EFFECT OF KISPEPTIN-10 AS AN ALTERNATIVE TO ECG IN ESTRUS SYNCHRONIZATION PROTOCOL ON IMPROVING THE REPRODUCTIVE PERFORMANCE OF KARADI EWES

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ABSTRACT

This study was conducted to examine the effect of Kisspeptin-10 as an alternative to equine chorionic gonadotropin (eCG) in estrus- synchronization protocol for improving the reproductive performance of Karadi ewes. Forty adult ewes of 3-7 years old and 65–70 kg live body weight were equally divided into four equal groups. Animals were inserted with a progestagen (60 mg MAP)-impregnated sponges for 13 days as estrus synchronization protocol. Following withdrawal of sponges, first group was served as control (C) and intramuscularly injected with normal saline only. The second group was intramuscularly injected with 250 IU of eCG (eCG), while those of third and fourth groups were intravenously injected with 4 and 8 µg /kg body weight of Kisspeptin-10, referred as Kisspeptin1 and Kisspeptin2 groups respectively. Serum progesterone and LH concentrations did not significantly differ among groups before estrus synchronization protocol and eCG or Kisspeptin treatments. The two Kisspeptin-injected groups attained better ($P \leq 0.01$) overall mean estrus (100%), fertility (90%), conception (90 and 100%, respectively), lambing (90%), and twinning (10%) rates with lesser barrenness (10%) as compared with the other groups. Moreover, eCG and Kisspeptin groups exhibited greater ($P \leq 0.01$) percentage of increasing fecundity (233%) and extra lambs born (0.7) than the control group. In conclusion, treatment of Karadi ewes either with Kisspeptin-10 or eCG hormones enhanced the overall reproductive performance, but did not affect serum progesterone and LH concentrations.

Keywords: Kisspeptin; eCG; Hormones; Reproductive Performance; Karadi ewes.

تأثير هرمون Kisspeptin-10 كبديل لهرمون eCG في برنامج توحيد الشياع لتحسين الأداء التناسلي للنعاج الكرادي

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المستخلص

اجريت هذه الدراسة لبيان تأثير هرمون Kisspeptin-10 كبديل لهرمون المناسل المشيمي للخيول (eCG) في برنامج توحيد الشياع لتحسين الاداء التناسلي لدى النعاج الكرادي. استخدمت في هذه الدراسة اربعون من نعاج الكرادي البالغة بعمر 3 – 7 سنوات ومعدل وزن حي 65 – 70 كغم وتم تقسيمها الى اربع مجاميع متساوية. تم دفع الاسفنجات المهبلية المشبعة بالمشابه الصناعي للبروجستيرون (60 ملغم من مركب MAP) للنعاج جميعها لمدة 13 يوم بهدف توحيد الشياع. وبعد سحب الاسفنجات المهبلية، عدت المجموعة الأولى كمجموعة سيطرة (C) وحقنت بالمحلول الفسيولوجي فقط، في حين حقنت المجموعة الثانية عن طريق العضلية بهرمون eCG بجرعة مقدارها 250 وحدة دولية، في الوقت الذي حقنت فيه المجموعتين الثانية والثالثة عن طريق الوريد بهرمون Kisspeptin-10 بجرعة مقدارها 4 و8 مايكروغرام / كغم من وزن الجسم ورمز لهما Kisspeptin 1 و Kisspeptin 2 على التوالي. لم يختلف تركيز هرموني LH والبروجستيرون معنوياً بين المجاميع المختلفة سواء قبل توحيد الشياع او بعد المعاملة بهرموني eCG و Kisspeptin-10. من جانب آخر، حققت المجموعتين الثالثة والرابعة المعاملتين بهرمون Kisspeptin-10 افضل ($P \leq 0.01$) نسبة ظهور للشياع (100 %) والخصوبة (90 %) والأخصاب (90 و100 % على التوالي) والولادات (90 %) والتوائم (10 %) واقل نسبة للتفويت (10 %) مقارنة ببقية المجاميع. كما حققت المجاميع المعاملة بهرموني eCG وKisspeptin-10 اعلى ($P \leq 0.01$) نسبة زيادة في الخصب (233 %) واعلى عدد حملان اضافية مولودة (0.7) مقارنةً بمجموعة السيطرة. يمكن الأستنتاج بان معاملة النعاج الكرادي بهرموني eCG وKisspeptin-10 حسنت من الاداء التناسلي الكلي للنعاج لكنها لم تؤثر على تركيز هرموني LH والبروجستيرون.

الكلمات المفتاحية: Kisspeptin، eCG، الهرمونات، الأداء التناسلي، النعاج الكرادي.

