PLASMA CORTISOL LEVEL AND MAIN METABOLISM EVOLUTION IN PREGNANT EWE

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Abstract

The purpose of this research was to determine the role of glucocorticoid hormones in enhancing metabolic processes characteristic of gestation in ewe. In this respect a group of pregnant ewes was monitored in terms of evolution of plasma cortisol and metabolism during a period of about 14 weeks from the time of pregnancy diagnosis. Parallel determinations were made in a control group consisting of non pregnant ewes. It was determined the evolution of plasma cortisol, plasma glucose, triglycerides, cholesterol, proteins, urea, uric acid, fibrinogen, creatinin, amylase, calcium, phosphorus and magnesium. Results relived progressively increasing of plasma cortisol concentrations in pregnant ewes compared with control. In parallel it was found different evolution of determined blood parameters by comparing with control, meaning different metabolic evolution of the studied parameters in pregnant vs. control ewes. The metabolic evolution of the blood determined parameters in pregnant ewe correlated to an increased secretion of cortisol.

Key words*: cortisol, ewe, pregnancy, metabolism.*

INTRODUCTION

The pregnancy status of a mammalian in marked by a very intensive and varied metabolic dynamic. This metabolic dynamic is monitored by nervous system as well as endocrine system. In addition to protecting and carrying consecrated pregnancy hormones (e.g. progesterone, estrogen), a special place and role they hold other metabolic hormones, including glucocorticoid hormones (Campbell, 1987; Reisman şi Matheny, 1968; Wood, 1988).

MATERIAL AND METHODS

To reach the objectives of this research the following activities were performed: firstly, it was identified a farm which agreed the collaboration in these experiments. Then, a group of pregnant ewes (experimental) and non-pregnant (control) ewes were diagnosed. The experimental and control groups were constituted by ewes of Spanca, Karakul and Tzigae crossed

breed, two years aged, second gestation. The pregnant ewes were diagnosed using an ultrasound method (LOGIQ 100 TM PRO echograph). This device allows the diagnostic of gestation and the number of fetuses. The groups were clinically monitored for the evolution of gestation and health. Both ewe groups, experimental and control were kept in the herd and fed *ad libitum* using an alpha alpha and grass hay based forage.

Blood was sampled for a period of gestation monitoring: 14 weeks from the moment of pregnancy diagnose.

For blood sampling, only p.v.c. EDTA-type anticoagulant vacuutainers were used, tight locked, that were slowly agitated for homogenization. Sampling was done 14 times, at about one week intervals, from November (from the moment when the diagnostic of pregnancy was technically possible) to February.

The tubes were positioned on 45° angle for a fast velocity sedimentation / separation, stored at laboratory temperature, and transported in time for laboratory determinations, which were done no later than two hours from the moment of sample collection.

Determination of serum hydrocortisone was done by an enzymatic method according to Dima Gesellschaft für Diagnostik. Blood biochemical parameters (glucose, total lipids, cholesterol, total protein, urea, uric acid, fibrinogen, creatinin, calcium phosphorus and magnesium and amylase activity) were determined according to Manta *et al*. (1976).

The obtained data were statistically processed and expressed as mean ± standard error of mean of each analyzed group. The differences between the obtained values from the two groups were statistically analyzed by paired Student't test, according to Tacu (1968). The differences between group were considered significant when $P < 0.05$.

