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The Clinicopathological Picture of Some Postpartum Disorders in Barki Ewes

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ABSTRACT

Postpartum disorders are a real threat on the animal husbandry, usually result in high mortalities and low fertility rates and ages in the affected ewes. So, this research aimed to investigate the most important clinicopathological alterations in the normal postpartum stage and some postpartum disorders in barki ewes and evaluate the importance of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) as biomarkers for postpartum disorders in barki sheep. 80 barki ewes were used were used for this study, 20 apparently healthy non-pregnant ewes (control group), 20 had a normal postpartum stage, 20 had inflammatory postpartum conditions and 20 had non-inflammatory postpartum conditions. Blood samples were collected from the control group and from other ewes (before and after parturition). The results of this work cleared that the postpartum stage as well as postpartum disorders associated with a significant ($P<0.05$) normocytic hypochromic anemia, neutrophilia, lymphocytopenia, hypoglycemia, hypolipidemia, decreased minerals, electrolytes, trace elements and total antioxidant capacity (TAC) concentrations, increased liver enzymatic activities (AST, ALT, ALP) and renal function tests (blood urea, creatinine). While, the matrix metalloproteinases (MMP-2 and MMP-9) significantly ($P<0.05$) increased in both normal postpartum stage and inflammatory postpartum disorders (endometritis and mastitis) at the pre and postpartum stage. However, they significantly ($P<0.05$) decreased in non-inflammatory postpartum disorders (before and after parturition). Conclusion: The awareness with the clinicopathological changes in the postpartum period and the postpartum disorders is helpful in reducing the postpartum disorders incidence and improving our treatment and prophylactic programs against them. MMP-2 and MMP-9 estimation before and after parturition may be valuable in prediction and indication of some postpartum disorders.

1. INTRODUCTION:

Postpartum period is a real challenge for different livestock breeders including sheep breeders. Whereas the physiological stressors occur at this period facilitate the incidence of many inflammatory and non-inflammatory disorders such as endometritis, mastitis and hypocalcaemia, that may cause unaffordable economic losses for the livestock owners represented by the high mortality rates in ewes and their newly born, lowering both fertility ages as well as rates for the affected ewes and the cost of the treatment (Tóthová et al., 2014; Sharma et al., 2015; Ahmed et al., 2018). Unfortunately, the shortage of the available information about the biochemical and hematological changes of the ovine postpartum stage and related disorders is an obstacle in improving our veterinary mangemental plans.

Matrix metalloproteinases (MMPs) are proteolytic enzymes, their principle mechanism of action is extracellular tissue matrix degradation and modulation. Therefore, they share in cellular proliferation, migration, angiogenesis, apoptosis and body defense mechanisms. They were known as sensitive markers for different human diseases especially malignant and inflammatory conditions (Li et al., 2016; Guo et al., 2017). Besides, they are involved in different physiological conditions such as menstruation, maintaining pregnancy and facilitating

parturition (Alali et al., 2018). Although they were widely studied in human medicine, there are only few studies about them in veterinary medicine.

Hence, this research aimed to study the most important clinicopathological alterations in the normal postpartum stage and in some postpartum disorders in barki ewes with special reference to the value of MMP-2 and MMP-9 as indicators for postpartum disorders in barki sheep.

2. MATERIAL AND METHODS

This study was carried out on 80 adult barki ewes (3-4years), they were kept at sustainable development centre for Matrouh resources, DRC, ministry of agriculture and land reclamation, Egypt. 20 ewes from them were apparently healthy non-pregnant ewes and considered as control group (CG). The other sixty ewes were prepartum apparently healthy ewes (2 weeks before parturition), they didn`t show any abnormal signs before parturition. They were observed till parturition and 2 weeks after parturition, then divided into 3 groups:

Normal postpartum group (NPG): 20 ewes had a normal parturition and normal postpartum period (normal appetite, temperature, no uterine discharge, normal udder). Inflammatory postpartum disorders group (IPG): 20 ewes suffered from endometritis (10 ewes with high temperature, persistent uterine discharge, off food, depressed) or mastitis (10 ewes feverish, reduced appetite, swollen udder, painful in examination, abnormal milk color and odor, teat cracks) during the postpartum stage. Non-inflammatory postpartum disorders group (NIPG): 20 ewes suffered from dystocia (5 ewes, usually primiparous females with large fetus), hypocalcaemia (5 ewes with tremors, recumbancy, cold extremities, stand shortly after calcium supplement), uterine prolapse (5 ewes) and retained placenta (5 ewes) during the postpartum stage.

Samples collection: two blood samples were collected from all ewes (one sample from CG) by jugular vein puncture. The first sample was 2 weeks before parturition and the second sample was 2 weeks after parturition in NPG while, the IPG and NIPG the second sample was collected before any intervention. Each sample was separated into two parts: one part was collected on EDETA and used for hematological parameters evaluation according to Feldman et al. (2000) method. The other part was collected in plain clean tube and was let to coagulate and was centrifugated at 3000 r.p.m. for 20 minutes at 37 ºC then serum was harvested in a clean eppendorf tube and kept at -80 ºC for estimation of the biochemical parameters spectrophotometrically by using Biodiagnostic Company® commercial kits and serum MMPs concentrations were measured by Cloud- Clone Corp company® ELISA kits (all manual instructions were applied).

