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THE EFFECT OF OVINE PREGNANCY TOXEMIA ON ACID BASE BALANCE, OXIDATIVE STRESS, SOME HORMONAL ASSAYS AND MATRIX METALLOPROTEINASES

***Asmaa Abdallah Ragb Darwish**

Researcher in Department of Animal and Poultry Health, Desert Research Center, Mataria, Egypt.

***Corresponding Author: Asmaa Abdallah Ragb Darwish**
Researcher in Department of Animal and Poultry Health, Desert Research Center, Mataria, Egypt.

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ABSTRACT

Background: Ovine pregnancy toxemia (PT) is a serious metabolic disorder, widely affects the late pregnant ewes form all species, resulting in high stillbirth and mortality rates. **Objective:** this research aimed to study the effect of the PT and the late pregnancy on the acid base balance, anion gap, oxidant-antioxidant status, some hormonal assays and matrix metalloproteinases (MMPs) and evaluate the diagnostic and prognostic value of MMP-2 and MMP-9 in PT and their relations with the other estimated parameters. **Material and method:** sixty barki ewes aged from 3-4 years, were divided equally into 3 groups: the healthy control group, late pregnant group and pregnant toxemic group. Blood samples were collected from the three groups and different biochemical parameters were measured. **Results:** both of toxemic ewes and late pregnant ewes suffered from a significant drop in the blood pH, PCO_2, HCO_3, minerals, electrolytes, antioxidants, FT3, FT4 and insulin levels. On contrast, they had increased blood levels of PO_2, base deficit, K, anion gap, free radicals, cortisol, GH, TSH and MMPs. Furthermore, both of MMP-2 and MMP-9 yielded 100% sensitivity as well as NPV and close values of specificity, LR, PPV, accuracy rate and percentage of increase in the toxemic ewes when compared to the late pregnant ewes. **Conclusion:** the monitoring of the acid base balance, anion gap, oxidant-antioxidant status and hormonal assays in the late pregnant ewes as well as the toxemic ewes is important to reduce the PT morbidities and mortalities respectively. MMP-2 and MMP-9 are valuable tools for the early detection and prediction of PT.

KEYWORDS: Pregnancy toxemia, acid base balance, oxidative stress, hormonal stress, matrix metalloproteinases.

INTRODUCTION

Pregnancy toxemia (PT) is defined as a group of sequential metabolic disorders, usually observed in the late pregnant ewes. It is originally connected with the inappropriate nutrition at the last trimester of pregnancy. As the majority of the fetal growth takes place at this stage, so the energy requirements are duplicated especially in case of multifetal pregnancy. If the late pregnant ewe hasn`t suitable feed amounts especially carbohydrates to fulfill the fetal growth requirements and her own requirements, the subsequent hypoglycemia will result in a serial of metabolic disorders, end with a state of ketoacidosis, coma and death.[1,2] Unluckily, PT affects all ovine species worldwide causing high morbidities and high mortalities among the diseased ewes. Absence of the early detection of the disease due to the insufficient information about its pathogenesis and the lack of efficient diagnostic tools are the main obstacles in the way of improving our prophylactic as well as therapeutic policies against the disease.[3]

Matrix Metalloproteinases (MMPs) expression refers to a collection of zinc containing proteolytic enzymes, synthesized and secreted by different cells as inactive zymogen then activated exracellurally. According to their structures, they are listed in 6 subgroups (collagenases, gelatinases stromelysins, matrilysins, matrilysins and membranous MMP). They are concerned with extracellular matrix (ECM) remodeling and degradation by splitting one or more of its component.[4] Although of their importance as sensitive markers for different human diseases such as arthritis, atherosclerosis, GIT disorder, pulmonary diseases, malignancy, diabetes mellitus, diabetic ketoacidosis and some gynecological problems as preeclampsia and early labor[5], there is only a little information about their value in detection of different animal diseases.