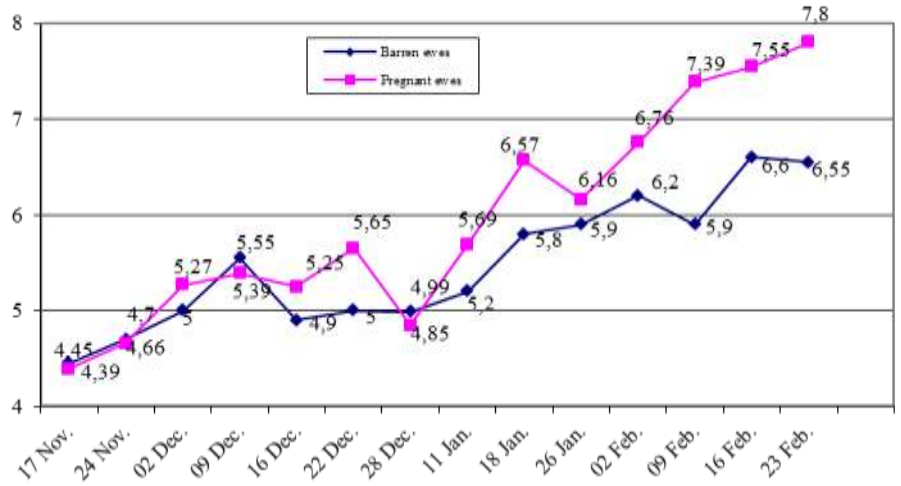
RESULTS AND DISCUSSION

The data presented in Figure 1 show the following: mean plasma cortisol concentrations were not constant over the considered period, ie, November 17, 2009 to February 23, 2010. The values varied between 4.39 and 8.45 ng / mL of plasma in the experimental group (pregnant ewes).

There is a general tendency to increase plasma levels of cortisol, both in the group of pregnant ewes and ewes in the control group (barren, non-pregnant). In this respect it is found that the cortisol levels of November, after installing the latest pregnancy, were located at around 4.45 ng / mL in the control group and 4.39 ng / mL in the experimental group without

statistic significant differences between groups. These values increased to 4.99 ng / mL in the control group and 4.95 ng / mL in the experimental group in mid-December, then to 5.82 ng / ml in the control group and 6.57 ng / mL in the experimental group mid-January.
In mid-February, values of plasma cortisol levels were 6.60 ng / mL in the control group and 7.55 ng / mL in the experimental group. It appears that this growth has characterized both the control group and experimental group. On the other hand it was found a statistic significant difference ($P < 0.05$) between mean plasma cortisol in the experimental group compared with the control, starting from January. This has become a distinct difference statistically significant ($P < 0.01$) in February, after which the mean plasma cortisol of the control group amounted to 6.55 ng / mL, while those in the group of pregnant ewes values were 7.80 ng / mL. Increased plasma cortisol in the group of barren ewes could be explained by the gradual cooling of the weather and switch to maintenance in stalls, which may create some discomfort and stress in a row, that can be reflected in increased cortisol secretion (Damjanovic, 2008).

Figure 1. The evolution of the plasma cortisol concentration in pregnant ewes vs non pregnant ewes along a period of 14 weeks of gestation, from the moment of diagnose (in ng/mL)



For the characterization of metabolic pathways in pregnant ewes, and if this trend is related or not with cortisol level it was monitored the development of the blood plasma concentration of glucose, triglicerides, proteins, urea, uric acid, fibrinogen and amylase. It was also monitored

blood levels of certain minerals: calcium, phosphorus and magnesium. Findings of metabolic pathways of calcium, phosphorus and magnesium were carried out based on monitoring the evolution of these elements in serum. The results are presented in Table 1.

Table 1

The evolution of the main blood biochemical parameters in pregnant ewes vs. non pregnant ewes along a period of about 14 weeks of gestation, from the day of pregnancy diagnostic

Item	Values of reference	Data of blood sampling and the obtained values											
		17 Nov.		09 Dec.		28 Dec.		11 Jan.		26 Jan.		23 Feb.	
		C	P	C	P	C	P	C	P	C	P	C	P
Glucose (mg/dL)	40–80*	54± 21	112± 34a	78± 32	157± 12 a	74± 32	147± 16 a	87± 23	143± 31a	76± 29	132± 43 a	77± 29	132± 33 a
Total lipids (mg/dL)	~300	132± 21	420± 150 a	229± 99	309± 176 a	140± 54	300± 45 a	134± 17	350± 134 a	143± 54	409± 135 a	152± 44	454± 143 a
Cholesterol (mg/dL)	60-150*	98± 32	143± 22	207± 35	232± 43	190± 109	154± 43	132± 31	143± 87	176± 39	219± 86	194± 52	136± 40
Total proteins (g/L)	58-60***	79± 43	165± 71aa	68± 21	113± 15	80± 23	143± 29	87± 28	145± 43	75± 28	140± 54	79± 31	158± 32a
Albumins (g/L)	42% from total***	39± 16	69± 20	30± 6	50± 8	46± 18	60± 21	40± 8	68± 21	36± 19	65± 22	38± 21	77± 21
Globulins (g/L)	58% from total***	40± 12	96± 25	38± 4	63± 14	43± 16	83± 15	47± 16	77± 29	41± 9	85± 32	41± 9	81± 20
Urea (mg/dL)	35	29± 3	44± 10	49± 12	40± 5	24± 20	37± 10	39± 12	46± 12	41± 10	52± 20	34± 11	54± 15

