SEMINAL PROTEINS PROFILE BY GRADIENT SDS-PAGE ELECTROPHORESIS IN NARI SUWARNA RAMS

PRATHIBHA KAIMAL R[1*] M.K. TANDLE[2] AND S. SELVARAJU[3],

[1,2] Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, India

[3] National Institute of Animal Nutrition & Physiology, Adogudi, Bengaluru, India

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ABSTRACT

A study on seminal proteins profile using 6-16% gradient SDS-PAGE electrophoresis was conducted in NARI Suwarna rams. The majority of seminal plasma proteins were observed to be less than 100 kDa. Among these 26 kDa were abundant followed by 68 kDa and 23 kDa proteins. Identification of these proteins will be useful in relating the semen quality and fertility.

Key words: NARI Suwarna, seminal proteins, SDS-PAGE, electrophoresis

INTRODUCTION

Several studies have pointed out actions of different proteins of semen and related them to the acquisition of fertilizing capacity of the spermatozoa in mammals (Carballada and Esponda, 1998). Such proteins affect a wide range of sperm-related phenomena, including sperm capacitation and the acrosome reaction (Therien and Manjunath, 2003), formation of oviduct reservoir (Gwathmey et al., 2006), sperm motility (Yoshida et al., 2008) and fertilization and embryonic development (Goncalves et al,. 2008a,b). Despite the information obtained from those studies, there is lack of information in literature; so, a study was conducted to identify profile of seminal proteins by using 6-16% gradient SDS-PAGE electrophoresis of NARI Suwarna rams.

MATERIALS AND METHODS

The study was carried out on six mature NARI Suwarna rams (60% Deccani, 30% Madgyal and 10% Garole) at Department of Veterinary Gynaecology & Obstetrics, Veterinary College, Bidar. This strain of sheep breed was developed by the Nimbkar Agricultural Research Institute, Phaltan, Satara, Maharashtra, India. The NARI Suwarna breed of sheep has over 60% of ewes with twins instead of single lambs by introducing the 'FecB' gene from Garole breed of Sunder ban of West Bengal which is prolific but rare breed of small sheep found in the hot and humid region. The semen samples were collected with an artificial vagina (AV) from all the six NARI Suwarna rams and seminal plasma was separated after centrifugation as per standard procedure and stored at -200C. The stored seminal plasma samples were thawed, centrifuged at 7000g for 10 minutes at 4°C. Supernatant obtained was transferred without disturbing sperm pellet to another tube and repeated centrifugation for two more times to ensure seminal plasma free from sperm cells and somatic cells. Finally protein concentration was estimated by Bradford's method and stored at -80°C for protein profiling.

The SDS-PAGE was carried out as per the protocol mentioned by (Laemmli, 1970). The seminal plasma and sperm membrane proteins were separated by using 6-16% linear gradient gel. The seminal proteins (10 µg/ well) were loaded with 2x sample buffer with 1:1 ratio and equal quantity of samples were loaded in all wells along with protein molecular weight marker (6.5-212 kDa, Bio labs, India). The gel was subjected to pre run for 2-4 minutes at 50 volts prior to loading samples. After loading of samples, gel was run at 40 volts for 10 minutes followed by 100 volts until the sample buffer lane reaches the bottom of the gel. The gel was stained with silver stain (Gromova, 2006) and the gel images were captured in doc (G-BOX, Syngene, USA) using gene snap image acquisition software and protein bands intensity was analyzed by using gene tools from Syngene gel analysis software.

RESULTS AND DISCUSSION

The majority of the seminal plasma proteins were observed to be less than 100 kDa. The proteins such as 94, 68, 57, 49, 45, 42, 37, 34, 31, 26, 23, 22, 21, 19, 18, 15, 14 and 11 kDa were prominent and commonly seen in the sheep seminal plasma (Table 1). In one of the rams, proteins 34, 31 and 18 kDa were absent. Among commonly seen proteins 26 kDa proteins were abundant followed by 68 kDa and 23 kDa proteins in seminal plasma of the NARI Suwarna rams (Figure 1, 2 and 3).

The presence of these proteins was similar to the observation reported earlier by other research workers. Though the abundant protein 26 kDa was identified in the present study, the protein could be either Alpha monosidase 2c1 or 14-3-3 Protein Zetaas as reported in ram seminal plasma (Soleilhavoup et al., 2014). Such molecular weight proteins were also identified as prostaglandin D synthase in bovine (Gerena et al., 1998). This protein could be involved in sperm maturation in the epididymis (Leone et al.,2002) and fertility (Killian et al., 1993).

