



Association of Cortisol with Litter Size and Lamb Weight in Awassi Sheep

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Abstract | Reproductive performance is influenced by various factors, including litter size, nutrition, and stress. Stress could contribute to economic losses in the sheep farming industry by decreasing the lambing rate and litter size. Therefore, this study investigated the association of cortisol hormone with litter size and lamb weight in Awassi ewes. The study evaluated 100 sexually mature and healthy ewes aged between 3 and 4 years. Body weight and age were recorded for each animal. Serum samples were collected to measure cortisol hormone levels using ELISA kits from ELK Biotechnology. The study revealed that litter size significantly ($P \leq 0.05$) influences the body weight and cortisol hormone levels of Awassi ewes. Twin-born ewes were heavier and had higher cortisol hormone levels, as determined by an ANOVA test. However, they had lower lamb weights at birth and weaning compared to ewes with single births, as indicated by the t-test. The highest Pearson correlation ($P \leq 0.05$) was found between cortisol hormone levels, litter size, and body weight, as confirmed by univariate regression analyses. In conclusion, cortisol hormone levels increased in Awassi ewes with twin births. Study findings reveal a valuable connection between cortisol levels and the economic impacts on sheep farming in terms of litter size and lamb weight. Consequently, cortisol may be a promising candidate for further research in sheep breeding and reproduction to enhance prolificacy.

Keywords | Growth traits, Pregnancy, Sheep, Stress hormone, Twinning rate, Weaning

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INTRODUCTION

Litter size is a critical factor in determining the economic traits of sheep. Increasing the litter size could enhance productivity (Ali *et al.*, 2022; Alkhammas and Al-Thuwaini, 2023). Ewe productivity could be improved by increasing litter size, the numbers of lambs, and lamb weights within a specified period (Duguma *et al.*, 2002; Al-Jumaili

et al., 2023; Bayraktar *et al.*, 2024). A lamb's birth weight is directly correlated with its weaning weight and mature live weight, playing a vital role in sheep production (Kuchtík and Dobes, 2006; Petrovic *et al.*, 2011). The mortality risk for lambs with intermediate birth weights is lower than that for lambs with either small or extremely large birth weights (Piwczyński *et al.*, 2012). Consequently, farmers and animal breeders are keen to understand the factors that

influence litter size and birth weight, as these elements are crucial to their economic viability. Additionally, identifying the factors that affect early weight gain may enhance herd management practices (Mousa *et al.*, 2013).

A variety of factors influence litter size, including hormones, cytokines, enzymes, and growth factors (Al-Jarayan *et al.*, 2023). While cortisol is commonly recognized as the stress hormone, it also serves several other essential functions. Cortisol is vital for the proper functioning of the central nervous system, immune response, and reproductive processes (Zeinstra *et al.*, 2023). According to Gootwine *et al.* (2007), elevated cortisol levels facilitate normal organ development and the timing of birth. This hormone also plays a critical role in regulating blood pressure, blood sugar, and sleep-wake cycles, as well as in the metabolism of fats, proteins, and carbohydrates (Zeinstra *et al.*, 2023). Furthermore, adverse environmental conditions, such as thermal stress, can impact cortisol concentrations in both ewes and lambs during pregnancy (Sathisha *et al.*, 2021). The pregnancy status of the mother may also affect the developing fetus. When an animal carries multiple offspring, competition for nutrients and space occurs within the uterus can lead to increased cortisol levels (Gootwine *et al.*, 2007). Recent research has demonstrated a correlation between cortisol levels and litter sizes in Swifter and Targhee ewes, with postpartum ewes exhibiting higher wool cortisol levels (Zeinstra *et al.*, 2023; Manuel *et al.*, 2024). However, the practicality of using wool hormones as a diagnostic tool for producers may be limited. Alon *et al.* (2021) also found that multi-fetal pregnancies elevate maternal plasma and wool cortisol levels during late pregnancy due to increased metabolic stress in Afec-Assaf ewes.

In livestock animals, however, the cortisol hormone has not yet been linked to litter size and birth weight, and no studies in Awassi sheep have been conducted. An effective study in Awassi ewes can fill in these knowledge gaps and provide valuable insights. The Awassi sheep breed is prevalent in many Middle Eastern countries (Al-Thuwaini and Al-Hadi, 2022). Despite its reputation for thriving in unfavorable conditions, this breed reproduces at a lower rate than other local breeds, such as Karakuls and Assafs (Abd Al-Jabar and Al-Thuwaini, 2024). Middle Eastern breeders are increasingly concerned about the breed's low reproductive capacity, which has become a focal point for breeding efforts. Research questions for investigating cortisol hormone levels in Awassi ewes concerning litter size and lamb weight. It is hypothesized that cortisol levels increase with litter size and ewes with multiple births have higher cortisol levels than single births. Therefore, this study aimed to determine the correlation between cortisol hormone levels with litter size and lamb weight in Awassi ewes, highlighting the significant role of cortisol hormone in sheep reproduction.

