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## Comparative electrophoretic studies on serum proteins of murrah buffaloes at various stages of reproduction

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#### Abstract

A comparative study on serum proteins through SDS-PAGE was carried out in pregnant, estrus, anestrus and regular cyclic Murrah buffalo cows. A total of 24 healthy Murrah buffalo cows, aged 3-6 years were selected and they were split into four groups, each group comprising of six animals (n=6) (Group - I : pregnant; Group-II: estrus; Group-III: anestrus and Group-IV: regular cyclic). Animals were vaccinated and dewormed as per the farm schedule. Blood samples were collected in heparinised vacutainer and immediately transferred to the laboratory. The blood samples were centrifuged at 3000 rpm for 15 min. Serum samples were separated and kept at -20 °C in deep freezer until further use. The serum samples were subjected to SDS- PAGE analysis (10 % gel: discontinuous method) under non-reducing conditions. In group I, major bands observed at 240, 200, 70, 66 and 30kDa. In Group II, three prominent bands (120, 66 and 30kDa) and three minor bands (120, 70, 35 kDa) were observed. In Group III, only two prominent (200 and 66 kDa) bands were observed. In Group IV, three prominent bands (200 and 100, 66 kDa) and two minor bands (120, 75 kDa) were observed. The 70 and 30 kDa proteins were found exclusively in pregnant buffalo bows but not in other groups. It was concluded that these two proteins could be used as the biomarkers of early pregnancy detection in Murrah buffalo cows. Moreover, the anestrus group had less protein expression as compared to all other groups and the group of estrus and regular cyclic buffaloes had similar protein pattern of expression.

**Keywords:** SDS-page, serum proteins, estrus, anestrus, pregnant murrah buffaloes

#### Introduction

In India, buffalo (*Bubalus bubalis*) is a chief livestock resource, providing milk, meat and draught power in several ecologically poor agricultural systems, so it is being the basis in rural economy contributing dairy and meat industry. Early pregnancy diagnosis is an important part of sound reproductive management to decrease the inter-calving period. Ruminant placenta produces pregnancy-associated glycoproteins (PAGs) during pregnancy<sup>[1]</sup>. The pregnancy specific proteins are released at the trophoblast-endometrial interface during pregnancy to disrupt normal cyclic luteal regression thereby augment embryonic survival and nourishment of pregnancy<sup>[2, 3]</sup>. Pregnancy marker(s) is (are) embryo specific and depicts presence and/or viability of the embryo and should be considered as an ideal candidate for developing pregnancy diagnostics<sup>[4]</sup>. The discovery of early pregnancy factor in the serum of pregnant cattle, pig and sheep has raised the possibility of accurate diagnosis of early pregnancy in domestic animals<sup>[5]</sup>. However, pregnancy of these animals has not been diagnosed properly using this early pregnancy factor. Reports of serum proteins at various reproductive stages of buffalo are very scanty. Hence, the present study was designed to find out the pregnancy marker and compared the electrophoretic pattern of serum proteins through SDS-PAGE in serum samples of estrus, anestrus, pregnant and regular cyclic buffalo cows.

#### Materials and Methods

This study was conducted at the Department of Veterinary Physiology and Biochemistry, TANUVAS - Veterinary College and Research Institute, Orathanadu, Tamil Nadu, India.

#### Experimental animals

A total of 24 healthy Murrah buffaloes (*Bubalus bubalis*), aged about 3-6 years with good body condition (score 5-6) were selected from the herd of an organized farm, Orathanadu,

Thanjavur, India and were maintained under uniform feeding, housing and managemental conditions. The experimental buffalo cows were grouped into four groups according to their reproductive status by reproductive history, assessing the smoothness of ovary and palpable structures through per rectal examination. Each group consisting of six animals (n=6) viz. group I: pregnant, group II: estrus, group III: anestrus and group IV: regular cyclic buffalo cows. Animals were vaccinated and dewormed as per the farm schedule.

