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The Impact of Administering Kisspeptin-10 Peripherally on FSH Release and Reproductive Performance in Estrus Synchronized Ewes

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Abstract

The aim of this study was to elucidate the impact of administering kisspeptin-10 through injection on gonadotropin profile (FSH) in treated ewes, to evaluate the effect of CIDR and kisspeptin-10 injection on ovarian cyclicity (estrus activity), estrus response, estrus phase length, gestation period, Pregnancy Rate, fertility Rate (%) and litter size in adult ewes. Twenty four local Awassi non-pregnant and non-lactating ewes and 3 adult Rams of proven fertility (2-4 years old) were used in the study. The duration of study was for 9 months from March 2022 to December 2022. The Experimental animals were divided into three groups, each consisting of 8 sheep. The First group (G1) served as control, the second group (G2) Estrus synchronization using CIDR only for 12 days and third group (G3) Estrus synchronization using CIDR for 12 days and at CIDR withdrawal with an injection of kisspeptin-10 (5µg/kg B.W. The investigation encompassed the examination of estrus synchronization and subsequent natural mating of the animals with proven rams. Blood samples were collected at 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. Results reviewed duringApril and May 2022 , indicated that all the ewes in G1 control group didn't show any estrus signs during this period (out of breeding season), but the % of ewes that exhibited estrusin G2 and G3 was 75% and 100% respectively. Significant differences were seen in estrus phase length between G2and G3($P<0.05$) (27.50± 1.59, 47.12±5.71hours) respectively. The observed gestation period was 149.50±0.64 days in G2 and 149.38±0.80 days in G3 which is non-significant. Pregnancy Rate and Fertility Rates were 66.6 and 100%and litter size rates were 125and 137.5% in G2 and G3 respectively. Regarding the activity of FSH, the concentration increased after 15minutes of kisspeptin injection. In conclusion, treatment of Awassi ewes with Kisspeptin did not show any effect on reproductive performance and serum FSH concentrations.

Key words : Kisspeptin, reproductive, Estrus, Ewes.

Sheep and goats represent among the earliest animals to be domesticated by humans, primarily for their diverse utility in providing meat, milk, dairy products, fleece, and skin (Joshi *et al*., 2020). One significant rationale behind rearing these animals on farms was the convenience in producing meat, due to the ease of keeping, raising, and slaughtering livestock whenever the need for meat arose (Fournié *et al*., 2017; Mazinani and Rude, 2020).

Iraqi sheep are considered seasonal polyestrous animals, and their reproductive activity aligns with the ideal time of the year when ample pasture or food resources are available during the spring and summer (Hussain *et al*., 2017, Hatif and Younis, 2018). Notably, variations exist among different sheep breeds in terms of the timing and duration of their breeding seasons (Maquivar *et al*., 2021).

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Estrous synchronization in farm animals can be achieved by regulating the secretary activity of the corpus luteum and ovulation (Abecia *et al*., 2012; Aqwaan, 2023). In sheep, the most effective method for estrous synchronization involves manipulating the lifespan of the corpus luteum (Arya *et al*., 2023), thereby inducing females to express estrus almost simultaneously, ensuring a sufficient number of estrual females available for mating (Zhao *et al*., 2010).

Various synthetic P4 analogues such as CIDR, MAP, and FGA have been found to yield comparable outcomes in terms of estrous synchronization/induction, according to studies. During both breeding as well as non-breeding seasons, the addition of eCGto P4-based protocols has been shown to improve the response of estrus and the rate of pregnancy (Hussein, 2007).

Alternately, PG is fruitful for successful estrous synchronization, but specifically during the season of breeding (Reddene*t al*., 2023). It is typically administered in two injections, 11 or 14 days aside, along with P4-based protocols to lyse ovine corpus luteum (CL) when it is receptive to PG, approximately 3 days post-ovulation. Additionally, the "ram effect," as demonstrated in a study by AL-Mutar, (2017) andHameed *et al*. (2021), has the potential to enhance the effectiveness of P4-based protocols and can serve as a substitute for eCG in ewes.

