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Electrophoretic profile of bull sperm membrane proteins as a tool for selection of breeding bull

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ABSTRACT

Proteins present in the seminal plasma and sperm influence sperm function and fertilization. The present study was carried out to characterize the electrophoretic profile of bull sperm membrane proteins and to correlate with bull fertility. Semen samples were collected from 20 breeding bulls (10 Jersey and 10 Jersey crossbred) and the sperm membrane proteins were extracted by Triton X. Heparin binding proteins were eluted with heparin column, and the molecular weight of the proteins was assessed by discontinuous sodium dodecyl sulphate polyacrylamide gel elecrophoresis (SDS-PAGE). To assess the field fertility of the bulls, 50 frozen semen straws/bull were used for insemination. Results indicated that only 50 % of the bulls screened had 28– 30 kDa heparin binding proteins in their sperm and these bulls had 12 % higher conception than the bulls lack the protein. So, it can be concluded that the bulls positive for of 28– 30 kDa heparin binding proteins in sperm membrane protein had higher chance of fertility and screening for its presence can be used as a tool in addition to routine criteria for selecting bulls.

Key words: Bull, Fertility, Heparin binding proteins, Seminal plasma, Sperm

Seminal plasma, a complex mixture of secretions from testis, epididymis and accessory sex glands contained factors that modulate the fertilizing ability of sperm (Yanagimachi 1994). It was suggested that seminal proteins mediate the binding of sperm cells to oviductal epithelium and preserve membrane integrity, exerting inhibiting effects on the mitochondrial activity and metabolism to conserve energy needed until fertilization as well as to minimize the production of reactive oxygen species and lipid peroxidation of sperm membrane (Schoneck *et al*. 1996). The proteins were topographically reorganized into specific regions of the sperm surface (Wolf and Voglmayr 1984) and changed the properties of the sperm membrane by binding to it and/or modifying the structure or the arrangement of the existing membrane molecules. Proteins such as osteopontin, prostaglandin D synthase, bovine seminal plasma proteins (BSP A_1, A_2, A_3) and heparin binding proteins (HBP) were reported as indicators of bull fertility (Killian *et al.* 1993, Bellin *et al.* 1994, Gerena *et al*. 1998, Sprott *et al*. 2000, McCauley *et al*. 2001, Moura *et al*. 2006). 28 – 30 kDa HBP of sperm membrane was considered as one of the genetic markers for male fertility and heritable character. The present study was conducted to study the electrophoretic profile of sperm membrane proteins, heparin binding proteins (HBP) of sperm membrane and to correlate with field fertility.

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MATERIALS AND METHODS

Semen ejaculates were obtained by artificial vagina method from 20 adult trained bulls (10 Jersey and 10 Jersey crossbred). Seminal plasma and sperm cells were separated immediately after collection by centrifugation (560g for 10 min at 5°C). The sperm pellets were transported in ice to the laboratory and stored at –80°C until extraction of protein.

Sperm membrane proteins were extracted as per Nass *et al.* (1990) and heparin binding proteins were isolated using heparin-sepharose affinity chromatography (heparin- CL agarose prepacked column) as per Manaskova *et al*. (2002). Discontinuous sodium dodecyl sulphate polyacrylamide gel elecrophoresis (SDS-PAGE) was performed according to Laemmli (1970) to characterize the proteins. The gel was stained with coomassie brilliant blue and the apparent

molecular mass was determined by using molecular weight markers and gel documentation and analysis system. Frozen semen straws (50) having more than 50% post-thaw progressive forward motility were procured for each bull and AI was carried out to assess fertility of each bull. Semen for extraction of sperm membrane proteins and for preparation of frozen semen straws for artificial insemination were collected on different days from each bull.

RESULTS AND DISCUSSION

Protein bands in the molecular weight ranging from 3 to 205 kDa were observed in the SDS-PAGE of bovine sperm membrane proteins (Table 1). Protein bands of 66 and 15/14 kDa were present in sperm membrane proteins of all 20 bulls. Protein bands in the molecular weight ranging from 15/14 to 205 kDa were observed in the SDS- PAGE of heparin binding proteins of bovine sperm membrane (Table 1). A total of 10 heparin binding proteins with the molecular weight range of 15 kDa to 205 kDa were observed in sperm membrane. Fertility related heparin binding protein with molecular weight 15/14 kDa was present in all the 20 bulls (100 %) and 28 kDa protein was present in 9 out of 20 bulls (45 %). Bulls which were positive for 28 kDa heparin binding proteins in sperm membrane were also positive for the other fertility associated proteins in total sperm proteins such as 55 kDa, 28 kDa, 26 kDa, 15/14 kDa. Based on the presence of 28 kDa heparin binding protein in the sperm membrane bulls were categorized into 2 groups, group 1 bulls positive for the protein and group 2 bulls negative for the protein. Out of 1000 cows inseminated 38 cows were sold out during the experimental period and the remaining cows were checked for pregnancy; 57.11 % (277 out of 485) cows that were inseminated with semen of group1 bulls were pregnant while only 44.86 % (214 out of 477) cows that were inseminated with semen from group 2 bulls were pregnant.

