Effect of ECG and GnRh hormones at two different periods on Awassi ewes treated with vaginal sponges on some reproductive and biochemical traits

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Abstract

The aim was to assess hormonal changes and hematological characteristics after using two techniques (vaginal sponge insertion and intramuscular injection of GnRH and ECG) in Awassi ewes.

The study included 32 Awassi ewes, age range 1 to 4 years,. The ewes were divided into four groups (n=8),. The first group was a short-term ECG treatment, the second group was a long-term ECG treatment, the third group was a short-term GnRH treatment, and the fourth group was a long-term GnRH treatment. The short-term treatment was 6-8 days, while the long-term treatment was 12-14 days. The results showed that the ewes treated with ECG improved some productive traits, including average birth weight and the number of offspring (twins). The results also showed that the Awassi ewes treated with ECG for the short period led to higher blood progesterone levels compared to the GnrH treatment. The results also showed a significant increase in zinc levels in the ewes treated with ECG and GnrH for the short period compared to the long period.

Introduction

Sheep reproduction follows a seasonal pattern, leading to variations in product availability throughout the year to meet consumer demand. In this context, local sheep breeds play an important role in providing meat and dairy. Local sheep are characterized by their tolerance to environmental and climatic conditions, although they have lower production efficiency compared to other sheep breeds [3]. Improving sheep reproduction is a research priority to increase productivity and quality. Therefore, it is necessary to induce reproduction outside the traditional season. Recent studies have explored the possibility of using vaginal sponges and injections of exogenous hormones to facilitate the stimulation and synchronization of estrus during the estrus period to control the reproductive activity of sheep [9]. GnRH is an important factor in regulating the reproductive cycle, playing a key role in regulating puberty and fertility in the hypothalamus [15]. At the onset of puberty, GnRH activates the gonadal axis, as it is secreted pulsatile from the hypothalamus to the anterior pituitary gland, enhancing the synthesis and secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [16]. Equine chorionic gonadotropin (ECG) is a hormone that may play a role in improving mating outcomes and supporting pregnancy. Vaginal sponges are commonly used with ECG to improve fertility rates by stimulating follicular growth and thus increasing ovulation rates [4]. Therefore, the current study aims to estimate the hormonal changes and hematological

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characteristics after using two techniques (vaginal sponge insertions and intramuscular injection of GnRh hormone and ECG) for two types of ewes (Awassi and Krishi.(

Materials and Methods

The study was conducted at Al-Faihaa Station and the laboratories of the Animal Production Technology Department at the Technical College/Al-Musayyab. Thirty-two Awassi ewes, aged between 1 and 4 years, were used in this study. All ewes were under veterinary supervision.

The ewes were divided into four groups, each containing eight ewes. The first group was treated with ECG for a short period (please, add the intervals), the second group was treated with ECG for a long period (please, add the intervals), the third group was treated with GnRH for a short period(please, add the intervals), and the third group was treated with GnRH for a long period (please, add the intervals.(

Traits studied

Hematological traits

Five ml of blood was drawn from the jugular vein of experimental animals in the morning, at three times a day: early pregnancy, mid-pregnancy, and late pregnancy, using a Vacationer tube containing an anticoagulant (heparin). The blood samples were centrifuged at 3,000 rpm for five minutes to separate the plasma from the blood and stored in a freezer (-20 ^{o}C) until serum zinc analysis was performed.

:1Zinc.

:2Progesterone determination.

Progesterone levels were determined using an ELISA device (Minivides, France), using the KIT Biosystem, a US company, according to the method of [12.[

Productive Traits

:1Average Birth Weight

:2Percentage of Births (Twins(

Statistical Analysis

The Statistical Analysis System [21] program was used to analyze the data to study the effect of various factors (sheep type and treatment) on the studied traits. This was done according to a completely randomized design (CRD). Significant differences between means were compared using the [11] multinomial test.

eij: Random error with a normal distribution with a mean equal to zero and a variance of σ2e.

The second mathematical model: to study the effect of treatment

$Yij = \mu + Ti + eij$

Where:

Ti: Effect of treatment i (ECG: short interval, ECG: long interval, GnRH: short interval, GnRH: long interval.(

Results and Discussion

The effect of using ECG and GnRH hormones for two periods on blood progesterone concentrations in female Awassi sheep.

