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Serum biochemical indicators and leptin alterations associated with some risk factors in Egyptian Baladi ewes

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ABSTRACT

The present study was conducted on 100 Egyptian Baladi ewes, including 80 pregnant ewes and 20 post-parturient ewes. The aim of this study was to evaluate the biochemical and hormonal parameters in ewes during early, mid, and late pregnancy and post-partum conditions as well as in single and twin pregnancies. Additionally, the impact of age and shearing on inflammatory and stress biomarkers was assessed. Serum samples were collected from ewes for assessing the concentration of interleukin -2 (IL-2), interlukin-6 (IL-6), cortisol, GPx, sodium, creatinine, urea, AST, ALT, and leptin. Results showed a significant decrease ($P < 0.05$) in IL-6 with a significant increase ($P < 0.05$) of cortisol in the mid and late stages of pregnancy. In addition, there was a significant increase in GPx during late pregnancy; there was a significant decrease ($P < 0.05$) in serum sodium at mid and late pregnancy, while there was a significant increase in creatinine at late pregnancy compared to early pregnancy. In addition, there was a significant decrease in urea in mid and late pregnancy compared to the postpartum stage. Serum AST and ALT were significantly increased in late pregnancy compared to other groups. The mean values of Gpx and cortisol were significantly increased in ewes carrying twins compared to single fetuses. Furthermore, significant differences were found between young and senile ewes in the mean values of IL-6, cortisol, and urea, with increases observed in pregnant senile ewes. Moreover, the study revealed a significant increase in pregnant ewes after shearing in the mean values of IL2, IL6, cortisol, Gpx, and leptin. In conclusion, the study shows that the biochemical and hormonal profiles of ewes are significantly impacted by pregnancy stage, litter size, age, and shearing. Therefore, improved sheep management and health monitoring are required.

1. INTRODUCTION

Sheep play a pivotal role in supporting the livelihoods of numerous families, particularly in rural areas within the tropics. However, various management practices and physiological processes can significantly impact the well-being and productivity of these animals. Studies have highlighted the detrimental effects of stressful management procedures on the production performance of sheep (Caroprese et al., 2009) .

Stressful conditions can lead to the excessive production of free radicals, resulting in oxidative stress, characterized by an imbalance between oxidants and antioxidants at the cellular level (Piccione et al., 2011) .

Pregnancy induces substantial metabolic changes in ewes to support gestation and fetal development, with increasing nutrient requirements and metabolic stress as gestation progresses (Kandiel et al., 2016). Parturition and the associated metabolic problems are potentially stressful events. One method an animal might express stress is to activate an acute phase response, which is characterized by increased synthesis of acute phase proteins in the liver (Kovac et al., 2011).

Leptin, because of its role in the regulation of energy metabolism, has been implicated in the coordination of maternal adaptations during pregnancy and lactation (Ehrhardt et al.,2001). Previous reports indicated that young and old ewes have distinct metabolic and inflammatory responses during the transition phase. Older multiparous ewes are more susceptible to metabolic stress than younger sheep. A negative energy balance may affect leucocyte function (Greguła et al., 2021). As with age, the blood becomes more inflammatory, with higher levels of TNF-α and IL-6. This also affects the concentration of acute phase proteins. Furthermore, IL-6 plays an important role in the production of acute phase responses that restore the body's homeostasis following infection, inflammation, wounds, or other stressors (Brüünsgaard and Pedersen, 2003).

Twinning was also related to oxidative stress in the feto-placental unit, which may lead to fetal development limitation. These findings emphasize the significance of maternal nutrition, particularly for ewes bearing multiples, and offer up new avenues for nutritional or antioxidant therapies to avoid fetal hypoxia and oxidative stress (Francisco et al., 2018).

Shearing alters oxidative parameters and disrupts the homeostatic balance of sheep, resulting in oxidative stress (Piccione et al., 2011). Shearing can be stressful, resulting in increased cortisol, MDA, IL-6, IL-2, and TNF-α levels,

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reduced GSH-R and CAT levels, and clinical and behavioral abnormalities (Hefnawy et al., 2018).
Cortisol's bidirectional influence on sheep's immune systems is proportional to the size and duration of the stressor. Physiological cortisol levels following acute stress had a detrimental effect on IL-6 secretion by PBMC, resulting in an increase in cell proliferation. Under chronic stress circumstances, the same cortisol concentration had opposing effects, resulting in a decrease in cell proliferation triggered by higher levels of IL-6 and augmented by the coordinated action of IL-6 and IL-10 (Ciliberti et al., 2016).
Thus, the aim of this study was to evaluate changes in certain biochemical indicators and Leptin during different stages of pregnancy as well as in the single and twin pregnancy. Moreover, the impact of age and shearing on inflammatory and risk factors in Baladi ewes.

