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Clinical pregnancy toxaemia diagnostic indicators and therapeutic evaluation in goats

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Abstract
Out of 516 adult non descriptive does brought to Veterinary University Peripheral Hospital, Madhavaram Milk Colony, Chennai – 51, during the period October 2016 to September 2018, 264 (51.16%) were treated for various medical conditions. Among the does treated, 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 beta hydroxybutyric acid (BHBA) concentration by means of a portable blood ketone and glucose monitoring system and qualitative urinalysis using urine dip stick. Does with beta hydroxybutyric acid level > 1.6 mmol/L were classified as clinical pregnancy toxaemic group (n = 12). The control animals were selected from adult Tellicherry does in the age group of 2 to 4 years maintained at a private goat farm (ECR Goat Farm), Injambakkam, Chennai. The clinical pregnancy toxaemic group (n = 12) were resorted to treatment with intravenous glucose therapy (5% Dextrose), parenteral therapy of Vitamin B_1, B_6 & B_{12} and oral administration of glycerine @ 25 ml twice daily. Of the twelve animals treated only four showed signs of improvement to therapy with a cure rate of 33%, while mortality was present in four does (33%) and the remaining four (33%) did not show any sign of recovery to therapy and hence the owners resorted to disposal of their animal. Reliable diagnostic indicators for detection of pregnancy toxaemia under field conditions include presence of ketone body in urine and blood BHBA (≥ 0.8 mmol/L).

Keywords: clinical pregnancy toxaemic goats, diagnostic indicators, therapeutic evaluation

Introduction
Periparturient mortality in goats have a great economic impact on the livelihood of marginal farmers. Pregnancy toxaemia also called as gestational ketosis, twin-lamb disease, ketosis of pregnancy, kid disease, lambing sickness, kidding paralysis and lambing or kidding ketosis is a metabolic disease affecting pregnant ewes and does after a period of negative energy balance (NEB) and impaired gluconeogenesis (Lima *et al.*, 2012) [20]. Pregnancy toxaemia normally occur 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 foetuses (Schlumbohm and Harmeyer, 2008) [29]. 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) [15]. 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 concentration of ketone bodies (hyperketonaemia) and high mortality rate (Van Saun, 2000) [30]. The mortality rate can attain 100% even with the initiation of treatment due to severe irreversible organ damage. In goat farming reliable diagnostic indicators of negative energy balance in the primary stage of the disease are the need of the hour for better herd health management.

Materials and Methods
The study was carried out at Veterinary University Peripheral Hospital (VUPH), Madhavaram Milk Colony, Chennai – 6000 051 and a private goat farm (ECR Goat Farm), Injambakkam, 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 ECR Goat Farm, Injambakkam, Chennai. Non pregnant does (n = 12) and pregnant does (n = 12) carrying twins / triplets, without exhibiting signs of pregnancy toxaemia throughout gestation served as control. Does in their last six weeks of gestation carrying twins / triplets presented with the

history of off feed and dullness to Veterinary University Peripheral Hospital, Madhavaram Milk Colony, Chennai were subjected to determination of blood beta hydroxybutyric acid (BHBA) concentration by means of a portable blood ketone and glucose monitoring system and qualitative urinalysis using urine dip stick. The pregnant does were subjected to radiography for conformation of pregnancy and assessment of fetal numbers and ultrasonography to assess the stage of pregnancy. Does with BHBA level > 1.6 mmol/L were classified as clinical pregnancy toxaemia.

Parameters included in the Study

Clinical Signs

The clinical signs exhibited by the pregnant does were recorded.

Blood BHBA concentration

The blood 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) [25].

Fig 1: Portable Blood ketone monitoring system

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) [4]. The urine samples were analyzed using Multistix 10 SG reagent strip (Siemens Healthcare Private Limited, India) for qualitative determination of ketone bodies, glucose and protein (Emam and Galhoom, 2008) [12]. 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. (Fig 2).

Fig 2: Urinalysis using Multistix 10SG reagent strip in sub clinical pregnancy toxaemic doe

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) and X = Fetal parameter (cm) in case of crown rump length and Y = 2.675 + 3.229 X where Y = Gestational age (wks) and X = Fetal parameter (cm) in case of bi-parietal diameter (Abdelghafar *et al.*, 2011) [2].

