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Assessing the Influence of Administration of Kisspeptin-10 on LH Release and Reproductive performance in estrus synchronized ewes

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THE aim of this study was to elucidate the impact of administering kisspeptin through injection on gonadotropin profile (LH) in treated ewes, to evaluate the effect of CIDR and kisspeptin injection on ovarian cyclicity (estrus activity), pregnancy rate and fecundity in adult ewes. Twenty four local Awassi non-pregnant and non-lactating ewes with three adult Rams of proven fertility of (2-4) years old used in the study, the period of study was extended from March 2022 to December 2022. The Experimental animals, which were sheep in this case, were randomly divided into three groups, each consisting of eight ewe: The First group of control (G1), The control (G1), Estrus synchronization group (G2); utilizes CIDR for estrus synchronization for 12 days+ kisspeptin-10 at time of CIDR withdrawal, and the third group (G3) using only for 12 days. The investigation encompassed the examination of estrus synchronization and the subsequent natural mating of the animals with proven rams. Blood samples were collected 60 and 30 minutes prior to the administration of kisspeptin and CIDR withdrawal, and 15, 30, 60, 90, 120, 180, 240, 300, and 360 min after kisspeptin injection. The results indicated that the response duration in G2 and G3 was 49.75 and 60.12 minutes, respectively. These findings revealed no significant differences in duration response. Additionally, there were no significant differences in conception rates between G2 and G3, with rates of 100% and 66.6%, respectively. In terms of lambing rates, G2 achieved 100% while G3 reached 66.6%. Fecundity rates were recorded as 137.5% for G2 and 83.3% for G3, respectively. Regarding the activity of LH, the concentration increase after (15) min of kisspeptin injection. In conclusion, treatment of Awassi ewes with Kisspeptin not effect on reproductive performance and significant affect serum LH concentrations.

Keywords: Awassi ewes, kisspeptin-10, synchronization

Introduction

The domestication of sheep, which occurred between 11,000 and 9,000 BC, marked a significant milestone in human-animal interaction. One of the earliest animals to be domesticated for meat, milk, and wool production was the sheep [1]. Sheep are generally categorized as seasonal polyestrous animals and short-day breeders [2- 4].

Sheep breeds in subtropical, temperate, and high-latitude regions experience fluctuations in their reproductive activity throughout the seasons. Typically, the breeding season commences in autumn and concludes in winter, with a period of anoestrus occurring during spring and summer [5-8]. An endogenous circannual rhythm governs the initiation and termination of the breeding season, with its regulation and synchronization being influenced by the annual photoperiod cycle [9]. However, the timing and duration of the breeding season can be influenced by interactions between the photoperiod and factors such as breed, geographical origin, nutritional [10-13] and lactational status, social interactions [14], season of parturition [15] and genetic factors [16,17]. On the other hand, Ahmad and his team [18] highlight that seasonal breeding in sheep poses challenges to improving productivity, as there is still incomplete understanding of the mechanisms involved. Consequently, effective strategies for enhancing the lamb crop remain limited.

Estrous synchronization in Sheep is achieved through the manipulation of photoperiod, ram effect [19, 15], and various hormonal regimens [20,21].

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Exogenous hormones utilized for this purpose encompass prostaglandins [22,23], progesterone [24,25], equine chorionic gonadotropin (eCG) [25-27], melatonin [28], and human chorionic gonadotropin (hCG) [29]. These hormone interventions have been employed for synchronizing sheep estrus over an extended period [30].

On another hand, kisspeptin, a neuropeptide encoded by the kisspeptin 1 (KISS1) gene, plays a significant role in reproduction by acting on the hypothalamic-pituitary-gonadal (HPG) axis [31-33]. Kisspeptins are crucial for the maturation and proper functioning of the reproductive system in both males and females [34, 35]. Kisspeptin, also referred to as metastin, has been discovered to stimulate the release of gonadotropin-releasing hormone (GnRH) and subsequently induce luteinizing hormone secretion [36]. In ruminants, the neuroendocrine control of reproduction ultimately results in the release of luteinizing hormone (LH) from the anterior pituitary gland. [37,38].