Statistical analysis:

All presented parameters were displayed as mean ± standard deviation (SD). SPSS® program version 23 used to assess the differences between the estimated parameters means (one-way ANOVA test) and the post hoc differences between means (a multiple comparison Tukey`s HSD test). A difference was considerable significant at $P< 0.05$.

Graphed prism version 5 program was used to evaluate the cut off points, sensitivity, specificity and likelihood ratio (LR) for the measured MMPs (before and after parturition) between IPG, NIPG and NPG.

The positive predictive value (PPV), negative predictive value (NPV), accuracy rate and percentage of increase for them were calculated (before and after lambing) according the next equations:

$$PPV = True\ positive \div Total\ positive \times 100.$$

$$NPV = True\ negative \div Total\ negative \times 100.$$

$$Accuracy\ rate = (True\ positive + True\ negative) \div Total\ population \times 100.$$

$$Percentage\ of\ increaese = (The\ mean\ value\ of\ the\ marker\ concentration\ in\ IPG\ or\ NIPG - The\ mean\ value\ of\ its\ concentration\ in\ NPG) \div The\ mean\ value\ of\ its\ concentration\ in\ NPG \times 100.$$

3. RESULTS:

3.1. Hematological changes: table (1, 2) displayed a significant ($P<0.05$) decrease in RBCs, Hb, PCV, MCH, MCHC and lymphocytes between NPG, IPG, NIPG and CG before and after lambing, between IPG, NIPG and NPG after lambing only. No significant changes were detected in red blood cell parameters or lymphocytes between IPG, NIPG and NPG before parturition. On the contrary, the TLC and neutrophils significantly ($P<0.05$) increased in the pre and postpartum stage in NPG, IPG, NIPG compared to CG, in IPG, NIP compared to NPG and in IPG compared to NIPG.

3.2. Biochemical changes:

Table (3, 4) clarified a significant (P<0.05) hypoproteinemia, hypoalbuminemia and decreased A/G ratio between NPG and CG before and after lambing. While, the globulin levels significantly (P<0.05) raised before parturition and significantly (P<0.05) declined after parturition. On the other hand, a significant (P<0.05) hyperproteinemia was determined between IPG and CG, NPG (before and after parturition), between IPG and NIPG (before parturition only), between NIPG and NPG (before and after parturition) while, the total protein concentrations significantly (P<0.05) decreased in NIPG in relation to CG before parturition and significantly (P<0.05) increased after parturition. There was a significant (P<0.05) hypoalbuminemia, hyperglobulinemia and decreased A/G in IPG, NIPG compared to CG, NPG and in IPG compared to NIPG in the pre and postpartum stages (albumin demonstrated non-significant changes between NIPG and NPG in the prepartum stage).

Additionally, a significant (P<0.05) hypoglycemia, hypertriglyceridemia, hypocholesterolemia , HDL-hypocholesterolemia and LDL-hypocholesterolemia were observed between NPG, IPG, NIPG and CG (before and after parturition), between IPG, NIPG and NPG (after parturition only), but the total lipids concentrations significantly (P<0.05) increased before parturition in NPG, IPG, NIPG compared to CG and significantly (P<0.05) decreased after parturition in NPG compared to CG, in IPG, NIPG compared to CG, NPG. A significant (P<0.05) decline was also observed in all estimated minerals (Ca, Mg, P), electrolytes (Na, Cl), trace elements (Cu, Zn), total antioxidant capacity (TAC) in NPG, IPG, NIPG related to CG (before and after parturition), in IPG, NIPG related to NPG (after parturition only), except K as it significantly (P<0.05) elevated in NPG, IPG, NIPG in relation to CG before parturition and significantly (P<0.05) reduced in NPG, IPG, NIPG in relation to CG as well as in IPG, NIPG in relation to NPG after parturition. On the contrary, Liver enzymatic activities (AST, ALT, ALP), renal function tests (blood urea, Cr) significantly (P<0.05) increased in NPG IPG, NIPG compared to CG (before and after parturition) and in IPG, NIPG compared to NPG (after parturition only).

There are no significant (P<0.05) changes in glucose, total lipids, triglycerides, cholesterol, HDL-hypocholesterol, LDL-hypocholesterol, minerals, electrolytes, trace elements, TAC, liver enzymatic activities, kidney functions between NIPG, IPG and NPG before parturition. On the other hand, Ca concentrations significantly (P<0.05) decreased in NIPG when compared to IPG and TAC significantly increased (P<0.05) in NIPG related to IPG after parturition.