The results of Table (1) showed highly significant differences ($P \leq 0.01$) in progesterone levels, with the T1 treatment outperforming the other treatments, recording (8.93 ± 0.26). Progesterone is a steroid hormone, synthesized from cholesterol and produced by the yellow cells in the corpus luteum, the placenta, and the adrenal gland [6]. Progesterone performs several functions, the most important of which is preparing the endometrium for implantation and maintaining pregnancy by increasing the activity of the endometrial gland and inhibiting the secretion of luteinizing hormone (LH) at high levels[9,10,22] . Synthesized progesterone is used to normalize estrus in small ruminants, especially sheep, and depends primarily on increasing the level of progesterone. It prevents the feedback of estradiol and inhibits

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the secretion of FSH and LH, giving a blood hormonal profile similar to the luteal phase in females[4 (Alvarez et al., 2007; Dogan et al., 2008). After that, the concentration of progesterone in the blood is reduced and the inhibitory effect on the hypothalamus is removed. The pituitary gland begins to secrete GnRH, which is then stimulated by the secretion of FSH and LH, leading to the development of follicles and the production of sufficient amounts of estradiol, thus triggering estrus and ovulation [1 .[

Several researchers have indicated that administering progesterone through various methods has yielded favorable results regarding reproductive parameters, particularly when used with the mare's serum eCG or human gonadotropin (hCG) after removal of the corpus luteum. This enhances the action of FSH, or when PGF2α is injected 24 or 48 hours before removal, which leads to the removal of the corpus luteum, inhibiting its activity, and reducing the duration of sexual diapause, which can be very prolonged in females if left to reproduce naturally[18,23] (Tamer and Al-Hamedawi, 2013; Moura et al., 2014.(

Table 1: Effect of using ECG and GnRH hormones for two periods on the concentration of progesterone in the blood of female Awassi sheep.

Treatment	Mean ± standard error of progesterone (ng/ml)
	Awassi females
T1 ECG Short interval	0.26± 8.93 A
T2 ECG Long interval	0.20± 8.17 B
T3 GnRH Short-term	0.12± 7.00 C
T4 GnRH hormone long term	0.22± 7.04 C
Significant level	**

Means with different letters are significantly different from each other.
** (P≤0.01).

The effect of two periods of ECG and GnRH supplementation on blood zinc concentrations in female Awassi and Krishi sheep.

The results in Table 2 showed highly significant differences (P≤0.01) in zinc levels, with T1 and T3 outperforming the other treatments, recording (16.05 ± 0.24) and (15.91 ± 0.18), respectively.

These results can be explained by zinc's role in the functioning of the hypothalamus, pituitary gland, and gonads. Furthermore, zinc is also involved in sperm fertilization. Therefore, low zinc concentrations negatively affect sex hormone concentrations [13]. Adrenal

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hormones have many functions in the body, the most important of which are metabolism and maintaining body temperature. Zinc helps the thyroid gland perform optimally by secreting Thyroid Releasing Hormone (TRH) [26]demonstrated that the defect in sex hormone production is the result of a decrease in zinc concentration, and that zinc deficiency leads to a defect in follicle growth in females and sperm formation in males due to a defect in LH [8]. Zinc deficiency causes enzymes to lose their function. These enzymes play an important role in the production of certain steroid hormones, such as gonadotrophin-releasing hormone (GnRH) in animals, or by affecting the secretory cells of the pituitary gland, which play a role in the secretion of FSH, LH, and prolactin, which regulate the production of sex hormones. Sex hormone levels are linked to blood zinc levels [7]. Studies have demonstrated the importance of zinc for normal cell growth, especially immune cells, neutrophils, natural killer cells, phagocytic cells, and cytokine production [20]. B and T cells are affected by zinc deficiency. Zinc is essential for DNA and RNA synthesis, cell division and activation, and the regulation of programmed cell death (apoptosis). Therefore, a deficiency in this element negatively impacts cytokine function [21[

Table 2: Effect of two periods of ECG and GnRH on blood zinc concentrations in female Awassi sheep.

Treatment	Mean ± standard error of zinc concentration (ppm)
	Awassi females
T1 ECG Short interval	0.24± 16.05 A
T2 ECG Long interval	0.16± 15.03 B
T3 GnRH Short-term	0.18± 15.91 A
T4 GnRH hormone long term	0.12± 15.14 B
Morale level	**
Means with different letters are significantly different from each other. ** (P≤0.01).	

The effect of using ECG and GnRH hormones for two periods on the birth rate in Awassi sheep.

