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Seminal Plasma Proteins as Biomarkers of Fertility and Semen Traits in Egyptian Barki Rams

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Abstract

The objective of this study was to identify seminal plasma proteins associated with semen traits (mass motility (MM; %), live sperm (LS; %), abnormal sperm (AS; %), hypo-osmotic swelling test (Host; %), pH, colour, viscosity (Visco), ejaculate volume (VOL; ml), normal chromatin (NC; %), live sperm with reacted acrosome (LR; %), live sperm with unreacted acrosome (LU; %), dead sperm with reacted acrosome (DR; %), dead sperm with unreacted acrosome (DU; %)) in Egyptian Barki rams. Depending on MM%, the animals were divided into three different groups (high (≥ 80% MM), medium (50-80% MM) and low-fertility (≤50% MM) group). The seminal plasma proteins were separated using one-dimensional sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) electrophoresis technique based on their molecular weight. The actual means for MM, LS, AS, Host, pH, colour, Visco, VOL, NC, LR, LU, DR and DU were 69.93%, 94.40%, 6.76%, 20.14%, 6.94, 3.30, 3.04, 0.75 mL, 53.52%, 44%, 15.78%, 19.36% and 20.86%, respectively. The correlation coefficients between MM and each of LS (0.61) and NC (0.89) were highly positive and significant ($P<0.05$). Semen samples displayed a total of 8 distinct protein bands in the SDS-PAGE of seminal plasma proteins. The identified protein bands ranged in molecular weight from 14 to 248 kDa. The high and medium fertility ram groups showed six of the bands. However, all eight protein bands were seen in the group of low-fertility rams. It is notable that the two bands solely identified in this group are those with molecular weights of 29 and 35 kDa. Negative and high correlations ($P<0.05$) were observed between these two bands and each of MM and LS, these correlations were -0.87 and -0.36 for the 29 kDa band and were -0.62 and -0.56 for the 35 kDa band, respectively. The reduced motility and low fertility of the rams in this group may be caused by these two bands of protein. In contrast, there were positive and significant correlation between the bands of 14 kDa, 25 kDa, and 248 kDa and MM, LS, NC, and LR ($P<0.05$). Relevant correlations between mass motility and other seminal characteristics were found in Barki rams. Potential seminal plasma proteins have identified as biomarkers for sperm mass motility and other related seminal and fertility associated traits.

Keywords: Sheep, semen, seminal plasma proteins, fertility, SDS-PAGE.

Introduction

Sheep make up around 6.4% of Egypt's total red meat production (Abousoliman et al., 2020). Ossimi, Rahmani, and Barki are the three most prevalent indigenous sheep breeds in Egypt (Galal et al., 2005). Barki sheep is medium size and light colour desert breed well adapted to survival in the hot arid environment and can produce a considerable amount of meat, wool, and milk in these conditions (El-Wakil, et al., 2008). Semen quality traits are the main factors that limit the male reproductive efficiency. Due to the fact that each male can be mated with a large number of females, so, the fertility of ruminant male is more important than the female fertility in the breeding programs (Oliveira et al., 2012). Moreover, prediction of ram fertility is critical in order to decrease conception failures caused by low fertility rams. Ram fertility has an influence on flock performance and reproductive efficiency, using highly fertile rams is crucial for increasing sheep production (Fathy et al., 2021). Seminal plasma is a complex mixture of fluids from the testicle, epididymis, and accessory sex glands that includes components that affect sperm fertilization ability (Somashekar et al., 2015). Both the male and female reproductive characteristics of animals are significantly influenced by the complicated seminal plasma composition (Fuentes-Albero et al., 2021). In many species, seminal plasma proteins have been identified as fertility indicators (Yue et al., 2009; Kadoom et al., 2016). Seminal plasma has proteins and peptides that play a role in the control of the fertilization process, notably through their capacity to bind diverse types of ligands including polysaccharides, lipids, and ions (Mogielnicka-Brzozowska and Kordan, 2011). Through the multifunctional effects of organic and inorganic components, seminal plasma may both inhibit and stimulate sperm activity, motility and fertility (Maxwell et al., (2007); Leahy et al., (2019)). Seminal plasma also protects sperm from peroxidative damage and inhibits early sperm capacitation (Schöneck et al., 1996). It also affects the control of acrosome response (Cross, 1993), sperm Ca^2 uptake (Clark et al., 1993) sperm membrane protection and antimicrobial activity (Moura et al., 2007). Seminal plasma proteins have also been reported to be associated with sperm motility (Yoshida et al., 2008) and directly related to

