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Exploring the Impact of Kisspeptin-10 on the Fecundity Traits of Anestrus Iraqi Cows

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ABSTRACT

The inactive ovaries of cattle caused a significant economic loss, so the current study is intended to recover and enhance the fertility of dairy cattle suffering from anestrus by kisspeptin-10. Forty Holstein Friesian cows, aged 3.5-6.8 years with inactive ovaries for 60-70 days of postpartum, were distributed randomly into four equal groups. The control (C) group administered 5 mL intramuscularly (I.M) of normal saline, Gn group administered 0.5 mg/animal I.M of GnRH. K1 and K2 were administered with 6 μg/kg BW I.M and 12 μg/kg BW I.M of kisspeptin-10, respectively. After heat signs were detected, artificial insemination was performed. Blood samples were collected at 0, 2, 6, 24, and 72 hours post-hormonal injection to estimate fertility hormones, and fecundity features were demonstrated after hormonal treatment and after twelve months. The current results noted a significant ($p<0.05$) increment in estradiol, FSH, and LH in K1 and K2 cows compared to Gn and C at 6-24 h post hormonal treatment, while progesterone showed a significant ($p<0.05$) drop in K1, Gn, and K2 in comparison with C. Also, the calving and fertility rates were significantly higher ($p<0.05$) in K2 and K1 compared with Gn and C after hormonal treatment and after one year. The kisspeptin-10 injection improved the fertility of anestrus cows by enhancing the reproductive hormonal profile and fertility traits for long-term effects and without requiring a second kisspeptin-10 injection in Holstein Friesian cows; therefore, administration of kisspeptin-10 can be regarded as an alternative application of using some hormones like GnRH.

***Keywords:** anestrous; dairy cow; fecundity; GnRH; kisspeptin-10*

INTRODUCTION

In several countries, industrial livestock is a valuable source of animal production; this depends on reproductive abilities like fecundity, which are regarded as essential economic characteristics to increase the animal population (Gogaev *et al.*, 2019). Bovine products represent the cornerstone of the agricultural economy for meat and dairy products in Iraq; there is a relative deficit in beef production with a continuous increment in the Iraqi population (Ritter *et al.*, 2019). Many factors could reduce bovine fertility, such as reproductive disorders and insufficient cows' nutrition after parturition, that can negatively influence production by altering the growth and development of ovarian follicles and luteal functions (Zhao *et al.*, 2019). The absence of estrus behavior in cows is referred to an anestrus, which can be influenced by many factors such as nutrition, suckling, season, dystocia, uterine palpation, postpartum, and low gestation rate. Furthermore, anestrus is responsible for reducing dairy production, lowering bovine beef production, and causing financial losses (AL-Nuaimi *et al.*, 2020).

In certain Asian countries, cattle are reared in confined spaces or compact farms and exposed to substantial stressful factors, such as malnutrition, climate alteration, parasitic disease, and poor hygiene, that affect ovarian functions, as documented in Galina & Geffroy (2023). Therefore, many researchers attempted to improve the bovine industry to raise their fecundity values by using hormonal or non–hormonal techniques like immunization against some hormones, polyherbal preparation, and nutritional flushing (Kafaji *et al.*, 2017; Khafaji, 2018; Amin *et al.*, 2022). Among the hormones that could be applied are progestin and pregnant mare serum gonadotropin (PMSG), which could enhance the fertility in anestrous lactating cows and improve the reproductive hormones and pregnancy rate with side effects on maternal and calf health (Al-Hamedawi *et al.*, 2016; Leonardi *et al.*, 2020). GnRH is a neuroregulatory of animal reproduction; the external administration of GnRH analogues caused luteinization after activating the hypophyseal GnRH receptors leading to LH release (Mohammadzadeh *et al.*, 2020). Studies research conducted by AL-Nuaimi and colleagues in 2020 reported that injection of GnRH causes detrimental effects in cows with ovarian dysfunction, leading to disruption in their reproductive hormone levels that negatively affect fecundity.

Interestingly, researchers discovered products deriving from a common 145 amino acids precursor as prepro-kisspeptin by proteolytic process, which acts as a metastasis suppressor of cancer cells, previously known as metastin and its current common name is kisspeptin (Ulasov *et al.*, 2019). Kisspeptin is a product of the "Kiss1" gene; it belongs to the neuropeptide family with the feature Arg-Phe-NH2 motif. The general form of kisspeptin "kp" in the bloodstream is kisspeptin-54, which is further split into 10, 14, and 13 peptides (Masumi *et al.*, 2022).

Initially, the upregulation of Kiss1 expression was noted in the placenta, then it was observed in the small intestine, pancreas, and testis (Fratangelo *et al.*, 2018; Yeo & Colledge, 2018). Additionally, kisspeptin-encoding genes are expressed in ovarian granulosa cells and the endometrium's luminal and glandular cells, so kisspeptin plays a central role in reproduction regulation (Yeo & Colledge, 2018). The Kiss1 gene is distributed throughout the brain, particularly in the preoptic area and arcuate nucleus of the hypothalamus (Kükürt *et al.*, 2020). Kisspeptin exerts its action via a G-protein-coupled receptor 'kisspeptin 1 receptor' also known as "GPR53", which can trigger the release of gonadotropin-releasing hormone, GnRH, then follicle stimulating hormone, FSH, and luteinizing hormone, LH, in various mammals. Kiss1 is regarded as both an activator and a regulator of the surge and pulsatile nature of GnRH/or LH (Leonardi *et al.*, 2022).