Radiography

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

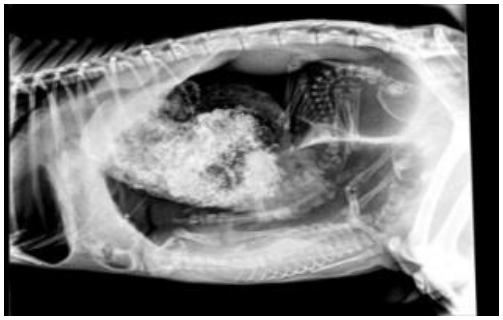

Fig 3: Radiography in pregnant doe – Twins

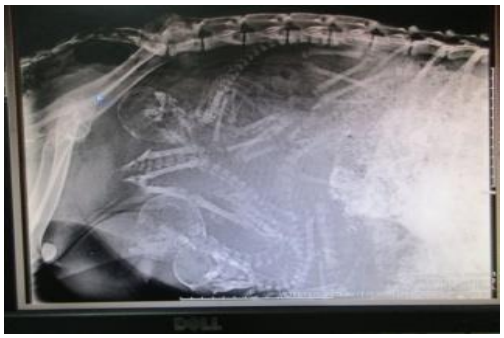

Fig 4: Radiography in pregnant doe - Triplets

Haematology

Haematological investigation with automated haematology analyzer (Mindray BC 2800 Vet): haemoglobin (g/dL, packed cell volume (%), red blood cell ($X10^6$/cmm), white blood cells (/cmm) and differential 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 (A 15 Random Access 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 (Diestro 103 AP)**.**

Serum Metabolites

The serum was stored at – 20º C until analysis of levels of serum metabolites namely beta hydroxybutyric acid (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 (My Bio

Source Inc., USA) while the level of serum cortisol (nmol/L) was analyzed by Enzyme Linked Immunosorbent Assay (ELISA) method 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) and oral administration of glycerine for 3-4 days @ 25 ml twice daily supported with parenteral Vitamin B_1, B_6 & B_{12} therapy. The response to therapy was evaluated 3-5 days post initiation of therapy and the efficacy was assessed 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 were 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 recorded in clinical pregnancy toxaemia 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 (Fig. 5) in 9 (67%), sternal recumbency (Fig. 6) in 6 (50%) and lateral deviation of neck (Fig. 7) in 5 (42%).

