Biochemical Changes in Glutathione Redox System and Glucose Regulation in Late Pregnant Ossimi Ewes

Ali Hafez El-Far[*1], Mohamed K. Mahfouz[2] and Hussein A. Abdel maksoud[2]

[1] Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Damanhour Branch (Al-Bostan), Egypt.
[2]Department of Biochemistry, Faculty of Veterinary medicine, Moshtohor, Banha University, Egypt.
[*]aboufares90@yahoo

Abstract: Pregnancy is the more prevalent stress in under feeding small ruminant with multiple bearing. Fifty Ossimi ewes of two years old and their body weight ranging between 35 and 50 kg were allotted into three groups; Group I: contains ten non pregnant non lactating ewes were used as control group. Group II: contains twenty single pregnant ewes* and Group III: contains twenty twin pregnant ewes used as experimental animals. Our study focused on the comparison between single and twin bearing ossimi ewes in the last four weeks of pregnancy and the day of parturition by measurement of reduced glutathione (GSH) level and the activities glutathione peroxidase (GSH-Px); glutathione reductase (GR-ase); glutathione-S-transferase (GST) and total superoxide dismutase (t-SOD) in erythrocytic haemolysate. In addition, glucose, non esterfied fatty acid (NEFA), Beta hydroxyl butyric acid (BHBA), cortisol, insulin and protein electrophoric patterns were measured in serum. Our results concluded that, In erythrocytic haemolysate the mean values of GSH-Px and GST in group II and III during the period of 2nd and last week before parturition and at the day of parturition were high significantly increased. While, GSH and t-SOD were high significantly decreased ($P<0.01$) and GR-ase activities were significantly decreased. While serum insulin level decreased while serum NEFA, BHBA and cortisol were increased in single and twin but in twin the values is more significant. The data showed that twin bearing ewes are more susceptible to pregnancy toxemia than single bearing that may be influence the productivity and performance of those animals.
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1. Introduction:

Pregnancy, parturition, and lactation represent a physiological load to the female body. Where pregnancy toxemia (gestational ketosis) caused by negative energy balance in late gestation is commonly observed in ewes and does (Kulcsar et al, 2006), in beef cows (Rook, 2000), and also in monogastric species as rabbits, guinea pigs, dogs and in ferrets (Lewington, 2007). The background of the disease is the result of the fetal carbohydrate- or energy-demand exceeding maternal supply during the last trimester of pregnancy.

In ruminants, dietary carbohydrates provide well over one half of the energy needs for maintenance, growth and production. Glucose is a primary energy source for certain animal tissues and a precursor for lactose synthesis in the mammary gland. Consequently, understanding carbohydrate digestion and absorption, dietary glucose availability, and the involvement of gluconeogenesis in the regulation of glucose homeostasis is essential for the manipulation of the production and quality of agricultural foods (Rafael and Donald, 2007). Lipid digestion in ruminants is unique in that after ingestion feed lipids are placed into a hydrolytic and reductive environment. The result is that glycerol from triacylglycerols and phospholipids are fermented to VFA and those unsaturated fatty acids which are hydrogenated to mostly saturated fatty acids before absorption (Van Saun, 2000). In ewes, number of fetuses plays role in keeping the homeostasis. The last trimester of pregnancy is very demanding for that homeostasis, because fetuses gain over half of their weight in this period (Seidal et al., 2006). Pregnancy toxemia is a metabolic disease that commonly affects pregnant ewes with multiple fetuses and does during late gestation. It is characterized by hypoglycemia, increased concentrations of ketone bodies in the blood and elevated plasma concentrations of free fatty acids is the result of energy demand exceeding maternal supply during the last trimester of pregnancy (Kulcsar et al., 2006).

The endocrine system especially the pancreas probably is involved in the development of ruminant ketosis. Insulin inhibits ketogenesis when free fatty acids levels are high, as well as growth hormone secretions inhibited by cortisol and free fatty acids. Insulin also appears to be important in regulating the utilization of ketone bodies as the uptake of β-hydroxybutyrate and acetate (Abd-Elghany et al., 2010).

Cortisol is a regulator of glucose in ruminants, which acts to increase gluconeogenesis from amino acids. In starving ruminants the gluconeogenesis is maintained by elevated levels of glucocorticoids (Azab and Abdel-Maksoud, 1999). In lactating ruminants the rate of hepatic gluconeogenesis and the relative concentrations of glycogenic precursors regulate the level of milk production (Huntington, 1990).

