



Original Research

Isolation, Purification and Immuno-Biochemical Characterization of Gir and Jersey Seminal Plasma Fertility Associated Proteins

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Abstract

The objective of the present study was an attempt has been made to throw some light on the reproductive performance of Gir and Jersey bull in terms of evaluation of polypeptide characteristics of seminal plasma as well as mass activity, motility and morphology of spermatozoa. Seminal plasma from apparently normal semen containing viable sperm was collected for isolation; purification and immune-biochemical characterization of fertility associated proteins 26 kDa, 55 kDa, 14.3 kDa, 12 kDa and 16 kDa in bulls are a prominent immuno-dominant protein and have pivotal role in fertility. Research conducted in bovine seminal plasma showed that the abundance of specific protein in semen is related to fertility. Two seminal plasma protein 26 kDa and 55 kDa were more abundant in semen of high fertility bulls and two different proteins 16 kDa (pI 6.7) and 16 kDa (pI 4.1) were more abundant in lower fertility bulls. This paper reviews comparatively our current knowledge on the growing field of proteomics of the seminal plasma proteins and its relevance in relation to the in vivo situation, for the sake of reproductive biology, diagnostics and treatment. Proteins of the seminal plasma have an ample panorama of action and some appear responsible for establishing fertility.

Key words: Gir Bull, Immuno-Dominant, Jersey Bull, Fertility Associated Protein, Seminal Plasma

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Introduction

The present paper attempts to view the aspects of the composition of the seminal plasma of bulls, with a particular focus on its proteomics and the differential functions this fluid would play in relation to sperm function and signaling to the female, with an ultimate focus on its role in modulating fertility. The ability to select high fertility sires results in the production of semen samples with optimal quality that will,





ultimately improve conception rates. Currently, routine semen analysis based on motility and morphology provides useful, but limited information about fertility indexes in the male. Proven, high-use bulls from artificial insemination centers still show differences in non-return rates by as much as 20 to 25 %, but these results are not explained by routine semen analysis (Larson and Miller, 2000). Thus, the existence of sub fertile sires that appear to show normal semen quality is an important observation and has stimulated the study of other markers of fertility, such as molecular components of the seminal plasma (Braudmeyer and Miller, 2001).

The potential influence of seminal plasma and the success of artificial insemination also depend upon the ability to screen for semen with high fertilization potential (Holt *et al.*, 2007). Thus, given the biological and economic importance of knowing with certainty the potential fertility of the semen for artificial insemination before insemination, it becomes essential to explore aspects that relate to fertility. Although the most conclusive evidence of fertility from freeze-thawed semen is made on the basis of pregnancy rate in the females served, semen evaluation offers predictive information on expected performance of the male. This study summarizes the physiological functions of proteins of seminal plasma and their relation to bull fertility.

Materials and Methods

For this research work fresh semen samples were collected from Gir and Jersey bulls of sound reproductive health maintained and ejaculate was transported to the lab in ice-cold condition by semen shipper for immediate evaluation of semen characteristics in terms of mass activity, motility and morphology of spermatozoa as per method described by Shastri, A. and Rama Rao, P. (1983). The semen samples having poor quality of sperm were discarded. For frozen semen thawing of the semen was carried out by immersing straws in a water bath at 37°C for 15 seconds (Rasul *et al.*, 2000). The semen having apparently normal spermatozoa was taken into consideration for collection of seminal plasma. The seminal plasma was separated by centrifugation at 3000 rpm for 20 minutes at 4°C. The supernatant was collected very carefully and concentrations of the seminal plasma were determined (Lowry *et al.*, 1951). Then the samples were pulled and kept at -20°C until further use. 10% SDS-PAGE (Laemmli, 1970) were performed to detect the polypeptide bands of the seminal plasma. Fertility associated polypeptides of crude seminal plasma antigen were purified by gel filtration chromatography in a column on Sephacryl S-200 (2.1 cm diameter and 43 cm in length) in a buffer containing PBS (pH 7.2), PMSF (0.03mM) and 0.02% sodium azide at a flow rate of 20 ml per hour. The elutes were collected in 36 fractions of 4 ml each. The distribution of protein was monitored by taking the absorbance at 280 nm in a UV/VIS spectrophotometer (SYSTRONICS-119). Then the 4 pooled fractions were concentrated by dialysis against sucrose using dialysis membrane (cut off value 12,000). The protein concentrations of the 4 pooled fractions of seminal plasma were determined (Lowry





