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Seminal Plasma Protein Profile in Indigenous Jacks

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Abstract: **The seminal plasma is of testicular or epididymal origin and its proteomes reflect spermatogenesis and epididymal sperm maturation. The present study was designed to investigate the seminal plasma proteins profiles in the indigenous jacks. Nine clinically healthy, docile, adult indigenous jacks of non-descript breed were used for semen collection. Ejaculation for semen collection was done using artificial vagina (Colorado model), filtrated the semen and immediately the seminal plasma was separated by centrifugation for further analysis. Seminal plasma proteins were evaluated by poly acrylamide gel electrophoresis (PAGE) and identified the banding pattern of existed proteins. Twelve bands were observed in the range of 10.0 – 67.0 kDa (i.e. 10.0, 12.0, 15.0, 17.0, 18.5, 19.5, 31.5, 36.0, 40.0, 45.0, 56.5 and 67.0 kDa) in the seminal plasma of indigenous jacks. The majority of these proteins were with a molecular weight <50 kDa. The study from obtained results concluded that at least 12 bands of protein having different molecular weight are present in seminal plasma of indigenous jacks.**

Index Terms: **Docile, Epididymis, Polyacrylamide gel electrophoresis, Seminal plasma proteins**

INTRODUCTION

Seminal plasma is the fluid part of the ejaculate and it consists of liquids secreted by various male accessory glands that vary according to animal species. It is a complex secretion mainly originating from the epididymis and accessory sex glands and significantly contributes to semen volume. Epididymal proteins are considered to play roles in final maturation of sperm. Accessory sex gland proteins, in turn, contribute to sperm capacitation and motility, acrosome reaction and protection of sperm against oxidative stress and immune responses (Intasqui et al., 2016; Kňažická et al., 2020). Such proteins also mediate sperm interaction with oviduct epithelium and fertilization (Kelly et al., 2006; Moura et al., 2007; Moura et al., 2010; Rego et al., 2014; Yanagimachi, 1994). Studies on different mammalian species suggest that seminal plasma contains many distinct components which are important for spermatozoa function. Recently, many seminal plasma proteins have been identified and characterized (Ayyagari et al., 1987; Frazer and Bucci, 1996) while several have been associated with fertility in various species (Ayyagari et al., 1987; Brandon et al., 1999; Killian et al., 1993; Talluri et al., 2017). So far, there is no information on the seminal plasma proteins and their profile of the indigenous donkeys. However, little literature is available on Indian horse breeds namely Marwari and Zanskari stallions only (Arangasamy and Bhure, 2009; Talluri et al., 2012; Talluri et al., 2017). The present study was aimed to document the seminal plasma proteins profiles in the indigenous jacks.

MATERIALS & METHODS

A. Animals

The present study was conducted in the month of July, 2016 at NRCE, Bikaner. Nine adult indigenous jacks of different age groups having sound body condition score, docile, non-descript breed and semen with more than 70% of morphologically normal sperm were taken under experiment. The jacks were chosen on the basis of their height (85 to 105 cm) and body weight (100–120 kg) with no fertility record. The study was approved by Animal Ethics Committee.

B. Semen Collection & Processing

All the jacks were maintained under uniform conditions of feeding and management. One ejaculate each from all the jacks was collected using artificial vagina (Colorado model). Gel portion of the semen was removed by filtration and immediately the seminal plasma was separated by centrifugation at 3000 rpm for 30 minutes to remove any cell debris followed by storage at -200C until further analysis. The seminal plasma was evaluated for protein profile as well as for total protein contents in each sample as shown in Fig.1

