Research Article

Relationship of Protein Profile in Blood and Seminal Plasma with Semen Quality of Egyptian Buffalo Bulls

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Abstract | The current study aimed to determine the possible relationship between blood plasma (BP) proteins and seminal plasma (SP) proteins in low and high fertile buffalo-bulls. Also, the relationship between protein profile in BP of adult bulls and bull calves were studied. Blood samples were taken from 3 calves (128.33±7.58 kg and ageing 6 mo.) and 10 bulls (400±37.5 kg and 24-25 months of age) and semen samples from bulls. Semen was collected once a week from all bulls which were divided according to semen quality to high and low fertile bulls (5 for each). Reaction time and semen characteristics were determined. Blood from bulls and calves, and seminal plasma of bulls were separated by centrifugation, and protein analysis was performed by using SDS-polyacrylamide gel electrophoresis. Results showed the presence of the molecular weight of proteins of 71, 52 and 31 kDa in BP and SP; 63, 33, and 19 kDa-proteins in SP, and 28 - 30 kDa-proteins in BP is in association with bull fertility (high semen quality). Also, 48, 18 and 16 kDa-proteins were present in the BP and SP of low fertile bulls. Proteins with 16, 30, 34, 35, 36, 37, 63, 64, 175, and 316 kDa were observed in the BP of bull calves. Proteins of BP and SP are related to bull fertility. Determination of protein profile in BP may be considered as a strategy for selection of semen quality of buffalo-bulls used for natural or artificial insemination. There is a relationship between protein profile in BP of buffalo-bulls and buffalo-bull calves which may suggest an early prediction of semen quality in bull calves in future studies.

Keywords | Buffalo-bulls, Blood, Semen, Protein, Fertility.

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INTRODUCTION

Fertility is closely related to the ability of bull to produce sperm that can fertilize the oocyte then produce a new calf. There are many factors affecting bull fertility, such as management, age, nutrition, genetics, and disease. In bovine, the important indices of semen quality that correlated with the fertilization rate included ejaculate volume, sperm cell concentration, live and abnormal sperm proportions, and sperm motility (Fiaz et al., 2010).

Sperm proteins have an important role related to fertility, such as morphological integrity and sperm function, including motility, capacitation, fertilization, oocyte acti-

vation, and embryonic development (Parisi et al., 2014). In different species, seminal plasma (SP) proteins may be involved in energy metabolism, cell communication, spermatogenesis, and motility of spermatozoa. The SP proteins are considered as an indicator of male fertility. Bulls with higher expression of SP proteins have high fertility (Peddinti et al., 2008). The proteins of SP are produced and secreted from the seminal vesicles and ampullae (Moura et al., 2006 a, b). The SP contains organic and inorganic compounds affecting the quality of sperm cells (Foxcroft et al., 2008). The SP proteins in bovine have several biological properties (Manjunath and Therien, 2002). In sheep, SP proteins revert the cold-shock damage on the membrane of sperm cells (Jobim et al., 2004). In domestic bulls, the bovine seminal plasma contains a family of major proteins (40–50% of the total proteins) with apparent molecular weights between 15 to 17 kDa, and 28 to 30 kDa (Chacur, 2012). In buffalo bulls, the electrophoretogram of semen revealed 24 protein bands ranging between 6.0 to 200 kDa. Also, Sharma et al. (2014) revealed 25 protein bands on SDS-PAGE analysis.

In bovine, SP proteins may modulate sperm properties and molecular weights of SP proteins predominate is different in low and high fertile bulls (Moura et al., 2006 a, b). Osteopontin is an acidic glycoprotein of about 41.5 kDa, separated from the bone of different species (rat, human and bovine bone) and contains amino acids (aspartic, glutamic and serine) and monosaccharides (Butler, 1989). Bull fertility rate is related to contents of 55 kDa osteopontin (Cancel et al., 1998; Moura et al., 2006b).