SHEEP POPULATION AND PHENOTYPIC MEASUREMENTS

The study was performed between May 2022 and September 2023 at Al-Qasim Green University with approval from the ethics committee (Agri, No. 07, 5, 22). There were 100 ewes of the Awassi breed, aged 3 to 4 years, mature sexually and healthy. Ewes were randomly selected from Karbala station as a subsample from the total population of available ewes. Within the studied flock, 10 to 12 rams were randomly assigned to mate with approximately 20 to 25 ewes per ram. Non-pregnant, pregnant with singleton birth, and pregnant with twin birth were classified as 30, 37, and 33, respectively, after parturition. Study participants included ewes in the fifth month of pregnancy and gave birth in October and early November. A single or twin-bearing ewe was identified by ultrasound on day 40 of gestation. The animals were raised at the Karbala station, situated at a longitude of 32.6027° N, a latitude of 44.0197° E, and 32 m above mean sea level. Semi-open pens designated for housing sheep were distributed among the animals, with 40% being roofed and 60% open. The barn measured 50 m x 20 m. Each barn was equipped with manholes that were 15 and 30 m long, as well as water troughs. The sheep were managed according to a comprehensive program that included feeding, preparation for the feeding season, preparation for pregnancy and childbirth, and health care. The animals were fed the same concentrated feed and had access to fresh water at all times.

The sheep's external jugular vein was punctured with a disposable needle of 18 gauge to collect blood (5 ml). An analysis of hormone levels was made by centrifuging blood for 15 minutes at 2,000 xg and storing the serum at -20°C. The most common methods for quantifying hormones such as cortisol were competitive binding immunoassays, specifically radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). ELISA was preferred due to its high sensitivity and specificity, and it was generally more accurate than other techniques, including RIA. For assessing the precision of an ELISA kit, it was important to consider the coefficients of variation, which were 8% for inter-assay precision and 10% for intra-assay precision (Kinn Rød *et al.*, 2017). An ELISA kit provided by ELK Biotechnology CO., LTD (Wuhan, China) was used to measure cortisol levels (ELK8817). Cortisol sensitivity was 0.93 ng/mL, and the intra- and inter-assay coefficients of variation were 8% and 10%, respectively. The assay range of leptin concentrations was 3.13 ng/mL to 200 ng/mL. A suspended spring balance was used in the morning to measure the weight of Awassi ewes and lamb weight (kg) (Kadhem and Al-Thuwaini, 2022).

DATA ANALYSIS

To analyze the association of cortisol hormone with litter

Table 1: The effect of cortisol on litter size and lamb weight in Awassi ewes.

Indices	Progeny type (LSM $\pm$ SE)			P-value
	Non-pregnant (30)	Pregnant with single (37)	Pregnant with twin (33)	
Ewe live body weight (Kg)	46.60 $\pm$ 1.32 ^c	50.15 $\pm$ 1.12 ^b	52.53 $\pm$ 1.29 ^a	0.01
Age (year)	3.40 $\pm$ 0.24	3.50 $\pm$ 0.11	3.60 $\pm$ 0.13	0.72
Lamb weight at birth (kg)	-	3.98 $\pm$ 0.29 ^a	3.65 $\pm$ 0.49 ^b	0.001
Lamb weight at weaning (kg)	-	22.15 $\pm$ 2.75 ^a	18.95 $\pm$ 2.09 ^b	0.01
Cortisol (ng/ml)	3.73 $\pm$ 0.36 ^c	7.36 $\pm$ 0.48 ^b	9.32 $\pm$ 0.58 ^a	0.005

LSM $\pm$ SE: Least square means $\pm$ Standard error; ^{a,b,c} Significant differences in means represent differences in the same row within each classification.

size and lamb weight in Awassi ewes, SPSS v23.0 was used. To compare the two groups (pregnant with single and pregnant with twin), a student's t-test was used, and a one-way ANOVA was used to identify differences in the measured characteristics for the three groups (non-pregnant, pregnant with single, and pregnant with twin). Bonferroni test with a significance level of 0.05 was used to compare means. The Kolmogorov-Smirnov test was used to determine normality. According to the Kolmogorov-Smirnov statistic, the obtained value was 0.84, indicating that the data were normally distributed. The correlation was analyzed using the Pearson correlation coefficient, and significance was set at $P \leq 0.05$. Logistic regression analysis was used to examine the relationship between cortisol and phenotypic traits.