et al., 1951). The concentrated peak fractions were then preserved at -20°C in aliquots for further use. 10% SDS-PAGE (Laemmli, 1970) were performed to detect the purified fertility associated protein. The crude and purified samples of Gir and Jersey bull of fresh and frozen seminal plasma were analyzed by One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) in vertical mPAGE (AE-6530) and vertical slab gel electrophoresis chamber (AE-6200) along with power supply and gel casting apparatus (ATTO Corporation, Japan).

Molecular weights were determined by SDS-PAGE using protein markers by gel documentation systems (Bio-Rad). Two healthy New Zealand white rabbit with an average weight of 1.5 kg of each (Purified seminal plasma proteins of Gir and Jersey bull) respectively were taken for raising of Hyperimmune Serum. DID test was performed according to Ouchterlony (1963) with some modifications. The proteins were separated by SDS-PAGE (Laemmli, 1970) and then the resultant proteins were electro-blotted to nitrocellulose filter paper (Immobilon- NC) from gel according to the method of Towbin *et al.* (1979) and Kataria *et al.* (2000). Indirect ELISA for detection of sero-reactivity crude and purified seminal plasma protein of Gir and Jersey bulls was performed according to the method of Sarkar, *et al.*, (2003) with some modifications. Statistical analysis was done by t-test for Indirect ELISA using homologous and heterologous hyper immune serum (Snedecor and Cochran, 1967).

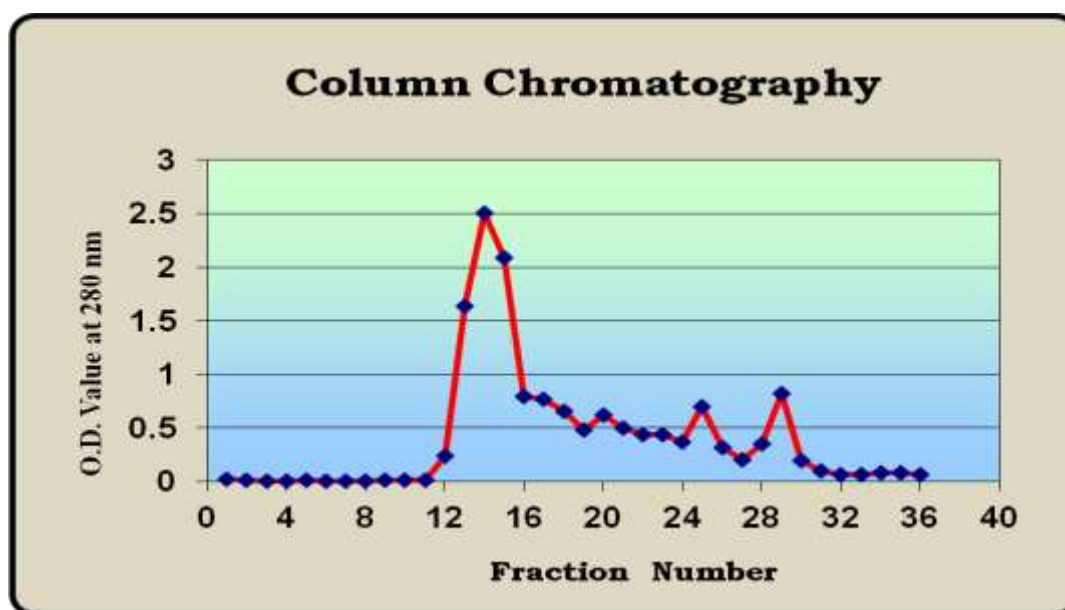
Results and Discussion

In this study, attempt has been made to purify and characterize this fertility associated protein from Gir and Jersey bull which give some valuable information to understand the status of these proteins. The semen evaluation was done in terms of mass activity moderate wave noticed (++). The motility of individual spermatozoan was more than 60%. The morphology of spermatozoa was normal at the level of 80%, when stained with Rose Bengal.

