CORRELATION BETWEEN FERTILITY AND LEVELS OF PROTEIN IN SEMINAL PLASMA OF EGYPTIAN BUFFALO BULLS

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ABSTRACT

The Objective of this study was to measure the protein profiles of seminal plasma in Egyptian buffalo bulls semen and to examine their correlation with semen characteristics of buffalo bulls in Mehalet Mousa . Semen of 20 buffalo bulls (divided into high and low fertile bull, 10 for each group, according to records of insemination) were collected twice weekly for 12 weeks by artificial vagina . Semen characteristics (ejaculate volume, motility , abnormalities, viability and sperm cell concentration were recorded . Seminal plasma was seperated by centrifugation , treated with cold ethanol and then underwent SDS-polyacrylamide gel electro phoresis (PAGE) . Fourteen protein bands were Identified on the gel of these protein band . 25 KDa was significantly correlated with all semen parameters studied in high fertile group , while 63 KDa was significantly correlated with sperm motility and sperm abnormalities in low fertile Group **.**

INTRODUCTION

Sperm morphology and motility, the number of sperm per insemination, percentage of reacted spermatozoa and invitro fertilization have been extensively evaluated as an indication of ability of spermatozoa to fertilize an egg. Seminal plasma , which in a complex mixture of secretion of testis, epididymis and accessory glands ,contains a variety of proteins that modulate the fertilizing ability of bovine spermatozoa (**Henault et al ., 1995**).

A number of mammalian seminal plasma protein have been investigated which act as molecular markers of fertility in different species. The protein composition of mammalian seminal plasma has important effects on sperm function as sperm motility (**Henricks et al . , 1998**), viability (**Brandon et al., 1999**) and freezability, (**Asadpour et al ., 2007**) .

Buffaloes have less physiological adaptation to externs of heat and cold than the cattle due to dark skin, sparse coat or hair , and less dense sweat glands (one-sixth of the density than cattle) (**Marai and Haeeb, 2010**). It is suggested that the season was not only affect semen parameters but may also affected by the composition of

seminal plasma proteins of buffalo bulls. The aim of the present study was to assess the protein profile of the Egyptian buffalo seminal plasma in high and low fertilizer bulls by using SDS-PAGE and to investigate semen physical characteristics and a possible relationship between these proteins and the semen characteristics of buffalo bulls .

MATERIAL AND METHODS

- **The experimental animals.**

Twenty sexually mature Egyptian buffalo bulls (4-5 years old) were randomly selected from Mehalet Mousa Station . The selected bulls were maintained in nearly identical nutritional and management conditions throughout the study period and regularly vaccinated against infections and contagious diseases as per standard schedule. The period of study was three months (December 2013 to February 2014) . According to the filed fertility (obtained from records maintained at the station) animals were divided into two groups as following highly fertile bulls of average fertility rate above 70% and low fertile bulls of average fertility rate below 40 % .

- **Semen evaluation and preparation of seminal plasma**. Ejaculates were collected towice weekly from each bull at (8.00 to 9.00 am) using sterilized artificial vagina . Ejaculate volume was determined by using graduated tube, and sperm concentration was measured using standard hemocytometer methods, (**Sorensen , 1979**) . The percentage of viable spermatozoa was estimated by viewing 200 spermatozoa under 400x magnification using eiosin negrosin staining method (**Hancock, 1951**). Mass motility was estimated on percentage score according to **Melorse and Laing (1970**). The sperm abnormalities was evalvated by standared method of eosim-nigrosin staining.

Fresh semen was centrifuged at 5000 r.p.m for 10 min (**Clements,2000**) .The supernatants were transfered into 1.5mp tubes, recentrifuged at 1200 r.p.m for 20 min at 4^{o}c to eliminate the remaining cells. After total protein determination, nine volumes of cold ethanol (-20^{o}c) were added and left with constant stirring for 90 min at 4^{o}c to precipitate the proteins. Proteins were then recovered by centrifugation at 10.000 r.p.m for 10 min re-suspended in phosphate buffered saline (PBS) and stored at -20^{o}c until further analysis of seminal plasma proteins.