the male fertility (Oliveira et al., 2012). These proteins may have either negative (Iwamoto et al., 1995; Schöneck et al., 1996; La Falci et al., 2002) or positive (Somlev et al., 1996; Qu et al., 2007) effects on sperm motility. Bull fertility was shown to be correlated with four seminal plasma proteins (Killian et al., 1993). Two of these proteins, 26 kDa and 55 kDa were more prevalent in high-fertility bulls, while 16 kDa and 6.7 kDa were more frequent in low-fertility bulls. In the Egyptian sheep, Abdel-Mageed and Dessouki (2018) comparing Ossimi to Ossimi crossbred and Assaf breeds, found that the relative concentration of the 150-159, 40, and 29 KDa protein bands was significantly lower in Ossimi (0.33). Additionally, they suggested that these protein bands may represent protein types that are associated with ram fertility and the considerable decrease in sperm parameters and concentration of Ossimi rams compared to Ossimi crossbred and Assaf breeds may be explained by the relative high protein content of 54-57 and 47 KDa protein bands in Ossimi breed. The studies concerning the relationship between seminal plasma proteins and semen quality traits in sheep scarce. Thus, the aim of this work was to search for seminal plasma proteins as biomarkers associated with semen traits and fertility in the Egyptian Barki rams.

Materials and Methods

Animals

Twelve mature and healthy Barki rams were randomly selected for this study during the period between September and November 2020 from the sheep herd at the Experimental Station of the Animal Production Department, Faculty of Agriculture, Menoufia University, Shibin El-Kom. The experimental animals aged between 2 and 3 years, were kept indoor under natural ventilation and artificial lighting and fed *ad libitum* on a balanced ration to conform to rams' requirements according to the National Research Council (NRC, 2001). Throughout the duration of the experiment, fresh water and salt blocks were freely available.

Semen collection and evaluation

Prior to the start of the experiment, semen from the intended rams was collected and discarded for two weeks. Semen was collected twice weekly at 8 AM from the experimental rams utilizing an electroejaculator with a ram rectal probe (Blackshaw, 1954). The ejaculate volume (mL) was directly measured in milliliters to the closest 0.1 ml using a graduated glass tube. Collected semen samples were immediately transferred in a water bath (37°C) to the laboratory for further semen evaluation. Semen mass motility was calculated using the proportion of spermatozoa wave motion in a drop of semen placed on a glass slide (%). The live sperm (%), abnormal sperm (%) were measured according to the procedure adopted by Blom (1983) and Barbas and Mascarenhas (2009). Each semen sample was assessed for colour and appearance, viscosity, pH immediately after collection. The ejaculates with abnormal colors (in case of the presence of blood or urine) were discarded. The acrosome reaction and spermatozoa response to the hypo-osmotic swelling test HOST were also assessed as described by Mosaferi et al. (2005). Mass sperm motility in sheep is a reliable indicator of fertility (David et al., 2015). So, semen samples were characterized and grouped into three categories based on the percentage of mass motility as follow:

Group A (High-fertility rams):	Only ejaculates with ≥ 80% mass motility
Group B (Medium-fertility rams):	Only ejaculates between 50-80% mass motility
Group C (Low-fertility rams):	Only ejaculates with ≤ 50% mass motility

Ejaculates from each group (A, B, and C) were collected and each semen sample was centrifuged for 15 minutes at 3000 rpm. Until analysis, the supernatants (seminal plasma) were kept in glass vials and preserved at a temperature of -20 °C. The hyaluronidase enzyme's activity was assessed, the integrity of the sperm chromatin was determined, and the seminal plasma proteins were also identified.

Assessing the spermatozoa's chromatin integrity

According to Liu and Baker (1994) methodology, the chromatin integrity of sperm was evaluated using the Acridine Orange test (AOT) as follows: The sperm pellet from each group was centrifuged before being resuspended in 0.5 mL of phosphate buffered saline. The sperm solution was then glass smeared into a tiny aliquot (50 µL). Each sample was divided into three smears on glass slides, which were then air dried and fixed with Carnoy's solution (3 methanol: 1 acetic acid) overnight. The slides were cleaned, air dried, and then stained for 5 minutes with freshly prepared acridine orange (AO) stain as follows: To a combination of 40 ml of 0.1 M citric acid and 2.5 ml of 0.3 M Na2HPO47H2O, 10 ml of 1% AO in distilled water was added. For four weeks, the AO solution was kept at 4°C in a dark location. The slides were cleaned, dried, and then examined under a fluorescence microscope (Leitz, Germany; excitation of 450-490 nm). Sperm that had intact chromatin or normal DNA content fluoresced in the colour green, whereas sperm that had damaged or abnormal DNA content fluoresced in a spectrum ranging from yellow-green to red.

Measurement of hyaluronidase activity

The activity of hyaluronidase is measured turbidometrically according to Pukrittayakamee et al. (1988). The assay mixture consisted of 0.5 ml 0.2 M acetate buffer, pH 5.5 containing 0.15 M NaCl, 50 µg hyaluronic acid and Sperm. It was incubated for fifteen minutes at 37°C then stopped by adding 1 ml of 2.5% cetyltrimethylammonium bromide (CTAB)

in 2% NaOH and absorbance was read at 400 nm. The quantity of hyaluronidase required to hydrolyze 50% of the hyaluronic acid was referred to as turbidity reduction units. One unit of hyaluronidase was required to achieve a 50% decrease in turbidity.

