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Assessment of Hemato-biochemical, mineral and oxidative changes in postpartum anoestrous (true anoestrus) Murrah Buffaloes

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Abstract
The present study was aimed to assess the hemato-biochemical, mineral and oxidative changes in postpartum anoestrous Murrah buffaloes. A total of 30 multiparous buffaloes of almost same age and body weight were used for the experiment. The experimental group I (GI) consisted of 15 postpartum anoestrous buffaloes (PPA) and control group II (GII) consisted of 15 normal cyclic buffaloes. Blood samples were collected by jugular vein puncture and analyzed for hematological parameters. Further, the serum was separated from another aliquot of blood sample and utilized for biochemical, mineral, MDA and SOD activity. The mean Hb, PCV, glucose, cholesterol and phosphorous values were significantly ($p< 0.05$) lower in PPA compared to cyclical buffaloes. The SOD activity and MDA concentration was significantly ($p< 0.05$) higher in PPA compared to cyclical buffaloes. In contrast, no significant ($p>0.05$) difference was observed in total protein and calcium concentration between groups. From the present study, it was concluded that postpartum anoestrous can be attributed to oxidative stress, which induces significant changes in hemato-biochemical and mineral profile.

Keywords: Postpartum anoestrous, biochemical, malondialdehyde, superoxide dismutase, Murrah buffaloes

Introduction
One of the major constraints for profitable dairy farming is reproductive inefficiency, which may be due to poor metabolic profile, nutritional deficiencies or improper management [1]. As the contribution from buffaloes to the total milk production in India is more than 50%, production potential of the buffaloes is of utmost importance [2]. However, buffaloes are poor breeders due to low reproductive efficiency as they are inherently susceptible to environmental stress; which might be due to poor thermoregulatory mechanisms [3]. The low reproduction efficiency in buffalo is the major constraint to obtain maximum production potential. Regular cyclicity of the animals is of utmost importance for profitable dairying [4].

Anoestrous is one of the commonly occurring reproductive disorders in dairy animals which affect the productivity and economy [5]. While postpartum anoestrous (PPA) is the major reproductive limitation in buffaloes [3], there are many contributing factors that are responsible for this condition. Individually or combined all these factors complicate and make it a complex phenomena [2]. The factors include nutrition, mineral deficiencies [6], season [7], infection [8] and so on. Macro minerals like calcium and phosphorous play a role in regulating the metabolic profile in buffaloes and their deficiencies may lead to reproductive malfunction [3]. The hemato biochemical parameters are key indicators of the physiological condition of the animals; which also reflect production and reproduction. There might be variation in regular cyclic and acyclic animals [3]. There were reports of high incidence of repeat-breeding and anestrous that are associated with the deficiencies of cholesterol [9] glucose [10] and total protein [11] etc. These physiological stressors may increase the production of free radicals that impair folliculogenesis, steroidogenesis and lead to impaired fertility [12]. In this context it is necessary to estimate the various hemato-biochemical, mineral and oxidative changes in relation to PPA condition in order to evaluate some blood biochemical markers associated with anoestrous condition.

Materials and Methods

The present study was carried out at Buffalo Research Station, Vekataramannagudem of Sri Venkateswara Veterinary University and laboratory profiling of different analytes was performed at Department of Veterinary Physiology, NTR College of Veterinary Sciences, Gannavaram. A total of 30 multiparous buffaloes of almost same age and body weight were used in the experiment. Based on farm records buffaloes that had shown anestrous signs for more than 90 days were subjected to rectal palpation and ultrasonographic examination at ten days interval, buffaloes with quiescent ovaries were considered as true anoestrous (GI, n=15), While those animals that had shown normal estrus behavior during the period of study were selected under control (GII, n=15).

Blood samples were collected by jugular vein puncture on day 0 and day 10 from GIand 0 day (day of estrus) from GII into heparinized and serum tubes for hematological analysis and biochemical analysis respectively. Hemoglobin (Hb) was estimated by using Drabkins method [13] and packed cell volume (PCV) by micro haematocrit method [14]. The serum tubes were subjected for centrifugation at 3500 rpm for 10 minutes at room temperature and serum was stored at -20 ºC for estimation of biochemical parameters. The serum glucose was estimated by glucose oxidase (GOD/POD) method(15), total plasma proteins by biuret method [16], cholesterol by phenol amino antipyrine method [17], total calcium by o-cresol phenophthalein complexone method [18], phosphorous by ammonium molybdate method [19], erthyocytic malondialdehyde (MDA) [20] and superoxide dismutase (SOD) [21] were estimated. The average mean values obtained on day 0 and 10 for GI was compared with GII using unpaired t-test [22]. The whole data was analysed using computerized software programme SPSS Ver.20.0.