With respect to the MMPs activity in the current work, a significant (P<0.05) elevation was recorded in MMP-2 and MMP-9 activity in NPG compared to CG and in IPG compared to CG, NPG before and after parturition. On the contrary, a significant (P<0.05) decrease was reported in their activity in NIPG compared to CG, NPG and IPG before and after parturition. Table (5) explained that both MMP-2 and MMP-9 yield a 100% sensitivity and NPV, but the specificity, LR, PPV and accuracy rate values of MMP-9 were better than MMP-2 values for IPG and NIPG at the prepartum stage and for NIPG only at the postpartum stage. While, MMP-2 achieved a higher specificity, LR, PPV and accuracy rate than MMP-9 for IPG at the postpartum stage. The percentages of increase were in the sake of MMP-2 in the inflammatory conditions before and after lambing but the percentages of decrease of MMP-9 in the non-inflammatory conditions were better than those of MMP-2 during the pre and postpartum stages.

Table (1): Comparison between the hematological parameters in the studied groups (before parturition).

Parameter	CG	NPG	IPG	NIPG
RBCs ($\times10^6$/µl)	12.05±0.73[d]	10.76±0.14[a]	10.85±0.90[a]	10.65±0.02[a]
Hb (g/dl)	13.84±0.86[d]	10.37±1.06[a]	10.47±0.49[a]	10.37±0.26[a]
PCV (%)	33.05±0.83[d]	29.63±0.45[a]	29.53±0.51[a]	29.58±0.41[a]
MCV (fl)	27.52±1.72	27.57±1.04	27.35±1.75	27.77±0.30
MCH (pg)	11.48±0.28[d]	9.93±1.08[a]	9.70±0.72[a]	9.74±0.28[a]
MCHC (%)	41.88±2.73[d]	36.02±3.62[a]	35.48±1.83[a]	35.07±0.17[a]
TLC ($\times10^3$/µl)	7.80±0.30[d]	8.50±0.25[a]	9.57±0.19 [a,b]	8.99±0.21[a,b,c]
Neutrophils ($\times10^3$/µl)	2.64±0.23[d]	3.58±0.21[a]	4.65±0.13[a,b]	4.06±0.04[a,b,c]
Lymphocytes($\times10^3$/µl)	4.16±0.26[d]	3.96±0.06[a]	3.96±0.06[a]	3.96±0.06[a]
Monocytes ($\times10^3$/µl)	0.53±0.07	0.52±0.08	0.43±0.07	0.44±0.08
Eosinophils ($\times10^3$/µl)	0.44±0.06	0.42±0.0.08	0.57±0.09	0.57±0.09
Basophils ($\times10^3$/µl)	0.04±0.05	0.04±0.05	0.04±0.05	0.05±0.05

[a](significant with CG), [b](significant with NPG), [c](significant with IPG), [d](significant between the four groups), considered significant when considered statistically significant at $P<0.05$.

Table (2): Comparison between the hematological parameters in the studied groups (after parturition).

Parameter	CG	NPG	IPG	NIPG
RBCs ($\times10^6$/µl)	12.05±0.73[d]	10.65±0.14[a]	8.83±0.06[a,b]	8.85±0.02 [a,b]
Hb (g/dl)	13.84±0.86[d]	10.37±0.26[a]	8.03±0.04 [a,b]	8.04±0.03[a,b]
PCV (%)	33.05±0.83[d]	29.58±0.41[a]	24.49±0.21 [a,b]	24.56±0.18 [a,b]
MCV (fl)	27.52±1.72	27.77±0.30	27.74±0.18	27.76±0.17
MCH (pg)	11.48±0.28[d]	9.74±0.28[a]	9.10±0.04 [a,b]	9.09±0.03 [a,b]
MCHC (%)	41.88±2.73[d]	35.07±1.01[a]	32.81±0.19 [a,b]	32.73±0.17[a,b]
TLC ($\times10^3$/µl)	7.80±0.30[d]	8.50±0.25[a]	11.61±0.69 [a,b]	10.86±0.27 [a,b,c]
Neutrophils ($\times10^3$/µl)	2.64±0.23[d]	3.58±0.21[a]	7.31±0.82 [a,b]	6.58±0.11 [a,b,c]
Lymphocytes($\times10^3$/µl)	4.16±0.26[d]	3.96±0.06[a]	3.27±0.16[a,b]	3.23±0.04[a,b]
Monocytes ($\times10^3$/µl)	0.53±0.07	0.52±0.08	0.43±0.07	0.44±0.08
Eosinophils ($\times10^3$/µl)	0.44±0.06	0.42±0.0.08	0.57±0.09	0.57±0.09
Basophils ($\times10^3$/µl)	0.04±0.05	0.04±0.05	0.04±0.05	0.05±0.05

[a](significant with CG), [b](significant with NPG), [c](significant with IPG), [d](significant between the four groups), considered significant when considered statistically significant at $P<0.05$.

Table (3): Comparison between the biochemical parameters in the studied groups (before parturition).