Heparin binding proteins were secreted from the prostate, seminal vesicles and bulbourethral glands into seminal fluid and bind to sperm at ejaculation (Miller *et al*. 1990, Nass *et al*. 1990). Bulls with increased fertility produced sperm with greater affinity to bind heparin like complex sugars that were commonly found in the reproductive tract of females (Marks and Ax 1985). Heparin binding proteins promote capacitation of sperm cells by increasing the number of heparin binding sites on the sperm surface (Therien *et al*. 1995) and stimulating cholesterol release from the membrane (Therein *et al*. 1998). In female reproductive tract, heparin binding proteins bound sperm interacted with oviductal components like high density lipoproteins which stimulated a second cholesterol efflux resulting in capacitation (Therien *et al*. 1998). Thus, a positive effect of heparin binding proteins on fertility could be linked to its ability to mediate these events which are crucial for successful fertilization. The 28 – 30 kDa heparin binding protein in sperm membrane is a heritable trait (Ax 2004). Bellin *et al*. (1996) and Sprott *et al*. (2000) observed that beef bulls positive for the protein had increased fertility by 9 to 40 % under natural service and artificial breeding. Bellin *et al*. (1998) reported that the percentage of bulls that were negative for 28 kDa heparin binding proteins in sperm membrane among 44 herds ranged from zero to 50 % (average, 12 %; n = 2191 bulls). Given this wide range, the chance of having bulls negative for the protein may be relatively high in some herds. Sprott *et al*. (2000) suggested that screening semen samples for fertility associated antigen (FAA) after a breeding soundness examination for all bulls is prudent whether natural mating or AI is to be employed and FAA negative bulls should be eliminated regardless of

Table 1. Electrophoretic profile of bovine sperm membrane proteins and heparin binding proteins assessed by SDS-PAGE

Protein molecular weight (kDa)	Jersey (n, 10)		Jersey crossbred (n,10)		Total bulls (n, 20)	
	Sperm membrane proteins	HBP of sperm membrane	Sperm membrane proteins	HBP of sperm membrane	Sperm membrane proteins	HBP of sperm membrane
205	5 (50.00)	8(80.00)	9 (90.00)	8(80.00)	14 (70.00)	16(80.00)
160	1 (10.00)	9(90.00)	8 (80.00)	6(60.00)	9 (45.00)	15(75.00)
97	5 (50.00)	9(90.00)	10 (100.00)	6(60.00)	15 (75.00)	15(75.00)
80	0 (0)	0 (0)	2 (20.00)	0 (0)	2 (10.00)	0 (0)
66	10 (100.00)	8(80.00)	10 (100.00)	4(40.00)	20 (100.00)	12(60.00)
55	5 (50.00)	0(0)	4 (40.00)	0(0)	9 (45.00)	0(0)
43	8 (80.00)	8(80.00)	3 (30.00)	4(40.00)	11 (55.00)	12(60.00)
36	4 (40.00)	4(40.00)	6 (60.00)	0(0)	10 (50.00)	4(20.00)
28	9 (90.00)	5(50.00)	10 (100.00)	4(40.00)	19 (95.00)	9(45.00)
26	6 (60.00)	0(0)	6 (60.00)	0(0)	12 (60.00)	0(0)
17	2 (20.00)	0 (0)	3 (30.00)	0 (0)	5 (25.00)	0 (0)
15/14	10 (100.00)	10(100.00)	10 (100.00)	10(100.00)	20 (100.00)	20(100.00)
6.5	6 (60.00)	0 (0)	4 (40.00)	0 (0)	10 (50.00)	0 (0)
3	0 (0)	0 (0)	1 (10.00)	0 (0)	1 (5.00)	0 (0)

Figures in parentheses indicate percentage to total.

whether they are to be used in natural or artificial breeding programme. In the present study bulls which were positive for 28 kDa heparin binding proteins in sperm membrane were also positive for the other fertility associated proteins in such as 55 kDa, 28 kDa, 26 kDa, 15/14 kDa. It was also noticed that bulls positive for 28– 30 kDa protein in their sperm membrane had about 12 % more fertility than the negative bulls.

As the 28 kDa heparin binding protein in the sperm membrane is a heritable character, the bull positive for the protein will also be able to transmit the trait to its progeny and it can be used as a marker for fertility.

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