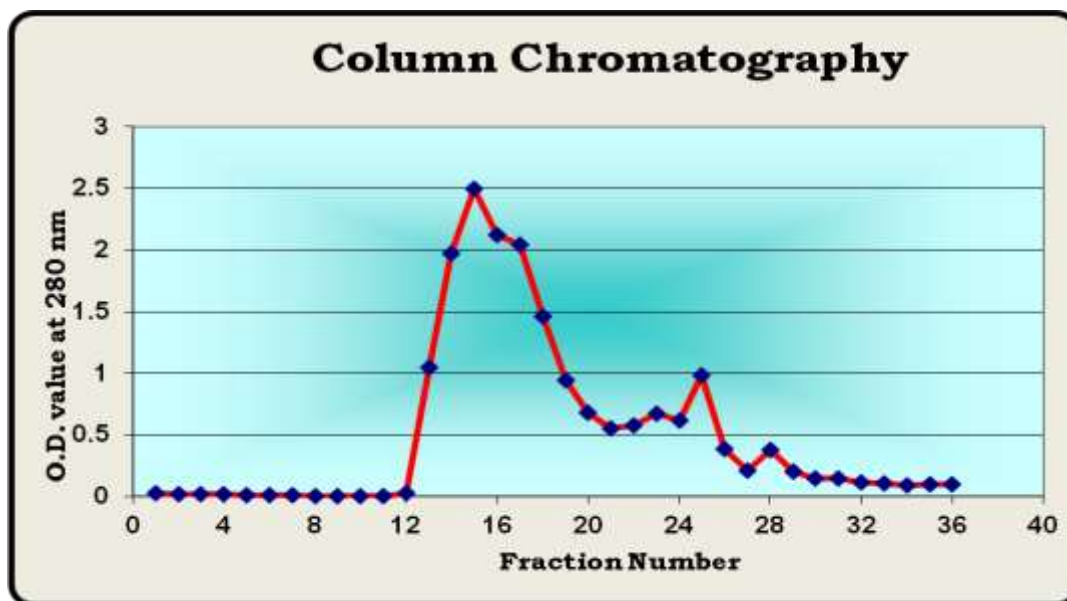
Seminal plasma protein has important relatively specific for the regulation of sperm function (Villemure *et al.*, 2003 and Strezezek *et al.*, 2002). Seminal plasma protein composition varies among species and some of seminal plasma proteins have influence on sperm motility, viability and fertilization (Sanchez-Luengo *et al.*, 2004). The protein concentration of crude and frozen after thawing seminal plasma determined by Lowry *et al.* (1951) was 25.45 mg/ml and 24.57 mg/ml respectively and after thawing Gir frozen and Jersey frozen 20.15 mg/ml and 19.64 mg/ml respectively. After that SDS-PAGE of crude seminal plasma was carried out and several polypeptide bands were identified which in correlation with the findings of the (Asadpour *et al.*, 2007) molecular weight of the polypeptide bands could not be evaluated as the existence of seminal plasma protein might be aggregated forms in normal physiological condition. This is in support of the findings of Manaskova *et al.* (2003) who reported the aggregation of boar seminal plasma protein as they are mostly glycoprotein in nature.

