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CHARACTERISTICS OF SEMINAL PLASMA PROTEINS IN RELATION TO FERTILITY OF SHEEP UNDER EGYPTIAN CONDITIONS

I.I. Abdel-Mageed and SH.M. Dessouki

Animal Production Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt, E-mail: sherifd2002@agr.cu.edu.eg

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SUMMARY

This study aimed to investigate the relationship between the protein profile of seminal plasma and fertility rate of some subtropical sheep breeds .By using artificial vagina three ejaculates were collected from ten mature rams(Ossimi (3), crosses of Ossimi × Finnish Landrace and Suffolk (3), and Assaf (4) breeds).Semen characteristics e.g. (ejaculate volume, semen density, mass motility, individual motility (%), live sperm (%), abnormalities (%) and sperm concentration were determined after collection. Seminal plasma proteins were identified by one dimensional polyacrylamide gel electrophoresis (SDS-PAGE) method. Semen density and sperm concentration were significantly ($p<0.05$) higher in both Assaf and Crossbreds of Ossimi. Abnormal sperm percentage revealed a significant increase in Ossimi semen (9.80%) compared to its crossbreds and Assaf semen (7.83 and 6.97%, respectively).A total of 14 protein bands were visualized in ram seminal plasma samples. Protein bands 78-85 and 97-107 KDa were present in all studied breeds. However, the protein band of 40 KDa showed an increase in the percent of appearance in Ossimi crossbreds and Assaf (100%) compared to Ossimi breed (33%).It is clear that Assaf rams proved a potential capability for their breeding ability under Egyptian conditions. Therefore, it may be recommended to incorporate this breed in breeding programs of sheep in Egypt.

Keywords: rams, Ossimi, Assaf, semen, motility, protein, electrophoresis

INTRODUCTION

Sheep production has a distinguished position in the agricultural system in Egypt. Its population reached 5,450,000 head in 2013 and contributed to the local production of red meat in Egypt by about 7 % (FAO, 2013). Following selection and crossbreeding trials conducted in various countries, the Assaf provide a set of genotypes that are adapted to Middle East conditions and it can be tailored to a range of management conditions (Galal *et al*., 2008).

Male fertility in ruminants is much more important in reproductive programs than female fertility (Oliveira *et al*., 2012), as a result of its capability for mating a large number of females. This focuses to the importance of male rams on the breeding strategies of ruminants on the national level. One important element for selection of breeding rams for either natural mating or artificial insemination relies upon semen quality evaluation (Rodríguez-Martínez, 2003).Semen quality is very important factor affecting rams reproduction efficiency (Aller *et al.,*2012).

Seminal plasma is important to maintain spermatozoa motility, viability and fertility in bulls (Baas *et al*., 1983; Cross, 1993; Killian *et al*., 1993; Bellin *et al.*, 1996 and Jobim *et al*., 2004) and rams (Ashworth *et al.,* 1994; Graham, 1994 and Maxwell *et al.,* 1997) and for resisting cold shock damage (Pursel *et al*., 1973; Vischwanath and Shannon, 1997 and Barrios *et al.,* 2000). In addition, seminal plasma prevents premature capacitation of sperm (Eng and Oliphant, 1978) and protects sperm from peroxidative damage (Schoneck *et al*., 1996).In addition, it has also implications in the regulation of acrosome reaction (Cross, 1993; Florman and First 1988) and in the regulation of Ca^2 uptake by sperm (Clark *et al.,*1993).

Baas *et al.* (1983) and Jobim *et al.* (2004) concluded that seminal plasma has a deleterious effect on bovine spermatozoa during storage at ambient temperatures. However, it had no related effect on freezability. Decreasing sperm-bound bovine seminal plasma proteins (BSP) after cryopreservation; may indicate its' role during cryopreservation process (Nauc and Manjunath 2000).

Seminal plasma proteins of several species were separated, described and analyzed using Two-Dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE) (Killian *et al.,* 1993; Frazer *et al.,*1996; Mortarino *et al*., 1998 and Brandon *et al*., 1999). Protein composition of seminal plasma has important effects on sperm function and varies according to genotype (Cross, 1993; Mortarino *et al*., 1998; Villemure *et al*., 2003 and Boisvert *et al*., 2004).

