RESEARCH PAPER
AL-ANBAR JOURNAL OF VETERINARY SCIENCES
Vol. 12 issue: 1 2019 L- ISSN :1999-6527

The effect of hormonal treatment with kisspeptin, GnRH and hCG on semen characteristics in buck Cyprus goats during non-breeding season as compared with breeding season

Maad Hasani AL-Ameri *, Talal Anwer Abdulkareem ** and Ahmed Alaa Eldin Taha***

***College of Veterinary Medicine/ University of Fallujah**

**** College of Agriculture/ University of Baghdad**

***** Directorate of Agricultural Researches/ Ministry of Agriculture**

Abstract

The current study aimed to determine the effect of hormonal treatment with kisspeptin, GnRH and hCG on plasma testosterone concentration and semen characteristics in buck Cyprus goats during non-breeding season as compared with breeding season. This study was executed at the Ruminant Researches Station pertaining to the Directorate of Agricultural Researches, Ministry of Agriculture, Abu-Ghraib, Baghdad (latitude 33˚20' N) for the period from November 15th, 2012 to December 31st 2013. A total of 20 buck Cyprus goats 2 years old and averages 55 kg body weight. During non-breeding season bucks were randomly divided into five equal groups (4 bucks / group). The first group (A1) was regarded as a control group, i.m injected with normal saline, whereas, the second (A2) and third (A3) groups were i.v injected with 4 and 8 µg / kg body weight of Kisspeptin-10 respectively. The fourth (A4) and fifth (A5) groups were i.m injected with hCG (250 IU / buck) and GnRH (20 µg / buck) respectively. Plasma testosterone concentrations were significantly (P≤ 0.01 and P≤ 0.05) increased at 20, 30, 40 and 50 min post-treatment in A5, A2 and A3 groups as compared with control A1. Mass motility and individual motility were significantly (P≤ 0.01) in A5, A4, A2 and A3 as compared with control during non-breeding season. In conclusion, our results show that Kisspeptin-10, GnRH and hCG can stimulate the quiescent hypothalamic–pituitary–gonadal axis of bucks during non-breeding season by increasing plasma testosterone concentration post treatment that leads improving some semen characteristics during non-breeding season.

تاثير المعاملة الهرمونية kisspeptin وGnRH وhCG على صفات السائل المنوي لذكور الماعز القبرصي خلال فترة السكون الجنسي ومقارنته بالموسم التناسلي

معد حساني العامري*، طلال انور عبد الكريم ** واحمد علاء الدين طه***

*** كلية الطب البيطري / جامعة الفلوجة ، ** كلية الزراعة / جامعة بغداد ، ***دائرة البحوث الزراعية / وزارة الزراعة**

الخلاصة

هدفت الدراسة الحالية لمعرفة تاثير المعاملة الهرمونية kisspeptin وGnRH وhCG في تركيزبلازما التستوستيرون وصفات السائل المنوي لذكور الماعز القبرصي خلال موسم السكون الجنسي ومقارنته بالموسم التناسلي. نفذت هذه الدراسة في محطة ابحاث المجترات – دائرة البحوث الزراعية – وزارة الزراعة في منطقة ابو غريب ، بغداد (دائرة عرض 33 20˚ شمالا) للفترة من 15 تشرين الثاني 2012 الى 31 كانون الاول 2013 . استخدمت 20 ذكر من الماعز القبرصي بعمر 2 سنة وبمعدل وزن 55 كغم. خلال موسم السكون الجنسي وزعت الذكور بشكل عشوائي الى خمسة مجاميع (4 ذكور / مجموعة) .مجموعة الاولى (A1) تمثل مجموعة السيطرة، حقنت بالعضلة بالمحلول الفسلجي ، بينما المجاميع (A2) و(A3) حقنت بالوريد (4 و 8 مايكروغرام / كغم وزن الجسم) من Kisspeptin-10 على التوالي. المجموعتين الرابعة (A4) والخامسة (A5) حقنت بالعضلة بهرمون hCG (250 وحدة دولية / ذكر) و20 مايكروغرام لكل ذكر بهرمون GnRH على التوالي. ازداد معنويا تركيز بلازما التستوستيرون (P≤ 0.01 and P≤ 0.05) بالاوقات 20، 30،40 و50 دقيقة بعد المعاملة الهرمونية لدى المجاميع A5، A2 و A3 مقارنة بمجموعة السيطرة A1. الحركة الجماعية والحركة الفردية كانت معنوية (P≤ 0.01) لدى المجاميع A5 ، A4 ، A2 و A3 مقارنة بمجموعة السيطرة A1 خارج الموسم التناسلي. نستنتج من الدراسة الحالية بان kisspeptin وGnRH وhCG تحفز محور تحت المهاد – النخامية – الغدد الجنسية لدى الذكور خلال موسم السكون الجنسي بزيادة تركيز بلازما التستوستيرون بعد المعاملة التي ادت الى تحسين بعض صفات السائل المنوي خلال موسم السكون الجنسي.