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INTRODUCTION

Increasing sheep productivity by increasing lambing frequency and ⁄ or fecundity is considered important in the development of sheep production in world. On the other hand, increasing rate of fecundity in sheep offers the opportunity to increase the efficiency of lamb meat production (26). Estrous synchronization is a valuable management tool which has been successfully employed to enhance reproductive efficiency, particularly in ruminants (27, 28). Estrus in the ewe is a less obvious event than in other ruminants (37). Hence, a detailed detection of estrous stages becomes crucial in these species, particularly in hand-mating or artificial insemination (AI) protocols. The eCG injection is required to stimulate follicular growth, leading to a higher ovulation, lambing and twinning rates by anestrous ewes in and out-breeding seasons (1,7, 8, 21). Kisspeptins are a recently discovered family of neuropeptides with central role in regulating the reproductive functions of livestock animals. They are cleaved from a single 145 amino acid precursor to peptides ranging from 54 to 10 amino acids in length with equivalent biological activity (10, 25, 32). Kisspeptins are released from neurons in the hypothalamus and via their receptor (Kiss1r), which is present on GnRH neurons, stimulate GnRH release with a subsequent rise in circulating LH, FSH along with the progesterone in laboratory and livestock animals (5, 6, 13, 30, 31). Although GnRH neurons are a critical component of the reproductive axis, Kisspeptin peptides have been identified recently as vital upstream regulators that integrate central and peripheral signals with GnRH release, thereby playing a pivotal role in the control of reproduction (16, 34). Recently, it was discovered that the majority (~90%) of GnRH neurons located in the ovine hypothalamus are immunoreactive for Kisspeptin (36). This is unique to the sheep and has not been observed in other non-ruminant species. Co-localization of GnRH and Kisspeptin within cells of the hypothalamus and GnRH neurosecretory terminals of the median eminence suggested that the two peptides might be co-secreted into the hypophyseal portal blood to act on the pituitary gland (48). Using a more specific antibody (11, 19, 24) it was found that Kisspeptin cells are ocated predominantly in the arcuate nucleus (ARC) of the ovine and rat brains and co-produce dynorphin and neurokinin B (NKB), two other factors that regulate reproduction. Taken together with the knowledge that 100% of the cells co-producing Kisspeptin dynorphin-NKB also express estrogen receptor alpha (ESR1), they are likely to be key mediators of GnRH secretion (48). Moreover, Kisspeptin not only induced gonadotrophe activation and LH release, but also stimulated growth hormone (GH) secretion by a small subset of somatotrophes (22). Thus, there is the possibility that Kisspeptin might regulate both Luteinizing hormone (LH) and GH, a basic requirement for proposing Kisspeptin as a coordinator of the reproductive and somatotrophic control mechanisms suggesting that Kisspeptin may serve as an integrator between metabolism and reproduction (14). Limited results concerning the influence of Kisspeptin on hormonal profiles and ovulation rate of ewes were recorded (11, 12, 45, 46, 47). However, the effect of Kisspeptin as GnRH promoter post-progestagen synchronization protocol in comparison with equine chorionic gonadotropin (eCG) hormonal protocol on blood profile and reproductive performance of sheep in general and Karadi breed in particular were not previously investigated. Therefore, this study was undertaken to examine if the Kisspeptin-10 be an alternative to equine chorionic gonadotropin (eCG) in estrus synchronization protocol for improving the reproductive performance and inducing hormonal profile (LH and progesterone) of Karadi ewes.

MATERIALS AND METHODS

Experimental animals

This experiment was conducted at the private farm located in Qamtaran village belonging to Ranya district, 130 kilometers North West of Sulaimani city, North of Iraq. Forty adult Karadi ewes of 3-7 years old and 65–70 Kg live body weight, 1.5-2 months post-lambing stage were used in this study. Animals were allowed to graze on natural pasture constituted of legumes, grasses and sunflower residues for 10-15 hours daily from June until mid of

December. After that (mid of December until first of March),animals were fed on barley grains (400-500 g per day ⁄ animal) and meadow hay indoors following grazing time, due to the low pasture quality during this period. Post-lambing, ewes were fed only on barley grains and meadow hay indoors. Free access of water was available for all animals. Rams were left with the ewes for 3 estrus cycles (51 days).

Kisspeptin hormone preparation

Kisspeptin-10 (Metastin 45-54) manufactured by AnaSpec, Inc., USA as a powder was used in this study. It is composed of ten amino acids (Histidine-Tyrosin-Asparagine Tryptophan-Asparagine-Serine-Phenylalanin-Glycine-Leucine-Arginine-Phenylalanin-NH2). The hormone was prepared as the company instructions, by dissolving 40 mg of Kisspeptin102 10 in one milliliter of dimethyl sulfoxide (DMSO, (CH3)2SO) in two phases (20 mg / 0.5 milliliters per phase) to facilitate the dissolving process. The volume was completed to eight milliliters using normal saline (0.9 % NaCl) solution. Dialysis of the solution was performed using dialysis tube to get rid of the toxic sulfur element existed within the DMSO constituents. Glass beaker containing the dialysis tube was put on magnetic stirrer mixer for 18 hours at 4°C condition to speed up the dialysis process. The dialysis tube was offloaded in a glass beaker and complete the volume to 40 milliliters using normal saline solution and be ready for injection process.