Results and Discussion

Although buffaloes are considered as black gold in major part of the world owing to their role in supply of animal protein, these animals are reputed for silent estrus, mildovarian activity, long calving interval and reproductive problems [23]. The reproductive performances in buffaloes are reflected by the hematological and biochemical changes (4). Moreover the severity of reproductive disorders in dairy cows is related to oxidative stress characterized by generation of reactive oxygen species (ROS) [23]. On this background the present study focused on assessment of changes in hemato-biochemical parameters in PPA animals. In the present study, the Hb concentration was significantly ($p< 0.05$) lower in PPA compared to cyclic buffaloes, with mean values of 8.63±0.17 and 12.14±0.25 g/dl respectively (Fig. 1). The present findings corroborates with the reports of [24], where they observed significantly higher Hb values (12.63±0.49 vs 9.81±0.21 g/dl) in cyclic than in acyclic buffaloes. Similar observation was also made by (25) where the Hb concentration was significantly higher in cyclical (13.34±0.43 g%) compared to anoestrous buffaloes (11.38±0.56 g%). Low Hb in PPA could be attributed to nutritional factors. Low levels of Hb in acyclic buffaloes might suppress tissue oxygenation of the reproductive tract, which would in turn affect the ovarian activity [26]. The PCV observed in our present study was found to be 28.24±0.64 and 37.01±0.70% in PPA and cyclical buffaloes with significantly ($p< 0.05$) lower PCV in PPA compared to cyclic buffaloes (Fig 2). Similar findings were put forward by [4] where a PCV of 40.28±6.06 vs 36.80±4.30% were observed. The low PCV in acyclic buffaloes is indicative of low red blood cell count and anemia, which results low oxygenation to ovarian tissue.

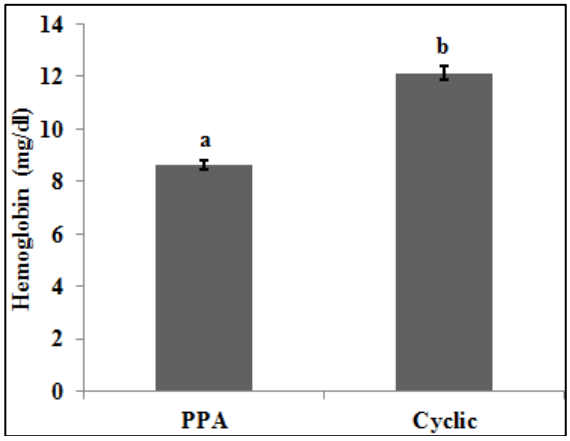

Fig 1

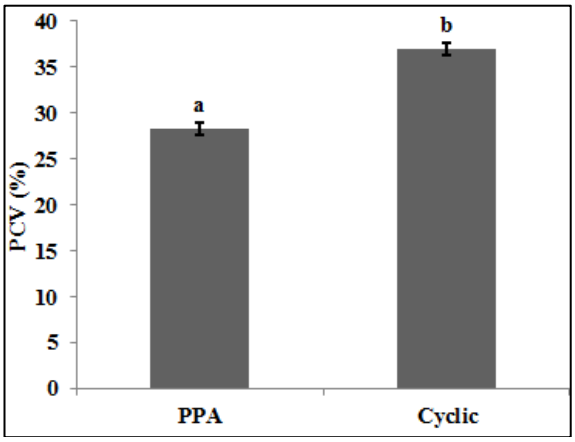

Fig 2

Fig 1 and 2: Changes in hemoglobin (Hb) and packed cell volume (PCV) in postpartum anoestrous (PPA) and cyclic Murrah Buffaloes (n=15). Bars with different superscript (a, b) differ significantly, $p< 0.05$.

