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Diagnostic Indicators and Therapeutic Evaluation of Pregnancy Toxaemia in Goats

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ABSTRACT

Background: Periparturient mortality in goats have a great economic impact on the livelihood of marginal farmers. Pregnancy toxaemia, a metabolic disease in small ruminants occurs as a result of negative energy balance consequent to enhanced requirement for glucose by the developing fetuses in the last trimester (last 6 to 4 weeks) of gestation. The present study was aimed to identify diagnostic and prognostic indicators of pregnancy toxaemia.

Methods: During the period October 2016 to September 2018, a total of 516 adult non descriptive does were brought to Veterinary University Peripheral Hospital, Madhavaram Milk Colony, Chennai - 51, of which 264 (51.16%) were treated for medical conditions. Among the does treated for various medical conditions, 72 does were in their last six weeks of gestation carrying twins/triplets and presented with the history of off feed. They were subjected to determination of blood β-hydroxybutyric acid (BHBA) level by means of a portable blood ketone and glucose monitoring system and qualitative urinalysis using urine dip stick. Does with BHBA level >0.8 mmol/L and <1.6 mmol/L were classified as sub-clinical pregnancy toxaemic group (n=12) and BHBA level >1.6 mmol/L were classified as clinical pregnancy toxaemic group (n=12) and subjected to therapy while the remaining 48 does had BHBA levels <0.8 mmol/L. The control animals were selected from adult Tellicherry does in the age group of 2 to 4 years maintained at Livestock Farm Complex (LFC), Madhavaram Milk Colony, Chennai-600 051.

Result: All the twelve does of sub-clinical pregnancy toxaemic group recovered completely with a cure rate of 100%, while in the clinical pregnancy toxaemic group the cure rate was only 33%. Reliable diagnostic indicators of pregnancy toxaemia include blood β-hyroxybutyric acid concentration (≥0.8 mmol/L) and presence of ketone body, glucose and protein in urine, while hypergly-caemia in advanced pregnancy toxaemic does indicate fetal death.

Key words: Diagnostic, Goats, Indicators, Pregnancy toxaemia, Therapeutic evaluation.

INTRODUCTION

Pregnancy toxemia also called gestational ketosis, twin-lamb disease, ketosis of pregnancy, kid disease, lambing sickness, kidding paralysis and lambing or kidding ketosis (Rook, 2000) is a metabolic disease affecting pregnant ewes and does after a period of negative energy balance (NEB) and impaired gluconeogenesis (Lima *et al.,* 2012). Pregnancy toxaemia normally occurs in the last trimester (last 6 to 4 weeks) of gestation in goat and sheep as a result of negative energy balance consequent to enhanced requirement for glucose by the developing fetuses (Schlumbohm and Harmeyer, 2008). Risk factors include multiple fetuses, poor quality of ingested energy, decreased dietary energy level, genetic factors, obesity, lack of good body condition, high parasitic load, stress factors and multiple pregnancies (Hefnawy *et al.,* 2011). The disease is characterized by hypoglycaemia, low concentrations of hepatic glycogen, increased fat catabolism leading to high plasma concentration of non-esterified fatty acids (NEFA), high concentrations of ketone bodies (hyperketonaemia) and high mortality rate (Van Saun, 2000). The mortality rates can attain 100% even with the initiation of treatment due to severe irreversible organ damage. Hence diagnostic indicators in the primary stage of the disease and prognostic indicators of pregnancy toxaemia are the need of the hour for better herd health management. Hence, this study was aimed to identify diagnostic and prognostic indicators of pregnancy toxaemia in goats.

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MATERIALS AND METHODS

The study was carried out at Veterinary University Peripheral Hospital (VUPH) and Livestock Farm Complex (LFC),

Madhavaram Milk Colony, Chennai during the period October 2016 to September 2018. The control animals were selected from adult Tellicherry does in the age group of 2 to 4 years maintained at Livestock Farm Complex (LFC) of the University. Non-pregnant does (n=12) and pregnant does (n=12) carrying twins/triplets and without exhibiting signs of pregnancy toxaemia throughout gestation served as control. A total of 516 adult non descriptive does were brought to Veterinary University Peripheral Hospital, Madhavaram Milk Colony, Chennai- 51 of which 264 (51.16%) were treated for medical conditions namely bloat in 14 (5.30%), mastitis in 14 (5.30%), respiratory tract infections in 48 (18.18%), enteric infection in 42 (15.91%), simple indigestion in 54 (20.45%), acidosis in 68 (25.75%), sub-clinical pregnancy toxaemia in 12 (4.55%) and clinical pregnancy toxaemia in 12 (4.55%). Among the does treated for medical conditions, 72 does were in their last six weeks of gestation carrying twins/triplets and presented with the history of off feed. The pregnant does were subjected to ultrasonography and radiography for confirmation of pregnancy, to assess the stage of pregnancy and fetal numbers. They were subjected to determination of blood β-hydroxybutyric acid (BHBA) and glucose concentration and qualitative urinalysis. Does with BHBA level >0.8 mmol/L and <1.6 mmol/L were classified as sub-clinical pregnancy toxaemic group (n=12) and BHBA level >1.6 mmol/L were classified as clinical pregnancy toxaemic group (n=12) and subjected to therapy while the remaining 48 does had BHBA levels <0.8 mmol/L.

Parameters included in the study

Clinical signs

The clinical signs exhibited by the pregnant does were recorded.