Even though, many studies proved a correlation between seminal plasma proteins and fertility of the male in different species of domestic animals such as buffalo-bulls (El-Shamaa et al., 2016), bovine bulls (Asadpour et al., 2007), rams (Almadaly et al., 2016) and goats (Villemure et al., 2003). However, little information is available regarding the relationship of blood plasma proteins with semen characteristics and fertility in Egyptian buffalo bulls.

Therefore, this study aimed to evaluate the relationship of the seminal plasma proteins with blood proteins of Egyptian buffalo bulls with different semen qualities which can be used as a selection indicator for male fertility. Also, the relationship between protein profile in BP of adult bulls and bull calves were studied.

MATERIALS AND METHODS

The experimental work was conducted at Mahlt Mouse Animal Production Station, belonging to Animal Production Research Institute (APRI), Agricultural Research Center, Egypt.

This experiment was conducted in accordance with the Directive 2010/63/EU for animal protection that used for scientific purposes (Official Journal of the European Union, 2010). All effort has been made to reduce animal suffering.

Animals

A total of ten healthy Egyptian buffalo-bulls weighing 400±37.5 kg and 24-25 months old were divided into high and low fertile bulls (5 bulls in each) based on semen quality (Abdel-Khalek et al., 2001; El-Shamaa et al., 2016). Also, 3 buffalo-bull calves weighing 128.33±7.58 kg and ageing 6 mo. were used in this study. The experimental animals were kept under the same conditions of housing (individually in semi-open sheds), environmental and managerial conditions.

Feeding system

The experimental animals received an individual diet containing concentrate feed mixture (CFM), berseem hay (BH) and rice straw (RS) according to Kearl (1982). Feeds were offered at 7 a.m. and 4 p.m. Drinking water was offered all daytime. Ingredients and chemical analyses of different feedstuffs are presented in Table 1.

Semen collection and evaluation

Semen was collected once a week from 5 high fertile and 5 low fertile bulls using an artificial vagina (IMV, France) for five weeks (25 ejaculates/group). The collected semen of each group was individually placed in a water bath (37°C) and then taken immediately to the laboratory for evaluation of fresh semen. During the collection period, the reaction time (RT) was recorded in terms of time elapsed (second) from exposing each bull to a teaser up to complete ejaculation.

Ejaculate volume was measured in milliliters in a graduated collection glass tube. Percentages of progressive motility, livability, abnormality, and acrosomal status of sperm cells were performed in fresh semen according to Amann and Hammerstedt (1980), Hackett and Macpherson (1965), Blom (1983), and Yanagimachi (1982), respectively.

The integrity of the sperm membrane was determined by hypo-osmotic swelling test (HOS-t, 50 mOsm/l for 30 minutes) in terms of percentage of spermatozoa with coiled tail in microscopic field of 200 sperm cells, the percentage of response to HOS-t was calculated according to El-Sherbieny (2004). Sperm cell concentration (SCC $\times 10^9$/ml) was determined by Haemocytometer (Khan, 1994) to calculate total sperm output (TSO) per ejaculate. TSO = Ejaculate volume (ml) × SCC/ ml.

After evaluation, the semen sample was centrifuged at

Table 1: Chemical analysis of CFM, BH and RS in the basal diet of the experimental bulls.

Item	DM	Chemical analysis (%, on DM basis)					
		OM	CP	EE	CF	NFE	ASH
Concentrate feed mixture	91.50	88.74	15.85	4.71	13.66	54.53	11.36
Rice straw	92.30	79.63	3.47	1.41	35.10	39.65	20.37
Berseem hay	89.00	85.96	15.96	2.92	28.20	38.88	14.04

DM= Dry matter, OM= Organic matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber, NFE= Nitrogen free extract.

5000 rpm for 10 minutes to separate seminal plasma (Dixit et al., 2016) for determining the contents of proteins.

Blood samples

Blood samples were taken before morning feeding from high-fertile bulls (n=3), low-fertile bulls (n=3), and bull calves (n=3). Blood samples were aspirated from the jugular vein into tubes with anticoagulant (EDTA) and kept at 4°C. Blood plasma was separated from the whole blood by centrifugation (3000 rpm for 15 min), stored at -20°C until performing the analysis (Windusari et al., 2017).