Parameter	CG	NPG	IPG	NIPG
Total protein (g/dl)	6.11±0.09[d]	5.11±0.03[a]	6.39±0.06[a,b]	5.96±0.04[a,b,c]
Albumin (g/dl)	3.99±0.20[d]	2.05±0.03[a]	1.88±0.05[a,b]	2.05±0.03[a,c]
Globulin (g/dl)	2.12±0.21[d]	3.07±0.04[a]	4.51±0.08[a,b]	3.91±0.05[a,b,c]
A\G	1.91±0.22 [d]	0.67±0.02[a]	0.42±0.02[a,b]	0.52±0.01[a,b]
Glucose (mg/dl)	85.10±2.73[d]	68.15±2.25[a]	68.65±3.47[a]	68.15±2.25[a]
Blood urea (mg/dl)	24.74±0.73[d]	28.50±0.75[a]	29.50±4.72[a]	30.00±6.57[a]
Cr (mg/dl)	0.75±0.11[d]	1.30±0.15[a]	1.35±0.29[a]	1.35±0.25[a]
AST (U/L)	26.74±1.61[d]	33.30±1.03[a]	33.80±2.43[a]	33.30±1.03[a]
ALT (U/L)	36.74±1.60[d]	43.45±0.49[a]	43.95±2.72[a]	43.45±1.09[a]
ALP (U/L)	28.54±0.30[d]	29.69±0.49[a]	29.69±0.49[a]	30.19±2.48[a]
Total lipids (mg/dl)	353.90±6.10[d]	378.18±5.65[a]	379.92±8.26[a]	381.18±12.81[a]
Triglycerides (mg/dl)	73.17±2.12[d]	114.17±2.12[a]	115.52±3.61[a]	115.77±4.77[a]
Phospholipids (mg/dl)	159.54±5.64[d]	185.55±53.19[a]	185.55±53.19[a]	185.55±53.19[a]
Cholesterol (mg/dl)	121.19±1.98[d]	77.86±3.71[a]	78.86±6.44[a]	79.86±8.50[a]
HDL-cholesterol (mg/dl)	86.90±1.39 [d]	54.36±2.81[a]	54.86±5.01[a]	55.36±6.86[a]
LDL-cholesterol (mg/dl)	34.29±1.40 [d]	23.50±1.29[a]	24.00±2.94[a]	24.50±1.14[a]
Ca (mg/dl)	10.73±0.42[d]	8.31±0.12[a]	8.26±0.33[a]	8.31±0.21[a]
Mg (mg/dl)	3.71±0.50[d]	2.63±0.08[a]	2.60±0.16[a]	2.63±0.08[a]
P (mg/dl)	6.35±0.27 [d]	5.37±0.27[a]	5.32±0.37[a]	5.37±0.27[a]
Cl (mmol/L)	105.23±2.64[d]	92.62±0.80[a]	92.12±2.54[a]	92.62±0.80[a]
Na (mmol/L)	142.40±2.80[d]	127.37±1.37[a]	126.37±4.77[a]	127.37±1.23[a]
K (mmol/L)	3.38±0.26[d]	4.52±0.17[a]	4.47±0.27[a]	4.52±0.17[a]
Cu (µmol/L)	23.55±1.31[d]	18.69±1.51[a]	18.69±1.51[a]	18.69±1.51[a]
Zn (µg/dl)	155.72±7.65[d]	126.60±1.61[a]	122.60±5.11[a]	122.60±1.61[a]
TAC (Mm/L)	0.90±0.04[d]	0.52±0.03[a]	0.50±0.03[a]	0.52±0.02[a]
MMP-2 (ng/ml)	17.09±1.30[d]	30.26±1.31[a]	38.13±2.53[a]	12.33±0.84[a,b,c]
MMP-9 (ng/ml)	23.07±0.94[d]	48.52±2.25[a]	54.57±1.68[a]	18.15±1.09[a,b,c]

[a](significant with CG), [b] (significant with NPG), [c] (significant with IPG), [d] (significant between the four groups), considered significant when considered statistically significant at $P<0.05$.

Table (4): Comparison between the biochemical parameters in the studied groups (after parturition).

