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Electrophoretic characterization of the serum proteins associated reproductive disorders in cross bred cows

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Abstract
The present study was conducted to establish a suitable marker for diagnosis of reproductive disorder of cross bred cows by characterizing the serum proteins, at Department of Veterinary Biochemistry, College of Veterinary Science & AH, Bhubaneswar from the time period between December 2016 to October 2017. In this study, five groups of animals (each having six animals) such as repeat breeder (RBC), heifer anestrus (HA), repeat breeder heifer (RBH), post-partum anestrus (PPA) cows and normal cycling cows (NC) were taken for experimental purpose. Serum protein from each group was isolated and characterized through one dimensional electrophoresis and subsequent densitometry studies. The electrophoretic characterization revealed that there is an expression of one extra band of proteins (155 kDa) in HA and PPA, but RBH and RBC showed two extra protein bands of 34, 20 kDa and 116, 110 kDa approx. respectively. So it can be concluded that these extra band might be responsible for the reproductive failure and would be a better platform to act as a marker for diagnosis of reproductive disorder.

Keywords: electrophoretic study, reproductive disorder, serum proteins

1. Introduction
In the current scenario, the livestock sector contributes the major economy growth to the farmers as well as to our country [1]. With an annual production of about 130 million tons, India is the highest milk producer in the world contributing 15% of the total world milk production [2], but occurrence of different reproductive disorder which is considered as a major cause of economic loss in dairy herds, affects 10-30% loss of annual milk production in 3-6% of herd animals of developed countries [3]. Different reproductive disorder such as anoestrous, aberration of the normal estrus cycle, estrus behavior, delayed maturity, repeats breeding, which are the major devastating problems in crossbred cows at present scenario, are mainly due to different factors such as environmental, hormonal, infectious and managemental practices [4]. Anestrus impacts a major economic loss through increased intercalving interval, poor net calf crops, production loss, treatment expenses and cost of replacing mature animal with first calving heifer, causes an estimated loss of Rs.193.00 per day in cow [5]. In the present context, there is a need of a reliable diagnostic strategy for development of suitable therapeutic regimen against these reproductive disorders to combact the economic loss. So different serum proteins may act as a biomarker for diagnosis of the aforesaid disorders which needs a thorough characterization of all these proteins. It has been shown that the occurrence of low levels of serum protein such as leptin in cross bred cows are associated with reproductive disorders [6] which prompts to identify certain similar other proteins in those cows to forecast the ailments. Furthermore, the physiological electrophoretic pattern of serum proteins in ruminants can be used as major elite for the diagnosis of different diseases [7]. It has been shown that a 40 kDa protein band of bovine oviductal fluid as a haptoglobin-like protein, associated with ovarian and oviductal tissues [8]. So electrophoretic identification of different proteins in serum are associated with fertility that provide an understanding of contribution of fertility-related proteins to the fertility phenotype of an individual and provide insights into potential applications to improve the fertility of cattle [9]. Keeping the present view in mind, this current study will provide not only for identification of the serum proteins but also for development of suitable management practices against the reproductive disorders in crossbred cows. This study would provide a better platform for other researchers to develop a suitable

biomarker against different reproductive diseases in dairy cattle in nearest future.