Hyaluronidase activity staining on polyacrylamide gel

Samples were run on a 7% native PAGE gel that co-polymerized with 0.17 mg/ml hyaluronic acid (Smith, 2013). After electrophoresis, the gel was incubated for three hours at 37°C in 20 mM Tris-HCL buffer solution, pH 7.5, containing 150 mM NaCl, and then incubated overnight at 37°C in 100 mM sodium acetate buffer, pH 4.0. The gel was then stained for two hours at room temperature with 0.5% alcian blue in 3% acetic acid and immaculate in 70% acetic acid. The activity in the gel was identified by a lack of colour in the digested area against a backdrop of blue that is characteristic of undigested hyaluronic acid (Guntenhöner et al., 1992).

Identification of seminal plasma proteins

Using SDS-PAGE with 12% PAGE, which is frequently used to evaluate the proteins in complex extracts as described by Laemmli (1970), seminal plasma proteins were identified in supernatants of each group. The ability to identify proteins using Coomassie Brilliant Blue G-250 depended on the presence of Coomassie dye in both red and blue colour variants. On dye-protein binding, the red color was converted to blue. 0.5 mL of the protein reagent (Coomassie dye) was added to 0.5 mL dH2O and the protein sample. The absorbance was recorded at 595 nm against a blank control. Using bovine serum albumin (BSA) as a standard protein, a calibration curve was created (Bradford, 1976).

Statistical analysis

Prior to the analysis, all percentages and data on semen traits were transformed using the arcsine transformation after being checked for normality. Using the General Linear Model procedure (Proc GLM), the following statistical model was used to analyze the data using one-way analysis of variance: $Y_{ij} = \mu + G_i + e_{ij}$ where Y_{ij} is the observation; μ is the overall mean; G_i is the fixed effect of the rams' group (3 levels; High-fertility, Medium-fertility and Low-fertility group); and e_{ij} is the residual of the model. Duncan's Multiple Range Test (Duncan, 1955) evaluated the significance ($P < 0.05$) of the differences between rams' group means. Pearson's correlation test using CORR procedure was applied to determine the phenotypic correlation among semen quality traits and between these trait and seminal plasma proteins. Statistical analyses were performed by using SAS software (SAS, Version 9.1.3. SAS Institute Inc., Cary, NC, 2014).

Results and Discussion

Actual means and variation of semen traits

Actual means, standard deviations (SD) and coefficients of variation (CV%) for semen traits (mass motility (MM; %), live sperm (LS; %), abnormal sperm (AS; %), hypo-osmotic swelling test (Host; %), pH, colour, viscosity (Visco), ejaculate volume (VOL; ml), normal chromatin (NC; %), live sperm with reacted acrosome (LR; %), lives sperm with unreacted acrosome (LU) ; %, dead sperm with reacted acrosome (DR; %), dead sperm with unreacted acrosome (DU; %)) of Barki rams were presented in Table 1. The overall means for MM, LS, AS, Host, pH, colour, Visco, VOL, NC, LR, LU, DR and DU were 69.93%, 94.40%, 6.76%, 20.14%, 6.94, 3.30, 3.04, 0.75 mL, 53.52%, 44%, 15.78%, 19.36% and 20.86%, respectively.

Table 1. Actual mean, standard deviation (SD), minimum (Min), maximum (Max) and coefficient of variation (CV%) for semen traits in Egyptian Barki rams.

Trait	Mean	SD	Min	Max	CV%
Mass Motility	69.93	27.92	0.00	95.00	39.92
Live sperm	92.40	4.04	79.00	99.00	4.37
Abnormal sperm	6.76	4.13	1.00	21.00	61.07
Host	20.14	8.37	9.00	45.00	41.58
pH	6.94	0.29	5.90	7.50	4.16
Colour	3.30	1.21	1.00	5.00	36.73
Visco.	3.04	0.94	0.50	4.50	30.90
Vol.	0.75	0.68	0.10	3.00	89.75
NC	53.52	7.38	42.00	67.00	13.78
Category of acrosome reaction (%)					
LR	44.00	14.54	22.80	74.40	33.06
LU	15.78	10.82	0.90	55.10	68.56
DR	19.36	8.38	4.80	36.80	43.27
DU	20.86	11.54	3.20	43.70	55.33

Host: Hypo-osmotic swelling test, NC: normal chromatin, LR: Live sperm with intact (reacted) acrosome, LU: Live sperm with detached (unreacted) acrosome, DR: Dead sperm with intact (reacted) acrosome, DU: Dead sperm with detached (unreacted) acrosome.