Many seminal plasma proteins have been identified and characterized (Ayyagari *et al.,* 1987; Frazer *et al.*, 1996 and Moreau and Manjunath, 1999). Some of the seminal proteins were found to be associated with fertility in various species (Ayyagari *et al.,* 1987; Manjunath and Sairam, 1987 and Kraus *et al.,* 2001). Bovine seminal plasma (BSP) contains a family of major proteins, designated BSP-A1/-A2

and BSP-A3, with apparent molecular masses ranging from 15 to 17 kDa, and the BSP-30 kDa protein with molecular mass of 28–30 kDa, collectively called BSP proteins (Manjunath, 1984). The biological properties of BSP proteins have been extensively studied (Manjunath and Therien, 2002). However, there is a little information available regarding to buffalo seminal plasma proteins.

Four seminal plasma proteins were found to be associated with bull fertility (Killian *et al*., 1993). Two of these proteins, 26 kDa (p*I* 6.2) and 55 kDa (p*I* 4.5), were associated with high-fertility bulls, whereas the other two, 16 kDa (p*I* 4.1) and 6.7 kDa, respectively, were more frequent in low-fertility bulls. The 55 kDa (p*I* 4.5) bovine seminal plasma protein was identified as an Osteopontin (Cancel *et al*., 1997) and the 26 kDa (p*I* 6.2) protein as a lipocalin-type prostaglandin-D synthase (Gerena *et al*., 1998). Seminal plasma ribonuclease (14 kDa) was present in higher concentrations in bulls with poor post-thaw semen quality (Roncoletta *et al*., 2002).

Voglmayr *et al*. (1982) and Hammerstedt and Parks (1987) demonstrated that the changes in sperm membrane proteins and glycoproteins of rams during epididymal transit have been documented by SDS-PAGE studies however, the ovine seminal plasma proteins are still poorly understood and described in previous studies with SDS-PAGE. Barrios *et al.* (2000) mentioneda total of 20 bands in ovine seminal plasma. The objective of this work is to correlate between the seminal plasma protein and fertility rate of some subtropical sheep breeds.

MATERIALS AND METHODS

Ten rams, related to a commercial sheep flock, located in Delta region of north Egypt [Ossimi (3 rams), Ossimi crosses with Finnish Landrace and Suffolk (3 rams), and Assaf (4 rams)] were used in this study. Age of such rams ranged between 2 and 4 years. Ossimi ewes came in heat were introduced to the rams to be hand-mated twice, 12-hours apart. Animals were kept loose in semi-shade pens, where drinking water was made available all day time. Clover hay plus concentrate feed mixture (12% CP and 65 %TDN) were offered to animals according to their body weights (NRC, 1985).

Semen was collected from rams by using artificial vagina (three samples) each two weeks apart immediately before mating. Physical characteristics of ram semen were determined (ejaculate volume-semen density-mass motility-individual motility%–live%-sperm abnormalities%- sperm concentration) immediately after collection. Fresh semen samples were centrifuged at 5000 rpm for 10 minutes. The supernatants were transferred into glass vials and stored at -20°C until biochemical analysis. Seminal plasma proteins were recognized for each ramusing one dimensional polyacrylamide gel electrophoresis.

Samples of frozen seminal plasma (n=30) were used after thawing at room temperature to determine molecular weight of seminal plasma protein. For protein denaturation, 950 µ of sample buffer (3.55 ml deionized water, 1.25 ml 0.5 M Tris-Hcl, pH 6.8, 2.5 ml Glycerol and 2 ml 10 % (w/v) Sodium Dodecyl Sulphate SDS) was added to 50 µ of β-Mercaptoethanol. Fifteen u of seminal plasma samples were added to 2u of loading buffer (3.55 ml deionized water, 1.25 ml 0.5 M Tris-Hcl, pH 6.8, 2.5 ml Glycerol, 2 ml 10 % (w/v) SDS and 0.2 ml 0.5 % (W/V) Bromophenol blue) was prepared. Running buffer was prepared by using 30.3 gm Tris base, 144 gm Glycine and 10 gm SDS up to 1000 ml of deionized water.

The gel was prepared using 7.2 ml H_2O, 7.5 ml of 1.5 M Tris-HCl, pH 8.8, 0.15 ml 20% (w/v) SDS, 15.0 ml Acrylamide/Bis-acrylamide (30%/0.8% w/v), 0.15 ml of 10% (w/v) ammonium persulfate (APS) and 0.02 ml TEMED.

The gel was placed in Petri dish for staining with Comassie brilliant stain (40% methanol 20 ml , 10% acetic acid 5 ml, 0.025 % Comassie 0.012 gm and up to 50 ml of deionized water) and was shaken for 6 hours. The molecular weight of each band representing specific protein in each lane was determined using Uvidoc software (version 12.4, England).