Hence, this study aimed to study the effect of the ovine PT as well as the late pregnancy on the acid bas balance, anion gap, oxidant-antioxidant status, some hormones assays and MMPs and evaluate the diagnostic and

prognostic value of MMP-2 and MMP-9 in PT and their relations with the acid bas balance, anion gap, oxidant-antioxidant status and hormonal stress.

MATERIAL AND METHODS

Animals

This is a cohort study was carried out on sixty barki ewes aged from 3-4 years, in the period from 1-11-2018 to 1-12-2018, at the farm of the Sustainable Development Center for Matrouh Resources, in accordance to the legislation and approval of Desert Research Center, Ministry of Agricultural and Land Reclamation, Egypt. They were divided equally into 3 groups:

The control group (CG): contained twenty non-pregnant apparently healthy ewes. The late pregnant group (LPG): contained twenty late normal pregnant ewes (2-4 weeks before parturition, normal body temp (38-39ºC), respiration rate (20-30 breaths/min) and pulse rate (70-80 beat/min), normal appetite, normal eye and ear reflexes, flying response, standing with the rest of the flock and beta hydroxy buyrate (BHB) < 3 Umo/L). These ewes were observed until the parturition and during postpartum stage to ensure that they have a normal lambing as well as postpartum period. The toxemic group (PTG): contains twenty late pregnant ewes (2-4 weeks before parturition, they suddenly lost their appetite, sensation, flying response and stood away from the other ewes. After that, they became dull, weak, emaciated, unable to stand and dropped their head. By clinical examination, acetone breath and rapid respiration (35-45 breaths/min), low body temp (35-36ºC) and pulse rates (60-65 beat/min) were detected. Some of them suffered from advanced hypoglycemic encephalopathy, and showed nervous symptoms like defenses, blindness, tremors, muscle convulsions, smashing mouth, excess salivation, coma and death. Dystocia and stillbirth were a common finding in these cases as well. The dead ewes postmortem examination showed multiple fetus in her uterus, minimal body fats and fatty hepatic degeneration). This signs directed the primary diagnosis towards the PT and the biochemical analysis confirmed this diagnosis.[2,3]

Samples

Blood samples were obtained from the three studied groups ewes by jugular vein puncture carefully with avoiding hemolysis. Each sample was divided into 2 tubes: the first tube contains heparin (20 IU/ ml) and was split into 2 portions: the first one immediately sent to the lab for the blood gases analysis in the whole blood. While, the second portion was centrifugated at 3000 r.p.m for 20 min at 37º C for getting plasma for estimation of plasma BHB and non-esterified fatty acids (NEFAs). The second tube was clean plain tube and blood was left to coagulate then, it was centrifugated at 3000 r.p.m for 20 min at 37º C for getting serum for estimation of the rest biochemical parameters.

Biochemical analysis

- Serum glucose, minerals (Ca, P, Mg), electrolytes (Na, Cl, K), total antioxidant capacity (TAC), antioxidants (catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), ascorbic acid) and free radicals (nitric oxide (NO), malondialdehyde (MDA)), trace elements (Zn, Cu) concentrations were detected spectrophotometrically using commercial kits of Biodiagnostic Company® following the manual instructions.

- BHB and NEFAs were measured spectrophotometrically using commercial kits of gamma trade® company following the manufactur`s instructions.

- Rapid point 340® Blood Gas Analyzer (England) was used for estimation of blood pH, partial carbon dioxide tension (PCO_2) and partial oxygen tension (PO_2) at 37ºC by using kits supplied by Synbiotics Corporation®, 11011 via Frontera, San Diego, according to the manufacturer's instructions. While, the bicarbonate (HCO_3) levels and the base excess or deficit were calculated automatically by the blood gas analyzer.

- Serum hormonal assays (cortisol, TSH, Free T3 (FT3), Free T4 (FT4), insulin and growth hormone (GH)) were estimated by chemiluminescence immunoassay (CLIA) using kits supplied by Diasorin®, Italy Company following the manual instructions.