الكلمات المفتاحية : كيسبيبتين 10 ، هرمون محرر لهرمونات الغدد ، هرمون الغُدَدِالتَناسُلِيةِ البَشَرِية ، الهرمون الذكري ،صفات السائل المنوي ،ذكر الماعز
Keyword: kisspeptin-10 , GnRH , hCG, bucks, testosterone, semen characteristics

RESEARCH PAPER
AL-ANBAR JOURNAL OF VETERINARY SCIENCES
Vol. 12 issue: 1 2019 L- ISSN :1999-6527

Introduction

Goat breeds living in subtropical, middle and high latitudes regions show seasonal changes in reproductive activity (1). Photoperiod is an important environmental factor that controls the timing of sexual activity may have occurred through changes in the endogenous annual rhythm of reproduction by photoperiod (2). Consequent occurred the increase of testosterone concentration during the natural breeding season (summer and autumn), followed by a significant decrease during the non-breeding season (winter and spring) (3,4,5) indicating ability to use bucks for semen collection and for natural breeding due to the most semen quality parameters were better during breeding season (6). The seasonality of reproduction in the herd is dependent on both bucks and does. Therefore, attempts to control sexual activity in goats during non-breeding season based on simulating activity of hypothalamic-pituitary-gonadal (HPG) axis by manipulation of sexual activity to offer meat and milk over the year and increase the income of farmers.

Nowadays, researchers are using kisspeptin which is considered gatekeeper of the hypothalamic-pituitary-gonadal (HPG) axis in which the hypothalamic GnRH plays a crucial role. Kisspeptin-10 can stimulate this axis of ewes in anestrus (7) consequently the release of LH and FSH (8) at 20- 30 min after the second injection in anestrus does (9). On the other hand, kisspeptin -10 studied in men (10) monkey (11) rats (12) mice (13,14) calves (8,15) to stimulate secretion of LH and testosterone. There is no available information about the effect of hormonal treatments kisspeptin10, GnRH and hCG on testosterone concentrations and improving semen characteristics in buck Cyprus goats during non-breeding as compared with the breeding season.

Materials and methods

Animals: The present study was conducted at Ruminant Research in the Department of Agricultural Research Station / Ministry of Agriculture, Abu Ghraib /Baghdad (latitude 33 20 'º N). A total of 20 buck Cyprus goats 2 years old and averages 55 kg body weight.

Bucks were trained to collect semen for 45 days before starting the experiment (mid-November-end of December in 2012) using an artificial vagina. The process of collecting semen from all bucks until has stopped at the end of the breeding season, giving conclusive evidence of the start of the animal non-breeding season.

Bucks were kept indoors at night and outdoors most of the day, and allowed field grazing pasture near the station on day. Indoors, bucks were fed hay and concentrated diet. All males had free access to water and trace mineral salt blocks.

Experimental design: The study period which extended from June 1^{st} 2013 to December 31^{st} 2013. During non-breeding season in June 1^{st} 2013 bucks were divided randomly into five groups (4 bucks /group). The First group (67.8 ± 14.14 kg) control (A1) injected i.m. (0.9% NaCl) is divided into two doses (2 ml / animal / dose). A second group (64.5± 1.55kg) (A2) injected i.v. Kisspeptin-10 (Ana Spec, Inc., USA) (4 µg / kg/ animal). A third group (55.3± 2.05kg) (A3) injected i.v. Kisspeptin-10 (8 µg / kg/ animal). Divided doses based on preliminary results (8.9). Fourth group (62.3± 6.22kg) (A4) injected i.m. hCG (Chorulon, Intervet International BV, Boxmeer, Holland) 250 IU / animal, divided two doses. A fifth group (58.0± 2.54kg) (A5) injected i.m. GnRH (Receptal, Intervet International BV, Boxmeer, Holland) 20 µg/ animal and divided two doses. All bucks received two doses 2h intervals.

Blood sampling and assay: Blood samples collected 0 (pretreatment) and 20, 30, 40 and 50 min after the second dose injected by the recommendations of (9). The blood samples collected from the jugular vein using vacutainer tubes, container inhibitor coagulant (EDTA) for testosterone. The blood sample immediately (10 ml) collected via vacutainer tubes, and plasma was harvested following centrifugation of the samples (3000 RPM for 20 minutes) and stored under -20°C until assay. Enzyme-linked immunosorbent assay, ELISA used to measure the plasma testosterone concentration (ng/ml). The kit provided by Kit (Testosterone AccuBind ELISA from producer Monobind Inc. Company Lake Forest, USA). Inter- and intra-assay coefficients of variation (CV %) were 9.7% and 4.8% respectively.

Semen collection and evaluation: During non-breeding season (June, July, August and September) and breeding season (October, November and December). Semen collected from each buck twice a month, with an artificial vagina and at a temperature of 41 ˚C at (8.00h). Immediate examinations, ejaculate volume recorded using a graduated collection tube. pH measured directly using indicator papers range 5.6 - 8.0 (Madaus GmbH, Koeln, Germany). Microscopic examinations included mass motility (16,17) individual motility (18), dead sperm percentage (19), sperm concentration (20) was determined using a haemocytometer following dilution of an aliquot of semen with 0.05% formal saline (1:400). The overall number of sperm per ejaculate calculated (volume × concentration). Abnormal sperm percentage (21) classified to sperm head abnormalities, sperm tail midpiece abnormalities and sperm tail principal and terminal abnormalities (22).

