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Hepatic B-mode Ultrasonography for the Diagnosis of Does Subclinical Pregnancy Toxemia with Special Reference to Hematological Alterations

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ABSTRACT

The aim of our study was to describe the B- mode hepatic ultrasonography of does with subclinical pregnancy toxemia and shed the light on the hematological and protein profile alterations. Based on the blood serum levels of glucose, pregnant does were classified into; Group 1: Thirty pregnant does were used as controls (Their glucose levels were more than 40 mg/dL). Group 2: Twenty-five does were diagnosed as subclinical pregnant toxemic (SCPT) since their glucose values were <40 mg/dL. B- mode of the diagnostic ultrasound scanner was used. Special kits were used for the determination of glucose, total protein and albumin. Liver B- mode ultrasonography of does with SCPT indicated by fatty infiltration among hepatic parenchyma. Rounded margins which were indicated by hepatic enlargement. subclinical pregnancy toxemic does showed significant decreases of RBCs, Hb, MCV, MCH and MCHC and significant increases of PCV, leukocytes, neutrophils and lymphocytes than healthy control pregnant does($P \leq 0.05$). A significant decrease of serum total protein, albumin, and A/G ratio were recorded in subclinical pregnant toxemic does when compared to healthy control one.

Key words: Beta-hydroxybutyrate, Does, Ultrasonography and Sub-clinical pregnancy toxemia.

INTRODUCTION

In Egypt, goats are regarded as one of the most important economic incomes, where many native productive breeds are reared for obtaining milk or meat (Khameis et al., 2018**).** Pregnancy toxemia is a metabolic disease in goats occurs as a result of negative energy balance consequent to enhanced requirement for glucose by the developing fetuses in the last stage of gestation (Kelay and Assefa, 2018), which is characterized by disturbance in energy, protein and kidney functions, the mortality rates often exceed 80% (Simpson et al., 2019), while the subclinical form is asymptomatic form which is detected laboratory by higher concentration of beta hydroxy butyrate and hypoglycemia (Basavanagouda et al., 2021**).** Poor nutrition because of the high energy demands of fetuses during the final stage of pregnancy and poor body condition led to ketonemia which is related to reduced glucose and increased lipid mobilization (Andrews et al., 1997**).**

Undetected cases of pregnancy toxemia could lead to higher mortality rates (Rook, 2000**),** poor prognosis to the treatment protocol, and lower survival rate of offspring's born from pregnant toxemic

goats (Weaver et al., 2021)**,** so the early diagnosis of any pathological condition is essential, which can be depended on the diagnostic ultrasonography (Mattoon et al., 2020)**.** B-mode hepatic ultrasonography is a satisfactory sensitivity mirror for the diagnosis of any abnormal focal lesions in the liver parenchyma (Feeney et al., 2008), The previous research suggests a potential link between hematological factors and indicators of low energy status in ruminants, which resulted in pregnancy toxemia (González et al., 2012)**.**

The aim of this study was to describe the B- mode ultrasonography in the detection of subclinical pregnancy toxemia in does in parallel with hemogram and protein changes.

MATERIALS AND METHODS

Animals

Fifty- five pregnant does at the late stage of pregnancy, which were diagnosed ultrasonographyaged (3-5) years and 22–35 kg body weight, were chosen from the Farm of the Faculty of Veterinary Medicine, University of Sadat City, Egypt, all animals were clinically examined. Pregnant does were classified on the basis of blood serum glucose level into 2 main groups as following:

Group 1: 30 pregnant does had glucose concentrations >40 mg/dL and were included as controls.

Group 2: The remaining 25goats had glucose concentrations <40 mg/dL and were categorized as having subclinical pregnancy toxemia.

Blood samples

Blood samples were taken from jugular vein by a sterile syringe from each doe under the experiment. A portion was collected in a dry, clean, labelled vacutainer tube containing EDTA as anticoagulant for the determining of hemogram, and the other portion was drawn into an anticoagulant-free, dry-clean-labeled vacutainer and allowed to clot for 20 minutes at room temperature in a sloping posture, then centrifuged for ten minutes at 3000 rpm, and the clean, non-hemolyzed serum samples were carefully aspirated into clean, dry, labelled Eppendorf tubes and kept at -20°C for detection of glucose and protein profile analysis.