SDS-polyacrylamide gel electrophoresis (SDSPAGE)

Blood or seminal plasma samples were taken from three animals in high and low fertile bulls were subjected to SDSPAGE analysis. Each seminal or blood plasma sample was used in duplicate. Fresh samples of seminal plasma or blood plasma of the same bull were centrifuged (1500 *g* for 15 min), conditioned in cryotubes, and stored (–20°C) until further processing. In a sample of seminal or blood plasma (200 µL), proteins were extracted in 2 mL of extraction buffer (0.625 M Tris-HCl, pH 6.8, 2% SDS, 5% β-mercaptoethanol and 20% of glycerol). Quantification and electrophoresis of proteins were performed according to the methods of Bradford (1976) and Laemmli (1970), respectively. Briefly, gels were fixed with isopropanol: acetic acid: water (4:1:5 v/v) for 30 min, and stained in the same solution containing Comassie Blue R250 (2%). A spectrophotometer PF-901 (Chemistry Analyzer Lab-systems) was used for determining the protein concentration. To a photo documentation system (Bio Doc-IT and Visidoc-IT Gel Documentation systems, UVP), gels were submitted and analyzed by Doc-IT-LS 6.0 software.

Statistical analysis and image analysis

T-test of IBM SPSS (2017) statistical program version 25 was used to set the differences in semen characteristics between high and low fertile bulls. Before the statistical analysis, all percentage values were transformed to arcsine values, then the actual values were tabulated as mean ± SE with its probability (P-value). Gel images were analyzed to determine molecular weight and relative protein content using the Gel doc system. Personal correlation coefficient was estimated within the SPSS program.

RESULTS

Reaction time, semen traits and sperm output of buffalo-bulls

Results presented in Table 2 showed that ejaculate semen volume did not differ significantly in high and low fertile buffalo bulls. However, the sexual desire in terms of reaction time and all semen-quality parameters of buffalo-bulls were significantly higher (P<0.001) in high fertile than in low fertile buffalo-bulls.

Protein profile in plasma of blood and semen of buffalo-bulls

Protein molecular weights in blood plasma (BP) and seminal plasma (SP) of high and low fertile bulls were identified by SDS PAGE, being between 6.5 and 270 kDa (Tables 3 and 4). In this study, more than 57 protein fractions were detected in BP and SP of high and low fertile bulls with molecular masses ranging from 5 to 316 kDa. The protein bands of 35, 49, 36, 60, 67 and 76 kDa were commonly observed in both BP and SP of high and low fertile bulls, protein bands of 35 and 67 kDa were predominant in all samples with a high percentage (Tables 3 and 4). The protein bands of 5, 31, 52, 62, 71 and 175 kDa were commonly observed in BP and SP of high fertile buffalo bulls (Table 3). Each of 16, 18, 48 and 162 kDa-proteins was commonly assayed in both BP and SP of low fertile bulls (Table 4). In high fertile bulls (Table 3) protein bands molecular weights of 259, 249, 189, 182, 154, 127, 95, 64, 58, 51, 37, 30, 28, 14, 13 and 6.5 kDa were found only in BP, while 19, 33, 63, 70, 136, 143 and 221 kDa-proteins were found in SP. In low fertile bulls (Table 4), 204, 196, 65, 34, 25, 23 and 9 kDa were found in BP, while those of 61, 98, 147, 230 and 316 kDa-proteins were presented in SP.

Protein profile in plasma of blood and semen of buffalo-bull calves

In bull calves (Table 5), protein bands of 35 and 36 kDa were observed in BP which were observed also in the BP of high and low fertile bulls. On the other hand, protein bands of 30, 37, 63, 64 and 175 kDa in BP of high fertile bulls, and those of 16, 34 and 316 kDa in BP of low fertile bulls were detected in BP of bull calves.

Table 2: Sexual desire and semen parameters of high and low fertile buffalo-bulls.

