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Evaluation on serum glucose, BHB, urea and cortisol concentrations in pregnant ewes

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Summary

A study was conducted to determine the distribution of subclinical pregnancy toxemia by the evaluation of serum glucose, BHB, urea, and cortisol concentrations in pregnant ewes. Eight hundred and nine blood samples were collected from 497 pregnant, 242 lambed, and 70 aborted ewes. The concentrations of BHB, glucose and urea were assessed by spectrophotometer and cortisol by ELISA. Mean urea and BHB concentrations in pregnant ewes were greater and glucose was lower than in lambed and aborted ewes. Cortisol concentrations in pregnant ewes were higher than lambed but lower than aborted ewes. Except for BHB, the mean comparison of parameters showed a significant difference ($P < 0.01$) in that the concentrations were similar in all groups. A negative correlation ($P < 0.05$) in urea concentration was observed between pregnant and lambed ewes. There were correlations ($P < 0.01$) between BHB and glucose in pregnant ewes, BHB and cortisol in lambed ewes and BHB and urea in aborted ewes. Regarding the references, the concentration of BHB > 0.7 mmol/l is considered as subclinical pregnancy toxemia; the distribution and percent of ewes in pregnant, lambed and aborted groups were 61 (12.3%), 17 (7%) and 6 (8.57%), respectively. Similarly, for glucose < 20 mg/dl were 76 (15.3%), 38 (15.7%), 10 (14.3%) and for cortisol > 52 ng/ml were 28 (8.54%), 1 (0.6), 5 (10.2%), respectively. Significant differences were found for the BHB and cortisol groups. The highest subclinical pregnancy toxemia occurred among pregnant and aborted ewes. Mean comparison of parameters in the group with BHB > 0.7 mmol/l showed a significant difference ($P < 0.01$) for urea and cortisol concentrations and in the group of glucose < 20 mg/dl and cortisol > 52 ng/ml showed differences only for the BHB concentration. Thus it is concluded that hypoglycemia, uremia and high cortisol concentration in late pregnancy could be considered as subclinical pregnancy toxemia up to 12% that should be seriously considered in order to support prevention or enact treatment.

Keywords: ewes, BHB, urea, cortisol

Blood glucose, BHB, urea and cortisol concentrations were mainly recommended to diagnose pregnancy toxemia in ewes and does. These substances are the sources of energy, final product of fat, protein metabolisms and stress phenomenon, respectively, which are predisposing factors of pregnancy toxemia. Determination of these parameters and their correlations will facilitate the therapeutic and prevention methods for pregnancy toxemia in pregnant ewes.

Concentrations for blood glucose, ketone bodies, cortisol and urea in non-pregnant ewes were reported 35-45 mg/dl (15), 0.7 mmol/l (17), 10 ng/ml (7) and 6.92 mmol/l (23), respectively. The values were varied according to pregnancy, parturition and lactation (3), stresses (7) and pregnancy toxemia (22). Blood urea in pregnant ewes is higher than lactating and non-pregnant ones (22). Blood glucose in pregnant ewes was lower than non-pregnant and lactating ewes (6). The values for ketone bodies were also different (16), but for cortisol values were not (8). There were correlations between glucose and BHB (16, 22), blood glucose and urea (3) and ketone bodies and urea in pregnancy toxemia (16). Présence of the correlations between parameters in pregnancy toxemia not only show their diagnostic importance, but also creates essential attention in the priority of the disorders occurs in this disease. Schlumbohm and Harmeyer (2003) believe that hyperketonemia occurs simultaneously by hypoglycemia and hypocalcemia. The same results for urea, glucose and cortisol were concluded by others (1, 4, 21).

With the consideration of the reported results and values for relevant parameters either in subclincal or clinical pregnancy toxemia and in order to present an appropriate diagnosis, treatment, prevention and prognosis for pregnancy toxemia in ewes, this study was conducted by following objectives: 1) Determination and comparison of the concentrations of blood gluco-

se, BHB, cortisol, and urea in pregnant, lambed and aborted ewes. 2) Determination of a relationships between intragroup and intergroups. 3) Distribution and percentage of probable subclinical pregnancy toxemia and 4) Determination of priority of parameter abnormalities could happen in subclinical pregnancy toxemia.

Material and methods

Flocks and ewes. A total of 809 blood samples was taken from 497 pregnant ewes (in the last month of pregnancy), 242 lambed ewes (one week after lambing) and 70 aborted ewes that were selected among 145 flocks located at forty five village in Urmia suburbs in 2005. In average five pregnant and three lambed ewes were selected from each herd. If there were aborted ewes in the flocks, blood samples were also collected from them. Blood samples were taking mainly early in the morning when they were in the pen or around the village. They were fed Lucerne and grass hay.

