IRANIAN JOURNAL OF RUMINANTS HEALTH RESEARCH 2017, 2(2): 1-22
Doi: 10.22055/ijrhr.2019.23724.1013

Circulating metabolic biomarkers and hormones and their relationships at different pre- and post-parturition periods of Ghezel ewes

Chalmeh, A*.; Pourjafar, M.; Badiei, K.; Mirzaei, A.; Mazrouei Sebdani, M.; Saadat Akhtar, I.;Z arei, M. H.

Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

*Corresponding Author: achalmeh81@gmail.com

Abstract

Information regarding metabolic profile in different physiological periods of ewes can assist veterinarians to monitor the herd health and productive performance. Furthermore, the relationships among the metabolic parameters can be used to detect the effect of each parameter on another one. Five clinically healthy adult Ghezel ewes were selected at 4 weeks before parturition and evaluated at 4 and 2 weeks before, 2 and 4 weeks and 2, 3 and 4 months after parturition. Blood samplings were performed from each animal at the above mentioned periods, in the meantime, serum concentrations of glucose, BHBA, NEFA, cholesterol, TG, HDL, LDL, VLDL, insulin, T3, T4, prolactin, cortisol and IGF-1 were measured in all specimens. In all studied periods, glucose was negatively correlated with Insulin, BHBA, and NEFA. The positive significant correlation was detected between glucose and cortisol. BHBA had a positive and significant correlation with NEFA in all ewes. BHBA and NEFA were significantly correlated, negatively and positively, with insulin and cortisol, respectively. Cortisol had both significant and positive relationships with TG, cholesterol, HDL, LDL, and VLDL. Insulin had significant negative and positive correlations with cortisol and both IGF-1 and prolactin, respectively. Moreover, there was a negative significant correlation between cortisol, IGF-1 and prolactin in all ewes. Significant positively relationship was detected between IGF-1, and prolactin at all groups. T3 and T4 were significantly positive related together. Information regarding the correlations among circulating metabolic parameters can be used to estimate the changing patterns of each metabolic parameter via evaluating the others.

Keywords: Metabolic profile, Endocrinology, Normal level, Correlation, Ghezel ewe.

Introduction

Evaluating the circulating metabolic biomarkers and hormones plays an important role in the diagnosis of ovine metabolic functions. One of the important characteristics of metabolic disorders is the increment or reduction in blood biochemical parameters. The majority of these disorders are characterized by certain changes in the concentration of blood parameters (Chalmeh *et al.* 2016; Roubies *et al*. 2017). Therefore, understanding the normal values of blood biochemical parameters would be the useful indices in the determination of the physiological aspects in pregnant, non-pregnant, lactating or non-lactating ewes.

The magnitude of negative energy balance during the last stage of pregnancy is considered as an important indicator of the development of metabolic diseases (Sahoo *et*

al. 2009; Taherpour & Mirzaei, 2012). Circulating metabolic parameters are the most important factors involved in setting and modulating the basal metabolic rate in target tissues, such as liver, heart, kidney, and brain (Saicic *et al.* 2006).

Based on the other studies, different results have been reported for circulating parameters when ewes were in different physiological status (Firat & Ozpinar, 2002; West, 1996) and different relationships have been reported among some blood biochemical parameters in ewes (Ford *et al.* 1990; West, 1996) and cows (Chalmeh *et al.* 2016). Ghezel ewes as a fat-tailed breed, have different physiological and metabolic characteristics due to their fat-tail which can be used as an energy source for this breed; however, to the best of our knowledge, information regarding circulating metabolic biomarkers and hormones in different pre- and post- parturition periods of Ghezel ewes is rare. Different results from the previous studies regarding circulating parameters and correlations among them in each breed show that determination and standardization of serum metabolic parameters and hormones for native ewes will be a useful and reliable index in the understanding of physiological status of these animals (Firat & Ozpinar, 2002; Ford *et al.* 1990; West, 1996). Hence, the aims of the present study were presenting the basal concentrations of some serum metabolic parameters as well as hormones including insulin, cortisol, prolactin, T3, T4 and IGF-1 at different pre- and post- parturition periods of Ghezel ewes and evaluating the possible relationships among them. The relationships among these parameters together can represent the effect of each parameter on the others from 4 weeks before to 4 months after parturition in Ghezel sheep.

Materials and methods

Animals

The present study was carried out in winter 2015 on 5 adult Ghezel ewes collected from a farm around Shiraz, Southwest of Iran. Ewes were housed in open-shed barns with free access to water and shade. The ration included alfalfa hay, corn silage, wheat straw, barley grain, wheat bran, cottonseed meal, urea, calcium carbonate, common salt (NaCl) and vitamin-mineral premix. All the animals were clinically healthy, had no history of a debilitating disease and were free from internal and external parasites due to routine antiparasitic programs at this farm. Their body condition score (BCS) was estimated based on the 0-5 system and

the state of pregnancy was assessed by ultrasonography. These 5 sheep were selected approximately at 4 weeks before their parturition and were followed 2 weeks before, 2 and 4 weeks and 2, 3 and 4 months after parturition.