Body condition score (BCS)

Body condition score was assessed using 5 point scale (1.0 -5.0) by evaluating the animals visually and by palpating the region of lumbar vertebrae and sternum (Villaquiran *et al.,* 2012).

Blood β-hydroxybutyric acid (BHBA) concentration

The blood β-hydroxybutyric acid (BHBA) concentration was determined using a portable blood ketone and glucose monitoring system (Fig 1) (Free Style Optium Neo H - Abbott ®) (Pichler *et al.,* 2014). The ear vein was punctured with a sterile 23 G needle and the monitoring system attached with blood ketone strip was directed towards the drop of blood. Sufficient quantity of blood droplet was absorbed by capillary action at the tip of the strip and within 10 seconds the blood BHBA concentration was displayed on to the digital meter.

Urine sample

Urine samples were obtained after a voluntary micturition or induced by covering the nose and mouth of does for a few seconds (Albay *et al.,* 2014). The urine samples were analyzed using Multistix 10 SG reagent strip (Fig 2) (Siemens Healthcare Private Limited, India) for qualitative determination of ketone bodies, glucose and protein (Emam and Galhoom, 2008). The test strips were dipped into the collected urine and immediately compared with the colour chart provided on the label of the urine test strip container to determine the presence of ketone, glucose and protein in the urine.

Ultrasonography

The pregnant does were subjected to ultrasonography to assess the stage of gestation and the viability of the fetuses. The estimated gestational age of the fetus in weeks was calculated using the formula:

$$Y = 4.712 + 0.445\ X$$

Where,
Y= Gestational age (wks).
X= Fetal parameter (cm) in case of crown rump length.

$$Y = 2.675 + 3.229\ X$$

Where,
Y= Gestational age (wks).
X= Fetal parameter (cm) in case of bi-parietal diameter (Abdelghafar *et al.,* 2011).

Radiography

To confirm pregnancy and assess the fetal numbers (Fig 3 and 4).

Haematology

Haematological investigation was done with an automated haematology analyzer for haemoglobin (g/dL), packed cell volume (%), red blood cell ($\times 10^6$/cmm), white blood cells (/cmm) and differential leucocyte count.

Serum biochemistry

Serum biochemical parameters - blood urea nitrogen (mg/dL), creatinine (mg/dL), aspartate aminotransferase (IU/L), alanine aminotransferase (IU/L), glucose (mg/dL) and total protein (g/dL) were estimated in an automated biochemical analyzer.

Serum electrolytes

The serum electrolytes - sodium (mmol/L), potassium (mmol/L), calcium (mg/dL), magnesium (mg/dL) and chloride (mmol/L) were estimated in an automated electrolyte analyzer.

Fig 1: Portable Blood ketone and glucose monitoring system (Free Style Optium Neo H - Abbott®).

Fig 2: Urinalysis - Multistix 10SG reagent strip (Siemens Healthcare Pvt. Ltd.).

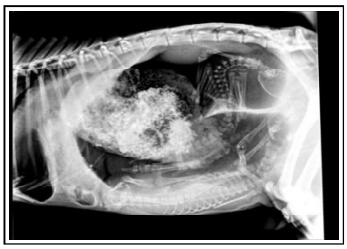

Fig 3: Radiography in pregnant doe - Twins.

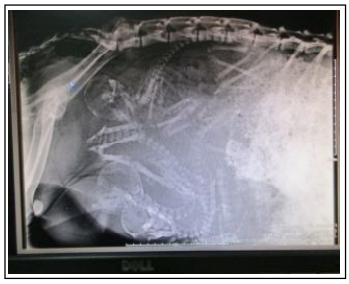

Fig 4: Radiography in pregnant doe - Triplets.

Serum metabolites

The serum was stored at- 20°C until analysis of serum metabolites namely BHBA (µmol/L) and non-esterified fatty acid (NEFA) (µmol/L) by Enzyme Linked Immunosorbent Assay (ELISA) method using goat specific BHBA and NEFA ELISA kits (MyBio Source Inc., USA), while serum cortisol (nmol/L) concentration was analyzed by using goat specific Cortisol ELISA kit (Cusabio Biotech Co. Ltd.) as per the manufacturer's instruction and the optical density value was read in the ELISA microplate reader at 450 nm.

Therapy

The pregnancy toxaemic does were treated with intravenous glucose therapy (5% Dextrose) supported with parenteral therapy with Vitamin B_1, B_6 and B_{12} and oral administration of glycerine @ 25 ml twice daily. The response to therapy was evaluated based on the clinical signs, haematology, serum biochemistry, metabolic and hormonal parameters.

Cure rate and case fatality rate

The cure rate and case fatality rate was evaluated based on the response to treatment.

Statistical analysis

The data collected were statistically analyzed by One Way Analysis of Variance (ANOVA) using Statistical Software IBM® SPSS® Version 20.0 for Windows® and critically discussed.

RESULTS AND DISCUSSION

The clinical signs observed in the sub-clinical pregnancy toxaemic does were anorexia (100%), dullness in 10 (83%) and bruxism in 7 (58%). All the does were in a standing posture with normal carriage of head and neck and normal voiding of dung. In the clinical pregnancy toxaemic does the clinical signs observed were anorexia (100%), dullness in 10 (83%), bruxism in 7(58%), scanty dung in 12 (100%), acetone odour from mouth in 11 (92%), standing posture in 6 (50%), stargazing in 9 (67%), sternal recumbency (Fig 5) in 6 (50%) and lateral deviation of neck (Fig 6) in 5 (42%).