Statistical analysis: The statistical analysis system –SAS 2012 program was used (CRD) to study the effect of hormonal treatments on plasma testosterone concentrations for different periods and comparison semen characteristics between two seasons. Duncan's multiple range tests were used for significant differences between means in this study (23).

Results and Discussion

Plasma testosterone concentrations

The results showed the plasma testosterone concentrations in Cyprus bucks in different times during non-breeding season (Table 1). There were no significant differences pretreatment in different groups. Plasma testosterone concentrations were significantly ($P \leq 0.01$) higher in A5 group after 20, 30, 40 and 50 min as compared to A1 group. These results agreed with findings (25) in male Japanese Shiba goats and with (25) in Damascus, Mountain and hybrid bucks and agree with (26).

Increased testosterone concentration in A5 group after treated GnRH due to increase steroidogenic capacity regulatory (StAR) protein and steroidogenic enzymes in testis that it responsible for producing steroid hormones such as Cholesterol Side -chain cleavage cytochrome P450 and 3 beta-hydroxysteroid dehydrogenase / delta in the Leydig cells (27) or increased ICSH receptors (28) and increase GnRH receptors on the pituitary gland lead to stimulate secretion of ICSH and affect to release testosterone greater amounts.

Plasma testosterone concentrations increased in bucks treated with kisspeptin-10 in different times (Table 1). However, highest concentrations of testosterone in A2 group at 50 min and A3 group at 40 min. These results agreed with (13,24,15). The effect of kisspeptin increased testosterone concentration due to it stimulate secretion of GnRH neurons synthesis in the hypothalamus as observed in humans (29), mice (30), rats

(31, 32), monkeys (33), Sheep (34) and Goats (9), because of exist receptors of Kisspeptin in neurons synthesis GnRH in the hypothalamus (35, 36, 37) that reflected to increase secretion of ICSH from the pituitary gland (38, 39,40) which effect on Leydig cells and stimulating secretion of testosterone (41).On the other hand, Zhang et al (42) reported that kisspeptin depolarizes GnRH neurons from 5 to 22 mv and making it more permeable to Kisspeptin and led to increase GnRH firing rates (43, 44). Depolarization process may last for 30 minutes after the disappear of the effect of Kisspeptin, that explains concentration of testosterone is high in groups A2 and A3 as compared with A1 during 20-50 minutes after treatment. In the present study, increase concentration testosterone (Table 1) in A4 group treated hCG at 30 minutes (7.58 ± 0.49 ng / ml) and 40 minutes (8.02 ± 0.26 ng / mL) as compared with A1 due to the role of hCG in stimulating leydig cells on secretion larger amounts of testosterone into the blood stream, because of LH and hCG are closely related in sequence, and these two hormones bind to the same receptor and elicit identical biological responses (45, 46).

Semen

A clear effect of hormonal treatment to improve some semen characteristics observed during non-breeding season as compare with control, mass motility (Table 4) individual motility (Table 5) sperm concentration (Table 7) in bucks. On the other hand, bucks treated GnRH (A5) distinguished that better results in semen characteristics as compare with groups. These results agree with (47) who found that injection Nili-Ravi buffalo bulls 2 ml of GnRH (Dalmarelin-Fatro) at weekly interval, 12 hours prior to semen collection. Similarly, Azawi et al **(**48) in a study with Awassi rams in Iraq found injection 50 µg im of GnRH (Cystorelin) weekly for 3 months lead to improve semen quality after GnRH treatment. The dose used in our current study (20 µg / animal) was lower than that used in previous studies. Therefore, a cost-effective method for owners that can use to improve the sperm quality of bucks during non-breeding season. In addition, that using different GnRH analogues in the different studies may give different results to semen quality studied due to the different effectiveness of their effect (49).

Results of the present study showed that bucks treated kisspeptin (4 and 8 µg / kg/ animal) during non-breeding season led to improve mass motility (Table 4) individual motility (Table 5) and sperm concentration (Table 7). The role of kisspeptin and its receptor play in the regulation of the reproductive axis (35, 36, 37) and necessary for GnRH secretion and release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) (38, 39,40) affecting the cells of Leydig and stimulating to secrete testosterone (41) and activate spermatogenesis process (50) On the other hand, there were no significant difference between bucks treated kisspeptin as compare with control (A1) in dead sperm percentage (Table 6), sperm head abnormalities (Table 9), sperm tail midpiece abnormalities (Table 10) and percentage sperm total abnormalities (Table 12) may have not affected treatment due to the low dose of kisspeptin-10 In the current study, Which selected based on a few previous studies, focused mostly on the study of the effect of kisspeptin in the concentrations of LH and testosterone exclusively did not treat the role in improving the characteristics of sperm of any farms animal, and the use of high doses in future studies may give better results in improving quality of sperm of bucks in the non-breeding season without adversely affecting the body's hormonal system. (51) Reported that High doses (50 nmol / day) of Kisspeptin-54 for three consecutive days lead to desensitization of hypothalamus-pituitary-testis axis to the