- MMPs serum levels were evaluated by ELISA kits supplied by Cloude-Clone Corp Company® according to the manufacturer's instructions.

$$-Anion\ gap = (Na+ + K+) - (Cl- + HCO3-).$$

Statistical analysis

- The observed power of the study was 1 and the effect size (eta squared) was more than 0.8 for all measured parameters. They were calculated using SPSS program version 20.

- All estimated parameters were expressed as mean ± standard deviation (SD). SPSS program version 20 was used for comparing between the means of the three groups by one-way ANOVA test, detection of the post hoc differences between means by a multiple comparison Tukey`s HSD test and estimation of correlation between the estimated parameters by Pearson's simple correlation method. Differences at 0.05 level of probability were considered significant.

- Cut off points, sensitivity, specificity and likelihood ratio (LR) for MMP-2 and MMP-9 were evaluated between the late pregnant ewes and the toxemic ewes by graphed prism version 5 program.

- The positive predictive value (PPV), negative predictive value (NPV) and accuracy rate for the measured MMPs is the outcome of dividing the number of true positive or true negative or sum of both on the number of total positive or total negative or total population respectively, then multiplied in 100.

-The percentage of increase for each MMP is calculated by subtracting the mean value of its concentration in the late pregnant ewes from the mean value of its concentration in the toxemic ewes then, dividing the result on the mean value of its concentration in the late pregnant ewes then multiplied in 100.

RESULTS

The data of table (1) exhibited a significant decline in the blood glucose, pH, PCO_2, HCO_3, Ca, P, Mg, Na and Cl levels between the three studied groups, between LPG and CG and between PTG and LPG. On contrast, the blood concentrations of BHB and NEFAs, base deficit, PO_2, K and anion gap were significantly increased.

With respect to the oxidant-antioxidant status and trace element concentrations, table (2) demonstrated a significant increase in the free radicals serum concentrations and a significant decrease in TAC, antioxidants, Zn and Cu concentrations in the LPG and PTG when compared to CG and LPG respectively.

Concerning with the hormonal changes and MMPs activity in LPG and PTG, they had a significant increase in the cortisol, TSH, GH, MMP-2 and MMP-9 when compared to CG and LPG respectively. On the contrary, they had a significant decrease in FT3, FT4, insulin assays (table 3).

Table (4) cleared that both MMP-2 and MMP-9 yielded equal sensitivity and NPV (100%). Meanwhile, MMP-9 had higher specificity, LR, PPV and accuracy rate than MMP-2, but MMP-2 had a higher percentage of increase than MMP-9.

Table (5 and 6) illustrated a negative correlation between the serum glucose and BHB, NEFAs, PO_2, K, anion gap, free radicals, cortisol, TSH, GH, MMP-2, MMP-9 as well as a positive correlation between glucose and blood pH, PCO_2, HCO_3, Ca, P, Mg, Na, Cl, antioxidants, trace elements, FT3, FT4, insulin in PTG and LPG. While, a negative correlation between MMP-2, MMP-9, BHB, NEFAs and glucose, blood pH, PCO_2, HCO_3, Ca, P, Mg, Na, Cl, antioxidants, trace elements, FT3, FT4 and insulin as well as a positive correlation between MMP-2, MMP-9, BHB, NEFAs and BHB, NEFAs, PO_2, K, anion gap, free radicals, cortisol, TSH and GH was also determined in both groups.

Table (1): The comparison between glucose, BHB, NEFAs, acid base balance, minerals, electrolytes concentrations and anion gap in the three studied groups. Values are mean ± SD.

