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Haemato-biochemical and blood metabolite monitoring in pregnant does for pregnancy toxaemia

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Abstract
Pregnancy toxaemia in small ruminants occur as a result of negative energy balance consequent to enhanced requirement for glucose by the developing foetuses in the last trimester (4 to 6 weeks) of gestation. The present study was carried out in the pregnant Tellicherry does maintained at Instructional Livestock Farm Complex, Madhavaram Milk Colony, Chennai – 51, and their body condition score, qualitative urinalysis using urine dip stick, haemato-biochemical parameters and serum metabolites were monitored periodically at monthly interval starting at 60 day of gestation until 30 days post kidding for the presence of subclinical form of pregnancy toxaemia. Early indicators of subclinical form of pregnancy toxaemia in does include any deviation in body condition score (≤ 2.0), presence of ketone body in urine and blood β-hyroxybutyric acid concentration (≥ 0.8 mmol/lt). However in the present study the pregnant animals were maintained in a positive energy balance which reflected as haemato-biochemical parameters to be in par with that of the control animals and within the reference range.

Keywords: Pregnant does, screening, pregnancy toxaemia

Introduction
Pregnancy toxemia also called as gestational ketosis, twin-lamb disease, ketosis of pregnancy, kid disease, lambing sickness, kidding paralysis and lambing or kidding ketosis (Rook, 2000) [16] is a metabolic disease affecting pregnant ewes and does after a period of negative energy balance (NEB) and impaired gluconeogenesis (Lima *et al.*, 2012) [13]. Pregnancy toxaemia normally occur in the last trimester (4 to 6 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) [17]. 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) [11]. 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) [20]. The morbidity and mortality rates can reach 20% and 80% respectively during severe outbreaks (Andrews, 1997) [3] thereby having a significant economic impact on goat and sheep enterprises due to the loss of fetuses, veterinary cost and loss of the dam. Prognosis of pregnancy toxemia is generally very poor and hence early detection is essential for its successful treatment, both to save the life of the dam and the fetuses (Ismail *et al.*, 2008) [12]. The mortality rates can attain 100 % even with the initiation of treatment due to severe irreversible organ damage. Hence the present study was carried out in the pregnant Tellicherry does maintained at Instructional Livestock Farm Complex, Madhavaram Milk Colony, Chennai - 51, and their body condition score, qualitative urinalysis using urine dip stick, haemato-biochemical parameters and serum metabolites were monitored periodically at monthly interval starting at 60 day of gestation until 30 days post kidding for the presence of subclinical form of pregnancy toxaemia.

Materials and Methods
The present study was carried out in pregnant Tellicherry does (n=8) aged between 2 to 4 years maintained at Instructional Livestock Farm Complex, Madhavaram Milk Colony,

Chennai - 600 051. Ultrasonography was used to confirm that all does included for the study had a minimum of two foetus. Adult non-pregnant Tellicherry does (n=8) in the age group of 2 to 4 years served as control.

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 as suggested by Villaquiran *et al.* (2012) [20].

Blood β-hydroxybutyric acid (BHBA) concentration
The blood β-hydroxybutyric acid (BHBA) concentration were determined using a handheld portable human blood ketone and glucose monitoring system (Free Style Optium Neo H – Abbott ®) as suggested by Dore *et al*. (2013) [5] and Pichler *et al.* (2014) [15].

Urine sample
Urine samples were obtained after a voluntary micturition or induced by covering the nose and the mouth of does for a few seconds (Albay *et al.*, 2014) [2]. The urine samples were analyzed using Multistix 10 SG reagent strips (Siemens Healthcare Private Limited, India) for the qualitative determination of ketone bodies, glucose and protein (Emam and Galhoom, 2008; Gurdogan *et al.*, 2014) [6, 10].

Haematology and Serum Biochemistry
Blood samples were obtained by jugular venipuncture using sterile 20G needle and vacutainer with K_3 EDTA (2ml capacity) and clot activator (4ml capacity). Haematological investigation were done with an automated haematological analyzer and the following parameters were analyzed: haemoglobin (g/dl), packed cell volume (%), red blood cell ($X10^6$/cmm), white blood cells (/cmm) and differential count.
Blood sample collected in clot activator tubes were centrifuged at 3000 rpm for 10 minutes. Serum samples were carefully harvested and the following biochemical parameters namely blood urea nitrogen (mg/dl), creatinine (mg/dl), aspartate aminotransferase (IU/dl), alanine aminotransferase (IU/dl), glucose (mg/dl) and total protein (g/dl) were analyzed in a semi automated biochemical analyzer.

Statistical analysis
Statistical analysis of data was done as per Snedecor and Cochran (1994) [18].