2. MATERIALS AND METHODS

Ethical approval
The ethics committee of Benha University approved this study with the approval number BUFVTM 09-05-23.

Animals

This study was carried out between January 2023 till January 2024 in a farm at Quliobyia Province on 100 pregnant baladi ewes (20 at early pregnancy, 40 at mid-pregnancy, 20 at late pregnancy, 20 post-partum). The 40 pregnant ewes at mid-pregnancy divided into two groups as follows: The first group (Young) 30 pregnant ewes of age 1- 2 years. The second group (Senile) 10 pregnant ewes of age 5 -7 years.
The first group (young) of 30 pregnant ewes was divided into two groups as follows: The first group consisted of 20 pregnant ewes with a single newborn. The second group, which included twins, consisted of 10 pregnant ewes, as depicted in Figure 1. Data on physiological and hormonal parameters were recorded 5 days before and after shearing on 20 pregnant ewes of single newborns. All the ewes in the study were apparently clinically healthy.

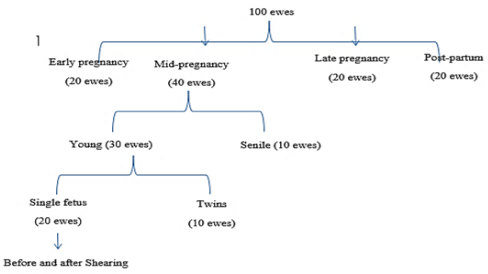

Figure 1 Animal experimental design

Samples
Blood samples (8 mL) were collected from each ewe's vena jugularis and placed in vacuum containers with no anticoagulant (VACUETTE® TUBE 8 ml Z Serum Sep Clot Activator) before being centrifuged at 3000 rpm for 10 minutes. The serum was separated and maintained at -20 °C until analysis time.
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Serum biochemical analysis

Blood samples were frozen at -80°C and tested for IL-2 and IL-6 using an immunoassay (ELISA). Plasma cortisol levels were measured using an ELISA kit (Eucardio Laboratory, Inc., Encinitas, CA 92024, USA). Na concentrations were determined using a flame spectrophotometer (Corning Flame Photometer 410; Corning Medical and Scientific, Corning, NY, USA). Sera were tested using commercially available kits and a UV spectrophotometer to measure urea and creatinine (Slim SEAC model, Florence, Italy). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes and glutathione peroxidase (GPX) were measured at 37°C using an automated analyzer (Synchron CX5 Clinical Systems; Beckman Coulter) using manufacturer-supplied reagents and calibrators.
A solid-phase double-antibody sheep leptin ELISA test kit from SinoGene-clon (Hangzhou, China) was used for the leptin analysis (Krawczyńska et al., 2022).

Statistical Analysis

The statistical analysis was performed using one-way ANOVA and the T-test in SPSS Statistics for Windows, version 25.0. Armonk, NY: IBM Corp. The data were processed as a full randomization design, according to Steel et al. (1997). Multiple comparisons were performed using the Duncan test, with a significance level of $P < 0.05$.

3. RESULTS

Table 1 showed serum biochemical and hormonal indicators in pregnant ewes in different stages in Egyptian Baladi ewes. There was no significant change observed during early, mid, late, and post-partum pregnancy of IL-2. A significant increase in serum IL-6 in early pregnancy ($P < 0.05$). A significant increase in serum cortisol ($P < 0.05$) was observed in mid- and late-pregnancy. Significant increase in GPx ($P < 0.05$) at late pregnancy compared to early, mid, and post-partum pregnancy. Significant decrease in serum sodium (Na+) at mid and late pregnancy ($P < 0.05$) compared to early and post-partum pregnancy, while there was a significant increase ($P < 0.05$) in creatinine at late pregnancy compared to early pregnancy. In addition, there is a significant decrease in urea ($P < 0.05$) in mid and late pregnancy compared to the postpartum stage. Serum AST and ALT were significant increases in late pregnancy ($P < 0.05$) compared to other groups. No significant effect in leptin was observed during early pregnancy and post-partum stages ($p > 0.05$), while a significant increase occurred in mid and late pregnancy.
Table 2 showed serum biochemical and hormonal indicators in single and twin pregnant ewes. A significant increase ($P < 0.05$) was found only between single and twin mean values in Gpx and cortisol, where they were increased in pregnant twins. In addition, there was no significant difference between single and twin pregnancies.