Parameters	CG	LPG	PTG
Glucose (mg/dl)	92.40±7.09[c]	75.10±3.06[a]	45.50±2.95[a,b]
BHB (Umo/L)	0.33±0.16[c]	1.26±0.03[a]	8.47±1.45[a,b]
NEFAs (Umo/L)	0.45±0.04[c]	1.05±0.02[a]	2.84±0.02[a,b]
Blood pH	7.48±0.01[c]	7.43±0.01[a]	7.27±0.01[a,b]
PCO_2 (mm Hg)	37.61±0.51[c]	35.91±0.77[a]	33.86±0.20[a,b]
HCO_3 (m mol/L)	26.90±0.46[c]	23.10±0.40[a]	15.62±1.19[a,b]
Base excess or Base deficit (m mol/L)	3.20±0.51[c]	-0.88±0.50[a]	-10.52±0.73[a,b]
PO_2 (mm Hg)	68.01±1.45[c]	73.1±1.45[a]	85.5±2.95[a,b]
Ca (mg/dl)	10.53±0.52[c]	8.31±0.21[a]	7.55±0.17[a,b]
P (mg/dl)	6.35±0.27[c]	5.37±0.27[a]	4.56±0.33[a,b]
Mg (mg/dl)	3.71±0.50[c]	2.63±0.08[a]	1.44±0.26[a,b]
Na (mmol/L)	142.40±2.80[c]	127.36±1.23[a]	115.45±2.71[a,b]
Cl (mmol/L)	105.23±2.64[c]	92.61±0.80[a]	78.73±3.13[a,b]
K (mmol/L)	3.38±0.26[c]	4.52±0.17[a]	5.86±0.08[a,b]
Anion gap (m mol/L)	13.80±3.76[c]	16.17±1.08[a]	26.96±1.87[a,b]

[a] (significant with CG), [b] (significant with LPG), [c] (significant between the three studied groups) considered statistically significant at $P<0.05$.

Table (2): The comparison between oxidant-antioxidant status and trace elements concentrations in the three studied groups. Values are mean ± SD.

Parameters	CG	LPG	PTG
TAC (Mm/L)	0.84±0.04[c]	0.52±0.02[a]	0.15±0.04[a,b]
CAT (U/L)	412.25±14.64[c]	193.00±13.99[a]	127.00±6.78[a,b]
GPx (mU/ml)	1005.45±0.04[c]	810.45±3.03[a]	605.50±2.95[a,b]
GSH (U/L)	17.40±1.15[c]	11.65±0.11[a]	11.10±0.06[b]
Ascorbic acid (mg/L)	18.00±1.45[c]	12.50±1.15[a]	8.56±1.15[a,b]
MDA (nmol/ml)	12.10±1.15[c]	24.78±2.03[a]	35.75±2.35[a,b]
NO (µmol/L)	27.00±1.45[c]	39.49±0.69[a]	42.73±1.47[a,b]
Zn (µg/dl)	155.72±7.65[c]	123.60±1.61[a]	85.69±7.44[a,b]
Cu (µmol/L)	23.55±1.30[c]	18.69±1.51[a]	14.18±0.88[a,b]

[a] (significant with CG), [b] (significant with LPG), [c] (significant between the three studied groups) considered statistically significant at $P<0.05$.

Table (3): The comparison between the hormones and MMPs concentrations in the three studied groups. Values are mean ± SD.

Parameters	CG	LPG	PTG
Cortisol (µg/dl)	1.79±0.16 [c]	3.00±0.34 [a]	7.22±1.34 [a,b]
TSH (µIU/ml)	0.01±0.00 [c]	0.03±0.01 [a]	1.11±0.04 [a,b]
FT3 (Pg/ml)	1.74±0.15 [c]	1.08±0.03 [a]	0.67±0.17 [a,b]
FT4 (ng/dl)	0.85±0.08 [c]	0.50±0.03 [a]	0.32±0.06 [a,b]
Insulin (µIU/ml)	8.41±0.15 [c]	6.54±0.15 [a]	4.54±0.15 [a,b]
Growth hormone (ng/dl)	3.30±0.15 [c]	7.32±1.60 [a]	13.30±0.15 [a,b]
MMP-2 (ng/ml)	15.39±0.75 [c]	30.50±1.31 [a]	47.00±3.27 [a,b]
MMP-9 (ng/ml)	22.75±1.08 [c]	48.84±2.72 [a]	74.25±3.36 [a,b]

[a] (significant with CG), [b] (significant with LPG), [c] (significant between the three studied groups) considered statistically significant at $P<0.05$.