These means were within the range reported by the previous studies for Egyptian sheep breeds (Mahmoud, 2013; Shamiah et al., 2015; Abdel-Khalek et al., 2018; Abdel-Mageed and Dessouki, 2018; Mansour, 2021). Shamiah et al. (2015) stated that means of VOL, MM, LS, AS, LR, LU, DR and DU were 0.79 mL, 81.88%, 90%, 2%, 30.38%, 45.90%, 6.56% and 17.13% in Rahmani rams, respectively and were 0.86 mL, 84.06%, 93.60%, 2%, 28.50%, 47.13%, 6.30% and 18.10% in Ossimi rams, respectively. Comparing Ossimi and Assaf sheep breeds raised in Egypt, Abdel-Mageed and Dessouki, (2018) reported that means of VOL, LS and AS were 1.50 mL, 70.90% and 9.80%, in Ossimi rams, respectively and were 1.75 mL, 89.87% and 6.97%, in Assaf rams, respectively. Comparing Barki and Rahmani breeds, Mansour (2021) revealed that means of VOL, MM, LS, AS in Barki rams were 0.68 mL, 68.36%, 81.55% and 15.83%, respectively, the same author added that Barki breed was higher than Rahmani breed in all the studied semen characteristics. The present results were superior than those of Abdel-Khalek et al. (2018) who stated that actual means of VOL, MM, LS and AS were 0.21 mL, 45.5%, 41.9% and 18.1% in Ossimi rams, respectively. In another Egyptian sheep breed named Sohagi, Solouma (2013) found that means of MM, LS, AS, LR were 80.20%, 60.92%, 11.52% and 58.92%, respectively.

The differences between the present means of semen traits and those reported by various researchers working on different sheep breeds could be attributed to differences in genetic make-up, reproductive and health status of rams, age of rams, frequency of collection, collection teamwork, nutrition, season and year of collection, and management conditions. The coefficients of variation (CV%) for the most common semen traits (MM, LS, AS and VOL) were low to high and ranged from 4.37 to 89.75%. The highest CV% for ejaculate volume in the present study (89.75%) represents the wide variation (Table 1) in semen volume between rams of the different groups as shown in Table 2.

Semen traits as affected by ram fertility

Table 2 represent the least-square means and their standard errors for semen and acrosome reaction traits as affected by the ram's fertility level. As the experimental rams were already categorized into three groups (High-fertility, Medium-fertility and Low-fertility group) depending on the mass motility, there were significant ($P<0.05$) differences between these groups for MM (Table 2). Relevant differences between the three groups for LS were observed with means of 93.94%, 91.35 and 87.45% in high, medium and low fertility group, respectively. This result is compatible with the highly positive correlation (0.61) between MM and LS observed in this study (Table 5). Moreover, these results are in accordance with those of David et al. (2015) who stated that seminal mass motility is a good sign and predictor of ram fertility. Means of the percentage of normal chromatin in the three ram groups were 62.00%, 53.00% and 45.57% for high, medium and low fertility groups with significant differences between these groups.

Table 2. Least-square means ± standard errors for semen traits in Egyptian Barki rams as affected by ram's fertility level.

Fertility level / Trait	High-fertility rams	Medium-fertility rams	Low-fertility rams
Mass Motility (%)	87.55^{a}±0.78	58.82^{b}±1.30	11.82^{c}±1.62
Live sperm (%)	93.94^{a}±0.34	91.35^{b}±0.57	87.45^{c}±0.71
Abnormal sperm (%)	5.68^{b}±0.39	9.76^{a}±0.65	6.72^{b}±0.81
Host (%)	20.08^{a}±1.17	19.00^{a}±1.99	21.44^{a}±1.99
pH	6.93^{a}±0.03	6.91^{a}±0.06	7.03^{a}±0.06
Colour	3.47^{a}±0.14	2.91^{a}±0.26	3.10^{a}±0.27
Viscosity	3.14^{a}±0.11	3.09^{a}±0.20	2.55^{b}±0.21
Vol. (mL)	0.87^{a}±0.09	0.63^{a}±0.16	0.57^{a}± 0.20
NC (%)	62.00^{a} ±0.65	53.00^{b}±0.70	45.57^{c}±0.78
Category of acrosome reaction (%)			
LR (%)	52.73^{a}±2.98	45.58^{a}±2.82	34.71^{b}±2.98
LU (%)	22.09^{a}±2.32	15.39^{b}±2.32	10.45^{b}±2.20
DR (%)	12.26^{c}±1.44	18.75^{b}±1.44	26.31^{a}±1.36
DU (%)	12.92^{c}±2.29	20.29^{b}±2.61	28.53^{a}±2.17

*Means within each row superscripted by different letters are significantly different (P<0.05), Host: Hypo-osmotic swelling test, NC: normal chromatin, LR: Live sperm with intact (reacted) acrosome, LU: Live sperm with detached (unreacted) acrosome, DR: Dead sperm with intact (reacted) acrosome, DU: Dead sperm with detached (unreacted) acrosome.