Parameter	CG	NPG	IPG	NIPG
Total protein (g/dl)	6.11±0.09[d]	5.05±0.03[a]	7.06±0.02[a,b]	6.87±0.55[a,b]
Albumin (g/dl)	3.99±0.20[d]	3.41±0.25[a]	1.94±0.03[a,b]	2.76±0.32 [a,b,c]
Globulin (g/dl)	2.12±0.21[d]	1.64±0.32[a]	5.12±0.03[a,b]	4.49±0.63[a,b,c]
A\G	1.91±0.22 [d]	1.81±0.42[a]	0.38±0.01[a,b]	0.63±0.14[a,b,c]
Glucose (mg/dl)	85.10±2.73[d]	76.85±1.98[a]	45.30±1.13[a,b]	45.20±1.20[a,b]
Blood urea (mg/dl)	24.74±0.73[d]	29.15±1.15[a]	38.09±2.67[a,b]	37.87±2.80[a,b]
Cr (mg/dl)	0.75±0.11[d]	1.06±0.03[a]	1.74±0.15[a,b]	1.74±0.15[a,b]
AST (U/L)	26.74±1.61[d]	32.73±1.69[a]	40.89±1.77[a,b]	40.80±1.88[a,b]
ALT (U/L)	36.74±1.60[d]	42.76±1.88[a]	50.26±1.84[a,b]	50.17±1.89[a,b]
ALP (U/L)	28.54±0.30[d]	32.38±1.51[a]	51.29±2.07[a,b]	51.24±2.10[a,b]
Total lipids (mg/dl)	353.90±6.10[d]	337.66±5.85[a]	332.58±6.70[a,b]	331.99±6.23[a,b]
Triglycerides (mg/dl)	73.17±2.12 [d]	83.17±2.12[a]	94.06±2.10[a,b]	93.85±1.44[a,b]
Phospholipids (mg/dl)	159.54±5.64	159.44±5.64	159.34±6.17	161.04±4.99
Cholesterol (mg/dl)	121.19±1.98 [d]	94.95±2.31[a]	78.94±2.52[a,b]	78.10±2.99[a,b]
HDL-cholesterol(mg/dl)	86.90±1.39 [d]	66.90±1.39[a]	56.68±2.31[a,b]	55.32±2.46[a,b]
LDL-cholesterol(mg/dl)	34.29±1.40 [d]	28.05±1.85[a]	22.20±0.89[a,b]	22.78±1.14[a,b]
Ca (mg/dl)	10.73±0.42[d]	8.69±0.12[a]	7.77±0.10[a,b]	7.30±0.96[a,b,c]
Mg (mg/dl)	3.71±0.50[d]	3.20±0.06[a]	2.51±0.05[a,b]	2.52±0.06[a,b]
P (mg/dl)	6.35±0.27 [d]	5.34±0.15[a]	4.41±0.05[a,b]	4.42±0.05[a,b]
Cl (mmol/L)	105.23±2.64[d]	95.23±2.64[a]	85.83±2.47[a,b]	84.67±2.07[a,b]
Na (mmol/L)	142.40±2.80[d]	133.72±2.51[a]	126.63±1.30[a,b]	126.85±1.37[a,b]
K (mmol/L)	3.38±0.26[d]	2.67±0.24[a]	1.53±0.31[a,b]	1.45±0.26[a,b]
Cu (µmol/L)	23.55±1.31[d]	20.45±0.27[a]	17.38±0.94[a,b]	17.44±0.91[a,b]
Zn (µg/dl)	155.72±7.18[d]	136.16±2.88[a]	127.21±2.16[a,b]	128.00±2.15[a,b]
TAC (Mm/L)	0.90±0.04[d]	0.75±0.03[a]	0.31±0.03[a,b]	0.45±0.02[a,b,c]
MMP-2 (ng/ml)	17.09±1.30[d]	25.49±1.09[a]	54.29±1.64[a,b]	13.53±0.83[a,b,c]
MMP-9 (ng/ml)	23.07±0.94[d]	42.22±3.33[a]	74.32±3.23[a,b]	20.95±1.03[a,b,c]

[a] (significant with CG), [b] (significant with NPG), [c] (significant with IPG), [d] (significant between the four groups), considered significant when considered statistically significant at $P<0.05$.

4. DISCUSSION:

The postpartum stage is a critical physiological stage in the barki ewes' life. It is normally connected with a normocytic hypochromic anemia indicated in the current study by the significant decrease in all red cell parameters and indices (except MCV) in NPG before and after parturition. Whereas, theses parameters physiologically decline during the gestation period especially the last trimester and remain low during the early postpartum and the blood lose during lambing may take a part in this anemia mechanism also (Ahmed et al., 2018). No doubt that, the activated cytokines (such as TNF-α and interleukins) and the anorexia related to both inflammatory and non-inflammatory postpartum disorders helped in augmentation the severity of this anemia in both IPG and NIPG after lambing (Heidarpour et al., 2014; Sarvesha et al., 2016). As the pro-inflammatory cytokines stimulate hepcidin release which prevents the intestinal dietary iron absorption and increase ferritin and hemosiderin formation. Otherwise, their direct toxic effects on the erythroid precursors, which inhibit erythropoietin and stem cell factor expression causing inhibition of erythropoiesis and shortening of erythrocyte life span. Furthermore, their role in free radical production and subsequent oxidative stress may be involved in red blood cell destruction and dysfunction. Similar results were obtained before in sheep and other species in uterine prolapse (Ahmed et al., 2005; Purohit et al., 2018), mastitis (El-Deeb, 2013), metritis (Bhuyan et al., 2017), endometritis (Sheldon and Owens, 2017), retained placenta

(Jovanovic et al., 2013) and dystocia (Bansal et al., 2011).

On the other hand, the obtained neutrophilia and subordinate leukocytosis in NPG before and after parturition, occurred due to its role in lambing preparation in the prepartum stage and in healing, inflammation and uterine involution during postpartum stage (Ahmed et al., 2018), and was assigned in IPG and NIPG to the pro-inflammatory cytokines activity and its action as innate immunity generator in similar conditions (Bansal et al., 2011; El-Deeb, 2013; Jovanovic et al., 2013; Bhuyan et al., 2017; Sheldon and Owens, 2017; Purohit et al., 2018). Logically, this neutrophilic leukocytosis is more intensive in IPG than NIPG due to presence of infection and consequent stronger immune response. Interestingly, the significant neutrophilic leukocytosis between IPG, NIP and NPG, and between IPG and NIPG before lambing suggested TLC and DLC as a rapid field test for postpartum disorders predication before parturition particularly inflammatory disorders.