Table (4): Cut off points (ng/ml), sensitivity, specificity, LR, PPV, NPV, accuracy rate and percentage of increase for MMP-2 and MMP-9 in PTG compared to LPG.

Parameter	MMP-2	MMP-9
Cut off	31.85	52.65
Sensitivity	100%	100%
Specificity	80%	90%
LR	5	10
PPV	83.33%	90.91%
NPV	100%	100%
Accuracy rate	90%	95%
% of increase	35.11%	34.22%

Table (5): The correlation between the glucose, BHB, NEFAs, MMPs and acid base balance, minerals, electrolytes, anion gap, free radicals, antioxidants, trace elements, hormones concentrations in PTG (Pearson's correlation test, values = r).

	Glucose	BHB	NEFAs	MMP-2	MMP-9
Glucose	1	-0.985*	-0.990*	-0.998*	-0.992*
BHB	-0.985*	1	0.985*	0.986*	0.984*
NEFAs	-0.990*	0.985*	1	0.990*	0.989*
Blood pH	0.985*	-0.948*	-0.976*	-0.980*	-0.974*
PCO_2	0.853*	-0.846*	-0.892*	-0.859*	-0.881*
HCO_3	0.923*	-0.864*	-0.898*	-0.917*	-0.907*
Base deficit	-0.076	0.136	0.094	0.078	0.098
PO_2	-0.999*	0.985*	0.990*	0.998*	0.992*
Ca	0.918*	-0.877*	-0.907*	-0.920*	-0.920*
P	0.956*	-0.942*	-0.958*	-0.950*	-0.940*
Mg	0.878*	-0.844*	-0.828*	-0.866*	-0.839*
Na	0.971*	-0.968*	-0.984*	-0.974*	-0.981*
Cl	0.977*	-0.970*	-0.974*	-0.978*	-0.974*
K	-0.977*	0.953*	0.956*	0.973*	0.962*
Anion gap	-0.957*	0.932*	0.948*	0.955*	0.950*
TAC	0.931*	-0.892*	-0.900*	-0.922*	-0.904*
CAT	0.915*	-0.890*	-0.874*	-0.907*	-0.892*
GPx	0.999*	-0.985*	-0.990*	-0.998*	-0.992*
GSH	0.979*	-0.936*	-0.969*	-0.975*	-0.971*
Ascorbic acid	0.965*	-0.958*	-0.958*	-0.960*	-0.953*
MDA	-0.985*	0.972*	0.985*	0.984*	0.986*
NO	-0.854*	0.817*	0.812*	0.843*	0.821*
Zn	0.969*	-0.960*	-0.957*	-0.962*	-0.947*
Cu	0.929*	-0.942*	-0.942*	-0.927*	-0.927*
Cortisol	-0.981*	0.942*	0.966*	0.974*	0.963*
TSH	-0.828*	0.816*	0.860*	0.837*	0.790*
FT3	0.953*	-0.955*	-0.963*	-0.953*	-0.958*
FT4	0.976*	-0.946*	-0.964*	-0.972*	-0.966*

Insulin	0.985*	-0.948*	-0.976*	-0.980*	-0.974*
GH	-0.985*	0.948*	0.976*	0.980*	0.974*

Statistical significance of correlations * was recorded at ($P < 0.05$).

Table (6): The correlation between the glucose, BHB, NEFAs, MMPs and acid base balance, minerals, electrolytes, anion gap, free radicals, antioxidants, trace elements, hormones concentrations in LPG (Pearson's correlation test, values = r).

