Heparin binding proteins and their correlation with *in vitro* sperm characters of Black Bengal buck semen

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Introduction

- Selection of breeding male with high fertility is essential to get optimal conception rate upon artificial insemination (AI)
- Currently breeding soundness examination (BSE) is carried out to select breeding males
- Bulls which had passed through BSE, had difference of 20-25 % conception rate (Larson and Miller, 2000)
- Difference in fertility is not addressed by regular laboratory tests

- Seminal plasma influences the sperm functions and fertility

- Seminal proteins
  - mediate the binding of sperm cells to oviductal epithelium (Moura et al., 2006)
  - preserve sperm membrane integrity (Karunakaran et al., 2016)
  - anti-apoptotic (Rangaswami et al., 2006)
  - controls oxidative stress
  - promotes sperm capacitation (Therein et al., 1998)

- Seminal proteins –
  - Osteopontin
  - prostaglandin D synthase
  - BSP A_1, A_2, A_3
  - HBPs- markers of bull fertility (Sprott et al., 2000; McCauley et al., 2001; Moura *et al*., 2006; Karunakaran et al., 2016)

Black Bengal goat

- Precious germplasm of WB, Bangladesh, Odisha, Jharkhand and NE states

- Known for fertility, fecundity, adaptability and meat quality

AI in goat is gaining importance

- 5000 – 6000 AI/ month in WB

- To get optimal conception rate upon AI, the buck selected as semen donor should have high fertilizing potential

Objectives

- To study the *in vitro* sperm characters of Black Bengal buck
- To isolate and characterize seminal plasma and sperm proteins of Black Bengal buck
- To study the correlation between seminal proteins and *in-vitro* sperm characters and freezability of Black Bengal buck semen

Methodology

- 9 Black Bengal bucks maintained at Eastern Regional Station of ICAR-NDRI, Kalyani

- Semen ejaculates were collected by AV method

- A total of 20 ejaculates (10x2) from each buck were used

Evaluation of neat semen- Volume, Sperm cell concentration, Mass motility, Individual motility, Functional membrane integrity, Morphology

In vitro characters studied after dilution with buffer, equilibration, freeze- thaw

i). Progressive forward motility

ii). Functional membrane integrity using osmotic resistance test

iii). Estimation of lipid peroxidation compound malondioldehyde (MDA) using TBA-TCA reagent

2. Isolation and characterization of seminal proteins

- Seminal plasma proteins were extracted by ice cold ethanol method
- Sperm proteins were extracted by Triton X detergent extract method
- Heparin binding proteins from sperm and seminal plasma were isolated using heparin-sepharose affinity chromatography
- SDS-PAGE was performed using total proteins as well as heparin binding proteins

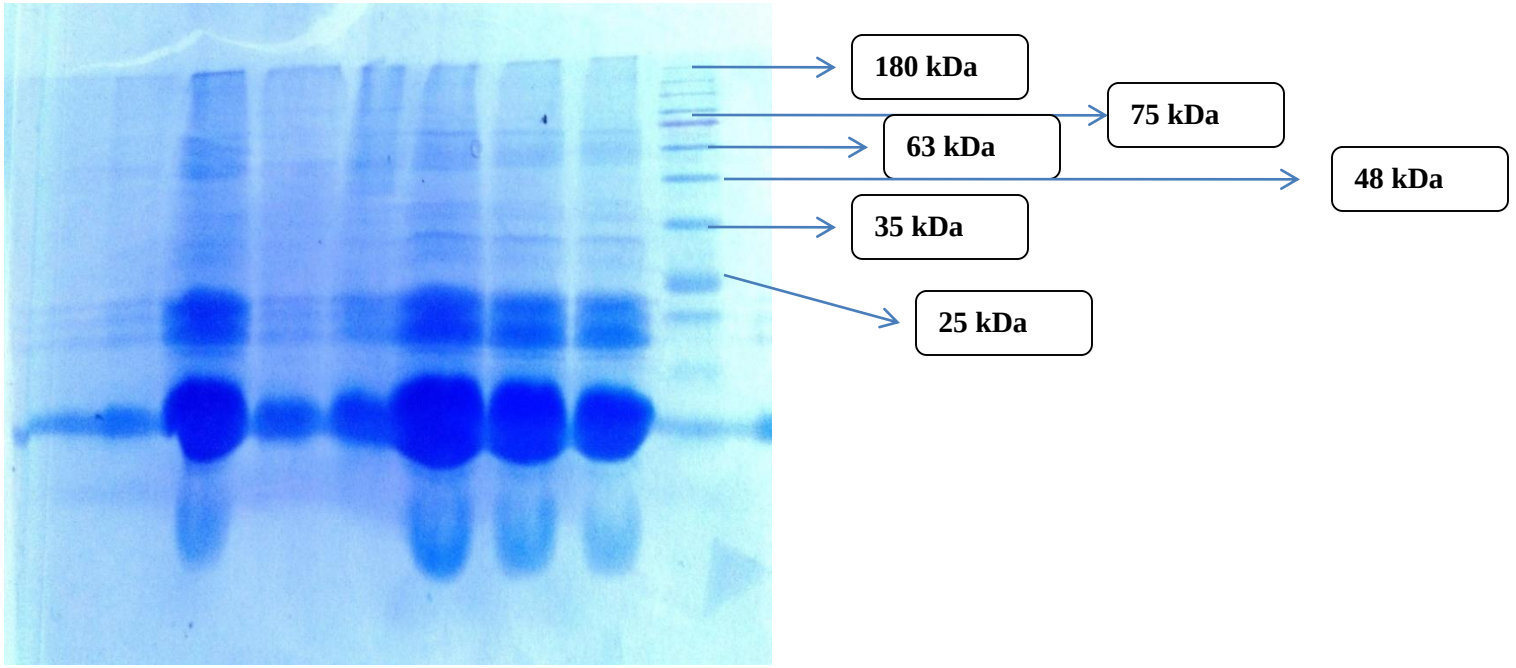
RESULTS- In vitro sperm characters (mean ± SEM)

