University of Thi-Qar Journal of agricultural research

Thi-Qar Journal of agricultural research

ISSN Onlin:2708-9347, ISSN Print: 2708-9339 Volume 15, Issue 1 (2026) PP 128-135

https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/semrcx23

Effect of HCG hormone Supplement after the Natural Mating on Some Reproductive Performance in Local Iraqi Ewe

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Abstract

This study evaluated the effect of human chorionic gonadotropin on reproductive performance and progesterone concentration in Iraqi ewes following natural mating. The experiment was conducted at Al-Asimah Station, located 25 km northwest of Baghdad, from November 2024 to May 2025. Fourteen healthy, non-pregnant ewes aged 2–3 years were randomly allocated into two equal groups (n=7 each) after estrus synchronization. Group 1 received 300 IU HCG intramuscularly at mating and again on day 4 post-mating, while Group 2 served as an untreated control. Estrus response, onset, and duration were recorded. Blood samples were collected on days 0, 5, and 17 to determine progesterone and estrogen levels. Ultrasonography was performed on days 30 and 45 to assess conception and pregnancy rates, followed by recording lambing rate, litter size, and number of offspring at parturition. Results demonstrated a significant reduction ($p \leq 0.01$) in estrus duration in G1 compared to G2. Although conception rate did not differ significantly between groups, pregnancy rate increased significantly ($p \leq 0.05$) in G1. Moreover, HCG treatment significantly improved lambing rate ($p \leq 0.01$), litter size ($p \leq 0.05$), and the incidence of twinning. Progesterone concentrations were significantly higher in the treated group, indicating enhanced luteal function. In conclusion, HCG administration at and after mating improved reproductive efficiency in Iraqi ewes by increasing pregnancy rate, lambing rate, litter size, and progesterone levels.

Key words: Ewe, estrus synchronization, HCG hormone, natural mating.

I. Introduction

This study aims to evaluate the effect of human chorionic gonadotropin (hCG) supplementation after natural mating on reproductive performance in local Iraqi ewes. Estrus synchronization in ewes is a key technique to enhance reproductive efficiency and improve the utilization of ewes by shortening the generation interval **(Gómez-Brunet et al., 2007)**. A major factor that limits the reproductive performance of farm livestock is embryonic mortality. Embryonic losses during the first 3 weeks of pregnancy account for approximately 25–40% of fertilized eggs in cattle and sheep, resulting in reduced conception rate and litter size **(Khan et al., 2007)**. Embryonic death occurs during early stages of pregnancy and represents one of the most common causes of economic loss in the sheep industry. Nearly 40% of sheep embryos fail to survive during the first month of gestation, with 70–80% of these losses occurring between days 8 and 16 after conception **(Rostami et al., 2017)**. Inadequate luteal function is a major cause of pre-implantation losses in sheep **(Khan et al., 2003)**. Progesterone (P4) plays a critical role in regulating endometrial secretions necessary for embryonic growth and is essential for the establishment and maintenance of pregnancy **(Spencer et al., 2006; Hussain et al., 2016)**. Numerous pharmacological and management strategies have been developed to improve reproductive efficiency in sheep **(Mirzaei and Asadi, 2014)**. Among these, human chorionic gonadotropin (hCG) has been widely used due to its luteinizing hormone (LH)-like activity, which induces ovulation, enhances luteal development, and increases endogenous progesterone secretion **(Nephew et al., 1994)**. Post-mating administration of hCG has been shown to elevate progesterone levels, promote placentation, and support embryonic and fetal development. Additionally, hCG treatment during seasonal anestrus has been

University of Thi-Qar Journal of agricultural research

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https://jam.utq.edu.iq/index.php/main https://doi.org/10.54174/semrcx23

associated with improved lambing rates **(Khan et al., 2007; Al-Zubaidi, 2017)**. The significance of this study is particularly relevant to Iraqi sheep production systems, where indigenous breeds such as the Awassi often exhibit suboptimal reproductive performance. Under local conditions characterized by heat stress, semi-arid environments, and fluctuating nutritional availability, ewes frequently experience inadequate luteal function and increased embryonic loss. These challenges contribute to reduced fertility rates, lower lambing percentages, and economic inefficiencies for farmers. Early embryonic mortality, especially during the critical period of maternal recognition of pregnancy, represents a major constraint to flock productivity in Iraq **(Al-Mutar, 2017)**. Therefore, the application of hCG as a practical hormonal intervention may help stabilize progesterone levels, enhance embryo survival, and improve overall reproductive outcomes. By addressing these limitations, this study provides a valuable approach to improving flock productivity, supporting farmer livelihoods, and contributing to food security in Iraq.