The body condition score (BCS) of pregnant does in control group ranged between 2.5 to 3. Among the sub-clinical pregnancy toxaemic does, eight (67%) had a BCS of 2.0 and four (33%) had 2.5, while in the clinical pregnancy toxaemic does, nine (75%) had a BCS of 2.0 and 2.5 in three (25%). The reason for the pregnancy toxaemic does to have a body condition score of 2.0 to 2.5 may be due to increased fat and protein catabolism as a result of severe under nutrition (Rook, 2000). Body condition score should be included for effective monitoring of feeding and herd health management for the development of a healthy and productive herd (Russel, 1984).

The BHBA concentration in blood of control group ranged between 0.2 mmol/l to 0.4 mmol/l, between 0.9 mmol/l to 1.5 mmol/l in sub-clinical pregnancy toxaemic does and between 2.1 mmol/l to 7.9 mmol/l in clinical pregnancy

Fig 5: Sternal recumbency with star gazing.

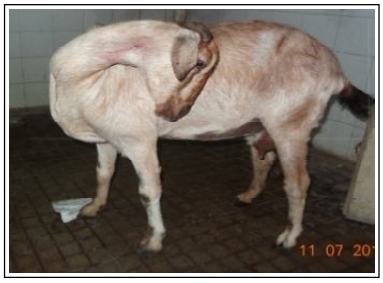

Fig 6: Standing posture with lateral deviation of neck.

toxaemic does which were in accordance to Andrews (1997). The values obtained in the portable ketone meter were immediate, reliable and highly useful in screening does for pregnancy toxaemia in field conditions. The portable human ketone meter can be successfully applied to estimate BHBA levels in field conditions due to the non-availability of other reliable spot tests (Yadav *et al.,* 2016).

Urinalysis of control group revealed absence of ketone bodies, glucose and protein, while presence of ketone bodies, protein and glucose are diagnostic for pregnancy toxaemia. Trace quantities of ketone bodies in the urine of 9 does (75%) and small quantities in 3 does (25%) of sub-clinical pregnancy toxaemic group, while trace in 2 (17%), moderate in 2 (17%), small in 4 (33%) and large in 4 (33%) in the urine of clinical pregnancy toxaemic group were observed. Presence of ketone bodies in urine might be due to the increased fat hydrolysis (Cleon, 1988). Protein was completely absent in the urine sample of sub-clinical pregnancy toxaemic group, while the protein grading were 1+ in 3 does (25%), 2+ in 4 does (33%) and 3+ in 5 does (42%) in the clinical pregnancy toxaemic group. The glucose grading were trace in 6 does (50%) and 1+ grading in 6 does (50%) in the sub-clinical group, while the grading in clinical pregnancy toxaemic group were trace in 2 does (17%), 1+ in 1 doe (8 %), 2+ in 5 does (42%) and 3+ in 4 does (33%). The qualitative analysis of urine samples for the presence of ketone bodies, glucose and protein under field conditions can be carried out with accuracy and reliability using Multistix 10 SG reagent strips (Emam and Galhoom, 2008).

The mean±SE values of haemoglobin, packed cell volume, red blood cells and white blood cells in control, sub-clinical and clinical pregnancy toxaemic groups are presented in (Table 1). Highly significant ($p \leq 0.01$) difference was observed in the haemoglobin, packed cell volume and red blood cell values between the sub-clinical and clinical pregnancy toxaemic groups compared to that of control group. The significant increase of the above values in the pregnancy toxaemic does may be due to haemoconcentration and dehydration (Hefnawy *et al.,* 2011).

The mean±SE values of differential count in control, sub-clinical and clinical pregnancy toxaemic groups are presented in (Table 2). Neutrophilia observed in sub-clinical and clinical pregnancy toxaemic group might be due to the increased cortisol level, which created a movement of granulocytes from the bone marrow to the peripheral blood (Alidadi *et al.,* 2012). The lymphopenia in sub-clinical and clinical pregnancy toxaemic group might be due to the toxic and sub-toxic concentration of BHBA and acetoacetate in blood, which inhibit the lymphocytic proliferation (Franklin and Young, 1991) or may be due to increased cortisol level (Alidadi *et al.,* 2012). With respect to basophils a significant ($p \leq 0.05$) difference was observed between the sub-clinical and clinical pregnancy toxaemic group compared to that of control.

The mean±SE values of blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose and total protein in control, sub-clinical and clinical pregnancy toxaemic groups are presented in (Table 3). A highly significant ($p \leq 0.01$) difference was observed between sub-clinical and clinical pregnancy toxaemic groups compared to control in BUN and creatinine levels. Elevated levels in sub-clinical and clinical pregnancy toxaemic groups concurred with Hefnawy *et al.* (2011) and might be due to severe kidney dysfunction due to the elevated ketone bodies in general circulation (El-Sayed and Siam, 1994), or reduced glomerular filtration due to fatty infiltration in tubular epithelium of kidney (Barakat *et al*., 2007) or due to death and decomposition of fetuses (Radostits *et al*., 2000).