negative feedback of testosterone, as a result of the significant increase in its secretion, thus a decrease GnRH and subsequent reduction LH and FSH from the pituitary gland. The negative feedback of testosterone plays a significant regulatory role to secrete GnRH from neurons produced by the hypothalamus (52). Our results showed that sperm tail principal and terminal abnormalities (P≤ 0.05) starts to increase in A3 group as compare with A1 during non-breeding season as well increased in A1 as compare with A 5 and A3 during breeding season (Table 11). On the other hand, sperm tail principal and terminal abnormalities showed difference significance (P≤ 0.01) in A3, A2 and A5 groups during non-breeding season as compare with breeding season (Table 11). However, the hormonal treatments did not effect on percentage sperm total abnormalities and others during non-breeding season and breeding season. In addition the percentage of sperm head abnormalities and sperm total abnormalities remained within their acceptable natural range agricultural animals 20% (53). On the other hand, absence significant differences in most of the sperm abnormalities during non-breeding season and breeding season confirms the effectiveness of hormonal factors in sustaining seminiferous tubules in the testis for a long period until they enter the breeding season. These results agree with (54) who found that a greater number of natural sperm are available to fertilize the female reproductive system so that they can penetrate the ova and fertilize in a natural way. In addition, applicable to the percentage of sperm abnormalities applies to all the characteristics of semen microscopic, whose values did not differ between the non- breeding season and breeding season showing the role of long-term factors maintenance good quality of the sperm. In conclusion, our results show that Kisspeptin-10, GnRH and hCG can stimulate the quiescent hypothalamic–pituitary–gonadal axis of bucks during non-breeding season by increasing plasma testosterone concentration post treatment that leads improving some semen characteristics during non-breeding season.

RESEARCH PAPER
AL-ANBAR JOURNAL OF VETERINARY SCIENCES
Vol. 12 issue: 1 2019 L- ISSN :1999-6527

Table 1. The effect of hormonal treatments on plasma testosterone concentrations (ng / ml) in bucks for different periods during non-breeding season (mean ± S.E.).

Period / Treatment	Pre treatment	20 min after treatment	30 min after treatment	40 min after treatment	50 min after treatment	Level of significance
A1	5.73±1.90 A a	1.45±0.61 B c	0.94±0.41 B d	0.94±0.38 B d	3.67±1.51 AB b	P≤ 0.05
A2	2.20±0.43 B a	5.37±0.67 A b	5.30±0.62 A c	5.81±0.50 A c	5.95±0.45 A ab	P≤ 0.05
A3	2.50±1.04 C a	5.26±0.31 B b	6.03±0.57 B c	7.18±0.69 AB b	6.95±0.63 AB ab	P≤ 0.01
A4	3.34±1.82 B a	6.49±0.93 AB ab	7.58±0.49 AB b	8.02±0.26 A ab	6.35±1.48 AB ab	P≤ 0.05
A5	4.06±1.35 B a	8.31±0.65 A a	9.34±0.16 A a	9.18±0.20 A a	8.17±1.05 A a	P≤ 0.01
Level of significance	NS	P≤ 0.01	P≤ 0.01	P≤ 0.01	P≤ 0.05	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between periods within treatment.
A1 = 2ml (0.9% NaCl) (control), A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 2. The effect of hormonal treatments on ejaculate volume (ml) in bucks during two seasons (mean ± S.E.).

season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	1.58±0.25 A a	1.64± 0.42 A a	NS
A2	1.86± 0.18 A a	1.70± 0.21 A a	NS
A3	1.68± 0.29 A a	1.62± 0.19 A a	NS
A4	0.13 ± 1.92 A a	1.50± 0.19 A a	NS
A5	1.60± 0.20 A a	1.56± 0.22 A a	NS
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 3. The effect of hormonal treatments on pH of semen in bucks during two seasons (mean ± S.E.).

season / Treatment	Non-breeding Season	Breeding season	Level of significance
A1	6.72±0.04 A a	6.80± 0.03 A a	NS
A2	6.77±0.04 A a	6.75± 0.03 A a	NS
A3	6.63±0.07 A a	6.77± 0.02 A a	NS
A4	6.64± 0.06 A a	6.77± 0.01 A a	NS
A5	6.63± 0.09 A a	6.80± 0.03 A a	NS
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 4. The effect of hormonal treatments on mass motility in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding Season	Breeding season	Level of significance
A1	47.91± 10.27 A b	68.33± 3.81 A a	NS
A2	75.00± 2.21 A a	72.91± 2.71 A a	NS
A3	72.27± 2.72 A a	67.91± 3.91 A a	NS
A4	78.18± 1.54 A a	72.08± 1.78 B a	P≤ 0.01
A5	80.00± 1.82 A a	72.08± 3.56 A a	NS
Level of significance	P≤ 0.01	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 5. The effect of hormonal treatments individual motility in bucks during two seasons (mean ± S.E.).