- **Molecular weight determinations . (SDS_PAGE)**

The stored samples were taken and processed for protein analysis by SDS-polyacrylamide gel electrophoresis. Sodium dodecyl sulphate - PAGE was used for separation and determination of molecular weight

(MW) of seminal plasma proteins (**Laemmli, 1970**) by using a 12% polyacrylamide gel. Electrophoresis was run at constant voltage of 60v at room temperature for 30 min through the stacking gel and at voltage 80v through the separating gel till tracking dye front reached close to the bottom of gel slab. At the end of electrophoresis, the gels were stained with 0.1% coomassie Brilliant Blue G solution for 2h and then destained with three change of destaining solution (water :methanol :acetic acid) at room temperature. The relative molecular weight were determimed using the Bio-Rad (GS700 IMIGAING DENSITIMETER) By molecular analysis software .

Gel images were analysed to determine molecular weight of protein bands and relative protein fractions (protein %) using the gel doc system .

-Data analysis .

Data analysis was performed usiny SPSS software computer program . The correlations of seminal plasma protein with all parameters of the semen were tested by multiple linear regression test .

-Results.

The result of the semen evaluation of 20 buffalo bulls are summarized in Table (1) , and depicted as mean ± SE. The mean value obtained for semen volume , sperm motility and sperm cell concentration of highly fertile buffalo semen significantly were ($p<0.05$) higher compared with low fertile buffalo bulls semen . Also , the viability of highly fertile group was higher and abnormal morphology was lower than the low fertile buffalo group but the differeneces were not significant.

Table (1): Semen characteristics of high and low fertile buffalo bulls

Trait	High Fertile	Low Fertile
No of bulls	10	10
Ejaculate volume	3.90^{a}±0.15	3.67^{b}±0.10
Sperm motility	75.4^{a}±0.71	62.3^{b}±0.82
Abnormalities	14.4±0.43	18.9±0.44
Viability	86.5±0.43	79.9±0.49
Sperm Concentration x 10^6	1.44^{a}±0.31	1.30^{b}±0.24

Mean values denoted by letters (a and b) are significantly different ($p < 0.05$) .All values Are mean ± SE

Seminal plasma proteins of different groups (High vs. low fertile buffalo groups) were fractionated with the help of SDS-polyacryalamide gel. The gel resulted with 14 protein bands in the molecular weight range from 5 to 245 KDa shown in Fig (l) and (2) . The protein bands of 1,2,5,6,8,9,10,11, 14,15,25,40,43,45, 49,50,52,58,59, 60 and 67 KDa molecular weights were observed

common in semsinal plasma of high and low fertile buffalo bulls. While protein bands of 3,16, 19,21,26,31,36,38,44,51,54,55, 65,66,72,73, 87,214 KDa were observed only in high fertile buffalo seminal plasma. In addition, protein bands of 7,18,22,23,28, 35,37,39, 42,47,48,53,84,99,128,157,219 KDa molecular weigts were observed only in low fertile group. 65 KDa protein and 54-59 KDa proteins were prominent (60 and 70% of the bands in high fertile group) while 58 KDa and45-49 KDa proteins were promiment (60 and 80 % of the band in low fertile group , respectively) .

Of these protein fractions , 25 KDa was significantly correlated with ejaculate volume ($p < 0.05$) ,sperm motility($r=1$), abnormal sprerm ($r=-1$), sperm concentration ($r=1$) and life spermatozoa ($r=1$) while 20KDa band as correlated with ejaculate volume ($r=0.93$) and sperm concentration ($r=-0.82$) in high fertile group. In addition, 63KDa protein fractions were significantly ($p<0.05$) correlated with sperm motility ($r=-0.72$) and sperm abnormalities ($r=0.66$) in seminal plasma of low fertile buffalo bulls.