A highly significant ($p \leq 0.01$) difference in AST and ALT levels was observed between sub-clinical and clinical pregnancy toxaemic groups compared to that of control. Elevated activity of the enzymes in sub-clinical and clinical pregnancy toxaemic does correlated with Barakat *et al.* (2007) and might be due to the damage of the hepatic cells and release of cellular enzymes into circulation as a result of fatty infiltration of the liver due of adipolysis and hepatic ketogenesis following energy deficit (Nassif *et al*., 2005).

With respect to glucose level a highly significant ($p \leq 0.01$) difference was observed between the clinical

Table 1: Mean±SE of haemoglobin, packed cell volume, red blood cells and white blood cells in group 1 (control), group 2 (sub-clinical pregnancy toxaemia) and group 3 (clinical pregnancy toxaemia).

Parameters	Group 1 (Control)			Group 2 (n=12)	Group 3 (n=12)	'F' value
	Non-pregnant does (n=12)	Pregnant does (n=12) gestation in days				
		120 days	150 days			
Haemoglobin (g/dL)	$8.43^{a}\pm0.10$	$8.55^{a}\pm0.07$	$8.53^{a}\pm0.05$	$9.0^{b}\pm0.15$	$9.06^{b}\pm0.13$	8.13**
Packed cell volume (%)	$23.22^{a}\pm1.67$	$24.4^{ab}\pm0.84$	$26.32^{bc}\pm0.83$	$27.75^{c}\pm0.10$	$28.13^{c}\pm0.24$	9.68**
Red blood cells ($\times10^{6}$/cmm)	$14.27^{a}\pm0.84$	$15.34^{a}\pm0.73$	$16.04^{a}\pm0.73$	$18.66^{b}\pm0.08$	$17.95^{b}\pm0.19$	8.10**
White blood cells (/cmm)	19025±1235.45	19112.5±2046.28	20250±1399.74	22091.67±166.27	22683.33±235.43	1.06^{NS}

NS: Not Significant; **Highly significant ($p \leq 0.01$).

Means bearing the same superscript within the same row do not differ significantly.

pregnancy toxaemic group and pregnant does of control group. The hypoglycemia observed in sub-clinical pregnancy toxaemic group might be due to long periods of starvation (Andrews, 1997) or due to the increased demand for glucose by the developing twins or triplets or due to decreased hepatic gluconeogenesis and hypoglycemic effect by the increased level of BHBA level in blood, which can suppress endogenous glucose production and reduction in food intake (Marteniuk and Herdt, 1988; Schlumbohm and Harmeyer, 2004). In the clinical pregnancy toxaemic group the glucose level was higher and equal in comparison to that of non-pregnant does. Four does (33%) of this group were presented in sternal recumbency with lateral deviation of neck and found to be >140 days pregnant with the aid of ultrasound. The fetal heart beats were absent in these four does which indicated fetal death. The blood β-hydroxybutyric acid and glucose levels monitored indicated BHBA levels >7 mmol/L (Fig 7) and abnormally high glucose levels (Fig 8). This correlated with Lima *et al.* (2012), who stated hyperglycemia to occur with fetal death in advanced pregnancy toxaemia and the reason were attributed to the removal of the suppressing effect of the fetus on hepatic gluconeogenesis (Wastney *et al.,* 1983; Lima *et al.,* 2012) or due to the increased serum cortisol level (Ford *et al.,* 1990). The mean glucose levels for the remaining eight does were 23.87±0.48 mg/dL, indicating hypoglycemia and correlated with Rook (2000) and Hefnawy *et al.* (2011).

A highly significant ($p \leq 0.01$) difference was observed in protein levels between the control and pregnancy toxaemic group. Decreased protein levels observed in sub-clinical and clinical pregnancy toxaemic does correlated with Barakat *et al.* (2007) and Hefnawy *et al.* (2011) and might be due to anorexia and reduction in albumin synthesis due to hepatic insufficiency and albuminuria (Yarim and Ciftci, 2009) or malnutrition resulting in inadequate provision of amino acid substrate for general protein production (Nasr *et al.,* 1997).

The mean±SE values of sodium, potassium, calcium, magnesium and chloride in control, sub-clinical and clinical pregnancy toxaemic groups are presented in (Table 4). A highly significant ($p \leq 0.01$) difference in sodium levels was observed between the sub-clinical and clinical pregnancy toxaemic groups compared to that of control. Hyponatremia

Table 2: Mean±SE of differential count in group 1 (control), group 2 (sub-clinical pregnancy toxaemia) and group 3 (clinical pregnancy toxaemia).

Parameters	Group 1 (Control)			Group 2 (n=12)	Group 3 (n=12)	'F' value
	Non-pregnant does (n=12)	Pregnant does (n=12) gestation in days				
		120 days	150 days			
Neutrophils (%)	$33.5^{a}\pm1.03$	$32.12^{a}\pm0.63$	$32.0^{a}\pm0.46$	$40.91^{c}\pm1.03$	$53.58^{d}\pm1.68$	58.04**
Lymphocytes (%)	$62.0^{cd}\pm0.80$	$63.62^{d}\pm0.41$	$63.37^{d}\pm0.26$	$55.16^{b}\pm0.96$	$42.66^{a}\pm1.68$	65.82**
Monocytes (%)	2.75±0.16	2.5±0.18	2.5±0.26	2.41±0.14	2.5±0.15	0.50^{NS}
Eosinophils (%)	1.5±0.26	1.5±0.26	1.75±0.25	1.5±0.19	1.25±0.13	1.39^{NS}
Basophils (%)	$0.25^{ab}\pm0.16$	$0.25^{ab}\pm0.16$	$0.37^{ab}\pm0.18$	$0^{a}\pm0$	$0^{a}\pm0$	2.47*

NS: Not Significant; *Significant ($p \leq 0.05$); **Highly significant ($p \leq 0.01$).