	Neat semen						After Equilibration			Post Freeze- Thaw		
	Volume (µl)	Mass motility	Individual motility (%)	Functional membrane integrity (%)	Sperm concentration (millions/ml)	Abnormal count (%)	Motility (%)	FMI (%)	MDA (µ mol/ml)	Motility (%)	FMI (%)	MDA (µ mol/ml)
Mean	397.40±8.52	4.2±0.07	69.6±0.96	71.8±0.87	2517.5±17.86	4.8±0.11	56±0.58	60±0.68	0.17±0.04	44.3±0.57	41.1±0.51	0.3±0.01
Buck No.	**	**	**	**	**	**	**	**	**	**	**	**
46	490.20±25.36^{a}	4.6±0.21^{a}	82.4±2.85^{a}	75.3±2.58^{bc}	2533.1±53.13^{bc}	3.7±0.32^{b}	60±1.98^{a}	55±1.82^{a}	0.33±0.024^{a}	54.5±1.71^{a}	51.3±1.52^{a}	0.57±0.033^{a}
48	485.20±25.36^{a}	3.4±0.21^{b}	62.4±2.85^{bc}	58.0±2.58^{de}	2594.1±53.13^{ab}	5.0±0.32^{ab}	45±1.98^{b}	45±1.82^{b}	0.23±0.024^{a}	30.0±1.71^{b}	27.7±1.52^{b}	0.32±0.033^{a}
51	485.20±25.36^{a}	3.1±0.21^{bc}	60.9±2.85^{bcd}	73.2±2.58^{a}	3020.1±53.13^{a}	4.7±0.32^{ab}	42±1.98^{b}	44±1.82^{b}	0.25±0.024^{a}	26.5±1.71^{b}	27.2±1.52^{b}	0.28±0.033^{a}
52	415.20±25.36^{a}	2.9±0.21^{bc}	49.9±2.85^{e}	61.4±2.58^{bcd}	2339.6±53.13^{c}	4.9±0.32^{ab}	40±1.98^{b}	45±1.82^{b}	0.21±0.024^{b}	28.5±1.71^{b}	25.3±1.52^{b}	0.38±0.033^{a}
53	425.2±25.36^{a}	2.7±0.21^{c}	49.9±2.85^{e}	62.6±2.58^{bcd}	2417.4±53.13^{c}	4.4±0.32^{ab}	42±1.98^{b}	41±1.82^{b}	0.22±0.024^{b}	33.0±1.71^{ab}	30.8±1.52^{ab}	0.45±0.033^{b}
55	455.2±25.36^{a}	4.8±0.21^{ab}	77.9±2.85^{ab}	81.7±2.58^{ab}	2406.6±53.13^{c}	4.7±0.32^{ab}	66±1.98^{a}	66±1.82^{a}	0.21±0.024^{a}	50.5±1.71^{a}	46.4±1.52^{a}	0.63±0.033^{a}
57	290.2±25.36^{b}	2.8±0.21^{c}	63.4±2.85^{ab}	60.7±2.58^{cd}	2486.6±53.13^{bc}	4.8±0.32^{ab}	48±1.98^{ab}	44±1.82^{b}	0.07±0.024^{b}	34.5±1.71^{ab}	27.1±1.52^{b}	0.13±0.033^{b}
59	270.2±25.36^{b}	2.7±0.21^{c}	54.4±2.85^{de}	51.5±2.58^{e}	2329.6±53.13^{c}	5.7±0.32^{a}	45±1.98^{ab}	41±1.82^{ab}	0.08±0.024^{b}	39.0±1.71^{ab}	34.3±1.52^{ab}	0.13±0.033^{b}
67	260.2±25.36^{b}	2.8±0.21^{c}	55.4±2.85^{cde}	51.8±2.58^{e}	2530.1±53.13^{bc}	5.7±0.32^{a}	44±1.98^{ab}	40±1.82^{ab}	0.07±0.024^{b}	35.5±1.71^{ab}	29.2±1.52^{ab}	0.13±0.033^{b}