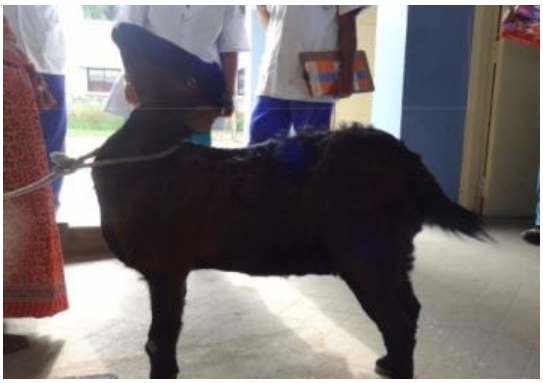

Fig 5: Star gazing

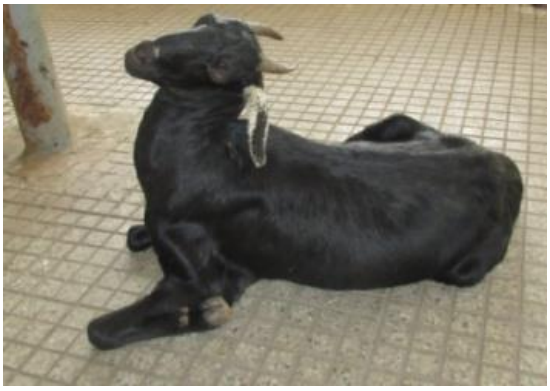

Fig 6: Sternal recumbency

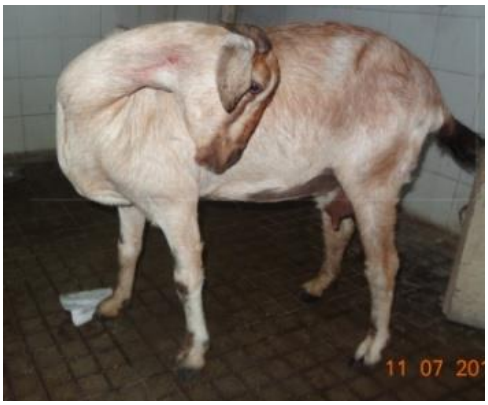

Fig 7: Lateral deviation of neck

The BHBA concentration of blood recorded in control group ranged between 0.2 mmol/l to 0.4 mmol/l (Fig. 8) and between 2.1 mmol/l to 7.9 mmol/l in clinical pregnancy toxaemic does (Fig. 9) concurred with Andrews (1997) [7]. The values obtained in the portable ketone meter were immediate, reliable and highly useful in screening does for pregnancy toxaemia under 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) [33].

Fig 8: Blood BHBA concentration in healthy non pregnant doe

Fig 9: Blood BHBA concentration in Clinical Pregnancy Toxaemic Doe

Urinalysis in control group indicated absence of ketone bodies, glucose and protein while in the pregnancy toxaemic group, presence of ketone bodies, protein and glucose are diagnostic. The ketone bodies grading were trace in 2 does (17%), moderate in 2 does (17%), small in 4 does (33%) and large in 4 does (33%). The protein grading were 1 + in 3 does

(25%), 2 + in 4 does (33%) and 3 + in 5 does (42%), while the glucose grading were trace in 2 does (17%), 1 + in 1 doe (8%), 2 + in 5 does (42%) and 3 + in 4 does (33%) respectively. 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 which concurred with the findings of Emam and Galhoom (2008) [12].

The Mean ± S.E. of Haemoglobin, Packed Cell Volume, Red Blood Cells and White Blood Cells in control and clinical pregnancy toxaemic group are presented in Table 1. The haemoglobin, packed cell volume and red blood cell values in clinical pregnancy toxaemic group were higher than the control group. Highly significant ($P \leq 0.01$) difference was observed in the above values between the clinical pregnancy toxaemic group and that of control group. The significant increase of the above values in the pregnancy toxaemic does may be due to hemoconcentration and dehydration as stated by Hefnawy *et al.* (2011) [15].

Table 1: Mean ± S.E. of Haemoglobin, Packed Cell Volume, Red Blood Cells and White Blood Cells in Control and Clinical Pregnancy Toxaemic Group

Parameters	Control			Clinical Pregnancy Toxaemic Group (n = 12)	'F' value
	Non Pregnant Does (n = 12)	Pregnant Does (n = 12) Gestation in days			
		120 days	150 days		
Haemoglobin (g/dL)	$8.31^{a} \pm 0.07$	$8.45^{a} \pm 0.07$	$8.45^{a} \pm 0.04$	$9.06^{b} \pm 0.13$	8.13**
Packed Cell Volume (%)	$22.36^{a} \pm 0.54$	$22.80^{a} \pm 0.87$	$23.23^{a} \pm 0.83$	$28.13^{c} \pm 0.24$	9.68**
Red Blood Cells ($X10^{6}$/cmm)	$14.20^{a} \pm 0.64$	$15.19^{a} \pm 0.69$	$15.99^{a} \pm 0.61$	$17.95^{b} \pm 0.19$	8.10**
White Blood Cells (/cmm)	21325 ± 457.45	20741.66 ± 1773.3	20558.33 ± 1496.93	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

The Mean ± S.E. of Differential Count in control and clinical pregnancy toxaemic group are presented in Table 2. Neutrophilia was observed in clinical pregnancy toxaemic group compared to that of control group. The neutrophilia might be due to the increased cortisol level which created a movement of granulocytes from the bone marrow to the peripheral blood as stated by Alidadi *et al.* (2012) [5]. The Lymphocytes in the clinical pregnancy toxaemic group was lower than the control group. Lymphopenia in clinical pregnancy toxaemic does might be due to the toxic and subtoxic concentration of beta hydroxybutyrate and acetoacetate in blood which inhibit the lymphocytic proliferation (Franklin and Young, 1991) [14] or may be due to increased cortisol level as stated by Alidadi *et al.* (2012) [5]. With respect to Basophils significant ($P \leq 0.05$) difference was observed between the clinical pregnancy toxaemic group and control.