Within sheep, kisspeptin neurons are situated in the preoptic area as well as the arcuate nucleus (ARC), with the latter playing a role in both the positive and negative feedback regulation of GnRH by estradiol. [39,40]. In addition, sheep are seasonal breeders, with an annual cycle controlled by changes in the pulsatile secretion of GnRH [41,18]. Kisspeptin neurons also play a crucial role in this phenomenon by exhibiting heightened expression and forming terminal connections with GnRH neurons during the breeding season. During the nonbreeding season, the diminished expression of kisspeptin can be counteracted through the administration of kisspeptin, which induces ovulation in seasonally acyclic females [42]. The study aimed to clarify how administering kisspeptin via injection affects the gonadotropin profile, specifically LH levels in treated ewes. It also to assess the impact of CIDR and kisspeptin injections on ovarian cyclicity, pregnancy rates, and fecundity in adult ewes.

Material and Methods

Experimental animals

The present study was conducted at private field in Kirkuk governorate. Twenty four local Awassi non-pregnant and non-lactating ewes with (3) adult Rams of proven fertility of (2-4) years old used in the study, all ewes are in good health and free from any clinical signs of internal or external parasites. The animals were accommodated in partially opened shade, receiving a concentrated diet three times a day along with supplemental straw and green food, all under natural daylight conditions.

Experimental design

The period of study was extended from March 2022 to December 2022. During breeding season. Ultrasonographical examination, all ewes were examined by ultrasound (at the beginning of experiment) in two occasion 15 days apart to confirm non pregnancy by application Ultra sound machine, B mode (SonoSite) USA (liner probe).

The Experimental animals (sheep) were divided randomly into three groups (8 sheep /group). The First group of control (G1). A second group (G2) Estrus synchronization using CIDR (CIDR for Sheep Vetosider® made in Iran) it incorporates 0.3 grams of progesterone within a molded silicone structure covering a flexible spine, which is further extended by a nylon tail) for 12 days and at CIDR withdrawal injection of kisspeptin-10 (5µg/kg B.W.) (Kisspeptin hormone, also called (Metastin) Wuhan Senwayer Century Chemical Co.,Ltd) China. A third group (G3) Estrus synchronization using CIDR only for 12 days.

Blood sampling and assay

Blood samples collection before and after kisspeptin treatment Blood samples were collected at various time intervals, including 60 and 30 minutes before the administration of kisspeptin, as well as 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes after the kisspeptin injection. These samples were drawn from the jugular vein using vacutainer tubes, with immediate processing of a 10 ml blood sample within the vacutainer tubes. Subsequently, serum was extracted following centrifugation of the samples at 3000 RPM for 15 minutes and then stored at -20°C until the assay. An Enzyme-Linked Immunosorbent Assay (ELISA) technique was employed to measure the concentration of serum luteinizing hormone (ng/ml), utilizing a kit provided by Kit (ELK Biotechnology, China).

Statistical analysis

The data underwent statistical analysis using SAS (Statistical Analysis System - version 9.1) [43]. A repeated measures ANOVA was employed, which is a research design involving multiple measurements of the same variable taken on the same or matched subjects, either under different conditions or over two or more time periods. For instance, in a longitudinal study, repeated measurements are collected to assess changes over time.

To evaluate significant differences among means, post hoc tests using the Least Significant Differences (LSD) method were conducted. Chi-square analysis was also utilized to assess significant differences among percentages.

Results and Discussion

Table 1 presents that there was no significant difference between the two groups in the duration of response (in hours) following the withdrawal of CIDR. However, it is noteworthy that the estrus duration in G3 (60.12±4.90 hours) appeared to be slightly longer ($p<0.07$) than in G2 (49.75±1.76 hours). These findings align with Texeira *et al.* [44].