Semen trait	High fertile bulls	Low fertile bulls	P-Value
Reaction time (second)	57.96±1.47	105.80±1.99	0.000***
Ejaculate volume (ml)	3.39±0.21	3.18±0.17	0.434NS
Progressive sperm motility (%)	82.92±0.95	60.56±0.67	0.000***
Live sperm (%)	84.96±0.67	62.20±0.83	0.000***
Abnormal sperm (%)	12.68±0.59	25.60±0.60	0.000***
Acrosomal damage (%)	16.08±0.62	25.32±0.69	0.000***
Membrane integrity (%)	76.08±1.48	60.16±0.62	0.000***
Sperm cell concentration (x10^9/ml)	1.584±0.09	0.710±0.07	0.000***
Total sperm output (x10^9/ejaculate)	5.512±0.51	2.362±0.30	0.000***

NS: Non-significant, *** Significant at P<0.001.

Table 3: Molecular weight and abundance (%) of proteins in the blood and seminal plasma of high fertile bulls.

Protein marker MW	High fertility (Blood)						High fertility (Seminal)					
	Lane 1		Lane 2		Lane 3		Lane 1		Lane 2		Lane 3	
	MW	Ab%	MW	Ab%	MW	Ab%	MW	Ab%	MW	Ab%	MW	Ab%
270	270	4	249	10	259	6	292	5	221	8	292	14
175	175	6	182	8	189	6	213	8	166	7	175	6
130	130	8	127	4	154	5	158	7	136	8	143	6
95	95	7	76	28	71	27	130	6	70	32	71	29
66	66	10	64	12	62	10	67	29	62	13	63	11
52	71	21	58	6	49	5	60	11	35	25	52	6
37	52	9	51	8	35	10	49	6	33	4	36	14
30	37	13	36	11	31	4	35	14	19	6	33	12
16	30	10	28	4	13	3	31	3	5	3	-	-
6.5	6.5	6	14	4	5	8	19	7	-	-	-	-
-	-	-	-	-	0.36	10	-	-	-	-	-	-

MW: Molecular weight (kDa). Ab: Abundance.

Table 4: Molecular weight and abundance (%) of proteins in the blood and seminal plasma of low fertile bulls.

Protein marker MW	Low fertility (Blood)						Low fertility (Seminal)					
	Lane 1		Lane 2		Lane 3		Lane 1		Lane 2		Lane 3	
	MW	Ab%	MW	Ab%	MW	Ab%	MW	Ab%	MW	Ab%	MW	Ab%
270	270	5	270	5	270	6	316	6	316	8	292	5
175	196	7	204	7	213	8	213	3	147	17	230	9
130	162	12	158	5	166	6	162	5	66	35	147	2
95	67	40	130	5	65	35	130	6	61	13	98	21
66	48	7	65	25	60	9	67	36	35	15	76	13
52	34	15	60	8	49	7	48	3	18	8	67	22
37	25	1	48	6	35	20	35	8	-	-	49	12
30	16	1.7	34	16	18	3	16	15	-	-	36	12
16	9	2	23	3	-	-	0.36	11	-	-	-	-
6.5	2	5	16	1	-	-	-	-	-	-	-	-
-	-	-	1.9	8	-	-	-	-	-	-	-	-

MW: Molecular weight (kDa). Ab: Abundance.

Table 5: Molecular weight and abundance (%) of proteins in the blood plasma of bull calves.

Protein marker MW	Bull calves (Blood)					
	Lane 1		Lane 2		Lane 3	
	MW	Ab%	MW	Ab%	MW	Ab%
270	304	9	304	12	316	14
175	116	6	175	4	171	5
130	75	19	118	7	120	8
95	64	10	78	22	80	23
66	55	7	64	13	63	13
52	36	10	37	11	57	7
37	33	2	16	30	35	9
30	16	21	-	-	30	5
16	3.6	3	-	-	16	14
6.5	0.88	7	-	-	-	-

MW: Molecular weight (kDa). Ab: Abundance.

DISCUSSION

The current study aimed to determine the possible relationship between blood plasma (BP) proteins and seminal plasma (SP) proteins in low and high fertile buffalo bulls. Also, the relationship between protein profile in BP of adult bulls and bull calves were studied.

