymorphism in the promotor region of TNF- α of water buffalo (*Bubalus bubalis*) which is associated with disease resistant. Molecular techniques have been used recently in the diagnosis of endometritis in different livestocks. Several researches have focused on the transcriptional profile of TLRs and proinflammatory cytokines in peripheral blood or tissues in cows and, mares and there was a significant correlation between the expression of these genes and the presence of endometritis [44]. TLR2 and TLR4 are members of the TLR family which is highly expressed in the endometrial cells of the uterus and they play a key link between endometritis and immune system response, therefore they can be considered as detection markers for endometritis [45]. Toll-like receptors play an essential role in innate immunity through recognition of pathogen- associated molecular patterns (PAMPs) and activation of proper innate and adaptive immune responses against pathogens [46, 47].

In conclusion, chronic endometritis is usually associated with disturbed blood constituents such as steroids, total lipids, enzymes, trace elements and protein electrophoresis. For future strategy to increase productivity and to improve reproductive efficiency of Egyptian buffalo, it is very important to identify a specific genotypes associated with resistance and susceptibility to occurrence of endometritis which help in genetic selection in breeding program.

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