There were no significant differences in The conception rate between G2 and G3 groups, the conception rates in CIDR+ Kisspeptin and CIDR alone were 100 % and 66.6 %, respectively and these results are disagree with Garoussi *et al.[*45] and these differences may returns to the differences in the breed of sheep or the season of experiment.

There were no significant differences in the lambing rate between G2 and G3 groups, the lambing rates in CIDR+ Kisspeptin and CIDR alone were 100 % and 66.6 %, respectively and these results are disagree with Garoussi *et al.* [45] and Abdulkareem *et al*. [46] and these differences may returns to the differences in the breed of sheep or dose of P4 that use in estrus synchronization

Fecundity, percentage there are great when we use the kisspeptin group when compere with CIDR alone but this result was non-significant differences.

TABLE 1. Reproductive performance of estrus synchronized ewes by CIDR alone and combination of CIDR and kisspeptin hormone.

Trait	CIDR & kisspeptin	CIDR alone	Chi-square	P Value
Duration of response (hr)	49.75±1.76	60.12±4.90		0.07NS
conception rate %	100%	66.6%	3.11	0.07NS
lambing rate	100%	66.6%	3.11	0.07NS
Fecundity rate	137.5 %	83.3%	0.43	0.07NS

Serum LH concentrations: The tables (2 and 3) refers to the groups, time and (time- group interaction) have a significant effects on serum (LH) profile, and this results are agreement with Al-Amri [47] when they found the Variations in plasma LH levels where a statistically significant treatment-time interaction was observed. Both the vehicle-treated and kisspeptin-treated groups were assessed at each time point. The significance threshold was set at 5%.

TABLE 2. Depicts Repeated Measures Analysis of Variance to test the hypothesis between groups in effecting on serum (LH) profile.

Source of variation	DF	Type III SS	Mean square	F-value	P Value
Groups	2	2474049	1237024	10.08	<0.01
Error	12	1473050	122754		

TABLE 3. The Repeated Measures Analysis of Variance to test the hypothesis within time effecting on serum (LH) profile.

Source of variation	DF	Type III SS	Mean square	F-value	P
Time	10	480541	48054	5.30	<0.0001
Time*group	20	893031	44651	4.92	<0.0001
Error	120	1088982	9074		

In the Table (4) refer to the LH level in control group don't effective by the time of blood collection, this result because the animals in the non-breeding season also in the CIDR alone group the time of blood collection (15-360 min) after CIDR with drawl non-significant effect on the LH concentration value And that's because shorter time required to collect blood samples this result agreement with [48] found the LH surge The onset of the surge occurred later (39.4 - 61.2 h) after CIDR withdrawal [48], and they observed that the surges of LH were more variable and occurred later, typically commencing between 44 to 60 hours after the removal of progesterone CIDR.) If the time required for blood collection was more than

the time for our experiment, there would be an effect of progesterone on the LH hormone.

On the other hand doses of Kp10 effectively stimulated LH release within 15-min after injection, with maximal levels ranging 7-13 fold higher than those before injection, these results are due to effect of kisspeptin on gonadotropins this result agreement with some studies[50-52].

TABLE 4.Values of circulating serum (LH) with instruction between time and groups in estrus synchronized Ewes (ng/ml).

Time /Minute	Control G1	CIDR G2	CIDR+KIIS G3
Before 60	A1.60±0.19^{a}	A1.68±0.11^{a}	A1.65±0.12^{e}
Before 30	A1.57±0.12^{a}	A1.62±0.16^{a}	A1.43±0.10^{e}
After 15	A1.73±0.64^{a}	A1.87±0.13^{a}	B14.35±4.18^{bc}
After 30	A1.65±0.31^{a}	A1.75±0.16^{a}	B8.54±3.23^{d}
After 60	B1.68±0.46^{a}	B1.75±0.53^{a}	A17.01±4.52^{a}
After 90	B1.88±0.13^{a}	B1.97±0.18^{a}	A13.004±4.751^{c}
After 120	B1.74±0.50^{a}	B1.96±0.23^{a}	A16.59±4.34^{b}
After 180	B1.53±0.68^{a}	B1.63±0.86^{a}	A10.96±3.40^{d}
After 240	B1.82±0.78^{a}	B1.91±0.23^{a}	A9.92±3.29^{d}
After 300	C1.49±0.11^{a}	B1.81±0.31^{a}	A8.01±2.89^{d}
After 360	B1.71±0.18^{a}	B1.95±0.21^{a}	A8.05±2.60^{d}
LSD	2.46		