SDS-PAGE was performed to observe the polypeptide profiles of crude seminal plasma and different partially purified fractions separated by gel filtration chromatography and molecular weight was determined. The resolution of fraction for Gir bull (Graph 1), F1 (fractions of test tube no. 12, 13 & 14 of ascending loop of first peak), F2 (fractions of test tube no. 15, 16 & 17 of descending loop of first peak) in 10% SDS-PAGE revealed major polypeptide bands of 55 kDa, 26 kDa and with minor polypeptide band 16 kDa of molecular weight (Fig. 2). For Jersey bull (Graph 2) fertility associated seminal plasma proteins as F1 (fractions of test tube no. 13, 14 & 15 of ascending loop of first peak), F2 (fractions of test tube no. 16, 17 & 18 of descending loop of first peak) in 10% SDS-PAGE revealed molecular weight were 26 kDa and with minor polypeptide bands 14.3 kDa and 12 kDa (Fig. 1).

Single precipitin line was observed in double immune-diffusion test, when each protein was run against homologous antibodies. Cross reactivity with heterologous antibody raised in rabbit by purified seminal plasma protein of Gir and Jersey bull were also found.



Graph 1: Line diagram showing O.D. values of purified fertility associated seminal plasma proteins of Gir bull by Gel Filtration Chromatography on Sephacryl S200



Graph 2: Line diagram showing O.D. values of purified fertility associated seminal plasma proteins of Jersey bull by Gel Filtration Chromatography on Sephacryl S200

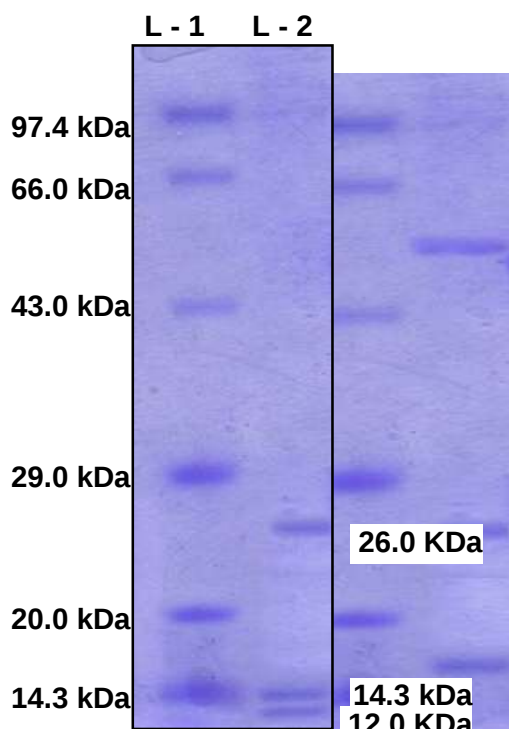


Fig. 1



Fig. 2

Fig. 1 & 2: SDS-PAGE (10% gel) showing purified fertility associated seminal plasma proteins of Jersey and Gir bull respectively along with protein molecular weight marker (Coomassie blue stain).

Lane-1: Protein marker (range: 14.3-97.4 kDa).

Lane-2: Purified fertility associated seminal plasma proteins of Jersey & Gir bull respectively