Ketone bodies serve as an alternative fuel for many tissues, but they probably do not or only to a minor extent contribute to energy supply of the fetus (Battaglia and Meschia, 1988). Glucose remains most important metabolite for fetal and placental growth. The ability of the ewe to provide a sufficient amount of glucose to the fetus from dietary sources is limited because about 70 to 75% of the dietary carbohydrate is converted in rumen into nonglucogenic products. The remaining fraction of digestible carbohydrate provides 40 to 60% of the circulating glucose through propionate. During periods of a negative energy balance and increased demand for glucose, up to 23% of the glucose may be synthesized from liberated glycerol from the adipose tissue. Along with this glucogenic precursor, a larger amount of fatty acids is released into circulation that may give rise to an increased rate of ketone body formation (Schlumbohm and Harmeyer, 2004). Our study aimed to investigate carbohydrate and fat metabolic changes in single and twin bearing ossimi sheep.

2. Materials and methods

A. Experimental design

The present study was carried out in field farm of Veterinary medicine, Moshtohor, Banha University. Fifty apparently healthy, multiparous Ossimi sheep, of two years old and their body weight ranging between 35 and 50 kg. All animals were kept at the same environmental and nutritional conditions. All over the experimental period, the ewes were allotted into three groups as following:

Group I: included ten ewes (non pregnant non lactating) were used as control group.

Group II: included twenty single pregnant ewes used as experimental animals.

Group III: included twenty twin pregnant ewes used as experimental animals.

Animals were fed free in feedlot. Concentrate feed mixtures were adjusted to the changing of body weight every two weeks. Concentrate mixtures were given twice daily at 10 a.m. and 2 p.m. while wheat straw was offered (*ad lib*.).

B. Blood samples

The blood samples were collected from jugular vein of all animals in the examined groups in the early morning with one week interval during the last month of pregnancy and the day of parturition. Blood samples were divided into two portions; The first portion was collected in heparinized Tube contained 20 I.U. heparin for one mL blood for preparation of haemololysate by using digitonin and washing by physiological saline according to (Kornburg and Korecker 1955).

Table (1) Chemical and cell wall constituents of feed concentrate mixture and corn stalks (on DM basis)

Items	Feed concentrate mixture ϴ	Wheat straw
Chemical composition		
DM	91.51	93.48
OM	89.64	89.58
CP	14.34	3.26
CF	8.47	40.23
EE	2.24	1.32
NFE	64.59	44.77
ASH	10.36	10.42
Cell wall constituents		
NDF	34.62	78.24
ADF	16.24	54.13
Hemi cellulose	18.38	24.11
NFC*	38.44	6.76

*NFC: Non fibrous carbohydrates= 100 - % (CP+ NDF + EE + ASH) (Calsamiglia et al., 1995).

ϴ Feed concentrate mixture consists of 18% undecorticated cotton seed meal, 4% soybean meal, 36% yellow corn, 36% wheat bran, 3% Vinass, 1.5 % limestone, 1.4% sodium chloride and 0.1% common salts.

This was used for estimation of erythrocytic GSH (Sedlak and Lindsay, 1968); t-SOD (Misra and Fridovich, 1972); GSH-Px (EC: 1.11.1.9) (Chiu et al., 1976); GR-ase (EC: 1.6.4.2) (Bergmayer, 1983); GST (EC: 2.5.1.18) (Vessey and Boyer, 1984). The second one was collected without anticoagulant for obtaining a clear non-hemolyzed serum by centrifugation of the blood sample at 3000 r.p.m for 5 minutes. The clear sera were freshly used for determining of blood glucose (Trinder, 1969), non esterified fatty acid (NEFA) and Beta hydroxyl butyric acid (BHBA) (Duncombe, 1964), Commercial radioimmunoassay kits were used to measure concentration of cortisol and insulin (Tietz, 1968 and Wilson and Miles, (1977). C. Electrophoretic pattern of serum protein by SDS-PAGE which performed according to the method of (Laemmli, 1970). D-Statistical analysis was done by (SAS, 1996).

3. Results

The data presented in (Table 2) revealed a high significant increase (P<0.01) in the mean values of GSH-Px and GST in group II and III during the period of 2nd and last week before parturition and at the day of parturition. In contrast, GSH and t-SOD were high significantly decreased (P<0.01) and GR-ase activities were significantly decreased (P<0.05) at the same period of experiment.