For each ram, the mean of all three collections was estimated. Data of the semen evaluation were analyzed using the SAS GLM procedure (SAS, 2004). Fertility rate at lambing was analyzed using the chi-square CATMOD procedure. Duncan's multiple range test was used to detect differences among means. The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

Semen characteristics and fertility rate of the different breeds and crosses are shown in Table (1). The results showed insignificant increase in Assaf semen volume compared to Ossimi breed or Ossimi crosses. Many authors **(**Tabbaa *et al*., 2006; Mahmoud, 2013 and Miloud and Karima, 2015) recorded lower values of semen volume compared to those recorded in this study. Mahmoud (2013) recorded lower semen volume (1.1 ml) in Ossimi rams. Moreover, Tabbaa *et al*. (2006) on Awassi breed and Miloud and Karima (2015) on Ouled Djellal breed reported lower values for semen volume (1.12 - 1.21 ml). On the other hand, Assaf showed similar volume (1.75 ml) to that recorded by Olah *et al.* (2013) using Ile de France breed in autumn and spring, but it was lower than that recorded in winter (1.89 ml).

Semen density (0-3) and sperm concentration (10^9/ml) were high ($P<0.05$) in both Assaf and Ossimi crossbreds. Olah *et al* .(2013) reported lower value of Suffolk semen density (2.35) in summer compared to that recorded on Ossimi crossbreds in the present study (2.83). Moreover, sperm concentrations (2.10 – 3.40 x10^9/ml) (Table 1) were lower than the number (4.9 ×10^9/ml) recorded by

Miloud and Karima (2015). Also, Mahmoud (2013) on Ossimi breed, recorded higher concentration (2.9 $\times 10^9$/ml) than that recorded for Ossimi rams in the present study (2.1×10^9/ml).

Table 1. Semen characteristics and fertility rate of subtropical sheep as affected by breed of rams

Semen Traits	Breed		
	Ossimi	Ossimi crossbreds	Assaf
Volume(ml)	1.50±0.15	1.67±0.15	1.75±0.13
Semen Density (0-3)	1.83±0.15 [b]	2.83±0.15 [a]	2.87±0.13 [a]
Mass Motility (0-5)	1.67±0.19 [c]	3.17±0.19 [b]	4.75±0.17 [a]
Individual Motility (%)	60.00±2.81 [c]	80.00±2.81 [b]	91.25±2.44 [a]
Live Sperm (%)	70.90±1.40 [c]	81.67±1.40 [b]	89.87±1.21 [a]
Abnormal Sperm (%)	9.80±0.64 [a]	7.83±0.64 [b]	6.97±0.56 [c]
Concentration (10^9/ml)	2.10±0.26 [b]	3.40±0.26 [a]	3.07±0.22 [a]
Fertility %, (ewes lambed/ewes mated, n)	52.60±7.17 [b]	46.50±7.17 [b] (47/101)	75.00±6.21 [a]

Columns with different mean superscripts differ significantly from each other ($p<0.05$).

Values of mass motility (0-5), individual motility and live sperm percentages were the best in Assaf semen, and they were significantly higher ($p<0.05$) in the semen of Ossimi crossbred rams compared to Ossimi breed. Mass motility value of Ossimi breed in the present study (1.67) was lower than those recorded by Mahmoud (2013) (4.2) on Ossimi rams and (4.3) by Miloud and Karima (2015) on Ouled Djellal rams. Mahmoud (2013) recorded (73.9%) on Ossimi rams of live sperm, which is in agreement with that reported in the present study on Ossimi breed (70.90%), but it is lower than that recorded on Ossimi crosses (81.67%). On the same trend, low percentage of live sperm (65%) was reported by Miloud and Karima (2015) on Ouled Djellal rams.

Abnormal sperm percentage revealed a significant increase in Ossimi semen compared to Ossimi crossbreds and Assaf semen. The percentages of abnormal sperm in the present study (6.97 – 9.80 %) were lower (Table 1) than that stated (19.7%) by Mahmoud (2013), but it is higher than the value (4.6%) recorded by Miloud and Karima (2015).

The fertility rate (ewes lambed per ewes mated) was significantly ($p<0.05$) higher in Assaf rams (75%) compared to Ossimi and Ossimi crossbred rams (52.6 and 46.5 %, respectively, Table 1). Higher fertility rates (62 – 77 %) were previously recorded for Ossimi sheep (Aboul-Naga *et al*.,1992; Hassan *et al*.,1992; Abdel-Mageed and Abo El-Maaty, 2012). While, Abdel-Mageed (2011) recorded lower rate (47.5%) which agreed with the fertility rate of Ossimi sheep and its crosses in this study (46.4 – 52.6 %). The proportion of Assaf sheep lambing (68.2 – 74.2 %) reported by Palacin *et al*. (2008)is matched with the fertility rate that achieved in the current study.