While, the lymphocytopenia in NPG, IPG and NIPG before and after parturition is closely linked to the lymphocytes escape from circulation to the uterus in the prepartum and the early postpartum stage and site of infection or inflammation in IPG (Bhuyan et al., 2017), while the pain and stress and connected hpercortisolemia mostly is the cause of this lymphocytopenia in NIPG (Ahmed et al., 2005; Mordak et al., 2017).

Table (5): Cut off points (ng/ml), sensitivity, specificity, LR, PPV, NPV, accuracy rate and percentage of increase for MMP-2 and MMP-9 in IPG and NIPG compared to NPG (before and after parturition).

Statistical parameter	MMP-2				MMP-9			
	IPG		NIPG		IPG		NIPG	
	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum	Prepartum	Postpartum
Cut off	31.5	27	28.5	24.5	50.90	46	45.70	38.5
Sensitivity	100%	100%	100%	100%	100%	100%	100%	100%
Specificity	80%	95%	80%	70%	90%	85%	85%	85%
LR	5	20	5	3.33	10	6.67	6.67	6.67
PPV	83.33%	95.24%	83.33%	76.92%	90.91%	86.96%	86.96%	86.96%
NPV	100%	100%	100%	100%	100%	100%	100%	100%
Accuracy rate	90%	97.5%	90%	85%	95%	92.5%	92.5%	92.5%
% of increase or decrease	26.01%	112.99%	-59.25%	-46.92%	12.36%	76.03%	-62.59%	-50.38%

Concerning the protein profile, the demonstrated hypoglobulinemia and dependant hypoprotienemia in NPG after lambing in the present data were because of immunoglobulins (especially IgG) migration to the mammary glands in order to produce colostrum (Sharma et al. 2015). On the contrary, the different pro-inflammatory cytokines and acute phase proteins release and their participation in the parturition mechanism are the precursor of the described hyperglobulinemia in NPG before parturition (Kaneko et al., 2008). In the same line, the enhanced pro-inflammatory cytokines and acute phase proteins production and immunolglobulins generation are reasonable causes for the outstanding hyperglobulinemia and linked hyperproteinemia and decreased A/G in IPG and NIPG in the pre and postpartum stage (El-Deeb, 2013, Ali et al., 2016, Sarvesha et al. 2016, Kaya et al., 2017, Mordak et al., 2017). Rationally, the observed increase in total protein and globulin concentrations in IPG in relation to NIPG referred to presence of microbial infection and subsequent immune response amplification either innate or humeral. It worth to mention that, the detected hyperproteinemia and hyperglobulinemia between IPG, NIPG and NPG, between IPG and NIPG before parturition cleared the importance of the protein profile estimation in expecting the postpartum disorders especially inflammatory ones.

The improper amount and composition of introduced rations for the ewes physiological status, ignorance of the importance of feed additives for healthy ewes and supportive treatment for diseased animals, hyperthermia and anorexia associated with different postpartum pathological conditions are fundamental causes for the noted hypoalbuminemia (resulted in hypoproteinemia in NPG, NIPG before parturition), hypoglycemia, HDL-hypocholesterolemia and LDL-hypocholesterolemia, hypocholesterolemia (hypolipidemia in NPG, IPG, NIPG after parturition), decreased minerals and electrolytes concentrations, decreased Zn and Cu levels and subordinate decline in TAC (Zn and Cu are necessary cofactors in antioxidants enzymes formation) in NPG, IPG, NIPG before and after parturition (Kaneko et al., 2008). Meanwhile, the hyperkalemia recorded in the NPG, IPG and NIPG before parturition was because of the physiological blood acidosis connected with the late pregnancy, whereas the accumulated H+ is pumped intracellularly in exchange with K+ extracellularly to maintain electroneutrality which known as potassium efflux (Kaneko et al., 2008). The acute phase response and the oxidative stress associated with the investigated physiological and pathological conditions in the current research are another possible reasons for the distinguished hypoalbuminemia in NPG, IPG and NIPG in the pre and postpartum stages. Whereas, albumin a negative acute phase reactant and antioxidant as well (Kaneko et al., 2008; Bansal et al., 2011; El-Deeb, 2013; Kaya et al., 2017; Mordak et al., 2017). Interestingly, the oxidative stress may play a role in the above-described HDL-hypocholesterolemia in the diseased groups, as HDL-hypocholesterol has potent antioxidant characters (Kaneko et al., 2008). The decreased TAC and in IPG compared to NIPG after parturition was mainly attributed to the stronger activation of the pro-inflammatory cytokines due to presence of infection and more tissue destruction. Therefore, the immune response in the inflammatory conditions will be more prominent and the free radicals production will be more harmful (Kaneko et al., 2008; El-Deeb, 2013; Kaya et al., 2017). The detected hypocalcemia in NIPG in relation to IPG is basically linked to presence of hypocalcaemia cases in the group.