Therefore, it plays a major role in puberty and maturity-onset, ovulation, implantation, metabolic regulation at parturition, and fertility (Jamil *et al.*, 2017; Kükürt *et al.*, 2020). Therefore, scientists attempted to regulate the seasonal fecundity in some animal species by kisspeptin, and researchers reported an increase in sexual activity is associated with the elevation of hypothalamic expression of kisspeptin during long photoperiod in Syrian hamsters (Shashank *et al.*, 2018; Abdulkareem *et al.*, 2021).

A kisspeptin injection, either peripherally or centrally, can induce synthesis and secretion of gonadotropins in humans (Kükürt *et al.*, 2020), rats (Azizi *et al.*, 2020), goats (AL-Ameri, 2019), ewes (Abdulkareem *et al.*, 2021), heifers (Macedo *et al.*, 2019), and postpartum dairy cattle (Amin *et al.*, 2022). Several studies reported that when injected kisspeptin-10 intravenously, intracerebroventricularly, or subcutaneously, it can influence the hypothalamus-hypophysis-gonad axis and increase GnRH secretion, in turn, LH and FSH secretions (Picard-Hagen *et al.*, 2015; Narayanaswamy *et al.*, 2016; Azizi *et al.*, 2020). Additionally, a continuous infusion of kisspeptin-10 has the potential to trigger ovulation in ewes during non-breeding season and enhance puberty onset in prepuberty rats (Beltramo & Decourt, 2018). Furthermore, kisspeptin promotes the release of the growth hormone (GH), associated with LH in prepubertal cattle (Daniel *et al.*, 2015; Leonardi *et al.*, 2022).

To our knowledge, the kisspeptin effects as a regulator and/or an activator of FSH and LH in comparison with GnRH on fertility performances and reproductive hormones in anestrus cattle were not previously studied, so the current research was aimed to explore the impact of kisspeptin-10 on the fecundity traits of anestrus Iraqi cows as an alternative strategy to GnRH application.

MATERIALS AND METHODS

Ethics Approval

This experiment was approved by the ethics committee (ECVM) at the Veterinary Medicine College, Al-Qasim Green University (approval ECVM No. 112022).

Animals

Forty lactating Holstein Friesian cows, aged 3.5-6.8 years and weighing 335- 420 kg, were housed in a semi-opened system at Barakat AL Redah station in Babylon government from 16/February/2021 to 15/February/2023.

The animals have green grass and hay. Water was provided as free according to the station system "without restriction of feed and water". All cows have suffered from inactive ovaries and anestrus for 60-70 days postpartum. The clinical investigations were done before the study to check its health; additionally, vaginal inspection and rectal palpation of the uterus and ovaries were done to confirm that each cow is nonpregnant and has soft, small, non-functional, and non-cystic ovaries, as well as an empty and soft uterus that was confirmed by ultrasonography.

Experimental Treatments

Forty anestrous Holstein Friesian Iraqi cows were distributed randomly into four groups (n=10). For synchronization, all animals were treated with a control intravaginal drug-releasing dispenser, CIDR, with 1.38 mg progesterone for eight days (Chacher *et al.*, 2017). All cows were treated as follows: C was referred to

Table 1. Feedstuffs and chemical analysis of ration

Feedstuffs		Chemical analysis		
Ingredients	g/kgfeed	Items (%)	Ration	Straw
Wheat bran	398	Dry matter	92.98	93.67
Barly grain	402	Crude protein	17.15	2.94
Soy bean meal	71	Crude ash	5.78	9.90
Yellow corn	104	Ether extract	2.71	0.64
Urea (42%N)	5	Crude fiber calculated*	6.55	38.38
Salt (NaCl)	10	Nitrogen free extract	58.11	41.81
Limestone (CaCO3)	10	Calculated metabolized energy (ME)* (Kcal/kg feed)	2754.00	1375.00

Note: *Calculated from chemical analysis tables for Iraqi feed stuffs (Al-Khawaja *et al.*, 1978).

the control group and administered 5 mL of normal saline intramuscularly (I.M) after removal of CIDR. Gn was referred to 2nd group treated with 0.5 mg/animal of GnRH I.M (Fertagyl, Intervet, Holand) after the withdrawal of CIDR immediately (Khamas, 2011). K1 and K2 were referred to the third and fourth groups administered intramuscularly with kisspeptin-10, at 6 and 12 μg/kg body weight (BW) doses in 5 mL normal saline post-removal CIRD, respectively. Kisspeptin-10 is a lyophilized powder (Biotechpeptides, Inc, USA); it is soluble in DMSO (dimethylsulfoxide); it thawed in 5% DMSO according to the manufacturer instrument immediately before injection; the kiss 1-10 dosage was performed at approximately according to Macedo *et al*. (2019).

Estrus Investigation and Artificial Insemination

After detecting the heat signs in cows, the proficient veterinarian inseminated cows artificially with thawing frozen semen; artificial insemination (AI) was done once only. Pregnancy was diagnosed after 45-55 days of artificial fertilization by rectal palpation.

Blood Collection and Hormonal Estimation

A day of injection with GnRH and kisspeptin-10 is considered as 0 day. Blood was collected from the jugular vein in a test tube at 0, 2, 6, 24, and 72 h post hormonal injection, then centrifuged to obtain sera that was stored in -20 °C for FSH, LH, Estradiol -17 beta, and Progesterone detection by ELISA techniques according to the manufacturing instructions of Biotech Fine Co., Ltd., Wuhan kits.