2. Materials and Methods

The present study was carried out in crossbred cows and heifers with a history of anestrus/ postpartum anestrus and repeat breeding at Department of Veterinary Biochemistry, College of Veterinary Science and Animal Husbandry, OUAT from time period between December 2015 to August 2016. This study was undertaken making them into five groups such as Heifer anestrous (HA) [Group-I], Repeat breeding heifers (RBH) [Group-II], Repeat breeding cows (RBC) [Group-III], Post-partum anestrous (PPA) [Group-IV] and Normal cycling (NC) [Group-V] containing six animals each by primary observing the physical and clinical symptoms and the serum was collected from all animals by centrifuging at 3000 rpm for 10 minutes in centrifuge; separated aliquots were made for each vial and stored at -20 °C deepfreeze for further analysis. One dimensional sodium dodocyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried for the each collected serum sample by the standard protocol Laemmli [10] in mini protein Tetra system Protean® (Biorad) with 5% and 10% stacking and separating gel respectively. Serum samples with dilution of 1:5 [taking 5 µl of sample mixed with 20 µl of distilled water then mixed with 25 µl sample buffer (Laemmli buffer)] were run in the other wells with marker protein (Spectra BR, Thermo Scientific) in first well, with known molecular weight standard (260kDa, 140kDa, 95kDa, 72kDa, 52kDa, 42kDa, 34kDa, 26kDa, 17kDa and 10kDa), at constant voltage mode of 30 volt/slab for 20 minutes and increased to 60 volt/slab subsequently till tracking dye reached the lower end of the gel in suitable electrophoresis buffer. Coomassie Blue staining solution was used for staining the gel which was stored in 7% acetic acid solution after distaining, till photographed. Densitometric analysis was done by taking the gel images with gel scanner (GS-800 Calibrated Densitometer). The protein profiling of the bands was carried by using gel analyzer 2010 software and molecular weight of protein bands was determined by using "Quantity 1" software.

3. Results and discussion

The electrophoretic characterization of the serum protein is shown in the Fig 1. The study showed that many and variable numbers of protein bands were appeared in each lane under one-dimensional electrophoresis (1DE) which may be due to abrupt changes in protein expression at the end of pregnancy and early post-partum [11]. Visual comparison of gel pictures gave a platform for differentiation of normal cycling cows from other reproductive disease cows which showed extra protein bands in post-partum anestrus, heifer anestrus, repeat breeder cows and also in heifers from normal cycling cows [12].

