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INVESTIGATION OF BIRTH TYPE IN AWASSI SHEEP AND THEIR CORRELATION TO REPRODUCTIVE HORMONES AND OXIDATIVE/ANTIOXIDANT INDICES

SUMMARY

Many factors affect reproductive performance, including litter size, season, nutrition, management, oxidative stress, and genetics. Therefore, this study investigated the relationship between number of lambs and oxidative status in Awassi ewes. The present study was conducted between July and September 2022 and included 130 sexually mature, healthy Awassi ewes (57 singletons and 73 twins). The ewes weighed 45-50 kg and were between 3 and 4 years old. The blood samples (5 mL) were collected from the sheep's jugular vein in the one day of birth and continued until the weaning period and were centrifuged for 15 minutes at 2,000 g to separate the serum, which was subsequently stored at -20°C for hormone analysis. Hormonal analysis was performed for measurement of estradiol, progesterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), superoxide dismutase (SOD), malondialdehyde (MDA), and glutathione (GSH). Statistical analysis were employing the general linear model and correlation was analyzed using the Pearson correlation coefficient, and significance was set at $P \leq 0.05$. The results revealed that Awassi ewes at birth and weaning showed a significant higher levels ($P \leq 0.05$) in estrogen (1.37±0.10 and 1.25±0.16 (pg/ml)), progesterone (0.68±0.07 (ng/ml)), FSH (1.13±0.11 and 0.96±0.12 (ng/ml)), LH (0.89±0.12 (ng/ml)), SOD (0.61±0.06 (nmol/L)), glutathione (0.56±0.07 (nmol/L)), and MDA (0.63±0.12 (nmol/L)) in twins birth type. The highest correlations between reproductive hormones and oxidative/antioxidant indices were observed at birth and weaning in Awassi ewes. In conclusion, Awassi ewes exhibit a significant difference in concentrations of reproductive hormones and antioxidant levels at birth and weaning.

Keywords: Antioxidant, Awassi ewes, litter size, pregnancy, reproductive hormones, weaning

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INTRODUCTION

A sheep is a small ruminant that significantly contributes to the protein content of human diets worldwide (Al-Thuwaini and Al-Hadi 2022; Kadhem and Al-Thuwaini 2022). Higher ewe productivity and reproductive performance are profitable for sheep producers (Habtegiorgis *et al*. 2022; Mohammed *et al.* 2022). Reproductive performance is influenced by many factors, including litter size, season, nutrition, management, and genetics (Ajafar *et al.* 2022). Moreover, the interaction between reproduction and oxidative stress affects pregnancy in goats (Celi *et al.* 2010) and sheep (Soriano *et al*. 2015). Oxidative stress happens when the body cannot neutralize the excessive production of pro-oxidants (free radicals) through antioxidant defenses (Lykkesfeldt and Svendsen 2007). The antioxidant defense mechanisms in aerobic biological systems neutralize or minimize the damaging effects of free radicals and reactive oxygen species (Majlesi *et al.* 2021). Catalase, superoxide dismutase (SOD), and glutathione peroxidase (GSH) enzymes are the most studied antioxidants in sheep during pregnancy (Soriano *et al*. 2015).

During pregnancy, changes in the body make mothers more susceptible to oxidative stress (Aköz *et al.* 2017). Physiological regulation in the uterus during pregnancy involves the increased generation of reactive oxygen species (ROS) linked to oocyte maturation, luteolysis, and embryo implantation, all occurring during pregnancy. Placental functions are negatively affected by oxidative stress, which can lead to an adverse pregnancy outcome (Mutinati *et al*. 2013). Oxidative stress may also develop in late pregnancy due to a negative energy balance (Nawito *et al.* 2016). Lipid peroxidation may increase, while antioxidant activity may decrease, contributing to complications (Rejitha and Karthiayini 2014). The peroxidation of lipids could produce various aldehydes, including MDA (Ayala *et al.* 2014). Elevated oxidative stress with increased metabolic demands during pregnancy and lactation correlates with higher blood levels of MDA, which may increase the risk of ewes and lambs mortality (Bhale *et al*. 2014; Fassah *et al.* 2015). Both ewes and lambs are likely to be affected by oxidative stress during pregnancy (Mutinati *et al*. 2013). Consequently, overall antioxidant status prevent reactive oxygen species concentrations from reaching harmful levels that can cause oxidative damage to follicles and oocytes (Gonzalez-Maldonado *et al.* 2018). Antioxidants may also improve endometrial receptivity, decrease levels of ROS, and relieve uterine inflammation (Zheng *et al.* 2022). An experiment was conducted to evaluate the effect of antioxidants on goat reproductive performance in early pregnancy (Fu *et al*. 2022).