The Experimental treatments

Ewes were randomly divided into four equal groups (10 ewes each). Animals were inserted with progestagen (60 medroxy progesterone acetate, MAP)-impregnated sponges (SINCRO-GEST, OVEJERO Group, Spain) for 13 days as (E.S.) protocol. Following withdrawal of sponges, first group was served as control and intramuscularly injected with normal saline only (Placebo group).The ewes in group two were intramuscularly injected with 250 IU of equine chorionic gonadotropin, eCG (Serigan, OVEJERO Group, Spain), while those of groups three and four were intravenous injected with4 (Kisspeptin1) and 8 (Kisspeptin2) µg / Kg body weight (BW) of Kisspeptin-10 respectively. The Kisspeptin administration was divided into two doses, two hours apart, according to Ezzat et al (18), Hashizume et al (23) and Al-Ameri (5) protocols. Blood samples were collected from five ewes / group selected randomly, at sponges withdrawal and two hours post hormonal treatment for serum progesterone and luteinizing hormone (LH) assay. Estrus was monitored by the shepherd during day and night. Time of estrus and mating were recorded carefully. Pregnancy was also detected by ultrasonography at 30-40 days post mating.

Blood sampling and assay: The blood sample (10 milliliters) was collected via jugular venipuncture from five ewe / group selected randomly. Serum was harvested by centrifugation (3000 rpm for 15 minutes) after allowing the blood samples to clot for 24 hours at 4°C, and then kept frozen (- 20˚C) until assay.

Serum progesterone assay: Electro-chemiluminescence immunoassay (ECLIA) method was used to measure the serum progesterone concentration (nmol / L) using Cobas e 411 automated analyzer. A commercial kit was used (Progesterone II, Roche Diagnostic GmbH, and Mannheim, Germany). The analytical sensitivity of the assay was 0.095 nmol / L; whereas, intra-assay and inter-assay coefficients of variation were 3.7 and 4.6 % respectively. The cross-reactivity (specificity) was 100, 20.7, 1.30, 0.858 and 0.211% with progesterone, 5β Dihydroprogesterone, 17α-Hydroxyprogesterone 5α-Pregnanedione and 5β-Pregnanedione 138 respectively. The cut-off value of progesterone level used to detect pregnant ewes was 3.18 139 nmol / L.

Serum LH assay: Electro-chemiluminescence immunoassay (ECLIA) method was used to measure the 142 serum progesterone concentration (mIU/ml) using Cobas e 411 automated analyzer. A 143 commercial kit was used (LH, Roche Diagnostic GmbH, Mannheim, Germany). The assay was carried out at the Laboratory of Meran Veterinary Company, Erbil. The analytical sensitivity of the assay was 0.100 mIU/ml; whereas, intra-assay and inter-assay coefficients of variation were 1.9 and 2 % respectively. The cross-

reactivity (specificity) was 100 and 0.20 % with LH and hCG respectively.

Determination of ewe's reproductive performance: Fertility percentage, litter size and the rates of estrus, conception, lambing and twinning 150 along with barrenness were calculated according to Al-Saigh and Alkass (4) at the first, 151 second and third cycles, as well as overall mean. Fecundity, percentage of increasing 152 fecundity (gain %) and extra lambs born were calculated using Palacin et al (35) equations.

Statistical analyses: Statistical computations were performed using General Linear Model (GLM) procedure in the SAS program (40), using CRD with interactions to examine the influence of hormonal treatments (eCG or Kisspeptin) and time of sampling on serum progesterone and LH concentrations. The statistical model for analysis of variance was as follows:

$Y_{ijk} = \mu + T_i + P_j + e_{ijk}$

Where:

Y_{ijk} = dependent variable (serum progesterone, LH concentrations).

μ = overall mean

T_i = effect of treatment (eCG and Kisspeptin hormones).

P_j = effect of sampling time (Before and two hours after treatment).

e_{ijk} = error term

Differences among means were computed using the Duncan multiple range test (17).Chi-square test was used to compare the differences among the reproductive performance traits (44).