Blood sampling and biochemical analyses

Blood samplings were performed from each animal at the periods mentioned earlier, 1 hour after the morning meal, at about 06:00 a.m. Immediately after blood collections, sera were separated by centrifugation for 10 minutes at 3,000g and stored at -22°C until assayed. Glucose was assayed by an enzymatic (glucose oxidase) colorimetric method (ZistChems, Tehran, Iran). Beta-hydroxybutyric acid (BHBA) (Ranbut, UK) and non-esterified fatty acid (NEFA) were assayed by a colorimetric method (NEFA®, UK). The sera were analyzed for cholesterol by a modified Abell-Kendall/Levey-Brodie (A-K) method (Abbel *et al.* 1952; Burtis & Ashwood, 1994), and triglyceride (TG) by the enzymatic procedure of McGowan *et al.* (1983). Lipoproteins were isolated using a combination of precipitation and ultra-centrifugation. HDL-cholesterol (high density lipoprotein-cholesterol) was measured using the precipitation method. In the first step, the precipitation reagent (sodium phosphotungstate with magnesium chloride) was added to the serum to aggregate non-HDL lipoproteins which were sedimented by centrifugation (10,000×g for 5 min). The residual cholesterol was then measured by the enzymatic method (Burtis & Ashwood, 1994). LDL-cholesterol (low density lipoprotein-cholesterol) was calculated as the difference between the total cholesterol measured in the precipitate and in the HDL fraction minus 0.2×triglyceride (LDL=total cholesterol–HDL cholesterol–0.2×TG). VLDL-cholesterol was estimated as one-fifth of the concentration of triglycerides (Friedewald *et al.* 1972). Insulin was measured by ovine insulin ELISA kit (Cusabios, China, specificity 100%, and precision) [1]. Serum triiodothyronine (T3) concentrations were determined using a competitive enzyme immunoassay kit (Padtan Elm Co., Tehran, Iran)[2]. The sensitivity of the test was 0.2 ng/mL. Serum thyroxine (T4) concentrations were measured using a competitive enzyme immunoassay kit (Monobind Inc., CA,

1 - Intra-assay and inter-assay CV < 8% and 10%, respectively.
2 - Intra- and inter-assay CVs of the assays were 12.6% and 13.2%, respectively.

U.S.A)[3]. The sensitivity of the test was 0.4 µg/dL. Prolactin was measured by competitive enzyme immunoassay technique using commercial ovine prolactin ELISA Kit (Wuhan Huamei Cusabio Biotech Co., Ltd, China). Serum cortisol concentrations were determined by Enzyme Immunoassay Colorimetric method (AccuBind® ELISA kit; Monobind Inc., CA, USA). The sensitivity of the test was 0.25 µg/dL. Serum levels of IGF-1 were evaluated by ELISA kit (ImmunoDiagnosticSystem®, Boldon, UK) with the sensitivity equal to 3.1 µg/L.

Statistical analysis

All data are presented as mean ± standard deviation (SD). One way ANOVA and LSD post hock test was used to evaluate the differences among the same parameters in different sampling periods. Repeated Measures ANOVA was used to evaluate the changing patterns of different parameters at each period. Pearson's correlation test was also used to evaluate the relationship among different metabolic biomarkers and hormones at each production period by using SPSS software (SPSS for Windows, version 20, SPSS Inc, Chicago, IL, USA). The level of significance was set at $P<0.05$.

Results

Normal levels (Mean±SD) of circulating metabolic biomarkers and hormones in different physiological states of Ghezel ewes are presented in Table 1. As is shown, Glucose increased before and subsequently decreased after parturition ($P<0.05$). Its decreasing pattern continued to the last period. Insulin decreased from 4 weeks before to 2 weeks after parturition and then significantly increased to 4 months after parturition ($P<0.05$). Although the highest levels of BHBA and NEFA were detected at 4 weeks before parturition, there were no significant changing patters during the studied periods ($P>0.05$; Table 1).

The lowest serum concentrations of TG and VLDL were detected at peri-parturition periods. Their levels increased at 4 weeks after parturition but subsequently decreased to 4 months after parturition ($P<0.05$). Cholesterol significantly increased from 4 weeks before to 2 months after parturition and then decreased to the last sampling, significantly ($P<0.05$). There was a significant increasing pattern of LDL from pre to post parturition periods but it

3 - Intra- and inter-assay CVs of the assays were 3.0% and 3.7%, respectively.

subsequently decreased from 2 to 4 months after parturition, ($P<0.05$). There was no significant changing pattern in HDL levels ($P>0.05$; Table 1).

T3 and T4 significantly decreased from 4 weeks before to 4 weeks after parturition and then increased to the last studied period ($P<0.05$). Prolactin and IGF-1 decreased significantly before parturition and then increased up to 3 months after parturition. Their concentrations decreased after weaning of the lambs, subsequently ($P<0.05$). There was no significant changing pattern in cortisol levels during all studied periods ($P>0.05$; Table 1).

The correlations among circulating metabolic parameters in studied animals at different pre- and post- parturition periods are presented in Tables 2 to 8. It should be noted that the relationships among all studied parameters at different groups followed the same patterns. In all studied groups, glucose had a significantly negative correlation with Insulin, BHBA and NEFA. The significant positive correlation was detected between glucose and cortisol (Tables 2 to 8). BHBA had a significant positive correlation with NEFA in all ewes. BHBA and NEFA had a significantly negative and positive correlation with insulin and cortisol, respectively. The positive and non-significant correlations were detected among BHBA and NEFA and lipid profile in all ewes (Tables 2 to 8). Insulin, IGF-1 and prolactin were negatively correlated with all lipid profile constituents but cortisol had significant positive relationships with TG, cholesterol, HDL, LDL and VLDL. Insulin had significant negative and positive correlations with cortisol and both IGF-1 and prolactin, respectively. There were negative significant correlations between cortisol, IGF-1 and prolactin in all ewes. A significant positive relationship was detected between IGF-1 and prolactin in all groups. There were positive and non-significant relationships among both T3 and T4 and all studied parameters in different groups (Tables 2 to 8); furthermore, these hormones had a significantly positive relation with each other.

Table 1- Normal levels (Mean±SD) of circulating metabolic biomarkers and hormones in different pre- and post-parturition periods of Ghezel ewes.