Fecundity traits: Estrous onset, fertility, and calving rates have been recorded after hormonal treatment and post-twelve months later. The conception rate was estimated as the number of cows pregnant after AI / number of cows were artificially inseminated x 100. The calving rate was estimated as the number of calves born / number of cows were artificially inseminated x 100. The fertility rate was estimated as the number of cows calving / number of cows artificially inseminated x 100 (Abdulkareem *et al.*, 2012).

Statistical Analysis

Two-way variance analysis (ANOVA II) was performed in the present data to compare among and within treatment groups and periods. The chi-square was also applied in the existing article to detect the significant alteration; the significant changes were estimated at the probability 0.05 level (SAS, 2001).

RESULTS

The statistical evaluation of kisspeptin action on fertility hormones and fecundity feature were established in the existing article. The kisspeptin-10 action on FSH was explained in Figure 1. At 2 and 6 h after hormonal injection, the current results revealed a significant ($p<0.05$) gradual rise in FSH values in cows injected 6 and 12 μg/Kg of Kiss-10 in comparison with Gn and C (Figure 1B & 1C), while Gn1 recorded a significant ($p<0.05$) elevation in comparison with C at 24 & 72 h (Figure 1D & 1E). However, they did not record a significant difference among Gn, K2, and K1 at 24 h (Figure 1).

At the same time, there is a gradual significant rise ($p<0.05$) in serum FSH value in Gn at 24 h post-treated (133.880 ± 12.818). While K1 and K2 registered a significant value at 6 h post-treatment with values (140.180 ± 1.063) and (146.040 ± 1.907), respectively, compared to their values at 0, 2, and 72 h post-treated (Figure 1F).

The results of mean values of LH of anestrus Iraqi cows administered with GnRH and kisspeptin-10 show a significant ($p<0.05$) increase in K2 at 2, 6, and 24 h after hormonal injection when compared with K1, Gn, and C (Figure 2B, 2C, & 2D). In addition, Gn recorded a significant aggregate ($p<0.05$) at 72 h in comparison with K1, K2, and C (Figure 2E). Within the same time, K1 and K2 documented significant ($p<0.05$) elevation at 24 h when compared with their values during periods interval 0, 2, 6, and 72 h with values (2.287 ± 0.088) and (2.848 ± 0.136), respectively, as well as LH reported high significant ($p<0.05$) in Gn at 72 h with value (2.043 ± 0.048) in compared with its value during 0, 2, 6, and 24 h also (Figure 2F).

Besides, the efficacy of kisspeptin-10 and GnRH on serum estradiol -17 beta (pg/mL) in anestrous cows is explained in Figure 3. The differences among groups within the same period referred to elevate significantly ($p<0.05$) in serum values of estradiol -17 beta in the cows of K2 at 2, 6, 24, and 72 h periods when compared with the other treated groups (Figure 3B, 3C, 3D, &3E), whereas cows administered 6 μg/Kg of kiss-10, K1, showed a significant elevation in serum estradiol -17 beta at 2, 6, and 24 h in comparing with Gn and control (Figure 3B, 3C, & 3D). On the other hand, when compared among periods for each group, the statistical results noted that GnRH caused a significant ($p<0.05$) increment in serum estradiol -17 beta values at 24 h post hormonal treatment (8.563 ± 0.087) when compared with its values in the other periods. In addition, anestrus cows in K1 and K2 reported a significant elevation ($p<0.05$) at 24 h (11.585 ± 0.357) and (15.834 ± 0.090), respectively, in comparison with their values in different periods intervals (Figure 3F).

In Figures 4C and 4D, there is a significant decline in serum progesterone levels in K2 and K1 at 2, 6, and 24 h when compared with their values in the control cow, while at 72 h, there is a significant elevation in serum progesterone levels in K2 and K1 cows in comparison with Gn and C cows (Figure 4E). At the same time, the K1 and K2 results registered a significant rise in serum progesterone values at 72 h compared with their values at 0, 2, 6, and 24 h. At the same time, Gn showed a significant decline during these periods.

Table 2 shows the statistical results of the kisspeptin effect on fecundity features in Iraqi cows. After hormonal treatment, the current results reported a significant raising in conception, calving, and fecundity rates in cow-administered kisspeptin-10 when compared with GnRH and control cows, as well as

