SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS OF EQUID SEMINAL PLASMA PROTEINS

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ABSTRACT

Twelve and eight semen ejaculates were obtained from three Marwari and two Exotic Jack (*Martina franca*) stallions, respectively. They were subjected to Plasma separation immediately after collection by centrifugation at 2000 rpm for 20 minutes and stored at -20° C until analysis. In the seminal plasma the total protein concentration was estimated by lowry method and molecular weight was determined by SDS-PAGE analysis. The overall mean values of total protein were recorded as 29.12±2.47 and 34.29±1.44 mg/ml, respectively, in marwari and exotic stallion. Thirteen total protein bands were observed in the range of 17-95 kDa in the Marwari stallion seminal plasma. Thirteen total protein bands were observed in the range of 17-101 kDa in the exotic Jack seminal plasma. It can be concluded that among the total proteins, eight protein bands are commonly seen in both the stallion.

Keywords : Marwari, Stallion, Exotic Jack, Seminal plasma, Total protein

INTRODUCTION

Seminal plasma is the fluid fraction of semen, which is a complex mixture of secretions from testis (Kato et al., 1985), epididymis (Turner and Reich, 1987), vas deferens (Feng et al.,1995) and seminal vesicles (Manjunath et al., 1994). Seminal plasma contains a number of substances and the absolute concentration of many of the components varies with individual stallions and with circulating levels of testosterone, as well as the amount of use of stallion, the time of year and the semen fraction collected (Davis Morel, 1999). The level of total proteins present in the fresh semen is an important characteristic for freezability and fertility of cattle and buffalo bulls semen. Unlike cattle and buffalo bulls in the equine species, the seminal plasma is separated immediately after collection, to protect the sperm cells motility from further damage during dilution and cryopreservation. Seminal plasma composition and its effects on the fertilizing ability of sperm seems to vary depending on the fertility of an individual animal (Henault and Killian, 1996; Brandon et al., 1999). Previous studies have shown that proteins content of equine seminal plasma is relatively low (10 mg/ml), when compared to other mammals containing about 20-60 mg/ml (Topfer-Petersen et al., 2005). Proteins of molecular masses between 13 and 122 kDa were detected by Amann et al. (1985). Brandon et al. (1999) resolved 14 proteins by two-dimensional electrophoresis. Some of these proteins have been found to correlate with the fertility of the individual stallion (Brandon et al.,1999). The isolated proteins, designated as horse seminal plasma proteins (from HSP-1 to HSP-8) had low molecular masses of 14-30 kDa. With exception of HSP-4, all of them showed sperm-binding properties and could be isolated from ejaculated, washed sperm (Calvete et al., 1994). In a number of mammalian species, including cattle (Miller et al., 1990) and horse (Varner et al., 1993), heparin binds to ejaculated sperm and modulates the capacitation process, thereby, enhancing the ability of sperm to undergo the zona pellucida-induced acrosome reaction (Florman and Babcock, 1991:Manjunath and Therien, 2002). Therefore, in this present investigation we aimed to document the protein profiles of equine seminal plasma with SDS-PAGE of Marwari and exotic jack (*Martina franca*) stallion seminal plasma.

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MATERIALS AND METHODS

Semen collection and seminal plasma separation : Ejaculates from three Adult Marwari stallions and two Exotic Jack (*Martina franca*) stallions were obtained from the Equine production campus, National Research Centre on Equine, Bikaner, India (Four ejaculates each from five stallion) by Artificial Vagina method. All the stallions were maintained under uniform standard conditions for feeding and management. The seminal plasma was separated immediately after collection by centrifugation at 2000 rpm for 20 minutes and stored in -20° C until analysis. The stored seminal plasma was thawed at ambient temperature and recentrifuged (10000 rpm, 45 minutes at 5° C) to remove cell debris, if any. Part of three individual stallion and two jack stallion seminal plasma were randomly aliquoted and stored for total plasma estimation. The rest of seminal plasma from the three stallions and two jack stallions were pooled together before protein isolation.

Total proteins in seminal plasma : Total protein concentrations in the stallion and jack stallion seminal plasma was estimated as per the method given by Lowry et al. (1951).

Molecular weight determination: Electrophoresis was performed (Laemmli, 1976) in 12.5% polyacrylamide gels. Electrophoresed gels were stained with Coomassie Brilliant Blue R-250. The apparent molecular mass was determined by using (Molecular weight markers of Bangalore Genei India) Gel documentation & analysis-system (Gel-Doc. Model-Alpha ImagerTM1220, Alpha Innotech Corporation, USA). All other chemicals used were of analytical grade and were obtained from commercial suppliers (Sisco Research Limited, India).