Means with a different small letter in the same column are significantly different (P<0.05)
Means with a different capital letter in the same row are significantly different (P<0.05).

Conclusions

The current study has showed that the CIDR-kisspeptin regimen demonstrated superior results in enhancing reproductive performance, including the percentage of responsive animals, conception rate and fecundity rate. This study also appeared the effective role of kisspeptin treatment in estrus synchronization, particularly in increasing the serum levels of LH.

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Conflicts of interest

There are no conflicts of interest to be declared.

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تقييم تأثير أعطاء كيسبيبتين -10 على إطلاق الهرمون اللوتيني والأداء الإنجابي في النعاج المتزامنة الشبق

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هدفت هذه الدراسة الى توضيح تأثير إعطاء هرمون الكيسبيبتين عن طريق الحقن على تركير موجهة الغدد التناسلية (الهرمون اللوتيني) في النعاج المعالجة وكذلك لتقييم تأثير حقن هرمون الكيسبيبتين مع على دورة المبيض (نشاط الشبق)، ومعدل الحمل والخصوبة في النعاج البالغة. تم استخدام أربعة وعشرون نعجة عواسي محلية غير حامل وغير مرضعة مع (3) كباش بالغة ذات خصوبة مؤكدة بعمر (2-4) سنوات، امتدت فترة الدراسة من مارس 2022 إلى ديسمبر 2022. قسمت حيونات التجربة عشوائياً إلى ثلاث مجموعات، كل مجموعة تتكون من 8 أنعاج. المجموعة الأولى مجموعة السيطرة (G1). المجموعة الثانية (G2) استخدم فيها محرر العقار المستمر الداخلي CIDR يحتوي على 0.3 جرام من البروجسترون لمدة 12 يومًا وعند سحب CIDR تم حقن كيسبيبتين-10 (5 ميكروجرام / كجم من وزن الجسم) والمجموعة الثالثة (G3) استخدم فيها محرر العقار المستمر الداخلي CIDR فقط لمدة 12 يومًا. تم جمع عينات الدم قبل 60 و 30 دقيقة من إعطاء كيسبيبتين وسحب CIDR، و15، 30، 60، 90، 120، 180، 240، 300، و 360 دقيقة بعد حقن كيسبيبتين، وكذلك مراقبة الحيوانات التي تضهر علامتات الشبق أظهرت النتائج أن مدة الاستجابة واضهار الشبق في المجموعتين G2 وG3 كانت (49.75 و60.12) على التوالي وكانت هذه النتيجة فروق غير معنوية، وكذلك فروق غير معنوية في معدل الحمل بين G2 وG3 كانت (100٪ و66.6٪) على التوالي، وكانت نسبة الحمل 100٪ في G2 و66.6٪ في G3 اما نسبة الخصوبية فكانت (137.5٪ و 83.3 ٪) في G2 وG3 على التوالي. وفيما يتعلق بنشاط LH، فإن تركيزه يزداد بعد (15) دقيقة من حقن كيسبيبتين. نستنتج من ذلك أن معاملة النعاج العواسية بالكيسبيبتين لم تؤثر على الأداء التناسلي ولها تأثير معنوي على تركيز LH في مصل الدم.

الكلمات الدالة: الاغنام العواسية،10 Kisspeptin ، توحيد الشبق.