Ethical Approval

Prior to conducting any experiments, the experimental protocol and design utilized in the present study were reviewed and sanctioned by the Ethics Committee at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq (Number P. G/534 on 03/03/2026).

II. Martial and methods

Animals and experimental design

The study was done at a specific area identified Al-Asimah Station, which is located 25 kilometers northwest of Baghdad, specifically in Khan Dhari. The local Iraqi sheep herd consisted of fourteen ewes, aged between 2 – 3 years determined by breeding record and verified by dental formula with 4 fertile rams used in this study. The animals subjected to careful clinical examination and ultrasonography examination to determine that they are non-pregnant with healthy and free from diseases. The animals kept at semi opened shade shelter supplemented with drinking water ad libitum daily per ewe according to **Naji, and Hussein, (2024).** The experiment was extended from November of 2024 to May of 2025.

Animals were randomly divided into two equal groups, each consisting of seven ewes, following the protocol used after estrus synchronization. Group 1 (G1) received an intramuscular injection of 300 IU of human chorionic gonadotropin (hCG) at the time of mating, with a repeat dose on day 4 post-mating. Group 2 (G2) served as the control group and did not receive any progesterone or hCG supplementation after mating. This division allowed for a controlled comparison of the effects of hCG treatment on reproductive outcomes.

Estrus detection after synchronization and hand mating

The animals had been evaluated for estrus appearance to record the animal's response percentage to synchronization protocol, the duration of response after treatment, and the duration of the estrus phase. The timing of estrus detection was two hours each day, in the morning and evening, with the introduction of rams of established fertility**, Naji and Hussein (2024).** The ewe that exhibited indicators of estrus activity after treatment was evaluated visually to make sure they were genuinely in the estrus period. The active rams serviced (hand-mated) all estrus females in the estrus phase according to **Al-Hamedawi *et al. (2016)* and Fesseha and Degu (2020).**

Blood samples to assay the progesterone hormone

All groups had blood samples taken at the time of mating (day 0), day 5, and day 17. Subsequently, 5 milliliters of blood are extracted from the jugular vein into vacuum tubes (Gel and Clot Activator); these tubes are kept cold until centrifugation **(Naji and Hussein, 2024).** Before progesterone concentrations were measured, the serum was separated by centrifugation after being collected for 15 minutes at 3000 RPM and then refrigerated at -18°C according to **Bowen and Remaley (2014).** The ELISA Test System was used to assay the concentration of progesterone and estrogen hormones.

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Pregnancy detection and follow up until parturition

Pregnancy was diagnosed by the application of an ultrasonographic examination with a transabdominal sector approach for early pregnancy (6 MHz) 30 days after mating, and the examination was repeated at 45 days after mating to confirm the pregnancy in the ewes in the study by **Alwan (2016).** The pregnant animals were followed up until parturition, and the different parameters of reproductive performance were recorded and evaluated using variables as defined below **(Quintero-Elisea *et al*., 2011; Hussein, 2017).**

Estrus response: The number of ewes showing standing estrus/total number of ewes in each treatment group X 100

Duration response: days interval from end of treatment to the time when ewes expressed standing estrus (heat).

Estrus duration (heat duration): time (hours) between the first and last accepted mount.

Non-return to estrus is the failure of a female to show signs of heat after breeding, serving as an initial indicator of pregnancy.

Time to conception: time of nonreturn to estrus and confirmed at lambing

Pregnancy rate: Number of ewes lambed/number of ewes mated X100.

Lambing rate: Number of ewes lambed/number of pregnant ewes in each group X 100.

Litter size: Number of total lambs/numbers of lambing ewes in each group

Statistical analysis and reproductive performance equations

The SAS Statistical Analysis System (2018) software was used to evaluate the effect of different groups on the study parameters. To determine significant differences between means, the least significant difference (LSD) method was applied. Additionally, the Chi-square test was employed to assess the statistical significance of percentages at probability levels of 0.05 and 0.01 (Bartlewski et al., 1999; Vlahek, 2023).

III. Result and discussion

Estrous response, duration of Response and Duration of estrus in local Iraqi ewes

The results of the current study in table (1) revealed the estrus response that 6/7 (85.71%), and 6/7 (85.71%) After estrous induction regimens, the ewes in (G1and G2, respectively) and no significant differences between groups. The estrus response after used intra vaginal sponge impregnate with progesterone 60 mg 12 days and injection the eCG 48 hours before vaginal sponges withdraw, they ranged between 85 .71%. This result in current study agreement with **Ozyurtlu *et al.* (2010)** the result of his study agreement with my current study when record the estrus response range between 87% -90% when study the characterization of estrus induction in awassi ewes during the non- breeding in turkey.