Semen quality is the main factor affecting male fertility in buffalo bulls (El-Sheshtawy et al., 2008). The composition of buffalo semen plays an important role in sperm membrane stability and subsequent fertility (El-Harairy et al., 2005). Also, Hafez and Hafez (2000) classified males into fertile and sub-fertile based on semen quality parameters. In accordance with the results of Abdel-Khalek et al. (2001) and El-Shamaa et al. (2016), the results showed that the sperm motility percentage and sperm cell concentration were higher in high than in low fertile buffalo bulls. The present results showed protein with molecular weight of 3 to 73 kDa in high fertile bulls, and those with molecular weight from 7 to 53 kDa in low fertile bulls (Nauk and Manjunath, 2000). However, protein bands ranged from 1 to 67 kDa in SP of high and low fertile buffalo bulls. Protein bands ranging between 3 and 214 kDa were observed only in SP of bulls with high fertility. Meanwhile, protein bands were 7-219 kDa molecular weights in low fertile buffalo bulls (El-Shamaa et al., 2016). Based on SDSPAGE analysis as illustrated in Figure 1, the molecular weights of 71 kDa-proteins were predominant in the BP and SP of buffalo-bulls characterized by high fertility, while this feature was nadir in BP and SP of low fertile bulls. In South Indian Jersey and Hybrid bulls, Vickram et al. (2016) found that the content of 72.45 kDa-protein in SP positively correlated with sperm cell concentration. Incomparable with our results, Arangasamy et al. (2005) detected about 18 molecular weights of protein bands (12 -127 kDa) with the majority of protein bands of <25 kDa in SP of buffaloes and protein bands of 80.5 kDa as the highest molecular weight proteins. Also, 14.4 kDa (3 protein fractions), 24.5 kDa (5 protein bands) and 35.5 kDa (7 bands).

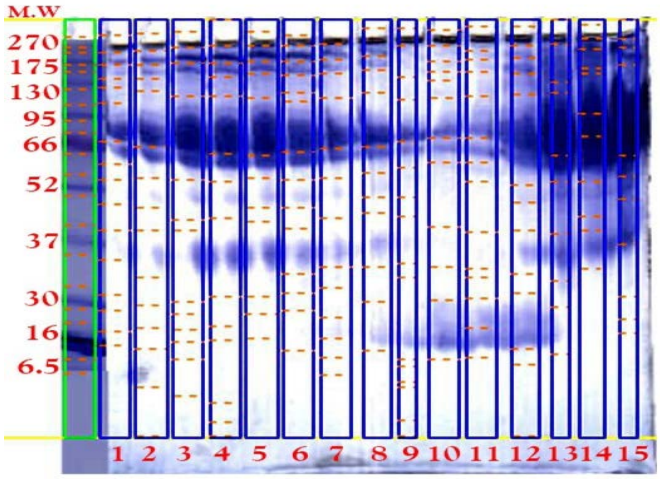

Figure 1: SDSPAGE analysis showing the protein bands in the blood plasma of high fertile (1-3), blood plasma of low fertile (4-6), seminal plasma of high fertile (7-9), blood plasma of bull calves (10-12), and seminal plasma of low fertile buffalo-bulls (13-15).

The present results showed that 52 and 62 kDa-protein was found in BP and SP of high fertile buffalo-bulls only. This protein (55 kDa) was correlated (P<0.012) with the percentage of sperm viability in fresh semen of high fertile buffalo bulls (Asadpour et al., 2007). Also, 55 kDa-proteins determined by osteopontin, was reported to be more prevalent in SP of Holstein bulls with high fertility (Cancel et al., 1998). Moreover, Moura et al. (2006b) found that 55 kDa-proteins in SP of high-fertile bulls were higher by about 2.3 and ≥4 times more than in above- and below-av-

erage fertile bulls, respectively. They added that a secreted form of phospholipase (58 kDa) presented in the fluid of the accessory gland was more dominant in high-fertile bulls. In *Bos taurus indicus* bulls, 55 and 66 kDa-peptides were observed in SP of animals with excellent sperm motility and vigour, while proteins of 16 and 36 kDa were present in SP of bulls characterized by poor semen parameters (Chacur et al., 2011).