This result was consistent with the highly positive correlation (0.89) between MM and NC as shown in Table 5. In this regard, it has been established that sperm morphological abnormalities are related to chromatin integrity loss (Tavalaee et al., 2009), progressive motility and viability loss (Ozmen et al., 2007). The percentage of live-reacted spermatozoa (LR) for the three ram groups were 52.73%, 45.58% and 34.71% for high, medium and low fertility ram groups, respectively, with significant (*P<0.05*) differences between the two groups of high and low-fertility (Table 2). These values were somewhat higher than those reported by Shamiah et al. (2015) who stated that means of LR were 30.38% and 28.50% in Rahmani and Ossimi rams, respectively. The current findings support earlier research indicating that the acrosome reaction test is a reliable indicator of sperm activity and may be used to predict fertilization success (Maji et al., 2010). The hypo-osmotic swelling test (HOST) has shown to be an effective method for determining the integrity of the membrane surrounding the sperm of various domestic animals (Nalley and Arifiantini, 2013). Moreover, the sperm membrane's capacity to control the flow of electrolytes and non-electrolytes as well as the integrity of the sperm plasma membrane are assessed by HOST, whereas vital stains only assess the membrane's integrity and not its biochemical activity (Brito et al., 2003). The present values of HOST were 20.08, 19.00 and 21.44% for the high, medium and low-fertility group, respectively, however, the differences between the three fertility groups were not significant.

Table 3 show the total protein and hyaluronidase enzyme activity as affected by the rams' fertility group. The means of total protein were 0.98, 0.96 and 0.89 mg/ml of the seminal plasma for high, medium and low-fertility groups. Hyaluronidase enzyme in sufficient amounts is necessary for effective fertilization in order to strip the ovum of follicular cells and enable a spermatozoon to make contact with the ovum (Rowlands, 1944). Therefore, semen hyaluronidase activity may be a measure of the success of fertilization (Bozkurt et al., 2004). The present means of hyaluronidase enzyme activity for the high, medium and low-fertility groups were 64.08, 59.64 and 50.24 Unit/ ml, respectively.

Table 3. Least-square means ± standard errors for Total protein, Hyaluronidase activity and Hyaluronidase Specific activity in Egyptian Barki rams' seminal plasma as affected by ram's fertility level.

Fertility level Trait	High-fertility rams	Medium-fertility rams	Low-fertility rams
Total protein (mg/ml)	0.98^{a}±0.06	0.96^{a}±0.06	0.89^{b}±0.07
Hyaluronidase enzyme activity (Unit/ ml)	64.08^{a}±2.59	59.64^{a}±2.61	50.24^{b}±2.89

*Means within each row superscripted by different letters are significantly different (P<0.05).

Correlations between semen traits

The simple correlations between semen traits in Barki rams were shown in Table 5. The significant (*P<0.05*) correlations were between MM and each of LS, Visco, NC, LR, LU and DR. These correlations may explain the superiority of the high-fertility ram group in LS, NC and LR (Table 2), since the rams of this group had ≥80% MM. Therefore, MM could be used as a simple parameter easy to measure and highly correlated with most semen quality traits in Barki rams. Moreover, the relationship between MM and other seminal traits was assessed by previous studies, since MM is an important factor determining the semen quality and the ability to fertilize the oocyte (Geraci and Giudice, 2005). Almadaly et al. (2016) in Barki rams, reported that the correlations (*P<0.001*) between MM and each of LS and LR were 0.95 and 0.84, respectively and the correlation between LS and LR was 0.86. They added that the correlations (*P<0.001*) between Host and each of MM, LS and LR were 0.75, 0.78 and 0.68, respectively. Meanwhile, the associations between MM and each of AS, pH, DR, and DU were all negative (Table 5). Additionally, Braundmeier and Miller (2001) reported that sperm motility and morphological problems will decrease the number of sperm that can reach the oocyte, and they also noted that there is only a weak correlation between sperm chromatin structure, morphological issues that do not impair movement, and fertility.