* Normal values in adult ewe, according to Reece, 1996.
** Normal values in adult ewe, according to Kolb, 1974.
*** Normal values in adult ewe, according to Rudas, 1996.
C = Group of control, non-pregnant ewes (n = 8)
P = Experimental, pregnant ewes (n =12)

a P<0,05 (statistic processed vs. control in the same day)
aa P<0,01

The evolution of blood glucose values in the group of pregnant ewes were significantly higher (P <0.05) than non-pregnant ewes throughout the monitoring period, varying between 112 and 145 mg / dL of plasma, maintaining relatively constant higher an showing an increase of the metabolism of this metabolic fuel.

Table 1 (continued)

A similar situation is recorded concerning the plasma total lipids, to whom

Item	Values of refer-ence	Data of blood sampling and the obtained values											
		17 Nov.		09 Dec.		28 Dec.		11 Jan.		26 Jan.		23 Feb.	
		C	P	C	P	C	P	C	P	C	P	C	P
Uric acid (mg/dL)	0,1-2*	0.5± 0.1	4.4± 0.5	2.3± 1.2	3.5± 0.5	1.8± 0,8	1.1± 0.3	1.1± 0.5	3,2± 0,3a	1.5± 0.3	3.3± 1.2	1.5± 0.4	3.9± 0.9a
Fibrinogen (mg/dL)	250-450**	244± 121	232± 65	324± 85	251± 132	386± 174	321± 200	210± 44	238± 39	200± 75	222± 32	236± 213	231± 65
Creatinin (mg/dL)	1-2*	1.0± 0.3	1.2± 0.4	0.4± 0.2	0.9± 0.3	0.8± 0.5	1.5± 0.5	1.8± 0.8	2,0± 1,0	1.1± 0.2	1.8± 0.6	2.0± 0.5	2.5± 1.2
Amylase (mg/dL)	200-500*	230± 76	300± 109	180± 55	180± 76	420± 210	430± 289	350± 210	460± 321	240± 209	360± 210	140± 90	260± 213
Calcium (mg/dL)	4.5 – 6.0*	4.0± 0.3	7.6± 2.1	4.9± 1.5	6.5± 0.5	4.2± 2.2	6.6± 2.6	4.5± 2.2	7.0± 3.0	4.4± 1.6	7.5± 2.3[a]	3.8± 2.7	8.4± 2.2a
Phosphorus (mg/dL)	2 – 7*	2.4± 0.1	3.0± 0.8	2.8± 0.1	3.2± 1.0	23± 0.2	3.1± 0.6	2.5± 1.1	2.5± 0.9	2.9± 1.0	3.1± 1.6	3,0± 1.8	2.9± 2.0
Ca/P	1 – 2	1.6	2.5	1.7	2.0	1.8	2.1	1.8	2.8	1.5	2.4	2.6	2.8
Magne-sium (mg/dL)	1.8 – 2.3*	1.4± 0.7	2.0± 0.4	3.2± 0.7	1.8± 0.2	2.6± 1.7	2.2± 1.0	2.0± 1.1	2.1± 0.6	1.9± 0.7	1.6± 0.5	2.2± 0.6	1.9± 0.5

Legend:

* Normal values in adult ewe, according to Reece, 1996.
** Normal values in adult ewe, according to Kolb, 1974.
*** Normal values in adult ewe, according to Rudas, 1996.
C = Group of control, non pregnant ewes (n = 8)
P = Experimental, pregnant ewes (n =12)
[a] P<0,05 (statistic processed vs. control in the same day)

metabolism was found significantly higher (P <0.05) in pregnant ewes compared with non-pregnant. Lipid levels ranged between 300 ± 45 and 454 ± 143 mg / dL of plasma in pregnant ewes and 124 ± 21 and 152 ± 44 in barren ewes. The high level of lipids illustrates their high tissue depot mobilization, which is due at least in part, to the high levels of plasma cortisol in pregnant ewes, as just it was related.