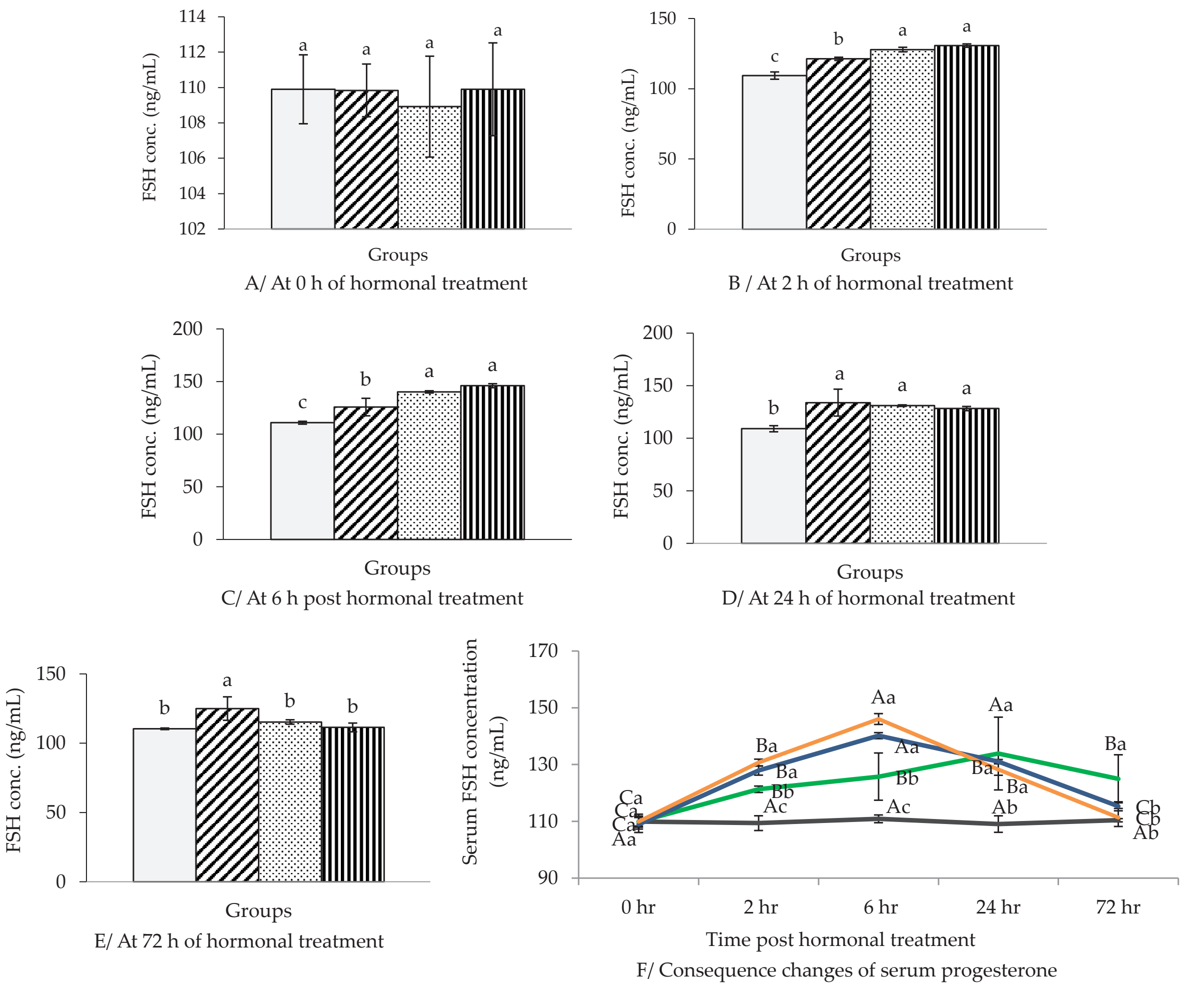

Figure 1. Serum FSH concentration (ng/mL) at 0, 2, 6, 24, and 72 h post treatment in Iraqi cows. The values represented means±SD. C= control group, Gn= injected 0.5 mg/animal intramuscularly, K1= injected 6 µg/kg bw intramuscularly of kisspeptin-10, and K2= injected 12 µg /kg bw intramuscularly of kisspeptin-10. Small letter denoted to comparison among groups within same period at (p<0.05). Capital letter denoted to comparison throughout periods per each group at (p<0.05). □C ▨Gn ▩K1 ▥K2 —C —Gn —K1 —K2

post-one year later, there was a significant increment in fertility, conception, and calving rates in K1 and K2 in comparing with C and Gn cows.

DISCUSSION

Several strategies have been applied to resolve anestrus in Iraqi Holstein cattle with various hormones like progesterone with gonadotropins and equine chorionic gonadotropin, eCG, and PMSG that were very cost and have side effects, like abortions, defect in the fetus, and formation of antibody when applied a second time (Al-Hamedawi *et al.*, 2016; Kafaji *et al.*, 2017; Kanasaki *et al.*, 2021), as the current research documented a comparison of two various hormones, GnRH and kisspeptin-10, on reproductive events in Holstein Friesian anestrous cows in Iraq. Additionally, it provides a novel reproductive conception. The current study established that kisspeptin-10 stimulates the secretion of FSH and LH that positively reflected on folliculogenesis, pregnancy, and calving rates because of the ability of kisspeptin-10 to resolve the decline of their levels via enhancement of gonadotropin and ovarian responses in anestrous animals (Hermiz & Hadad, 2020) consequent growing and development the follicles then ovulation of graafian follicle as a result of LH action on the ovary (Abdulkareem *et al.*, 2021).

Furthermore, Sébert *et al*. (2010) discovered that the IV kisspeptin-10 infusion caused FSH and GH elevation associated with LH surge. These findings were consistent with current results, which showed a gradual rise in serum FSH levels in anestrous cows post kisspeptin-10 treatment and peaked after 6 h. In contrast, its level in anestrous cow injected GnRH delay to reach the peak values after 24 h post-I.M injection of GnRH. This result is attributed to the stimulating action of kisspeptin-10 on hypothalamic-pituitary-gonad axis that activated GnRH neurons to secrete GnRH consequently FSH, triggering estrous cycle and estradiol secretion from growing follicles, which effecting negatively and positively FSH and LH, respectively (Hernández-Hernández *et al.*, 2021; Macedo *et al.*, 2021) leading to start decreasing of FSH level at 24 h post kisspeptin-10 intramuscular injection associated with significant elevation of LH level at 24 h in K1 and K2 anestrous cow. These findings have been supported by *in vivo* and *in vitro* documents (Kanasaki,