Seminal plasma proteins

Semen samples displayed a total of 8 distinct protein bands in the SDS-PAGE of seminal plasma proteins (Figure 1 and Table 4). This number of bands is lower than those previously reported by Barrios et al. (2000) on Rasa aragonesa and Jobim et al. (2004) on Hamphsire Down, Corriedale × Texel and Abdel-Mageed and Dessouki (2018) on Ossimi, Ossimi crossbreds and Assaf breeds who recorded 20, 21 and 14 bands, respectively. In the present study, the molecular weights of the detected protein bands varied from 14 to 248 kDa. Six of the bands were observed in the high and medium fertility ram groups. However, the group of low-fertility rams displayed all the eight protein bands. It is noteworthy that the two bands exclusively determined in this group are those of 29 and 35 kDa molecular weight. These two bands could be types of proteins responsible for the low motility and fertility of the

rams belonged to this group. This result is consistent with the negative correlation between these two bands and each of MM and LS (Table 6). These two bands of protein can significantly decrease semen pH value (negative correlation; -0.31 and -0.39 for 29 and 35 kDa, respectively) are harmful to spermatozoa survival. Contrarily, the bands of 14 kDa, 25 kDa and 248 kDa were positively correlated (*P<0.05*) with MM, LS, NC and LR. Moreover, in another study on Suffolk rams Bergeron et al. (2005) reported that alcohol-precipitated ram seminal proteins were subjected to SDS-PAGE analysis, which revealed the presence of roughly 25 proteins with molecular weights ranging from 14 to 120 kDa, considering that a subset of proteins with molecular weights of 15–16 and 22–24 kDa was more prevalent. Yue et al. (2009) stated that a total of 15 protein bands, ranging in molecular weight from 15.13 to 116.20 kDa, were found in German merino rams and concluded that certain proteins found in ram seminal plasma are linked to fertility and semen characteristics. Differences in the molecular weight variation between the different studies may be attributed to season, breed, and seminal plasma collection and preparation techniques. Holstein dairy bulls were shown to have two seminal plasma proteins (16 and 16 kDa) associated with low fertility and two (26 and 55 kDa) associated with high fertility that were detected using two-dimensional gel electrophoresis (Cancel et al., 1997). In bulls, 26 kDa (Killian et al., 1993) and 55 kDa (Chacur et al., 2009) seminal plasma proteins have observed to be link to increased fertility.

Table 4. SDS-PAGE of seminal plasma protein polymorphism within the studied Egyptian Barki rams.

RF*	Protein molecular weight (kDa)	High-fertility rams	Medium-fertility rams	Low-fertility rams
0.029	248	1	1	1
0.059	180	1	1	1
0.249	58	1	1	1
0.308	50	1	1	1
0.423	35	0	0	1
0.528	29	0	0	1
0.670	25	1	1	1
0.884	14	1	1	1

*: Relative front of bands from 1 (B1) to 8 (B8), respectively, 0: absent, 1: present, kDa: Kilodalton