	Glucose	BHB	NEFAs	MMP-2	MMP-9
Glucose	1	-0.856*	-0.984*	-0.961*	-0.982*
BHB	-0.856*	1	0.850*	0.841*	0.828*
NEFAs	-0.984*	0.850*	1	0.956*	0.986*
Blood pH	0.971*	-0.832*	-0.966*	-0.994*	-0.981*
PCO_2	0.910*	-0.826*	-0.903*	-0.960*	-0.913*
HCO_3	0.956*	-0.798*	-0.957*	-0.981*	-0.967*
Base deficit	-0.922*	0.835*	0.915*	0.974*	0.926*
PO_2	-0.971*	0.832*	0.966*	0.994*	0.981*
Ca	0.966*	-0.922*	-0.960*	-0.952*	-0.956*
P	0.875*	-0.905*	-0.866*	-0.921*	-0.881*
Mg	0.931*	-0.812*	-0.903*	-0.927*	-0.940*
Na	0.952*	-0.914*	-0.942*	-0.948*	-0.951*
Cl	0.924*	-0.844*	-0.914*	-0.958*	-0.946*
K	-0.670*	0.822*	0.688*	0.689*	0.669*
Anion gap	-0.986*	0.854*	0.977*	0.944*	0.985*
TAC	0.978*	-0.838*	-0.979*	-0.955*	-0.987*
CAT	0.976*	-0.863*	-0.972*	-0.985*	-0.988*
GPx	0.985*	-0.851*	-0.986*	-0.972*	-0.993*
GSH	0.959*	-0.829*	-0.951*	-0.943*	-0.964*
Ascorbic acid	0.707*	-0.687*	-0.674*	-0.786*	-0.733*
MDA	-0.917*	0.750*	0.901*	0.877*	0.934*
NO	-0.815*	0.842*	0.792*	0.869*	0.828*
Zn	0.986*	-0.942*	-0.979*	-0.972*	-0.986*
Cu	0.959*	-0.842*	-0.949*	-0.979*	-0.964*
Cortisol	-0.979*	0.850*	0.979*	0.987*	0.992*
TSH	-0.879*	0.669*	0.878*	0.827*	0.884*
FT3	0.981*	-0.888*	-0.982*	-0.976*	-0.989*
FT4	0.945*	-0.849*	-0.937*	-0.995*	-0.954*
Insulin	0.971*	-0.832*	-0.966*	-0.994*	-0.981*
GH	-0.971*	0.832*	0.966*	0.994*	0.981*

Statistical significance of correlations * was recorded at ($P < 0.05$).

DISCUSSION

PT is the most common ovine prepartiurient disturbance, mainly started with a developing hypoglycemia followed by subsequent catabolic alterations include augmented lipolysis, liver gluconeogensis, ketoacidemia and may end with coma and death.[2] In accordance with this description, the metabolic profile of LPG and PTG in the current work demonstrated a prominent hypoglycemia and increased BHB, NEFAs concentrations. Whereas, the fetal growth at this critical stage of pregnancy is rapid and massive, so the maternal energy needs multiply and the onset of hypoglycemia is predictable. Unfortunately, if the late pregnant ewe doesn`t have enough feed supplements, the constant hypoglycemia will elicit lipolysis of the body fats and induce hepatic gluconeogensis to get energy from another source instead of glucose. That way, the ketone bodies mainly BHB and NEFAs concentrations will be amplified in the toxemic ewes blood.[6]

In connection with the acid base balance of PTG, the present data depicted a state of primary metabolic acidosis accompanied with hypocapnia, hyperoxemia, decreased HCO_3 levels and intense base deficit. As the above-mentioned accumulated BHB and NEFAs will result in a progressive drop in the toxemic ewes' blood pH. So, the lungs will activate to correct this metabolic acidosis by expelling CO_2. Therefore, the hypocapnia and the hyperoxemia will arise and a deep fast respiration will be observed on the diseased cases.[3] On the other side, the decreased HCO_3 levels and the intense base deficit in the blood of toxemic ewes refer to absence of the expected renal compensate mechanism and the kidney dysfunction related to the disease pathogenesis.[3] On the other hand, the acid base balance in the late pregnancy usually tends towards primary respiratory alkalosis. As the high levels of progesterone, stimulate hyperventilation, which leads to a remarkable fall in PCO_2 and a noticeable rise in PO_2. These changes mainly