Means bearing the same superscript within the same row do not differ significantly.

Table 3: Mean±SE of serum biochemical parameters in group 1 (control), group 2 (sub-clinical pregnancy toxaemia) and group 3 (clinical pregnancy toxaemia).

Parameters	Group 1 (Control)			Group 2 (n=12)	Group 3 (n=12)	'F' value
	Non-pregnant does (n=12)	Pregnant does (n=12) gestation in days				
		120 days	150 days			
Blood urea nitrogen (mg/dL)	$28.53^{b}\pm1.08$	$26.02^{ab}\pm1.10$	$26.73^{ab}\pm1.14$	$39.70^{c}\pm0.56$	$47.74^{d}\pm1.16$	79.02**
Creatinine (mg/dL)	$0.79^{a}\pm0.13$	$0.62^{a}\pm0.04$	$0.76^{a}\pm0.04$	$1.45^{b}\pm0.09$	$1.69^{c}\pm0.09$	32.30**
Aspartate aminotransferase (AST) (IU/L)	$90.50^{a}\pm6.63$	$121.5^{c}\pm3.92$	$122.25^{c}\pm1.79$	$131.51^{d}\pm1.12$	$144.66^{e}\pm3.57$	39.83**
Alanine aminotransferase (ALT) (IU/L)	$29.0^{ab}\pm2.01$	$24.12^{a}\pm1.24$	$26.12^{ab}\pm0.66$	$49.23^{c}\pm2.02$	$76.70^{d}\pm2.44$	76.76**
Glucose (mg/dL)	$53.97^{b}\pm0.96$	$25.25^{a}\pm2.15$	$29.25^{a}\pm1.66$	$32.0^{a}\pm0.69$	$57.0^{b}\pm11.57$	6.03**
Total protein (g/dL)	$6.6^{bc}\pm0.06$	$6.57^{abc}\pm0.25$	$6.77^{cd}\pm0.07$	$6.36^{ab}\pm0.07$	$6.25^{a}\pm0.04$	10.56**

**Highly significant ($p \leq 0.01$).

Means bearing the same superscript within the same row do not differ significantly.

Table 4: Mean±SE of serum electrolytes in group 1 (control), group 2 (sub-clinical pregnancy toxaemia) and group 3 (clinical pregnancy toxaemia).

Parameters	Group 1 (Control)			Group 2 (n=12)	Group 3 (n=12)	'F' value
	Non-pregnant does (n=12)	Pregnant does (n=12) gestation in days				
		120 days	150 days			
Sodium (mmol/L)	144.81^{c}±0.93	142.2^{b}±0.45	154.45^{d}±1.04	141.33^{b}±0.35	136.1^{a}±0.59	65.42**
Potassium (mmol/L)	5.03^{b}±0.13	5.37^{c}±0.15	5.43^{c}±0.10	4.89^{b}±0.04	4.34^{a}±0.05	14.78**
Chloride (mmol/L)	108.62^{ab}±0.77	108.38^{ab}±0.56	109.61^{b}±0.76	111.50^{c}±0.17	112.53^{c}±0.17	16.27**
Calcium (mg/dL)	9.88^{a}±0.56	12.71^{c}±0.61	12.17^{c}±0.17	9.75^{a}±0.11	9.13^{a}±0.20	20.05**
Magnesium (mg/dL)	2.93^{b}±0.11	3.03^{b}±0.05	3.03^{b}±0.04	2.57^{a}±0.04	2.62^{a}±0.06	12.01**

**Highly significant (p≤0.01).

Means bearing the same superscript within the same row do not differ significantly.

Fig 7: Blood β-hydroxybutyric acid concentration in clinical pregnancy toxaemic doe.

Fig 8: Blood glucose concentration in clinical pregnancy toxaemic group.

observed in the sub-clinical and clinical pregnancy toxaemic groups correlated with Hefnawy *et al.* (2011) and might be attributed to the decrease in feed intake, dehydration or large quantity of sodium loss in the renal excretion of acetoacetate and BHBA (Judith and Thomas, 1988).

A highly significant (p≤0.01) difference in potassium levels was observed between sub-clinical and clinical pregnancy toxaemic groups compared to that of control. Hypokalemia observed in sub- clinical and clinical pregnancy toxaemic groups correlated with Albay *et al.* (2014) and might be attributed to the decrease in feed intake and dehydration (Judith and Thomas, 1988) or inadequate feed intake and incomplete renotubular absorption of potassium (Henze *et al.*, 1998), or lowered feed intake and loss of potassium ions in the urine as observed in human patients with ketonuria and ketoacidosis (Lima *et al.,* 2016).

A highly significant (p≤0.01) difference was observed in calcium levels between sub-clinical and clinical pregnancy toxaemic groups compared to the pregnant does of control. The hypocalcemia observed in pregnancy toxaemic does correlated with Hefnawy *et al.* (2011) and may be due to the disturbance in the electrolytes and minerals which might be due to stress of starvation, dehydration, electrolyte imbalance or due to enhanced lipolysis (Judith and Thomas, 1988). Alternate reasons might be due to the high demand of calcium by the developing offspring at the late stage of gestation, enhanced lipolysis as a result of high cortisol level in circulation, or fatty liver interfering with hydroxylation of Vitamin D and decreased intestinal absorption of calcium as pointed by Andrews (1997) or anorexia and disturbance of acid base balance (acidosis) with the excretion of calcium ions in urine or might be the sequelae to renal insufficiency (Rook, 2000).

