Research Article

Association Between Thyroid Metabolic Hormones and Awassi Sheep Reproductive Performance

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Abstract | The thyroid gland in sheep plays a vital role in reproduction by regulating the secretion of reproductive hormones in the hypothalamus, which influences gonadotropin neurons. This study aimed to determine whether thyroid hormones are associated with litter size. A total of 100 healthy, sexually mature ewes, aged 3 to 4 years, participated in the research. The ewes were divided into three groups: non-pregnant ewes (28), pregnant ewes with singletons (42), and pregnant ewes with twins (30). Blood samples were collected from the sheep and analyzed for hormone levels after collection. The findings revealed that lamb weight and hormone levels varied significantly ($P \leq 0.05$) with litter size among both non-pregnant and pregnant ewes. Awassi ewes with twin births exhibited significantly higher ($P \leq 0.05$) follicle-stimulating hormone levels (1.13 ± 0.11 ng/ml) compared to those with single births. Conversely, lamb weights at birth (4.08 ± 0.27 kg) and weaning (22.25 ± 1.41 kg), as well as triiodothyronine (T3) hormone levels (369.40 ± 18.80 pg/ml), were significantly higher ($P \leq 0.05$) in Awassi ewes with single births. Univariate regression analyses confirmed the association between litter size and phenotypic traits in Awassi ewes. In conclusion, Awassi ewes with twin births had lower thyroid hormone levels. These results provide valuable insights into the relationship between litter size, thyroid hormone, and lamb growth. In this context, further investigation into thyroid hormones may enhance understanding of sheep breeding and reproduction.

Keywords | Birth type, Lamb weight, Pregnancy, Reproduction, Sheep, Thyroid hormones

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INTRODUCTION

Thyroid glands are considered one of the three glands essential for nearly all physiological processes in sheep (Chang *et al*., 2023). The primary function of the thyroid gland is to support normal growth and development of the body by producing triiodothyronine (T3) and thyroxine (T4) (De Blasio *et al*., 2006; Sogut *et al*., 2017). These hormones, which are iodine-containing compounds derived from tyrosine, stimulate nuclear receptors (Chang *et al*., 2023). Additionally, they play crucial roles in regulating various physiological functions, including reproductive processes (Colodel *et al*., 2010; Sogut *et al*., 2017). Over the years, research has demonstrated that thyroid hormones regulate seasonal reproductive functions in several species (Colodel *et al*., 2010; Shinomiya *et al*., 2014). Animal reproduction is heavily dependent on thyroid hormones (Sogut *et al*., 2017; Chang *et al*., 2023; Yari *et al*., 2023). The reproductive performance of domestic animals relies on the proper functioning of the thyroid gland and the activity of its hormones (El-Tawab *et al*., 2022). Furthermore, thyroid hormones both stimulate and restrict anterior pituitary hormones, which are responsible for regulating sexual function (Sogut *et al*., 2017). According to Gy *et al*. (2002), thyroid hormones play a role in regulating specific ovarian functions, particularly in postpartum dairy cows. As T3 and T4 stimulate the production of androstenedione, they may indirectly enhance estradiol production by providing essential estrogen precursors to granulosa cells. In sheep, thyroid hormones are crucial for at least one aspect of the endogenous reproductive: The neuroendocrine changes that occur during the transition from anestrus to estrus (Skipor *et al*., 2010).