RESULTS AND DISCUSSION

Serum progesterone concentrations

Serum progesterone concentrations did not significantly differ among groups before estrus synchronization protocol and eCG or Kisspeptin treatment (Figure 1), as ranged between 0.53 172 ± 0.06 and 4.69 ± 2.81nmol / L. Similarly, the differences in serum progesterone 173 concentrations among groups post treatment lacked significance (Figure 1), however, it tended to be numerically higher (+ 89%) in Kisspeptin1 group (5.15 ± 3.30 nmol / L) as 175 compared with Kisspeptin2 group (0.54 ± 0.06 nmol / L). By comparing the concentrations 176 between the two periods together, it did not significantly alter in all groups (Figure 1).

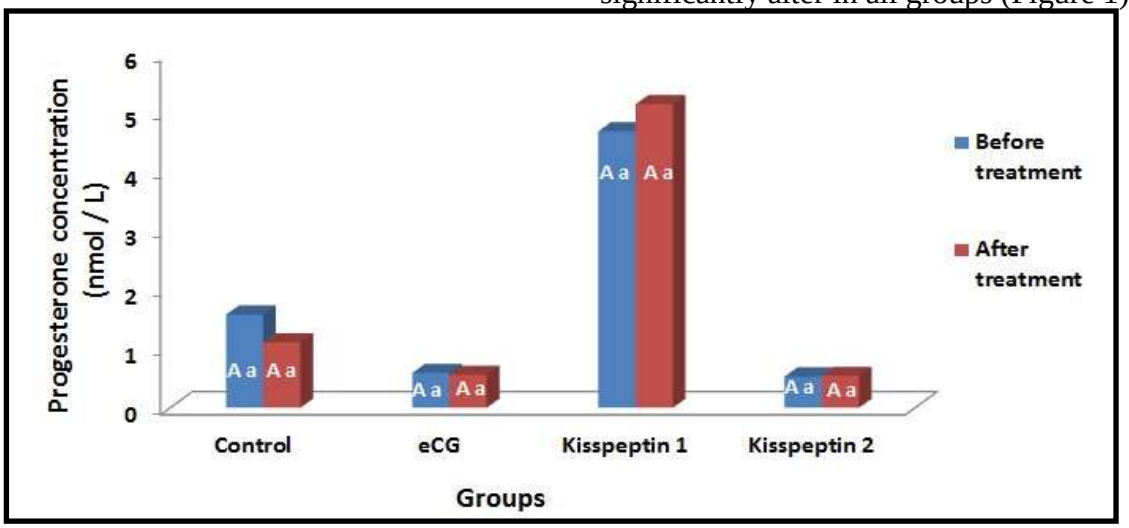

Figure 1. Serum progesterone concentrations (nmol / L) of Karadi ewes before and after treatment with equine chorionic gonadotropin (eCG) and Kisspeptin hormones post estrus synchronization

Means with small superscripts indicate comparison among treatments and capital superscripts indicate comparison between periods within each treatment. eCG = Equine chorionic gonadotropin.

Serum LH concentrations: Concomitant with the serum progesterone concentrations, the differences among groups in serum LH concentrations lacked significance either before or after estrus synchronization 180 protocol and hormonal treatments (Figure 2), ranged between 0.07 ± 0.01 and 0.21 ± 0.14 181 mIU/ml before treatment and 0.07 ± 0.01 and 0.09 ± 0.01 mIU/ml after treatment. None – significant differences were observed between two periods studied for all groups, being similar before and after hormonal treatment. (Figure 2).