Data shown all mean ± SEM (n = 10)

Means in a column with different superscripts a, b, c, d and e differ significantly at P < 0.01

Characterization of seminal proteins



Sperm proteins

Electrophoretic profile of seminal plasma proteins

10 protein bands with Mol. wt ranging from 17 to 180 kDa were observed in the SDS-PAGE of seminal plasma proteins

Protein Band	Presence (%)
75 kDa, 62- 49 kDa, 20, 17 kDa	100 %
180-136 and 134-101 kDa	55.55%
48 kDa	33.33%
47 – 36, 35 and 34- 25 kDa	44.44%

Electrophoretic profile of sperm proteins

9 bands starting from 17 to 134 kDa

Protein Band	Presence	Buck numbers
75, 20 and 17 kDa	100 %	46, 48, 51, 52, 53, 55, 57, 59, 67
134-101 kDa	44.44%	46, 55, 57, 59
100 kDa	77.77 %	46, 48,51,52,53 57, 67
62-49 kDa	66.66%	46, 48, 51, 52, 53, 67
63 kDa	55.55 %	52, 53, 55, 57, 59
47 – 36 kDa	55.55%	46, 51, 55, 57, 59
35 kDa	33.33%	46, 51, 57

Heparin binding proteins of seminal plasma

- 8 Protein bands of molecular weight 17 to 180 kDa

Protein Band	Presence	Buck numbers
75 kDa, 62-49, 20 and 17 kDa	100%	46, 48, 51, 52, 53, 55, 57, 59, 67
180-136 kDa	55.55%	46, 48,51,52,55
134-101 kDa	77.77%	48, 51, 52,53, 55,57, 67
47-36 kDa	88.88%	46, 51, 52, 53, 55, 57, 59, 67
35-25 kDa	22.22%	46 and 55

Heparin binding proteins of sperm

- 7 protein bands of 17kDa to 134 kDa

Protein Band	Presence	Buck numbers
17 kDa and 20 kDa	100%	46, 48, 51, 52, 53, 55, 57, 59, 67
134-101 kDa	33.33%	46, 48, 55
100 kDa	55.55%	46, 48, 51, 52, 67
75 kDa	66.66%	46, 52, 53, 55, 57, 59
62-49 kDa	88.88%	46, 48, 51, 52, 53, 55, 59, 67
47-36 kDa	33.33%	46, 52, 67

Correlation between proteins and in vitro sperm characters

180 -136 kDa Heparin binding protein of seminal plasma showed

- In neat semen high correlation with Mass Motility(0.711) , HOST(0.699) and moderate correlation with Volume(0.491)and Individual Motility(0.581) .
- In equilibration period high correlation with HOST (0.707) and negative correlation with MDA (-0.825) ,moderate correlation with Individual Motility(0.51)
- In post thaw parameters moderate correlation with HOST(0.532)

134-101 kDa Heparin binding protein of sperm showed-

- In neat semen high correlation with Mass Motility (0.741) and moderate correlation with individual motility (0.491) and moderate negative correlation with abnormal count (-0.462)
- In the examination of equilibration parameters it showed high correlation with individual motility (0.653) and moderate correlation with HOST(0.485) ,
- In the post-thaw analysis it shows high correlation with HOST (0.675) moderate correlation with Individual Motility (0.44)

Conclusion

- Seminal proteins influence the in vitro sperm characters and freezability

- Further studies on characterization of proteins and conception rate study needs to be carried out to find whether these proteins can be used as marker for buck selection.

Thank you