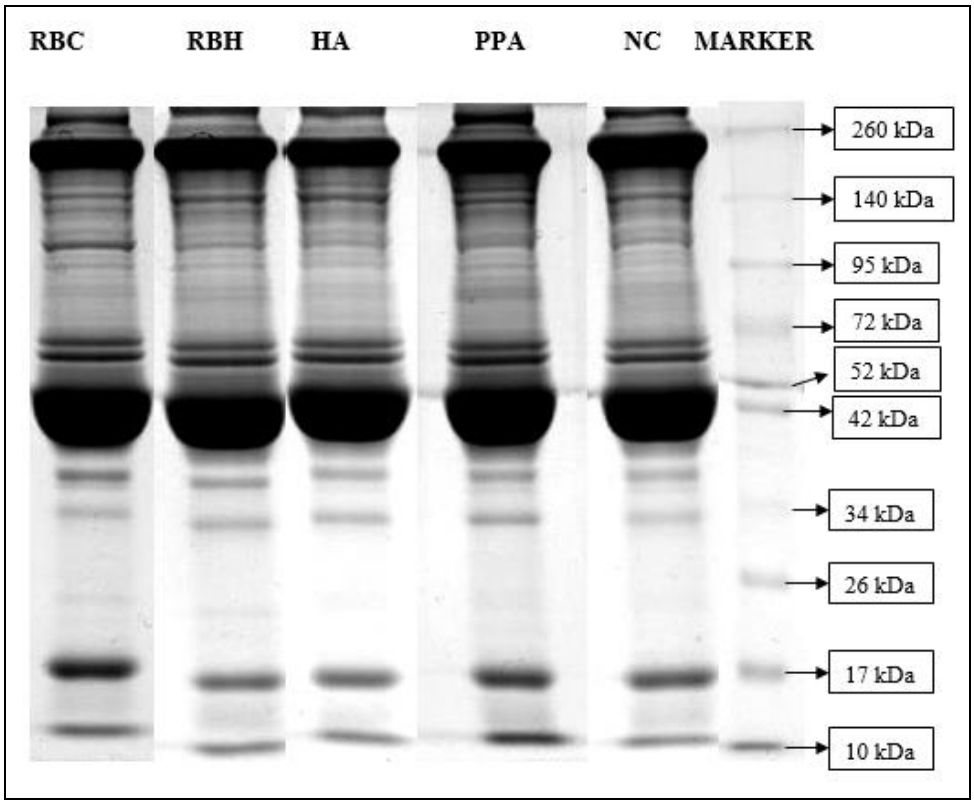

Fig 1: Showing the protein profile of the serum samples 10% 1D SDS-PAGE

The densitometric analysis of protein bands for each of the 5 groups of animals are given in Fig-2. A minimum of 10 peaks were identified for each animal with further subdivision of some fractions giving 11 peaks in heifer anestrus and post-partum anestrous; 12 peaks in repeat breeding heifers and repeat breeding cows. The peaks indicate some Low molecular weight (LMW) proteins that are in the molecular range of 42 kDa, 32 kDa, 24 kDa, 19 kDa, and 5kDa whereas HMW proteins were found in precipitates at the range of 156kDa, 115 kDa, 102 kDa, 95 kDa, 72 kDa, 69 kDa, 58 kDa, 43 kDa. Moreover, there was expression of certain extra protein of molecular weight approximately 155 kDa, 116 kDa, 100 kDa, 34 kDa and 15 kDa which might be responsible for infertility, sterility or reducing reproductive efficiency of repeater as well as anestrous cows [13].