Statistical analysis : The data were analyzed with ANOVA as per the method described by Snedecor and Cochran, (1994). Protein levels are expressed as Mean ±S.E.

RESULTS AND DISCUSSION

Total proteins in seminal plasma : The overall mean values of total proteins level has been presented in table 1. The seminal plasma and secretions of the accessory reproductive organs of several species have been shown to contain proteins that are capable of binding to the spermatozoal surface (Mann.1981). This involvement of seminal plasma proteins in the capacitation process and acrosome reaction is beyond doubt Shivaji et al. (1990). Part of this process actually involves removal of decapacitating factors, which may include seminal plasma proteins (Manjunath et al., 1994; Shivaji et al., 1990). Variability in fertilizing ability of different semen samples from the same animal may be due to altered level of secretion by the various accessory sex glands (Little and Holyoak 1992). In the present study, the observed overall mean values of total proteins in stallion seminal plasma are higher than the earlier works reported by Topfer-Petersen et al. (2005) and Amann et al. (1987). This variation might be due to the breed difference. The observed proteins level is higher in exotic jack than the Marwari breed in the current study, which is non-significant. This observation was similar like the difference observed between the cattle and buffalo bulls by Kulkarni et al. (1995). The low level of seminal plasma protein profiles in stallion as compared to bovine species could be attributed to the species difference. Our recent results indicate that exotic jack stallion seminal plasma contain more level of total proteins and also showed good freezability with higher post thaw motility, when compared to Marwari stallion (unpublished data).

Elecrophoretic profiles of seminal plasma proteins : Thirteen protein bands were observed in the range of 17-97 kDa (i.e 17,18,19,21,22,24,27,28,31,50, 58 72 and 95 kDa) in the Marwari stallion seminal plasma (Fig-1, lane 2). Thirteen protein bands were observed in the range of 17-101 kDa (i.e 17,18,19,20, 21,22,24,27,29,31,51,63 and 101 kDa) in the exotic jack stallion seminal plasma (Fig-1, lane 3). The total protein bands observed in the marwari stallion in the present study supports the earlier finding reported by Frazer and Bucci. (1996). The majority of these proteins were

with a molecular weight <50 kDa, and was similar as reported by Frazer and Bucci. (1996). Similar kind of observation were noticed in the present study in the case of exotic jack stallion (eight protein bands are common), five protein bands (20,29,51,63 &101 kDa) are different with Marwari stallion. The studies on total proteins is meager and no supporting literature was traced out during the persual regarding the concentration of total proteins in exotic jack stallion. Recently, a number of proteins constituting minor components of equine seminal plasma have been described, e.g. lactoferrin (Inagaki et al., 2002), which may promote the longevity of sperm (Lackey et al., 2002; Champion et al., 2002) and various enzymes, such as lipase (Carver and Ball, 2002), á 1.4-glucosidase (Dias et al., 2004) and an angiotensin-converting enzyme (Ball et al., 2003) and heparin binding proteins (from HSP1 to HSP-8) had molecular masses of 14-30 kDa and 13-71 kDa (Calvete et al.,1994). The present study showed that eight proteins of stallion seminal plasma are common with exotic jack stallion and the variation in the present study regarding the number of common and uncommon total proteins observed might be due to the inherent character of species difference between horses or donkey (or) it might be due to methodology involved in protein studies. Aggregations product of low molecular weight proteins (or) a degradation product of high molecular weight proteins.

The study observed 13 total protein bands in Marwari stallion as well as exotic jack (*Martina franca*) stallion seminal plasma. Future studies are needed to isolate and separate the individual fertility related protein in stallion and jack seminal plasma in order to examine their effects on in *in vitro* and *in vivo* conditions.

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Table 1: Concentration of total protein (mg/ml) in equid seminal plasma

Breed	Mean±S.E	N
Marwari	29.12±2.47	13
Exotic Jack	34.29±1.44	6

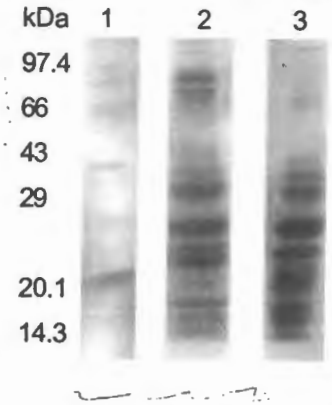

Fig.1. Electrophoretic profiles of equid seminal plasma

Lane 1 : Standard protein marker
Lane 2 : Stallion seminal plasma
Lane 3 : Exotic Jack seminal plasma

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