Table (1): Serum biochemical and hormonal indicators in different stages of pregnancy in Egyptian Baladi ewes (mean ± SE).

Parameter	Early (n=20)	Mid (n=40)	Late (n=20)	Post Part (n=20)	p-value
IL2 (pg/ml)	0.87 ± 0.01^{A}	0.82 ± 0.01^{A}	0.83 ± 0.02^{A}	0.84 ± 0.03^{A}	0.322
IL6 (pg/ml)	3.73 ± 0.15^{A}	2.47 ± 0.09^{B}	2.53 ± 0.12^{B}	1.90 ± 0.06^{C}	0.000
Cortisol (µg/dl)	6.07 ± 0.26^{C}	7.90 ± 0.41^{B}	10.20 ± 0.15^{A}	5.87 ± 0.23^{C}	0.000
Gpx (U/ml)	5.97 ± 0.15^{C}	5.37 ± 0.22^{C}	9.25 ± 0.14^{A}	6.93 ± 0.23^{B}	0.000
Na (mmol/L)	140.33 ± 1.20^{A}	127.67 ± 1.76^{B}	121.94 ± 0.24^{C}	139.35 ± 2.59^{A}	0.000
Creat (mg/dl)	0.91 ± 0.02^{B}	1.01 ± 0.07^{B}	1.19 ± 0.03^{A}	0.87 ± 0.04^{B}	0.005
Urea (mg/dl)	39.62 ± 0.85^{A}	34.49 ± 0.78^{B}	27.61 ± 0.39^{C}	40.40 ± 0.84^{A}	0.000
AST (U/L)	52.00 ± 3.61^{C}	57.88 ± 2.40^{B}	67.83 ± 1.58^{A}	45.21 ± 1.34^{C}	0.001
ALT (U/L)	35.46 ± 2.03^{C}	45.90 ± 2.19^{B}	55.07 ± 3.56^{A}	35.39 ± 1.14^{C}	0.001
Leptin (ng/dl)	5.30 ± 0.10^{C}	8.90 ± 0.21^{A}	7.27 ± 0.14^{B}	5.73 ± 0.18^{C}	0.000

Data in table represent mean ± SE. Values with different superscripts letter within the same row were statistically significant at (P<0.05)

Table (2): Serum biochemical and hormonal indicators in single- and twin- pregnant ewes (mean ±SE).

Parameter	Single (n=20)	Twins (n=10)	P-value
IL2 (pg/ml)	0.81±0.01	0.84±0.01	0.477
IL6 (pg/ml)	2.88±0.21	3.07±0.24	0.628
Cortisol (µg/dl)	7.87±0.41	9.77±0.15*	0.012
Gpx (U/ml)	7.10±0.08	8.52±0.23*	0.005
Na (mmol/L)	139.93±4.56	140.23±2.64	0.957
Creat (mg/dl)	1.02±0.07	1.03±0.09	0.873
Urea (mg/dl)	34.39±0.78	34.30±0.76	0.872
AST (U/L)	57.86±2.4	59.33±1.86	0.657
ALT (U/L)	45.8±2.19	47.50±2.36	0.646
Leptin (ng/dl)	8.90±0.21	9.27±0.15	0.222

Data in table represent mean ± SE. *: Significant (P<0.05)

Table 3 illustrated the serum biochemical and hormonal indicators in young and senile pregnant ewes. A significant difference ($P < 0.05$) in the mean values of cortisol, IL-6, and urea was observed between young and senile ewes during mid-pregnancy. These parameters were significantly increased only in pregnant senile ewes.

Table 4 showed serum biochemical and hormonal indicators in shearing before and after shearing in pregnant ewes. There was a significant increase ($P < 0.05$) in IL2, IL6, cortisol, GPx, and leptin after shearing. However, the levels of Na, creatinine, urea, AST, and ALT in the sheared ewes showed no significant differences.

Table (3): Serum biochemical and hormonal indicators in young and senile pregnant ewes (mean ±SE).

Parameter	Young age(n=30)	Senile age(n=10)	P-value
IL2 (pg/ml)	0.82±0.01	0.82±0.03	0.862
IL6 (pg/ml)	2.91±0.21	4.50±0.17*	0.004
Cortisol (µg/dl)	7.85±0.41	9.40±0.31*	0.042
Gpx (U/ml)	7.54±0.33	8.07±0.07	0.230
Na (mmol/L)	139.90±4.56	141.67±3.18	0.771
Creat (mg/dl)	1.01±0.07	1.14±0.11	0.399
Urea (mg/dl)	34.29±0.78	38.00±0.58*	0.022
AST (U/L)	57.78±2.40	59.33±3.76	0.761
ALT (U/L)	45.90±2.19	51.33±1.86	0.132
Leptin (ng/dl)	8.80±0.21	8.57±0.23	0.346

Data in table represent mean ± SE. *: Significant (P<0.05)

Table (4): Serum biochemical and hormonal indicators before and after shearing in pregnant ewes (mean ± SE).