### Sample collection

Blood samples were collected in a heparinised vacutainer and immediately transported to the laboratory. The blood samples were centrifuged at 3000rpm for 15 minutes. Serum was separated and stored at -20°C in deep freezer until further use. The protein content of the sample was estimated by Lowry method [6]. A standard curve was built using bovine serum albumin (BSA) as standard. The photometric estimation was carried out with the help of ELICO SL 207 Mini Spectrophotometer.

### SDS PAGE and analysis

To compare the serum proteins, SDS-PAGE was carried out as described by the method of Laemmli [7]. The resolving gel (8%) was co-polymerized with 0.3% gelatin solution (final concentration of gelatin in gel was 0.15 %) and the electrophoretic run was carried out at 100 V until tracking dye reaches to the bottom. Then renaturation was carried out with renaturation solution (2.5% Triton X -100) for 3 hours on a mechanical shaker with mild agitation. Then developing was carried out by incubating the gel in developing buffer (10 mm CaCl<sub>2</sub>, 0.15 M NaCl and 50 mm Tris (pH 7.5) for 18 hours at 37°C and then stained with 0.25% Coomassie blue for 2 hours followed by destaining for 1 hour with destaining solution and then further destaining was carried out with distilled water.

### Results and Discussion

The present study was carried out to compare the serum proteins through SDS-PAGE on all the four groups of buffaloes at various stages of reproduction. Serum samples of each group was subjected into discontinuous method of SDS-PAGE (10% gel) and results are depicted in the Fig 1.

In group I, on SDS-PAGE (Lane 8, 9), it was revealed five major bands at 240, 200, 70, 66 and 30 kDa range. The results were in agreement with the previous works of various authors [5, 8, 9]. In bubaline species, through SDS-PAGE, nine secretory proteins were observed at >97, 97, 75, 66, 43, 30, 29, 27 and 20 kDa in both oocytes and embryos respectively and concluded that two proteins of 75 and 29 kDa have pregnancy specific as they were found only in estrus and pregnant buffalo cows [8]. These proteins could be used as biomarkers for prediction/diagnosis of early pregnancy in buffaloes [8]. To concur with the present study, Malakar and Majumdar demonstrated that 66, 55 and 45 kDa (embryo) and 95 kDa protein bands were present in mid luteal, estrus and early pregnant goat serum [10]. Similarly, Singh *et al.* [5] reported six polypeptide bands (78, 67, 53, 42, 33 and 26 kDa) from the placental extracts of buffalo. In buffaloes, during early pregnancy, four glycoproteins (86, 67, 56 and 51 kDa) and five glycoproteins (86, 75, 67, 56 and 38 kDa) were identified in the mid and late stages of pregnancy. This might be due to more number of proteins are synthesized during pregnancy and secreted into peripheral circulation [9]. In another study, Glycoproteins isolated from placental extract corresponding

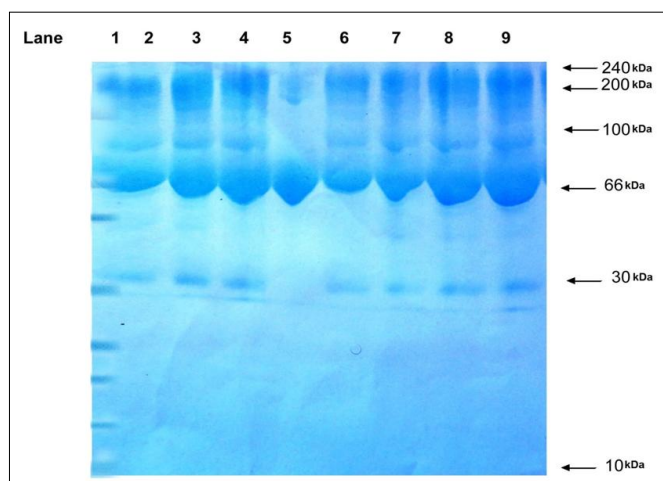
to 75 kDa band on SDS PAGE gel was a mixture of PAG-7 and PAG-11, which may be used as pregnancy diagnosis marker. It may be inferred that placenta synthesizes number of glycoproteins during pregnancy which come into the maternal circulation and can serve as biomarkers of pregnancy [9].