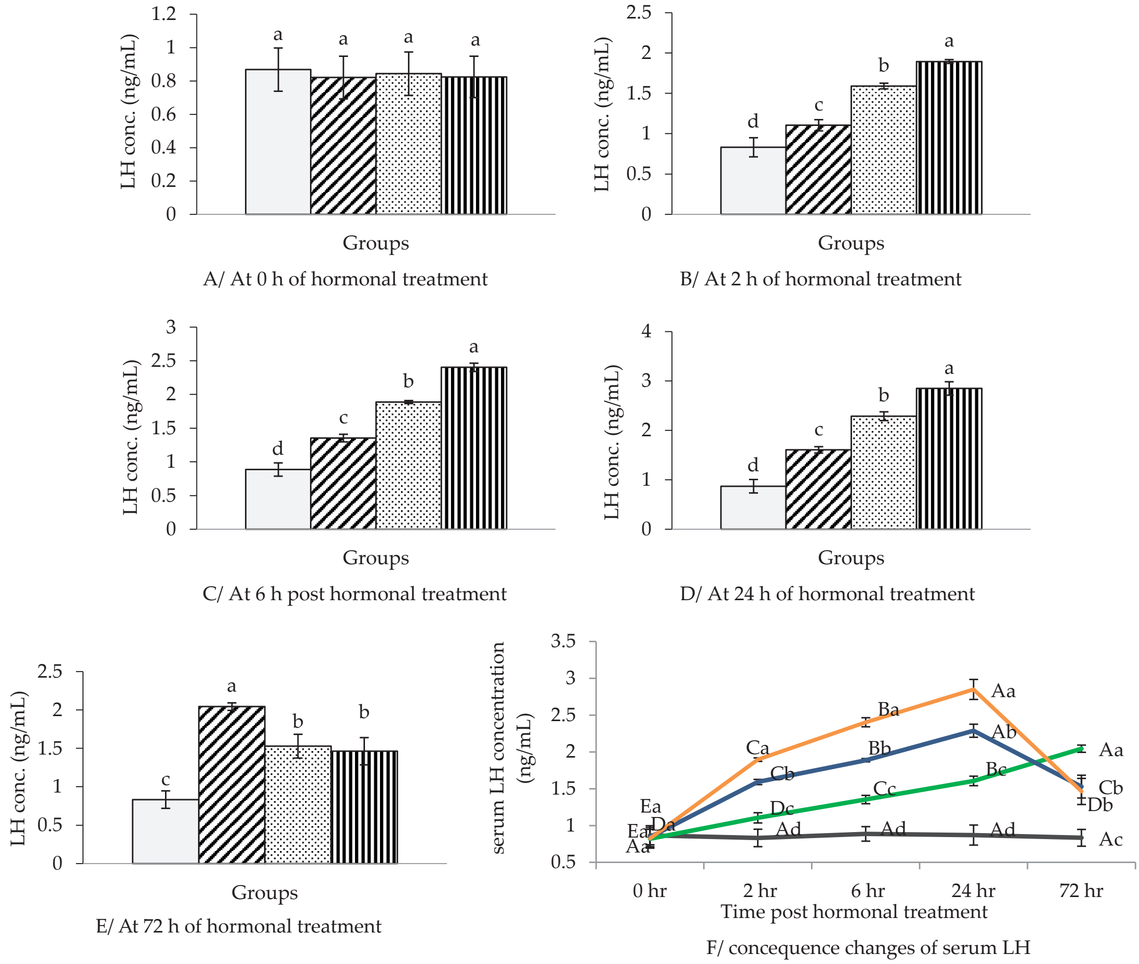

Figure 2. Serum LH concentration (ng/mL) at 0, 2, 6, 24, and 72 h post treatment in Iraqi cows. The values represented means±SD. C= control group, Gn= injected 0.5mg/animal intramuscularly K1= injected 6 µg /kg bw intramuscularly of kisspeptin-10, and K2= injected 12 µg/kg bw intramuscularly of kisspeptin-10. Small letter denoted to comparison among groups within same period at ($p<0.05$). Capital letter denoted to comparison throughout periods per each group at ($p<0.05$). ☐C ▨Gn ⊡K1 ▥K2 —C —Gn —K1 —K2

2021). Also, kisspeptin-10 has another potential pathway for stimulating LH secretion via its direct action on gonadotropic cells because the latter cells have GPR54 (Hassaneen *et al.*, 2016).

In addition, the changes in the levels of LH in K1 and K2 cows were consistent with the finding of Naniwa *et al*. (2013), who demonstrated that the intravenous injection of full-length kisspeptin-53 in adult beef Japanese black cows enhanced follicular growth and development and then released more LH depending on kisspeptin doses. Additionally, a previous study concluded that the injection of kisspeptin-10 could induce more FSH and LH secretion, leading to stimulating folliculogenesis and ovulation in female calves (Daniel *et al*., 2015). In addition, Leonardi *et al*. (2018) recorded that the IV injection of kisspeptin-10 can induce follicle growth and release LH dose-dependently during the luteal phase in cattle. This concentration peak occurred 15 min post 1, 10, or 15 mg of kisspeptin-10 treatment, then returned to its baseline level for one h, supporting the current results.

