Impact of Different Hormonal Estrus Synchronization Protocols on Serum Progesterone Concentrations and Biochemical Parameters in Iraqi Awassi Ewes.

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Abstract

This study investigated the use of kisspeptin-10 injections in Awassi ewes and compared them to GnRH injections to demonstrate their effect on improving progesterone levels and biochemical blood indicators during the breeding season, which influence estrous synchronization and improve reproductive performance. The ewes a 28-day nutritional induction program, after which they were randomly divided into four groups (N = 10). The first group received intravenous injections of kisspeptin-10 at a dose of 6 µg/live weight on days 0 and 9, and PGF2 on day 7. The second group received intravenous injections of GnRH at a dose of 2 mg on days 0 and 9, and PGF2 on day 7. The third group (control) received intravenous injections of 0.9% NaCl on days 0, 7, and 9. The fourth group received vaginal sponges containing 40 mg of FLogestone Acetate for 9 days from the beginning of experimental The results indicated that all hormonal treatments were superior in terms of progesterone levels. Progesterone hormone increased blood biochemical indicators of globulin, amino acids and fatty acids, but did not affect the level of total protein, albumin, cholesterol and triglycerides compared to the control treatment .

Keywords: Kisspeptin-10, GnRH , progesterone, biochemical indicators , Awassi ewes

Introduction

Sheep are generally classified as polyestrous animals characterized by high fertility; some breeds exhibit twinning, indicating that the ovaries remain in a state of active physiological function. However, since some sheep are restricted breeders, it is more accurate to describe them broadly as seasonally polyestrous meaning they undergo multiple estrous cycles, but only during specific times of the year in certain regions. The primary factor regulating the reproductive process in sheep is the ratio of night length to day length **[5]**. Strategies for estrus synchronization in ewes include manipulating photoperiod, ram exposure, and the use of exogenous hormones such as progesterone, prostaglandins gonadotropins, and melatonin. However, strategies that offer precise synchronization , acceptable fertility levels, cost-effectiveness, and ease of application are considered optimal for field conditions **[24], [36]**, **[11]**, **[26]**. The Ovsynch protocol is one such program; it has been widely used in recent years due to its effectiveness in synchronizing estrus in cattle and sheep eliminating the need for estrus detection and for its role in increasing fertility and lambing rates **[35]**, **[28]**. This was reflected in improved reproductive

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efficiency within these herds; various programs and strategies were employed with the primary aim of controlling the lifespan of the corpus luteum, regulating the growth and development of ovarian follicles, and inducing estrus **[25]**, **[10]**, **[3]**, **[17]**. Kisspeptin-10 is a neuropeptide derived from the primary translation product of the KISS1 gene, located on chromosome 1; it was discovered approximately 30 years ago **[21]**. It has been observed that GnRH-producing neurons are located in close proximity to kisspeptin-producing neurons in higher mammals, monkeys, and sheep **[15]**. The role of kisspeptin in stimulating the secretion of gonadotropic hormones in both humans and mice was elucidated in late 2003; kisspeptin-producing neurons act as the principal relay for mediating steroid hormone feedback on GnRH secretion. Kisspeptin is now considered crucial for regulating reproductive activity in seasonally breeding animals by increasing levels of luteinizing hormone (LH), as evidenced by observations of enhanced oocyte maturation in sheep in vitro through elevated levels of FSH and LH **[9]**, **[16]**. Gonadotropin-releasing hormone (GnRH) is the primary regulator of the reproductive axis. Its pulsatile secretion determines the secretory patterns of the gonadotropins follicle -stimulating hormone (FSH) and luteinizing hormone (LH) which, in turn, regulate endocrine function and gamete maturation within the gonads. Recent years have witnessed rapid advancements in our understanding of the regulation of GnRH secretion, following the discovery of the kisspeptin neurokinin -dynorphin neuronal network in the hypothalamus. This network mediates the positive and negative feedback control exerted by sex hormones on GnRH secretion, acting in concert with other neuropeptides and neurotransmitters **[22]**. When administered during the luteal phase, these hormones directly or indirectly affect the ovary, inducing the formation of an accessory corpus luteum (acc-CL) and increasing blood progesterone concentrations **[19]**, **[20]**. Progesterone is known as the "pregnancy hormone" and plays a crucial role in the early stages of pregnancy in ruminants, including sheep. In recent years, the role of this hormone has attracted significant research interest; **[8]** .noted that while research previously focused primarily on progesterone levels during the luteal phase, attention has now shifted to hormone levels during the initial days following insemination. Therefore, the aim of this study is to demonstrate the effect of using Kisspeptin, GnRH, or vaginal sponges containing Flogestone Acetate hormone in stimulating the ovary to secrete progesterone and increasing the concentration of biochemical blood indicators before inseminating Awassi ewes