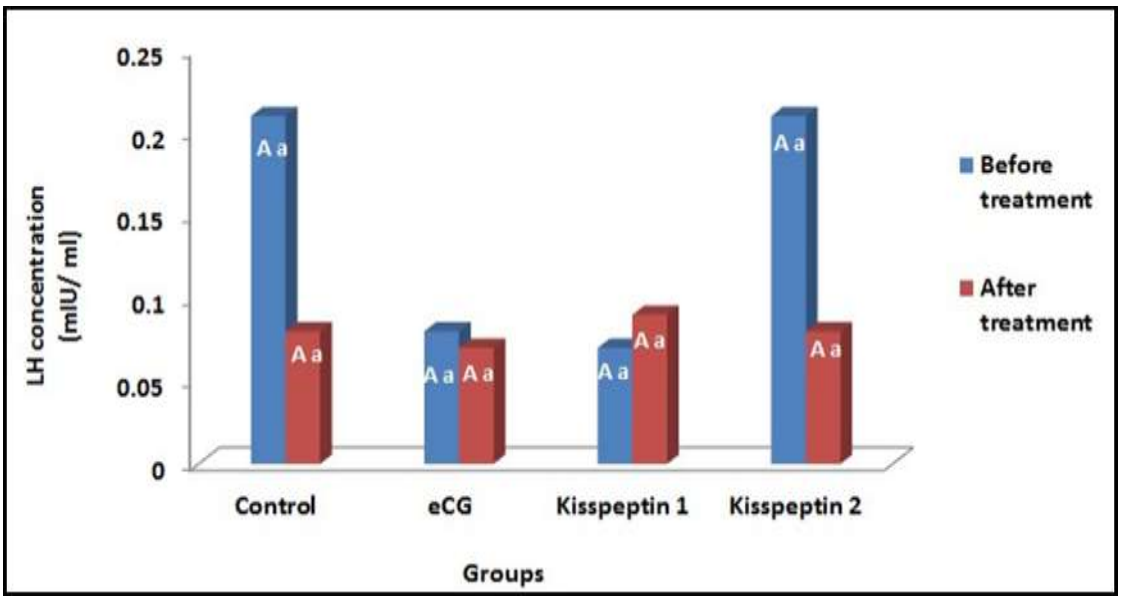

Figure 2. Serum LH concentrations (nmol / L) of Karadi ewes before and after treatment with equine chorionic gonadotropin (eCG) and Kisspeptin hormones post estrus synchronization.

Means with small superscripts indicate comparison among treatments and capital superscripts indicate comparison between periods within each treatment. eCG = Equine chorionic gonadotropin.

The non-significant differences in serum progesterone and LH concentrations in all groups following estrus synchronization protocol and eCG and Kisspeptin treatments may returns to the short time of blood sampling post treatment (2 hrs.). Longer time is needed to enable the treatment to stimulate LH surge along with large dominant follicles and corpus haemorraghicum formation consequently able to secrete a considerable amount of progesterone (43). The time selected for the blood sampling was performed according to previous studies of Ezzat et al (18) in cattle and Hashizume et al (23) in goat. However, Hashizume et al (23) observed an increasing LH secretion, 20-30 minutes following intravenous injection of native Japanese goat does with 1.5 and 10 µg of Kisspeptin-10 / kg body weight. Similarly, Saito et al (39) found a considerable amount of LH and FSH concentrations, 20-30 minutes after intravenous injection of Japanese Shiba goat does with 1, 5 and 10 µg of Kisspeptin-10 / kg body weight. On the other hand, Foradori et al (20) reported that progesterone treatment of OVX ewes had no effect on pre-prodynorphin mRNA expression in the arcuate nucleus which would have included KNDy neurons, and so there may have been insufficient progesterone receptors to allow progesterone to have much of an effect. However, the evidence regarding progesterone action on KNDy neurons in sheep is poor (42). It is worthy to mention that the current doses have attained a pronounced fertility, lambing and twinning rates along with an obvious increasing in fecundity as compared with the control group. This study was agree with Al-Ameri (5) findings in Cyprus goat, who noticed non-significant differences inplasma progesterone, 20-50 minutes following treatment with either Kisspeptin-10, hCG or GnRH hormones.

Reproductive performance

At the first cycle: Greater (P≤ 0.01) estrus rate was observed in eCG-treated group (70%) at the first estrus cycle, followed by Kiss2 (60%) and Kiss1 (40%) groups respectively, concomitant with estrus disappearance noticed in the control group (Table 1). Similarly, the eCG-treated ewes exhibited greater (P≤ 0.01) fertility rate (70%) as compared to the other groups, followed by Kisspeptin 2 (50%) and Kisspeptin1 (40%) groups respectively (Table 1). The eCG group exhibited greater (P≤ 0.01) conception rate in comparison with the other groups, namely 70%, followed by 60% for Kisspeptin2 and 40% for Kisspeptin1 groups. One ewes was aborted pertaining to Kiss2 group were recorded (Table 1).An obvious (P≤ 0.01) lambing rate was noticed for eCG-treated ewes (90%) as compared with the remaining groups. The Kisspeptin2 and Kisspeptin1 groups attained 60 and 50% lambing rates respectively (Table 1). Treatment with eCG post estrus

synchronization caused greater (P≤ 0.01) twinning rate (20%) than Kisspeptin1 and Kisspeptin2 groups (10%), exhibiting 100% increasing rate (Table 1). Litter size tended to be numerically greater in eCG group (1.30) than Kisspeptin1 (+4%; 1.25) and Kisspeptin2 (+8%; 1.20) groups (Table 1). Concomitant with fertility rate results, the eCG groups recorded lesser (P≤ 0.01) barrenness (30%) as compared with the Kisspeptin1 (60%) and Kisspeptin2 (50%) groups (Table 1).It is worthy to mention that the control group did not exhibit any reproductive performance during this cycle (Table 1).