In sheep, estrous synchronization is achieved through various means, including manipulation of photoperiod, the "ram effect," and the use of exogenous hormones such as prostaglandins, progesterone, eCG, melatonin and Bromocriptine during both breeding and non-breeding seasons in ewes (Tamer and Al-Hamedawi, 2013, Al-Hamedawi *et al*., 2016, Al-Hamedawi *et al*., 2020; Abas *et al*., 2022) and Does (Kadhim *et al*., 2014). Photoperiod manipulation is effective for inducing estrus during the non-breeding season, while both short and long durations of progesterone administration in combination with gonadotropins are considered for fixed-time artificial insemination (AI) and estrous synchronization in sheep (Ungerfeld and Rubianes, 2002, Zeleke *et al*., 2005; Olivera-Muzante *et al*., 2011). Progesterone treatment has proven effective in synchronizing estrus in sheep during both the breeding and non-breeding seasons (Abdul Hussain *et al*., 2017).

The administration of progesterone (P4) treatments typically involves the use of controlled internal drug-releasing (CIDR) devices, intravaginal sponges, or injectable forms. These treatments may be prescribed for varying durations, such as short periods (5-9 days) or extended periods (14 days), often in conjunction with gonadotropins as observed in studies by Texeira *et al*. in 2016 and Martinez-Ros *et al*. in 2019.

Kisspeptin binds to a G-protein-coupled receptor known as GPR54/KISS1R, which stimulates the release of gonadotropin-releasing hormone (GnRH) by hypothalamic neurons. This, in turn, leads to the secretion of pituitary gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as sexual steroids. These hormones subsequently act on the gonads to regulate the production of gametes (Zohar *et al*., 2010, Trevisan *et al*., 2018; López *et al*., 2022). Kisspeptin is recognized for its pivotal role in regulating reproductive functions (Roa *et al*., 2008). It is considered the primary factor responsible for governing the hypothalamic–pituitary–gonadal axis, initiating puberty, and overseeing estrus and fertility. The influence of kisspeptin extends to a multitude of processes, including steroidogenesis, follicular maturation, and ovulation (as demonstrated in studies by Szeliga and Meczekalski in 2022 and Dai *et al*. in 2022. Furthermore, it has been observed to stimulate the release of gonadotropin-releasing hormone (GnRH) and the subsequent secretion of luteinizing hormone. (Goodman *et al*., 2022; Xu *et al*., 2023).

The control of neuroendocrine of reproduction in ruminants ultimately reaches the climax in the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. Studies have shown that Kp-10 stimulation in ewes leads to increased circulating concentrations of GnRH in the cerebrospinal fluid (as observed in the research by Daniel *et al*. in 2015). Loss of kisspeptin signalling causes hypogonadotrophichypogonadism in humans and other mammals (Clarke *et al*.,

2015; Szeliga *et al*., 2021). In sheep, kisspeptin neurons are located in the preoptic area and the arcuate nucleus (ARC) (Hellier *et al*., 2023), with the latter involved in both oestradiol positive and negative feedback regulation of GnRH. In addition, sheep are seasonal breeders, with an annual cycle controlled by changes in the pulsatile secretion of GnRH. kisspeptin neurons show increased expression and terminal opposition to GnRH neurons. Reduced kisspeptin expression through the nonbreeding season could be overcome by administering kisspeptin, leading to ovulation in seasonally acyclic females (as described in the study by Smith *et al*. in 2014). Kisspeptin exerts significant influence on various processes, including steroidogenesis, follicular maturation, and ovulation (as demonstrated in studies by Szeliga and Meczekalski in 2022 and Dai *et al*. in 2022).

Materials and Methods

Experimental animals

This study was conducted in a private field in Kirkuk Province 240 kmnorth of Baghdad on twenty-four native, non-pregnant, non-lactating Awassi ewes and three fertile adult rams aged between2 to 4 years. All sheep used in the study were healthy and clinically free of external and internal parasites. Animals were kept in semi-open shade and fed concentrated feed supplemented with straw and forage three times daily in natural daylight.

Experimental design:

The study periodwas for 9 months from March 2022 to December 2022, excluding the breeding season.

Estrus detection: was performed by introduction of aproned ram to the herd two times a day (morning and evening) for half an hour to monitor estrus activity of ewes. This procedure was carried out during April and May. This procedure continued for three successive cycles (55 days) before applying estrus synchronization protocol

Ultrasound examination: All sheep were examined twice (at the beginning of the experiment), 15 days apart, to confirm absence of pregnancy using an ultrasound machine.