Parameters	4 weeks before parturition	2 weeks before parturition	2 weeks after parturition	4 weeks after parturition	2 months after parturition	3 months after parturition	4 months after parturition
Glucose (mg/dL)	62.40±0.24a	87.40±2.20b	72.80±3.18a	66.00±0.01a	76.40±0.97b	71.00±6.12a	52.00±7.34c
BHBA (µmol/L)	120.60±3.13a	98.60±9.83b	114.40±11.72a	94.00±0.01b	88.60±18.17b	116.80±12.29a	111.40±1.34a
NEFA (mmol/L)	161.00±11.90a	131.20±8.49b	149.80±18.62a	129.00±0.01b	147.20±10.57a	143.60±11.78a	147.60±15.93a
TG (mg/dL)	24.40±7.66a	22.00±2.23a	18.00±0.01b	29.00±0.01a	25.80±7.12a	21.80±3.83a	17.00±0.01b
Cholesterol (mg/dL)	61.20±4.31a	62.20±1.78a	63.80±7.85a	63.00±0.01a	72.80±5.33b	65.60±8.62a	51.80±7.52c
HDL (mg/dL)	1.30±0.01a	1.05±0.01b	1.18±0.10a	1.20±0.01a	1.10±0.01a	1.06±0.05b	1.16±0.05a
LDL (mg/dL)	58.02±7.77a	56.80±2.23a	59.02±5.96a	66.00±0.01b	69.54±5.91b	68.18±7.91b	57.24±7.58a
VLDL (mg/dL)	4.88±1.53a	4.40±0.44a	3.60±0.01b	5.80±0.01c	5.16±1.42	4.36±0.76a	3.40±0.01b
Insulin (µU/mL)	10.80±1.10a	10.10±0.54a	9.160±0.87b	10.40±0.01a	10.32±1.66a	10.08±1.49a	12.42±1.76c
Cortisol (ng/mL)	23.90±0.27a	13.66±0.53b	10.74±2.07b	11.80±0.01b	19.34±2.59a	29.16±2.16c	31.44±2.67c
IGF-1 (mg/dL)	127.20±6.57a	80.80±1.78b	85.60±10.28b	88.00±0.01b	100.40±12.19c	113.60±10.01a	91.40±7.60c
Prolactin (ng/mL)	115.20±13.67a	74.60±5.81b	94.20±19.76b	113.00±0.01a	159.40±14.56c	196.40±15.15d	132.20±13.30c
T3 (ng/dL)	126.60±7.10a	129.60±5.14a	126.60±4.40a	114.00±0.01b	134.60±9.55a	102.00±6.12b	128.80±9.06a
T4 (µg/dL)	5.51±0.46a	6.10±0.39b	5.01±0.14a	4.95±0.01a	7.01±0.44b	5.84±0.01a	7.47±0.56b

[a,b,c,d] Different letters indicate significant differences in each row ($P<0.05$).

Table 2- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 4 weeks before parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.721*												
NEFA	-0.642*	0.811*											
TG	-0.118	0.083	0.485										
Cholest	-0.355	0.449	0.642	0.214									
HDL	-0.329	0.047	0.176	0.414	0.252								
LDL	-0.482	0.365	0.138	0.314	0.793	0.567							
VLDL	-0.355	0.449	0.642	1.000*	0.214	0.414	0.314						
Insulin	-0.622*	-0.518*	-0.614*	-0.527	-0.468	-0.190	-0.533	-0.527					
Cortisol	0.581*	0.608*	0.709*	0.612*	0.579*	0.600*	0.730*	0.612*	-0.628*				
IGF-1	0.320	-0.125	-0.018	-0.314	-0.413	-0.313	-0.397	-0.314	0.711*	-0.579*			
Prolactin	0.212	-0.207	-0.347	-0.421	-0.317	-0.287	-0.276	-0.421	0.771*	-0.651*	0.618*		
T3	0.204	0.311	0.317	0.439	0.430	0.368	0.314	0.439	0.368	0.404	0.227	0.300	
T4	0.314	0.361	0.389	0.311	0.415	0.289	0.325	0.311	0.390	0.420	0.232	0.249	0.782*

*statistically significant correlations at P<0.05.

Table 3- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 2 weeks before parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.649*												
NEFA	-0.527*	0.824*											
TG	-0.214	0.178	0.406										
Cholest	-0.208	0.395	0.501	0.308									
HDL	-0.370	0.087	0.152	0.302	0.301								
LDL	-0.397	0.281	0.187	0.297	0.501	0.432							
VLDL	-0.381	0.397	0.432	1.000*	0.303	0.325	0.387						
Insulin	-0.711*	-0.612*	-0.712*	-0.501	-0.322	-0.201	-0.425	-0.501					
Cortisol	0.637*	0.709*	0.809*	0.729*	0.721*	0.781*	0.652*	0.729*	-0.742*				
IGF-1	0.428	-0.267	-0.127	-0.297	-0.311	-0.289	-0.221	-0.297	0.689*	-0.611*			
Prolactin	0.308	-0.281	-0.236	-0.342	-0.410	-0.320	-0.325	-0.342	0.821*	-0.690*	0.720*		
T3	0.297	0.267	0.208	0.441	0.472	0.258	0.401	0.441	0.287	0.342	0.309	0.427	
T4	0.361	0.270	0.328	0.297	0.325	0.297	0.422	0.297	0.301	0.325	0.285	0.329	0.811*

*statistically significant correlations at P<0.05.

Table 4- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 2 weeks after parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.511*												
NEFA	-0.612*	0.742*											
TG	-0.370	0.215	0.381										
Cholest	-0.372	0.281	0.431	0.401									
HDL	-0.298	0.127	0.211	0.192	0.296								
LDL	-0.279	0.211	0.261	0.311	0.316	0.420							
VLDL	-0.231	0.312	0.389	1.000*	0.401	0.309	0.420						
Insulin	-0.851*	-0.712*	-0.697*	-0.496	-0.312	-0.289	-0.394	-0.496					
Cortisol	0.722*	0.742*	0.811*	0.811*	0.902*	0.827*	0.611*	0.811*	-0.804*				
IGF-1	0.429	-0.311	-0.214	-0.310	-0.298	-0.379	-0.314	-0.310	0.728*	-0.639*			
Prolactin	0.311	-0.341	-0.319	-0.397	-0.501	-0.490	-0.389	-0.397	0.732*	-0.710*	0.771*		
T3	0.328	0.211	0.247	0.420	0.397	0.359	0.441	0.420	0.220	0.330	0.346	0.527	
T4	0.319	0.296	0.315	0.311	0.306	0.311	0.498	0.311	0.397	0.386	0.239	0.419	0.788*

*statistically significant correlations at P<0.05.