Results and Discussion
The Mean ± S.E. of β – hydroxybutyric acid (BHBA) level in blood, haematological and serum biochemical parameters of control and pregnant goats are given in Table 1, Table 2 and Table 3 respectively.

Table 1: Mean ± S.E. of β – hydroxybutyric acid (BHBA) level in blood of control and pregnant goats

Parameters	Reference Value #	Control (n = 8)	Pregnant goats (n=8)				
			60 days	90 days	120 days	150 days	30 days post kidding
BHBA (mmol / l)	< 0.8 (Normal)	0.28 ± 0.03	0.26 ± 0.03	0.26 ± 0.03	0.26 ± 0.03	0.28 ± 0.03	0.28 ± 0.03
	0.8 -1.6 (Subclinical form)						
	> 1.6 (Clinical form)						

Andrews, A. (1997) [3]

Table 2: Mean ± S.E. of Haematological parameters in control and pregnant goats

Parameters	Reference Value #	Control (n = 8)	Pregnant goats (n=8)				
			60 days	90 days	120 days	150 days	30 days post kidding
Haemoglobin (g/dl)	7 - 12	7.34 ± 0.51	7.46 ± 0.29	7.40 ± 0.30	7.34 ± 0.30	7.66 ± 0.31	7.21 ± 0.29
Packed Cell Volume (%)	22 - 38	23.23 ± 1.67	24.43 ± 0.79	24.2 ± 0.77	24.4 ± 0.85	26.33 ± 0.83	24.73 ± 0.75
Red Blood Cell ($X10^6$/cmm)	8 - 18	14.27 ± 0.84	15.52 ± 0.80	15.75 ± 0.72	15.34 ± 0.74	16.04 ± 0.74	15.37 ± 0..67
White Blood Cells (/cmm)	6000- 13,000	27775 ± 3478.71	19525 ± 1501.98	19162.5 ± 2229.68	19112.5 ± 2046.31	20250 ± 1399.76	19275 ± 699.43
Neutrophils (%)	30 - 48	33.5 ± 1.04	33.88 ± 0.95	33.25 ± 0.86	32.12 ± 0.64	32 ± 0.46	31.88 ± 0.40
Lymphocytes (%)	50 - 70	62 ± 0.80	61.25 ± 0.70	62 ± 0.65	63.63 ± 0.42	63.38 ± 0.26	63.63 ± 0.38
Monocytes (%)	0 - 4	2.75 ± 0.16	2.88 ± 0.23	3 ± 0.33	2.5 ± 0.19	2.5 ± 0.27	2.38 ± 0.26
Eosinophils (%)	0 - 1	1.5 ± 0.26	1.63 ± 0.18	1.38 ± 0.18	1.5 ± 0.27	1.75 ± 0.25	1.88 ± 0.30
Basophils (%)	0 - 1	0.25 ± 0.16	0.25 ± 0.16	0.38 ± 0.18	0.25 ± 0.16	0.38 ± 0.18	0.25 ± 0.16

Feldman et al. (2002) [7]

Table 3: Mean ± S.E. of Serum Biochemical parameters in control and pregnant goats

Parameters	Reference Value #	Control (n = 8)	Pregnant goats (n=8)				
			60 days	90 days	120 days	150 days	30 days post kidding
Blood Urea Nitrogen (mg/dl)	18 - 37	31.41 ± 2.91	29.95 ± 1.72	33.79 ± 1.43	37.27 ± 2.11	38.61 ± 1.71	38.08 ± 2.02
Creatinine (mg/dl)	0.9 – 2.0	0.79 ± 0.13	0.64 ± 0.03	0.65 ± 0.03	0.63 ± 0.04	0.76 ± 0.04	0.67 ± 0.03
Aspartate aminotransferase (AST) (IU/dl)	48.0 – 123.3	90.5 ± 6.63	99.88 ± 2.88	121.5 ± 3.93	108.63 ± 4.17	134.75 ± 7.46	109.84 ± 3.12
Alanine aminotransferase (ALT) (IU/dl)	14.8 – 43.8	29.0 ± 2.01	27.63 ± 0.98	27 ± 2.10	24.13 ± 1.25	48.63 ± 4.76	25.15 ± 0.71
Glucose (mg/dl)	44 – 81.2	38.98 ± 1.18	39.13 ±1.03	21.88 ±1.30	25.25 ± 2.15	29.25 ± 1.67	24.56 ± 1.24
Total Protein (g/dl)	5.9 – 7.9	5.9 ± 0.17	6.94 ± 0.22	6.28 ±0.23	6.58 ± 0.26	7.75 ± 0.06	6.81 ± 0.13