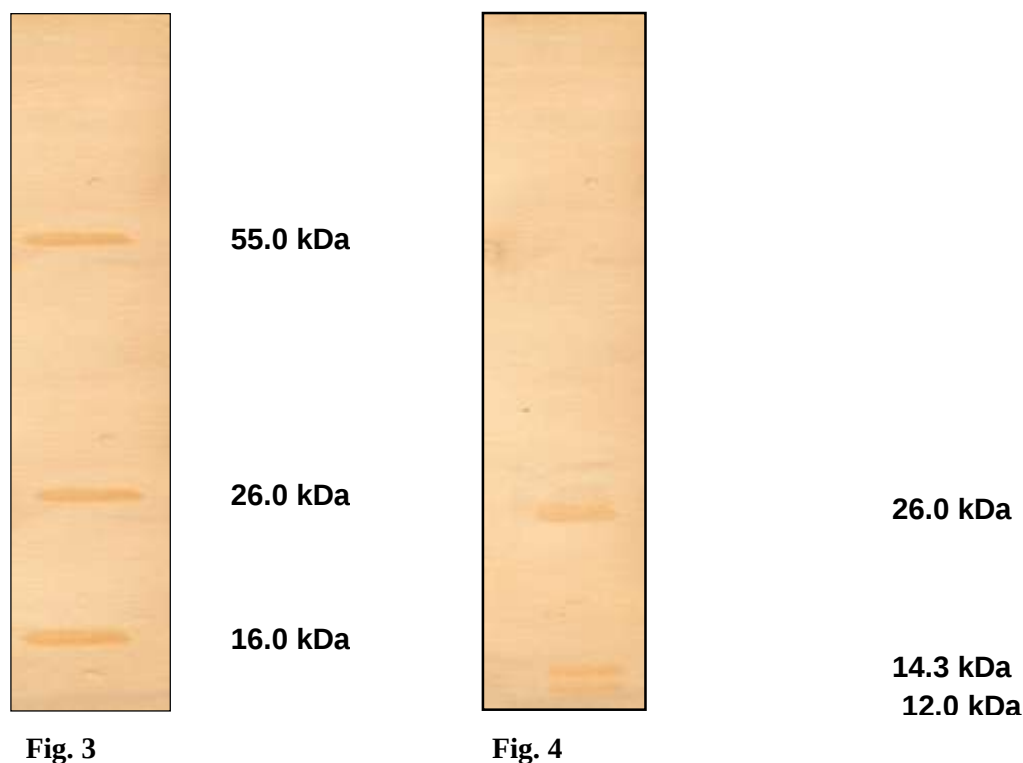


Fig. 3 & 4: Western Blot analysis of purified fertility associated seminal plasma proteins of Gir bull and Jersey bull respectively

The purified seminal plasma proteins were found immune-reactive against respective primary antibody in Western blot analysis Fig. 3 and 4. This may be due to antigenic similarity between the purified fertility associated proteins of Gir and Jersey bull. The hyper-immune serum raised against crude seminal plasma protein recognized the two partially purified polypeptides 55 kDa and 26 kDa in both species. This is in support of the findings of Cancel *et al.* (1999), who recognized the 55 kDa polypeptide in the bull seminal plasma fluids by western blot analysis using whole seminal plasma protein and recognized them as the accessory sex gland polypeptide and identified as Osteopontin. Gerena *et al.* (2000) analyzed a 26 kDa polypeptide from seminal plasma as Lipocalin type prostaglandin D synthase and identified its localization in epididymal tract of bull by western blot analysis and enhancement of sperm maturation. Desnoyers *et al.* (1994) reported that using specific antibodies, western blot of bovine seminal plasma proteins detected spots at 15 kDa and 16 kDa of gel filtration.

In Indirect ELISA (Table 1 & 2) Gir crude, frozen and purified seminal plasma protein showed higher ($P>0.01$) immunoreactive against homologous hyper immune serum compared to Jersey bull seminal plasma protein. There was no significant difference ($P<0.01$) observed in immune-reactive against heterologous hyper immune serum of crude and purified at the 1:400 dilution of Gir and Jersey bull seminal

plasma, which indicated that there might be some share epitopes between two breed of bull. The finding is supported by Ghosh *et al.* (2008) who showed specific immune-reactivity in DID, Western Blot and Indirect ELISA in case of 55 kDa fertility associated protein in seminal plasma.

Table 1: Comparative seroreactivity of crude, frozen and purified fertility associated seminal plasma proteins of Gir and Jersey bull by indirect ELISA using homologous hyperimmune sera raised in rabbit

