

Reproductive hormones and their association with the litter size in Kreishi ewes

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Abstract

This study evaluated the reproductive hormonal profile of Kreishi ewes during pregnancy and examined its association with litter size. In total, 100 ewes from the Kreishi breed were examined during their fourth and fifth months of pregnancy (46 with single pregnancies and 54 with twin pregnancies) and weighed between 75 and 95 kg. The levels of progesterone (P4), follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were measured and compared by litter size and age. The results revealed highly significant ($P \leq 0.05$) differences in hormonal concentrations between twin and single lamb-producing ewes. Ewes with twins displayed significantly higher progesterone, FSH, and LH concentrations (9.14 ± 0.91 , 37.24 ± 1.45 , 35.62 ± 1.55) than those with singletons (7.98 ± 1.19 , 25.04 ± 1.34 , 22.17 ± 1.20), suggesting increased endocrine activity. Age-related differences in progesterone levels were observed in 2-year-old ewes, but not in FSH and LH levels. Additionally, hormonal concentrations differed between the fourth and fifth months of pregnancy, reflecting physiological changes associated with pregnancy progression. Based on these findings, progesterone, FSH, and LH may serve as useful physiological indicators of reproductive performance in Kreishi ewes. This study provides valuable baseline information that may help improve prolificacy and lamb production in this indigenous sheep breed through future breeding and selection programs.

Keywords: Kreishi ewes, litter size, prolificacy, pregnancy, reproductive hormones.

Introduction

The hormonal options for controlling reproduction in small ruminants are well described; these are useful tools for increasing farm profitability [1]. Reproductive efficiency in ewes is effected by a range of variable related to the endocrine system such as ovarian activity rates, estrous cycle length and breeding

season, as well the effect of maternal age, number of birth and the timing of postpartum recovery of estrous activity [3]. Pregnancy is the cornerstone of species continuity but it places a significant metabolic burden on females, potentially making them more vulnerable to external environmental influences [16]. Progesterone (P4) and estrogens (E2), the primary hormones sustaining pregnancy, FSH (follicle-

ISSN 2072-3857

stimulating hormone), and LH (luteinizing hormone) are foundational gatekeepers of this process [20]. Progesterone is the main steroid hormone produced by placental cells to support the pregnancy in sheep after gestational day 50; before that, the pregnancy depends on P4 [23]. E2, P4, LH, and FSH are important reproductive hormones in the periodic activity of the ovary, acting via their receptors (ER α , ER β , PR, LHR, and FSHR), with LH as the main ovulatory hormone and regulator of corpus luteum function. FSH plays an essential role in the cyclic recruitment of the follicles [14].

The stability of pregnancy depends on the harmonious and dynamic hormonal changes throughout the three trimesters, which ensure an optimal uterine environment for embryo implantation. The first trimester of pregnancy is a critical period, during which implantation, placental formation, and the initiation of embryonic and placental growth all occur—crucial steps for a successful pregnancy and its continuation [5]. The concentration of progesterone (P4) in peripheral plasma rises gradually during the luteal phase in the first half of pregnancy, increases markedly at about 90 days post-mating, peaks at 125 days post-mating, and declines in the last few days before parturition. In sheep, during the first third of gestation, P4 is produced by the corpus luteum (CL); the placenta takes over production after about 50 days post-insemination [19]. In the second trimester, plasma progesterone showed a rising trend from conception to delivery. It controls uterine contractility and promotes maternal immune tolerance to the fetal semi-allograft. In the presence of progesterone, lymphocytes also release the progesterone-induced blocking factor (PIBF), a pivotal mediator of

progesterone-dependent immunomodulation [13]. The diameter of the corpus luteum and the progesterone concentration of pregnant ewes bearing a single fetus or twin fetuses displayed higher values than those found in non-pregnant ewes [7]. An increased number of corpora lutea may increase P4 and E2 secretion, thereby stimulating mammary gland growth in twin fetuses [15]. Furthermore, the reproductive success of the sheep population is largely determined by the maternal age of females. Ewes with mature hormonal profiles and enhanced uterine capacity have significant reproductive advantages [4]. According to research conducted in Indonesia, mature ewes (>3 years) consistently outperform younger ewes in key reproductive metrics [11]. Ibrahim et al. (2024) found that the rate of twins and singleton births at 1.5 years of age is higher than at other ages and drops with age. Other studies show that maternal age has no significant impact on reproductive parameters [4]. Despite significant advances in understanding the endocrinology of reproduction in sheep, information regarding the association between reproductive hormones and litter size remains scarce, particularly in indigenous breeds. To date, no study has comprehensively examined the relationships of serum progesterone, FSH, and LH concentrations with litter size in Kreishi ewes. Thus, this study was designed to fill the knowledge gap and provide baseline information on the hormonal factors associated with prolificacy.