A highly significant (p≤0.01) difference was observed in magnesium levels between sub-clinical and clinical pregnancy toxaemic groups compared to that of control. The hypomagnesemia observed in the pregnancy toxaemic does correlated with Hefnawy *et al.* (2011) and may be due to the disturbance in the electrolytes and some minerals related to stress of starvation, dehydration, involvement of the kidney or due to enhanced lipolysis (Judith and Thomas, 1988).

A highly significant (p≤ 0.01) difference in chloride levels was observed between sub-clinical and clinical pregnancy toxaemic groups compared to that of control. The hyperchloridemia observed in pregnancy toxaemic does correlated with Abdallah *et al.* (2015) and the reasons might be due to the metabolic acidosis as a result of proportionally smaller loss of chloride than bicarbonate and improved renal reabsorption of chloride in response to decreased bicarbonate (Kaneko *et al.,* 1997).

The mean±SE values of serum BHBA (µmol/L), NEFA (µmol/L) and cortisol (nmol/L) concentration in control, sub-clinical and clinical pregnancy toxaemic groups are presented in (Table 5). A highly significant (p≤0.01) difference

in serum BHBA concentration was observed between sub-clinical and clinical pregnancy toxaemic groups compared to that of control and correlated with Ismail *et al.* (2008). Elevated levels of BHBA might be attributed to the oxidation of long chain fatty acids into ketone bodies, *viz*., acetoacetate and beta hydroxy butyrate in the liver following lipolysis during periods of negative energy balance (Nassif *et al.,* 2005) or due to the reduction of acetoacetate produced by the liver to beta hydroxybutyrate by hydroxybutyrate dehydrogenase enzyme amounting to higher blood concentration of BHBA (Hefnawy *et al.,* 2011). Elevated levels of serum NEFA in the sub-clinical and clinical pregnancy toxaemic does correlated with Ismail *et al.* (2008). Elevated levels of NEFA might be the result of adipolysis during periods of negative energy balance (Vasava *et al*., 2016). A highly significant ($p \leq 0.01$) difference in serum cortisol concentration was observed between the sub-clinical and clinical pregnancy toxaemic groups compared to that of control. Increasing trend of cortisol concentration in pregnant and pregnancy toxaemic does correlated with Hefnawy *et al.,* 2011; Abdallah *et al.,* 2015. Increase in cortisol concentration might be due to hyperactivity of the adrenal glands as a result of hypoglycemia (Adel *et al.,* 2005) or due to reduced hepatic metabolism of cortisol (Radostits *et al.,* 2000) or increasing stress in the pregnant animals (Aly and Elshahawy, 2016).

The sub-clinical pregnancy toxaemic does responded to therapy and had a cure rate of 100%. The distribution of cases in clinical pregnancy toxaemic group is presented in (Table 6). Four does (33%) were presented in sternal recumbency with lateral deviation of neck and were found to be >140 days pregnant with the aid of ultrasound. The fetal heart beat were completely absent in these four does which indicated fetal death. The blood BHBA concentrations were >7 mmol/L (7.2 mmol/L, 7.6 mmol/L, 7.8 mmol/L and 7.9 mmol/L, respectively) and with abnormally high glucose levels (207 mg/dL, 78 mg/dL, 76 mg/dL and 132 mg/dL, respectively). The hypergly-caemia in advanced pregnancy toxaemic goats indicate fetal death and the reason were attributed to the removal of the suppressing effect of the fetus on hepatic gluconeogenesis (Wastney *et al.,* 1983; Lima *et al.,* 2012) or to the increased serum cortisol level (Ford *et al.,* 1990). They were resorted to treatment with intravenous glucose therapy (5% Dextrose) supported with Vitamin B_1 B_6 and B_{12} and antihistaminic drug chlorpheniramine maleate @ 0.5 mg/kg body weight intramuscularly on the day of presentation. The owners were advised caesarean section in order to save the dam, of which

Table 5: Mean±SE of serum BHBA, NEFA and Cortisol concentration in group 1 (Livestock Farm Complex), group 2 (Sub-clinical Pregnancy Toxaemia) and group 3 (Clinical Pregnancy Toxaemia).

Parameters	Group 1 (Control)		Group 2 (n=12)	Group 3 (n=12)	F' value
	Non-pregnant) does (n=12	Pregnant does (n=12) 120 days			
Beta hydroxybutyric acid (BHBA) (µmol/L)	275.0^{c}±31.34	312.5^{c}±29.51	1308.33^{a}±58.34	5058.33^{b}±652.81	8.86**
Non-esterified fatty acid (NEFA) (µmol/L)	406.56±49.23	434.42±77.14	534.52±89.17	641.37±61.16	2.03^{NS}
Cortisol (nmol/L)	295.61^{a}±54.53	348.32^{a}±33.98	600.76^{b}±111.55	737.36^{b}±69.02	6.13**

NS: Not Significant; **Highly significant ($p \leq 0.01$).

Means bearing the same superscript within the same row do not differ significantly.

Table 6: Distribution of cases in clinical pregnancy toxaemic group (n=12).