Parameter	Before shearing (n=10)	After shearing (n=10)	P-value
IL2 (pg/ml)	0.55±0.09	1.57±0.15*	0.004
IL6 (pg/ml)	4.53±0.5	12.88±0.72*	0.001
Cortisol (µg/dl)	9.03±0.42	14.22±0.71*	0.003
Gpx (U/ml)	7.00±0.29	11.09±0.3*	0.001
Na (mmol/L)	141.67±1.2	138.00±2.08	0.150
Creat (mg/dl)	1.19±0.03	1.09±0.15	0.583
Urea (mg/dl)	35.05±0.54	30.95±3.04	0.254
AST (U/L)	55.00±2.31	56.70±2.89	0.463
ALT (U/L)	50.33±1.45	47.33±2.6	0.371
Leptin (ng/dl)	7.37±0.09	9.23±0.15*	0.000

Data in table represent mean ± SE. *: Significant (P<0.05)

4. DISCUSSION

Stress is described as an animal's cumulative response to interaction with its environment via receptors; stress-induced impairment of biological activities can jeopardize animal well-being, health, and life (Fidan et al., 2009). To investigate the serum biochemical and hormonal parameters in pregnant ewes in different stages of pregnancy in Egyptian Balladi ewes, our findings showed that IL-2 has no changes in different stages of pregnancy. It has been found that IL-2 influences the immune response by increasing naïve CD4+ T cell development into T helper cells (Liao et al. 2013).

Our findings showed that there was a significant increase in serum IL-6 in early pregnancy ($p < 0.05$). IL-6 has both pro- and anti-inflammatory characteristics and plays a role in immune activation. There is an increase in endometrial IL-6 mRNA, which is a critical component of embryo-uterine interactions and aids in proper conceptus implantation in early pregnant pigs (Zhang et al., 2019). IL-6 is produced by extravillous and cytotrophoblast cells, and it is thought to regulate the invasiveness of embryonic ectoderm cells as well as the roles of extravillous and cytotrophoblast cells, which are required for effective placentation (Jiang et al., 2016). A significant increase in serum cortisol ($P < 0.05$) in mid and late pregnancy. The rise in cortisol concentrations during pregnancy supports the findings of Vaughan et al. (2016), who observed the normal increase in maternal cortisol concentrations in pregnant sheep. This might be linked to the dietary requirements of growing embryos, which aid in the development of fetal organs.

Significant increase in GPx ($P < 0.05$) at late pregnancy, which resulted in oxidative conditions and increased demands for antioxidant enzymes within the reproductive tissues (Fani et al., 2012). Significant decrease in serum sodium (Na^+) at mid and late pregnancy ($P < 0.05$) Regarding the decrease in Na levels, our findings may be explained by the mineralocorticoid impact of glucocorticoids, which leads to Na excretion and K retention. The animals with the highest blood cortisol levels showed the largest fluctuation in the level of these two minerals (Carcangiu et al., 2008).

Significant increase ($P < 0.05$) in creatinine at late pregnancy compared to early pregnancy. Creatinine excretion is largely influenced by urine flow velocity, and amounts higher than normal values have been seen when glomerular filtration rate declines (Ognik et al., 2015). Morsy et al. (2016) observed a mean serum creatinine of 0.49 mg/dL in late-pregnant Santa Inês ewes in Brazil, but it was 0.50 mg/dL in Awassi ewes 21 days pregnant (Gurgoze et al. 2009). In an assay with Sakiz-Awassi crossbreed sheep in early and late pregnancy, the values were higher in Santa Inês hair sheep at late gestation (Nascimento et al., 2015), reaching 0.95 mg/dL; for sheep diagnosed with pregnancy toxemia, serum creatinine concentration was 2.01 mg/dL (Varanis et al., 2021). All of the values reported above were within the physiological parameters indicated in our study.

In addition, significant decrease in urea ($P < 0.05$) in mid and late pregnancy, reduced feed intake in reaction to stress during late pregnancy, as well as hormonal changes during lambing, are related with lower blood urea concentrations (Bahman, 2010). Some scientists, on the other hand, propose that the maximum blood urea concentration occurs during lambing due to lower glomerular filtration rate and urea clearance (Raoofi et al., 2013) and increased protein metabolism during pregnancy (Gurgoze et al., 2009). According to González and Silva (2006), urea increase may also be associated with renal change.