All the experimental animals were randomly divided into three groups 8 ewes/group. First is a control group (G1). The second group (G2) Estrus synchronization via CIDR only for 12 days (CIDR for Sheep Vetosider®) contains 0.3 grams of progesterone in molded silicone over a flexible spine with a nylon tail) and the third group (G3) synchronization using CIDR for 12 days and at CIDR withdrawal given an injection of kisspeptin-10 (5µg/kg B.W. Kisspeptin used in this study was Metastin (Wuhan Senwayer Century Chemical Co., Ltd, China).

Blood sampling and assay:

Procedure for Collection of blood samples before and after kisspeptin treatment was as follows.

Samples of blood were collected 60 and 30 minutes prior to kisspeptin injection and 15, 30, 60, 90, 120, 180, 240, 300 and 360 minutes after kisspeptin injection. The samples of blood were collected from the jugular vein by vacuum tubes. The blood samples (10 mleach) were immediately collected into vacuum tubes, and serum was collected after centrifuging the samples at 3000 rpm for 15 minutesandstored at −20°C till thetime of analysis.

Enzyme-linked immunosorbent assay, ELISA was undertaken for measuring the concentration of Follicle-stimulating hormone (FSH)in serum (ng/ml) using the kit provided by ELK Biotechnology, China.

Statistical analysis:

Data collected were analysed using SAS (Statistical Analysis Systemversion 9.1). A "repeated measures design" was employed, includingvarious measurements of the same variable taken on identical or matched subjects under distinct conditions or over two or more periods of time. This approach has been often used in research to examine changes over time, as in a longitudinal study.

To assess significant differences among means, a post hoc analysis test known as the Least Significant Differences (LSD) was performed. Additionally, Chi-square test was employed to evaluate significant differences among percentages.

Results and Discussion

During Apriland May periods, the results indicated that all the ewes didnot show any estrus signs within this period confirming out of breeding season.This observation was not in agreement with the findings of Younis *et al*., 2019 who noticed estrus signs in ewes during Aprilmonth. Data collected in an experimental flock of 120Awassi ewes at the college of Agriculture, University of Baghdad, and data from state farms also showed that Awassi sheep are non-seasonal breeders (Al-Wahab *et al*.,1982). Such findings might be due to other non-genetic environmental factors other than the breed of sheep alone.

Table I summarises the data on reproductive performance of the ewes undergoing the present study.

Table I showsthat among the two experimental groups, all the ewes exhibited estrusafter CIDR withdrawal in CIDR + kisspeptin injection group and only 75% of ewes in CIDR alone group, but these differences are not significant. These results are different from the findings of Al-Amri, 2015; Abdulkareem *et al*., 2021and maybe due to the differences in the breed of sheep or the season of experiment or dose of kisspeptin.

Estrus phase length was 47.12±5.71 and 27.50±1.59 hours in CIDR+ kisspeptin group and CIDR alone group respectively and the Gestation periods (days) were 149.38±0.80 and 149.50±0.64 in CIDR+ kisspeptin group and CIDR alone group respectively. But all these differences are statistically non-significant showing that Kisspeptin injection had no significant effect on the estrus phase length and gestation period.

There were no significant differences between CIDR+ Kisspeptin and CIDR alone groups for pregnancy rate (100 % and 66.6 %, respectively) and these results disagreed with the results of Garoussi *et al*., 2020; Abdulkareem *et al*., 2021 which might again be due to the differences in the breed of sheep or the season of experiment.

There were no significant differences between CIDR+ Kisspeptin and CIDR alone groups in thelambing rates (100 % and 66.6 %, respectively) and these results are also not in agreement with that of Al-Amri, 2015; Abdulkareem *et al*.,2021 and these differences in the results of our study may be again due to the differences in the breed of sheep or dose of p4 that was used in estrus synchronization.

Serum FSH concentrations: The Tables II & III refer to the effects of groups, time andtime- group interaction which show significant effects on serum FSH profile, and these results are in agreement with (Caraty *et al*., 2007) who also found variations in plasma FSH levels.

Table I : Reproductive performance of estrus synchronized ewes by CIDR alone and combination of CIDR and kisspeptin hormone.

Group Trait	CIDR &kisspeptin(G3)	CIDR alone (G2)	Level of significance
No. exhibited estrus	100	75	_
Estrus phase length (hr)	47.12±5.71	27.50±1.59	0.05**
gestation period (day)	149.38±0.80	149.50±0.64	_
PregnancyRate%	100%	66.6%	_
FertilityRate%	100%	66.6%	_
Litter size%	137.5	125	_

** (P≤0.05).

Table II: Repeated Measures Analysis of Variance to test the hypothesis between groups effect on FSH profile.