Based on the above literature, no publications have investigated the association between oxidative status and litter size in Awassi ewes. The research was conducted to determine the association between the number of lambs born in one birth (twins and singletons) and oxidative status in Awassi ewes, emphasizing the significant role of the oxidative factor on reproductive performance in Awassi ewes.

MATERIAL AND METHODS

Sheep population and phenotypic measurements

An ethics committee approved the study at Al-Qasim Green University in July and September 2022 (Agri, No. 01, 7, 22). This study involved 130 Awassi ewes (57 singletons and 73 twins) that were sexually mature and healthy. The ewes weighed 45-50 kg and were between 3 and 4 years old. The study involved ewes in their fifth month of pregnancy, which gave birth between October and early November. On day 40 of gestation, ultrasounds were used to identify whether the ewe was carrying a single fetus or twins. A real-time B-mode ultrasound scanner equipped with a 3.5 MHz probe (EDAN DUS 6 Vet, China) was used to diagnose pregnancy. Food and water were withheld for 12 hours overnight before scanning early in the morning. Scanning was performed on the fleece-free inguinal region of the animal. The animals were fed 2.5% of their body weight in a grain concentrate consisting of 59% barley, 40% bran, and 1% salt at the Karbala station. Each animal also consumed three kilograms of alfalfa and one kilogram of straw daily, along with grazing once per day. Fresh water was available to all animals at all times. Their veterinary care included vaccination against foot-and-mouth disease, ivermectin treatment to combat parasites, and periodic treatments (Bayraktar *et al.* 2024).

Five milliliters of blood were collected by puncturing the sheep's jugular vein with an 18-gauge disposable needle. The blood samples were drawn at two time points postpartum (three times within a month) before the morning meal administration, and at three time points within a month after weaning the lambs. Hormone level measurements were conducted after centrifuging the blood for 15 minutes at 2,000 g and storing the serum at -20°C. An ELISA kits provided by Biotech Co., Ltd. (Shanghai, Korain) was used to measure the estradiol, progesterone, FSH, LH, SOD, MDA, and GSH (EA0016sh, EA0004sh, E0166sh, E0106sh, E0119Sh, E0039sh, and EA0011sh, respectively). 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).

Data analysis

This study was conducted using SPSS version 23.0, employing the general linear model:

$$Y_{ij} = \mu + L_i + e_{ij}$$

Y_{ij} = characteristics phenotypically, μ = overall mean, L_i = fixed effect of ith birth type (i=1, 2), and e_{ij} = random error associated with Y_{ij} observation and assumed to be NID (0, σ^2e). A significant difference was determined by the Tukey-Kramer test at the (0.05, 0.01) level. Pearson’s correlation coefficient (r) was calculated to quantify the degree of association among various phenotypic traits, indicating the extent to which two traits vary together, with significance set at P≤0.05. Statistical analyses of preliminary data suggest that seasonality, live body weight, parity, and factor interactions do not significantly affect phenotypic traits. Therefore, these variables were excluded from the general linear model.

RESULTS AND DISCUSSION

The effect of birth type on the concentrations of reproductive hormones and oxidative/antioxidant indices for Awassi ewes is shown in Table 1. We observed a significant higher levels (P≤0.01) in twins' birth type for FSH level at birth (1.13±0.11 (ng/ml), and in estrogen (1.25±0.16 (pg/ml)), progesterone (0.68±0.07 (ng/ml)), MDA (0.63±0.12 (nmol/L)), and glutathione (0.56±0.07 (nmol/L)) at weaning. At the same time, there were significant higher levels (P≤0.05) in estrogen (1.37±0.10 (pg/ml)) and SOD (0.61±0.06 (nmol/L)) at birth, and at weaning in FSH (0.96±0.12 (ng/ml)) and LH (0.89±0.12 (ng/ml)) in twin birth type. Whereas no significant difference was observed (P≥0.05) in other indices.