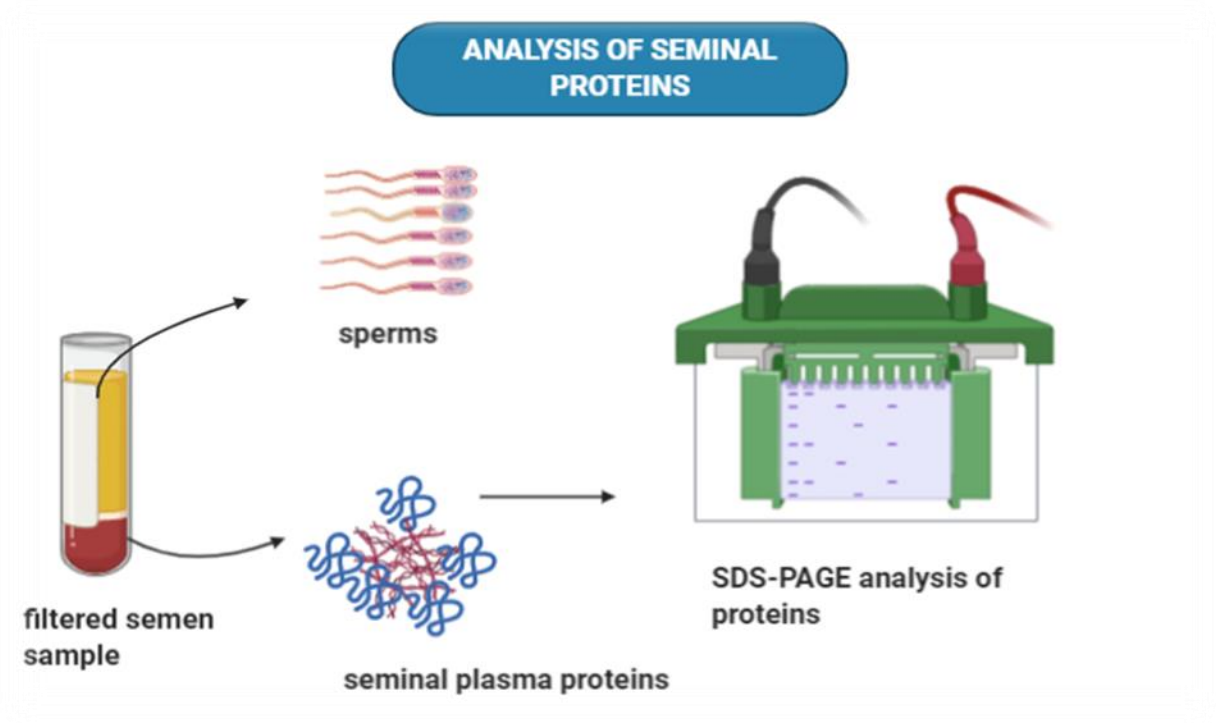

Fig. 1. Procedure of analysis of seminal proteins by SDS-PAGE

C. Semen Evaluation

All semen samples were evaluated immediately after collection (Fordyce et al., 2006; Lago-Alvarez et al., 2020). The volume was recorded and a drop of semen was analyzed by phase-contrast microscopy using a 37 ◦C stage set. Motility was assessed at 400× magnification under a pre-warmed cov er slip and the percentage forward progressive sperm estimated in increments of 5%. An aliquot of 20 µL was diluted into a 0.2% gluteraldehyde, phosphate-buffered saline solution for analysis of sperm morphology. Approximately 1 mL of semen was centrifuged at 700 × g for 15 min and supernatant was snap-frozen in liquid nitrogen for later analysis of seminal plasma proteins (Bucci et al., 2016; Lago-Alvarez et al., 2020; Rego et al., 2014).

1) Total Protein Estimation

Total protein was estimated in each seminal plasma sample as per the method of Lowry et al. (1951). This also helped in loading equal amount of protein for its profiling also.

2) Polyacrylamide Gel Electrophoresis (PAGE)

Electrophoresis was performed as per method suggested by Laemmli (1976) using 12 % polyacrylamide gels and loading 60µg protein per well after making their appropriate dilutions. Electrophoresis gels were stained with Coomassie Brilliant Blue G- 250 (with 50% methanol, 10% acetic acid) and destained using destain solution containing 5% methanol and 7.5% acetic acid. Dried gels were scanned using scanner with different settings. The apparent molecular mass was determined using gel documentation (molecular weight Markers of Genei India, kit was of SPIN REACT Company, Spain) & analysis-system (Quantity One software, Chemi Doc. BioRad). All other chemicals used were of molecular biology grade and were obtained from Commercial suppliers (Sisco Research Limited (SRL), India) as shown in Fig.2.