The mean glucose values were found to be 53.41±6.63 and 68.86±2.03 mg/dl with significantly ($p< 0.05$) lower values in PPA compared to normal cyclic animals (Fig. 3). Contrary to our results [25] reported no significant difference in glucose (65.27±5.07 vs 65.7±5.37) concentration between PPA and cyclic buffaloes. Significantly lower glucose in PPA could be attributed to lactation stress where there is increased utilization of glucose for milk production. The total protein values observed in the present study was found to be 7.82±0.42 and 8.28±0.15 g/dl in PPA and normal cyclic animals with no significant ($p>0.05$) difference between groups (Fig. 4). Contrary to our results, significantly higher protein (8.21±0.37 and 6.96±0.22 g/dl) was observed in cyclical compared to anoestrous buffaloes [25]. Moreover [2] also reported significantly higher total protein values of 8.034±0.12 in cyclical compared to7.54±0.11g/dl in anoestrous animals.

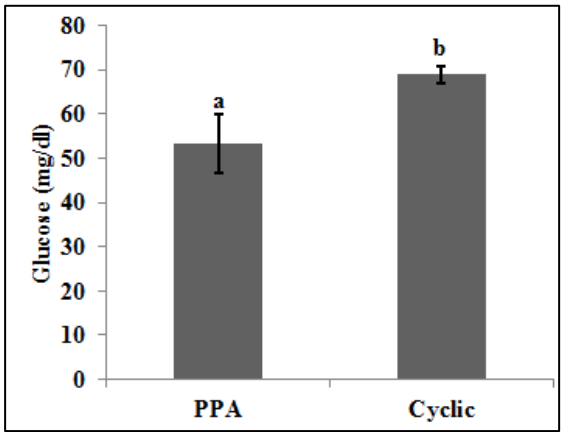

Fig 3

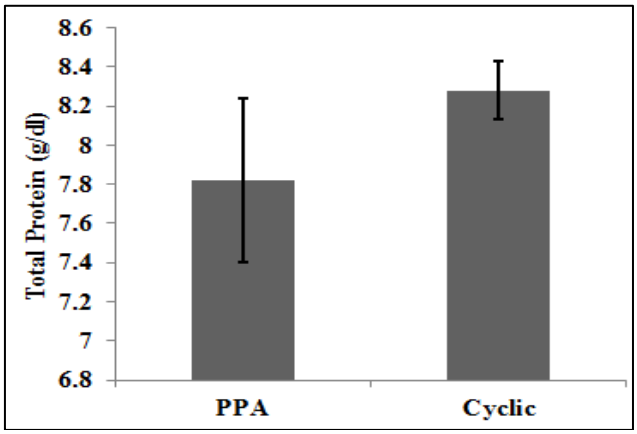

Fig 4

Fig 3 and 4: Changes in serum glucose and total protein in postpartum anoestrous (PPA) and cyclic Murrah Buffaloes (n=15). Bars with different superscript (a,b) differ significantly, $p< 0.05$.

The serum total cholesterol was significantly ($p< 0.05$) higher in cyclical animals (150.46±4.22 mg/dl) compared to PPA (75.97±9.59 mg/dl) buffaloes (Fig. 5). Our results are in agreement with the findings of [4] who reported significantly higher cholesterol concentration with a mean value of 142.85±7.43 and 88.84±5.33 mg/dl in cyclic and acyclic Niliravi buffaloes. Similarly, [24] also observed higher serum total cholesterol levels in cyclic than in acyclic buffaloes (132.49±3.39 vs 75.32±1.92 mg/dl). Contrary to our findings, [25] reported no significant difference in cholesterol concentration 108.16±7.43and 138.51±13.85 mg/dl between estrous and anestrous water buffaloes. Low cholesterol concentration might be due to lactation stress and partitioning of nutrients for milk production. Anoesturm could be attributed to low levels of cholesterol, a precursor for all sex steroid hormones and hence decreased synthesis of sex steroids [4]. In the present study, it was observed that the calcium levels were 8.58±0.76 and 10.16±0.27 mg/dl in PPA and normal cyclic animals with no significant ($p>0.05$) difference between groups (Fig. 6). Similar to our results, [25] reported a calcium concentration of 12.9±4.34 and 12.45±0.63 mg/dl in estrous and anestrous water buffaloes with no significant difference between groups. In contrary, [3] reported significantly lower levels of calcium in PPA compared to cyclical buffaloes (7.418±0.621 vs 11.756±0.860) mg/dl. Calcium influences the animals' ability to use other trace elements and may disrupt reproductive activity though its influence on certain enzyme systems [3]. This indicates that macrominerals like calcium and phosphorous are very important for regular cyclicity.