Research on thyroid hormones has highlighted their critical role in reproduction, seasonal breeding, lactation, and overall health (Todini, 2007; Ayoub *et al*., 2013). The central reproductive system receives input from thyroid hormones, which stimulate the pulsatile release of gonadotropin-releasing hormone (GnRH) (Brown *et al*., 2023). In turn, GnRH prompts the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from gonadotrophs in the anterior pituitary. These hormones act on theca and granulosa cells in the ovary, leading to the production of gonadal steroids and peptides, and promoting ovarian folliculogenesis (Chang *et al*., 2023). In seasonal breeding, thyroid hormones play a crucial role in regulating the expression of endogenous seasonal rhythms in neuroendocrine reproductive activity in sheep (Todini, 2007). During lactation, Crioula Lanada Serrana ewes produce lower levels of thyroid hormones. This reduction in thyroid hormone levels can occur when there is insufficient energy available in the extra-mammary tissues, as the mammary gland consumes a substantial amount of nutrients during lactation (Colodel *et al*., 2010). Furthermore, adult sheep with hypothyroidism exhibit reduced reproductive performance and increased susceptibility to infectious agents (Sipos *et al*., 2004). Despite this, no published research has examined the relationship between litter size and thyroid activity in Awassi sheep. Addressing these knowledge gaps can be addressed through a study of Awassi ewes would provide valuable insights into the species. The Awassi sheep breed is prevalent in Middle Eastern countries (Al-Thuwaini, 2021). Although this breed thrives in harsh conditions, it has a lower reproductive rate compared to other local breeds, such as Karakuls and Assafs (Abd Al-Jabar and Al-Thuwaini, 2024). Middle Eastern breeders are increasingly concerned about its low reproductive capacity. Therefore, this study aimed to investigate serum thyroid hormone levels in twin and singleton Awassi ewes, emphasizing the significant role of thyroid hormones in sheep reproduction.

MATERIALS AND METHODS

Animal and biochemical analyses

Al-Qasim Green University's research committee approved the study conducted between July 2022 and April 2023, which adhered to international standards for animal care and use (Agri, No. 015, 7, 22). This study involved 100 sexually mature ewes, aged between three and four years, with an average weight ranging from 45 to 55 kilograms. The ewes were randomly selected from the Babylon station as a subsample of the total population of available. Within the studied flock, 10 to 12 rams were randomly assigned to mate with approximately 20 to 25 ewes per ram. The ewes were classified as follows: 28 non-pregnant, 42 pregnant with singleton births, and 30 pregnant with twin births. Pregnant ewes were included in the study starting in the fifth month of gestation. A single or twin-bearing ewe was identified on day 40 via ultrasound. The comprehensive management program for the sheep encompassed feeding strategies, planning for the feeding season, preparation for pregnancy and childbirth, environmental conditions, and provision of health care. The animals were fed the same concentrated feed and had access to fresh water at all times.

The external jugular vein of the sheep was punctured with an 18-gauge needle, and 5 ml of blood was collected. The serum was collected by centrifuging the blood for 15 minutes at 2,000 x g and subsequently storing it at -20°C for hormone level analysis. Hormone quantification, including thyroid hormones, was traditionally performed using competitive binding immunoassays, such as radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays (ELISAs). The advantages of ELISA include its high sensitivity, specificity, and overall accuracy compared to other techniques, including RIA. The thyroid and reproductive hormones were measured with using ELISA kits manufactured by SunLong Biotech Co., LTD (Hangzhou, China) (T3: SL000082Sp, T4: SL00015Sp, FSH: SL00019Sp, and LH: SL00069Sp). The weights of Awassi ewes and lambs were measured in the morning using a suspended spring balance (kg) (Kadhem and Al-Thuwaini, 2022). Figure 1 summarizes the experimental design of the current study.

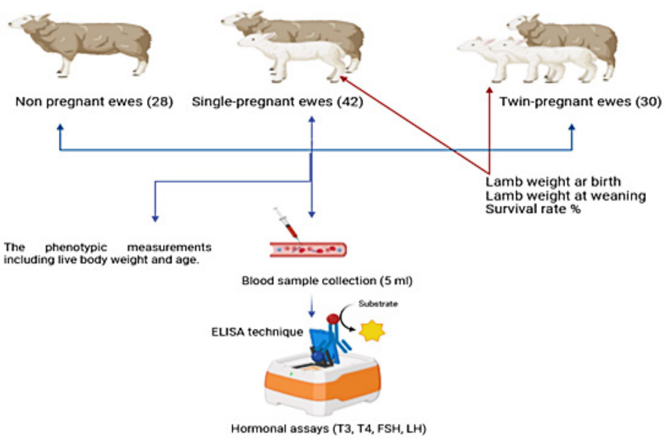

Figure 1: Experimental design. T3: triiodothyronine; T4: thyroxine; FSH: follicle-stimulating hormone; LH: luteinizing hormone.