Antigen	Homologous HIS				P Value
	0.180555556	0.319444444	0.597222222	1.152777778	
Gir Crude Seminal plasma	1.425 ^{xa} ±0.001	1.397 ^{wb} ±0.02	1.327 ^{wc} ±0.001	1.30 ^{wd} ±0.02	P<0.01
Gir Frozen Seminal plasma	1.422 ^{xa} ±0.05	1.389 ^{wb} ±0.07	1.325 ^{wc} ±0.07	1.26 ^{wd} ±0.04	P<0.01
Gir Purified seminal Plasma	1.416 ^{wa} ±0.02	1.386 ^{xb} ±0.04	1.312 ^{xc} ±0.05	1.254 ^{xd} ±0.05	P<0.01
Jersey Crude Seminal plasma	1.395 ^{ya} ±0.05	1.289 ^{yb} ±0.08	1.276 ^{yc} ±0.02	1.248 ^{yd} ±0.07	P<0.01
Jersey Frozen Seminal plasma	1.390 ^{ya} ±0.02	1.278 ^{yb} ±0.09	1.267 ^{yc} ±0.08	1.236 ^{yd} ±0.04	P<0.01
Jersey Purified seminal Plasma	1.312 ^{ya} ±0.05	1.268 ^{yb} ±0.04	1.245 ^{yc} ±0.05	1.232 ^{zd} ±0.07	P<0.01
P Value	P<0.01	P<0.01	P<0.01	P<0.01	

*a-d Mean and SE values bearing no common superscript in a row and w-z Mean and SE values bearing no common superscript in a column varies significantly (P<0.01).

Table 2: Comparative cross seroreactivity of crude, frozen and purified fertility associated seminal plasma proteins of Gir and Jersey bull by indirect ELISA using heterologous hyperimmune sera raised in rabbit

Antigen	Heterologous HIS				P Value
	0.180555556	0.319444444	0.597222222	1.152777778	
Gir Crude Seminal plasma	1.475 ^{wa} ±0.001	1.402 ^{wab} ±0.07	1.398 ^{wb} ±0.001	1.352 ^{wc} ±0.05	P<0.01
Gir Frozen Seminal plasma	1.462 ^{wa} ±0.001	1.398 ^{wb} ±0.05	1.392 ^{xc} ±0.001	1.348 ^{wd} ±0.05	P<0.01
Gir Purified seminal Plasma	1.460 ^{xa} ±0.02	1.390 ^{xb} ±0.02	1.385 ^{zbc} ±0.05	1.324 ^{zd} ±0.04	P<0.01
Jersey Crude Seminal plasma	1.356 ^{wxa} ±0.05	1.347 ^{wb} ±0.08	1.342 ^{xc} ±0.02	1.252 ^{wd} ±0.07	P<0.01
Jersey Frozen Seminal plasma	1.351 ^{wxa} ±0.02	1.345 ^{wab} ±0.09	1.339 ^{xc} ±0.08	1.248 ^{wd} ±0.04	P<0.01
Jersey Purified seminal Plasma	1.297 ^{wxa} ±0.05	1.254 ^{xb} ±0.04	1.248 ^{zc} ±0.05	1.240 ^{xd} ±0.07	P<0.01
P Value	P<0.01	P<0.01	P<0.01	P<0.01	

*a-d Mean and SE values bearing no common superscript in a row and w-z Mean and SE values bearing no common superscript in a column varies significantly

Conclusion

In the present study, Gir and Jersey bull seminal plasma was assessed for the detection of fertility associated protein. These proteins can be efficiently isolated and purified by gel filtration chromatography and purified fertility associated proteins were found immune-reactive. The molecular weights of Gir bull purified seminal plasma fertility associated proteins were 55 kDa, 26 kDa and 16 kDa. The molecular weights of Jersey bull purified seminal plasma fertility associated proteins were 26 kDa, 12 kDa and 14.3 kDa. These polypeptides isolated from Gir and Jersey bull crude seminal plasma were highly sero-reactive when studied by DID, Western Blot and ELISA. Fertility associated proteins of Gir and Jersey bull were immunochemically identified. It was evident that two different breeds of bull, Gir (indigenous breed) and



Jersey (exotic breed) semen characteristics were almost similar. It could therefore be summarized that, semen of the Gir could be the good substitute of Jersey semen used for artificial insemination but the duration of preservation would be less.

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