Materials and Method

This study was conducted at field sites in the Al-Nu'maniyah district (Wasit governorate), located approximately 40 km

north of the center of Al-Kut city. It lasted from September 2025 to April 2026. A total of 100 healthy, sexually mature, multiparous ewes aged 2-4 years participated in the study. Expert veterinarians confirmed that the ewes were clinically healthy. Ewes were randomly selected, including 100 Kreishi ewes (46 with single pregnancies and 54 with twin pregnancies) weighing between 75 and 95 kg. Seasonal green grass was provided for the sheep in spring and autumn, and they were housed indoors in winter. Concentrated feed was given to them twice daily at 2.5% of their body weight. This feed consisted of a barley center with 59% and 40% bran, and 1% salt. Green alfalfa and straw were provided per animal at 3 kg of green alfalfa and 1 kg of straw. Drinking water was available at all times.

Blood samples were collected from the ewes during pregnancy for hormonal testing. To ensure animal safety and accuracy, the ewes were carefully secured in a standing position, and blood samples were drawn directly from the jugular vein using sterile needles and collected in gel activator tubes for serum separation and hormonal analysis. The samples were centrifuged at 3000 rpm for 15 minutes, and the separated serum was then stored at -20°C until the hormonal assay. Reproductive hormones, including progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), were quantified using Enzyme-Linked Immunosorbent Assay (ELISA) kits ELK8876, ELK8853, and ELK8851 from ELK Biotechnology (Wuhan, China).

Statistical Analysis

The Statistical Analysis System (SAS) program (2018) was used to assess the effects of birth type, age, and month of

pregnancy on the parameters studied separately. A t-test, Duncan's (1955) test, and an ANOVA multiple range test were used to compare means at significance levels of 0.05 and 0.01. The following statistical model:

First model:

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

Where:

Y_{ij} : Observed value j corresponding to type of birth i.

μ : Overall mean of the trait.

T_i : Effect of the type of birth.

e_{ij} : Random error, assumed to be normally distributed with a mean of zero and a variance of σ^2_e .

Second model (Effect of the age of ewe on hormone levels):

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

A_i : Effect of the age of ewe (2, 3, and 4 years).

Third model (Effect of the pregnant month on hormone levels):

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

P_i : Effect of the pregnant month (4, 5 months).

Results and discussion

In Table 1, the results indicate significant differences ($P \leq 0.05$) in hormonal levels of progesterone, FSH, and LH between Kreishi ewes with twin and single births. Progesterone, FSH, and LH hormone levels reached (9.14 ± 0.91 , 37.24 ± 1.45 , 35.62 ± 1.55) in ewes with twin birth, compared to progesterone, FSH, and LH hormone levels

(7.98 ± 1.19 , 25.04 ± 1.34 , 22.17 ± 1.20) in Kreishi ewes with single birth.

Table 1: Effect of type of birth on the hormone levels of P4, FSH, and LH of Kreishi ewes

Hormones	Mean $\pm$ SE		Level of Sig.
	Twin	Single	
Progesterone (nmol/L)	9.14 ± 0.91	7.98 ± 1.19	0.04 *
FSH (mIU/mL)	37.24 ± 1.45^a	25.04 ± 1.34^b	0.0001 **
LH (mIU/mL)	35.62 ± 1.55^a	22.17 ± 1.20^b	0.0001 **
Means having the different letters in the same row differed significantly. * ($P \leq 0.05$), ** ($P \leq 0.01$).			

Kreishi ewes carrying twins had significantly higher levels of progesterone, FSH (follicle-stimulating hormone), and LH (luteinizing hormone) during the fourth and fifth months of pregnancy compared with those carrying a single lamb. These findings might indicate that prolific ewes are more efficient at reproduction and have stronger endocrine function. Progesterone concentrations may be higher in twin-bearing ewes due to a greater number of corpora lutea formed during multiple ovulations before conception [23]. In addition, twin pregnancies may provide enhanced endocrine support during pregnancy due to the larger fetoplacental unit. During pregnancy, progesterone stimulates uterine gland secretions, suppresses myometrial contractions, and promotes fetal development [8]. In twin-bearing ewes, progesterone levels may be higher due to the greater hormonal demands of multiple [6, 1]. Similarly, the P concentration of pregnant ewes bearing

twin fetuses appeared to be higher than that found in pregnant ewes bearing a single fetus [7]. Sheep litter size is primarily determined by follicular recruitment, maturation, and ovulation rate, which are determined by gonadotropins. High levels of FSH and LH are therefore likely to be associated with enhanced ovarian responsiveness and endocrine function in highly prolific (Herbison, 2016; Nestor et al., 2018). Further, genetic studies have shown that fecundity-related genes, such as BMP15 and GDF9, are strongly associated with higher ovulation rates and litter sizes in sheep. These genes are associated with improved follicular development and reproductive performance, which may explain the superior hormonal profile observed in twin-producing ewes in this study [22, 2]. Prolificity has been associated with reproductive hormones in highly productive sheep breeds, where elevated endocrine activity increases reproductive effectiveness and multiple births [1].