Source of variation	DF	Type III SS	Mean square	F-value	Level of significance
Groups	2	3022.82	1511.41	67.47	0.0001**
Error	12	268.87	22.40		

** (P≤0.001)

Table III : Repeated Measures Analysis of Variance to test the hypothesis within time effect on (FSH) profile

Source of variation	DF	Type III SS	Mean square	F-value	Level of significance
Time	10	348.88	34.88	6.89	0.0001**
Time*group	20	639.04	31.95	6.31	0.0001**
Error	120	607.93	5.06		

** (P≤0.001)

Since we noticed a highly statistically significant on treatment group effects, Repeated Measures analysis of variance for treatment-time interaction was performed where kisspeptin treated/ untreated groups were examined at each time point. The level of statistical significance was set at 5%. (Table III).

In the CIDR alone group the time of blood collection (15-360 min) after CIDR withdrawal showed no significant effect on the FSH concentration and this result is in agreement with Ben Said *et al.* (2007), the change in FSH concentration occurred much later (39.4 - 61.2 h) after CIDR withdrawal. Caraty *et al.* (2007) discovered concentration of FSH was more variable and commenced much later 44–60 h after progesterone CIDR removal.

On the other hand dosage of Kp10 effectively stimulated FSH release within 15-min after injection, with maximum levels ranging

Table IV : Values of circulating serum FSH levels (ng/ ml.)inestrus synchronized Ewes with interaction between time and groups.

Groups Time /Minute	Control	CIDR	CIDR+KIIS
Before 60	7.00±0.78	7.54±0.67a	7.85±0.83c
Before 30	7.12±0.75	7.36±0.62a	7.98±0.82c
After 15	B6.87±0.46	B8.05±0.69	A19.04±1.77ab
After 30	B6.86±0.69	B6.94±0.71	A17.77±2.49ab
After 60	B7.36±0.67	B7.76±0.54	A20.66±2.35a
After 90	B7.39±0.60	B7.31±0.54	A18.52±1.50ab
After 120	B7.17±0.54	B7.81±0.65	A20.57±1.94a
After 180	B7.20±0.58	B7.50±1.10	A16.89±1.02b
After 240	B6.82±0.80	B7.42±0.55	A17.61±0.81b
After 300	B7.14±0.62	B8.55±0.78	A16.76±1.49b
After 360	B7.28±0.71	B7.90±0.93	A17.26±2.55b

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

7-13 fold higher than those before injection, due to effect of kisspept in on gonadotropins which is in agreement with Uenoyama *et al*., 2019, Li *et al*., 2020; Hellier *et al*., 2023.

In Table IV, we can also notice that the FSH levels in control group were not affected by time of blood collection, which is because the animals are in the non-breeding season.

Conclusion

From the results of the present study, it can be concluded that Kisspeptin injection did not have any significant effect on the various reproductive performance parameters, but the serum FSH levels show an expected response to the CIDR withdrawal and Kisspeptin injection.

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Effect of Feeding Different Dietary Protein Levels on Meat Characteristics of Kadaknath Chicken

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Abstract

The effect of feeding different levels of crude protein on meat composition and fatty acid profile of Kadaknath chicken meat at 12th week of age was studied. The bird fed with four different dietary treatment groups with different crude protein levels (14, 16, 18and 20%) with constant metabolizable energy (2800 kcal/kg). Among different parameters of meat characteristics, crude protein showed highly significant ($P \leq 0.01$) difference between treatments. The other parameters viz. per cent moisture, ether extract, crude fibre, total ash and gross energy of meat did notfoundsignificant difference due to different dietary treatments. Significantly ($P \leq 0.01$) lower muscle protein was observed at 14% CP diet than other dietary treatments. The saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids showed non-significant difference due to different dietary treatments. The results concluded that Kadaknath chicken fed diet with 16% CP was adequate for higher muscle protein.

Key words: Meat composition, Fatty acids profile, Kadaknath chicken.

In India there are 19 registered breeds of native chicken as per ICAR-National Bureau of Animal Genetic Resources; Kadaknath is one among them. The specialty of the breed is the flesh and internal organs of this chicken that are black in colour. Kadaknath black meat

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