Table 5- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 4 weeks after parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.629*												
NEFA	-0.791*	0.701*											
TG	-0.421	0.230	0.441										
Cholest	-0.332	0.330	0.361	0.381									
HDL	-0.287	0.111	0.321	0.261	0.311								
LDL	-0.311	0.228	0.312	0.322	0.191	0.361							
VLDL	-0.289	0.326	0.299	1.000*	0.401	0.342	0.330						
Insulin	-0.742*	-0.829*	-0.721*	-0.304	-0.232	-0.290	-0.291	-0.304					
Cortisol	0.838*	0.831*	0.794*	0.799*	0.871*	0.714*	0.752*	0.799*	-0.832*				
IGF-1	0.399	-0.441	-0.230	-0.260	-0.305	-0.317	-0.367	-0.260	0.841*	-0.711*			
Prolactin	0.340	-0.421	-0.272	-0.371	-0.441	-0.321	-0.321	-0.371	0.890*	-0.829*	0.793*		
T3	0.333	0.361	0.231	0.431	0.287	0.281	0.411	0.431	0.328	0.401	0.428	0.413	
T4	0.340	0.316	0.399	0.372	0.342	0.298	0.432	0.372	0.297	0.310	0.299	0.490	0.809*

*statistically significant correlations at P<0.05.

Table 6- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 2 months after parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.732*												
NEFA	-0.799*	0.729*											
TG	-0.319	0.241	0.399										
Cholest	-0.318	0.391	0.210	0.429									
HDL	-0.299	0.297	0.366	0.355	0.281								
LDL	-0.328	0.311	0.411	0.411	0.211	0.401							
VLDL	-0.278	0.269	0.301	1.000*	0.399	0.378	0.111						
Insulin	-0.821*	-0.901*	-0.822*	-0.300	-0.411	-0.330	-0.274	-0.300					
Cortisol	0.760*	0.897*	0.891*	0.822*	0.766*	0.822*	0.821*	0.822*	-0.901*				
IGF-1	0.314	-0.561	-0.420	-0.311	-0.300	-0.420	-0.428	-0.311	0.725*	-0.811*			
Prolactin	0.320	-0.430	-0.320	-0.389	-0.501	-0.391	-0.330	-0.389	0.921*	-0.789*	0.820*		
T3	0.371	0.399	0.299	0.367	0.301	0.220	0.211	0.367	0.291	0.361	0.361	0.401	
T4	0.438	0.425	0.346	0.422	0.402	0.311	0.399	0.422	0.303	0.398	0.301	0.306	0.901*

*statistically significant correlations at P<0.05.

Table 7- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 3 months after parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.812*												
NEFA	-0.859*	0.819*											
TG	-0.301	0.311	0.301										
Cholest	-0.299	0.421	0.401	0.339									
HDL	-0.318	0.207	0.311	0.310	0.287								
LDL	-0.381	0.334	0.422	0.429	0.295	0.287							
VLDL	-0.411	0.271	0.391	1.000*	0.310	0.311	0.247						
Insulin	-0.910*	-0.708*	-0.905*	-0.328	-0.415	-0.417	-0.311	-0.328					
Cortisol	0.811*	0.761*	0.882*	0.817*	0.728*	0.792*	0.798*	0.817*	-0.822*				
IGF-1	0.229	-0.751	-0.394	-0.422	-0.308	-0.351	-0.322	-0.422	0.831*	-0.901*			
Prolactin	0.422	-0.384	-0.341	-0.399	-0.510	-0.374	-0.422	-0.399	0.867*	-0.811*	0.851*		
T3	0.412	0.352	0.421	0.408	0.311	0.351	0.328	0.408	0.314	0.369	0.299	0.344	
T4	0.400	0.401	0.411	0.377	0.258	0.387	0.418	0.377	0.408	0.371	0.345	0.402	0.882*

*statistically significant correlations at P<0.05.

Table 8- Correlations among circulating metabolic biomarkers and hormones in Ghezel ewes at 4 months after parturition.

	Glucose	BHBA	NEFA	TG	Cholest	HDL	LDL	VLDL	Insulin	Cortisol	IGF-1	Prolactin	T3
BHBA	-0.778*												
NEFA	-0.729*	0.874*											
TG	-0.314	0.325	0.214										
Cholest	-0.301	0.420	0.316	0.342									
HDL	-0.412	0.334	0.354	0.319	0.364								
LDL	-0.425	0.371	0.366	0.316	0.411	0.361							
VLDL	-0.389	0.411	0.311	1.000*	0.287	0.241	0.271						
Insulin	-0.921*	-0.801*	-0.841*	-0.299	-0.347	-0.369	-0.316	-0.299					
Cortisol	0.878*	0.661*	0.899*	0.911*	0.811*	0.824*	0.881*	0.911*	-0.922*				
IGF-1	0.274	-0.511	-0.411	-0.418	-0.241	-0.325	-0.360	-0.418	0.854*	-0.825*			
Prolactin	0.436	-0.411	-0.301	-0.390	-0.451	-0.361	-0.341	-0.390	0.861*	-0.911*	0.833*		
T3	0.384	0.336	0.447	0.427	0.399	0.304	0.322	0.427	0.224	0.422	0.210	0.311	
T4	0.335	0.478	0.449	0.335	0.281	0.357	0.430	0.335	0.367	0.314	0.341	0.411	0.930*

*statistically significant correlations at P<0.05.

Discussion

Information regarding metabolic profile in different physiological periods of ewes can assist to better monitoring the herd health and productive performance. Furthermore, the relationships among the metabolic parameters can be used to detect the effect of each parameter on another one. Hence, the present study showed the circulating normal values of metabolic parameters at different pre- and post- parturition periods of Ghezel ewes and their relationships with each other.

Several authors recorded plasma glucose concentrations to be higher during lactation than pregnancy in sheep (Balikchi *et al.* 2007; Takarkhede *et al.* 1999). In a study, peak plasma glucose levels were observed on the last day of pregnancy (Charismiadou *et al.* 2000). Serum glucose concentrations were significantly lower at pre-partum period than post-partum and reached the highest level at 21 days post-partum in the Taghipour *et al.'s* study (2010). Their findings may reflect the recovery of feed intake and improving

energy status of the ewe after lambing. Negative energy balance appears to be related to the glucose demands of the fetal-placental unit in pregnant ewes (Bauman & Currie, 1980). The results of the present study on serum glucose concentration were similar to those of Radostits *et al.'s* (2007).