Also, in table (1) reviled the duration of response / hours in G1 and G2 (40.03 ±1.71and 39.62 ±0.89 respectively) we show non-significant effect between all the two groups this result due to the use of vaginal sponges and eCG, which led to synchronized estrus in short time interval after sponge withdraw. This result explores due to the use of the same protocol in synchronizing estrus and then injecting eCG when vaginal sponges withdrawing and administration of eCG during estrus synchronization programs could increase the number of dominant follicles, prevent follicle regression, improve estrus expression. This result agreement with **Payan *et al.* (2022)** when record the interval between the last progesterone injections to the onset of estrus was shorter in those ewes that received eCG compared to control when study the time of eCG administration in progesterone injection-based estrus synchronization protocol could affect the time of estrus expression in ewes during non-breeding season.

Also, in table (1) the result of duration of estrus / hours in G2 (30.18 ±0.2) hrs. appeared highly significant increase value (P≤0.01) when compared with G1 (24.58 ±0.27 hrs.) this result explore the treatment groups (G1) administering HCG during mating accelerated ovulation and corpus luteum development. This result agreement with **Mırzaeı *et al.* (2014)** when study the reproductive performance after HCG or GnRH administration of long-term progestogen treatment of fat tailed ewes during seasonal anestrus, he explores the effect of hCG, which is similar to LH in function, can induce oocyte maturation and ovulation.

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Table 1: animal's response %, duration of response and duration of estrus / hours in different groups of treated Iraqi ewes.

Group NO.	NO. of ewes	NO. of estrus response %	NO. of non-estrus response %	Duration of Response (Mean± SE)	Duration of estrus (Mean± SE)
Group 1 (Intra-vaginal sponges 60 mg + hcg)	7	6 (85.71%)	1 (14.29%)	40.03 ±1.71 a	24.58 ±0.27 b
Group 2 (Control) Intra-vaginal sponges 60 mg	7	6 (85.71%)	1 (14.29%)	39.62 ±0.89 b	30.18 ±0.23 a
Total	14	**x^2 Value** = 7.027 **		L.S.D. = 3.450 NS	L.S.D. =0.945 **
Means having with the different letters in same column differed significantly. ** (P≤0.01), NS: Non-Significant.					

Effects of HCG injection on pregnancy rate and twining in local Iraqi ewe ewes:

In table (2) The result of current study fond significant effect between groups in (P≤0.05) the high percentage pregnancy rate in G1 (85.71%) than G2 (28.57%) conversely in non-pregnant rate the fond significant effect (P≤0.05) between groups the high percentage in control groupG2(71.43%) than G1 (14.29%) this result explore the role of HCG supplement, suggest that HCG act LH-like activity may increase the production of P4 before the critical time of maternal recognition of pregnancy (MRP). This result agreement with **Rostami *et al.* (2017)** when study the effect of post-mating hCG or progesterone administration on reproductive performance of ewes, he confirmed either progesterone or hCG induced P4 production after day 12 may increase IFN-τ production and prevent luteolysis by preventing PGF2α secretion.

Also, the result in table (2) reviled the percentage of single and twining rate after treatment with HCG after mating ewes. The result found significant effect increase in (P≤0.05) between groups the high percentage twining lamb in G1 than in control group G2(0.00%) conversely in single lambing high percentage in control group G2(100%) than G1 (50.00%) this results related to HCG act improvement the maternal concentration of P4. This result agreement with **Khan *et al.* (2007)** he explore the high percentage twining rate after used HCG to luteotropic stimulation of hCG may result from the conversion of small luteal cells to large luteal cells which consequently secrete higher concentrations of P4 or may even be due to ovulation or luteinization of growing follicles and the formation of accessory CLs.

4.3: Effects of HCG injection on pregnancy rate and twining in local Iraqi ewes

Group NO.	NO. of ewes	NO. of pregnant ewes %	NO. of non-pregnant ewes %	Single pregnancy %	Twining pregnancy %
Group 1	7	6 (85.71%)	1 (14.29%)	3 (50.00%)	3 (50.00%)
Group 2	7	2 (28.57%)	5 (71.43%)	2 (100%)	0 (0.00%)
Total	14	12 (57.14%)	9 (42.86 %)	7 (66.66 %)	5 (50 %)
x^2 Value		3.507 *		3.491 *	
*** (P≤0.05).**					

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Effects of HCG injection on Conception rate, Pregnancy rate and Lambing rate in different groups in Iraqi ewes:

