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Predominance of Some Blood Protein Gene Markers Associated with Early Pregnancy in Egyptian Buffalo-Cows

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Abstract: Pregnancy imposes numerous physiological changes that causes alterations in the blood biochemistry of the dam. This study investigated some blood protein gene markers associated with early pregnancy in Egyptian buffalo- cows. Blood samples were collected from 193 slaughtered buffalo-cows during the green season of the year. Samples from early pregnant cows with fetal CVR < 20cm. were subjected to some biochemical and immunogenetic analyses and were compared with normal cyclic non pregnant buffalo- cows. Results indicated that progesterone level was maintained at the luteal value in the early pregnant buffalo compared with non-pregnant cyclic animals. The imunogenetic analyses of the early pregnant buffalo-cows indicated that the albumin homozygotic genotype FF has superiority over the SS genotype which disappeared completely in the present study and this result leads to high frequency of Alb^F(0.684). Also, in post-albumin locus genotype (AA) was characterized by very high frequency of its genetic allele Pal^A(0. 833), the homozygotic transferrin (DD) also was distinguished by high frequency of its genetic allele Tf^D(0. 763). Concerning to the alpha-globulin genotype the given result showed that the homozygotic genotype (BB) predominate over the (AA) genotype with high frequency of $F\alpha_2^B$ (0.664) genetic allele. It could be concluded that the early pregnancy in buffalo-cows is associated with high frequencies of the: Alb^F, Pal^A, $F\alpha_2^B$ and Tf^D blood protein gene makers.

Key words: Buffaloes · Pregnancy · Genes marker · Blood biochemistry · Progesterone

INTRODUCTION

Water buffalo are important sources of food and fiber for the ever-increasing global human population. Despite valuable contributions to agriculture and human well-being, there are current and emerging challenges and opportunities. High resolution sequencing of breeds and comprehensive annotation of the genomes are not yet available. In addition, there is a lack of fundamental knowledge about economically important traits including longevity, disease resistance, milk production and quality, meat production and quality, growth and development, heat tolerance and fertility [1].

The major constraints to full exploitation of the productive potential of buffalo are its inherent low reproductive efficiency due to delayed puberty, higher age at first calving, long post-partum anestrous period, long intercalving period, silent heat coupled with poor expression of oestrus, seasonality in breeding and low conception rate [2].

Reproductive cycles in buffalo are regulated by endocrine-neuroendocrine interactions between hypothalamic, gonadotropic, gonadal and other hormones. To improve the buffalo reproductive efficiency, the investigation on endocrine aspects is useful to gain a better knowledge of buffalo reproduction. Since an understanding of the hormonal interaction is essential for relieving reproductive problems of endocrine origin, a considerable attention has been focused in the last two decades on utilizing reproductive endocrinology as a means to identify problems specific to this species and to devise means for improving reproductive performance [3].

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Pregnancy-associated glycoproteins (PAGs) are trophoblastic proteins belonging to the aspartic proteinase family secreted by different placental cells of many mammalian species. They play a pivotal role in placentogenesis, foetomaternal unit remodeling and implantation. The identification of the genes encoding those proteins will be helpful to unravel the intricate embryo genomic functions during pregnancy establishment [4].

Considering importance of these proteins, the present study was undertaken to characterize the predominating pregnancy associated gene markers in early pregnant Egyptian buffalo cows.

MATERIALS AND METHODS

Samples: Blood samples and genital organs were collected from 193 buffalo-cows (5-12 years) slaughtered at El Warak Abattoir, Giza, Egypt during the green season of the year (September – May). Samples from 19 animals with foetal crown rump length of <20 cm length were selected to represent the early stage of pregnancy. Plasma was separated by centrifugation at 3000 r.p.m for 15 minutes and then kept at – 20°C until biochemical and immunogenetic analyses.

Hormonal Analysis: Plasma progesterone levels were assayed using ELIZA micro wells technique, kits from Novotec, Germany [5] and ELIZA reader (AnthosZenyth 200rt).

Immunogenetic Analyses: Electrophoretic pattern of plasma proteins was done using polyacrylamide gel electrophoresis [6]. Gene frequencies were determined using Hardi-Wainberg formula [7].