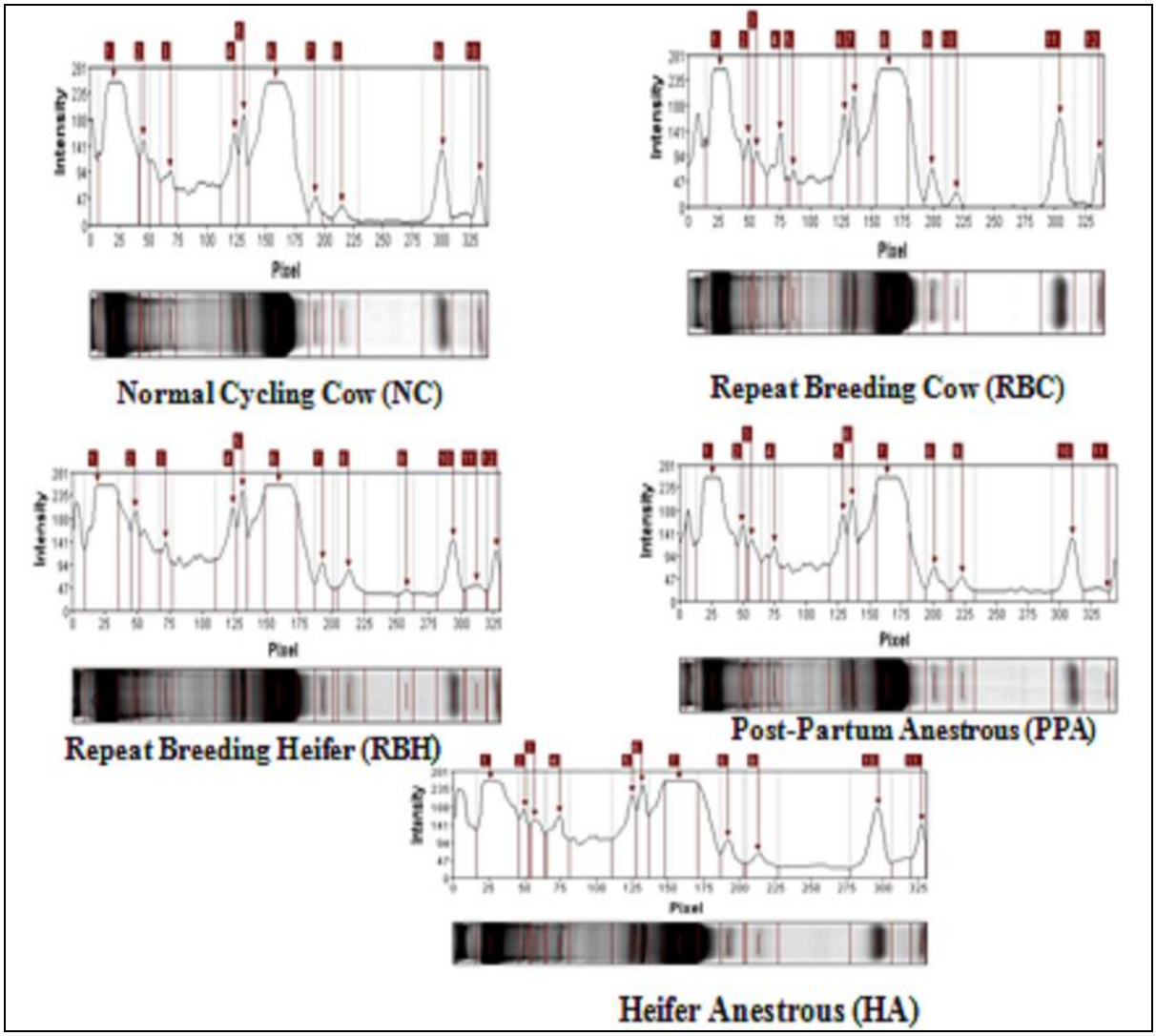

Fig 2: Showing the densitometric analysis of protein bands of all cases of animals

The molecular weights of protein bands of each group of animals were determined by gel scanner and Quantity 1" software shown in Fig-3 and Table-1

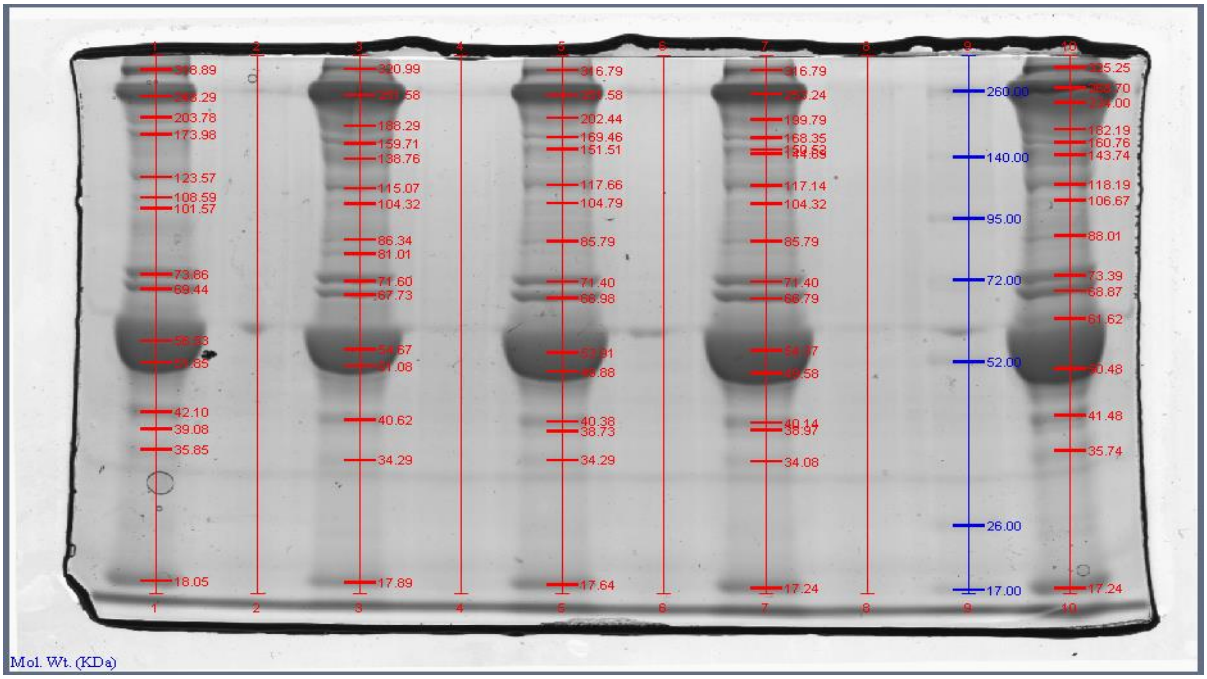

Fig 3: Molecular weight determination of separated proteins in gel by 'Quantity 1' software