Pregnant ewe plasma total protein values varied between 113 ± 14 and 165 ± 71 of plasma vs. 68±21 and 100±21 g / L in control (P <0.01). High

concentration of plasma proteins can be partly attributed to high levels of cortisol, but, given the physiological state of advanced pregnancy, probably other factors, maternal or fetal, such as increased protein synthesis capacity of the liver, could modify the metabolism of that large category of substances (Reisman and Matheny, 1998, Leach and Taylor, 2002). On the other hand it should be noted that the albumin / globulin ratio remained relatively constant. A special mention must be done on serum total proteins, which have exceeded in pregnant ewe twice the values in non-pregnant ewes

As a reflection of enhanced protein catabolism under the influence of cortisol it was found elevated serum urea and uric acid in pregnant ewe group, compared with barren ewes. In pregnant ewes, the values of the urea and uric acid concentrations were above the accepted physiological limits: 35 mg / dL urea and 0.1 - 2 mg / dL uric acid.

Concerning the developments of plasma fibrinogen and creatinine in pregnant ewes, they were not found significant differences compared to the group of barren ewes. A similar situation was found in the serum amylase activity whose value did not differ significantly between the group of barren ewes and pregnant ewes, along the entire monitored period.

With regard to calcium metabolism, such as it was presented, in advanced stage of pregnancy, birth and immediately postpartum, metabolism of this chemical element is experiencing a very intense dynamic, calcium (and phosphorus) showing a very high turn-over. According to literature data, glucocorticoid excess (Cushing's disease in the human, for example) causes muscle weakness, atrophy and loss of bone strength and bone matrix (Christiansen *et al*., 2007**;** Johnson *et al*., 1981). With an increased secretion of glucocorticoids, bone formation is reduced and less quantity of calcium is absorbed, but calcium is excreted in higher amount, which is reflected in the evolution of its plasma concentrations, exceeding in some way regulating functional systems for its homeostasis.

For these reasons we started to monitor calcium plasma concentrations together with other minerals (phosphorus and magnesium). Table 1 (continued) shows the evolution of serum calcium, phosphorus and magnesium levels in the group of pregnant ewes compared to non-pregnant ewes on the same monitoring period, from November up to February.

The data analysis presented in Table 1 (continued) shows that serum calcium was significantly higher ($P < 0.05$) for most data we have been done the determinations in the group of pregnant ewes vs. non-pregnant ewes throughout the all monitored period. Also, the Ca / P ratio was significantly

higher in the same batch of pregnant ewes compared with control. Plasma calcium concentration in non-pregnant ewes ranged from 3.8 ± 2.7 and 4.9 ± 1.4 mg / dL of plasma while in the group of pregnant ewes, oscillations ranged from 6.5 ± 0.5 to 8.8 ± 2.0 mg / dL of plasma.

The data analysis presents a constant rise in the concentration of plasma calcium in pregnant ewes, this growing being associated with intensive fetal skeleton growing.

Regarding phosphorus, serum phosphorus concentration is maintained within normal limits, but not so closed limits as those of calcium. In the young lambs, serum phosphorus levels are 3 to 4 times higher than in adult ewes. Also, plasma levels of phosphate shows large diurnal variations with maximal values at night.

In our measurements, the Ca / P ratio was between 1.5 and 2.6 in barren ewes and between 1.8 and 2.8 in pregnant ewes, which indicates increased metabolism of both calcium and phosphorus taken into account, which again can be considered as an effect of high plasma levels of cortisol, which in turn causes a high turn-over of these components.

CONCLUSIONS

During the period of gestation in sheep, progressively increasing plasma cortisol occurs, which indicates involvement of this hormone in the regulation of metabolic processes, exacerbated by the physiological state of pregnancy.

Intensification of metabolic processes such as protein, lipids, carbohydrate, calcium and phosphorus metabolisms during pregnancy may be at least in part attributed to increased secretion of cortisol.

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