A total of 14 protein bands were visualized in semen samples (Plate 1 and Table 2). This number is lower than those reported byBarrios *et al.* (2000)on *Rasa aragonesa* rams and Jobim *et al.* (2004) on Hamphsire Down, Corriedale × Texel who recorded 20 and 21 bands, respectively. The Molecular weights in this study ranged from 29 to 194 kDa. Eleven protein bands were determined in Ossimi samples. Meanwhile, semen of Ossimi crossbreds and Assaf rams had 13 protein bands. Protein bands ranged from 78-85 and 97-107 KDa appeared in almost all breeds. The type of protein 29-33 KDa was more frequent in crossbred rams compared to other breeds. This protein spot 13 (28–30 kDa, pI 3.3–3.5) probably corresponds to BSP 30 kDa, which isthe most acidic protein of the BSP family (Manjunath and Sairam 1987).

Table 2. The appearance frequency of seminal plasma proteins as affected by breed of rams

Molecular Weight(KDa)	Breed		
	Ossimi	Ossimi crossbreds	Assaf
190-194	0	0	0.50
186	0	0.33	0
151-159	0.33	0.67	1.00
109-114	0	0.33	1.00
97-107	1.00	1.00	1.00
78-85	1.00	1.00	1.00
61-65	0.67	1.00	1.00
54-57	1.00	0.33	0.50
47-58	1.00	0.33	0.50
45-49	0.67	1.00	0.25
40	0.33	1.00	1.00
36-37	0.67	1.00	0.75
33	0.67	1.00	1.00
29	0.33	1.00	0.75

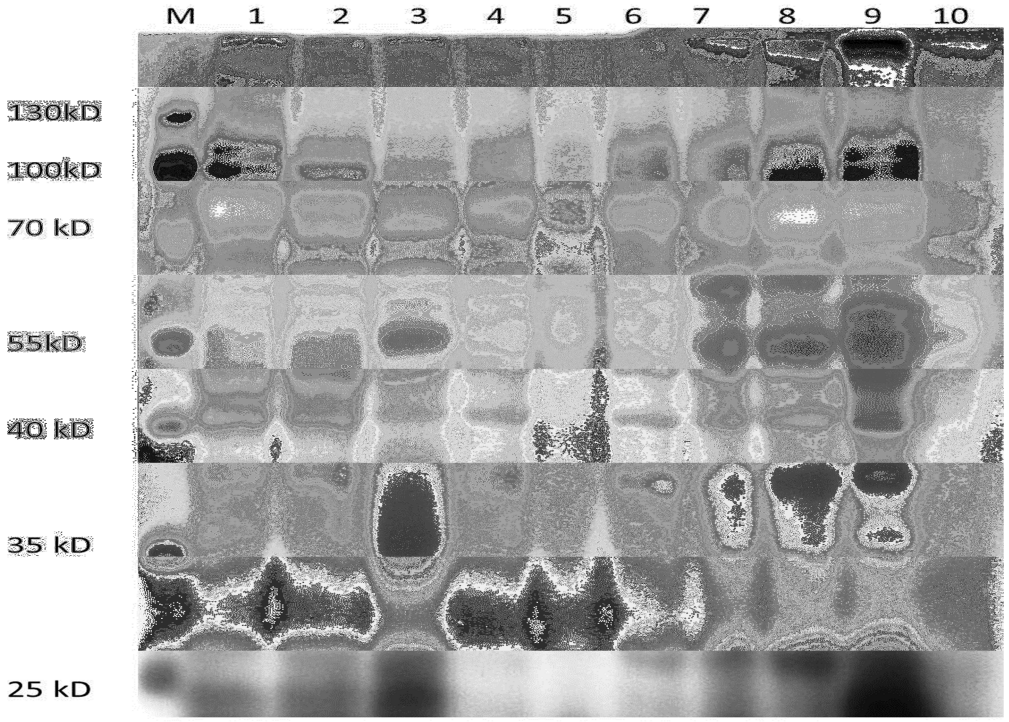

Plate 1. Polyacrylamide gel (SDS-PAGE) of subtropical sheep seminal plasma proteins.(M, Marker; 5, 6, and 10 for Ossimi rams; 4, 7 and 8 for Ossimi crossbreed rams; 1, 2, 3 and 9 for Assaf rams)

The relative content of twelve protein bands varied among the three different breeds. It is noticeable that the 190-194, 186 and 109-114 kDa protein bands cannot be detected in seminal protein samples of Ossimi breed. The relative content of 150-159, 40 and 29 KD protein bands were much lower (0.33) in Ossimi when compared to its content in Ossimi crossbred and Assaf breeds. So these protein bands could be types of protein which are correlated with the ram fertility. The relative high protein content of 54-57 and 47 KD protein bands in Ossimi breed could explain the significant decrease in sperm parameters and concentration compared to Ossimi crossbred and Assaf breeds.