AST and ALT were significant increases in late pregnancy ($P < 0.05$). AST has been widely utilized as a biomarker for hepatic damage. AST is regarded as a less specific diagnostic for liver disease than ALT since it is expressed in various organs such as the brain, cardiac cells, and skeletal muscle cells (Chien, T. L. et al., 2014). The normal amount of serum AST is generally between 8 and 45 units per liter of serum. Serum ALT levels increased considerably during mid-pregnancy as compared to other groups. Serum ALT levels should typically range from 7 to 56 units per liter of serum. However, if the amount of ALT in the patient's blood test exceeds 56 units/liter, this is considered a high level. Serum ALT values are used to measure liver function since elevated AST levels are linked to hepatic fatty infiltration (Lubojacka et al., 2005). (Nascimento et al., 2015) discovered a serum aspartate aminotransferase level of 53.7 U/L in Santa Inês sheep three weeks before lambing, whereas Yokus et al. (2006) found a mean of 46.23 U/L in Sakiz-Awassi crossbred sheep during early pregnancy. In Merino landschaf pregnant ewes on the 20th day before lambing, the serum AST content was (104.87 U/L).

A significant increase in leptin occurred in mid and late pregnancy, indicating its involvement in energy balance and metabolic adaptations. Leptin's function in the body pertains to regulating the balance between food intake and energy expenditure. Leptin is involved in the regulation of immune function and bone metabolism (Chan et al., 2005a; Chan et al., 2005b).

To investigate the serum biochemical and hormonal parameters in single- and twin-pregnant ewes, significant differences were found only between single and twin pregnancies in mean value in Gpx and cortisol, where they increased in pregnant twins. In addition, there was no difference between single and twin pregnancies. Adequate placental antioxidant status is essential for proper placental function and development, and its effectiveness against oxidative stress varies with the stage of placental development in sheep (Garrel et al., 2010). Hargreaves and Hutson, (1990) who reported a high level of cortisol in ewes carrying a single fetus compared to twins.

To investigate serum biochemical and hormonal parameters in young and senile pregnant ewes, a significant increase ($P < 0.05$) in the mean values of cortisol, IL-6, and urea was observed in senile than young ewes during mid-pregnancy; additional analyses were performed. By comparing the results between young and senile ewes, it becomes possible to discern potential age-related significant differences in biochemical and hormonal parameter levels. Furthermore, aging and oxidative stress are thought to be the primary causes of hypothalamic cell damage, including abnormal secretory activity of corticoliberin-producing (CRH) hormones (Vitale et al., 2013). Anterior pituitary dysfunction can impair systemic immune function by causing the adrenal cortex to generate corticosteroids. Corticosteroids have a permissive influence on acute phase reactions. Cortisol can increase the expression of IL-6 receptors in liver cells, boosting APP production (Greguła et al., 2021).

To investigate serum biochemical and hormonal parameters before and after shearing in pregnant ewes, there were significant increases in mean levels of IL2, IL6, cortisol, GPx, and leptin in pregnant ewes after shearing compared to those before shearing. Several studies have shown that sheep's plasma cortisol levels rise in response to stresses such as shearing (Fidan et al. 2009). The current investigation found significant increases in plasma cortisol concentrations following shearing, suggesting that shearing alone is a stressful event. Stressors can also influence the release of pro-inflammatory cytokines IL-2 and IL-6. Cortisol controls cytokine production and activities. The study identified a clear association between plasma cortisol and cytokine concentration (Caroprese et al., 2010). Results showed that significant increases in cortisol, IL-2, and IL-6 levels after shearing were regarded as stressful.

5. CONCLUSIONS

The study shows that the biochemical and hormonal profiles of Egyptian Baladi ewes are greatly influenced by a number of physiological parameters, including age, shearing, litter size, and pregnancy stage. In particular, there were noticeable alterations in the metabolic parameters (sodium, creatinine, urea, AST, ALT, leptin), stress indicators (cortisol), inflammatory markers (IL-2, IL-6), and antioxidant enzyme activity (GPx). The dynamic character of these parameters in response to physiological demands is highlighted by elevated cortisol levels after delivery, elevated GPx in late pregnancy, and elevated leptin levels in twin pregnancies. Furthermore, the impacts of shearing on stress and inflammation, as well as age-related changes in metabolic indicators, highlight how crucial it is to take these factors into account for improved sheep management and health monitoring. Overall, the findings provide valuable insights into the physiological adaptations of ewes, aiding in optimizing breeding and animal care practices.

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