Clinical examination of does

Does were inspected and given a thorough clinical and physical examination, which included measuring their temperature, breathing, heart rates, and ruminal motility using the techniques recommended by (Radostits et al., 2007)**.**

Ultrasonographic examination

B-mode of touch-screen DRAMISKI 4vet diagnostic ultrasound device with a 4–9 MHz micro–convex transducer (Poland) was used. Goats were securedat left lateral recumbency. The right 7^{th}–12^{th} intercostal areas of the liver were evaluated to look for focal or multifocal hepatic lesions. Hepatic ultrasound parameters such size, echogenicity, margin, vascularity and perihepatic abnormalitieswere routinely assessed(Gönencil et al., 2003)**.**

Hematological analysis

Complete blood picture including red blood cells count (RBCs), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells count (WBCs) and differential leucocytic count (lymphocytes, neutrophils, monocytes, eosinophils and basophils) was done by Benesphera Brand Hematology Analyzer (Harvey et al., 2012)**.**

Serum glucose and protein profile measurements

Glucose, total protein and albumin were determined spectrophotometrically according the manufactures of (*Bio-Diagnostic Company, Dokki, Egypt*) based

on the method specified.Typically, the total protein value is subtracted from the albumen value to get the total globulin fraction. The A/G ratio is calculated using the formula, A/G ratio= (Albumin value) / (Globulin value).

Statistical analyses

The comparison between the two groupswas done through t-test, the results were reported as means with standard deviations (Mean ± SD). Significant differencewas determined at $P < 0.05$. Data were analyzed using IBM SPSS Statistics 16 (IBM Corporation, Armonk, NY, USA).

RESULTS

Clinical examination of does

SCPT does showed no clinical symptoms of the clinical form of pregnancy toxemia, but some of them showed inappetence, dullness and grinding on the teeth. SCPT does showed no significant changes in rectal temperature, pulse and respiratory rates than controls ($p > 0.05$), the ruminal motility is staticallydeclined in SCPT than control ones at ($p < 0.05$). **(Table 1).**

Hematological analysis of control pregnant and SCPT does

Significant decreases of RBCs, Hemoglobin, MCV,MCHC and significant increases for PCV, WBCs, neutrophils, lymphocytes were recorded in subclinical pregnancy toxemic does when compared to healthy control one($P \leq 0.05$), while non-significant changes for monocytes, eosinophils and basophils were found in subclinical pregnancy toxemic does (Table 2).

Protein profile of control pregnant and SCPT does

Estimation of serum profiles in subclinical pregnancy toxemic and healthy control does by comparing the SCPT does with the healthy control one, there were a significant ($P \leq 0.05$) decrease of serum total protein, serum albumin and A\G ratio in SCPT does, while non-significant changes in the values of globulin was recorded between the two groups (Table3).

Ultrasonographic examination of the liver (B-mode)

Ultrasonographic examination of the liver of pregnant goats (control group) appeared as numerous echoes homogenously distributed over the entire region of the liver as seen in **fig (1A).** While in subclinical pregnancy toxemia, the liver appeared with fatty infiltration represented by hyper echoic areas, increased echogenicity of the liver parenchyma with rounded margins than the normal while the visibility of the hepatic vessels was still clearas shown in **fig (2B).**

Table (1): (Mean ± SD) of physical parameters in clinically healthy and subclinical pregnancy toxemic does:

Variables	Control pregnant does without SCPT (n=30)	Pregnant does with SCPT (n=25)
Rectal temperature (°c).	38.3 ± 0.18	38.1 ± 0.1
Respiratory rate (cycle/min).	19.2 ± 1.92	21.4 ± 1.82
Pulse rate (beat/min).	76.83 ± 1.83	79.5 ± 2.66
Ruminal motility (movement/2min).	2 ± 0.35	1.4 ± 0.2*

*$P < 0.05$.