season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	51.25±10.97 B b	75.41±2.78 A a	P≤ 0.05
A2	80.83±2.11 A a	79.58±2.25 A a	NS
A3	78.63±2.34 A a	74.58±3.56 A a	NS
A4	84.54± 1.84 A a	77.91± 1.68 B a	P≤ 0.01
A5	85.00±1.82 A a	78.33±3.44 A a	NS
Level of significance	P≤ 0.01	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 6. The effect of hormonal treatments on dead sperm percentage in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	19.25± 4.41 A a	29.66±2.57 A a	NS
A2	25.00±3.90 A a	28.00±2.97 A a	NS
A3	25.54±3.32 A a	30.75±4.16 A a	NS
A4	22.54±2.42 A a	27.41±2.75 A a	NS
A5	18.10±1.88 A a	27.50± 4.34 A a	NS
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 7. The effect of hormonal treatments on sperm concentration (x 10^9 /ml) in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	6.41±1.62 A b	8.68± 1.24 A a	NS
A2	8.72±0.88 A ab	10.66± 1.76 A a	NS
A3	8.82±0.64 A ab	10.14±1.38 A a	NS
A4	8.05±0.95 A ab	10.16±1.24 A a	NS
A5	10.07±0.65 A a	7.42± 0.81 B a	P≤ 0.01
Level of significance	P≤ 0.05	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 8. The effect of hormonal treatments on the overall number of sperm per ejaculate (x $10^{9)}$ in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	14.31±3.44 A a	12.60±2.00 A a	NS
A2	15.47±2.57 A a	18.97±3.38 A a	NS
A3	14.04±1.93 A a	16.80±3.59 A a	NS
A4	12.23±2.06 B a	19.80±2.83 A a	P≤ 0.05
A5	16.24±2.95 A a	11.33±1.52 A a	NS
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 9. The effect of hormonal treatments on sperm head abnormalities in bucks during two seasons (mean ± S.E.).

season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	6.08±1.38 B a	13.08±2.62 A a	P≤ 0.01
A2	8.58±1.73 A a	12.62±3.33 A a	NS
A3	6.50±1.05 B a	14.16± 3.14 A a	P≤ 0.05
A4	8.18±1.25 A a	8.37±1.41 A a	NS
A5	5.85±0.95 B a	16.79±4.20 A a	P≤ 0.05
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control), A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 10. The effect of hormonal treatments on sperm tail midpiece abnormalities in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	0.25± 0.16 A a	0.16±0.12 A a	NS
A2	0.16±0.09 A a	0.20±0.14 A a	NS
A3	0.09±0.06 A a	0.12±0.08 A a	NS
A4	0.22±0.18 A a	0.25±0.13 A a	NS
A5	0.15± 0.10 A a	0.08±0.08 A a	NS
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 11. The effect of hormonal treatments on sperm tail principal and terminal abnormalities in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	8.25±2.19 A b	11.08±1.63 A a	NS
A2	10.75±1.17 A ab	6.16±1.36 B b	P≤ 0.01
A3	14.40±2.30 A a	7.04±2.02 B ab	P≤ 0.01
A4	9.50±1.57 A ab	8.29±1.44 A ab	NS
A5	11.70±1.80 A ab	5.12±0.95 B b	P≤ 0.01
Level of significance	P≤ 0.05	P≤ 0.05	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

Table 12. The effect of hormonal treatments on percentage sperm total abnormalities of semen in in bucks during two seasons (mean ± S.E.).

Season / Treatment	Non-breeding season	Breeding season	Level of significance
A1	14.58± 3.54 B a	24.33± 2.60 A a	P≤ 0.05
A2	19.50± 2.41 A a	19.00± 3.17 A a	NS
A3	21.00± 3.04 A a	21.33± 3.10 A a	NS
A4	17.90± 2.54 A a	16.91± 1.86 A a	NS
A5	17.70± 2.29 A a	22.00± 4.09 A a	NS
Level of significance	NS	NS	

a,b,c Among columns, comparison between treatments.
A,B,C Among rows, comparison between seasons
A1 = 2ml (0.9% NaCl) (control) ,A2 (4 µg / kg/ animal) kisspeptin, A3(8 µg / kg/ animal) kisspeptin ,A4 (250 IU hCG / animal) and A5 (20 µg/ animal GnRH).

References

1) Gómez-Brunet, A.; Santiago-Moreno, J.; Toledano-Diaz, A. & López-Sebastián, A. (2012). Reproductive seasonality and its control in Spanish sheep and goats: A review. Tropical and Subtropical Agroecosystems., 15 (Suppl. 1): S47-S70.

2) Delgadillo, J, A.; Cortez, M, E.; Duarte, G.; Chemineau, P. & Malpaux, B. (2004). Evidence that the photoperiod controls the annual changes in testosterone secretion, testicular and body weight in subtropical male goats. Reprod. Nutr Dev; 44: 183–193.

3) Talebi, J.; Souri, M .; Moghaddam, A.; Karimi, I. & Mirmahmoodi, M.(2009). Characteristics and seasonal variation in the semen of Markhoz male in western Iran. Small Rumin. Res., 85:18–22.