Table 1. Effect of birth type on reproductive hormone and oxidative/antioxidant indices in Awassi ewes

Indices	LSM ± SE		*P*-value
	Twins	Single	
Estrogen at birth (pg/ml)	1.37 ±0.10	1.13 ±0.12	**0.04**
Progesterone at birth (ng/ml)	0.67 ±0.04	0.64 ±0.09	0.41
FSH at birth (ng/ml)	1.13 ±0.11	0.85 ±0.05	**0.001**
LH at birth (ng/ml)	0.94 ±0.08	0.81 ±0.09	0.53
MDA at birth (nmol/L)	0.51 ±0.04	0.41 ±0.03	0.15
SOD at birth (nmol/L)	0.61 ±0.06	0.47 ±0.02	**0.04**
Glutathione at birth (nmol/L)	0.58 ±0.05	0.51 ±0.07	0.23
Estrogen at weaning (pg/ml)	1.25 ±0.16	0.92 ±0.11	**0.003**
Progesterone at weaning (ng/ml)	0.68 ±0.07	0.47 ±0.03	**0.001**
FSH at weaning (ng/ml)	0.96 ±0.12	0.73 ±0.11	**0.02**
LH at weaning (ng/ml)	0.89 ±0.12	0.68 ±0.11	**0.01**
MDA at weaning (nmol/L)	0.63 ±0.12	0.31 ±0.06	**0.003**
SOD at weaning (nmol/L)	0.62 ±0.11	0.50 ±0.08	0.35
Glutathione at weaning (nmol/L)	0.56 ±0.07	0.39 ±0.03	**0.001**

LSM ± SE, Least square means ± Standard error. The P value with statistical significance is indicated in bold numbers. FSH, follicle-stimulating hormone; LH, luteinizing hormone; MDA, malondialdehyde; SOD, superoxide dismutase.

As shown in Table 2, the estimation of the correlation coefficient between reproductive hormones and oxidative/antioxidant indices at birth and weaning was recorded in Awassi ewes. At birth, there was a significant (P≤0.01) positive correlation coefficient between estrogen and progesterone with glutathione, and between FSH and LH with MDA and SOD. On the other hand, estrogen, FSH, and LH had significant correlation (P≤0.05) with MDA, SOD, and glutathione at weaning. Whereas progesterone level had significant (P≤0.05) correlation with glutathione and MDA.

Table 2. Correlation coefficients between reproductive hormones and oxidative/antioxidant indices at birth and weaning

	Variables	**MDA (nmol/L)**		**SOD (nmol/L)**		**Glutathione (nmol/L)**	
		r	*P*-value	r	*P*-value	r	*P*-value
Birth	Estrogen (pg/ml)	0.23	0.45	0.13	0.16	**0.82**	**0.002****
	Progesterone (ng/ml)	0.06	0.21	-0.04	0.62	**0.88**	**0.001****
	FSH (ng/ml)	**0.73**	**0.002****	**0.79**	**0.001****	0.26	0.18
	LH (ng/ml)	**0.65**	**0.006****	**0.75**	**0.001****	0.16	0.43
Weaning	Estrogen (pg/ml)	**0.39**	**0.001****	**0.38**	**0.004****	**0.81**	**0.003****
	Progesterone (ng/ml)	**0.30**	**0.04***	0.21	0.37	**0.93**	**0.001****
	FSH (ng/ml)	**0.85**	**0.001****	**0.91**	**0.001****	**0.31**	**0.04***
	LH (ng/ml)	**0.79**	**0.006****	**0.89**	**0.003****	**0.28**	**0.004****

(P≤**0.05), **(**P≤**0.01), the P value with statistical significance is indicated in bold numbers. FSH, follicle-stimulating hormone; LH, luteinizing hormone; MDA, malondialdehyde; SOD, superoxide dismutase.*

DISCUSSION

Awassi sheep are shown in Table 1 exhibit different reproductive hormones and oxidative/antioxidant indices depending on their litter size. Animal reproductive performance is strongly influenced by litter size (Nowak *et al.* 2020). Ewes capable of producing larger litter sizes tend to have better reproductive performance (Atoui *et al.* 2018). Among the most important economic traits in sheep production is litter size, which is influenced by reproductive hormones (Tesema *et al.* 2020). Additionally, the reproductive hormone of livestock can be used to assess reproductive performance (El Tahawy and Sharkawy 2014). The development and maturation of ovarian follicles are influenced by elevated reproductive hormone levels, leading to larger litter sizes (Luo *et al.* 2018). The results of this study align with the findings of Mohammed *et al.* (2022), which indicated that Awassi ewes with twin lambs exhibited higher levels of sex hormones compared to those with a single lamb. This is also consistent with the research conducted by Kartika *et al.* (2025), which found that Boer goats with multiple births had elevated levels of FSH and LH hormones.