Table 2: Mean ± S.E. of Differential Count in Control and Clinical Pregnancy Toxaemic Group

Parameters	Control			Clinical Pregnancy Toxaemic Group (n = 12)	'F' value
	Non Pregnant Does (n = 12)	Pregnant Does (n = 12) Gestation in days			
		120 days	150 days		
Neutrophils (%)	$37.0^{b} \pm 0.95$	$32.75^{a} \pm 0.46$	$33.16^{a} \pm 0.62$	$53.58^{d} \pm 1.68$	58.04**
Lymphocytes (%)	$59.66^{c} \pm 0.69$	$62.33^{cd} \pm 0.43$	$62.75^{d} \pm 0.50$	$42.66^{a} \pm 1.68$	65.82**
Monocytes (%)	2.33 ± 0.30	2.66 ± 0.22	2.75 ± 0.21	2.5 ± 0.15	0.50 NS
Eosinophils (%)	0.91 ± 0.25	1.66 ± 0.25	1.08 ± 0.31	1.25 ± 0.13	1.39 NS
Basophils (%)	$0.08^{a} \pm 0.08$	$0.50^{b} \pm 0.15$	$0.25^{ab} \pm 0.13$	$0^{a} \pm 0$	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

The Mean ± S.E. of Blood Urea Nitrogen, Creatinine, Aspartate aminotransferase, Alanine aminotransferase, Glucose and Total Protein in control and clinical pregnancy toxaemic group are presented in Table 3. A highly significant ($P \leq 0.01$) difference was observed between the clinical pregnancy toxaemic group and control in blood urea nitrogen and creatinine levels. Elevated levels observed in clinical pregnancy toxaemic does concurred with the findings of Hefnawy *et al.* (2011) [15]. The reason for increased blood urea nitrogen and creatinine levels may be due to severe kidney dysfunction due to the elevated ketone bodies in general circulation (El-Sayed and Siam, 1994) [11], or due to reduced glomerular filtration due to fatty infiltration in tubular epithelium of kidney (Barakat *et al.*, 2007) [8] or due to death and decomposition of fetuses (Radostits *et al.*, 2000) [26].

Table 3: Mean ± S.E. of Serum Biochemical Parameters in Control and Clinical Pregnancy Toxaemic Group

Parameters	Control			Clinical Pregnancy Toxaemic Group (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)	$28.45^{b} \pm 0.81$	$24.77^{a} \pm 1.13$	$24.90^{a} \pm 0.82$	$47.74^{d} \pm 1.16$	79.02**
Creatinine (mg/dL)	$0.74^{a} \pm 0.01$	$0.76^{a} \pm 0.04$	$0.73^{a} \pm 0.02$	$1.69^{c} \pm 0.09$	32.30**
Aspartate aminotransferase (AST) (IU/L)	$94.41^{a} \pm 1.08$	$105.5^{b} \pm 3.04$	$112.91^{b} \pm 0.99$	$144.66^{e} \pm 3.57$	39.83**
Alanine aminotransferase (ALT) (IU/L)	$30.5^{b} \pm 1.76$	$44.41^{c} \pm 2.14$	$45.41^{c} \pm 1.99$	$76.70^{d} \pm 2.44$	76.76**

Glucose (mg/dL)	$52.66^{b} \pm 1.33$	$31.08^{a} \pm 1.72$	$30.08^{a} \pm 1.15$	$57.0^{b} \pm 11.57$	6.03**
Total Protein (g/dL)	$7.06^{de} \pm 0.03$	$7.11^{e} \pm 0.13$	$7.12^{e} \pm 0.12$	$6.25^{a} \pm 0.04$	10.56**

**Highly Significant ($P \leq 0.01$)
Means bearing the same superscript within the same row do not differ significantly

A highly significant ($P \leq 0.01$) difference in aspartate aminotransferase and alanine aminotransferase levels was observed between the clinical pregnancy toxaemic group and control. Elevated activities of the enzymes observed in clinical pregnancy toxaemic group correlated with the reports of Barakat *et al.* (2007) [8]. The reasons for increased activities in the clinical pregnancy toxaemic group 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 to adipolysis and hepatic ketogenesis following energy deficit (Nassif *et al.*, 2005) [24].