4) Şogorescu, E .; Zamfirescu, S.; Roşoiu, N.; Anghel, A, H & Nadolu, D.(2011). Seasonal variations of plasma testosterone levels and testicular volume in Carpathian male. Afr. J. Agric. Res., 6(32) :6735-6740.

5) Ait Amrane, A.; Hammoudi, S, M.; Belhamiti, B, T.; Selles, S, M, A.; Benia, A, R. & Kaidi, R.**(** 2013**)** . Seasonal variation of plasma testosterone levels in Algerian male Arabia goats**.** Afr. J. Biotechnol., 12(48): 6785-6790.

6) Kridli, R, T.; Tabbaa, M, J . & Barakeh, F, S. (2007). Seasonal variation in scrotal circumference and semen characteristics of Black Bedouin and Black Bedouin-Damascus crossbred male. Asian-Aust. J. Anim. Sci., 20(3): 359 – 364.

7) Sebert, M,E.; Lomet, D.; Said, S,B.; Monget, P.; Briant, C.; Scaramuzzi, R,J.& Caraty, A. (2010). Insights into the mechanism by which kisspeptin stimulates a preovulatory LH surge and ovulation in seasonally-acyclic ewes: potential role of estradiol. Domest. Anim. Endocrinol.38: 289–298.

8) Ahmed, E, A.; Siato, H.; Sawada, T,; Yaegashi, T.; Yamashita, T.; Hirata, T.; Sawai, K.& Hashizume, T.(2009). Characteristics of the stimulatory effect of Kisspeptin-10 on the secretion of luteinizing hormone, follicle-stimulating hormone and growth hormone in prepubertal male and female cattle. J. Reprod. Dev., 55: 650-654.

9) Hashizume, T.; Saito, H.; Sawada, T.; Yaegashi, T.; Ahmed, E, A.; Sawai, K.& Yamashita, T.(2010) .Characteristics of stimulation of gonadotropin secretion by kisspeptin-10 in female goats. Anim. Reprod. Sci .,118: 37-41.

10) George, J, T.; Veldhuis, J, D.; Roseweir, A, K.; Newton, C, L.; Faccenda, E.; Millar, R, P.& Anderson, R, A.(2011). Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. J. Clin. Endocrinol. Metab.; 96: E1228–E1236.

11) Huma, T.; Ulla, F.; Hanif, F.; Rizaz, J, D. & Shahab, M.(2014).Peripheral administration of kisspeptin antagonist does not alter basal plasma testosterone but decreases plasma adiponectin levels in adult male rhesus macaques. Turk J Biol., 38: 1-7.

12) Tovar, S.; Vazquez, M, J.; Navarro, V, M.; Ferna´ndez-Ferna´ndez, R .; Castellano, J, M.; , Vigo, E.; Roa, J.; Casanueva, F, F.; Aguilar, E.; Pinilla, L.; Dieguez C. & Tena-Sempere, M.(2006). Effects of single or repeated intravenous administration of kisspeptin upon dynamic LH secretion in conscious male rats. Endocrinology., 147(6):2696–2704.

13) Mikkelsen, J, D.; Bentsen, A, H.; Ansel, L.; Simonneaux, V.& Juul, A.(2009). Comparison of the effects of peripherally administered kisspeptins. Regulatory Peptides., 152 : 95–100.