Sample collection procedure. Samples were taken by a five ml disposable syringe from Jugular vein of pregnant, lambed and aborted ewes. Blood were collected in the 10 ml test tubes and were carried immediately to the laboratory centre. Ewes were apparently health with no special clinical signs, aged over one year old and supplementary information as location of the village, breeder name, date of sampling and nutritional program was recorded. Samples were centrifuged at 3000 g for 15 minutes and serum was separated to assess the glucose, urea, BHB and cortisol concentrations. Blood from aborted ewes were tested for Brucellosis and were all negative.

Laboratory tests. Blood glucose (mg/dl), urea (mmol/l) and BHB (mmol/l) concentrations were measured by spectrophotometer (RA-1000, UK) using commercial glucose, urea (Pars Azemon Iran) and BHB (Runbut, UK) kits. Cortisol (ng/ml) was measured by ELISA using commercial cortisol kit (Human, Germany).

Statistical method. SPSS software program (Version 13) and case summaries were used to determine mean, standard deviation and standard error. ANOVA was used for comparison of the mean blood parameters among pregnant, lambed and aborted ewes. Student t-test was carried out for measuring the difference between blood parameters. Pearson correlation test was applied to establish relationship between parameters under study within and between groups. Assuming BHB < 0.7 mmol/l (Robinson 1980, Lacetra et al. 2001), glucose < 20 mg/dl (Ramin et al. 2005) and cortisol < 52 ng/ml (Henze et al. 1998), the distribution and percentage of ewes in each group was determined and analysed by Chi square test to find out the differences among those distributions. Mean and standard deviation of other parameters were measured and compared with ANOVA among those distributions.

Results

Mean ± SE for blood glucose, urea, BHB and cortisol concentrations were shown in fig 1. Mean Urea (fig. 1D) and BHB (fig. 1C) concentrations in pregnant ewes were greater and glucose (fig. 1A) was lower than lambed and aborted ewes. Cortisol concentration (fig. 1B) in pregnant ewes was higher than lambed ewes but lower than aborted ewes.

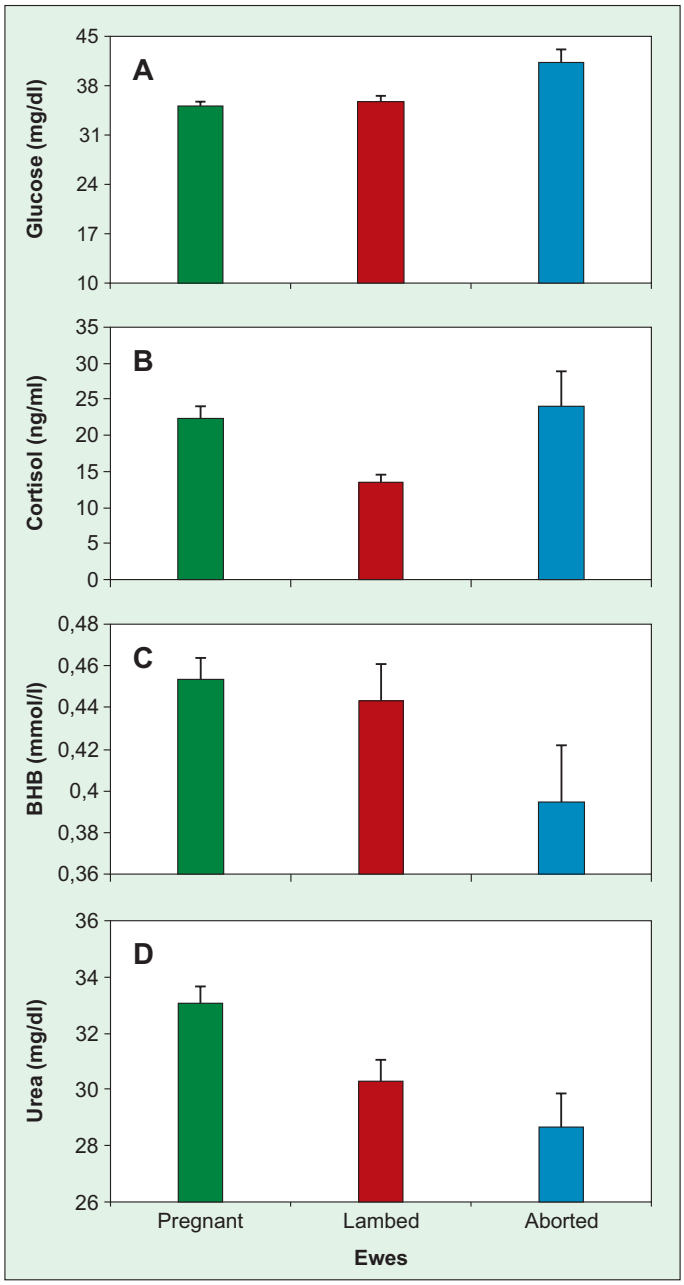

Fig. 1. Mean ± SE of blood parameter concentrations in pregnant, lambed and aborted ewes