CONCLUSION

It can be concluded that the majority of the seminal plasma proteins were observed to be less than 100 kDa. Among these 26 kDa were observed to be abundant followed by 68 kDa and 23 kDa proteins in NARI Suwarna rams. Further, identification of these proteins is essential to relate these proteins with semen quality and fertility.

Table 1: Similarity matrix in seminal plasma protein profile among NARI Suwarna sheep

Sl. No.	Molecular Weight	Ram no.5514	Ram no.5550	Ram no.4668	Ram no.5597	Ram no.5200	Ram no. 4931
1	94	P	P	P	P	P	P
2	68	P	P	P	P	P	P
3	57	P	P	P	P	P	P
4	49	P	P	P	P	P	P
5	45	P	P	P	P	P	P
6	42	P	P	P	P	P	P
7	37	P	P	P	P	P	P
8	34	P	A	P	P	P	P
9	31	P	A	P	P	P	P
10	26	P	P	P	P	P	P
11	23	P	P	P	P	P	P
12	22	P	P	P	P	P	P
13	21	P	P	P	P	P	P
14	19	P	P	P	P	P	P
15	18	P	A	P	P	P	P
16	15	P	P	P	P	P	P
17	14	P	P	P	P	P	P
18	11	P	P	P	P	P	P

Note: Among these proteins 26 kDa proteins were observed to be abundant followed by 68 kDa and 23 kDa proteins