Table 1: The effect of litter size on productive and reproductive traits in Awassi ewes.

Indices	Progeny type (LSM ± SE)			P-value
	Non-pregnant (28)	Pregnant with a single (42)	Pregnant with twin (30)	
Ewe live body weight (Kg)	45.57 ^{c} ± 1.22	49.42 ^{b} ± 1.30	52.64 ^{a} ± 1.53	0.01
Age (year)	3.53 ± 0.09	3.57 ± 0.08	3.62 ± 0.07	0.64
Lamb weight at birth (kg)	-	4.08 ^{a} ± 0.27	3.69 ^{b} ± 0.81	0.001
Lamb weight at weaning (kg)	-	22.25 ^{a} ± 1.41	19.54 ^{b} ± 1.18	0.04
Survival rate %	-	41 (97.61%)	57(95.00%)	0.27
T3 (pg/ml)	217.76 ^{c} ± 20.76	369.40 ^{a} ± 18.80	260.13 ^{b} ± 22.70	0.02
T4 (ng/ml)	11.03 ± 0.90	10.52 ± 0.39	11.23 ± 0.60	0.46
FSH (ng/ml)	0.89 ^{b} ±0.12	0.85 ^{b} ±0.05	1.13 ^{a} ±0.11	0.03
LH (ng/ml)	0.96 ±0.12	0.94 ±0.08	0.91 ±0.09	0.31

LSM ± SE, Least square means ± Standard error. [a,b,c] Significant differences in means represent differences in the same row within each classification.

Statistical analysis

An analysis of the associations between birth type and phenotypic traits was conducted using IBM SPSS version 23.0 (New York, USA). Student's t-test was employed to compare two groups: Those pregnant with a single fetus and those pregnant with twins. One-way ANOVAs were utilized to identify differences among three groups: non-pregnant individuals, those pregnant with a single fetus, and those pregnant with twins. The Bonferroni test was applied to compare means at a significance level of 0.05. Normality was assessed using the Kolmogorov-Smirnov test, which indicated that the data were normally distributed, with a statistic value of 0.84. During this study, the effects of lambing season, age, and interaction factors were examined, and their nonsignificant effects were excluded.

Table 2: Logistic regression analysis of litter size with phenotypic parameters in Awassi ewes.

Univariate logistic regression			Characteristic
P value	Odds ratio (95% Cl)	Estimate	
0.08	1.41 (0.92-4.20)	0.35	Ewe live body weight (Kg)
0.25	1.09 (0.88-3.74)	0.09	Age (year)
0.42	1.10 (0.96-3.93)	0.10	Survival rate %
0.04	1.58 (1.01-4.60)	0.46	Lamb weight at birth (kg)
0.03	1.37 (1.00-4.14)	0.32	Lamb weight at weaning (kg)
0.01	2.11 (1.08-5.36)	0.75	T3 (pg/ml)
0.11	1.49 (0.91-4.48)	0.40	T4 (ng/ml)
0.01	2.24 (1.08-5.37)	0.81	FSH (ng/ml)
0.31	1.33 (1.01-5.18)	0.29	LH (ng/ml)

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

RESULTS AND DISCUSSION

This study examined the associated progeny type and thyroid metabolic hormones as well as reproductive hormones in Awassi ewes. The least-squares means of phenotypic traits affected by the progeny type are presented in Table 1. Awassi ewes with twin births had significantly higher live body weights (52.64 ± 1.53 kg) and FSH levels (1.13 ± 0.11 ng/ml) ($P \leq 0.05$) compared to those with single births. In contrast, lamb weights at birth (4.08 ± 0.27 kg) and weaning (22.25 ± 1.41 kg), as well as T3 hormone levels (369.40 ± 18.80 pg/ml), were significantly higher ($P \leq 0.05$) in Awassi ewes with single births. However, the differences in the age of Awassi ewes, and the survival rate of lambs, T4, and LH hormone levels ($P \geq 0.05$) were not statistically significant. Univariate regression analyses further investigated the association between litter size and phenotypic traits in Awassi ewes (Table 2).