Blood parameters showed significant difference ($p < 0.01$) in glucose ($F = 6.9$), urea ($F = 7.3$) and cortisol ($F = 6.7$) concentrations but the difference in BHB among groups was insignificant. The t-test results revealed a difference in glucose concentration between pregnant and lambed ewes ($p < 0.01$) and in urea concentration between pregnant with lambed and aborted ewes ($p < 0.01$). Cortisol concentration in lambed ewes was significantly ($p < 0.01$) lower than aborted and pregnant ewes. BHB concentration demonstrated a significant difference between aborted and pregnant ewes ($p < 0.01$).

Tab. 1. Mean ± SE and frequency (No) of blood parameters in pregnant, lambed and aborted ewes with BHB > 0.7, glucose < 20 mg/dl and cortisol < 52 ng/ml concentrations

Parameters	BHB > 0.7 mmol/l			Glucose < 20 mg/dl			Cortisol < 52 ng/ml		
Ewes	Glucose	Urea	Cortisol	BHB	Urea	Cortisol	Glucose	BHB	Urea
Pregnant	31.8 ± 1.8 (61)	34.2 ± 1.7 (61)	22.2 ± 3.6 (41)	0.5 ± 0.03 (70)	32 ± 1.38 (76)	18.3 ± 3.3 (46)	37.5 ± 2 (28)	0.5 ± 0.1 (28)	35.4 ± 2.8 (28)
Lambed	37.7 ± 3.0 (17)	28.6 ± 2.3 (17)	16.3 ± 4.7 (13)	0.37 ± 0.1 (18)	30 ± 1.75 (17)	17.3 ± 3.1 (13)	52 (1)	1.09 (1)	17 (1)
Aborted	39.5 ± 3.6 (6)	45.2 ± 4.3 (6)	75.0 ± 3.5 (6)	0.37 ± 0.1 (8)	28 ± 3.27 (10)	29.0 ± 10.1 (5)	42.2 ± 8 (5)	0.3 ± 0.1 (5)	34.2 ± 4.3 (5)

Negative correlation was found in urea concentration between pregnant and lambed ewes ($r = –0.14$, $p < 0.05$). There were also correlations between BHB and glucose concentrations in pregnant ewes ($r = –0.16$, $p < 0.01$), between BHB and cortisol concentrations in lambed ewes ($r = 0.25$, $p < 0.01$) and between BHB and urea concentrations in aborted ewes ($r = 0.28$, $p < 0.05$).

If the concentrations of BHB > 0.7 mmol/l, glucose < 20 mg/dl and cortisol > 52 ng/ml considered as subclinical pregnancy toxemia, therefore, the distribution and percentage of ewes in pregnant, lambed and aborted groups was demonstrated in table 1. Chi-Square tests show significant differences for the BHB and Cortisol groups only. The highest subclinical pregnancy toxemia occurs among pregnant and aborted ewes. Mean comparison of parameters in group with BHB > 0.7 mmol/l showed significant difference ($p < 0.01$) for urea and cortisol concentrations and in group of glucose < 20 mg/dl and cortisol > 52 ng/ml showed differences just for BHB concentration (tab. 2).

Tab. 2. The results of ANOVA in ewes with BHB over 0.7 mmol/l, glucose less than 20 mg/dl and cortisol over 52 ng/ml concentrations

Parameters	Glucose		Urea		Cortisol	
	SS	F	SS	F	SS	F
BHB > 0.7	611	1.7	1652	5.6**	5854.7	5.6**
	BHB		Urea		Cortisol	
Glucose < 20	0.29	3.3*	225	0.85	574.8	0.69
	Glucose		Urea		BHB	
Cortisol > 52	249	0.9	327	1.2	0.56	4.8*

Explanation: * = $p < 0.05$, ** = $p < 0.01$

Discussion and conclusion

Regarding to glucose results being high in aborted and low in pregnant ewes approve the fact that continuation of pregnancy aggregates hypoglycemia. The values reported for glucose (20) was in agreement with this study for lambed and aborted ewes, but not in pregnant ones. The value below 20 mg/dl was considered as subclinical pregnancy toxemia (17), means ewes in this study with less than 20 mg/dl must be suspected to subclinical pregnancy toxemia. Blood glucose is known as metabolic profile test, thus, it has distinguishable value in pregnancy toxemia, retarded growth, weight loss, production and reproduction defects (16). It is varied in pregnancy, lactation and non-lactation (3, 6, 20). This variation is related to nutrition, production and reproduction (16). The differences in glucose concentration among pregnant, lambed and aborted ewes reveals the consumption of glucose by fetus and milk yield, so glucose administration before and after parturition results reduction in hypoglycemia and pregnancy toxemia (12).