Fractionation based on SDS-polyacrylamide gel in our study indicated that 33 and 19 kDa-proteins were found in SP, while two molecular weights 28 and 30 kDa-proteins were found in BP of high-fertile buffalo-bulls. In this context, Chacur (2012) found that 30 kDa-proteins in bovine SP make up 40–50% of the total proteins in domestic bulls; these proteins are present in products of the seminal vesicles and ampullae. In this way, the presence of 31 kDa-proteins in the SP of the bull may indicate high fertility of this bull (Ramteke et al., 2014). Also, 24.5 kDa-proteins were significantly higher in bovine (Jobim et al., 2004) and buffaloes (Asadpour et al., 2007) bulls with high fertility. Moreover, Manjunath and Therien (2002) reported that 26 and 55 kDa-proteins were predominating in high fertile bulls.

Our results revealed that BP and SP protein profiles in the buffalo of low fertility have 48, 18 and 16 kDa-proteins, which were not detected in high fertility. In accordance with these findings, Vickram et al. (2016) reported a similar result in SP of South Indian Jersey and Hybrid bulls with low fertility. These findings indicated the association of 71 and 52 kDa-proteins presence in BP and SP, proteins of 33 and 19 kDa presences only in SP, and 28 and 30 kDa-proteins presence only in BP with increasing semen quality in buffalo bulls. Incomparable with our results, El-Shamaa et al. (2016) reported that predominant (60-70% of the bands) of 65 and 54-59 kDa-proteins in high fertile bulls, and of 58 and 45-49 kDa-proteins (60 and 80 % of the bands) in low fertile ones. The correlation between the molecular weight of seminal plasma proteins and fertility was attributed to the coating SP proteins on the external surface of sperm cells after semen collection. Proteins of SP may play a vital role in the modification of sperm membrane during capacitation. In bovine, SP proteins determination on the sperm membrane may be taken as an indicator of individual fertilizing ability of bulls or membrane integrity of cryopreserved spermatozoa (Nauc and Manjunath, 2000). The current study indicated a highly significant correlation between protein profile in BP and SP ($r= 0.991$, $P<0.000$, unshown result) indicating a nearly closed relationship between proteins in BP and SP.

In addition, when protein bands in BP were compared in adult bulls (Tables 3 and 4) and bull calves (Table 5), proteins with 35 and 36 kDa were observed in BP in high and low fertile bulls, 30, 37, 63, 64 and 175 kDa only in high fertile, and 16, 34 and 316 kDa only in low fertile bulls (Figure 1). Unfortunately, there are no results in the literature on the blood protein profile in bull calves. Therefore, such results may suggest a relationship between protein profile in BP of adult buffalo-bulls and buffalo-bull calves (6 months of age).

CONCLUSION

According to the relationship between proteins profile in blood and seminal plasma of buffalo-bulls in reference with male fertility, determination of the molecular weight of proteins in blood plasma may be considered as a strategy for early selection of semen quality for buffalo-bulls used for natural services or artificial insemination. The relationship of blood plasma protein-profile between adult buffalo-bulls and buffalo-bull calves may suggest an early prediction of semen quality in bull calves in future studies.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

NOVELTY STATEMENT

According to the blood plasma protein-profile relationship between adult buffalo-bulls and buffalo-bull calves, it may be used as an early prediction of semen quality in bull calves.

AUTHORS CONTRIBUTIONS

All authors were contributed to design the experimental work. Sakr, A.M. and Wafa, W.M. were conducted the experimental procedures and collected data. El-Nagar, H.A. and Badwy, M.I. were performed the sample preparations for laboratory analysis. El-Nagar, H.A. conducted the statistical analyses. El-Nagar, H.A. and Wafa, W.M. were drafted the manuscript reviewed the draft paper and delivered recommendations.

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