According to the baseline values of circulating insulin and glucose concentrations at different pre and post parturition periods of studied ewes (Table 1), the highest and lowest significant levels of insulin and glucose were detected at 4 months after parturition ($P<0.05$). These findings are justifiable because insulin decreases glucose levels. However, energy demands of the studied animals at this period may be lesser than the other ones. The findings of the current study showed that there was a significant ($p<0.05$) negative correlation between glucose and BHBA, as well as glucose and NEFA in different studied groups. Anoushepour *et al.* (2014) also showed that serum glucose had significant negative correlations with NEFA and BHBA at pre- and post-partum periods. In the study conducted by Raoofi *et al.* (2013), blood glucose levels were negatively correlated with serum NEFA. Production of ketone bodies and NEFA is a result of energy limitations. Hence, reducing glucose can increase the need for energy and BHBA and NEFA are the subsequent byproducts of this event. Therefore, NEFA and BHBA increase following glucose reduction.

Ketone bodies resulted from fats catabolism where blood glucose level did not meet the energy requirements of the body. In the absence of glucose and oxaloacetic acid in Krebs cycle, BHBA increases in serum and result in the presence of ketone body in milk and urine in that called subacute (Lacetera *et al.* 2001) and acute pregnancy toxemia (Radostits *et al.* 2007). Measuring serum BHBA concentrations may serve as a useful method for monitoring the energy status in pregnant ewes (Edmondson & Pugh, 2009).

The increase in serum NEFA may be ascribed to the fact that the deposited fat is used to generate energy for the energy-restricted sheep and fetus and growth of the fetus which increases exponentially during the late pregnancy. The mean serum BHBA concentration for Ramin *et al.*'s study (2005) was 670 µmol/L. The normal values reported for BHBA level in sheep blood were between 700 µmol/L (Robinson, 1980), and 860 µmol/L (Lacetera *et al.*, 2001). But the range of BHBA in Ghezel

ewes was lower than their results (Table 1). Different unique physiological characteristics of this breed may explain this difference. Furthermore, the different rearing conditions such as climate, diet, etc. may be considered as the other probable causes. In the present study, at before and after parturition of ewes, NEFA had a significantly positive correlation with BHBA concentrations, which is in agreement with the other studies (Duehlmeier *et al.* 2011; Raoofi *et al.* 2013). Concerning periparturient BHBA and NEFA fluctuations, contradictory results are reported. More specifically, BHBA and NEFA concentrations of ewes before parturition were higher in comparison with post-partum values in low milk-yielding native Iranian breeds (Moghaddam & Hassanpour, 2008; Raoofi *et al.* 2013). BHBA concentrations were low before parturition and reached peak level on lambing; then, gradually decreased. However, these variations were not statistically significant.

Taghipour *et al.* (2010) revealed that serum cholesterol and triglyceride concentrations gradually decreased during pregnancy in Baloochi sheep and reached low levels after lambing. Some researchers reported that serum cholesterol and triglyceride concentrations to be higher in pregnant compared to non-pregnant sheep (Al- Dewachi, 1999; Hamadeh *et al.*, 1996). Nazifi *et al.* (2002) stated that pregnancy had a significant effect on the serum lipids and cholesterol concentrations of Iranian fat-tailed sheep, as with progression in the pregnancy period there was an increase in the cholesterol and triglyceride concentrations. These variations appear to reflect increased hepatic triglyceride synthesis and VLDL secretion, because of the activities of lipoprotein lipase and hepatic lipase, the enzymes responsible for the catabolism of VLDL and their remnants (Watson *et al.* 1993). In contrast to sheep, cholesterol concentrations are higher in lactating cows than pregnant non-lactating ones (Seifi *et al.* 2007). Negative energy balance is prevalent in dairy cows during early lactation because of the rapid increase in energy demands for milk production (Herdt & Gerloff, 2009). However, negative energy balance appears to be related to the energy demands of the fetal-placental unit in pregnant ewes (Watson *et al.* 1993). Ruminants have an inherently low capacity for synthesis and secretion of VLDL to export TG from the liver (Pullen *et al.*

1989), and a similar capacity to reconvert NEFA back to TG (Graulet *et al.* 1998). Moreover, the rate of production of TGs in the liver increased at the time of parturition (Grum *et al.* 1996).

In this study, positive correlations were found between the cholesterol and lipoproteins, and also between triglyceride, HDL, and VLDL concentrations. In addition, there was a significant negative correlation between the triglyceride and LDL concentration.

In ruminants, volatile fatty acids from the gastrointestinal tract are the major energy source rather than direct sources of glucose. Thus, insulin plays a slightly different role in ruminants vs. non-ruminants (Kahn, 1978). Elevating volatile fatty acid concentrations during lactation can interfere with glucose-induced insulin secretion (Bossaert *et al.,* 2008). Therefore, it has been reported that elevated circulating volatile fatty acid levels are one of the factors that may account for the impaired hepatic insulin extraction in non-ruminants (Lewis *et al.,* 2002). Other researchers also mentioned that both basal concentration of insulin and insulin response to endogenous glucose can be lower in lactating than in dry dairy cows (Sartin *et al.* 1985) and lower in high-yielding lactating cows compared to low yielders (Sartin *et al*. 1988). Faulkner and Pollock (1990) also suggested that the sensitivity of glucose utilization was increased in lactating sheep compared to non-lactating ones. However, Debras *et al.* (1989) reported that the insulin-stimulated glucose utilization above basal levels was greatly impaired during early lactation compared with the dry period in goats; they suggested that a decrease in insulin sensitivity in some insulin-sensitive tissues might occur.