Days of gestation	No. of does	Clinical signs	BHBA (mmol/L)	Blood glucose (mg/dL)	Fetal status	Dam recovery status
> 140 days	4(33%)	Sternal recumbency with lateral deviation of neck	7.2	207	Dead	Died
			7.6	78		
			7.8	76	Dead	Disposed
			7.9	132		
120 - 140 days	8(67%)	Standing posture with stargazing	3.6	27	Alive	Disposed
			3.8	22	Alive	
		Sternal recumbency	5.2	23	Feeble heart beat	Died
		Sternal recumbency with lateral deviation of neck	6.7	24		
		Standing posture, Anorexia, Dullness, Bruxism	2.1	21	Alive	Recovered
			2.2	22		
			3.1	27		
			3.5	26		

two of the owners did not accept and decided to dispose off, while the remaining two does died later in the evening before the owners decided to accept for the caesarean section. Parturition induction or caesarean section is the recommended treatment in advanced stages of pregnancy toxaemia or in pregnant does that did not respond to the treatment due to the high glucose demand or in fetal death to save the dam (Brounts *et al.,* 2004; Lima *et al.,* 2012). The remaining eight does (67%) were in between 120 to 140 days of pregnancy, among which four had blood BHBA concentration of 3.6 mmol/L, 3.8 mmol/L, 5.2 mmol/L and 6.7 mmol/L, respectively. Out of these four does, two had BHBA levels above 5 mmol/L and were presented in sternal recumbency and the one with BHBA level of 6.7 mmol/L had lateral deviation of the neck in addition to sternal recumbency. Both the does had a feeble fetal heart beat and were resorted to treatment with intravenous glucose therapy (5% Dextrose) supported with parenteral Vitamin B_1, B_6 and B_{12} therapy. However both the does died the next day. The remaining two had blood BHBA concentration of 3.6 mmol/L and 3.8 mmol/L, respectively, and were presented in standing posture with stargazing. They were resorted to above treatment and oral administration of glycerine for 3-4 days @ 25 ml twice daily. These two does did not show much sign of recovery even after three days of therapy and hence the owners resorted to disposal of their does.

The remaining four does of the group (between 120 to 140 days of pregnancy) had BHBA concentration of 2.1 mmol/L, 2.2 mmol/L, 3.1 mmol/L and 3.5 mmol/L, respectively and were presented in standing posture. They were resorted to standard treatment. These does showed signs of recovery from third day of treatment in the form of alertness and improved feed intake. Out of the twelve does of clinical pregnancy toxaemic group, only four does showed signs of improvement to therapy with a cure rate of 33%, while the mortality was present in four (33%). The remaining four (33%) did not show any signs of recovery to therapy and hence the owners resorted to disposal of their does.

CONCLUSION

The recovery rates were better when the therapy was initiated in the early stage of the disease compared to the advanced stages of the disease. All the twelve does of sub-clinical pregnancy toxaemic group recovered completely with a cure rate of 100%, while in the clinical pregnancy toxaemic group the cure rate was only 33%. The reliable on field diagnostic and prognostic indicators of pregnancy toxaemia include blood β-hyroxybutyric acid concentration (≥0.8 mmol/L) and presence of ketone body, glucose and protein in urine, while hypergly-caemia in advanced pregnancy toxaemic does indicate fetal death.

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REFERENCES

Abdallah, A.A.M., El-Deen, N.A.M.N. and Ibrahim, A.A. (2015). Early biomarkers for diagnosis and prognosis of pregnancy toxemia in goats. Zagazig Veterinary Journal. 43: 130-142.

Abdelghafar, R.M., Ahmed, B.M., Ibrahim, M.T. and Mantis, P. (2011). Prediction of gestational age by transabdominal real time ultrasonographic measurements in Saanen goats (*Capra hircus*). Global Veterinaria. 6: 346-351.

Adel, M.A., Sahar, S. and El-Hamied. A. (2005). Anion gap determination and its relationship to some serum biochemical indicators in pregnancy toxemia in does. Zagazig Veterinary Journal. 33: 162-168.

Albay, M.K., Karakurum, M.C., Sahinduran, S., Sezer, K. and Yildiz, R. (2014). Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Veterinary Medicine. 59: 336-342.

Alidadi, N., Rafia, S. and Moaddab, H. (2012). Outbreak of primary pregnancy toxemia in fat tailed ewes due to ultra sonographic misdiagnosis of pregnancy. Irish Journal of Veterinary Research. 13: 870-879.

Aly, M.A. and Elshahawy, I.I. (2016). Clinico-biochemical diagnosis of pregnancy toxaemia in ewes with special reference to novel biomarkers. Alexandria Journal of Veterinary Sciences. 48: 96-102.

Andrews, A. (1997). Pregnancy toxaemia in the ewe. In Practice. 19: 306-312.

Barakat, S.E.M., Al-Bhanasawi, N.M., Elazhari, G. and Bakhiet, A.O. (2007). Clinical and serobiochemical studies on naturally occuring pregnancy toxaemia in Shammia goats. Journal of Animal and Veterinary Advances. 6: 768-772.

Brounts, S.H., Hawkins, J.F., Baird, A.N. and Glickman, L.T. (2004). Outcome and subsequent fertility of sheep and goats undergoing cesarean section because of dystocia: 110 cases (1981-2001). Journal of American Veterinary Medical Association. 224: 275-281.