Materials and Methods

Ewe Management

The study was conducted at a private farm in Diyala Governorate, which was fully equipped to house the ewes, at the period from May 1, 2025, to November 1, 2025 (the breeding season). Fourty local Awassi ewes, aged 3–4 years and weight between 40 and 50 kg, were selected for the study. Standard management and field procedures were carried out, including shearing and the administration of required veterinary vaccines. The ewes were fed a ready-made ration for nutritional flushing as detailed in **Table (1)** at a rate of 0.5 kg per ewe for 28 days prior to breeding; this was supplemented by free grazing on available seasonal forage. A source of drinking water was provided throughout the duration of the experiment

Estrus Synchronization Protocols

After selection, the ewes were randomly divided into four groups (10 ewes for each group), with each group assigned a specific

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color code. In the first treatment group, ewes received intravenous injections of Chinese-made Kisspeptin-10 (6 µg/kg live weight) on days 0 and 9, and an injection of Dutch-made PGF2α (2 mg) on day 7. The second treatment group received intravenous injections of German-made GnRH (Receptal; 2.5 mL/2 mg) on days 0 and 9, and PGF2α (2 mg) on day 7. The third group (control) received intravenous injections of normal saline (2 mL) on days 0, 7, and 9. Finally, the fourth group was treated with Uruguayan-made vaginal sponges (Ovakon) containing (40) mg of Flogestone Acetate , which were left in place for 9 days. At the end of the experiment 9 day (4 mL) blood sample was drawn from the jugular vein using a sterile, disposable syringe to measure progesterone levels; an ELISA test was performed to analyze the hormone in the serum according to the manufacturer's instructions (Wuhan Fine Biotech). Serum proteins were measured using the method of **[14]**, cholesterol according to the method described by **[6]**. and triglycerides according to the method of **[13]**. Amino acids (lysine, methionine, arginine, leucine, and glutamic acid) were measured according to the method cited in **[2]** as were fatty acids (palmitic, oleic, linoleic, and linolenic). Following the method of **[23]**

Table 1: Composition of the concentrate feed used in the study (%)

Nutritional Ingredients	roportion in the Feed Ingredients Experimental Diet (%)
rushed barley	50
Wheat bran	35
Yellow corn	5
Soybean meal	7
Salt	1
Limestone	1
*Premix	1
Total	100

Crude protein content:14.5%

Crude protein was calculated according to the US National Research Council [27]

essential vitamins (A, D3, E, B12, B1, B6, Niacin), trace elements and minerals (magnesium, copper, iron, cobalt, iodine, manganese, zinc.

***The "Power Mix" premix manufactured by the Turkish company Siyalite - contains**

Experimental Design and Statistical Analysis

The obtained data were analyzed using SPSS **[32]** software based on a Completely Randomized Design (CRD). Duncan's Multiple Range Test **[12]** was used to test the significance of differences between means.

Results

Effect of Hormones on Biochemical Blood Parameters in Awassi Ewes

Table (2) presents the results of biochemical blood parameters prior to insemination. The findings indicate a significant difference ($P \leq 0.05$) in progesterone concentration for the first, second, and fourth treatment groups compared to the control group. While no significant differences were observed in total protein or albumin levels, a significant difference ($P \leq 0.05$) was found in globulin levels, favoring the vaginal sponge treatment

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group over the other groups. No significant differences were noted in cholesterol or triglyceride concentrations among the experimental treatment the vaginal sponge group was the most effective in synchronizing estrus compared with the other groups.

Table 2: Effect of hormonal injections on certain biochemical blood parameters in local Awassi ewes (Mean ± Standard Error).