14) Curtis, A, E.; Cooke, J, H.; Baxter, J, E.; Parkinson, J, R, C.; Bataveljic, A.; Ghatei, M, A.; Bloom, S, R. & Murphy, K, G.(2010). A kisspeptin-10 analog with greater in vivo bioactivity than kisspeptin-10. Am J Physiol Endocrinol Metab., 298: E296–E303.
15) Ahmed, E, A.; Haridy, M.; Kassab, A, Y.; Ahmed, H.; Senosy, W .; Toh-Ichi, H. & Hashizume, T. (2018). The Efficiency of Kisspeptin and GnRH as Stimulators of Gonadotrophins and Testosterone inPrepubertal Male Cattle . Zagazig Veterinary Journal., 46, (2): 1-10.
16) Blom, E.(1946). Kompartions, Kammeret Hjaeipe middle foberet mikroskopisk under sogelse of ufortyndet tyesperma. skand .Vet. Tidskr. For Bakteriol, Patologi, Samr. Koh Ock. Mjalk Iggrin. 613: Abst.Vet., 102: 252.
17) Salisbury, G, M.; Van Denmark, N, L.& Lodge, J, R.(1978).Semen evaluation. In: Physiology of Reproduction and Artificial Insemination of Cattle. 2nd ed. W. H. Freeman (Ed.), San Francisco., 326-353.
18) Walton, A.(1933).Technique of Artificial Insemination. Imp. Bur. Anim. Genet. 56, llius, Edinburgh.
19) Swanson, E, W.& Bearden, H, J.(1951). An eosin nigrosin stain differentiating live and dead bovine spermatozoa. J. Anim. Sci., 10: 981-987.
20) Salisbury, G, W.; Beck, G, H.; Elliet, I. & Willett, E, L.(1943). Rapid method of estimating the number of spermatozoa in bull semen. J. Dairy Sci., 26: 483-486.
21) Hancock, J, L.(1951). A staining technique for the study of temperature shock in semen. Nature., 167:323-324.
22) Melrose, D, R. & Laing, J, A.(1970). Characteristics of normal semen. In: Fertility and Infertility in the Domestics Animals. J. A. Laing (Eds.), Chapt. 4, Bailling Tindell and Cassell Press, London.
23) Duncan, D.B. (1955). Multiple Rang and Multiple F-test. Biometrics.,11:1-24.
24) Saito, H.; Sawada, T.; Yaegashi, T.; Goto, Y.; Jin, J.; Sawai, K.& Hashizume, T.(2012). Kisspeptin-10 stimulates the release of luteinizing hormone and testosterone in pre- and post-pubertal male goats. Anim. Sci. J., 83: 487-492.
25) Al-Omari, H, Y.(2012). Study of Testosterone concentration during breeding season of goat bucks and their crossbred under exogenous GnRH treatments. Asian. J. Anim. Vet. Adv., 7(8):693-701.
26) Schanbacher, B, D. & Lunstra, D, D.(1977). Acute and chronic effects of gonadotropin releasing hormone on reproductive characteristics of rams during the nonbreeding season. J. Anim. Sci., 44:650-655.
27) Aspden, W, J.; Rodgers, R, J.; Stocco, D, M.; Scott, P, T.; Wreford, N, G.; Trigg, T, E.; Walsh, J. & D'Occhio, M, J. (1998). Changes in testicular steroidogenic acute regulatory (StAR) protein, steroidogenic enzymes and testicular morphology associated with increased testosterone secretion in bulls receiving the luteinizing hormone releasing hormone agonist deslorelin. Domest. Anim. Endocrinol., 15: 227-238.
28) Melson, B, E.; Brown, J, L.; Schoenemann, H, M.; Tarnavsky, G, K. & Reeves, J, J. (1986). Elevation of serum testosterone during chronic LHRH agonist treatment in the bull. J. Anim. Sci; 62: 199-207.
29) Dhillo, W, S.; Chaudhri, O, B.; Thompson, E, L.; Murphy, K, G.; Patterson, M.; Ramachandran, R.; Nijher, G, K.; Amber, V.; Kokkinos, A. & Donaldson, M.(2007). Kisspeptin-54 stimulates gonadotropin release most potently during the

preovulatory phase of the menstrual cycle in women. J. Clin. Endocrinol. Metab., 92: 3958–3966.

30) Gottsch, M, L.; Cunningham, M, J.; Smith, J, T.; Popa, S, M.; Acohido, B, V.; Crowley, W, F.; Seminara, S.; Clifton, D, K. & Steiner, R, A.(2004). A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology., 145(9):4073-4077.

31) Navarro, V, M.; Castellano, J, M.; Fernandez – Fernandez, F, R.; Barreiro, M, L.; Roa, J.; Sanchez-Criado, J, E.; Aguilar, E.; Dieguez, C.; Pinilla, L. & Tena-Sempere, M. (2004). Developmental and hormonally regulated messenger ribonucleic acid expression of KiSS-1 and its putative receptor, GPR54, in rat hypothalamus and potent luteinizing hormone-releasing activity of KiSS-1 peptide. Endocrinology., 145(10):4565–4574.

32) Navarro, V ,M.; Castellano, J, M.; Fernandez-Fernandez, F, R.; Tovar, S.; Roa, J.; Mayen, A.; Nogueiras, R.; Vazquez, M, J.; Barreiro, M, L. & Magni, P.(2005). Characterization of the potent luteinizing hormone-releasing activity of KiSS-1 peptide, the natural ligand of GPR54. Endocrinology., 146:156–163.

33) Seminara, S, B. (2006). Mechanisms of disease: the first kiss-a crucial role for kisspeptin-1 and its receptor, G-protein-coupled receptor 54, in puberty and reproduction. Nat. Clin. Pract. Endocrinol. Metab., 2:328–334.

34) Caraty, A.; Smith, J, T.; Lomet, D.; Ben Saïd, S.; Morrissey, A.; Cognie, J.; Doughton, B.; Baril, G.; Briant, C.& Clarke, I, J.(2007). Kisspeptin synchronizes preovulatory surges in cyclical ewes and causes ovulation in seasonally acyclic ewes. Endocrinology., 148: 5258–5267.

35) Irwig, M, S.; Fraley, G, S.; Smith, J, T.; Acohido, B, V.; Popa, S, M.; Cunningham, M, J.; Gottsch, M, L.; Clifton, D, K.& Steiner, R, A.(2004). Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology., 80:264–272.

36) Han, S, K.; Gottsch, M, L.; Lee, K, J.; Popa, S, M.; Smith, J, T.; Jakawich, S, K.; Clifton, D, K.; Steiner, R, A. & Herbison, A, E.(2005). Activation of gonadotropin-releasing hormone neurons by kisspeptin as a neuroendocrine switch for the onset of puberty. J. Neurosci., 25:11349–11356.