Sheep litter sizes vary based on several factors, including age, season, management practices, nutrition, genetics, body condition score, uterine capacity, environmental conditions, and live body weight (Al-Thuwain and Al-Hadi, 2022). The live body weight of ewes can be used to predict the litter size; larger ewes are likely to give birth to more lambs than smaller ewes (Al-Jumaili *et al*., 2023; Hanna *et al*., 2023). Additionally, lambs born to mothers with higher body weights tend to have a correspondingly higher weights (Al-Thuwain and Al-Hadi, 2022). Momoh *et al*. (2013) have reported a significant association between birth type and both birth weight and weaning weight. According to McGovern *et al*. (2015) and McHugh *et al*. (2017), lambs born or raised from single litters exhibit greater birth weights, pre-weaning weights, and weaning weights compared to lambs born or raised from multiple litters. Smith (2022) also found that singletons have higher birth weights and weaning weights than twins. This may be attributed to the fact that single lambs occupy a greater volume within the uterus and they do not compete for nutrition in utero as twin lambs do (Babar *et al*., 2004). The weight advantage observed in individual lambs at weaning highlights the competition among twin births for both milk production and maternal care, contrasting with the findings regarding individual lambs reported by Kramarenko *et al.* (2021). Fassah *et al.* (2015) found that low birth weight in twin lambs is primarily due to competition for placental nutrients and maternal milk within the uterine environment. Furthermore, twins may experience greater nutritional challenges than singletons due to their higher fetus-to-placenta weight ratio (Al-Thuwaini, 2022). Kenyon *et al.* (2019) reported that fetal growth accelerates during the final 50 days of pregnancy, resulting in noticeable variations in feed requirements. Consequently, this study was conducted on ewes in late pregnancy, when lamb growth is particularly critical.

Litter size is influenced by reproductive and thyroid hormones (Medrano and Hua, 2016; Alkhammas and Al-Thuwaini, 2023). Research indicates that ewes with higher litter sizes exhibit elevated levels of reproductive hormones (Ajafar *et al.*, 2022; Goldansaz *et al.*, 2022). Increasing the concentration of reproductive hormones significantly impacts ovarian follicle development and maturation, thereby enhancing the number of offspring produced (Atoui *et al.*, 2018). Additionally, the thyroid gland and its hormones play a crucial role in maintaining the productivity and efficiency of domestic animals, particularly in growth, milk production, and reproduction (Medrano and Hua, 2016). The functions of T3 and T4 in sheep depend on the flexibility of hypothalamic ventricular membrane cells, which regulate the secretion of GnRH and influence the hypothalamic-pituitary-gonadal axis. This axis, in turn, affects reproductive processes during both the follicular and luteal phases (Chang *et al.*, 2023). Furthermore, GnRH may directly stimulate thyroid activity by promoting hypothalamic-pituitary-gonadal function, and indirectly by influencing the biosynthesis of sex steroid hormones (Behringer *et al.*, 2018; Silva *et al.*, 2018; Brown *et al.*, 2023). T3 and T4 regulate the secretion of LH and FSH by modulating the release of kisspeptins (KISS) and RFamide-related peptides (RFRPs) in the hypothalamus, thereby influencing the estrous cycle through their effects on GnRH neurons. Consequently, GnRH governs estrous cycle function by regulating LH and FSH secretion (Chang *et al.*, 2023). As thyroid hormone levels rise, the secretion of FSH and LH is subsequently affected, impacting their synthesis, secretion, circulation levels, and metabolism (Behringer *et al.*, 2018). The metabolic processes and milk yield in pregnant dairy cows are dependent on thyroid hormones (Fazio *et al.*, 2022).