Treatment	Progesteron ng/mL	Total Protein g/Dl	Albumin g/dL	Globulin g/Dl	Cholesterol mg/dL	Triglycerides mg/dL
T1	1.28 **ab** ±0.05	6.80 **a** ±0.55	3.00 **a** ±0.10	3.80 **ab** ±0.59	85.83 **a** ±0.97	33.80 **a** ±3.27
T2	1.73 **ab** ±0.18	6.90 **a** ±0.15	3.44 **a** ±0.08	3.45 **b** ±0.23	88.45 **a** ±0.72	32.45 **a** ±1.02
T3 (control)	.81 **b** 0 ±0.07	.22 **a** 7 ±0.34	.28 **a** 3 ±0.38	.94 **a** 3 ±0.45	.88 **a** 83 ±1.51	**a** 70.34 ±3.29
T4	1.92 **a** ±0.51	7.77 **a** ±0.06	3.67 **a** ±0.02	4.10 **a** ±0.04	88.05 **a** ±12.00	32.10 **a** ±3.28
Sig	*	N.S	N.S	*	**N.S**	N.S

Different letters within the same column indicate significant differences between the means
N.S. means no significant effects between the means in the ANOVA table.
***means significant effects (P ≤ 0.05) between the means in the ANOVA table.**

Effect of Hormones on Amino Acid Levels in Awassi Ewes

Based on the results presented in Table (3) which revealed highly significant differences (P≤0.01) among the treatment groups regarding pre-insemination amino acid levels—ewes in the first treatment group (Kisspeptin-10) exhibited significantly higher concentrations of most measured amino acids (Leucine, Lysine, Methionine, Glutamine, and Arginine) compared to the other groups. This group was followed by the second treatment (GnRH) and then the fourth treatment, whereas the control group (T3) recorded the lowest values

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Table 3: Effect of hormone injections on amino acid levels (mg/dL) in ewes prior to insemination in local Awassi ewes (Mean ± Standard Error).

Treatment	Arginine	Glutamine	Methionine	Lysine	Leucine
T1	8.07 **a** ±0.03	8.55 a ±0.02	2.61 **a** ±0.02	8.94 **a** ±0.03	8.05 **a** ±0.02
T2	7.85 **b** ±0.02	8.30 **b** ±0.01	2.32 **b** ±0.01	8.64 **b** ±0.17	7.86 b ±0. 03
T3 (control)	**d**066. 0.02±	**d**17.0 0.04±	**d** 21.2 0.01±	6.96 **d** ±0.04	**d**45.7 0.02±
T4	7.48 **c** ±0.02	7.94 **c** ±0.02	1.95 **c** ±0.02	8.15 **c** ±0.06	7.61 **c** ±0.02
Sig	**	**	**	**	**

Different letters within the same column indicate significant differences between the means

****means highly significant effects (P ≤ 0.05) between the means in the ANOVA table.**

Effect of Hormones on Fatty Acid Levels in Awassi Ewes

Table (4) reveals highly significant differences (P<0.01) in fatty acid levels among the hormonal treatments administered prior to insemination; specifically, levels of linoleic, palmitic, linolenic, and oleic acids increased compared to the control group. Among the hormonal treatments, the first treatment involving the administration of kisspeptin yielded the most favorable fatty acid levels.

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Table 4: Effect of hormone injections on fatty acid levels (mg/dL) prior to insemination in local Awassi ewes (Mean ± Standard Error).

Different letters within the same column indicate significant differences between the

Treatment	Linoleic Acid	Palmatic	Linolenic Acid	Oleic Acid
T1	8.54 **a** ±0.02	17.77 **a** ±0.01	3.40 **a** ±0.01	26.86 **a** ±0.03
T2	8.04 **c** ±0.02	16.24 **c** ±0.03	3.03 **c** ±0.02	26.04 **c** ±0.02
T3 (control)	**d** 17.5 ±0.02	15.93 **d** ±0.02	**d** 642. ±0.02	.65 **d** 25 ±0.02
T4	8.20 **b** ±0.008	16.50 **b** ±0.02	3.19 **b** ±0.01	26.43 **b** ±0.01
Sig	**	**	**	**

means

****means highly significant effects (P ≤ 0.05) between the means in the ANOVA table.**

Discussion

The current results indicate that hormonal treatments (kisspeptin-10, gonadotropin-releasing hormone, and vaginal sponges) stimulated coordinated hormonal and metabolic adaptations. Collectively, these led to improved reproductive efficiency parameters specifically estrus expression, fertility, and lambing rates in the ewes. Although no significant differences were observed in pre-insemination concentrations of cholesterol, triglycerides, total protein, and albumin, distinct effects were noted regarding serum progesterone and globulin levels. Furthermore, a marked increase in amino acid and fatty acid levels was recorded, particularly in the hormonally treated groups. These findings suggest that the employed protocols enhanced reproductive physiological efficiency without inducing metabolic stress. The rise in serum progesterone concentration particularly in ewes treated with progesterone-impregnated vaginal sponges, and to a lesser extent in groups treated with kisspeptin-10 and gonadotropin-releasing hormone (GnRH) indicates improved corpus luteum function. Adequate circulating progesterone levels during the pre-insemination period are essential for preparing the uterus to receive the fertilized ovum, facilitating endometrial glandular secretions, and supporting embryonic elongation, thereby reducing early embryonic mortality **[30]**. Elevated progesterone levels during the early luteal phase have consistently been associated