The previous studies demonstrated that the degree of basal hyperinsulinemia was positively correlated with the degree of obesity in ruminants (McCann & Reimers, 1986) and that obese ruminants were insulin resistant (McCann & Bergman, 1988). It was suggested that liver and muscle were the predominant sites of tissue resistance to the glucoregulatory effects of insulin in obese ruminants (McCann & Bergman, 1988) and obese humans (DeFronzo, 1982). It is likely that a greater pancreatic production rate of insulin and the accompanying peripheral hyperinsulinemia are related to the presence of insulin resistance since an increased concentration of insulin could compensate to

some extent for the reduced effectiveness of insulin in target tissues.

The results of the present study showed that the basal levels of insulin at 2 weeks post parturition were significantly lower than the other periods. The lower insulin level may be considered as a compensatory mechanism to activate the lipase for improving the negative energy balance in this period. Insulin was negatively correlated with glucose, NEFA, BHBA and lipid profile in all the studied groups which may reflect the role of insulin to modulate the metabolic pathways. Insulin can lead metabolic pathways to normal conditions, via better utilizing the energy sources.

Cortisol stimulates the gluconeogenesis in the liver by stimulating the breakdown of a substrate, for example promoting lipolysis. By the cortisol action, the insulin-mediated glucose uptake by muscle, adipose tissue and other tissues that use glucose is inhibited; this reduces the use of glucose in the body. Cortisol is necessary for epinephrine, growth hormone and other lipolytic substances stimulate the hydrolysis of stored lipids at maximal rates (Dunlop, 2004). The effect of cortisol on fat metabolism is an increase in cholesterol catabolism and serum concentration of free fatty acids. Hence, the significant positive correlations between serum cortisol and all lipid profile constituents were detected in pre and post parturition periods.

Anoushepour *et al.* (2014) revealed that the activity of cortisol has a strong positive correlation with BHBA ($r=0.831$, $p<0.01$) and NEFA ($r=0.647$, $p<0.01$) and also was negatively correlated with glucose concentration ($r=-0.563$, $p<0.01$). But, in the present study, the significant positive relationship was detected between cortisol and glucose in all studied groups. Hefnawy *et al.* (2010) and Bani Ismail *et al.* (2008) reported the presence of significant positive correlations between BHBA and cortisol concentrations in experimentally pregnant toxemic goats and subclinical pregnancy toxemic goat does, respectively.

Prolactin is a primary component of the galactopoietic complex of hormones. It may affect the lactogenesis in ruminants. Generally, blood concentrations of prolactin in ruminants seem to be effective for maximal milk yield. Some evidence indicated that a 2-hour infusion of prolactin into lactating goats starting 30 minutes after milking may result in a slight (2.6%) but a statistically significant increase in milk yield

(Jacquemet & Prigge, 1991). If prolactin plays a part in partitioning nutrients between body tissue and milk, it was not reflected in the differences in concentration of the hormone in plasma of high and low yielding cows, either during lactation or during the dry period according to a study by Hart (1973). Apparently, the concentration of prolactin in the circulation can be reduced without markedly affecting milk secretion. The administration of exogenous prolactin during lactation has little or no effect on the lactational performance of high yielding dairy cows (Plaut *et al.* 1987). The results of the current study showed that prolactin had negative correlations with NEFA, BHBA, lipid profile and cortisol in all the groups, which may indicate that the concentration of this hormone could decrease at negative energy balance and milk production reduces subsequently at this state.

According to the reports of Wasfi *et al.* (1987), there were no correlations between T4 and T3 and serum cholesterol concentration, which is in agreement with the previous reports in goats (Nazifi *et al.* 2002). In this study, we also showed non-significant positive correlations between T4 and T3 with plasma lipid profile level. An increase in VLDL and LDL is commonly associated with hypothyroidism (Chatterjea, 2004). These correlation coefficients are related to the complex effects of age, gender, nutrition, physiological, and other factors such as lactation and endocrine functions (Eshratkhah *et al.* 2010). Some thyroid gland diseases such as hyperthyroidism decrease serum triglyceride concentration (Ibrahim *et al.* 1984). T4 also had a positive correlation with serum lipid profile which indicates the effects of thyroid hormones on the stimulation of lipid synthesis (Eshratkhah *et al.* 2010).

IGF-1 concentrations were greater in non-pregnant ewes fed with energy-enriched diets (Sosa *et al.* 2006). In the same way, high IGF-1 concentrations were found in well-nourished pregnant ewes (Wallace *et al.* 1999). Furthermore, late gestational under-nutrition of twin-bearing ewes causes a decrease in plasma IGF-1 concentrations at parturition as well as during the late gestation (Kiani *et al.* 2011). IGF-1 is an anabolic hormone and its negative correlations with NEFA, BEBA, lipid profile, and cortisol (as indicators of negative energy balance) at all studied periods, may indicate the suppressive effects of high energy demand conditions on this hormone.

In conclusion, the results of the present study may provide the estimated values of studied parameters at different pre- and post-parturition periods of Ghezel ewes. These values may assist veterinarians to diagnose metabolic abnormalities by determining circulating metabolic parameters. Finally, information regarding the correlations among circulating metabolic parameters can be used to estimate the changing patterns of each metabolic parameter via evaluating another one.

References

Abell, L.L., Levy, B.B., Brodie, BB. & Kendall, FE. (1952). A simplified method for the estimation of total cholesterol in serum and determination of its specificity. J. Biol. Chem. 195, 357–366.

Al-Dewachi, O.S. (1999). Some biochemical constituents in the blood serum of pregnant Awassi ewes. Iraqi J. Vet. Sci. 12, 275–279.

Anoushepour, A., Mottaghian, P. & Sakha, M. (2014). The comparison of some biochemical parameters in hyperketonemic and normal ewes. Eur. J. Exp. Biol. 4, 83-87.

Bani Ismail, Z.A., Al-Majali, AM. & Amireh, F. (2008). Metabolic profiles in goat does in late pregnancy with and without subclinical pregnancy toxemia. Vet. Clin. Path. 37, 434-437.

Bossaert, P., Leroy, J.L., De Campeneere, S., De Vliegher, S. & Opsomer, G. (2009). Differences in the glucose induced insulin response and the peripheral insulin responsiveness between neonatal calves of the Belgian Blue, Holstein-Friesian, and East Flemish breeds. J. Dairy Sci. 92, 4404-4011.