Serum glucose level (Table 3) of single pregnant ewes showed significant (P<0.05) decrease than control during last 3 weeks of pregnancy. But of twin pregnant ewes was decreased significantly (P<0.05) during last 4 weeks of pregnancy.

Concentration of serum non esterified fatty acid (Table 3) of single pregnant ewe showed significant (P<0.05) increase than the control during the last 3 weeks of pregnancy as well as at the day of parturition. But of twin pregnant ewes showed significant (P<0.05) increase during the last 4 weeks of pregnancy. Serum BHBA level (Table 3) of single pregnant ewes showed significant (P<0.05) increase than the control during the last 3 weeks of pregnancy and the day of parturition. And twin pregnant ewes showed significant (P<0.05) decrease than the control during the last 4 weeks of pregnancy and the day of parturition. Serum insulin level (Table 3) of single and twin pregnant ewes showed significant (P<0.05) decrease than the control during the last 4 weeks of pregnancy and the day of parturition.

Serum cortisol level (Table 3) of single and twin pregnant ewes showed significant (P<0.05) increase than the control during the last 2 weeks of pregnancy as well as the day of parturition.

The electrophoretic pattern of serum protein revealed that albumin, alpha (α)-1-globulin, alpha (α)-2-globulin and gamma (γ) globulin of single pregnant ewes (Table, 4 and Figure, 1) were significantly decreased during the last week of pregnancy and the day of parturition. But, the concentration of serum beta (β) globulin showed significant (P<0.05) decrease during the last week of pregnancy as well as the day of parturition.

Table (2): Mean values of some biochemical parameters of group I (control), group II (single bearing ewes) and group III (twin bearing ewes)

Duration / Parameters / Groups			GSH (umol/mg protein)	t-SOD (U/g protein)	GSH-Px (U/g protein)	GR-ase (U/g protein)	GST (U/g protein)
Control			0.89 ±0.08	13.01 ±1.11	3.19 ±0.27	0.81 ±0.02	0.41 ±0.03
Gestation period	4th week	Group II	0.71 ±0.11	13.01 0.47±	4.01 ±0.32	0.61 ±0.10	0.59 ±0.01
		Group III	0.81 ±0.12	11.20 ±0.88	4.91 ±0.19*	0.73 ±0.09	0.67 ±0.09
	3rd week	Group II	0.79 ±0.11	10.11 ±0.49	4.19 ±0.31	0.60 ±0.11	0.91 ±0.11*
		Group III	0.77 ±0.09	9.75 ±0.17	6.11 ±0.32*	0.62 ±0.09	1.11 ±0.09*
	2nd week	Group II	0.57 ±0.11*	8.75 ±0.40	7.01 ±0.27*	0.59 ±0.11	1.10 ±.11*
		Group III	0.61 ±0.10*	8.97 ±0.51	7.33 ±0.29*	0.49 ±0.10*	1.25 ±0.21*
	1st week	Group II	0.42 ±0.11**	8.19 ±0.31*	7.41 ±0.11**	0.41 ±0.02*	1.28 ±0.12**
		Group III	0.55 ±0.10**	7.70 ±0.21*	7.51 ±0.31**	0.39 ±0.03*	1.39 ±0.20**
Day of parturition	Group II		0.39 ±0.10**	7.12 ±0.16**	8.31 ±0.70**	0.35 ±0.01*	1.75 ±0.21**
	Group III		0.40 ±0.11**	6.11 ±0.13**	9.55 ±0.29**	0.25 ±0.03*	1.90 ±0.13**

* Indicate significant difference from control at (P<0.05).
** Indicate high significant difference from control at (P<0.01).
GSH (reduced glutathione); t-SOD (total superoxide dismutase); GSH-Px (glutathione peroxidase); GR-ase (glutathione reductase) and GST (glutathione-S-transferase).