At the second cycle

The eCG-treated ewes exhibited greater (*P*≤ 0.01) estrus rate (66.66%) among the other groups, concomitantly with lesser rate recorded by Kisspeptin2 group (20%). On the other hand, Kisspeptin1 and control groups had 33.33 and 30% estrus rates respectively (Table 2). Fertility and conception rates were slightly greater (*P*≤ 0.05) in eCG and Kisspeptin1 groups (33.33%) in relation with the two remaining groups (20%; Table 2). Similar trend was observed for lambing rate, being slightly higher (*P*≤ 0.05) in eCG and Kisspeptin1 ewes (33.33%) in comparison with the control and Kisspeptin2 groups (20%; Table 2). None of the groups exhibited any twinning rate during this cycle, being 0% for all. The differences in litter size among groups lacked significance, namely 1 for all (Table 2).The barrenness was significantly (*P*≤ 0.05) lesser in eCG and Kisspeptin1 groups 215 (66.67%) as compared with the 80% in control and Kisspeptin2 groups (Table 2).

Table 1. Effect of post estrus synchronization equine chorionic gonadotropin (eCG) and Kisspeptin hormones on the first cycle reproductive performance of Karadi ewes

Group / Trait	Control	eCG	Kisspeptin 1	Kisspeptin 2	Chi-square
No. of ewes	10	10	10	10	---
No. of ewes exhibited estrus	0	7	4	6	---
Estrus rate (%)	0	70.00	40.00	60.00	12.407 **
No. of ewes lambed	0	7	4	5	---
No. of ewes aborted	0	0	0	1	---
Fertility rate[1] (%)	0	70.00	40.00	50.00	12.183 **
Conception rate[2] (%)	0	70.00	40.00	60.00	12.407 **
No. of lambs born	0	9	5	6	---
No. of twin born	0	2	1	1	---
Lambing rate[3] (%)	0	90.00	50.00	60.00	14.661 **
Twinning rate[4] (%)	0	20.00	10.00	10.00	8.255 **
Litter size[5]	0	1.30	1.25	1.20	NS
Barrenness[6] (%)	0	30.00	60.00	50.00	12.259 **

eCG = Equine chorionic gonadotropin; ** = (P<0.01); NS = Non-significant.

No. of ewes lambed/No. of ram-exposure ewes x100

No. of ewes lambed and aborted/No. of ram - exposure x100

No. of lambs born/No. of ram-exposure ewes x100

No. of lambs born/No. of ewe lambed

No. of twin born/No. of ewe lambed x100

100 - Fertility rate

Table 2. Effect of post estrus synchronization equine chorionic gonadotropin (eCG) and Kisspeptin hormones on the second cycle reproductive performance of Karadi ewes

Group / Trait	Control	eCG	Kisspeptin 1	Kisspeptin 2	Chi-square
No. of ewes	10	3	6	5	---
No. of ewes exhibited estrus	3	2	2	1	---
Estrus rate (%)	30	66.66	33.33	20.00	10.335 **
No. of ewes lambed	2	1	2	1	---
No. of ewes aborted	0	0	0	0	---
Fertility rate[1] (%)	20.00	33.33	33.33	20.00	5.173 *
Conception rate[2] (%)	20.00	33.33	33.33	20.00	5.173 *
No. of lambs born	2	1	2	1	---
No. of twin born	0	0	0	0	---
Lambing rate[3] (%)	20.00	33.33	33.33	20.00	5.173 *
Twinning rate[4] (%)	0	0	0	0	0.00 NS
Liter size[5]	1.0	1.0	1.0	1.0	NS
Barrenness[6] (%)	80.00	66.67	66.67	80.00	5.209 *

eCG = Equine chorionic gonadotropin; ** = (P<0.01); NS = Non-significant

No. of ewes lambed/No. of ram-exposure ewes x100

No. of ewes lambed and aborted/No. of ram - exposure x100

No. of lambs born/No. of ram-exposure ewes x100

No. of lambs born/No. of ewe lambed

No. of twin born/No. of ewe lambed x100

100 - Fertility rate

At the third cycle

Lesser ($P \leq 0.01$) estrus rate was found in eCG groups (50%) as compared with the other three remaining groups, which did not differ significantly among them, namely 10% (Table 3). The eCG and control ewes exhibited lesser ($P \leq 0.01$) fertility and conception rates (0 and 14.29%) in relation with the two Kisspeptin groups, recorded similar non-significant rates (75%, Table 3). Similar trend was noticed for lambing rate, being lesser ($P \leq 0.01$) in eCG and control groups (0 and 14.29%) in comparison with the two Kisspeptin groups, namely 75% (Table 3). None of the groups exhibited any twinning rate during this cycle, being 0% for all. The litter size did not vary among control, Kisspeptin1 and Kisspeptin2 groups (1.0). Though it significantly ($P \leq 0.05$) greater than those of eCG group (0%; Table 3). Concomitantly with the fertility rate results, lesser ($P \leq 0.01$) barrenness (25%) were found for Kisspeptin1 and Kisspeptin2 groups, as compared with the 87.5 and 100% rates noticed in the control and eCG groups respectively (Table 3).