Table (2): Mean ± SD of hematological parameters in healthy control and subclinical pregnancy toxemic does:

Variables	Control pregnant does without SCPT (n=30)	Pregnant does with SCPT (n=25)
RBCs ($\times10^6$/µL)	10.88 ± 1.05	8.68 ± 0.85*
Hb (g/dL)	13.73 ± 0.65	11.97 ± 0.79*
PCV (%)	23.88 ± 0.71	27.8 ± 0.75*
MCV (fL)	20.93 ± 0.23	18.73 ± 0.2*
MCH (pg)	9.03 ± 0.58	6.96 ± 0.86*
MCHC (g/dL)	36.1 ± 4.09	27.2 ± 2.69*
WBCs (x10^3/ µL)	10.19 ± 0.79	13.28 ± 0.66*
Neutrophils (x10^3/µL)	3.27 ± 0.07	5.38 ± 0.08*
Lymphocytes(x10^3/µL)	6.42 ± 0.02	7.34 ± 0.04*
Monocytes (x10^3/µL)	0.29 ± 0.1	0.33 ± 0.03
Eosinophils(x10^3/µL)	0.17 ± 0.03	0.2 ± 0.05
Basophils (x10^3/µL)	0.04 ± 0.01	0.03 ± 0.005

RBCS; red blood cells,
Hb: Hemoglobin,
PCV: Packed cell volume,
MCV; mean corpuscular volume,
MCH; mean corpuscular hemoglobin,
MCHC: mean corpuscular hemoglobin concentration,
WBCS; white blood cells,
*P < 0.05.

Table (3): Mean ± SD of serum total protein, albumin, globulin, and A\G ratio in pregnancy toxemic and healthy control does.

Variables	Control pregnant does without SCPT (n=30)	Pregnant does with SCPT (n=25)
Total Protein (g/dl)	6.86 ± 1.00	4.19 ± 0.69*
Albumin (g/dl)	4.37 ± 0.50	2.75 ± 0.35*
Globulin (g/dl)	1.94 ± 0.39	1.96 ± 0.21
A/G ratio	2.38 ± 0.35	1.33 ± 0.35*