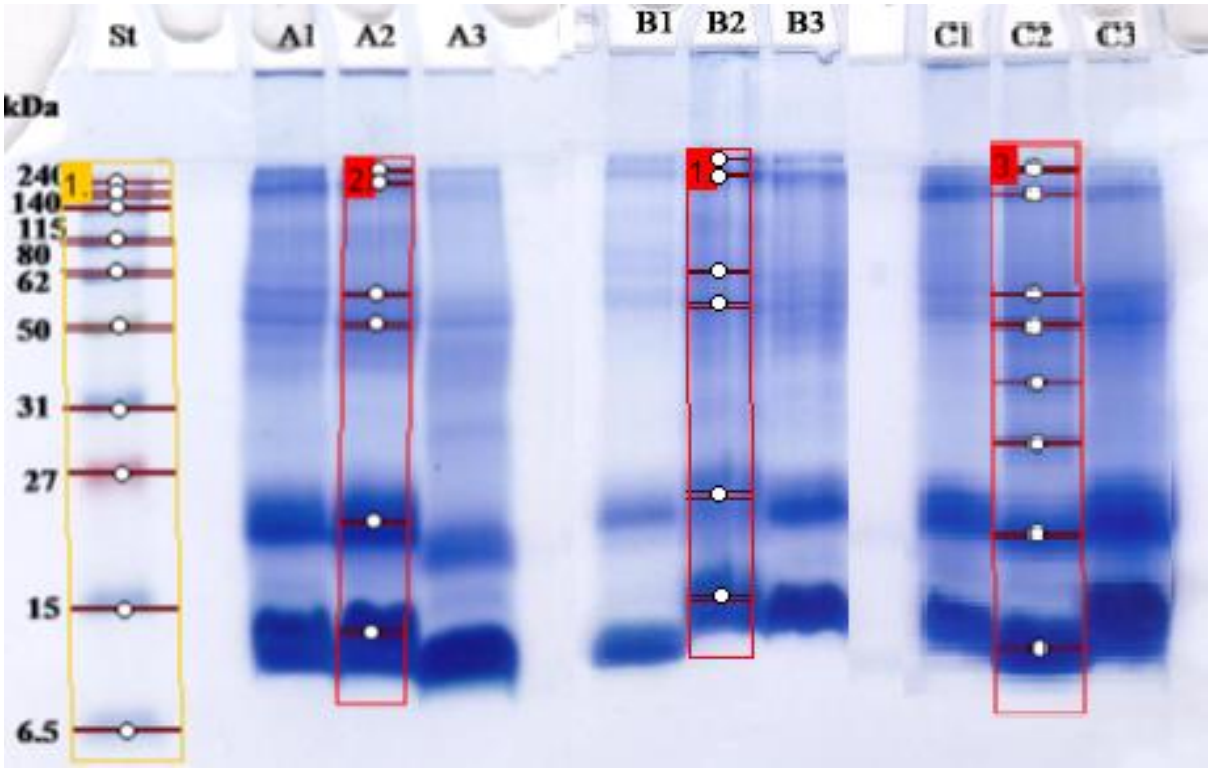

Figure 1. Polyacrylamide gel (SDS-PAGE) of the seminal plasma proteins in the Egyptian Barki rams (Group A: high-fertility ≥ 80% MM; Group B: medium-fertility 50-80% MM; Group C: low-fertility ≤ 50% MM).

Table 5. Pearson's correlation (r) coefficients between semen traits in Egyptian Barki rams.

	MM	LS	AS	Host	pH	Colour	Visco.	Vol.	NC	LR	LU	DR	DU
MM	1.00												
LS	0.61*	1.00											
AS	-0.20	-0.41*	1.00										
Host	0.01	0.20	-0.19	1.00									
pH	-0.11	-0.11	-0.05	-0.12	1.00								
Colour	0.18	0.22	-0.16	-0.14	-0.11	1.00							
Visco.	0.29*	0.26	-0.13	0.07	-0.18	0.59*	1.00						
Vol.	0.08	-0.18	0.17	-0.05	0.10	-0.14	-0.41*	1.00					
NC	0.89*	0.56*	-0.17	0.16	-0.44	0.29	0.23	0.28	1.00				
LR	0.29*	0.39	0.13	-0.24	-0.61*	-0.04	0.27	-0.55*	0.21	1.00			
LU	0.42*	0.31	-0.16	0.16	0.13	0.02	0.34	-0.05	0.45	-0.05	1.00		
DR	-0.69*	-0.42*	-0.03	-0.23	0.31	0.16	-0.16	-0.41	-0.70*	-0.25	-0.59*	1.00	
DU	-0.09	-0.32	-0.04	0.25	0.45*	-0.11	-0.52*	0.84*	0.12	-0.78*	-0.23	-0.03	1.00

MM: Mass Motility, LS: Live sperm, AS: Abnormal sperm, Host: Hypo-osmotic swelling test, NC: normal chromatin, LR: Live sperm with reacted acrosome, LU: Live sperm with unreacted acrosome, DR: Dead sperm with reacted acrosome, DU: Dead sperm with unreacted acrosome, * Significant correlation (*P<0.05*).

Table 6. *Pearson's correlation (r) coefficients between semen and acrosome reaction traits and seminal plasma proteins in Egyptian Barki rams.*

Protein molecular weight (kDa)	**MM**	**LS**	**AS**	**Host**	**pH**	**Colour**	**Visco.**	**Vol.**	**NC**	**LR**	**LU**	**DR**	**DU**
248	0.47*	0.66*	-0.22	0.51*	0.23	-0.01	0.19	-0.09	0.79*	0.52*	0.33	-0.16	-0.21
180	-.55*	-0.72*	0.19	-0.38*	-0.29	-0.05	-0.20	-0.11	-0.76*	-0.31	-0.28	0.59*	0.46*
58	-0.30	-0.12	-0.31	0.09	0.12	0.07	-0.13	-0.07	-0.44*	-0.21	-0.07	0.36*	0.28
50	-.82*	-0.47*	-0.05	0.08	0.14	-0.06	-0.21	-0.05	-0.79*	-0.32	-0.30	0.60*	0.47*
35	-.62*	-0.56*	0.15	-0.40*	-0.39*	-0.07	-0.21	0.13	-0.89*	-0.58*	-0.66*	0.63*	0.49*
29	-.87*	-0.36*	0.18	-0.54*	-0.31	-0.07	-0.18	0.17	-0.78*	-0.43*	-0.50*	0.58*	0.38*
25	0.20	0.33	-0.19	0.36*	0.19	0.08	0.21	0.09	0.57*	0.41*	0.33	-0.23	-0.18
14	0.83*	0.47*	-0.29	0.72*	0.14	0.06	0.21	0.11	0.84*	0.62*	0.30	-0.60*	-0.47*

MM: Mass Motility, LS: Live sperm, AS: Abnormal sperm, Host: Hypo-osmotic swelling test, NC: normal chromatin, LR: Live sperm with reacted acrosome, LU: Live sperm with unreacted acrosome, DR: Dead sperm with reacted acrosome, DU: Dead sperm with unreacted acrosome, kDa: Kilodalton, * Significant correlation (*P<0.05*).