$P^2 + 2pq + q^2 = 1$

P^2 is the number of homozygotic genotype (AA)
q^2 is the number of homozygotic genotype (BB)

Statistical Analysis: Data were analyzed using student (t) test between parameters in early pregnant buffalo- cows and normal cyclic one. Genetic equilibrium was determined by x^2 [8].

RESULTS

Hormonal Analyses: Table 1 reveals that progesterone level averaged 5.07±0.74ng/ ml in early pregnant buffalo-cows. It was evident in a slightly higher level compared to the luteal value of the non-pregnant buffalo-cows (4.21±0.52ng/ml).

Immunogenetic Parameters

Protein Polymorphism: The effect of early pregnancy on plasma proteins and their electrophoretic pattern are shown in Table 1. Results revealed low total protein ($p < 0.05$), post-albumin ($p < 0.01$), α-globulin ($p < 0.01$), transferrin ($p < 0.01$) and post -transferrin ($p < 0.01$) were obvious in early pregnant buffalo-cows compared to non pregnant cyclic buffaloes.

Protein Genotyping and Gene Frequencies: The distribution of blood protein genotypes for each genetic locus and the gene frequency of each allele is shown in Table 2. The results revealed that the most predominated gene markers in early pregnant buffalo-cows are: Alb^F(0.684), Pal^A(0. 833), $F\alpha_2{}^B$(0.664) and Tf^D(0. 763).

Table 1: Some biochemical parameters in early pregnant buffalo cows (Mean±S.E)

Parameter	Non-Pregnant	Early Pregnant
Progesterone (ng/mg)	4.21±0.52	5.07±0.74
Total Proteins (gm/dl)	6.52±0.13	6.17±0.12*
Prealbumin	8.55±1.98	5.07±1.77
Albumin	18.34±1.41	14.15±3.46
Post-albumin	5.82±0.36	2.85±0.07**
α globulin	6.03±1.02	1.37±0.44**
Transferrin	16.33±1.02	42.50±3.83**
Post- transferrin	12.28±1.47	6.22±1.90**

*<0.05 **P<0.01

Table 2: Distribution of blood protein genotypes and their gene frequencies in early pregnant buffalo-cows (N= 19)

Blood protein loci	Genotyping	Gene Frequency	X^2
Pre-albumin	AA 7 (5.2)	Pr^A=0.526	2.6**
(Pr)	AB 6 (9.5)	Pr^B =0.474	
	BB 6 (4.3)		
Albumin	FF 7 (8.9)	Alb^F=0.684	4.0*
Alb	FS 12 (8.2)	Alb^S=0.316	
	SS - (1.9)		
Post-Albumin	AA 13 (10.4)	Pal^A=0.833	1.2
Pal	AB 4 (4.2)	Pal^B=0.167	
	BB 2 (0.4)		
α globulin	AA 4 (1.9)	$F\alpha_2{}^A$=0.336	4.9*
$F_{\alpha 2}$	AB 4 (8.2)	$F\alpha_2{}^B$=0.664	
	BB 11 (8.9)		
Transferrin	DD 12 (11.0)	Tf^D=0.763	1.3
Tf	DE 5 (6.9)	Tf^E=0.237	
	EE 2 (1.1)		
Post-transferrin	AA 7 (5.8)	Ptf^A=0.553	1.2
Ptf	AB 7 (9.4)	Ptf^B=0.447	
	BB 5 (3.8)		

*P<0.05 ** P<0.01
In brackets: theoretical number of genotypes.

DISCUSSION

Pregnancy is established and maintained by the two-way communication between the conceptus and the mother. These intricate dialogues which are initiated after fertilization are crucial as these signals are considered potential markers for effective placental remodeling, pregnancy recognition and successful implantation. These interactions between the conceptus and maternal system emphasize the importance of both the components in maternal recognition of pregnancy and embryonic development [9, 10].

An early and precise pregnancy diagnosis is an important criterion for better reproductive management in livestock like cows and buffaloes. High reproductive efficiency is a prerequisite to realization of high life-time production from dairy animals. Early pregnancy diagnosis is crucial to shortening the calving interval through enabling the farmer to identify open animals so as to treat and/or rebreed them at the earliest opportunity [11].