Table 2: Correlation between cortisol and other variables in Awassi ewes.

Variables	Single		Twin	
	r	P-value	r	P-value
Litter size	0.44	0.03	0.65	0.001
Ewe live body weight (Kg)	0.33	0.05	0.47	0.01
Age (year)	0.03	0.52	-0.12	0.36
Lamb weight at birth (kg)	0.35	0.02	0.41	0.03
Lamb weight at weaning (kg)	0.29	0.01	0.38	0.02

r: correlation coefficient; $P \leq 0.05$: Significant; $P \geq 0.05$: Not significant.

RESULTS AND DISCUSSION

This study examined the relationship between cortisol hormone levels, litter size, and lamb weight in Awassi ewes. The least-squares means of cortisol hormone levels, influenced by the progeny type and lamb weight, are presented in Table 1. The ewe live body weight and cortisol levels of Awassi ewes with twin births significantly higher ($P \leq 0.05$) than those of ewes with single births. Conversely, lamb weight at birth and lamb weight at weaning were significantly greater ($P \leq 0.05$) in Awassi ewes with single births compared to those with twin births. However, the age of the Awassi ewes did not exhibit a significant difference ($P \geq 0.05$). The correlation coefficients between cortisol hormone levels

and the phenotypic characteristics of Awassi ewes are presented in Table 2. The highest and strongly positive correlations ($P \leq 0.05$) were observed between cortisol hormone levels with litter size ($r = 0.65$, $P = 0.001$), ewe live body weight ($r = 0.47$, $P = 0.01$), lamb weight at birth ($r = 0.41$, $P = 0.03$), and lamb weight at weaning ($r = 0.38$, $P = 0.02$), respectively. Univariate regression analyses further investigated the association of cortisol hormone levels with litter size and lamb weight, as shown in Table 3.

Table 3: Logistic regression analysis of cortisol with phenotypic parameters in Awassi ewes.

Univariate logistic regression			Characteristic
P-value	Odds ratio (95% CI)	Estimate	
0.01	2.36 (1.08-5.48)	0.86	Litter size
0.06	1.25 (0.88-4.18)	0.23	Ewe live body weight (Kg)
0.43	1.12 (0.96-3.98)	0.12	Age (year)
0.04	1.66 (1.01-4.32)	0.51	Lamb weight at birth (kg)
0.02	1.97 (1.11-5.14)	0.68	Lamb weight at weaning (kg)

The P-value with statistical significance is indicated in bold numbers; CI: confidence interval.

Litter size plays a crucial role in the economic viability of livestock production, and a comprehensive understanding of the factors influencing reproductive traits in livestock is essential for enhancing these traits (Ajafar *et al.*, 2022; Al-Jaryan *et al.*, 2023). In sheep, litter size is affected by their body weight, with ewes of greater live body weight exhibiting a higher twinning ratio. The weight of ewes serves as a general indicator of litter size, as heavier ewes tend to give birth to more lambs than their lighter counterparts (Al-Thuwain and Al-Hadi, 2022). Furthermore, the maternal weight of the dam influences lamb birth weight; heavier dams are typically better nourished and produce heavier lambs (Torres *et al.*, 2021). Momoh *et al.* (2013) found a significant effect of birth type on both birth weight and weaning weight. According to Aktas *et al.* (2014), birth type significantly impacts the growth of 'White Karaman' Turkish lambs. The current study identified a significant

difference in birth weight and weaning weight for single birth types ($P \leq 0.01$). Consistent with previous studies, lambs born and raised singly are consistently heavier at birth, pre-weaning, and weaning compared to lambs born and raised in multiples (McGovern *et al.*, 2015; McHugh *et al.*, 2017). Raoof (2021) also reports that single-born lambs are heavier at birth and weaning than twin lambs. The low birth weight of twin lambs may be due to competition between twins for nutrients from the placenta and milk from mothers (Fassah *et al.*, 2015). Babar *et al.* (2004) suggest that single births may be superior to their twin counterparts at birth because a single lamb occupies a larger space in the dam's womb and has no competition for nutrients in the womb than twin lambs do. Kramarenko *et al.* (2021) revealed that weight differences between twins and individual lambs reflect competition for milk production and care provided by dams. According to Kenyon *et al.* (2019), fetal growth accelerates during the final 50 days of pregnancy, leading to noticeable variations in feed requirements. Therefore, the study involved ewes in late pregnancy months since lamb growth is more critical during this period.