develop to ensure the blood gases diffusion from the mother to her fetus. In this investigation, the hypocapnia was adequately buffered by enhancing renal bicarbonate loss and H^+ retention, thereby the blood pH returned to its normal ranges with a slight reduction and a small base deficit was recorded.[7] Regarding the changes in the minerals and electrolytes concentrations in the present study, the considerable hypocalcaemia, hypophosphatemia, hypomagnesaemia, hyponatremia and hypocholremia obtained in LPG and PTG are parallel to several researches results.[8] These alterations were generally attributed in PT to the anorexia and the excess electrolytes and minerals loss due to renal damage as well as elevated cortisol levels linked to the increased BHB and NEFAs concentrations during the course of the disease.[1,9] Otherwise, the fatty liver related to PT may be a more specific reason for the determined hypocalcaemia due to the improper hydroxylation of vitamin D which is necessary for Ca gut absorption.[9] No doubt that, these deficiencies in minerals and electrolytes particularly the hypocalcaemia and hypomagnesaemia were responsible for muscle convulsions and dullness distinguished on the diseased animals. While, these deficiencies were returned in LPG to the augmented fetal demands at this crucial stage for different minerals and electrolytes, predominately Ca which is massively mobilized from the mother to her fetus to cover his bone development requirements. In addition to that, the inadequate unbalanced rations and the hormonal changes connected with pregnancy that may interfere in some minerals and electrolytes gut absorption and excretion during different pregnancy stages.[1,9] Unlike the other minerals and electrolytes in this research, K^+ levels showed a marked increase in LPG and PTG. This hyperkalemia probably is a part of the acid base balance, as the body cells try to eliminate the excess H^+ ions by pumping them intracellularly in exchange with K^+ ions extracellularly (K^+ efflux).[10] Reasonably, this hyperkalemia and the aforementioned decreased HCO_3 concentrations and hypocholremia, resulted in widening the anion gap in both groups.[11] Logically, the negative correlations between the serum glucose and BHB, NEFAs, PO_2, K, anion gap, free radicals, cortisol, TSH, GH, MMP-2, MMP-9 as well as the positive correlation between glucose and blood pH, PCO_2, HCO_3, Ca, P, Mg, Na, Cl, antioxidants, trace elements, FT3, FT4 and insulin which determined in PTG here, confirm that the hypoglycemia is the engine of all shifts demonstrated in the acid base balance, anion gap, oxidant-antioxidant status and hormonal assays. Interestingly, the presence of the same relations in LPG supports the fact that these alterations are already exist in the last stage of pregnancy but minimized, and under certain stressors this alterations maximized and facilitate the incidence of PT.[12]

In connection with the oxidant-antioxidant status, both of LPG and PTG in this investigation suffered from oxidative stress. As the free radicals, particularly NO, are physiologically liberated during pregnancy to maintain the placental blood flow from mother to fetus and vice versa, stimulate endothelial cell proliferation and reduce apoptosis. While, MDA is released in large amounts because of the high fetal oxygen requirements and the accumulated circulating lipids in the mother blood.[13] Normally, these free radicals controlled by different antioxidants but in our results, they overwhelm the neutralizing capacity of different antioxidants causing the outstanding oxidative stress. These findings coincide of other researchers opinion that the late pregnancy in sheep mostly accompanied with oxidative stress (directly proportional with the fetuses number and pregnancy stage), and If not managed well, it will predispose for PT or at least increase its intensity.[14,15] Another possible explanation for this oxidative stress is that the accumulated NEFAs and BHB observed in LPG and PTG in this work stimulate the pro-inflammatory cytokines production, which subsequently enhance the free radical release. Therefore, the antioxidants will be consumed and the oxidative stress will begin.[12,15] This postulate was approved by the positive correlation between BHB, NEFAs and free radicals as well as the negative correlation between BHB, NEFAs and antioxidant that detected in both groups. Besides that, the distinguished trace elements deficiency in both groups in the current data, may be involved in the oxidative stress as Cu and Zn are important cofactors in the synthesis of many proteins and antioxidant enzymes.[8,16]