Overall mean

An overwhelming ($P \leq 0.01$) estrus rates (100%) were obtained for eCG, Kisspeptin1 and Kisspeptin2 groups in relation with control group (80%). However, the three hormonal233 treated groups did differ significantly among them (Table 4). Both Kisspeptin groups exhibited greater ($P \leq 0.01$) fertility rates (90%), followed by 80% for eCG group and 30% for control groups, being the lesser rate (Table 4).Similar trend was observed for the conception rate, being obviously higher ($P \leq 0.01$) in Kisspeptin2 (100%) and Kisspeptin1 (90%) as compared to the eCG and control groups, namely 80 and 30% respectively (Table 4). Ninety percent lambing rate were obtained for both Kisspeptin groups, being significantly ($P \leq 0.01$) greater than those of eCG (80%) and control (30%) groups (Table 4). The eCG group attained an overwhelming ($P \leq 0.01$) twinning rate of 20% in comparison with 10% for both Kisspeptin groups and 0% for the control group (Table 4).Concomitant with the twinning rate results, greater ($P \leq 0.05$) litter size was found in eCG-treated group (1.25) followed by 1.10 for both Kisspeptin groups and the smallest size was 1.0 for the control group (Table 4). Treatment with Kisspeptin hormone resulted in lesser ($P \leq 0.01$) barrenness rates during the experimental period, namely 10% as compared with 20 and 70% for the eCG and control groups (Table 4).

Fecundity, percentage of increasing fecundity (gain %) and extra lambs born

Greatest ($P\leq$ 0.05) fecundity rates were obtained for eCG and Kisspeptin-treated groups (1.0) as compared with the control groups, which recorded the lowest value (0.3 ;Table 5). 250 Furthermore, greater ($P\leq$ 0.01) increasing fecundity percentage (233 %) was obtained for eCG and Kisspeptin-treated groups in relation with 0% for the control group (Table 5). Accordingly, greater ($P\leq$ 0.01) extra lambs born were attained for eCG and Kisspeptin groups, which were 0.7 with none extra lambs obtained for the control group (Table 5).

Table 3. Effect of post estrus synchronization equine chorionic gonadotropin (eCG) and Kisspeptin hormones on the third cycle reproductive performance of Karadi ewes

Group / Trait	Control	eCG	Kisspeptin 1	Kisspeptin 2	Chi-square
No. of ewes	7	2	4	4	---
No. of ewes exhibited estrus	5	1	3	3	---
Estrus rate (%)	71.43	50.00	75.00	75.00	8.912 **
No. of ewes lambed	1	0	3	3	---
No. of ewes aborted	0	0	0	0	---
Fertility rate[1] (%)	14.29	0.00	75.00	75.00	13.446 **
Conception rate[2] (%)	14.29	0.00	75.00	75.00	13.446 **
No. of lambs born	1	0	3	3	---
No. of twin born	0	0	0	0	---
Lambing rate[3] (%)	14.29	0.00	75.00	75.00	13.446 **
Twinning rate[4] (%)	0	0	0	0	0.00 NS
Liter size[5]	1.0	0.0	1.0	1.0	*
Barrenness[6] (%)	87.71	100.00	25.00	25.00	12.649 **

eCG = Equine chorionic gonadotropin ; ** = (P<0.01); NS = Non-significant

No. of ewes lambed/No. of ram-exposure ewes x100

No. of ewes lambed and aborted/No. of ram - exposure x100

No. of lambs born/No. of ram-exposure ewes x100

No. of lambs born/No. of ewe lambed

No. of twin born/No. of ewe lambed x100

100 - Fertility rate

Table 4. Effect of post estrus synchronization (eCG) and Kisspeptin hormones on the overall reproductive performance of Karadi ewes

Group / Trait	Control	eCG	Kisspeptin 1	Kisspeptin 2	Chi-square
No. of ewes	10	10	10	10	---
No. of ewes exhibited estrus	9	10	10	10	---
Estrus rate (%)	80	100	100	100	8.255 **
No. of ewes lambed	3	8	9	9	---
No. of ewes aborted	0	0	0	1	---
Fertility rate (%)[1]	30	80	90	90	13.751**
Conception rat (%)[2]	30	80	90	100	13.925**
No. of lambs born	3	10	10	10	---
No. of twin born	0	2	1	1	---
Lambing rate (%)[3]	30	80	90	90	13.751**
Twinning rate (%)[4]	0	20	10	10	8.251 **
Liter size[5]	1.00	1.25	1.10	1.10	*
Barrenness (%)[6]	70	20	10	10	13.053**

eCG = Equine chorionic gonadotropin ; ** = (P<0.01).