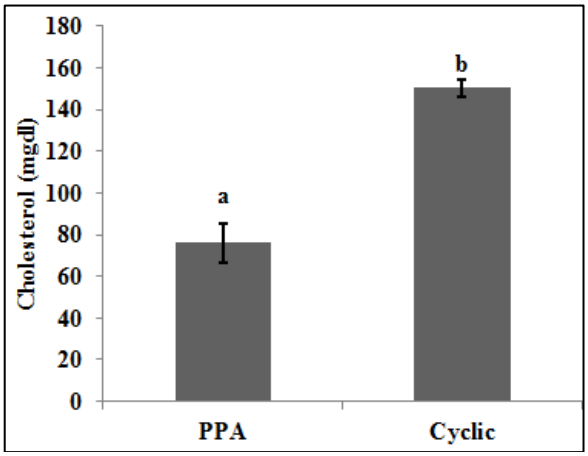

Fig 5

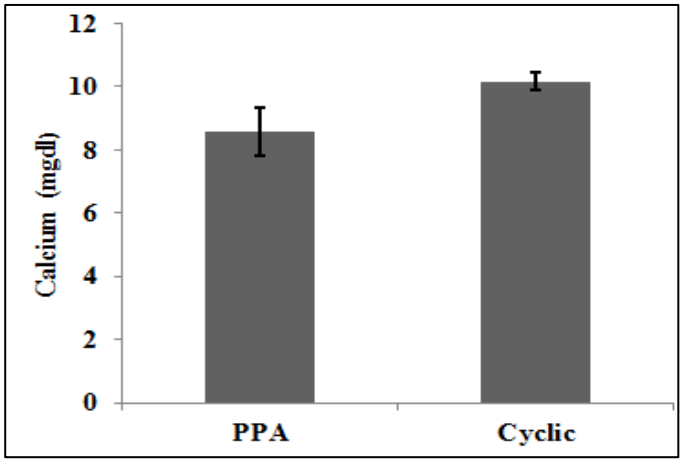

Fig 6

Fig 5 and 6: Changes in cholesterol and calcium in postpartum anoestrous (PPA) and cyclic Murrah Buffaloes (n=15). Bars with different superscript (a,b) differ significantly, $p< 0.05$.

In the present study, it was observed that the phosphorous levels were 4.35±0.24 and 5.69±0.16mg/dl in PPA and normal cyclic animals with significantly ($p< 0.05$) lower values in PPA compared to cyclical buffaloes (Fig. 7). Similar to our findings, [3] reported significantly lower phosphorus levels in PPA compared to cyclical buffaloes (5.937±0.301 vs 6.980±0.348 mg/dl). In contrary to the present findings, no significant difference in the concentration of phosphorus between PPA and oestrous buffaloes observed by [25] with values of 4±0.20 and 4.12±0.36 mg/dl in estrous and anestrous water buffaloes. Phosphorous and other minerals influence the onset of estrous [27].

The SOD levels observed in the present study was significantly ($p< 0.05$) higher in PPA compared to cyclic buffaloes with a mean value of 3.20±0.19 and 1.27±0.16mM respectively (Fig. 8).Contrarily, [2] reported no significant difference in SOD concentration between PPA and cyclic buffaloes(1.160±0.203 vs 0.968±0.160units/ml).SOD is an antioxidant enzyme that catalyzes the dismutation of two molecules of superoxide anion to hydrogen peroxide (H_2O_2)

and molecular oxygen, thus preventing the potentially harmful superoxide anion action on cell membranes. H_2O_2 thus generated is further catalyzed by another antioxidant enzyme catalase to water (H_2O) and oxygen (O_2) [28]. Significantly higher SOD in PPA compared to cyclic animals could be a defense mechanism to overcome ROS load.