with enhanced pregnancy stability and improved embryonic survival in ewes. The notable increase in globulin protein concentration observed in certain hormonally treated groups may reflect immunological adjustments associated with progesterone activity. Progesterone is known to modulate maternal immunity, thereby creating an environment conducive to embryonic implantation and survival **[31]**, this immunological adaptation is crucial for minimizing early embryonic loss .The super-estrous response observed in hormonally treated ewes may stem from the stimulation of the hypothalamic-pituitary-ovarian axis. Kisspeptin acts upstream of GnRH neurons, stimulating pulsatile GnRH secretion and subsequently increasing the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which enhances follicular maturation and estradiol production **[34]**.

Increased estradiol secretion explains the heightened expression of estrus signs, while improved ovulation synchronization likely contributed to higher fertilization rates. Similarly, GnRH administration directly stimulates LH release, thereby inducing ovulation and supporting corpus luteum formation. Furthermore, the use of a progesterone sponge followed by its withdrawal triggers a synchronized LH surge, enhancing both estrus detection and the precision of ovulation **[18]**. The metabolic profile supports these reproductive outcomes. Elevated levels of essential amino acids particularly leucine, arginine, glutamine, lysine, and methionine indicate enhanced capacity for cellular growth and proliferation. Leucine serves as a key activator of the mTOR signaling pathway, which regulates protein synthesis and cell proliferation within ovarian follicles and early-stage embryos

Arginine acts as a precursor to nitric oxide, thereby improving blood flow to the uterus and ovaries and supporting placental angiogenesis, which enhances fetal survival **[33]**. Glutamine provides an essential energy substrate for rapidly dividing embryonic cells, while methionine participates in methylation reactions crucial for gene regulation during early embryonic stages. Collectively, this increase in amino acid levels creates a biochemical environment conducive to fertilization and early embryonic development. Similarly, the rise in unsaturated fatty acids specifically oleic, linoleic, and linolenic acids indicates enhanced reproductive support provided by lipids .Unsaturated fatty acids are essential components of cell membranes and precursors to prostaglandins, which regulate the functions of the corpus luteum and the uterus. Increased availability of polyunsaturated fatty acids (PUFAs) has been linked to improved oocyte competence and embryonic development in ewes **[29]**. Oleic acid, in particular, has been shown to counteract the detrimental effects of saturated fatty acids on oocyte developmental competence **[1]**. These lipid-related changes may contribute to improving the quality of the follicular microenvironment and increasing ovulation rates, It can be stated that hormonal stimulation using kisspeptin, GnRH, and progesterone did not merely affect the reproductive axis; it also improved the metabolic environment (regarding amino acids and fatty acids), enhanced corpus luteum function, optimized the endometrial environment, and reduced early embryonic loss. Collectively, these factors explain the increases observed in estrus, fertility, and pregnancy rates, as well as the number of offspring produced. This confirms that the impact was physiologically multifaceted encompassing hormonal, metabolic, and uterine aspects rather than being limited to

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a localized effect on ovulation. These results align with recent evidence indicating that reproductive success in small ruminants is determined not only by ovarian stimulation but also by the synchronization of hormonal signals and metabolic support during the pre-conception period **[4],[7]** Consequently, the applied hormonal protocols may represent effective strategies for enhancing reproductive efficiency in Awassi ewes through coordinated physiological regulation.

Conclusion

The vaginal sponge group was the most effective in improving reproductive compared with the other groups by activating the hypothalamic-pituitary-ovarian axis and enhancing the ovulatory response. These treatments were associated with increased pre-insemination progesterone concentrations indicating improved corpus luteum function as well as elevated blood levels of amino acids and fatty acids, reflecting enhanced metabolic readiness for reproduction. Overall, these hormonal protocols promote hormonal balance and metabolic support, thereby contributing to improved reproductive performance in ewes

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Conflict of Interest:

The authors declare no conflict of interest.

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