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### Comparison of certain blood plasma biochemical profiles between normal and delayed pubertal buffalo heifers

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### Abstract

The aim of this study was to investigate concentrations of blood plasma biochemical composition of certain variables with reproductive importance (glucose, cholesterol, total protein, ceruloplasmin) in normal and delayed pubertal buffalo heifers. Buffalo heifers were screened for reproductive tract pathologies and 20 animals were selected. Animals were divided into two groups viz. Group-I (n=10; normal pubertal buffalo heifers) having average age of 2.5-3 years and the history of estrus exhibition at least once and Group-II (n=10; delayed pubertal buffalo heifers) having average age  $\geq 3$  years but without any history of estrus exhibition. All the four plasma biochemical variables measured were with in normal physiological range in both the groups. Mean±SEM values of plasma cholesterol and total protein are significantly higher (P < 0.05) in group II (delayed pubertal animal) compared to group I (normal pubertal animals). Non significantly higher (P > 0.05) mean±SEM values of plasma glucose and ceruloplasmin were recorded in group II than group I. Higher values of blood biochemical in delayed pubertal buffalo heifers may be attributed to higher body weight and body condition score compared to normal pubertal buffalo heifers. It was concluded that delayed pubertal buffaloes had differences in plasma biochemical profile that normal pubertal heifers which might be contributing for the underlying aetiology for delayed puberty.

Keywords: Delayed puberty, buffalo heifers, blood biochemical

### Introduction

India being the largest contributor to world's total buffalo population (54.6%) produces 91.8 million tons of milk per year (2018-19), comprising 49% of total Indian milk production <sup>[1]</sup>. Despite having numerous merits, buffaloes are often blamed for their poor reproductive efficiency due to late sexual maturity, poor summer estrus expression, distinct seasonal reproductive patterns and prolonged calving intervals <sup>[2]</sup>. Puberty and sexual maturity are closely related stages of life which have an enormous effect on the performance of dairy animals in reproduction.

Onset of puberty is credited to achieving target weight at a particular age rather than age alone. Puberty is the function of body weight and age where attainment of body weight strongly depends on nutritional status of the animal. Nutritional status of animal is reflected by plasma biochemical profiling. Among them, nutritional well-being of animal is best reflected by plasma glucose, plasma cholesterol, plasma total protein and plasma ceruloplasmin. Plasma glucose, plasma cholesterol and plasma total protein are some of the key determinants affecting the fertility and cyclicity in dairy animals <sup>[3, 4]</sup>. Copper is an important nutritional factor affecting by impairing conception rate, estrus cyclicity and delaying onset of puberty. Weak and silent estrus has been reported due to copper deficiency <sup>[5]</sup>. The activity of plasma copper and ceruloplasmin is highly correlated and ceruloplasmin represents 90 to 95% of the plasma copper content <sup>[6, 7]</sup>.

Authors read out literature suggested sparse information regarding plasma biochemical profile association with delayed puberty in buffalo heifers. Hence the study was formulated to study the comparison of certain blood plasma biochemical profiles between normal and delayed pubertal buffalo heifers

### **Materials and Methods**

The present study was carried out in Department of Veterinary Gynaecology and Obstetrics, College of Veterinary Sciences, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab. Twenty healthy buffalo heifers were selected from Teaching Veterinary Clinical Complex (TVCC) and those reared at private dairy farms in Ludhiana, Punjab. Animals were divided into two groups, group I includes ten pubertal buffalo heifers with the history of estrus exhibition at least, having body condition score (BCS) of 2.5-3 at 1-5 scale, average body weight  $\geq 250$ kg and age between 2.5-3 years and group II includes ten delayed pubertal heifers without any history of estrus exhibition despite having an age  $\geq$  3 years, body weight  $\geq$  250 kg and body condition score (BCS) of 2.5 to 3 at 1-5 scale. Blood samples were collected by jugular venepuncture into heparinised vials and plasma was separated by centrifuging the samples at 3000 rpm for 15 min. Plasma obtained was stored at -20 °C until biochemical analysis. Plasma samples were analysed for certain blood metabolites viz., glucose, cholesterol and total protein using automatic BPC based chemistry analyser and commercial biochemical kits (Transasia Bio- Medicals Ltd., ERBA diagnostics, Germany). Plasma ceruloplasmin was measured using commercially available diagnostic kit (Spin react, Spain) and analysed using automatic BPC based chemistry analyser. Values were tabulated and data thus generated were analysed by student "t" test using SPSS software version 16.0 and the differences were considered significant at P < 0.05.