Mean BHB in pregnant ewes is somewhat more than lambed and aborted ones. The BHB concentration should not be exceed 0.7 mmol/l (17) which is consisted in this study. The increase to 0.86 and 1.6 mmol/l will lead to subclinical and clinical pregnancy toxemia, respectively (4, 10). Hyperketonemia will not appear clinically unless it accompany by hypoglycemia and hyopcalcemia (13, 19). In other words, there is a negative correlation between blood BHB and glucose (15, 22). The signs of Hypoglycemia and hyperketonemia are the cause of abortion, brain defects, pregnancy toxemia and immune depression (10, 11). Some believes to neither correlation (1) nor differences among pregnant, lambed and lactating ewes (3, 7). The highest BHB distribution over 0.7 mmol/l being observe in pregnant ewes could be considered as subclinical pregnancy toxemia. Other factors affecting hyperketonemia include long term starvation, poor nourishment and reproduction disorders (18) in which nutrition was known the important one (22).

The highest urea concentration observed in pregnant ewes. The value was recorded up to 30 mg/dl (16, 23) which was consistent with our results. Urea production rises to 67% during pregnancy and fall to 36% following parturition and lactation (20) as demonstrates in this study. Dehydration (9) and starvation (5) result non-clinical uremia, while diarrhea (9), renal disorders (15) and pregnancy toxemia cause clinical uremia (16, 22). The reason for high urea concentration in pregnant ewes could be related to either high protein metabolism during pregnancy or nutritional management. In this study, in some pregnant and lambed ewes it reach up to 3 and 2 folds respectively which are together with hypoglycemia and hyperketonemia, would be considered as subclinical pregnancy toxemia.

Blood cortisol is recorded up to 10 ng/ml (4, 8) which was inconsistent with the result found in this study. It increases within hundred days of pregnancy, then reduce towards the end of pregnancy and early lactation (3). Cortisol products following pregnancy, abortion, parturition (3), poor nutrition (22) and veterinary handling as it can observe in this study. Henze et al. (1998) reported cortisol up to 52 ng/ml as subclinical pregnancy toxemia, thus some ewes in pregnant and aborted groups are suspected to disease. The differences in blood cortisol among groups underline the rule of stress in pregnancy and abortion. In individual surveys cortisol level in pregnant and aborted ewes rises up to 200 and 160 ng/ml, respectively as observed by Ford et al. (1990) in 80% of ewes with pregnancy toxemia. Cortisol terminates to hypoglycemia, hyperketonemia and uremia in pregnant ewes (4).

Results of correlations among parameters indicate a physiological relationship among hypoglycemia, uremia and cortisol in hyperketonemia. The mechanism could be the substitution of fats and proteins in energy production, results an increase in urea and BHB and depletion of glucose in blood, liver and muscles. These mechanisms are approved in bovine ketosis, pregnancy toxemia, fat cow syndrome and liver lipidosis (14), while, there is no correlation reported between glucose and BHB in non-pregnant ewes (16) but relationship between glucose and urea reports by others (2).

The ultimate objective of this study is to evaluate the rate of subclinical pregnancy toxemia based on the assessment of glucose, cortisol and BHB concentrations as reported in literatures. Urea changes seem to be not specific following disease. The highest distribution of subclinical pregnancy toxemia based on glucose and BHB levels are related to pregnant ewes and based on cortisol level is found in aborted ones. Chi-square result shows high susceptibility of pregnant ewes to subclinical pregnancy toxemia and also reveals that the relevant parameters are BHB and cortisol and then might be glucose and urea. Thus, it can be concluded that subclinical pregnancy toxemia rates in Urmia pregnant and aborted ewes are 12.3% and 10.2%. The glucose concentration in susceptible pregnant ewes declines to subnormal and causes abortion at the rate of 10.2%. Therefore, adjusting carbohydrates toward the end of pregnancy simultaneously monitoring BHB and cortisol tests would reduce the occurrence of subclinical pregnancy toxemia.

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