In a recent article, both Gy and Holstein, prepubertal heifers released LH in proportion directly to kisspeptin dose, while the quantities of LH secreted were always fewer than in the GnRH analogue treatment, as well as kisspeptin therapy can induce LH surge and ovulation in the same manner as that of a potent GnRH agonist, and LH began to be released earlier in heifer-treated kisspeptin than that in GnRH analogue (Macedo *et al*., 2019). The previous findings agree with the current data; LH reaches a peak at 24 h in anestrous cow K1 and K2 earlier than that in anestrous cow treated with GnRH reaches a peak at 72 h. These differences may be attributed to the potent role of kisspeptin/kpiss1 in the pituitary cell gland to regulate the function of the latter gland and the other relevant factors, as well as, brain and hypothalamus neurons, have been activated by kiss1, such as paraventricular and supraoptic nuclei (Kanasaki *et al*., 2021).

In particular, Kiss1 and its receptor are present in the arcuate nucleus and preoptic area (Yeo & Colledge. 2018). The arcuate nucleus has a specific set of cell bodies and fibers of neurons that play a significant role in synchronizing GnRH secretion. These neurons, KNDy neurons, co-expresses kisspeptin, neurokinin B, and dynorphin that act as output, stimulatory, and inhibito-

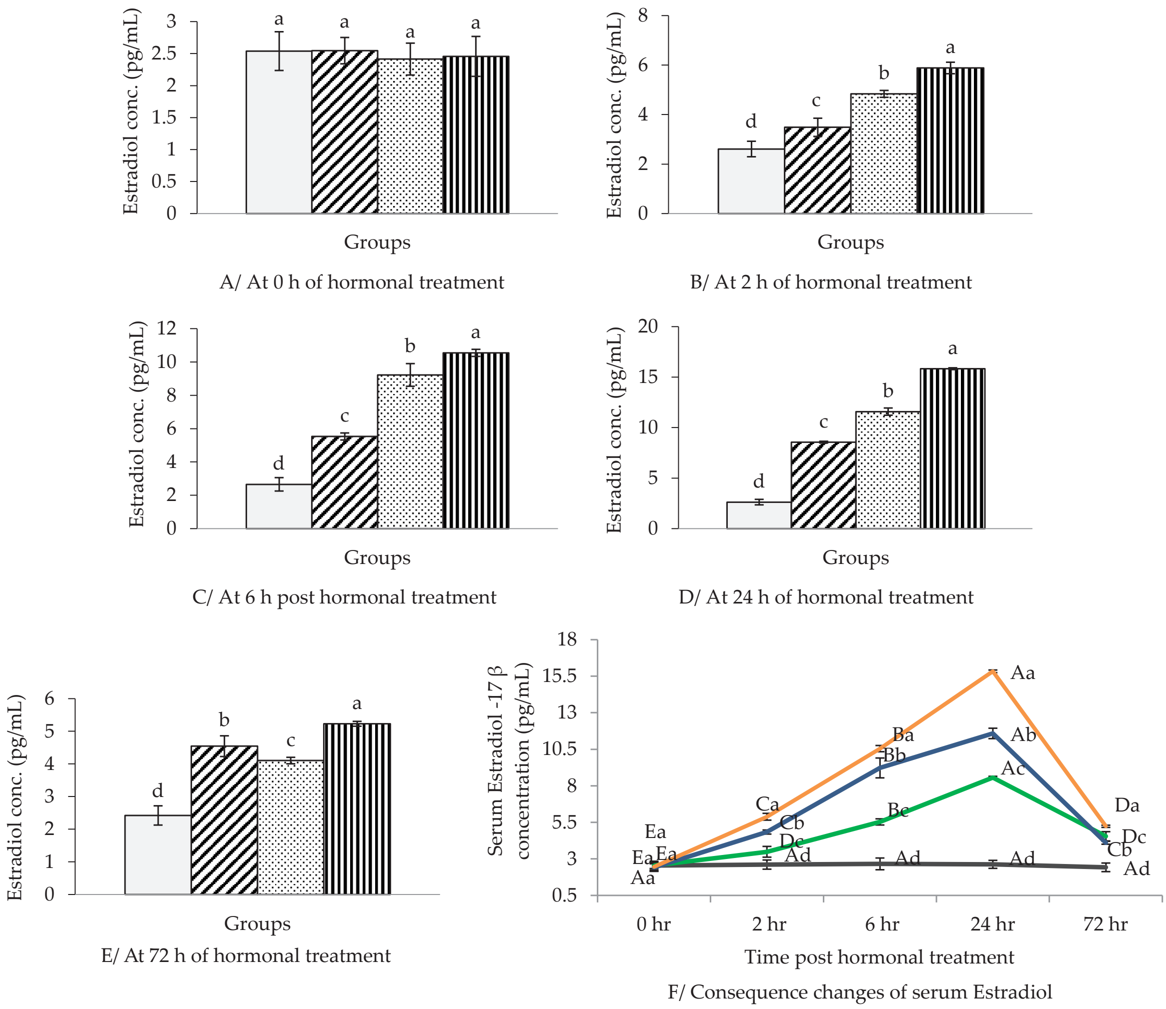

Figure 3. Serum Estradiol -17 β concentration (pg/mL) at 0, 2, 6, 24, and 72 h post treatment in Iraqi cows. The values represented means±SD. C= control group, Gn= injected 0.5mg/animal intramuscularly K1= injected 6 µg /kg bw intramuscularly of kisspeptin-10, and K2= injected 12 µg/kg bw intramuscularly of kisspeptin-10. Small letter denoted to comparison among groups within same period at ($p<0.05$). Capital letter denoted to comparison throughout periods per each group at ($p<0.05$). □C ▨Gn ▩K1 ▥K2 —C —Gn —K1 —K2