Balikci, E., Yildiz, A. & Gurdogan, F. (2007). Blood metabolite concentrations during pregnancy and postpartum in Akkaraman ewes. Small Rumin. Res. 67, 247–251.

Bauman, D.E. & Currie, W.B. (1980). Partitioning of Nutrients during pregnancy and lactation: A review of mechanisms involving homeostasis and hemeorhesis. J. Dairy Sci. 63, 1514-1529.

Burtis, C.A. and Ashwood, E.R. (1994). Tietz Textbook of Clinical Chemistry, second ed. W. B.

Saunders Com, Philadelphia. pp. 735–888.

Charismiadou, M.A., Bizelis, J.A. & Rogdakis, E. (2000). Metabolic changes during the perinatal period in dairy sheep in relation to level of nutrition and breed. I. Late pregnancy. J. Anim Physiol. Anim. Nutr. 84, 61–72.

Chalmeh, A., Pourjafar, M., Nazifi, S., Moomnifar, F. & Mohamadi Cheshmehgol, M. (2016). Relationships among circulating metabolic biomarkers in healthy high-producing Holstein dairy cows in different physiological states. Bulg. J. Vet. Med. 19, 317-327

Chatterjea, M.N. (2004). Text book of biochemistry for dental, nursing, pharmacy students, Jaypee, New Delhi.

Debras, E., orizard, J., Aina, E., Tesseraud, S., Qlampredon, C. & Amal, M. (1989). Insulin sensitivity and responsiveness during lactation and dry period in goats. Am. J. Physiol. 256, 295-302.

DeFronzo, RA. (1982). Insulin secretion, insulin resistance, and obesity. Int. J. Obesity. 6, 73-82.

Dunlop, RH. (2004). Pathophysiology of homeostatic and toxic disorders; In Veterinary Pathophysiology. Dunlop, R.H, Malbert, C.H, (Eds.), Black well Publishing, pp. 478-489.

Duehlmeier, R., Fluegge, I., Schwert, B., Parvizi, N. & Ganter, M. (2011). Metabolic adaptations to pregnancy and lactation in German Black-headed Mutton and Finn sheep ewes with different susceptibilities topregnancy toxaemia. Small Rumin. Res. 96, 178–184.

Edmondson, M.A. & Pugh, DG. (2009). Pregnancy toxemia in sheep and goats. In: Current veterinary therapy food animal practice. Anderson, D.E, Rings, D.M, Saunders, Philadelphia, pp. 144-145.

Eshratkhah, B., Sadaghian, M., Eshratkhah, S., Pourrabbi, S. & Najafian, K. (2010). Relationship between the blood thyroid hormones and lipid profile in Moghani sheep, influence of age and sex. Comp. Clin. Path. 19, 15–20.

Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. (1972).

Estimation of the concentration of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clin. Chem. 18, 499–502.

Faulkner, A. & Pollock, H.T. (1990). Metabolic responses to euglycaemic hyperinsulinaemia in lactating and non-lactating sheep in vivo. J. Endocrinol. 124, 59-66.

Firat, A. & Ozpinar, A. (2002). Metabolic profile of pre-pregnancy, pregnancy and early lactation in multiple lambing Sakiz ewes. 1. Changes in plasma glucose, 3-hydroxybutyrate and cortisol levels. Ann. Nutr. Metab. 46, 57–61.

Ford, E.J., Evans, J. & Robinosn, I. (1990). Cortisol in pregnancy toxemia of sheep. Br. Vet. J. 146, 539–542.

Graulet, B., Gruffat, D., Durand, D. & Bauchart, D. (1998). Fatty acid metabolism and very low density lipoprotein secretion in liver slices from rats and preruminant calves. J. Biochem. 124, 1212-1219.

Grum, D.E., Drackley, J.K., Younker, R.S., LaCount, D.W. & Veenhuizen, J.J. (1996). Nutrition during the dry period and hepatic lipid metabolism of periparturient dairy cows. J. Dairy Sci. 79, 1850-1864.

Hamadeh, M.E., Bostedt, H. & Failing, K. (1996). Concentration of metabolic parameters in the blood of heavily pregnant and nonpregnant ewes. Berl. Munch Tierarztl Wochenschr. 109, 81–86.

Hart, I.C. (1973). Effect of 2-bromo-a-ergocrytine on milk yield and the level of prolactin and growth hormone in the blood of the goat. J. Endocrinol. 57, 179-180.

Hefnawy, A., Shousha, S. & Youssef, S. (2011). Hematobiochemical profile of pregnant and experimentally pregnancy toxemic goats. J. Basic Appl. Chem. 1, 65-69.

Herdt, T.H. & Gerloff, B.J. (2009). Ketosis. In: Current Veterinary Therapy Food Animal Practice. Anderson, D.E, Rings, D.M, (Eds.), Saunders, St. Louis, 5th ed. pp. 141-144.

Ibrahim, RE., Maglad, M.A., Adam, S.E.I., Mirghani, TE. & Wasfi, I.A. (1984). The effect of altered thyroid status on lipid metabolism in Nubian goats. Com. Biochem. Physiol. B. 77, 507–512.

Jacquemet, N. & Prigge, E.C. (1991). Effect of increased postmilking prolactin concentrations on lactation, plasma metabolites, and pancreatic hormones in lactation goats. J. Dairy Sci. 74, 109-114.

Kahn, C.R. (1978). Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism. 12, 1893-1902.

Kiani, A., Nielsen, M.O. & Chwalibog, A. (2011). Late gestational undernutrition alters plasma IGF-1 concentration during subsequent lactation in ewe. Agric. Conspec Sci. 76, 361-364.

Lacetera, N., Bernabucci, U., Ronchi, B. & Nardone, A. (2001). Effects of subclinical pregnancy toxemia on immune response in sheep. Am. J. Vet. Res. 62, 1446.

