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Comparative seminal plasma fertility associated proteins profiling of cattle and buffalo bulls

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Abstract
This study was to compare the seminal plasma proteins profiles of Gir bull and Murrah buffalo bull to examine the reproductive performance in terms of seminal plasma polypeptide properties as well as semen concentration, Mass and Individual motility and shape of spermatozoa. Purification and immuno-biochemical characterization of fertility associated proteins in Murrah and Gir bulls are major immuno-dominant proteins that have a significant impact in fertility. Seminal plasma was collected from evidently normal semen comprising viable sperm for extraction. The Murrah buffalo and Gir cattle herds contribute the most to India's milk production, hence it is important to comprehend the proteomics of Gir bull seminal plasma proteins and to compare them to those of buffalo bulls in order to better understand their molecular characteristics. Therefore, this research compares and contrasts our existing understanding of the expanding field.

Keywords: Protein profiling, seminal plasma, immuno-dominant, Murrah buffalo bull, Gir bull

Introduction
To ensure optimal reproductive efficiency in the cattle and artificial insemination industries, production-tested sires with high fertilizing capacity are required. Focusing on the proteomics of this fluid and the different functions that it would have in regard to sperm activity and signaling to the female, the current paper attempts to view the aspects of the seminal plasma of Gir bulls and Murrah buffalo bulls with a focus on its role in modulating fertility as the final goal. The protein composition of seminal plasma varies in species and has important effect on sperm functions such as sperm motility, viability and freezability [1], sperm capacitation and fertilization [14] and also serve to protect sperm from damage or to maintain their longevity which were evident from the correlation observed among semen characteristic and seminal plasma proteins reported [16].
The ability to choose high fertility sires produces semen samples of the highest quality, which will ultimately increase the success rates. Molecular components of the seminal plasma are being studied as additional fertility markers because routine semen analysis has revealed the existence of sub fertile sires that seem to have normal semen quality [2]. BSP proteins biological characteristics have been thoroughly investigated. This fluid may also have an effect on bull fertility and sperm storage; however, its involvement in storage is remaining controversial [8]. The ability to screen for viable sperm is also necessary for the effectiveness of artificial insemination and for the potential impact of seminal plasma [7]. Therefore, it is crucial to look into fertility-related concerns given the biological and economic implications of knowing with certainty the potential fertility of the sperm for artificial insemination prior to insemination. However, there is only a limited amount of comparative data available on the seminal plasma proteins of the Gir and Murrah bull. The contrasting physiological roles of seminal plasma proteins are compared in this study, along with how they relate to fertility in the Murrah buffalo and Gir bull.

Materials and Methods
Sample collection and semen evaluation: The ejaculate was carried to the lab for immediate evaluation of semen characteristics in terms of mass activity, motility, and spermatozoa morphology in according to standard method [17].

Fresh semen samples were collected from six each Murrah buffalo bulls and Gir bulls of sound reproductive health maintained. Semen samples with sperm of poor quality were discarded.

Preparation of crude seminal plasma: The seminal plasma was separated at 3000 rpm for 20 minutes at 4 °C by centrifugation. The supernatant was carefully collected, and the seminal plasma concentrations were measured [12]. The samples were then extracted and stored at-20 °C until further use. The polypeptide bands of the seminal plasma were detected using 10% SDS-PAGE [11].

Preparation of purified seminal plasma antigens by Gel Filtration Chromatography: At a flow rate of 20 ml per hour, prepared of purified seminal plasma antigen by gel filtration chromatography in a column on Sephacryl S-200. The protein distribution was determined by measuring the absorbance at 280 nm with a UV/VIS spectrophotometer. The dialysis membrane was using to concentrate against sugar. The protein concentrations of seminal plasma fractions were measured as per standard method [12]. The concentrated samples were then stored in aliquots at-20 °C for future use.

Immunobiochemical characterization of purified fertility associated seminal plasma proteins: The purified fertility related protein was detected using 10% SDS-PAGE [11]. One-dimensional SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) was used to analyze crude and purified samples of Murrah and Gir bull seminal plasma. SDS-PAGE was used to determine molecular weights using protein markers using Gel Documentation systems (Bio-Rad).

Immunochemical Analysis: For the purpose of generating hyperimmune serum (purified seminal plasma proteins of Murrah and Gir bull, respectively) rabbits were chosen. Separation of proteins were done by some modified method [20, 9] and then performed western blotting in the resulting protein.

Indirect ELISA was used to determine sero-reactivity in crude and purified seminal plasma proteins from Murrah and Gir bulls, with some changes of standard method [15]. Homologous and heterologous highly immune serum was used in a t-test for indirect ELISA statistical analysis [18].

Results

Semen evaluation was done in terms of semen volume (ml), mass activity, motility percentage and semen concentration (Table No.1). When stained with Rose Bengal, the spermatozoa of Gir and Murrah bulls had a morphology that was 80% normal.

Table 1: Seminal Characters of Gir bull and Murrah bulls

Semen Parameters	Gir bull	Murrah bull
Semen Volume (ml)	4.14 ± 1.17^{a}	3.2 ± 0.24^{a}
Semen concentration (10^6 cells/ml)	1516.0 ± 186.0^{a}	1411.0 ± 267.10^{a}
Seminal Plasma Protein (mg/ml)	25.45 ± 0.45^{a}	21.57 ± 0.63^{b}

Means bearing at least one common superscript alphabet in one parameter did not differs significantly ($P\geq0.05$), otherwise significant at 5% level ($p<0.05$).

The statistical analysis of the results revealed that there was no significant difference in ejaculate volume and sperm concentration between buffalo and cattle bull semen, however there was a significant difference ($p<0.01$) in seminal plasma protein content between buffalo and cattle bull semen.