Cleon, V.K. (1988). Disease of Sheep. 3rd Edn, Lea and Febiger, Philadelphia, USA.

El-Sayed, R. and Siam, A. (1994). Pregnancy toxaemia in ewes. Alexandria Journal of Veterinary Sciences. 1: 31-35.

Emam, E.E. and Galhoom, K.I. (2008). Hormonal, haematological blood biochemical changes in pregnancy toxaemia in Balady goats (*Caprines caprina*) with trials of treatment as a field study. Egypt Journal of Comparative Pathology and Clinical Pathology. 21: 121-138.

Ford, E.J., Evans, J. and Robinson, I. (1990). Cortisol in pregnancy toxaemia of sheep. British Veterinary Journal. 146: 539-542.

Franklin, S.T. and Young, J.W. (1991). Effects of ketones, acetate, butyrate and glucose on bovine lymphocyte proliferation. Journal of Dairy Science. 74: 2507-2514.

Hefnawy, A.E., Shousha, S. and Youssef, S. (2011). Hemato-biochemical profile of pregnant and experimentally pregnancy toxemic goats. Journal of Basic Applied Chemistry. 1: 65-69.

Henze, P., Bickhardt, K., Fuhrmann, H. and Sallmann, H.P. (1998). Spontaneous pregnancy toxaemia (ketosis) in sheep and the role of insulin. Journal of American Veterinary Medical Association. 45: 255-266.

Ismail, Z.A.B., Al-Majali, A.M., Amireh, F. and Al- Rawashdeh, O.F. (2008). Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. Veterinary Clinical Pathology. 37: 434-437.

Judith, V.M. and Thomas, H.H. (1988). Pregnancy toxemia and ketosis in ewes and does. Veterinary Clinics of North America Food Animal Practice. 4: 307-315.

Kaneko, J.J., Harvey, J.W. and Bruss, M.L. (1997). Clinical Biochemistry of Domestic Animals. 5th Edn. Academic Press, California, U.S.A.

Lima, M.S., Pascal, R.A. and Stilwell, G.T. (2012). Glycaemia as a sign of the viability of the foetuses in the last days of gestation in dairy goats with pregnancy toxemia. Irish Veterinary Journal. 65: 136-148.

Lima, M.S., Silveira, J.M., Carolino, N., Lamas, L.P, Pascoal, R.A. and Hjerpe, C.A. (2016). Usefulness of clinical observations and blood chemistry values for predicting clinical out comes in dairy goats with pregnancy toxaemia. Irish Veterinary Journal. 69: 16-24.

Marteniuk, J.V. and Herdt, T.H. (1988). Pregnancy toxemia and ketosis of ewes and does. Veterinary Clinics of North America Food Animal Practice. 4: 307-315.

Nasr, M., Fauad, F.M. and El-Said, B. (1997). Studies on pregnancy toxaemia in does. Alexandria Journal of Veterinary Science. 13: 79-86.

Nassif, M.N., Hafez, A.M., Nasser, M.H. and Tahoon, A.M. (2005). Clinico-biochemical studies on experimentally induced pregnancy toxemia in does. Zagazig Veterinary Journal. 33: 31-41.

Pichler, M., Damberger, A., Amholdt, T., Schwendenwein, I. and Gasteiner, J. (2014). Evaluation of 2 electronic handheld devices for diagnosis of ketonemia and glycemia in dairy goats. Journal of Dairy Science. 97: 7538-7546.

Radostits, O.M., Blood, D.C. and Gay, C.C. (2000). A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. 9th Edn. W.B. Saunders, Canada.

Rook, J.S. (2000). Pregnancy toxemia of ewes, does and beef cows. Veterinary Clinics of North America Food Animal Practice. 16: 293-317.

Russel, A. (1984). Body condition scoring of sheep. In Practice. 6: 91-93.

Schlumbohm, C. and Harmeyer, J. (2004). Hyperketonemia impairs glucose metabolism in pregnant ewes. Journal of Dairy Science. 87: 350-358.

Schlumbohm, C. and Harmeyer, J. (2008). Twin pregnancy increases susceptibility of ewes to hypoglycemic stress and pregnancy toxemia. Research in Veterinary Science. 84: 286-299.

Van Saun, R.J. (2000). Pregnancy toxemia in a flock of sheep. Journal of American Veterinary Medical Asociation. 217: 1536-1539.

Vasava, P.R., Jani, R.G., Goswami, H.V., Rathwa, S.D. and Tandel, F.B. (2016). Studies on clinical signs and biochemical alteration in pregnancy toxemic goats. Veterinary World. 9: 869-874.

Villaquiran, M., Gipson, R., Merkel, R., Goetsch, A. and Sahlu, T. (2012). Body Condition Scores in Goats. Agriculture Research and Cooperative Extension. Langston University, Langston.

Wastney, M.E., Wolf, J.R. and Bickerstafle, R. (1983). Glucose turnover and hepatocyte glucose production of starved and toxaemic pregnant sheep. Australian Journal of Biological Sciences. 36: 271-284.

Yadav, S.N., Kalita, D.N., Phukan, A., Tamuly, S., Dutta, T.C. and Mahato, G. (2016). Diagnosis of caprine ketosis using human hand held ketone meter. Bangladesh Journal of Veterinary Medicine. 14: 179-181.

Yarim, G.F. and Ciftci, G. (2009). Serum protein pattern in ewe with pregnancy toxaemia. Veterinary Research Communication. 33: 431-438.