Fraser, C.M. (1991) [9]

The β – hydroxybutyric acid (BHBA) level in blood of pregnant does in the present study ranged between 0.2 mmol/l to 0.4 mmol/l which was within the normal range indicating that the pregnant does maintained in the farm were in a

positive energy balance throughout their gestation period. Andrews (1997) [3] reported β – hydroxybutyric acid (BHBA) level in normal does (< 0.8 mmol/l), subclinical form of pregnancy toxaemia (0.8 – 1.6 mmol/l) and in clinical form of pregnancy toxaemia (> 1.6 mmol/l),

In the present study qualitative examination of urine using Multistix 10 SG reagent strip (Siemens®) in different stages of pregnancy of the pregnant goats indicated absence of glucose, ketone and protein which indirectly indicated that the pregnant does maintained in the farm were in a positive energy balance throughout their gestation period. Emam and Galhoom (2008) [6] reported qualitative analysis of urine in pregnancy toxaemic goats wherein the concentrations of ketone bodies in urine varied according to the degree of disease. Glucosuria was detected in mild and severe cases, while proteinuria was present only in severe pregnancy toxemic does which may be attributed to renal insufficiency and albuminuria.

There was no significant difference in the haematological values between the control and pregnant goats throughout the study period and the values in both the groups were within the normal reference range. However significant decrease in total erythrocytic count, haemoglobin and packed cell volume were observed in light, moderate and severe cases of pregnancy toxaemic goats and the reasons were attributed to the deficiency of energy, protein and iron which are required for erythropoietin production and haemoglobin synthesis (Mohamed *et al*., 2004; Abdallah *et al.,* 2015) [14, 1].

Release of glucocorticoids in pregnancy toxemic goats produced leukocytosis, neutrophilia, lymphopenia and monocytopenia and the reasons were attributed to the increase in the movement of granulocytes from the bone marrow to the peripheral blood (Coles, 1986) [4]. Significant decrease in lymphocytes in pregnancy toxemic goats were attributed to the immunosuppressive effect of ketone bodies (beta hydroxybutyric acid and acetoacetate) (Hefnawy *et al.,* 2011) [11].

There was no significant difference in the biochemical parameters namely blood urea nitrogen, creatinine, aspartate aminotransferase, alanine aminotransferase and total protein values between the control and pregnant goats throughout the study period and the values in both the groups were within the normal reference range.

Increased serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels was observed both in mild and severe pregnancy toxaemic goats and attributed this 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 lipolysis and hepatic ketogenesis following an energy deficiency (Hefnawy *et al*., 2011) [11].

Likewise increased blood urea nitrogen and creatinine levels was observed both in mild and severe pregnancy toxaemic goats and was attributed to the increased protein catabolism by decomposing fetuses, reduced glomerular filtration as a result of extensive fatty infiltration of the kidneys or renal dysfunction as a result of extensive degenerative changes of the kidneys due to acidosis and increased ketone bodies in general circulation (Lima *et al*. 2012) [13].

Hypoproteinemia, hypoalbuminemia, hypoglobulinemia and increased albumin / globulin ratio were observed both in mild and severe pregnancy toxaemic goats and was attributed to malnutrition or inadequate provision of amino acid substrate for general protein production or to the reduction of albumin synthesis in hepatic insufficiency or due to albuminuria (Abdallah *et al.,* 2015) [1].

Significant reduction in blood glucose levels were observed as the stage of pregnancy advanced in the pregnant animal group when compared to the control group and this may be attributed to the increased glucose demand by the developing fetusus. Hypoglycaemia indicate that the foetuses are alive while hyperglycaemia in advanced pregnancy toxaemic goats indicate foetal death and this was attributed to the removal of the suppressing effect of the foetus on hepatic gluconeogenesis (Wastney *et al.,* 1983) [21] or to the increase in serum cortisol level (Ford *et al.,* 1990) [8].

Fig 1: Blood β-hydroxybutyric acid (BHBA) concentration determination using a handheld portable human blood ketone and glucose monitoring system (Free Style Optium Neo H – Abbott ®)

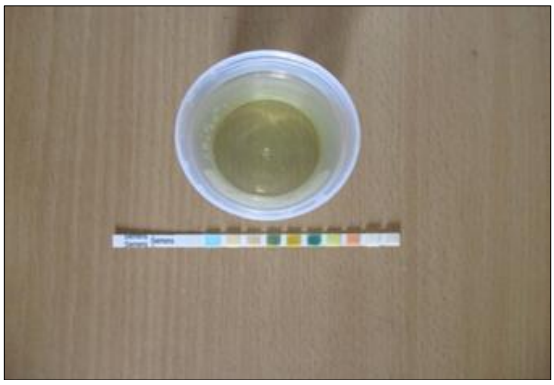

Fig 2: Urine samples were analyzed using Multistix 10 SG reagent strips (Siemens Healthcare Private Limited, India) for the qualitative determination of ketone bodies, glucose and protein

Conclusion

Early indicators of subclinical form of pregnancy toxaemia in does include any deviation in body condition score (≤ 2.0), presence of ketone body in urine and blood β-hyroxybutyric acid concentration (≥ 0.8 mmol/lt).