ry signals, respectively, for GnRH pulsatile production from GnRH neuron in bovine (Hassaneen *et al.*, 2016). Indeed, the pulsatile releasing of GnRH is controlled by the combined actions of these neuropeptides "neurokinin B, dynorphin, and kiss 1", as kiss1 can act as an autocrine, endocrine, and paracrine modulator to regulate anterior pituitary gland hormone secretion (Uenoyama *et al.*, 2021). Besides, Picard-Hagen *et al*. (2015) found that a single dose of GnRH analogue I.M injected during the luteal phase in Holstein heifers could induce the release of LH with a peak post 1-2 h and lasted six h, which is similar to the current results so GnRH injection can induce ovulation, as a result in a calving rate of 60% in animals. The present data is consistent with the finding of Leonardi *et al*. (2020), who demonstrated that the human kiss 10 administered IV over two h was able to promote ovulation in the dominant follicle after 36 h of injection with low progesterone level in heifers, which was the same as the ovulation rate in GnRH agonist and concluded decline in progesterone level post-kisspeptin treatment, in turn, ovulation occurred due to the action of kisspeptin-LH surge (Leonardi *et al.*, 2020).

Furthermore, murine kiss10 can elevate LH levels and sex steroid hormones in ovariectomized cows (Macedo *et al.*, 2021). Besides, the current results found a significant elevation in estradiol level and a decline in progesterone level in all treated anestrous cows, which may be due to the ability of kisspeptin to induce growth and development of follicles by the action of FSH (Macedo *et al.*, 2021). It promotes estradiol synthesis in the growing follicles; the latter hormone induces LH receptor expression on the follicular surface and is influenced by negative and positive feedback on FSH and LH, respectively (Leonardi *et al.*, 2022). As a result, ovulation occurs due to the action of LH; progesterone levels are inversely correlated with releasing LH; consequently, low levels of progesterone are closely linked with the increased activity of GnRH neurons and increased frequent pulse in gonadotropin hormone, in turn, promoted LH β transcription (Hassaneen *et al.*, 2016).

Besides, there is a correlation among kisspeptin, estradiol, and pregnancy outcome as reported by Latif & Rafique (2015), who concluded that the serum con-