A highly significant ($P \leq 0.01$) difference was observed between the clinical pregnancy toxaemic group and control with respect to glucose level. The glucose level in clinical pregnancy toxaemic group 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 beta hydroxybutyric acid and glucose levels were monitored using portable human blood ketone and glucose monitoring system which indicated beta hydroxybutyric acid levels > 7 mmol/L (7.2 mmol/L, 7.6 mmol/L, 7.8 mmol/L and 7.9 mmol/L respectively) and abnormally high glucose levels (207 mg/dL, 78 mg/dL, 76 mg/dL and 132 mg/dL respectively) (Fig. 10).

Fig 10: Blood Glucose concentration in Clinical pregnancy toxaemic group

This finding correlated with Lima *et al.* (2012) [20] who stated hyperglycemia to occur in pregnancy toxaemic does with fetal death. The mean ± S.E. of glucose levels for the remaining eight does were 23.87 ± 0.48 which indicated hypoglycemia and this correlated with Rook (2000) [27]. Hypoglycemia might be due to long periods of starvation as pointed by Andrews (1997) [7] or 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 beta hydroxybutyric acid level in blood which can suppress endogenous glucose production and reduction in food intake as pointed by Marteniuk and Herdt (1988) and Schlumbohm and Harmeyer (2004) [22, 28]. The hyperglycaemia in advanced pregnancy toxaemic goats indicate foetal death and the reason were attributed to the removal of the suppressing effect of the foetus on hepatic gluconeogenesis (Lima *et al.*, 2012) [20] or due to the increased serum cortisol level (Cleon, 1988) [10].

With respect to protein, decreased levels observed in clinical pregnancy toxaemic group compared to control correlated with Barakat *et al.* (2007) [8]. The reason for decreased total protein levels observed in the clinical pregnancy toxaemic group might be due to the anorexia and reduction in albumin synthesis due to hepatic insufficiency and albuminuria (Yarim and Ciftci, 2009) [34] or might be due to malnutrition resulting in inadequate provision of amino acid substrate for general protein production (Nasr *et al.*, 1997) [23].

The Mean ± S.E. of Sodium, Potassium, Calcium, Magnesium and Chloride in control and clinical pregnancy toxaemic group are presented in Table 4.

Table 4: Mean ± S.E. of Serum Electrolytes in Control and Clinical Pregnancy Toxaemic Group

Parameters	Control			Clinical Pregnancy Toxaemic Group (n = 12)	'F' value
	Non Pregnant Does (n = 12)	Pregnant Does (n = 12) Gestation in days			
		120 days	150 days		
Sodium (mmol/L)	$142.58^{b} \pm 0.38$	$146.35^{c} \pm 0.75$	$145.97^{c} \pm 0.48$	$136.1^{a} \pm 0.59$	65.42**
Potassium (mmol/L)	$5.09^{b} \pm 0.05$	$4.94^{b} \pm 0.09$	$5.08^{b} \pm 0.08$	$4.34^{a} \pm 0.05$	14.78 **
Chloride (mmol/L)	$108.17^{a} \pm 0.34$	$108.75^{ab} \pm 0.38$	$108.72^{ab} \pm 0.30$	$112.53^{c} \pm 0.17$	16.27**
Calcium (mg/dL)	$11.21^{b} \pm 0.19$	$11.35^{b} \pm 0.10$	$11.32^{b} \pm 0.15$	$9.13^{a} \pm 0.20$	20.05 **
Magnesium (mg/dL)	$2.91^{b} \pm 0.04$	$3.05^{b} \pm 0.05$	$3.09^{b} \pm 0.06$	$2.62^{a} \pm 0.06$	12.01**

**Highly Significant ($P \leq 0.01$)
Means bearing the same superscript within the same row do not differ significantly

A highly significant ($P \leq 0.01$) difference in sodium levels was observed between the clinical pregnancy toxaemic group and control. Hyponatremia observed in the clinical pregnancy toxaemic group correlated with Hefnawy *et al.* (2011) [15]. The hyponatremia observed might be attributed to the decrease in feed intake, dehydration or large quantity of sodium loss in the renal excretion of acetoacetate and beta hydroxybutyrate (Judith and Thomas, 1988) [18].