There are many direct and indirect diagnostic methodologies that were undertaken to confirm early pregnancy in bovine such as Early Conception Factor (ECF) [11], ultrasonography [12, 13], Estrone sulphate determination [12], Interferon (IFN) [14] and pregnancy associated glycoproteins (PAGs) [15]. On the other hand, there are some current researches based on biomarkers to detect early pregnancy. In this respect, it is very important to qualify any biomarker for pregnancy, because the candidate molecule should be able to accurately determine the pregnancy status as early as possible with minimum false positives or false negatives. Therefore, many authors suggested that proteomics are the most suitable diagnostic technique for early pregnancy as it provides an opportunity to simultaneously analyze thousands of proteins in a single experiment from a complex mixture of proteins in various body fluids [16]. This will help in identifying specific and sensitive biomarkers fulfilling the characteristics of uniqueness for a pregnancy diagnosis molecule [17].

In the present study, 6 blood proteins were studied, prealbumin, albumin, post albumin, alpha- globulin, transferrin and post- transferrin and all these blood protein loci used as genetic markers to detect the possible association between these loci and early pregnancy in buffalo-cows. Results revealed that the early pregnancy is associated with high frequency of the following gene markers: Alb^F(0.684), Pal^A(0. 833), $F\alpha_2{}^B$ (0.664) and Tf^D(0. 763). This result explained the polygenic effect of different gene marker on a trait and agree with finding of (Nicholas) [18] who reported that the genotypic value for any trait is obviously a reflection of the effect of an animal's genotype at all loci that affect the trait. In other words, genotype value reflects the way in which those genes are combined into its particular genotype.

In the present investigations the homozygotic transferrin genotype DD was predominates, this result confirms the finding of Zheing *et al*. [19] specially for Tf^D allele and also agree with those results obtained by Satish and Chakravarth [20]who reported that the increase of $beta_1$ band (Tf) is observed in iron deficiency anemia due to an increase level of free transferrin and also in pregnancy. In other researches the transferrin heterozygotic DE was superior [21] these results confirm the importance of serum transferrin locus in fertility. But, often results were conflicting regarding identification of superior genotypes or mating combination of specific alleles [22].

In the present study, not only transferrin locus has superiority in its gene frequency, but also post-albumin homozygotic genotype AA which gave the highest gene frequency among 6 studied loci), Pal^A(0. 833) and this result is agreeing with the finding of Billington [23] who reported the possibility of using immunogenetic markers to evaluate the feto- maternal interaction during pregnancy.

Barbato *et al*. [4] studied the Pregnancy-Associated Glycoproteins (PAGs) and they reported that these proteins are trophoblastic proteins belonging to the Aspartic proteinase family secreted by different placental cells of many mammalian species. These glycoproteins play a pivotal role in placentogenesis, foetomaternal unit remodeling and implantation. The identification of the genes encoding those proteins will be helpful to unravel the intricate embryogenomic functions during pregnancy establishment. The most important result in such study was the characterization of the pregnancy associated glycoprotein-1 gene of buffalo. In this respect, Luridiana *et al*. [24] studied the association between melatonin receptor 1A (MTNR1A) gene polymorphism and the reproductive performance of Mediterranean Italian buffaloes, they reported that, the association between the T/T genotype and reproductive activity during days with a long photoperiod indicates that this polymorphism may be considered a genetic marker to identify buffaloes that are able to reproduce out of the breeding season.

CONCLUSION

Early pregnancy diagnosis is an important aspect for optimizing dairy production, yet none of the present-day

methods qualifies as an ideal diagnostic due to limitations of accuracy, later stages of applicability and requirement for elaborate instrumentation and laboratory setup. This warrants further research on developing novel early pregnancy diagnostics for livestock species. Currently available methodologies of blood proteins gene markers and basis of molecular genetics, as well as proteomics techniques instill hope for finding molecules-exclusively related to intricate maternal metabolic alterations necessary to align with physiology of early embryonic development and it signaling for maternal recognition of pregnancy and continued survival.

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