Conclusions

Relevant associations were observed between mass motility and other seminal traits in Barki rams. Therefore, MM could be used as an ideal predictor for ram's fertility. Significant correlations exist between the majority of seminal plasma proteins and semen traits. Two protein bands of seminal plasma (29 and 35 kDa) were identified to be associated with low fertility. Further studies are needed to confirm the present findings and to identify specific proteins within the detected seminal protein bands directly related to spermatogenesis and fertility in rams. Moreover, to explore candidate genes codified and associated with these proteins.

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Conflict of Interests

The authors declare that they have no competing interests.

Animal Welfare Statement

This study was carried out according to the suggestions and guidelines of the Committee of Animal Care and Welfare, Menoufia University, Egypt.

Data Availability Statement

The datasets of the current study are available from the corresponding author upon reasonable request.

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بروتينات البلازما المنوية كواسمات حيوية للخصوبة وصفات السائل المنوي في كباش البرقي المصرية

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كان الهدف من هذه الدراسة تحديد بروتينات بلازما السائل المنوي المرتبطة بصفات السائل المنوي (الحركة الكلية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية الشاذة، إختبار Host، درجة الأس الهيدروجيني، اللون، اللزوجة، حجم القذفة، نسبة الحيوانات المنوية ذات الكروماتين الطبيعي، نسبة الحيوانات المنوية الحية ذات الأكروسوم المتفاعل السليم، نسبة الحيوانات المنوية الحية ذات الأكروسوم المنفصل الغير متفاعل، نسبة الحيوانات المنوية الميتة ذات الأكروسوم المتفاعل السليم، نسبة الحيوانات المنوية الميتة ذات الأكروسوم المنفصل الغير متفاعل) في كباش البرقي المصرية. تم تقسيم الحيوانات تبعاً لنسبة حركة الحيوانات المنوية الكلية إلى ثلاث مجموعات (مجموعة عالية الخصوبة ≤ 80% حركة كلية، مجموعة متوسطة الخصوبة 50–80% حركة كلية، مجموعة منخفضة الخصوبة ≥ 50% حركة كلية)، كما تم فصل بروتينات بلازما السائل المنوي على أساس الوزن الجزيئي بإستخدام تقنية الفصل الكهربي بإستخدام البولي أكريلميد جيل أحادي الأبعاد (SDS–PAGE). كانت المتوسطات الفعلية 69.93%، 94.40%، 6.76%، 20.14%، 6.94%، 3.30%، 3.04%، 0.75 ملل، 53.52%، 44%، 15.78%، 19.36%، 20.86% لصفات السائل المنوي السابق ذكرها على الترتيب. كانت معاملات الإرتباط بين نسبة الحركة الكلية للحيوانات المنوية وكلاً من نسبة الحيوانات المنوية الحية (0.61) ونسبة الحيوانات المنوية ذات الكروماتين الطبيعي (0.89) موجبة ومعنوية. أظهرت عينات السائل المنوي 8 حزم بروتينية مميزة في SDS–PAGE لبروتينات البلازما المنوية، وتراوحت حزم البروتين المحددة في الوزن الجزيئي من 14 إلى 248 كيلو دالتون. أظهرت مجموعات الكباش عالية ومتوسطة الخصوبة ستة من هذه الحزم البروتينية. ومع ذلك، شوهدت جميع الحزم البروتينية الثمانية في مجموعة الكباش منخفضة الخصوبة. من الجدير بالذكر أن الحزمتين الموجودين حصرياً فقط في هذه المجموعة والمميزين لها هما الحزمتين ذات الأوزان الجزيئية 29 و 35 كيلو دالتون. لوحظ وجود إرتباطات سالبة وعالية المعنوية بين هاتين الحزمتين وصفات نسبة الحركة الكلية للحيوانات المنوية ونسبة الحيوانات المنوية الحية. وكانت هذه الارتباطات −0.87 و −0.36 للحزمة 29 كيلو دالتون وكانت −0.62 و −0.56 للحزمة 35 كيلو دالتون، على التوالي. قد يكون سبب إنخفاض الحركة وإنخفاض الخصوبة للكباش في هذه المجموعة هو وجود هاتين الحزمتين من البروتين. في المقابل، كان هناك ارتباط معنوي وموجب بين الحزم البروتينية 14 كيلو دالتون و25 كيلو دالتون و248 كيلو دالتون وصفات الحركة الكلية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية ذات الكروماتين الطبيعي ونسبة الحيوانات المنوية الحية ذات الأكروسوم المتفاعل السليم. وجدت إرتباطات معنوية بين الحركة الكلية للحيوانات المنوية وصفات السائل المنوي الأخرى لكباش البرقي. تم تحديد بروتينات البلازما المنوية المحتملة كواسمات حيوية لحركة الحيوانات المنوية الكلية وغيرها من الصفات المرتبطة بالخصوبة وصفات السائل المنوي.