A highly significant ($P \leq 0.01$) difference in potassium levels was observed between the clinical pregnancy toxaemic group and control. Hypokalemia observed in clinical pregnancy toxaemic group correlated with Albay *et al.* (2014) [4]. The hypokalemia observed in pregnancy toxaemic does may be attributed to the decrease in feed intake and dehydration (Judith and Thomas, 1988) [18] or may be due to inadequate feed intake and incomplete renotubular absorption of potassium (Henze *et al.*, 1998) [16], or may be due to lowered feed intake and due to los of potassium ions in the urine as observed in human patients with ketonuria and ketoacidosis (Lima *et al.*, 2016) [21].

A highly significant ($P \leq 0.01$) difference was observed in calcium levels between the clinical pregnancy toxaemic group and control. The hypocalcemia observed in clinical pregnancy toxaemic group correlated with Hefnawy *et al.* (2011) [15]. The hypocalcemia observed in clinical pregnancy toxaemic does 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) [18]. Alternate reasons might be due to the high demand of calcium by the developing offspring at the late stage of gestation, due to 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 (Andrews, 1997) [7] 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) [27].

A highly significant ($P \leq 0.01$) difference was observed in magnesium levels between the clinical pregnancy toxaemic group and control. The hypomagnesemia observed in clinical pregnancy toxaemic group correlated with Hefnawy *et al.* (2011) [15]. Hypomagnesemia in pregnancy toxaemic does may be due to the disturbance in the electrolytes and minerals related to stress of starvation, dehydration, involvement of the kidney or due to enhanced lipolysis (Judith and Thomas, 1988) [18].

A highly significant ($P \leq 0.01$) difference in chloride levels was observed between the clinical pregnancy toxaemic group and control. The hyperchloridemia observed in clinical pregnancy toxaemic group correlated with Abdallah *et al.* (2015) [1]. The reasons for hyperchloridemia in clinical pregnancy toxaemic does might be attributed 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) [19].

The Mean ± S.E. of serum beta hydroxybutyric acid (µmol/L), non esterified fatty acid (µmol/L) and cortisol (nmol/L) concentration in control and clinical pregnancy toxaemic group assessed by ELISA method are presented in Table 5.

Table 5: Mean ± S.E. of Serum Beta hydroxybutyric acid (BHBA), Non Esterified Fatty Acid (NEFA) and Cortisol Concentration by ELISA method in Control and Clinical Pregnancy Toxaemic Group

Parameters	Control		Clinical Pregnancy Toxaemic Group (n = 12)	'F' value
	Non-pregnant Does (n = 12)	Pregnant does (n = 12) 120 days gestation		
Beta hydroxybutyric acid (BHBA) (µmol/L)	$275.0^{c} \pm 31.34$	$312.5^{c} \pm 29.51$	$5058.33^{b} \pm 652.81$	8.86**
Non-esterified fatty acid (NEFA) (µmol/L)	406.56 ± 49.23	434.42 ± 77.14	641.37 ± 61.16	2.03^{NS}
Cortisol (nmol/L)	$295.61^{a} \pm 54.53$	$348.32^{a} \pm 33.98$	$737.36^{b} \pm 69.02$	6.13**

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

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

A highly significant ($P \leq 0.01$) difference in serum beta hydroxybutyric acid concentration was observed between the clinical pregnancy toxaemic group and control correlated with Ismail *et al.* (2008) [17]. Elevated levels of beta hydroxybutyric acid in the blood might be attributed to the oxidation of long chain fatty acids into ketone bodies namely acetoacetate and beta hydroxybutyrate in the liver following lipolysis during periods of negative energy balance (Nassif *et al.*, 2005) [24] or to the reduction of acetoacetate produced by the liver to beta hydroxybutyrate by hydroxybutyrate dehydrogenase enzyme amounting to higher blood concentration of beta hydroxybutyrate (Hefnawy *et al.*, 2011) [15]. Elevated levels of serum non esterified fatty acid in the clinical pregnancy toxaemic does correlated with Ismail *et al.* (2008) [17]. Elevated levels of non esterified fatty acid might be the result of adipolysis during periods of negative energy balance (Vasava *et al.*, 2016) [31]. A highly significant ($P \leq 0.01$) difference in serum cortisol concentration was observed between the clinical pregnancy toxaemic group and control. Increasing trend of cortisol concentration in pregnant and clinical pregnancy toxaemic does correlated with Abdallah *et al.* (2015) [1]. Increase in cortisol concentration might be due to hyperactivity of the adrenal glands as a result of hypoglycemia (Adel *et al.*, 2005) [3] or due to reduced hepatic metabolism of cortisol (Radostits *et al.*, 2000) [26] or due to increasing stress in the pregnant animals (Aly and Elshahawy, 2016) [6].