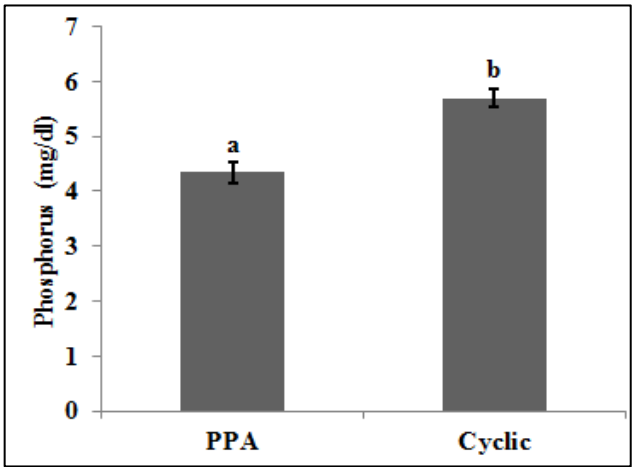

Fig 7

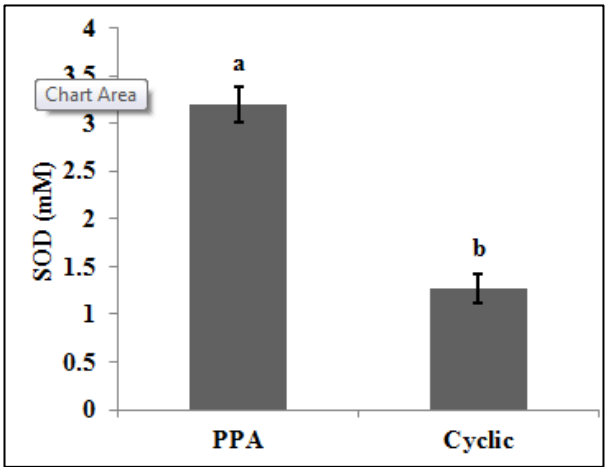

Fig 8

Fig 7 and 8: Changes in serum phosphorus and superoxide dismutase (SOD) in postpartum anoestrous (PPA) and cyclic Murrah Buffaloes (n=15). Bars with different superscript (a,b) differ significantly, $p< 0.05$.

In the present study, it was observed that the MDA levelsin PPA and normal cyclic animals were 72.37±1.55 and 55.25±1.23µM respectively. The MDA levels were significantly ($p< 0.05$) higher in PPA compared to control animals (Fig. 9). Similar to our results, [29] reported significantly higher levels in anestrous Cholistani cattle (2.54±0.22 vs 1.71±0.03nmol/ml). MDA is produced during the ROS mediated peroxidation of polyunsaturated fatty acids [30] thus it is an indicator of lipid peroxidation and is a widely used marker of oxidative stress. The increased MDA concentration in PPA compared cyclic buffaloes could be due to the increased production of ROS [31] and is indicative of oxidative stress.

The energy requirement of the animals increase during early lactation periods and thus makes the animals highly susceptible to negative energy balance causing increased level of oxidative stress. The higher SOD levels in PPA buffaloes might be due to increased free radicals generated due to the metabolic stress. Increased SOD was an indication of animal's effort to overcome the metabolic stress during early lactation periods. The increased concentration of MDA is indicative of the level of metabolic stress on the animal during early lactation periods.

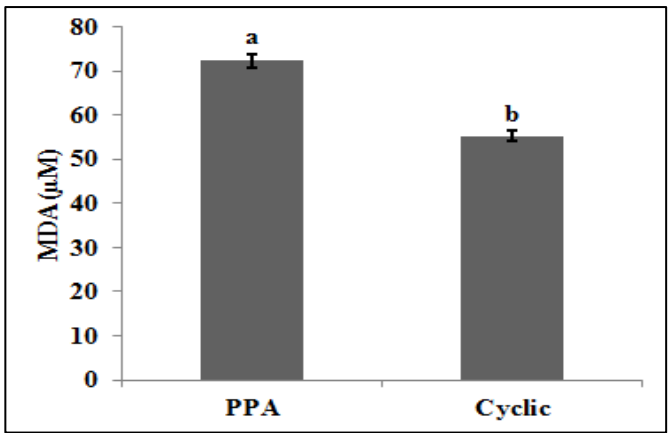

Fig 9: Changes in malondialdehyde (MDA) in postpartum anoestrous (PPA) and cyclic Murrah Buffaloes (n=15). Bars with different superscript (a,b) differ significantly, $p< 0.05$.

Conclusion

Anestrous is one of the commonly occurring impaired reproductive condition in buffaloes. Understanding the physiological status and changes in hemato-biochemical parameters could help us to adopt strategies for improvement of reproductive function. From the present study, we observed significantly, lower Hb, PCV, glucose, cholesterol, calcium and phosphorus while an increase in SOD and MDA levels in PPA compared to control animals is suggestive of oxidative stress. Low Hb derives low oxygen to tissues, low PCV is suggestive of reduced red cell count, hypoglycemia and hypercholesterolemia is indicative of lack of precursors for steroid hormone synthesis and high SOD and MDA is suggestive of oxidative stress. It may be concluded that PPA significantly reflects marked changes hemato-biochemical parameters. These changes could be used as markers of PPA.

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Conflict of Interest

The authors declare that there is no any conflict of interest for this manuscript.

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