37) Messager, S.; Chatzidaki, E, E.; Ma, D.; Hendrick, A, G.; Zahn, D.; Dixon, J.; Thresher, R, R.; Malinge, I.; Lomet, D.; Carlton, M, B.; Colledge, W, H.; Caraty, A. & Aparicio, S, A.(2005). Kisspeptin directly stimulates gonadotropin-releasing hormone release via G protein-coupled receptor 54. Proc. Natl. Acad. Sci. USA., 102:1761–1766.

38) Lapatto, R.; Pallais, J, C.; Zhang, D.; Chan, Y, M.; Mahan, A.; Cerrato, F.; Le, W, W.; Hoffman, G, E. & Seminara, S, B.(2007). Kiss1K/K mice exhibit more variable hypogonadism than Gpr54K/K mice. Endocrinology.,148:4927–4936.

39) d'Anglemont de Tassigny, X.; Fagg, L, A.; Dixon, J, P.; Day, K.; Leitch, H, G.; Hendrick, A, G.; Zahn, D.; Franceschini, I.; Caraty, A. & Carlton, M, B.(2007) Hypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. PNAS.,104: 10714–10719.

40) d'Anglemont de Tassigny, X.; Fagg, L, A.; Carlton, M, B.& Colledge, W, H.(2008) Kisspeptin can stimulate gonadotropin-releasing hormone (GnRH) release by a direct action at GnRH nerve terminals. Endocrinology., 149:3926–3932.

41) Senger , P, L.(2003). Regulation of reproduction: nerves, hormones and target tissues. In: Pathways to Pregnancy and Parturition. Chapter 5.

2nd revised edn. Current Conceptions Inc., Washington, USA., 102-127.
42) Zhang, C.; Roepke, T, A.; Kelly, M, J.& Rønnekleiv, O, K.(2008). Kisspeptin depolarizes gonadotropin-releasing hormone neurons through activation of TRPC-like cationic channels. J. Neurosci., 28:4423–4434.
43) Quaynor, S.; Hu, L.; Leung, P, K.; Feng, H.; Mores, N.; Krsmanovic, L, Z.& Catt, K, J.(2007). Expression of a functional G protein-coupled receptor 54-kisspeptin autoregulatory system in hypothalamic gonadotropin-releasing hormone neurons. Mol. Endocrinol., 21:3062–3070.
44) Dumalska, I.; Wu, M.; Morozova, E.; Liu, R.; van den Pol, A. & Alreja, M.(2008). Excitatory effects of the puberty-initiating peptide kisspeptin and group I metabotropic glutamate receptor agonists differentiate two distinct subpopulations of gonadotropin-releasing hormone neurons. J. Neurosci., 28:8003–8013.
45) McFarland, K, C.; Sprengel, R.; Phillips, H, S.; Kohler, M.; Rosemblit, N.; Nikolics, K.; Segaloff, D, L.& Seeburg, P, H.(1989). Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. Science., 245:494-499.
46) Ascoli, M.; Fanelli, F.& Segaloff, D, L.(2002). The lutropin/choriogonadotropin receptor, a perspective. Endocr. Rev., 23:141-174.
47) Sajjad, M.; Ali, S.; Akhter, S.& Ullah , N.(2007). Effect of gonadotropin releasing hormone on semen characteristics in Nili-Ravi buffalo bulls. Pakistan. Vet. J., 27: 153-154.
48) Azawi, O, I.; Al-Khashab, A, N, T, M. & Al-Kadoo, N, N, A.(2012). Effect of gonadotropin releasing hormone treatment on semen characteristics and enzymatic activities of Awassi rams in breeding and non-breeding seasons. Iranian J. Applied Anim. Sci., 2(1): 13-19.
49) Hayden, C. (2008). GnRH analogues: applications in assisted reproductive techniques. Eur. J. Endocrinol., 159: S17-S25.
50) Mei, H.; Walters, C.; Carter, R. & Colledge, W, H.(2011). Gpr54K/K mice show more pronounced defects in spermatogenesis than Kiss1K/K mice and improved spermatogenesis with age when exposed to dietary phytoestrogens. Reproduction., 141: 357-366.
51) Appleby, G, F.; Thompson, E, L.; Murphy, K, G.; Patterson, M.; Bewick, G, A.; Stamp, G, W, H.; Todd, J, F.; Ghatei, M, A.& Bloom, S, R. (2006). Continuous administration of kisspeptin-54 leads to desensitisation of the hypothalamo-pituitary-gonadal axis and testicular degeneration. 197th Meeting of the Society for Endocrinology, 6th – 7th Nov., London, UK. Endocrine Abstracts., 12: 92.
52) Tilbrook, A, J.& Clarke, I, J.(1995). Negative feedback regulation of the secretion and actions of GnRH in male ruminants. J. Reprod. Fertil. (Suppl.)., 49: 297-306.
53) Saacke, R, G.; Nadir, S.& Nebel, R, L. (1994). Relationship of semen quality to sperm transport, fertilization, and embryo quality in ruminants. Theriogenology., 41:45-50.
54) Vogler, C, J.; Bame, J, H.; DeJarnette, J, M.; McGilliard, M, L.& Saacke, R,G.(1993). Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. Theriogenology., 40: 1207-1219.