Contrariwise, the observed hypertriglyceridemia before and after parturition in all groups (lead to hyperlipidemia in all groups before parturition), indicated a negative energy balance and increasing body fat lipolysis in order to get energy and consequent triglycerides release (Tóthová et al., 2014). Therefore, the extra NEFAs and BHB will be liberated in the circulation and become a load on the liver and kidneys causing the depicted increase in hepatic and renal function tests values in NPG, IPG and NIPG before and after lambing. Additionally, the aforementioned oxidative stress and decreased TAC in NPG, IPG and NIPG before and after parturition, usually result in oxidative damages of different organ cells leading to an increase in organ function test values (Kaneko et al., 2008; Bansal et al., 2011; El-Deeb, 2013; Tóthová et al., 2014; Sarvesha et al., 2016; Kaya et al., 2017; Mordak et al., 2017; Shao et al., 2017).

MMP-2 and MMP-9 (gelatinases) are members of matrix metalloproteinases group, which are mainly activated to change the tissue structure through extra cellular matrix (ECM) degradation. They are controlled by tissue inhibitors of metalloproteinases (TIMPs). Their pronounced activity in the normal pre and postpartum stages in the present study pointed to their role in smoothing parturition and uterine involution (Nguyen et al., 2016). On the other hand, the remarkable increase in their concentrations in IPG before and after parturition return to the pro-inflammatory cytokines stimulatory effect on their secretion and reflect their action in the pathogenesis of different uterine and udder inflammatory diseases (Li et al., 2016; Guo et al., 2017; Alali et al., 2018). On contrast, MMP-2 and MMP-9 levels markedly reduced in NIPG before and after parturition, as they have an important role in fetal membrane separation thus facilitating the parturition process and their inhibition is closely attached to the appearance of dystocia, fetal membrane retention and uterine prolapse (Mosier et al., 2010, Patel and Parmar, 2016, Sundrani et al., 2012). In addition, they are Ca dependant enzymes and the hypocalcaemia is implicated in their inhibition (Cockeran et al., 2012). Regarding MMP-2 and MMP-9 value as markers for postpartum disorders, the data of table (5) cleared that both of them may be a good indicator and predictor for ovine postpartum disorders, but MMP-9 is better than MMP-2 in the prediction of the inflammatory and non-inflammatory postpartum disorders and in the indication of the non-inflammatory postpartum disorders. On the contrary, MMP-2 was better marker for the inflammatory postpartum disorders (Mosier et al., 2010; Cockeran et al., 2012; Sundrani et al., 2012; Li et al., 2016; Patel and Parmar 2016; Guo et al., 2017; Alali et al., 2018).

CONCLUSION:

Finally, it can be concluded that ovine postpartum period associated with dramatic clincopathological alterations, most of these changes start from the prepartum stage. The awareness with these changes is helpful in avoiding a part from the pathological disorders related to this stage. Following the clinicopathological changes associated with the postpartum disorders and modifying the treatment and nutrition strategies according to them, will enhance the recovery rates and reduce the subsequent economic losses. MMP-2 and MMP-9 increase in the prepartum and postpartum stage is an alarm for inflammatory postpartum disorders in sheep. While, their decrease draws attention towards the non-inflammatory postpartum disorders.

REFERENCES:

Ahmed, S., Ahmad, I., Lodhi, L.A., Ahmad, N., Samad, H.A. 2005. Clinical, Haematological and Serum Macro Mineral Contents in Buffaloes with Genital Prolapse. Pak. Vet. J. 25(4): 167-170.

Ahmed, M.N., Humide, A.O., Muhadi, M.J. 2018. Hematological state of ewes injected with some mediators during postpartum and lactation period. J. Pharm. Sci. Res. 10(8): 1921-1924.

Ali, A., Derar, D., Tharwat, M., Zeitoun, M.M., Al-Sobyil, F.A. 2016. Dystocia in dromedary camels: prevalence, forms, risks and hematobiochemical changes. Anim.Reprod. Sci. 170:149-156.

Alali, Z., Swan, K., Nothnick, W. 2018. Matrix Metalloproteinases and Endometriosis: Their Role in Disease Pathophysiology and Potential as Therapeutic Targets. Current Women`s Health Reviews. 14(2): 147 - 153.

Bansal, A.K., Singh, A.K., Cheema, R.S., Brar, P.S., Gandotra, V.K., Singh, P., Prabhakar, S. 2011. Status of oxidative stress and antioxidant enzymes in normally calved and dystocia affected buffaloes. Ind. J. Anim. Sci. 81(9): 915-918.

Bhuyan, M., Nath, K.C., Deka, B.C., Bhuyan, D., Goswami, S., Sarma, R.K. 2017. Comparative hematological and biochemical parameter study on normal and Metritic cows. Inter. J. Chem. Studies. 5(3): 77-79.

Cockeran, R., Mutepe, N.D., Theron, A.J., Tintinger, G.R., Steel, H.C., Stivaktas, P.I., Richards G.A., Feldman, C., Anderson, R. 2012. Calcium-dependent potentiation of the pro-inflammatory functions of human neutrophils by tigecycline in vitro. J. Anti. microb. Chemother. 67: 130-137.