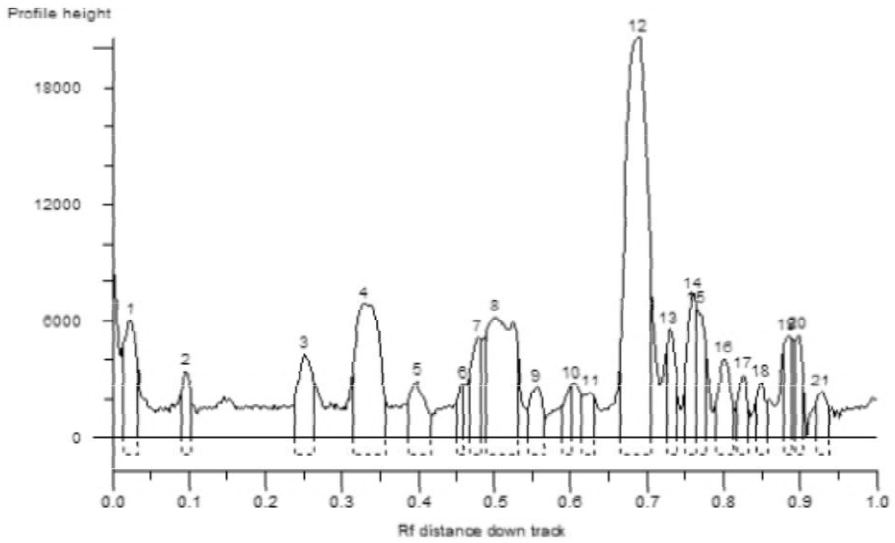

Figure 1: Seminal Plasma protein profile analysis using gel documentation system

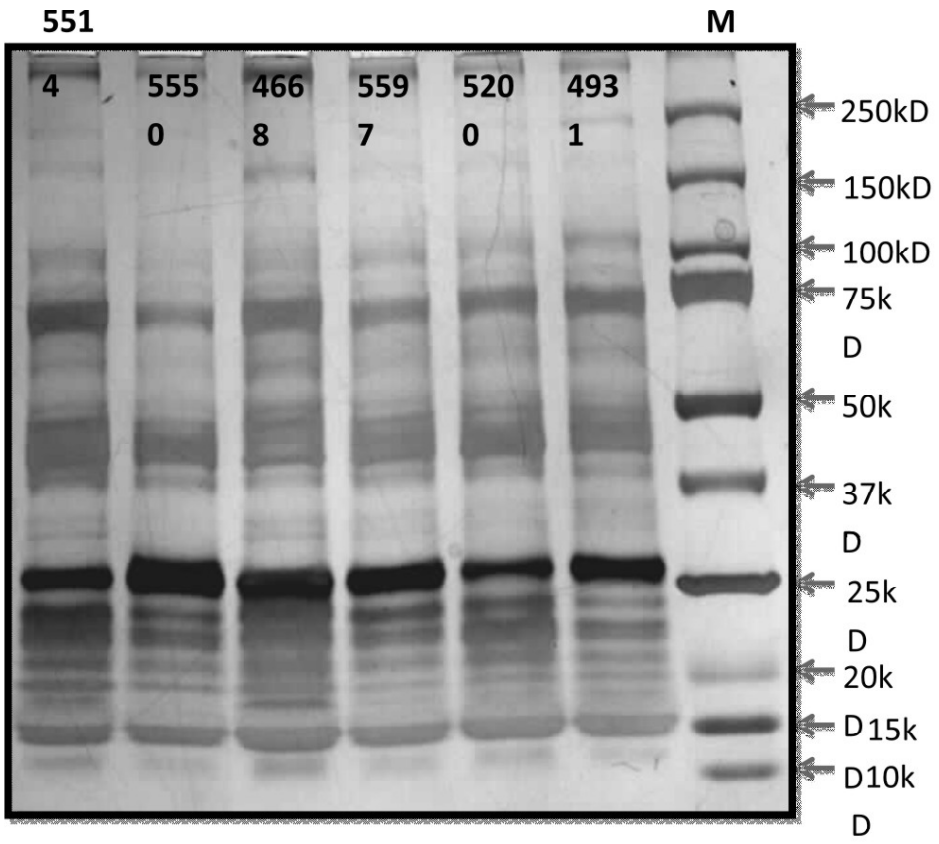

Figure 2: Profiling of NARI Suwarna seminal proteins using 6-16% Gradient SDS-PAGE electrophoresis

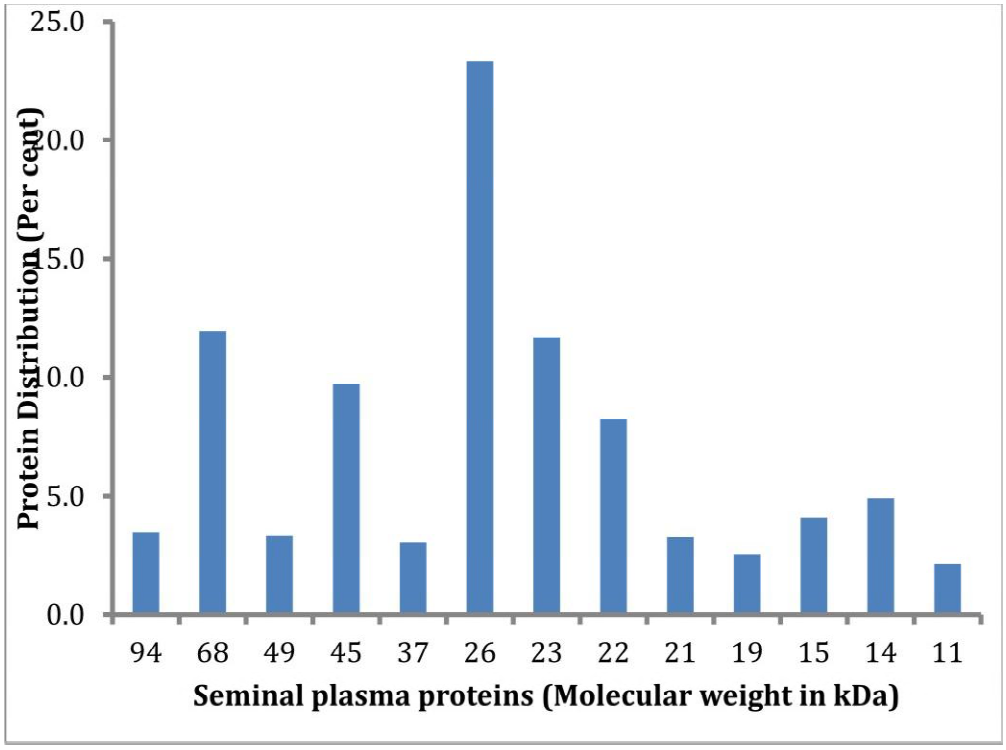

Figure 3: The seminal plasma proteins quantity distribution as analyzed by densitometric analysis

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