Additionally, this study showed that ewes with single births had the lowest cortisol concentrations, while ewes with twin births had the highest concentrations. A positive correlation between cortisol hormone and litter size was also revealed using univariate regression analysis. This study's results agree with previous research indicating that higher cortisol levels in wool during pregnancy and after delivery are associated with larger litter sizes (Alon *et al.*, 2021; Zeinstra *et al.*, 2023; Manuel *et al.*, 2024). Ewes experience stress when more lambs are born per ewe (Nurlatifah *et al.*, 2022). Large litters have higher plasma cortisol levels and metabolic stress since their offspring generally weigh less when born (Gootwine *et al.*, 2007). According to Gootwine (2013), ewes with large litters exhibit metabolic conditions similar to those of prolific ewes suffering from malnutrition. Ewes carrying multiple fetuses and experiencing elevated maternal cortisol levels may face adverse effects on fetal growth and metabolism, which can impact maternal metabolic and endocrine levels and alter placental structure (Keller-Wood *et al.*, 2014). In addition to affecting placental growth, cortisol also influences the transport of glucose and amino acids during pregnancy (Alon *et al.*, 2021). Livestock production processes expose animals to a wide range of physical and psychological stresses that can increase cortisol activity, resulting in increased susceptibility to disease, malnutrition, fetal mortality, and reproductive dysfunction (Narayan and Parisella, 2017). Furthermore, twin lambs and their mothers are found to have lower plasma nutrient concentrations in late gestation than single lambs (Gootwine *et al.*, 2007; Momoh *et al.*, 2013), implying that twin lambs are more susceptible to hypoxia and oxidative stress (Sales *et al.*, 2018). Nutritionally stressed ewes produced lambs with significantly lower birth weights

and fewer placental cotyledons than their unstressed counterparts (Meza-Herrera *et al.*, 2015). The effects of heat stress on fetal development are known to be adverse during pregnancy, as heat stress reduces the weight of the placenta by 54%, leading to low fetal weight. Similarly, cattle born to mothers under heat stress delivered smaller calves and weaned smaller calves (Tao *et al.*, 2012).

However, no research has been conducted on ewe cortisol, litter size, and lamb weight in Awassi sheep. In general, Awassi sheep reproduce less than Karakuls and Assaf (Ajafar *et al.*, 2022), despite their ability to survive in harsh conditions (Al-Thuwaini, 2021). The low fertility of most Awassi sheep, however, limits sheep breeding (Chen *et al.*, 2015). Thus, study findings may provide insight into sheep reproductive efficiency, as well as examine the effects of elevated cortisol on fecundity and lamb weight. Although this study is the first to report a relationship between the number of lambs born to ewes and cortisol hormone levels, it has some limitations. One limitation is that we did not measure cortisol levels during early pregnancy and lactation. The results may also be influenced by several factors, including environmental stressors, feed quality, and genetic variations within the sample population. Additionally, the small sample size poses another constraint on the findings. To gain a more comprehensive understanding of the relationship between cortisol hormone with litter size and lamb weight, further research should be conducted with a larger population of Awassi ewes.

CONCLUSIONS AND RECOMMENDATIONS

There was an association of cortisol hormone with litter size and lamb weight in Awassi ewes. A higher maternal cortisol level was associated with lower birth weights and reduced weaning weights in lambs. To promote optimal lamb growth, sheep breeders should ensure that twin-born ewes receive proper nutrition during both pregnancy and the postpartum, while also alleviate the environmental stresses during this time. Determining the association of cortisol hormone with litter size and lamb weight could improve various aspects of animal production and may provide further evidence that the cortisol hormone is a key regulator of reproductive function. However, this conclusion is based on a specific physiological status and a limited sample size. Future studies with larger sample sizes may provide additional insights into the effects of cortisol hormone on reproductive traits, including lambing rate, lambing interval, age at first lambing, and lamb survival rate.

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NOVELTY STATEMENTS

This study investigated the relationship between the number of lambs born to ewes and cortisol hormone levels for the first time. The Awassi ewes with twins exhibited elevated cortisol levels, making them potential candidates for further research in sheep breeding and reproduction.

AUTHOR'S CONTRIBUTIONS

Tahreer M. AL-Thuwaini; Conceptualization, Investigation, Writing - review & editing. Tamadhur H. Hussein; Investigation, Methodology. Layth H. Merzah; Investigation, Methodology. All authors checked and approved the final version of the manuscript for publishing in the *Advances in Animal and Veterinary Sciences*.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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