Table 1: Molecular weights (kDa) of expressed protein bands in Serum Samples

Molecular weight (KDa) of proteins present in serum sample in each group					
Normal Cycling	**HA**	**RBH**	**RBC**	**PPA**	**Range**
364	362	364	366	363	362-368
342	341	345	344	342	341-345
321	322	324	323	326	322-326
289	282	284	290	292	282-292
253	249	248	250	245	249-253
178	173	172	178	175	172-178
--	155	--	--	155	--
128	128	126	128	128	126-128
--	--	--	116	--	--
--	--	--	100	--	--
65	63	62	61	63	61-65
60	58	58	57	58	57-60
48	49	48	50	50	48-50
42	42	43	42	43	42-43
38	38	38	39	38	38-39
-	-	34	-	-	-
25	22	26	24	22	22-26
-	-	20	-	-	-
15	17	15	16	17	15-17

It has been seen that the normal cycling animals expressed 14 numbers of protein bands 364 kDa, 342 kDa, 321 kDa, 289 kDa, 253 kDa, 178 kDa, 128 kDa, 65 kDa, 60 kDa, 48 kDa, 42 kDa, 38 kDa, 25 kDa and 15 kDa and anestrous group of both heifers and post-partum cows showed 15 numbers of bands such as (362,363) kDa, (341,342) kDa, (322, 326) kDa, (282, 292) kDa, (249,245) kDa, (173,175) kDa, 155 kDa, 128 kDa, 63 kDa, 58 kDa, (49,50) kDa, (42,43) kDa, 38 kDa, 22 kDa and 21 kDa respectively. Similarly repeating heifers showed 16 numbers of bands such as 364 kDa, 345 kDa, 324 kDa, 248 kDa, 172 kDa, 126 kDa, 62 kDa, 58 kDa, 48 kDa, 43 kDa, 38 kDa, 43 kDa, 26 kDa, 20 kDa and 15 kDa and Repeat breeding cow showed a similar trend of 12 numbers of bands were 366 kDa, 344 kDa, 323 kDa, 290 kDa, 250 kDa, 178 kDa, 128 kDa, 116 kDa, 100 kDa, 61 kDa, 57 kDa, 50 kDa, 42 kDa, 39 kDa, 24 kDa and 16 kDa. It may be due to there is a differential expression 9 proteins (including transferrin, albumins, IgG, gamma globulins) between days 21 and 31 of pregnancy [14]. Comparison of proteomes revealed that the most abrupt changes in protein expression occur at the end of pregnancy and early post-partum. Increase in the expression of kininogen were observed until the fifth month of pregnancy, as well as abrupt changes of alpha-2-HS-glycoprotein and apolipoprotein A-IV that decreased at the end of pregnancy and early post-partum. A reverse tendency was observed for alpha-1-antichymotrypsin.Expression of orosomucoid and haptoglobin significantly increased during the last days of pregnancy and after parturition [15]. So the extra bands that were appeared after densitometry and gel scanning, in reproductive disorder animals, can be used as biomarker for diagnostic purpose.

4. Conclusion

This study can be concluded that certain extra bands of different molecular weight proteins identified in repeat breeders as well as anestrous cows as compared to normal cycling cows that might be responsible for the reproductive failure. The electrophoretic characterization revealed that anestrous heifer and post-partum anestrous had the expression of one extra band of protein (155 kDa approx.) as compared to the normal cycling. Similarly repeat breeding cow and repeater heifer had two extra bands of proteins having molecular weights of 34, 20 kDa approx. and 116, 110 kDa approx. respectively, as compared to normal cyclic cows which might be responsible for reproductive disorder there by reducing their productive efficiency and could be used as protein markers for reproductive disorders. Therefore, our results may be useful in screening and selection of apparently healthy cows with normal reproductive cycle which would increase the sustainability of livestock farms by reducing huge economic losses of the farmer.

5. Acknowledgement

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