In Group II, major bands at 120, 66 and 30 kDa (Lane 2, 3, 4) were observed and minor bands at 200 and 35 kDa were observed. The present study results were accorded with the results of various authors [8, 11, 12, 13]. Staros and Killian [11] who conducted one-dimensional SDS-PAGE with biotinylated oviductal fluid samples from dairy cows with normal estrus cycles and found biotinylated oviductal fluid proteins with clear molecular masses of 80, 74, 60, 45 and 30 kDa were found to be related with the zona pellucida. Similarly, Kalleshwarappa *et al.* [8] found a band of 27 kDa was expressed in estrus and regular cyclic animal groups and it is an oocyte specific protein, band 66 kDa is a serum albumin. In another study by Kumaresan *et al.* observed the protein pattern of oviductal fluid is generally similar to that of blood serum [12]. Similarly, the protein patterns of non-luteal, luteal, isthmic and ampullary fluids were almost similar among them as well as with serum. However, at least 4 proteins were specific to oviductal fluid with the molecular weight of 95, 43, 29 and 20 kDa. Similarly, Roy *et al.* [13]. Suggested that buffalo uterine secretion contained mainly serum (93.5 kDa) and several uterus specific proteins (560 to 14.3 kDa) and bands 38 and 19.2 kDa were luteal specific.

In group III, the serum samples contained only two prominent 200 and 66 kDa bands (Lane 5) were observed. In group IV, Lane 6, 7 showed major bands at 200, 100, 66 kDa and minor bands at 120 and 30 kDa. Our results are corroborated with the results of Pradeep *et al.* who demonstrated SDS-PAGE of follicular fluid in follicular, luteal, mid-luteal, late luteal and true acyclic phase of buffalo ovaries [14]. He and his co-workers further observed 7 protein bands in the molecular size range of approximately 145 kDa to 15 kDa. Two bands of approximately 107 and 37 kDa size present only in follicular fluid and two bands of approximately 38 and 35 kDa size only in serum samples. They further suggested that follicular fluid protein concentration did not differ significantly during different phases of estrus cycle, but was significantly lower in acyclic ovaries than that of cyclic ones and there might be expression of ovary specific proteins locally during different phases of estrus cycle in Murrah buffaloes.

It was concluded that 70 and 30 kDa bands were observed only in group I (in pregnant buffalo cows) but not in other groups. These two proteins 70 and 30 kDa bands are pregnant specific as they were solely present in blood circulation of pregnant buffaloes. These two proteins could be used as the biomarkers for prediction/diagnosis of pregnancy of buffaloes in future. Group II buffaloes and group IV buffaloes had the similar protein pattern of expression. As compared to all the experimental groups, the group III had lower expression of proteins as they expressed only two major proteins (200 and 66 kDa), it may be due to lower follicular protein concentration expressed in acyclic ovaries [14]. The minor variations in protein expression may be due to the method of reducing conditions, specific source for protein expression used in their study and reproductive status of animal. Further studies on characterizing the unique proteins in respective reproductive stage, purification, characterization and confirmation through immunoblotting techniques may be carried out and defining their role in uterine functions would

help to tackle the cause of low reproduction rate in bubaline species.



Lane 1: Low molecular weight protein markers

Lane 5: Anestrus buffaloes

Lane 8, 9: Pregnant buffaloes

Lane 2, 3, 4: Estrus buffaloes

Lane 6, 7: Regular cyclic buffaloes

**Fig 1:** SDS-PAGE analysis of serum proteins of Murrah buffaloes

### Conclusion

It was concluded that these two proteins could be used as the biomarkers of early pregnancy detection in Murrah buffalo cows. Moreover, the anestrus group had less protein expression as compared to all other groups and the group of estrus and regular cyclic buffaloes had similar protein pattern of expression.

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