Table (3): Mean values of some biochemical parameters of group I (control), group II (single bearing ewes) and group III (twin bearing ewes)

Duration	Parameters / Groups		Glucose (mg/dl)	NEFA (g/dl)	BHBA (µmol/L)	Insulin (µU/dl)	Cortisol (µg/dl)
	Control		66..41 ±0.36	11.53 ±0.22	3.57 ±0.07	2.54 ±0.02	2.04 ±0.04
Gestation period	4th week	Group II	42.88 ±0.85*	29.20 ±0.55*	10.35 ±0.89*	0.78 ±0.03*	3.64 ±0.07*
		Group III	41.56 ±0.70*	32.64 ±1.37*	12.72 ±0.95*	0.67 ±0.06*	3.73 ±0.13*
	3rd week	Group II	44..59 ±0.80*	22.70 ±0.73*	7.85 ±0.29*	0.94 ±0.05*	2.78 ±0.04*
		Group III	43.44 ±0.36*	22.63 ±1.31*	8.92 ±0.84*	0.87 ±0.12*	2.80 ±0.11*
	2nd week	Group II	47.57 ±0.49*	22.68 ±0.43*	7.10 ±0.33*	1.10 ±0.07*	2.32 ±0.06
		Group III	46.48 ±0.46*	21.87 ±0.37*	7.62 ±0.39*	1.17 ±0.03*	2.40 ±0.05
	1st week	Group II	52..29 ±0.53	18.92 ±0.18	6.21 ±0.25*	1.31 ±0.01*	2.04 ±0.02
		Group III	50.30 ±0.47*	19.11 ±0.36*	7.82 ±0.77*	1.29 ±0.006*	2.10 ±0.01
Day of parturition	Group II		40.50 ±0.63*	25.62 ±0.81*	7.52 ±0.32*	1.15 ±0.04*	5.50 ±0.18*
	Group III		40.12 ±0.25*	25.19 ±1.07*	9.36 ±0.27*	1.23 ±0.03*	8.02 ±0.11*

* Indicate significant difference from control at (P<0.05).

Table (4): Mean vales of serum protein fractions (g/dl) in control and twin ewes

The fractions	Control	The last week of pregnancy	The day of parturition
Albumin	3.15 ± 0.06	1.82 ± 0.11*	2.25 ± 0.17*
(α)-1-globulin	0.17 ± 0.004	0.08 ± 0.004*	0.08 ± 0.008*
(α)-2-globulin	0.56 ± 0.008	0.37 ± 0.006*	0.41 ± 0.006*
(β)-globulin	0.87 ± 0.006	0.74 ± 0.01*	0.75 ± 0.01*
(γ)-globulin	2.92 ± 0.01	1.87 ± 0.013*	2.17 ± 0.05*

* Indicate significant difference from control at (P<0.05).

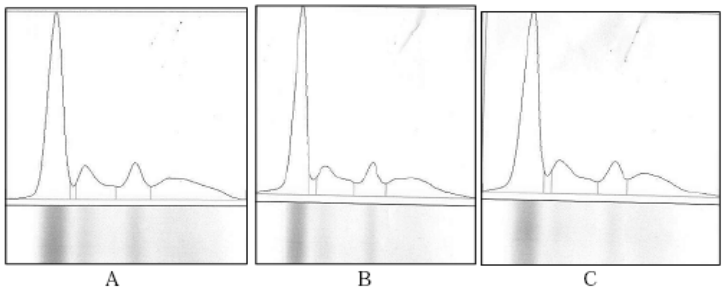

Figure (1): show the electrophoretic serum pattern of Control (A), the last week of pregnancy (B) and the day of parturition (C). In each picture, bands were arranged Albumin, Alpha (α)-1- globulin, Alpha (α)-2- globulin, Beta (β) globulin and Gamma (γ) globulin (From left to right).

4. Discussion

Our study revealed a high significant increase in the mean values of GSH-Px and GST in group II and III during the period of 2nd and last week before parturition and at the day of parturition. Also, showed high significantly decreased in GSH and t-SOD and a significant decrease in GR-ase. This result indicated that t-SOD activity decreased as it is a first line in antioxidant enzymes defense. In the second line, GSH-Px and GST were consuming GSH as a reductant cofactor. For that reason GSH-Px and GST activities were increased and GSH level was decreased. In addition, GR-ase activities were decreased because of GR-ase enzyme generates GSH (Mandour and Abou-El-Ela, 1999 and Abdel-Maksoud et al., 2000). As the glutathione assumes pivotal roles in bioreduction, protection against oxidative stress, detoxification of xenobiotics and endogenous toxic metabolites, transport, enzyme activity, and sulfur and nitrogen metabolism. Its biological significance comes from the free sulfhydryl moiety of the cysteine residue and nucleophilic properties. In cells, glutathione mainly exists in the reduced form (GSH), as the oxidized form (GSSG) (Taisuke et al., 2009). Erythrocytes are permanently in contact with potentially damaging levels of oxygen, but their metabolic activity is capable of reversing this injury under normal conditions. Erythrocytes are equipped by many defence systems representing their antioxidant capacity. This protective system includes superoxide dismutase (SOD), catalase (CAT), reduced glutathione, glutathione peroxidase (GPx), glutathione-S-transferase, and glutathione reductase (GR). However, the cellular antioxidant action is reinforced by the presence of dietary antioxidants (Nakbi et al., 2010).