Crude seminal plasma was purified by gel filtration chromatography and molecular weight was determined. Purified seminal plasma revealed polypeptide band of 12 kDa, 14.3 kDa, 16 kDa, 26.5 kDa, 55kDa. In buffalo bull 14.4 kDa; 24.5 kDa; 35.2 kDa; 45 kDa, 55 kDa and 85 kDa protein bands were recognized.

Western blot examination in both animals, the hyper-immune serum raised against crude seminal plasma protein recognized the two partially purified polypeptides 55 kDa and 26.5 kDa. In indirect ELISA (Tables 2 and 3), cattle crude and purified seminal plasma protein were more immunoreactive ($p<0.01$) against homologous highly immune serum than Murrah bull seminal plasma protein. There was no significant difference ($p<0.01$) in immuno-reactive against heterologous hyper immune serum of crude and purified at the 1:400 dilution of Gir and Murrah bull seminal plasma, indicating that the two breeds of bull may share epitopes.

Table 2: Comparative ELISA homologous seroreactivity of crude & purified FAS

Ag	Homologous HIS				P Value
	1:200	1:400	1:800	1:1600	
CCSP	$1.425^{xa}\pm0.001$	$1.397^{wb}\pm0.02$	$1.327^{wc}\pm0.001$	$1.30^{wd}\pm0.02$	$p<0.01$
CPSP	$1.416^{wa}\pm0.02$	$1.386^{xb}\pm0.04$	$1.312^{xc}\pm0.05$	$1.254^{xd}\pm0.05$	
BCSP	$1.395^{ya}\pm0.05$	$1.289^{yb}\pm0.08$	$1.276^{yc}\pm0.02$	$1.248^{yd}\pm0.07$	
BPSP	$1.312^{ya}\pm0.05$	$1.268^{yb}\pm0.04$	$1.245^{yc}\pm0.05$	$1.232^{zd}\pm0.07$	
P Value	$p<0.01$				

*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 ($p<0.01$).

(CCSP-Cattle Crude Seminal plasma CPSP-Cattle Purified seminal Plasma, BCSP-Buffalo Crude Seminal plasma, BPSP-Buffalo Purified seminal Plasma)

Table 3: Comparative ELISA heterologous serore activity of crude & purified FAS

Ag	Heterologous HIS				P Value
	1:200	1:400	1:800	1:1600	
CCSP	1.475^{wa}±0.001	1.402^{wab}±0.07	1.398^{wb}±0.001	1.352^{wc}±0.05	
CPSP	1.460^{xa}±0.02	1.390^{xb}±0.02	1.385^{zbc}±0.05	1.324^{zd}±0.04	
BCSP	1.356^{wxa}±0.05	1.347^{wb}±0.08	1.342^{xc}±0.02	1.252^{wd}±0.07	p<0.01
BPSP	1.297^{wxa}±0.05	1.254^{xb}±0.04	1.248^{zc}±0.05	1.240^{xd}±0.07	
P Value	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).

(Ag-Antigen, CCSP-Cattle Crude Seminal plasma CPSP-Cattle Purified seminal Plasma, BCSP-Buffalo Crude Seminal plasma, BPSP-Buffalo Purified seminal Plasma)

Discussion

The current study found that buffalo had lower ejaculate volume and sperm concentration than cattle bulls, which supports the findings [10] in Nilliravi buffalo and Holestein cow bulls. Despite the insignificance of differences semen parameters between the two species, buffalo bulls had lower values of ejaculate volume and sperm concentration than cow bulls (Table 1).Variations in sperm volume and concentration may be related to changes in collection frequency, season, diet, management, age, genetics and reproductive biology [19]. Variations can also be attributed to the skill of the sperm collector/attendant and the temperature of the AV.

SDS-PAGE of crude seminal plasma was performed and several polypeptide bands were identified, with corresponded to the findings [1]. However, the molecular weight of the polypeptide bands could not be determined because seminal plasma protein could exist in aggregated forms in normal physiological conditions. This supports the findings [13], who described the aggregation of boar seminal plasma protein, which is mainly glycoprotein in origin. In *Bos taurus* bulls simulates the findings of present study [5], which was also identified [1] the 55 kDa polypeptide in the bull in both species seminal plasma fluids by western blot analysis using whole seminal plasma protein and identified them as the accessory sex gland polypeptide and identified as Osteopontin. 26 kDa polypeptide was identified [4] from seminal plasma as Lipocalin type prostaglandin D synthase and identified its localisation in the epididymal tract of a bovine bull by western blot analysis and increase of sperm maturation. Particular antibodies [3] reported that western blot of bovine seminal plasma proteins found spots at 15 kDa and 16 kDa of gel filtration. In Western blot examination, the isolated seminal plasma proteins were revealed to be immune-reactive against the corresponding primary antibodies. This could be because the purified fertility related proteins of Gir and Murrah bull have antigenic similarities. Specific immune-reactivity in Western Blot was observed [6] and Indirect ELISA in the case of 55 kDa fertility related protein in seminal plasma.

Conclusion

Individual cattle bulls and buffalo bulls with high and low semen quality had different seminal plasma protein profiles, and seminal plasma proteins in cow bulls were similar to those described in other buffalo bulls. The disparity in protein profiles suggests that species differences may explain for the varying fertility and freezeability of buffalo and cow sperm. This finding lends credence to the theory that seminal plasma proteins influence sperm function in many ways. Additional research is needed to identify the proteins that alter sperm viability and the methods by which they act.

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Conflict of interest declaration

The authors declare that there is no conflict of interest in this research article.

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