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Seminal plasma protein profiles in three different breeds of equines

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ABSTRACT

Semen ejaculates (8) were obtained from 2 Marwari stallions, 2 Zanskari stallions and 2 exotic jacks (m*artina franca*). Gel portion of the semen was removed by filtration and immediately the seminal plasma was separated by centrifugation at 2000 rpm for 30 min and stored at –20°C until further analysis. Total protein from the seminal plasma was estimated by Lowry method and the molecular weight was analysed by SDS-PAGE. The total protein concentration was found to be 0.71, 2.24 and 2.21 g/dl in Marwari, Zanskari and exotic jack seminal plasma respectively. The differences in protein concentration were found to be significantly higher in Zanskari and jack stallions as compared to Marwari stallion. There was no significant difference of total protein values between Zanskari and jack stallions. A total of 12 protein bands were observed in the seminal plasma of both Marwari and Zanskari stallions and are in common, whereas, 15 protein bands were observed in the seminal plasma of exotic jacks. The seminal plasma proteins in Marwari and Zanskari stallions ranged from 11.45KDa to 130.23kDa and that of exotic jack ranged from 11.45kDa to 162.83kDa. Out of 15 protein bands observed, 3 were unique in jack stallions having molecular weight of 36.52 kDa, 39.25 kDa and 1602.83 kDa. It can be concluded from this study that at least 12 bands of protein were in common between the all the 3 breeds of equines, and seminal plasma protein profile of jack stallion is different from the Marwari and Zanskari stallions.

Key words: Exotic jack, Marwari stallion, Stallion seminal plasma protein, Total protein, Zanskari stallion

In mammals, the fertilization takes place by the interaction of spermatozoa from the male with the egg in the oviduct. The spermatozoa do not possess fertilizing capacity at the time of ejaculation, but acquire it during their transit through the female genital tract by a process known as capacitation. Studies on different mammalian species suggest that seminal plasma contains specific proteinaceous factors that influence the fertilizing ability of spermatozoa.

Seminal plasma proteins are secretory proteins originating mainly from the epididymis and the accessory sex glands. They are involved in remodelling of the sperm surface-which occurs during sperm transit through the male genital tract and continues later at ejaculation.

There are very few studies on the seminal plasma proteins of the Indian equine breeds. Literature in this context to Indian equine breeds like Marwari and Zanskari stallions is meagre and scanty. This present study was carried out to document the seminal plasma proteins in 2 Indian breeds of stallions and exotic jack.

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MATERIALS AND METHODS

Semen collection and seminal plasma separation: The semen was collected from the various stallions present at Equine Production Campus, National Research Centre on Equines, Bikaner, Rajasthan. All the stallions were maintained under uniform conditions of feeding and management. Ejaculates from 2 adult Marwari stallions, 2 exotic jack (*Martina franca*) stallions of known fertility status and from 2 Zanskari stallions were obtained using artificial vagina. Gel portion of the semen was removed by filtration and immediately the seminal plasma was separated by centrifugation at 2000 rpm for 30 minutes and stored at –20°C until further analysis. The stored seminal plasma was thawed at ambient temperature and again centrifuged at 10000 rpm for 30 min at 4°C to remove the cell debris, if any. Part of these various stallion seminal plasma was randomly aliquoted and stored for total plasma protein estimation. The rest of the seminal plasma from the stallions were pooled together before protein estimation.

Total protein estimation in seminal plasma: Total protein was estimated in seminal plasma sample so that an equivalent amount of protein could be used for gel preparation. Protein concentrations from each seminal plasma sample were estimated as per Lowry *et al.* (1951).

Molecular weight determination: Electrophoresis was performed as per Laemmli (1976), in 12.5% polyacrylamide gels. Electrophoresis gels were stained with coomassie brilliant blue R- 250. The apparent molecular mass was determined using gel documentation and analysis-system. All other chemicals used were of analytical grade and were obtained commercially.

Statistical analysis: The data were analysed using t test as per the method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

Total proteins in seminal plasma: Total protein concentration was estimated in seminal plasma sample of Marwari, Zanskari and exotic jacks. Total concentration of total protein was observed to be 0.71g/dl in Marwari Stallions and 2.24 g/dl in Zanskari stallions and 2.21 g/dl in that of exotic jacks. Total protein concentration was significantly lower in Marwari stallions as compared to Zanskari stallions and exotic jacks. The total protein concentration was almost same in Zanskari stallions and exotic jacks. But no significant difference was observed in the protein concentrations of Zanskari and exotic jack stallion seminal plasma samples.

Electrophoretic profiles of the seminal plasma proteins: Twelve bands were observed in the range of 11.45 - 130.23 kDa (i.e. 11.45, 13.11, 15.09, 17.36, 19.04, 20.87, 26.33, 28.87, 53.60, 58.11, 72.04 and 130.23 kDa) in both the Marwari and Zanskari stallion seminal plasma (Fig.1 lanes 1, 2 and 3 and 4). Fifteen protein bands were observed 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 exotic jack stallion seminal plasma (Fig.1, lanes 5and 6). The total protein bands observed in the Marwari stallion seminal plasma in the present study supports the earlier findings reported by Fazer 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). Same ranges of proteins were noticed in the seminal plasma of Zanskari stallions. In case of exotic donkeys there are variation in the number of proteins

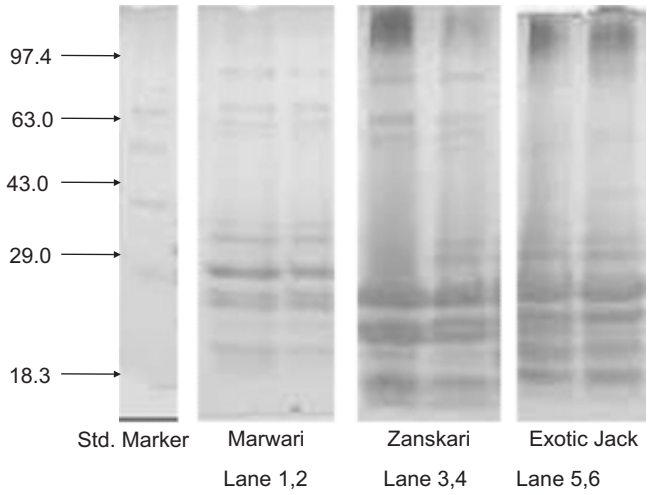

Fig.1. Comparative protein profile of Marwari, Zanskari stallions and exotic jack stallions.

and their molecular weight. A total of 15 bands appeared on the gel with the variation in molecular weight of 36.52,39.25 and 162.83 kDa at 3 proteins while other 12 bands were common with Marwari and Zanskari stallion seminal plasma.

The present study showed similar seminal plasma protein profile in Marwari and Zanskari stallions while there were different seminal protein profile in exotic jack seminal plasma profile, which may be due to the inherent character of the species difference between horses and donkeys (Arangasamy and Bhure 2009).

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