In Table 2, the results indicate significant differences in progesterone levels across age groups of Kreishi ewes. Progesterone levels were highest (10.46 ± 2.16) in 2-year-old ewes and decreased

at ages 3 and 4 (8.32 ± 0.82 , 6.55 ± 1.36), respectively. At the same time, there were no significant differences ($P \geq 0.05$) in FSH and LH levels between Kreishi ewes at different ages.

Table 2: Effect of age on hormone levels of P4, FSH, and LH of Kreishi ewes

Hormones	Age of ewes: Mean $\pm$ SE			Level of Sig.
	2 yr. (No. =9)	3 yr. (No =33)	4 yr. (No. =4)	
Progesterone (nmol/L)	10.46 ± 2.16^a	8.32 ± 0.82^b	6.55 ± 1.36^{ab}	0.04 *
FSH (mIU/mL)	30.49 ± 2.71	32.06 ± 1.70	28.09 ± 2.16	0.66 NS
LH (mIU/mL)	30.17 ± 1.97	28.65 ± 1.76	32.46 ± 0.35	0.80 NS
Means having the different letters in the same row differed significantly. * ($P \leq 0.05$), NS: Non-Significant.				

In 2-year-old ewes, progesterone concentrations were higher due to their greater reproductive efficiency and corpus luteum activity. Ewes at this age are generally at the beginning of their productive lives and exhibit optimal ovarian function, which may increase corpus luteum activity and progesterone secretion. With advancing age, progesterone concentrations may decrease due to gradual reductions in ovarian responsiveness, luteal function, or endocrine efficiency, potentially affecting progesterone production during gestation [6, 1]. Age-related changes in reproductive physiology have also been reported to affect ovarian steroidogenesis and hormone secretion. Younger ewes often exhibit greater reproductive performance and metabolic efficiency, thereby improving endocrine function during pregnancy. Accordingly,

2-year-old ewes may be more reproductively competent than older ewes due to their elevated progesterone concentrations [9].

In contrast, the absence of significant differences in FSH and LH levels across age groups suggests that age has minimal influence on pituitary gonadotropin secretion during gestation. This stability is likely due to the potent negative feedback mechanism exerted by progesterone on the hypothalamic-pituitary axis during pregnancy, which effectively suppresses fluctuations in gonadotropin secretion, regardless of the ewe's age [10, 17].

In the Table (3) the results indicate significant differences ($P \leq 0.05$) in hormonal levels FSH and LH level Kreishi ewes as pregnancy progressed

from the fourth to the fifth month, FSH hormone levels increased from (27.44 ± 1.71) to (33.32 ± 1.72) and LH hormone level from (25.91 ± 1.89) to ($30.77 \pm$

1.82), in contrast there were non-significant differences in progesterone level between fourth and fifth month

Table 3: Effect of pregnant month on the hormone levels of P4, FSH, and LH of Kreishi ewes

Hormones	Pregnant month: Mean $\pm$ SE		Level of Sig.
	Fourth month (No=15)	Fifth month (No=31)	
Progesterone (nmol/L)	10.07 ± 1.61	7.87 ± 0.76	0.16 NS
FSH (mIU/mL)	27.44 ± 1.71^b	33.32 ± 1.72^a	0.04 *
LH (mIU/mL)	25.91 ± 1.89^b	30.77 ± 1.82^a	0.04 *
* ($P \leq 0.05$), NS: Non-Significant.			

The elevation in serum FSH and LH concentrations observed during the fifth month of gestation may be attributed to alterations in hypothalamic-pituitary activity and specific endocrine adjustments. Furthermore, this significant increase in FSH and LH concentrations is attributed to the maturation of the fetal hypothalamic-pituitary-adrenal (HPA) axis toward the end of pregnancy. The subsequent influx of fetal cortisol stimulates placental enzymes to convert progesterone to estrogens. This change removes the negative feedback inhibition of maternal of pituitary gland, triggering the oscillatory release of gonadotropins (FSH and LH) to prepare the ewe for

lambing. Concurrently, progesterone levels remained relatively stable, underscoring the consistent luteal and placental support essential for the maintenance of pregnancy in ewes [6, 17].

In conclusion, there is a strong correlation between litter size and reproductive hormonal status during pregnancy in Kreishi ewes. The serum concentrations of progesterone, FSH, and LH were higher in twin-bearing ewes than in single-bearing ewes, suggesting superior reproductive efficiency and endocrine activity. There was a significant effect of age on

progesterone levels, but no significant effect on FSH or LH levels. The findings suggest that -reproductive hormones can be used as initial indicators of prolificacy in Kreishi ewes. To better

understand the genetic and endocrine mechanisms underlying prolificacy in Kreishi ewes, further studies with larger populations and molecular markers are recommended.

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