The present result on 40 KDa showed an increase in the frequency of appearance in Ossimi crossbred and Assaf rams compared to Ossimi rams which could affect the mass motility, sperm motility, live percentage and fertility rate. Jobim *et al.* (2005) revealed that, clusterin is the major glycoprotein in ram rete testis fluid, with an apparent molecular mass of approximately 40 kDa on SDS-PAGE and an acidic isoelectric point (3.6). Clusterin is found in the testis and epididymis with the highest concentrations and the testicular source of clusterin suggested being Sertoli cells (Blaschukz *et al*., 1983). The clusterin is found in the seminal plasma, sperm surface of humans and several mammalian species and involved in sperm maturation and possesses heparin-binding sites (Howes *et al*., 1998; Sylvester *et al.,* 1991 and Pankhurst *et al*., 1998). Jobim *et al*. (2004) concluded that cluster in is present in bull seminal plasma and it is related to high semen freezability.

Protein bands 97-107 KDa were present in all studied breeds(100%). Gatti *et al.* (1999) descried the transformation of this protein from 105-kDa in the fluid of the caput region to 94 kDa in the epididymis, the caudal fluid and in semen. Jobim *et al* (2004) suggested that the type of protein (105 KDa) correspond to the germinal form of the angiotensin I-converting enzyme (gACE).

It is clear from the results of the present study that Assaf rams proved a potential capability for their breeding ability under subtropical conditions. Therefore, it is recommended to widely use Assaf rams under Egyptian conditions as it has a great contribution in milk and meat productions.

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خصائص بروتينات بلازما السائل المنوى وعلاقتها بالخصوبة فى الأغنام تحت الظروف المصرية

إبراهيم إبراهيم عبد المجيد، شريف محمد دسوقى

قسم الإنتاج الحيوانى، كلية الزراعة، جامعة القاهرة

تم إجراء هذا البحث بغرض دراسة العلاقة بين بروتينات بلازما السائل المنوى والخصوبة فى بعض سلالات أغنام المناطق تحت الإستوائية. تم جمع عدد (٣) قذفة من عشرة كباش ناضجة جنسياً بإستخدام المهبل الإصطناعى وهى (٣) أوسيمى، (٣) من خلطان الأوسيمى مع كل من الفنلندى و السافولك ، (٤) عساف. تم قياس كل من الخصائص التالية للسائل المنوى بعد الجمع مباشرة (حجم القذفة، كثافة السائل المنوى، الحركة الكلية، نسبة الحركة التقدمية، نسبة الحيوانات المنوية الحية، نسبة الحيوانات المنوية الشاذة، وتركيز الحيوانات المنوية). كما تم تقدير بروتينات بلازما السائل المنوى بإستخدام طريقة التفريد الكهربائى البولى أكريلاميد. تم حساب نسب الخصوبة (عدد النعاج الوالدة إلى الملقحة) وربطها مع خصائص السائل المنوى للكباش. تزايدت كثافة السائل المنوى وتركيز الحيوانات المنوية بشكل معنوى ($p<0.05$) فى كل من سلالة العساف وخلطان الأوسيمى. تزايدت نسبة الحيوانات المنوية الشاذة (٩,٨%) فى السائل المنوى لسلالة الأوسيمى مقارنة بخلطانها والعساف (٧,٨٣ و ٦,٩٧% على التوالى). تم تقدير ١٤ نوع من البروتينات فى عينات بلازما السائل المنوى للكباش. ظهرت كل من البروتينات ذات الوزن الجزيئى ٧٨-٨٥ و ٩٧-١٠٧ ك. د. فى كل السلالات تحت الدراسة. بينما تزايد تكرار ظهور البروتين ذو الوزن الجزيئى ٤٠ ك.د. فى خلطان الأوسيمى والعساف بنسبة (١٠٠%) مقارنة بسلالة الأوسيمى بنسبة (٣٣%). سجلت سلالة العساف النسبة الأعلى للخصوبة (٧٥%) مقارنة بالأوسيمى وخلطانها (٥٢.٦ و ٤٦.٥ % ، على التوالى). أظهرت سلالة العساف كفاءة عالية تحت الظروف المصرية. لذلك من الموصى به إستخدام هذه السلالة للتربية تحت الظروف المصرية.