### **Results and Discussion**

Plasma metabolites and minerals, on acquiring a certain physiological concentration definitely initiate events necessary for bringing about puberty in buffalo heifers. High levels of glucose, amino acids and metabolic hormones in cow heifers are likely to serve as metabolic signals transmitting nutritional status information to the hypothalamic neurons, which leads to increased LH secretion and early onset of puberty <sup>[8]</sup>.

Parameter	Normal pubertal heifers		Delayed pubertal heifers	
	Mean ± SEM	Range	Mean ± SE	Range
Glucose (mg/dl)	38.33±6.94 <sup>a</sup>	11.02-87.39	39.43±2.64 <sup>a</sup>	29.59-59.53
Cholesterol (mg/dl)	44.36±5.36 <sup>a</sup>	24.3-75.5	60.72±3.94 <sup>b</sup>	37.0-75.0
Total protein (g/dl)	5.47±0.34 <sup>a</sup>	4.02-7.62	6.49±0.22 <sup>b</sup>	5.3-7.39
Ceruloplasmin (mg/dl)	$8.52\pm0.64^{a}$	5.22-11.54	$8.87 \pm 0.66^{a}$	6.68-13.6

 Table 1: Plasma biochemical changes in normal and delayed pubertal buffalo heifers

In rows of Mean  $\pm$  SEM (mg/dl) of glucose, cholesterol, total protein and ceruloplasmin, similar superscripts indicate no significant difference and dissimilar superscripts indicates significant difference at 1% level.

In the present study, all the biochemical variables measured were having values within normal physiological range in both normal and delayed pubertal buffalo heifers. There exist no significant difference (P>0.05) between the values of plasma glucose and plasma ceruloplasmin among both the groups. Mean plasma glucose value (39.4  $\pm$  2.6 vs. 38.3  $\pm$  6.9) and mean ceruloplasmin value ( $8.8 \pm 0.6$  vs.  $8.5 \pm 0.6$ ) was nonsignificantly higher (P>0.05) in delayed pubertal animals when compared to normal pubertal animals. Although the range of plasma glucose value was 11.02 mg/dl to 87.39 mg/dl in normal pubertal buffaloes whereas 29.59 mg/dl to 59.53mg/dl in delayed pubertal heifers and the range of plasma ceruloplasmin value was 5.2mg/dl to 11.54 mg/dl in normal pubertal buffaloes whereas 6.68 mg/dl to 13.6 mg/dl in delayed pubertal heifers. Similar values of blood glucose and total protein and higher values of cholesterol were reported by Sabasthin et al.<sup>[9]</sup> in repeat breeding buffaloes. Glucose and total cholesterol are within the reference range of buffalo heifers given by Abd Ellah et al. [10] (cholesterol reference range 34.92-76.82 mg/dl and glucose reference range 35.45–92.47 mg/dl). Mean plasma cholesterol (60.7  $\pm$ 3.9 vs.44.3  $\pm$  5.3) and mean plasma total protein (6.4  $\pm$  0.2 vs.5.47  $\pm$  0.3) value was significantly higher (p<0.05) in delayed pubertal animals compared to normal pubertal animals. The range of plasma cholesterol value was 24.3mg/dl to 75.5 mg/dl in normal pubertal buffaloes whereas 29.5mg/dl to 59.5 mg/dl in delayed pubertal heifers. The range of plasma total protein value was 4.02g/dl to 7.62 g/dl in normal pubertal buffaloes whereas 5.3 g/dl to 7.4 g/dl in delayed pubertal heifers. Higher values of blood glucose, cholesterol, ceruloplasmin and lower values of total protein in both normal and delayed categories were reported by Ahmed *et al.*<sup>11</sup> in Egyptian buffalo heifers. Higher values of blood glucose, cholesterol and similar values of total protein were reported by Maurya and Singh <sup>[12]</sup> in pooled buffaloes managed at field level.

In summary, the plasma cholesterol and protein were significantly higher in delayed pubertal buffalo heifers which may be attributed to higher body weight and body condition score compared to normal pubertal buffalo heifers. Malnourished heifers' only shows delayed puberty with sole cause of low plasma metabolite and mineral values. Delayed puberty attainment caused due to malnourishment during prepubertal growth phase caused delayed maturation of hypothalamic-hypophyseal-ovario-uterine axis. In organised dairy farms following feeding practices as per NRC recommendations delayed puberty may be attributed to other possible causes include hormonal imbalance caused by environmental stress factors.

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