Thyroid hormone receptors are found in ovarian tissue, specifically in oocytes, granulosa cells, ovarian stromal cells, and cumulus cells (Silva *et al.*, 2018). These receptors promote the development of preantral follicles and increase the ovulatory rate. A combination of T3 and FSH can enhance cell proliferation and reduce apoptosis in granulosa cells. T4 stimulates the FSH-induced production of estradiol in granulosa cells (Gy *et al.*, 2002). Furthermore, T3 and gonadotropic hormones interact to inhibit excessive androgen production by theca cells and stimulate aromatization, which leads to estrogen production by granulosa cells. By activating ERK1/2 signaling, thyroid hormones facilitate the maturation of preovulatory follicles and cumulus oophorus, as well as the meiotic maturation of oocytes (Silva *et al.*, 2018). As the fetus develops, thyroid hormones are essential for fetal growth and development, with further differentiation of fetal tissues occurring as the pregnancy progresses (Fereig *et al.*, 2023).

The thyroid hormone significantly influences tissue differentiation and fetal growth by stimulating growth and metabolism through specific nuclear receptors located in the ovary, uterus, and placenta (Chang *et al.*, 2023). Thyroid status affects pregnancy and placental development, promoting the function and development of the female reproductive tract while regulating placental and fetal growth (Brown *et al.*, 2023). Kiyohara *et al.* (2017) report that thyroid hormones play a crucial role in the functioning of the reproductive system's functioning, as well as in controlling metabolism, fetal growth, and development. This finding aligns with the research of Raoofi *et al.* (2017), which revealed that twin-bearing goats exhibit lower plasma T4 concentrations compared to single-bearing goats. Recently, elevated T4 concentrations and T4:T3 ratios have been linked to delayed ovulation in dairy cows. This delay results in a longer time to first service and fewer open days, ultimately contributing to lower conception rates (Yari *et al.*, 2023). Monitoring thyroid hormone levels is a valuable method for analyzing the anabolic and/or catabolic adaptations in Awassi ewes during functional periods, enabling the assessment of excess negative energy balance and metabolic disorders. Although this study is the first study to report a relationship between litter size and thyroid hormone levels, it has several limitations. Our research is limited by the absence of thyroid measurements taken during early pregnancy and lactation. Additionally, environmental stressors, feed quality, genetic variation, and the cross-sectional nature of the study within the sample population may have influenced the results. Another limitation is the small sample size. To gain a more comprehensive understanding of the association between thyroid hormone levels, litter size, and lamb weight, further research should be conducted with a larger population of Awassi ewes.

CONCLUSIONS AND RECOMMENDATIONS

There was an association between litter size and thyroid hormone levels in Awassi ewes, as well as lamb weight.

Ewes bearing twins exhibited higher live body weights but lower thyroid hormone levels, and their lambs were smaller at both birth and weaning. However, this conclusion is based on a limited sample size and a specific physiological condition. Further studies involving larger sample sizes may provide additional insights into the association between thyroid hormone levels and litter size. Additionally, longitudinal studies that monitor hormone levels throughout the reproductive cycle, along with experiments designed to manipulate thyroid hormone levels, could help elucidate direct effects.

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NOVELTY STATEMENT

This study investigated the relationship between the number of lambs born to ewes and thyroid metabolic hormones for the first time. The Awassi ewes with twins exhibited lower levels of thyroid hormones, and their lambs had smaller at both birth and weaning. This finding suggests that these ewes may be potential candidates for further research in sheep breeding and reproduction.

AUTHORS CONTRIBUTION

TMA-T: Conceptualization, investigation, writing review and editing. THH: Investigation, methodology. ZMAA: 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 have declared no conflict of interest.

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