According to the present study as well as prior studies[15,17], the last stage of pregnancy is physiologically associated with several endocrine changes due to the catabolic energy metabolism. Whereas, the usual hypoglycemia and accumulated BHB and NEFAs evoke the pro-inflammatory cytokines release which stimulate the concerned glands with energy regulation to reverse this catabolic energy balance and stop this hypoglycemia. The adrenal gland takes the most prominent action by increasing cortisol secretion to induce hyperglycemia through suppression of insulin release from the pancreatic islets and stimulate pituitary gland to secrete more growth hormone to decrease glucose uptake by different body cells and enhance both of hepatic and renal gluconeogensis and glycogenolysis and stimulate the adipose tissue lipolysis, thus finding a substitute for glucose. Moreover, the high cortisol levels inhibit the secretion of catabolic hormones as FT3 and FT4 to save energy.[18] Consequently, the decline of T3 and T4 blood levels induce the pituitary to produce more TSH. Rationally, these hormonal changes are more dramatic in PT, as in addition to the above-illustrated causes, the stress and the pain connected with the disease and the inability of the liver to metabolize the cortisol due to fatty degeneration result in accumulation of the hormone in the blood. Therefore, the cortisol levels are more pronounced in PT than the late pregnancy as well as the other related hormonal changes.[15,17] The positive correlation between BHB, NEFAs and cortisol, GH, TSH levels as well as the negative correlation between BHB,

NEFAs and FT3, FT4, insulin in PTG and LPG supports this assumption.

MMPs are the major tissue remodelers and destructors, produced under the pro-inflammatory cytokines control as a part of the inflammatory immune response in different diseases. In spite of their role in maintaining of pregnancy by remodeling uterine arteries to ensure vasodilatation and in facilitating the parturition by splitting the fetal membranes and separation of placenta from the uterus, their increased activity especially MMP-2 and MMP-9 in the last trimester of gestation, may be an alarm for several gynecological troubles as preeclampsia and premature labor.[5,19] The increased activity of serum MMP-2 and MMP-9 detected in the present work, in the late pregnancy and PT may be assigned to the high levels of liberated NEFAs and BHB associated with these conditions. They act as a catalyst for the pro-inflammatory cytokines release and the subordinate MMPs production.[5,20] This theory was strengthen in our research by the positive correlation between MMP-2, MMP-9 and NEFAs, BHB in both groups.

In regard with the diagnostic and prognostic importance of MMP-2 and MMP-9 in PT, table (4) suggested both of them as a good marker for it due to their high sensitivity and NPV. Meanwhile, the comparison between them was little confusing, as MMP-9 had higher values of specificity, LR, PPV and accuracy rate but MMP-2 had a higher percentage of increase. Thus, the combination between them will be more valuable in the early detection and prediction of severity of the disease. Additionally, their correlation with other changes in the acid base balance, anion gap, oxidant-antioxidant status and hormonal assays related to PT, make their estimation a good reflection to the other changes associated with the disease and propose them as a new target for therapeutic protocols.

At last, it can be concluded that both of ovine late pregnancy and PT associated with a prominent alterations in the acid base balance, anion gap, oxidant-antioxidant status and some hormonal assays. Monitoring of these alterations in the late pregnancy may decrease PT morbidities and mortalities. MMP-2 and MMP-9 are valuable tools for the early detection and prediction of PT and correlate efficiently with the acid base disturbances, anion gap, oxidative stress and endocrine changes associated with PT.

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