No. of ewes lambed/No. of ram-exposure ewes x100

No. of ewes lambed and aborted/No. of ram - exposure x100

No. of lambs born/No. of ram-exposure ewes x100

No. of lambs born/No. of ewe lambed

No. of twin born/No. of ewe lambed x100

100 - Fertility rate

Table 5. Effect of post estrus synchronization (eCG) and Kisspeptin hormones on the fecundity, percentage of increasing fecundity (gain %) and extra lambs born of Karadi ewes

Group / Trait	Control	eCG	Kisspeptin 1	Kisspeptin 2	Level of significance
Fecundity[1]	0.3	1.0	1.0	1.0	P≤0.05
entage of increasing fecundity (%)[2]	-	233.3	233.3	233.3	P≤0.01
Extra lambs born[3]	-	0.7	0.7	0.7	P≤0.01

eCG = Equine chorionic gonadotropin

Number of lambs born per ewe treated

Fecundity of treatment – Fecundity of control / Fecundity of control × 100

Fecundity of treatment – Fecundity of control.

It is clear that in animal production systems, reproductive success is critical to the operation of enterprises (sheep in particular), hence, a good understanding of the control of GnRH is necessary (42). Estrus synchronization in conjunction with eCG is a user-friendly technique to producers, require minimal labor and animal handling. Moreover, eCG is a commercially available gonadotropin in Iraq. Our results obtained successfully in private farm with large flock of Karadi ewes rose semi-extensively under north Iraqi conditions. Moreover, it is now clear that Kisspeptin has a crucial role in controlling the reproductive functions in mammals in general and sheep in particular through the systemic delivery of Kisspeptin-induced LH surge by activating estrogen negative feedback on gonadotropin secretion (41). The pronounced reproductive performance of eCG group during the first estrus cycle may attributed to the high eCG affinity for both FSH and LH receptors to the ovaries. On the granulosa and theca cells of follicle, eCG has long lasting LH and FSH effects that stimulate estradiol and progesterone secretion (15). Thus, eCG resulted in fewer atretic follicles, the recruitment of more small follicles showing an elevated growth rate, the sustained growth of medium and large follicles and improved development of the dominant and preovulatory follicles (43). The enhancement effect of eCG extended to the end of the experiment attaining good results of the overall fertility, conception, lambing and twinning rates. Accordingly, eCG has beneficial effects on embryo development and survival, which will positively increasing lambing rate ($81 per lamb at marketing) using one injection of eCG ($5 per dose). The current results were consistent with the previous reports in Awassi ewes (1, 2, 7, 8). However, the results were varied among these studies due to the different doses used (400-600 IU / ewe), and being greater than those used currently (250 IU / ewe). Differences in responsiveness of the ovaries to eCG [33], alteration in sperm transport within the female reproductive tract (29) or in the cleavage rate (3) may reflect the dissimilarities among studies. It is noteworthy, that this is the first study dealings with the effect of post estrus synchronization administration of Kisspeptin on the reproductive performance of Karadi ewes. The recognizable results of reproductive performance pertaining to the Kisspeptin1 and Kisspeptin2 groups may return to the pivotal role of Kisspeptin in stimulating GnRH synthesizing neurons to synthesis more GnRH quantities, that may consequently affected anterior pituitary to produce more LH and FSH necessary for ovulation and folliculogenesis (38), and improve lambing and twinning rates consequently. Concomitantly, Byri et al (9) found that addition of Kisspeptin (10 µg/ ml) to the FSH, LH and E_2-supplemented media enhanced the sheep oocyte maturation *in vitro*. On the other hand, Kisspeptin inhibits gamma amino butyric acid (GABA) activity, which inactivated GnRH-synthesizing neurons in the hypothalamus through its hyperpolarization. This would subsequently increase GnRH secretion (49). This pronounced lambing and twinning rates of Kisspeptin groups will positively reflect the greater economic impact ($81per lamb at marketing) using one injection of Kisspeptin ($10-20 per dose). In conclusion, Kisspeptin-10 (4 and 8 µg / kg body weight) could be an alternative to eCG in estrus synchronization protocol for improving the reproductive performance of Karadi ewes

and post progestagen-estrus synchronization administration with either eCG (250 IU / ewe) or Kisspeptin (4 and 8 µg / kg body weight) would be of greater economic advantage of sheep owners through marketing more lambs. This was confirmed by 0.7additional lambs borne and 233 % increasing in fecundity of treated groups as compared with the control group. Other studies are warranted to confirm this notion.

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