The results of Table (3) showed a significant difference (P≤0.05) in the birth rate. The T1 treatment increased with the short-term use of ECG, recording (1.29 ± 0.18), compared to treatments T3 and T4, which recorded (0.71 ± 0.18) and (0.571 ± 0.20), respectively.

The result can be explained by the possibility of obtaining a higher percentage of twins

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using a hormonal program that includes progesterone and eCG, compared to using a hormonal program that includes progesterone and pituitary follicle-stimulating hormone (P-FSH). The reason for this is that the half-life of eCG is (4-5) days, while that of follicle-stimulating hormone (FSH) does not exceed a few hours [22]. The use of vaginal sponges saturated with 60 mg medroxyprogesterone acetate for 13 days with 500 IU of eCG gave a twinning rate of 15%, while [24] showed that by treating Hamdaniya ewes with vaginal sponges for 14 days with injections of different doses of eCG 500, 600 and 750 IU for the three groups, respectively, the twinning rate was 12.4%, 15.6% and 16.75% for the three groups, respectively. [2] added that the twinning rate reached 66.2%, 36.4% and 20.2% by using vaginal sponges with different doses of eCG 700, 500 and 300 IU for the three groups and concluded that the twinning rate increases with the increase in the dose of gonadotropin, while researcher [14]reported a pregnancy rate of up to 50% with no twinning.

[25]observed that the estrus rate of ewes during the first 6 days of the breeding season increased significantly when using feed-grade progesterone (MGA) with PG-600, PG-600 alone, and MGA alone. The number of lambs born per ewe was 1.7, 1.5, and 1.6, respectively. Al-Naimi et al. (2009) achieved a twinning rate of up to 40% in their study on local ewes using progesterone as an intramuscular injection at a dose of 7 mg for 12 days, followed by 500 IU of eCG. [19] showed that hormonal treatment, including the use of different doses of eCG (350, 450, and 550 IU), had an effect on the twinning rate, reaching 50, 35, and 40% in the treatment groups, compared to 12% in the control group.

Table 3: Effect of using eCG and GnRH hormones for two periods on the birth rate (twins) in Awassi sheep.

Treatment	Mean ± Standard Error Birth Rate
	Awassi
T1 ECG Short interval	0.18± 1.29 a
T2 ECG Long interval	0.00± 1.00 ab
T3 GnRH Short-term	0.18± 0.71 b
T4 GnRH hormone long term	0.20± 0.571 b
Morale level	*
Means with different letters are significantly different from each other. * (P≤0.05), ** (P≤0.01), NS. Not significant.	

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The effect of using ECG and GnRH hormones for two periods on the average birth weight of Awassi sheep.

The results of Table (4) showed a significant increase (P≤0.05) in the birth weight of the Awassi breed. Treatments T1 and T2 outperformed, recording (4.27 ± 0.12) and (4.24 ± 0.07), respectively, compared to treatment T4, which recorded (2.40 ± 0.85.(

The result can be explained by the fact that the use of mare's serum hormone (MSH) is usually combined with vaginal sponges to improve reproductive efficiency in ewes, increase fertility rates, follicle growth and development, and increase ovulation rates and birth weight of the offspring [14]. The mare's serum hormone (MSH) affects reproductive performance in sheep, as treatment with 500 IU MSH outperformed the control group in terms of birth weight [13]. [17] found that treatment with mare's serum hormone (MSH) increased ovarian response and pregnancy rate at a dose of 400 IU, with estrus onset at 91.1%, pregnancy rate at 59.8%, and birth weight increases. Studies have shown that using MSH with HCG leads to a shorter estrus period, a significant increase in birth weight, and an increased rate of twinning. [5] reported a highly significant difference (P<0.01) in the duration of estrus when treating ewes with MSH. The birth rate in ewes was 93% compared to the control group (73%), both in and out of season, and an increase in birth weight .

Table 4: The effect of using ECG and GnRH hormones for two periods on birth weight in Awassi sheep.

Treatment	Mean ± Standard Error Average Birth Weight (kg)
	Awassi
T1 ECG Short interval	0.12± 4.27 a
T2 ECG Long interval	0.07± 4.24 a
T3 GnRH Short-term	0.81± 3.13 ab
T4 GnRH hormone long term	0.85± 2.40 b
Morale level	*
Means with different letters are significantly different from each other. * (P≤0.05).	

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