The table of (3) revealed the effect of HCG injection on conception rate, pregnancy rate and lambing rate and litter size. The result of current study found non-significant effect between G1 (85.71%) and control (85.71%) in conception rate. But in pregnancy rate we found significant effect between groups the high percentage in G1(85.71%) and low percentage in control group G2 (28.57%) also in lambing rate fond significant effect between treatment group than control group the high percentage in G1(128.57%) than control group G2 (28.57%). In litter size found significant effect increase in value($P \leq 0.05$) between treatment group G1 (1.50) than control group G2 (1.00).The result in the current study the ewe in treatment group and control group has fertilization during mating and not different in conception rate after diagnosis pregnancy in ultrasonography but after 45 day to detection pregnancy rate we found superior treatment group with HCG treatment than control group in pregnancy rate , lambing rate and litter size , this result returns to HCG supplement lead to improvement the reproduction performance in ewe treatment in study and early embryonic death occur in control group . My result agreement with **Rostami *et al*. (2017)** he confirms that HCG is effective to increase the plasma concentration of P4 and insufficient luteal function and consequently reduced concentration of P4 is one of the major concerns affecting embryonic survival in ewes. In sheep, MRP usually occurs around day 12 after breeding to establish and maintain the gestation. Progesterone has a critical role in preparing the uterine lumen for embryonic implantation and maintenance of pregnancy **(Spencer and Bazer, 2006)**. In addition to inducing P4 synthesis, hCG also plays a major role in placental vascularization and enhances reproductive efficiency in livestock **(Cole 2012).**

Table 3: Effects of HCG injection on Conception rate, Pregnancy rate and Lambing rate in different groups in Iraqi ewes:

Group NO.	NO. of ewes	Conception rate %	Pregnancy rate %	Lambing rate %	Litter size
Group 1	7	6 (85.71%)	6 (85.71%)	9/7 (128.57%)	9/6: 1.50
Group 3	7	6 (85.71%)	2 (28.57%)	2/7 (28.57%)	2/2: 1.00
Total	14	12	8	11	14/11
x^2 Value	--	0.121 NS	3.031 *	7.501 **	P-value = 0.0285 *
*** (P≤0.05), ** (P≤0.01), NS: Non-Significant.**					

Effects of HCG injection on progesterone concentrations in ewes at different times

Result of P4 level in (ng/ml) day 0, 5 days and 17 days of application different hormonal regimens for estrus synchronization in ewe are presented in table (4). The current study outcome that revealed serum progesterone concentration at day-0 there were no significant differences between all groups. At 5 days G1 (6.15 ±0.16) ng/ml demonstrated significant effect increase in ($P \leq 0.05$) when compared control group (2.86 ±0.11 ng/ml). At 17 days G1 (10.13 ±1.61) ng/ml demonstrated significant differences increase in ($P \leq 0.05$) when compared to G2 the progesterone value (**2.49 ±0.75** ng/ml). In G1 at 17 days (10.13 ±1.61) ng/ml there is highly significant increase at ($P \leq 0.05$) when compression in day-0 (0.662 ±0.04) ng/ml and 5 days (6.15 ±0.16), while the result in 5 days appear highly significant increase at ($P \leq 0.05$) when compression in day-0. In G 2 at 5 days and 17 days (2.86 ±0.11 ng/ml and 2.49 ±0.7 ng/ml respectively) showed highly significant increase at ($P \leq 0.05$) when compression in day-0 (0.702 ±0.05), and there is no significant differences between 5 days and 17 days. The result of current study show the progesterone hormone increased after mating in ewe treated with HCG than control this reason to ovulation in different follicle and formation of CL and accessory

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corpus luteum because HCG action like to LH hormone this result agreement with **Cam and Kuran, (2004),** when confirmed the hCG enhances progesterone production and increases uterine secretions that are embryotropic when supported by the fact that the P4 levels increased significantly more on day 7 in and that the levels were significantly higher in treatment group. Also, agreement with **Aslan *et al*. (2011)** when record the administration of hCG causes increasing progesterone levels because it causes the accessory corpus lutea to form increases the blood flow in the corpus luteum. In conclusion, the use of the estrous synchronization protocol and the injection of HCG after natural mating led to an increase in the pregnancy rate, birth rate, and litter size, as well as an increase in p4 concentration after progesterone. so recommended used hCG hormone after natural mating in synchronized ewe in Iraq.

Table 4. Effects of HCG and Progesterone injection on progesterone concentrations ng/ml at different times (Mean ± SE).

Group	Time of collection			LSD value
	Day-0	5 days	17 days	
Group 1	0.662 ±0.04 A c	6.15 ±0.16 A b	10.13 ±1.61 A a	1.906 *
Group 2	0.702 ±0.05 A b	2.86 ±0.11 B a	2.49 ±0.75 B a	0.885 *
LSD value	0.115 NS	0.367 *	3.747 *	--
Means having with the different big letters in same column and small letters in same row differed significantly. * (P≤0.05).				

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