Lewis, G.F., Carpentier, A., Adeli, K. & Giacca, A. (2002). Disordered fat storage and mobilization in the pathogenesis of insulin resistance and type 2 diabetes. Endocr. Rev. 23, 201-229.

McCann, J.P. & Reimers, T.J. (1986). Effects of obesity on insulin and glucose metabolism in cyclic heifers. J. Anim. Sci. 62, 772-782.

McCann, J.P. & Bergman, E.N. (1988). Endocrine and metabolic factors in obesity, In: Aspects of Digestive Physiology in Ruminants. Dobson, A., Dobson, M.J, (Eds.), Cornell University Press, Ithaca, NY.

McGowan, M.W., Artiss, J.D., Strandbergh, D.R. & Zak, B. (1983). A peroxidase coupled method for the colorimetric determination of serum triglycerides. Clin. Chem. 29, 538–542.

Moghaddam, G. & Hassanpour, A. (2008). Comparison of blood serum glucose, beta hydroxybutyric acid, blood urea nitrogen and calciumconcentrations in pregnant and lambed ewes. J. Anim. Vet. Adv. 7, 308–311.

Nazifi, S., Saeb, M. & Ghavami, S.M. (2002). Serum lipid profile in Iranian fat-tailed sheep in late pregnancy, at parturition and during the postparturition period. J. Vet. Med. A Physiol. Pathol. Clin. Med. 49, 9–12.

Plaut, K., Bauman, D.E., Agergaard, N. & Akers, R.M. (1987). Effect of exogenous prolactin on lactational performance of dairy cows. Domest. Anim. Endocrinol. 4, 279-90.

Pullen, D.L., Palmquist, DL. & Emery, R.S. (1989). Effect on days of lactation and methionine hydroxy analog on incorporation of plasma fatty acids into plasma triglycerides. J. Dairy Sci. 72, 49-58.

Ramin, A.G., Asri, S. & Majdani, R. (2005). Correlations among serum glucose, beta-hydroxybutyrate and urea concentrations in non-pregnant ewes. Small Rumin. Res. 57, 265–269.

Raoofi, A., Jafarian, M., Safi, S. & Vatankhah, M. (2013). Fluctuations in energy-related metabolites during the peri-parturition period in Lori-Bakhtiari ewes. Small Rumin. Res. 109, 64–68.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. & Constable, P.D. (2007). Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, W.B. Saunders Co, London, 10th Ed.

Robinson, J.J. (1980). Energy requirements of ewes during late pregnancy and early lactation. Vet. Rec. 106, 282–284.

Roubies, N., Polizopoulou, Z., Minas, A., & Papasteriadis, A. (2017). A pre- and postpartum study of selected biochemical parameters in ewes for the early detection of pregnancy toxemia. J. Hell. Vet. Med. 54, 11-20.

Sahoo, SS., Patra, R.C., Behera, PC. & Swarup, D. (2009). Oxidative stress indices in the erythrocytes from lactating cows after treatment for subclinical ketosis with antioxidant incorporated in the therapeutic regime. Vet. Res. Commun. 33, 281-290.

Saicic, Z.S., Mijalovic, DN., Nikolic, AL. & Blagojevic, S.M.B. (2006). Effect of thyroxine on antioxidant defense system in the liver of rats of different age. Physiol. Res. 55, 561-568.

Sartin, JL., Cummins, K.A., Kemppainen, R.J., Marple, D.N., Rahe, C.H. & Williams, I.C. (1985). Glucagon,

insulin, and growth hormone responses to glucose infusion in lactating dairy cows. Am. J. Physiol. 248, 108-114.

Sartin, JL., Kemppaben, R.J., Cllmminn, K.A. & Williams, J.C. (1988). Plasma concentrations of metabolic hormones in high and low producing dairy cows. J. Dairy Sci. 71, 650-657.

Seifi, H.A., Gorji-Dooz, M., Mohri, M., Dalirnaghadeh, B. & Farzaneh, N. (2007). Variations of energy-related biochemical metabolites during transition period in dairy cows. Comp. Clin. Path. 16, 253-258.

Sosa, C., Abecia, J.A., Forcada, F., Vinoles, C., Tasende, C., Valares, J.A., Palacin, I., Martin, GB. & Meikle, A. (2006). Effect of undernutrition on uterine progesterone and oestrogen receptors and endocrine profiles during the ovine oestrous cycle. Reprod. Fertil. Dev. 18, 447-458.

Taherpour, N. & Mirzaei, F. (2012). Wool characteristics of crossbred Baghdadi wild ram and Iran native sheep. Agricultural Sciences. 3, 184-186.

Takarkhede, R.C., Gondane, V.S., Kolte, A.Y. & Rekhate, D.H. (1999). Biochemical profile during different phases of reproduction in ewes in comparison to rams. Indian Vet. J. 76, 205–207.

Taghipour, B., Seifi, H.A., Mohri, M., Farzaneh, N. & Naserian, A. (2010). Variations of energy related biochemical metabolites during periparturition period in fat-tailed Baloochi breed sheep. I. J. V. S. T. 2, 85-92.

Watson, T.D.G., Burns, L., Packard, C.J. & Shepherd, J. (1993). Effects of pregnancy and lactation on plasma lipid and lipoprotein concentrations, lipoprotein composition and post-heparin lipase activities in Shetland pony mares. J. Reprod. Fertil. 97, 563-568.

Wallace, J.M., Bourke, D.A., Aitken, R.P. & Cruickshank, M.A. (1999). Switching maternal dietary intake at the end of the first trimester has profound effects on placental development and fetal growth in adolescent ewes carrying single fetuses. Biol. Reprod. 61, 101-110.

Wasfi, I.A., Hafez, A.M., Tayeb, F.M.A., Taher, A.Y., el-Tayeb, F.M.A. & eI-Taher, A.Y. (1987). Thyroid hormones, cholesterol, and triglyceride levels in the camel. Res. Vet. Sci. 42, 418.

West, H.J. (1996). Maternal under-nutrition during late pregnancy in sheep. Its relationship to maternal condition, gestation length, hepatic physiology and glucose metabolism. Br. J. Nutr. 75, 593–605.