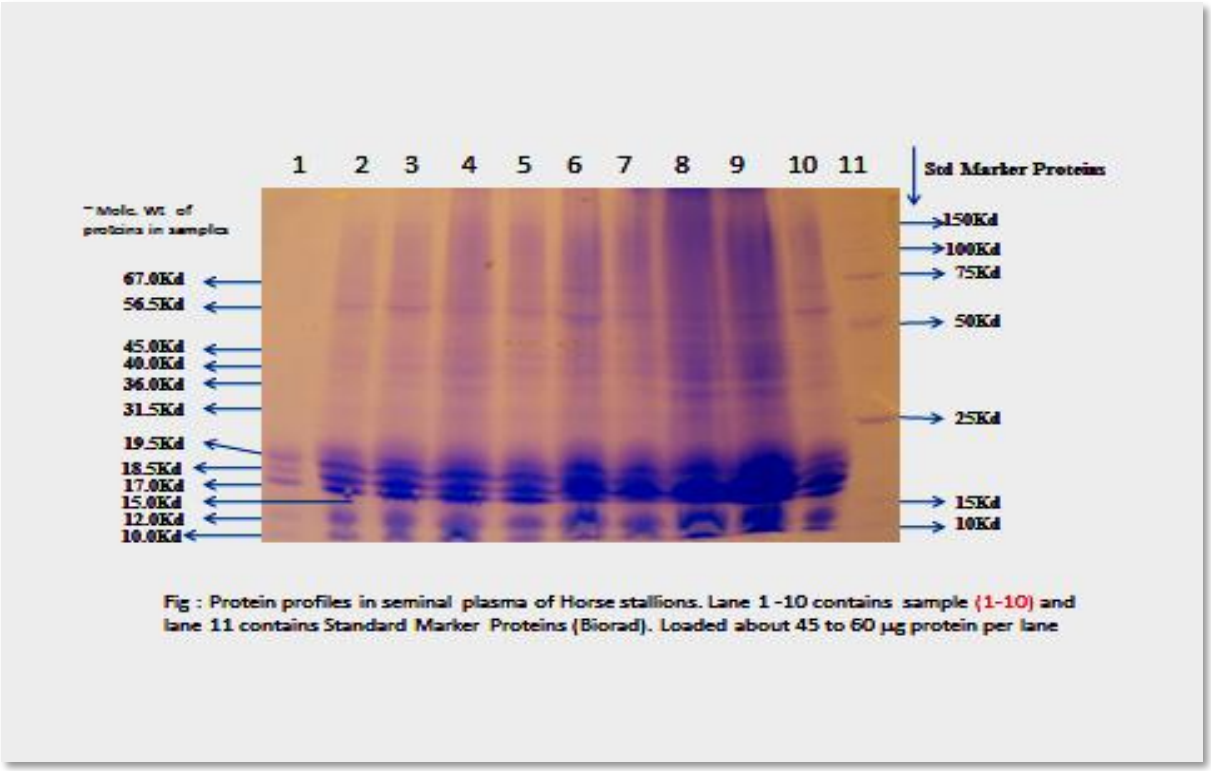

Fig. 2. Protein profiles in seminal plasma of Horse Stallions

3) Statistical Analysis

The data were analyzed as per the method described by Snedecor and Cochran, (1989).

RESULTS & DISCUSSION

A. Seminal Plasma Protein

Total seminal protein contents ranged from 2.1 to 9.6mg/dL with mean content as 5.41mg/dL of indigenous jacks. Significant variation in total protein values among the jacks was observed. There was Total protein concentration was observed very high in horse and donkey stallions (Pal et al., 2009; Talluri et al., 2012).

B. Electrophoretic Profiles of the Seminal Plasma Proteins

Total seminal protein contents ranged from 2.1 to 9.6mg/dL with mean content as 5.41mg/dL of indigenous jacks. Significant variation in total protein values among the jacks was observed. There was Total protein concentration was observed very high in horse and donkey stallions (Pal et al., 2009; Talluri et al., 2012).

Twelve bands were observed in the range of 10.0 – 67.0 kDa (i.e. 10.0, 12.0, 15.0, 17.0, 18.5, 19.5, 31.5, 36.0, 40.0, 45.0, 56.5 and 67.0 kDa) in the seminal plasma of indigenous jacks, whereas Talluri et al., (2012) reported fifteen protein bands in the range of 11.45 – 162.83 kDa (i.e. 11.45, 13.11, 15.09, 17.36, 19.04, 20.87, 26.33, 28.87, 36.52, 39.25, 53.60, 58.11, 72.04, 130.23 and 162.83kDa) in the seminal plasma of exotic jacks. The total protein bands observed in the indigenous jack seminal plasma in the present study is similar to the earlier findings reported in stallions by Frazer and Bucci, (1996) and Arangasamy and Bhure, (2009). The majority of these proteins were with a molecular weight <50 kDa, and correlated the earlier reports of Frazer and Bucci, (1996) and Arangasamy and Bhure, (2009). In case of Exotic Donkey there is variation the number of proteins and their molecular weight. A total of 15 bands appeared on the gel with the variation in molecular weight of 72.04, 130.23 and 162.83 kDa at three proteins other twelve bands were common with the observations of the present study.

CONCLUSION

The present study showed different seminal protein profile in indigenous jack than exotic jack seminal plasma profile as earlier reported by Talluri et al., (2012), which may be due to the inherent character of the donkey breeds. It can be concluded from this study that at least 12 bands of protein are present in seminal plasma of indigenous jacks.

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