El-Deeb, W.M. 2013. Clinicobiochemical investigations of gangrenous mastitis in does: Immunological responses and oxidative stress Biomarkers. J. Zhejiang Univ. Sci. B. 14: 33-39.

Feldman BF, Zinkl JC, Jain NC. "Schalm's Veterinary Hematology". 5th ed. London: Lippincott Williams & Wilkins, Philadelphia; 2000.

Guo, Y., Xu, N., Sun, W., Zhao, Y., Li, C., Guo, M. 2017. Luteolin reduces inflammation in Staphylococcus aureus-induced mastitis by inhibiting NF-kB activation and MMPs expression. Oncotarget. 8(17): 28481-28493.

Heidarpour, M., Mohri, M., Fallah-Rad, A.H., Dehghan, S.F., Mohammadi, M. 2014. Hematological changes before and after treatment in dairy cows with clinical and subclinical endometritis. Comp. Clin. Pathol. 23: 97-101.

Jovanovic, I.B., VeliIkovic, M., Vukovic, D., Milanovic, S., Vallic, O., Gvozdic, D. 2013. Effects of Different Amounts of Supplemental Selenium and Vitamin E on the Incidence of Retained Placenta, Selenium, Malondialdehyde, and Thyronines Status in Cows Treated with Prostaglandin F2α for the Induction of Parturition. J. Vet. Med. Volume 2013, Article ID 867453, 6 pages.

Kaneko JJ, Harvey JW, Bruss ML. Clinical Biochemistry of Domestic Animals. 6th ed. San Diego: Academic Press; 2008.

Kaya, S., Ögun, M., Özen, H., Kuru, M., Şahin, L., Kukurt, A., Kacr, C. 2017. The Impact of Endometritis on Specific Oxidative Stress Parameters in Cows. J. Hellenic Vet. Med. Society. 68(2): 231-236.

Li, H., Zheng, H., Li, L., Shen, X., Zang, W., Sun, Y. 2016. The Effects of Matrix Metalloproteinase-9 on Dairy Goat Mastitis and Cell Survival of Goat Mammary Epithelial Cells. PLoS ONE 11(8): e0160989.

Mordak, R., Nicpoń, J., Illek, J. 2017. Metabolic and mineral conditions of retained placenta in highly productive dairy cows: pathogenesis, diagnostics and prevention – a review. Acta. Vet. Brno. 86: 239-248.

Mosier, E., Lin, V.K., Zimmern, P. 2010. Extracellular Matrix Expression of Human Prolapsed Vaginal Wall. Neurourology and Urodynamics. 29: 582-586.

Nguyen, T., Shynlova, O., Lye, S. 2016. Matrix Metalloproteinase Expression in the Rat Myometrium During Pregnancy, Term Labor, and Postpartum. Biol. Reprod. 95(1): 1-14.

Patel, R.V., Parmar, S.C. 2016. Retention of Fetal Membranes and its Clinical Perspective in Bovines. Sch. J. Agric. Vet. Sci. 3(2): 111-116.

Purohit, G.N., Arora, A.S., Gocher, T., Gaur, M., Saraswat, C.S., Mishra, P. 2018. Uterine prolapse in buffaloes: A review. Asian Pacific J. Reprod. 7(6): 241-247.

Sarvesha, K., Satanarayana, M.L., Narayanaswamy, H.D., Rao, S., Yathiraj, S., Isloor, S., Mukartal, S.Y., Srikanth, M., Anuradha, M.E., Kamal, H. 2016. Effect of subclinical and clinical mastitis on haemato-biochemical profile and milk leukocyte count in indigenous cows. J. Cell and Tissue Res. 16(3): 5829-5834.

Shao, C., Wang, H., Wang, X., Jiang, S., Sun, J., Song, H., Li, J. 2017. Characterization of inflammatory responses by cervical cytology, cytokine expression and ultrastructure changes in a goat subclinical endometritis model. J. Vet. Med. Sci.79(1): 197-205.

Sharma, A., Kumar, P., Singh, M., Vasishta, N.K. 2015. Haemato-biochemical and endocrine profiling of north western Himalayan Gaddi sheep during various physiological/reproductive phases. Open Vet. J. 5(2): 103-107

Sheldon, I.M., Owens S.E. 2017. Postpartum uterine infection and endometritis in dairy cattle. Proceedings of the 33rd Annual Scientific Meeting of the European Embryo Transfer Association (AETE); Bath, United Kingdom.

Sundrani, D.P., Chavan-Gautam, P.M., Pisal, H.R., Mehendale, S.S., Joshi, S.R. 2012. Matrix Metalloproteinase-1 and -9 in Human Placenta during Spontaneous Vaginal Delivery and Caesarean Sectioning in Preterm Pregnancy. PLoS ONE. 7(1): e29855.

Tóthová, C., Nagy, O., Kovác, G. 2014. Changes in the concentrations of selected acute phase proteins and variables of energetic profile in dairy cows after parturition. J. Appl. Anim. Res. 42(3): 278-283.