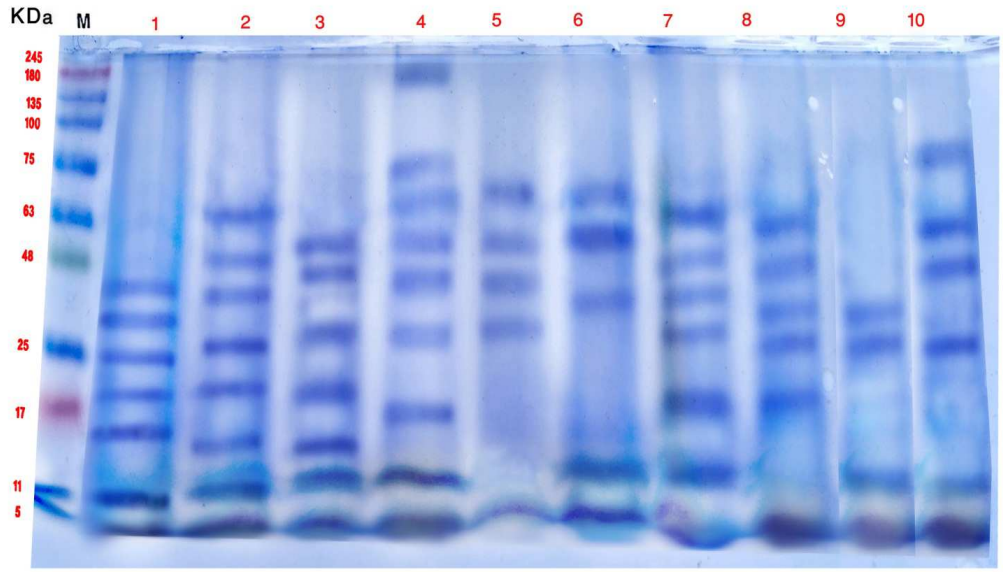

Figure (1): SDS – PAGE of seminal plasma protein of high fertile.

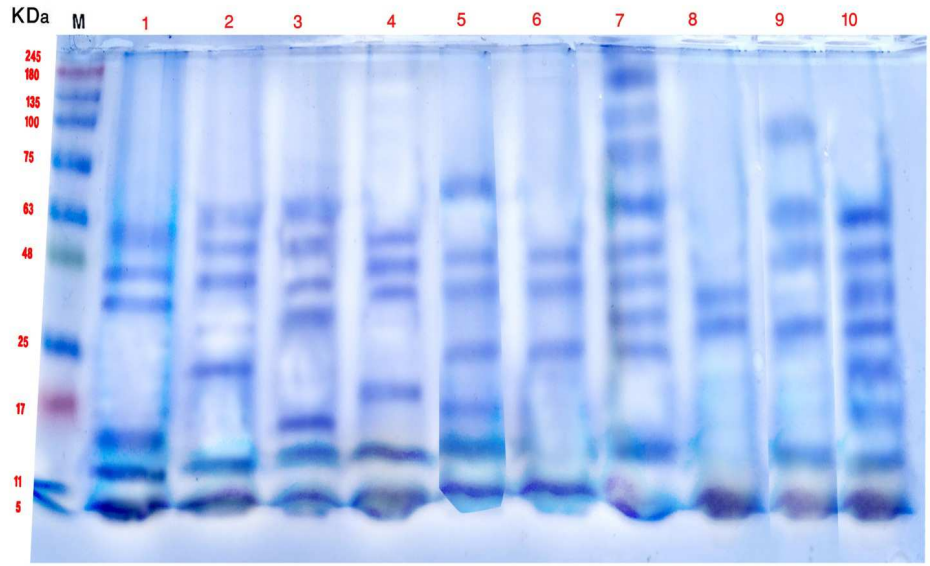

Figure (2): SDS – PAGE of seminal plasma protein of low fertile.