The present study showed that a significant decrease in the mean values of glucose of single and twin that lower plasma glucose levels came in accordance with (Seidal et al., 2006 and Balikci et al., 2007). The observed decrease in serum glucose level may be due to the, negative energy balance increases lipid mobilization, which results in hepatic lipidosis with subsequent impairment of hepatocellular function, glucose deficiency with intermittent hypoglycemia and accumulation of ketone bodies. The hypothesis that cows suffering from stress and/or painful diseases have elevated blood glucose levels due to an increase in serum cortisol (Forslund et al., 2010).

NEFA and BHBA concentrations in single and twin pregnant ewes were significantly increased than control but in twin were more significantly increased; these results were proved by (Nazifi et al., 2002 and Moghaddam and Hassanpour, 2008).

Serum cholesterol level of single and win pregnant ewes showed significant increase than the control during the last 2 weeks of pregnancy as well as at the day of parturition. The high cortisol level inhibits the growth of the axial skeleton in the sheep fetus during the late pregnancy which enhances the parturition process (Fowden et al., 1996).

Serum insulin level of single and twin pregnant ewes showed significant decrease than the control during the last 4 weeks of pregnancy as well as at the day of parturition. The decrease of insulin level may be attributed to negative energy balance which leads to decrease in glucose level and increase the liploysis (Faulkner and Pollock, 1990). The shift of energy metabolism in a catabolic direction is characterized by a wide range of endocrine changes, such as insufficient pancreatic β-cell function with a coinciding increase in insulin resistance.

The data illustrated in table (3) showed significant increase in cortisol level than the control during the last 2 weeks of pregnancy as well as the day of parturition in Single and twin pregnant ewes. This observation may be due to a hypothesis that the known relation between stress and/or painful diseases in high yielding dairy cows and pregnant ewes may be mediated through a concurrent increased cortisol secretion leading to hyperglycaemia (Rohrbach et al., 1999). On the other hand, Forslund et al., (2010) reported significantly low levels of cortisol in Cows with ketonemia (BHBA > 1.5 mmol/l). The significant increase in cortisol and presence of significant negative correlation between plasma glucose concentration and cortisol level and the significant positive relationship with β-hydroxybutyrate may be due to increased adrenal output or to impaired ability of the fatty liver, which was a consistent finding in pregnancy toxemia, to mobilize and excrete the hormone (Ford et al., 1990 and Abd-Elghany et al., 2010).

The concentrations of serum albumin, alpha-1-globulin, alpha-2- globulin, beta globulin and gamma globulin of single pregnant ewes showed significant decrease than the control during the last week of pregnancy as well as the day of parturition. These results may attributed to consequence increase in the mother's basal metabolic rate, the maximal nutrient requirements of the placenta and the growing fetus, together with the transfer of serum albumin, immune globulins, and amino acids from the blood stream to the mammary gland for synthesis of colostrums (Batavani et al., 2006).

5. Conclusion

Late pregnancy in ewes is a very stressful period specially the late period in which in erythrocytic haemolysate the mean values of GSH-Px

and GST were high significantly increased; GSH and t-SOD were high significantly decreased (P<0.01) and GR-ase activities were significantly decreased. While, serum glucose, total protein, albumin, globulin and insulin were decreased while serum NEFA, BHBA and cortisol were increased in single and twin but in twin the values is more significant. Our research results recommended that twin bearing ewes need a special care during pregnancy and after parturition by supplementation of ewes by a demands of appositive energy balance.

Corresponding author
Ali Hafez El-Far
Department of Biochemistry, Faculty of Veterinary Medicine, Alexandria University, Damanhour Branch (Al-Bostan), Egypt.
aboufares90@yahoo

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