References

1. Abdallah AAM, NAM N. El-Deen and A.A. Ibrahim, Early biomarkers for diagnosis and prognosis of pregnancy toxemia in goats. Zag. Vet. J, 2015; 43:130-142.
2. Albay MK, MC Karakurum, S Sahinduran, K Sezer, R Yildiz. Selected serum biochemical parameters and acute phase protein levels in a herd of Saanen goats showing signs of pregnancy toxaemia. Vet. Med., 2014; 59:336-342.
3. Andrews A. Pregnancy toxaemia in the ewe. In Pract., 1997; 19:306-312.
4. Coles EH. In: Veterinary Clinical Pathology. 4th ed., W. B. Saunders Company. Philadelphia, 1986.

5. Dore V, J Dubuc, AM Belanger, S Buczinski. Short communication: Evaluation of the accuracy of an electronic on-farm test to quantify blood β-hydroxybutyrate concentration in dairy goats. J Dairy Sci., 2013; 96:4505-4507.
6. Emam EE, KI Galhoom. Hormonal, haematological blood biochemical changes in pregnancy toxaemia in Balady goats (Caprines caprina) with trials of treatment as a field study. Egypt J Comp. Pathol. Clinic. Pathol., 2008; 21:121-138.
7. Feldman BF, JG Zink, NC Jain. Schalm's Veterinary Hematology. Lippincott Williams and Wilkins, Philadelphia, 2002.
8. Ford EJ, J Evans, I Robinson. Cortisol in pregnancy toxaemia of sheep. Br. Vet. J, 1990; 146:539-542.
9. Fraser CM. The Merck Veterinary Manual. A Handbook of Diagnosis, Therapy and Disease Prevention and Control for the Veterinarian. 7th ed. Merck and Co., New Jersey. 1991, 969-970.
10. Gurdogan F, E Balikci, A Yildiz. Some acute phase proteins, oxidative stress biomarkers and antioxidant enzyme activities in ewes with pregnancy toxemia, Ir. J Vet. Res., 2014; 15:297-299.
11. Hefnawy AE, S Shousha, Youssef S. Hematobiochemical profile of pregnant and experimentally pregnancy toxemic goats. J Basic Applied Chem., 2011; 1:65-69.
12. Ismail ZAB, AM Al-Majali, F Amireh, OF Al-Rawashdeh, 2008. Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. Vet. Clin. Pathol., 37:434–437.
13. Lima MS, RA Pascal, Stilwell GT. Glycaemia as a sign of the viability of the foetuses in the last days of gestation in dairy goats with pregnancy toxemia. Ir. Vet. J, 2012; 65:136-148.
14. Mohamed N, Z Mohamed, S Ahmed. Some clinico-pathological and hormonal studies on pregnancy toxaemia in goats (Field Study). Zag. Vet. J, 2004; 32:16-29.
15. Pichler M, Damberger A, Amholdt T, Schwendenwein I, J Gasteiner. Evaluation of 2 electronic handheld devices for diagnosis of ketonemia and glycemia in dairy goats. J Dairy Sci., 2014; 97:7538-7546.
16. Rook JS. Pregnancy toxemia of ewes, does, and beef cows. Vet. Clin. Nr. Am. Food Anim. Pract, 2000; 16:293-317.
17. Schlumbohm C, J Harmeyer. Twin pregnancy increases susceptibility of ewes to hypoglycemic stress and pregnancy toxemia. Res. Vet. Sci, 2008; 84:286-299.
18. Snedecor DW, WG Cochran. Statistical methods. 8th ed. Iowa State University Press, Iowa, 1994.
19. Van Saun RJ. Pregnancy toxemia in a flock of sheep. J Am. Vet. Med. Assoc., 2000; 217:1536-1539.
20. Villaquiran M, R Gipson, R Merkel, A Goetsch, T Sahlu. Body condition scores in goats. Agriculture Research and Cooperative Extension. Langston University, Langston, 2012.
21. Wastney ME, JR Wolf, R Bickerstafle. Glucose turnover and hepatocyte glucose production of starved and toxaemic pregnant sheep. Aust. J Biol. Sci, 1983; 36:271-284.