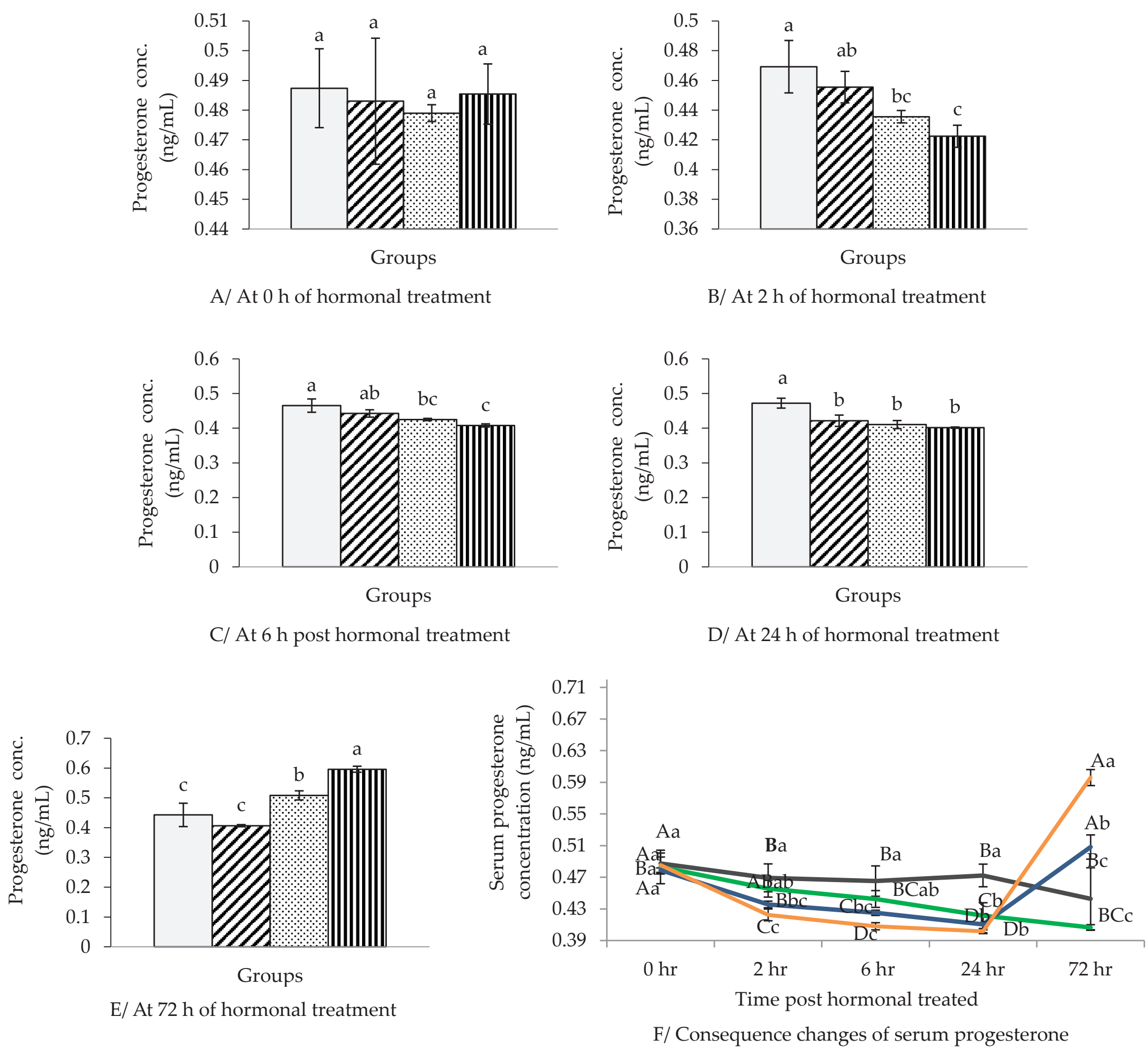

Figure 4. Serum progesterone concentration (ng/mL) at 0, 2, 6, 24, and 72 h post treatment in Iraqi cows. The values represented means±SD. C= control group, Gn= injected 0.5mg/animal intramuscularly K1= injected 6 µg /kg bw intramuscularly of kisspeptin-10, and K2= injected 12 µg/kg bw intramuscularly of kisspeptin-10. Small letter denoted to comparison among groups within same period at (p<0.05). Capital letter denoted to comparison throughout periods per each group at (p<0.05). ☐ C ◪ Gn ▦ K1 ▥ K2 — C — Gn — K1 — K2

Table 2. Efficacy of Kisspeptin 10 on fecundity traits in cattle

Duration interval	Fecundity traits	Treatments				Values of significance
		C (n=10)	Gn (n=10)	K1 (n=10)	K2 (n=10)	
After hormonal treatment	Fertility rate %	0.0 (0%)	0.3 (30%)	0.8 (80%)	10 (100%)	*
	Conception rate %	0%	30%	80%	100%	*
	Calving rate %	0%	30%	80%	100%	*
Post twelve months of hormonal treatment	Fertility rate %	0.0 (0%)	0.0 (0%)	0.7 (70%)	10 (100%)	*
	Conception rate %	0%	0%	70%	100%	*
	Calving rate %	0%	0%	70%	100%	*

Note: * Denote to significant differences at p<0.05. C= control group, Gn= injected 0.5 mg/animal intramuscularly, K1= injected 6 µg/kg.b.w. intramuscularly of kisspeptin-10, and K2= injected 12 µg/kg.b.w. intramuscularly of kisspeptin-10.

centration of estradiol and kisspeptin increased from the beginning of the follicular phase to preovulatory and luteal phases in a menstruating female, as other study proved that the level of serum estradiol could effect on the level of serum kisspeptin because there is a positive correlation between kisspeptin and estradiol and between pregnancy outcome and kisspeptin as found in Jamil *et al*. (2017) and Rehman *et al*. (2020), who also reported that the kisspeptin could enhance endometrium thickness, oocytes and embryo quantity and quality, and the possibility to become a blastocyst, inconsequence positive pregnancy and fertility, that is consistent with the current data in Table 2, which may explain the increasing values in conception, calving, and fertility rates due to the ability of injected kisspeptin-10 to improve oocyte maturity and embryo growth with success the pregnancy via improving the levels of progesterone during follicular development and pregnancy that affect pregnancy maintenance/loss (Martins *et al.,* 2018**;** Hameed & Alsalim, 2022; Masumi *et al*., 2022).

Additionally, Stevenson & Pulley (2016) suggested that when the concentration of estradiol was less than 4 pg/mL, whereas the concentration of progesterone was greater than 0.5 ng/mL at the time of AI, this resulted in suppression of pregnancy establishment via affecting ovulation. However, these findings support the current results of conception and calving rates in K and K2 groups, where the estradiol levels of each group at the time of AI exceeded 4 pg/mL up to 15 pg/mL, whereas progesterone was lowering that explained the elevated values of conception and calving rates in K2 and K1 groups counteract the effects observed in GnRH group.

Furthermore, kisspeptin showed a long-term effect on resolving inactive ovaries in bovine without requiring a second application or side effects such as abortion, poor fetus development, and defect in embryos as recorded when applied GnRH due to its short half-life that explains the higher calving rate after one year of injected kisspeptin in K1 and K2 cows compared with Gn and control cows. These results agree with Abdulkareem *et al*. (2021), who noticed higher rates in fertility, lambing, estrus, and conception at the third estrous cycle in ewes injected with kisspeptin compared with ewes injected eCG. In the future, several studies are required to investigate the ability of kisspeptin-10 to induce superovulation in ovine and bovine, as well as to study the role of kisspeptin-10 on certain factors that can cause early embryonic death and mortality with different causes.

CONCLUSION

The results achieved from the current study showed that kisspeptin-10 injection can improve and induce folliculogenesis and ovulation in lactating cows suffering from inactive ovaries by stimulating synthesis and releasing FSH, estradiol, and LH that positively reflect on calving rate and prolificacy for a long-term effect until a second estrous; therefore, kisspeptin considered as an alternative usage for GnRH to improve the fecundity in anestrous Iraqi cattle. The current experiment was regarded as the unique study to treat cattle suffering from inactive ovaries postpartum without requiring a second kisspeptin-10 injection, with long-term effects and a lower cost.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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