- **Discussion**

One factor affecting fertility is changes in semen quality induced by Individual buffalo bulls (**EL-Sheshtawy et al., 2008**). The ejaculate volume , sperm motility, viability and sperm concentrations ($X10^6$ /ml) were significantly ($P<0.05$) lower in low fertile buffalo bulls semen than high fertile one. This finding suggests that the composition of the buffalo semen parameters would play an important role in sperm membrane stability and subsequent fertility (**El-Harairy et al.,2005**). Even though , many studies proved a correlation between seminal plasma proteins and fertility of the male in some species of domestic animals such as bulls (**Asadpour et al ., 2007**), rams (**Al madaly et al .,2016**) and goats (**Villemure et al.,2003**). However,a little information is available regarding the relationship of seminal plasma proteins with semen characteristics and fertility in Egyptian buffalo bulls .

This study was designed to get some information in this field. In this study 14 proteins were detected in seminal plasma of buffalo bulls with molecular weights ranging from 1 to 245KDa by using SDS-PAGE technique which much lower than the finding of **Asadpour et al. (2007),** who found 25 protien fractions with molecular masses ranging from 14.4 To 80.5 KDa in Iranian buffalo bulls , but the present results are in partial agreement with the results of **Harshan et al . (2009)** and

Aramgasmy et al . (2005) on seminal plasma proteins of buffalo. They reported 19 (30-205KDA) and 18 protein bands (12-127 KDa) , respectively, in seminal plasma of different breeds of buffalo bulls. Few of the protein bands (approximately 20,24.5,32,35,44,55 and 70 KDa) observed in the present study were also reported by **Arangasamy et al. (2005) , Asadpour et al. (2007), Singh et al . (2013)** and **Sharma et al. (2014)** in buffalo seminal plasma wich substantiates the findings of the present study**.** These differences in the number of protein bands may be due to difference in protein extraction procedure applied prior to SDS-PAGE fractions (**Sharma et al , 2014**) . Statistical analysis of the present study showed that 25 KDa protein fraction was significantly ($P<0.05$) correlated with ejaculate volume (r=-1) sperm motility ,sperm abnormalilites , viability and sperm concentration in high fertile group . This is in agreement with those reported by **Jobin et al. (2004)** and **Asadpour et al.(2007)**, who found a significantly higher 24.5 KDa proteins in bulls and buffaloes , respectively.It partially agrees with the report of **Nauk** and **Manjunath (2000)**, who reported that two proteins of 26 and 55 KDa were predominate in higher fertility bulls.The majority of the differential expressed protein spots were in the range of 3 KDa to 73 KDa in high fertile bulls while the protein fraction ranged from 7 to 53 KDa in seminal plasma of low fertile group .

It was concluded that the differences in the seminal plasma proteins profile of high and low fertile Egyptian buffalo bulls were detected and seminal plasma proteins in buffalo bulls were similar to those reported in other animal species ; some of the seminal plasma proteins (25-63 KDa) are correlated with buffalo semen characteristics.

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العلاقة بين الخصوبه ومستويات البروتين في بلازما السائل المنوي لطلائق الجاموس المصري

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الملخص بالعربي

تهدف هذه الدراسة إلى تقييم بروتينات بلازما السائل المنوى لطلائق الجاموس المصرى واختبـــار علاقته بصفات السائل المنوى . تم تجميع السائل المنوى من 20 فحل جاموس (مقسمه على اساس سجلات الخصوبة إلى طلائق عاليه ومنخفضه الإخصاب) بواسطة المـهبل الصناعـــي مـــرتيـــن
اسبوعيا ولمدة 12 إسبوع . تم دراسة خواص السائل المنوى (حجم القذفه – حركه الحيوانات المنويـــــه - الحيوانات المنويه الشاذه والحيه وتركيز الحيونات المنوية) .
تم فصل بلازما السائل المنوى بالطرد المركزى بالمعاملة بالإيثانول المبرد والفرد عن طريق الإلكتروفريسس (SDS-PAGE) .تم تحديد 14 شريط علي عامود الجل. وقد وجد أن بروتينـــات 25KDa، ترتبط معنويا بكل صفات السائل المنوي المدروسه فى مجموعه الثيران عاليه الخصوبه بينما ,63KDa ترتبط معنويا مع حركه ونسبة الشواذ للحيوانات المنويه لمجموعة ثيران الجاموس المنخفضة الخصوبة.