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 beta hydroxybutyric acid and glucose levels were monitored using portable human blood ketone and glucose monitoring system which indicated blood beta hydroxybutyric acid concentration > 7 mmol/L (7.2 mmol/L, 7.6 mmol/L, 7.8 mmol/L and 7.9 mmol/L respectively) and abnormally high glucose levels (207 mg/dL, 78 mg/dL, 76 mg/dL and 132 mg/dL respectively).

Table 6: Distribution of cases in Clinical Pregnancy Toxaemic Group

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		

They were resorted to treatment with intravenous glucose therapy (5% Dextrose) supported with parenteral therapy of Vitamin B_1, B_6 & B_{12} and antihistaminic drug Chlorpheniramine maleate @ 0.5 mg/kg body weight intramuscularly on the day of presentation. However the owners were advised caesarean section to be performed in their does in order to save the dam. Two of the owners did not accept for caesarean section and decided to dispose off their animal, while the remaining two does died later in the evening before the owners accepted for the caesarean section. Caesarean section was the recommended treatment in advanced stages or in heavily pregnant does that did not respond well to treatment due to the high glucose demand or in fetal death to save the dam (Lima *et al.*, 2012) [20]. The remaining eight does (67%) were in between 120 to 140 days of pregnancy. Among the eight, four had blood beta hydroxybutyric acid concentration of 3.6 mmol/L, 3.8 mmol/L, 5.2 mmol/L and 6.7 mmol/L respectively. Out of the 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 therapy of Vitamin B_1, B_6 & B_{12}. However both the does died on the next day. The remaining two which had blood beta hydroxybutyric acid concentration of 3.6 mmol/L and 3.8mmol/L were presented in standing posture with stargazing. They were resorted to treatment with intravenous glucose therapy (5% Dextrose) supported with parenteral therapy with Vitamin B1, B6 & B12 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 decided to disposed off their does.

The remaining four of the group (between 120 to 140 days of pregnancy) had blood beta hydroxybutyric acid concentration of 2.1 mmol/L, 2.2 mmol/L, 3.1 mmol/L and 3.5 mmol/L respectively. They were presented in standing posture with anorexia, dullness and bruxism. They were resorted to treatment with intravenous glucose therapy (5% Dextrose) supported with parenteral therapy of Vitamin B_1, B_6 & B_{12} and oral administration of glycerine for 3-4 days @ 25 ml twice daily. These does showed signs of recovery from third day of treatment in the form of alertness, improved feed intake and absence of bruxism. 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 mortality were present in four (33%). The remaining four (33%) did not show any signs of recovery to therapy and hence the owners decided to disposed off their does. In the present study the cure rate in clinical pregnancy toxaemic does were only 33% as against 73% (Brounts *et al.*, 2004) [9].

Conclusion

The present study showed a cure rate of 33% in clinical pregnancy toxaemic does. The early indicators of pregnancy toxaemia include presence of ketone body in the urine and blood BHBA concentration (≥0.8 mmol/l). Hence the determination of blood BHBA concentration using a portable blood ketone meter and qualitative urinalysis using urine dip stick for the presence of ketone bodies are reliable indicators in the diagnosis of pregnancy toxaemia under field conditions for better herd health management.

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Conflict of Interest

The Author(s) declare(s) that there is no conflict of interest and certify that all authors have seen and approved the manuscript being submitted. We warrant that the article is the authors' original work and that the article has not received prior publication and is not under consideration for publication elsewhere. On behalf of all the co-authors, the corresponding author bears full responsibility for the submission.

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