Regarding oxidative status, oxidative stress plays a crucial role in key reproductive functions, including follicular development, oocyte maturation, ovulation, fertilization, and embryo implantation and development (Hardy *et al.* 2021). Spirulina supplementation at 15 g per ewe per day improved the oxidative status of the ewes' bodies and milk (Christodoulou *et al*. 2023). Dietary supplementation with Res enhanced the quality of colostrum in ewes and the growth of lambs, stimulated ovarian steroidogenesis, and improved antioxidant and immune indices across various reproductive stages (Li *et al.* 2024). According to Abd El-Hameed *et al*. (2018), multiple-bearing ewes and does exhibit higher malondialdehyde levels compared to singletons and non-pregnant animals. A study by Abdel-Ghani *et al.* (2016) concluded that elevated MDA levels indicate increased lipid peroxidation, particularly in animals with multiple births. Multiple-bearing animals also showed a significant increase in MDA levels alongside a decrease in total antioxidant activity, superoxide dismutase, and catalase activity, suggesting they may have experienced oxidative stress and lipid peroxidation (Al-Qudah, 2011). Twin pregnancies generate more reactive oxygen species than singleton pregnancies due to increased oxygen demand and heightened lipid peroxidation during gestation, combined with insufficient antioxidant defenses (Abd El-Hameed *et al.* 2018). Oxidative stress in the ovaries restricts the maturation of follicles and oocytes, resulting in fewer and less competent oocytes, as well as abnormally increased levels of follicle-stimulating hormone (Ra *et al.* 2023). Furthermore, higher ROS levels in follicular fluid can cause DNA damage, disruption of cellular scaffolding, and cell membrane damage, leading to abnormal oocyte development and infertility (Chen *et al.* 2023). These findings align with the research conducted by Lee *et al*. (2023) and Lee *et al.* (2024), which indicates that larger litter sizes elevate oxidative stress in sows during late gestation and lactation. However, no publications have examined the effects of reproductive hormones and oxidative/ antioxidant indices on litter size in Awassi ewes.

In terms of correlation analysis, the estimation of the correlation coefficient between hormones and oxidative/antioxidant indices at birth and weaning in Awassi ewes showed significant differences. The result of the current study agreed with the previous study results that explain the close relationship between estrogen and progesterone with the activity of the antioxidant enzymes (SOD, CAT, glutathione peroxidase (GSH-px), glutathione transferase (GSH-tr) and glutathione (GSH)) of cows (Khudhair *et al.* 2021). Additionally, correlations between reproductive hormones and antioxidants revealed positive relationships found between FSH and GSH (0.88; $P<0.05$), and E2 and GSH (0.90; $P=0.04$) in camel females (Zeitoun *et al.* 2022). In contrast, the correlation analysis of study by Cetin *et al.* (2021) indicated that there were no positive or negative correlations between hormones (P4 or E2) and oxidants (MDA) or antioxidants (SOD, GSH-Px, and total GSH activity) during different phases of pregnancy in hairy goats.

Although this study is the first to demonstrate a relationship between lambs born to ewes and oxidative status, it has several limitations. Oxidative status levels were not measured during early pregnancy or lactation, which restricts the scope of the findings. Additionally, various factors such as environmental stressors, feed quality, and genetic variation within the sample population may have influenced the results. The small sample size further limits the generalizability of the conclusions. Therefore, further research involving a larger population of Awassi ewes is necessary to better understand the relationship between oxidative status and litter size.

CONCLUSIONS

Awassi ewes bearing twins are subject to higher oxidative stress and hormonal fluctuations compared to those with single births. The study highlights the importance of antioxidant support during pregnancy, particularly in multiple-bearing ewes, to reduce oxidative damage and support better reproductive outcomes. Providing antioxidant supplements can help mitigate these effects by reducing oxidative stress and supporting hormonal balance. This approach may improve fetal development, increase lamb survival rates, and enhance the overall health of the ewes. Additionally, monitoring stress levels and adjusting nutrition can further optimize reproductive performance in Awassi sheep.

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