A/G ratio: Albumin/Globulin ratio; *P < 0.05

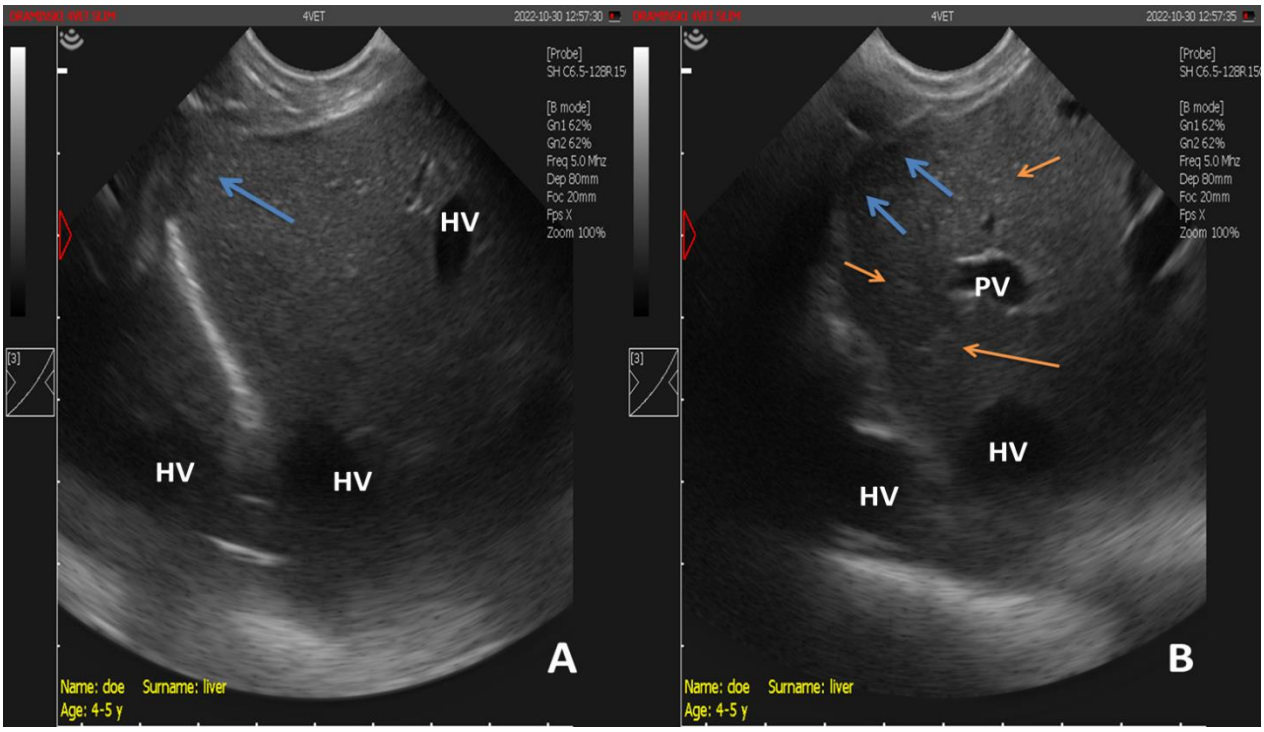

Figure (1): Ultra sonogram of normal hepatic parenchyma **(A)** Indicates normal liver echogenicity, hepatic vein (HV) showing mirror image artifact. The bluish arrows indicate pointed margins (normal liver size). Ultrasound scan of the liver in SCPT does **(B)** Indicates fatty liver infiltration represented by hyper echoic areas (orange arrows) showing increased echogenicity of the liver parenchyma than the normal with visible hepatic vessels; note the margins of the liver are rounded (blue arrows).

DISCUSSION

Small ruminants became much more vulnerable to different metabolic disorders (Sejian et al., 2021). Late stage of gestation in small ruminants have enormous energy demands that led to start fat tissue mobilization, which is internal sources because of the increased energy requirements of advanced pregnancy which resulted in negative energy balance, production of ketone bodies and ultimately severe hypoglycemia (Zamuner et al., 2020).

SCPT does had lower RBCs, Hb, MCV, MCH and MCHC and higher PCV,WBCs, neutrophils and lymphocytes values than control pregnant does, and no significant alterations in the values of monocytes, eosinophils and basophils. A nearly results were also reported by (Gupta et al., 2008; Tharwat and Al-Sobayil, 2014). Lower RBCs and Hb might be due to suppression of erythropoiesis in this metabolic disorder, while higher PCV of SCPT does might be due to hemoconcentration and dehydration (EL-DIN and EL-SANGERY, 2005).

Leukocytosis, neutrophilia and lymphocytosis might be related to metabolic acidosis (ketoacidosis), infection, hepatic lipidosis in which exposure of hepatocytes to fatty acids, localized inflammatory process and tissue necrosis of liver (Smith and Sherman, 2009).

SCPT does had lower total proteins, albumin and A/G ratio values than control group. Serum hypoproteinemia and hypoalbuminemia in SCPT does might be

attributed to hepatic degenerative changes and production of ketone bodies which led to hepatic insufficiency (Gupta et al., 2007).

The parenchymal pattern of the normal goat liver composed of numerous echoes homogenously distributed over the whole liver and the liver appeared more echogenic with pointed margins,as previously described by (Braun and Hausammann, 1992). Ultrasonogram of liver in SCPT goats revealed hyper echoic areas, round margin and lower deep attenuation which was associated with fat accumulation through liver. This result agrees with previously described by (Alsafy et al., 2013).

CONCLUSION

Ultrasonography is a helpful aid in diagnosing SCPT in does with fatty liver with decreased glucose, hemogram, hypoproteinemia, hypoalbuminemia, leukocytosis and neutrophilia.

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