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Impact of Both Growth Hormone and Gonadotropin Releasing Hormone on Puberty Based on Serum Progesterone and Insulin-Like Growth Factor-1 Level in Iraqi local Breed Ewe Lambs

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Abstract

OBJECTIVE: To investigate how Growth Hormone(GH) and Gonadotropic Releasing Hormone (GnRH) injection influence on the growth and onset of puberty in Iraqi crossbred ewe lambs. Methods: Two equal groups of fourteen Iraqi local breed ewes-lambs, with an average body weight of 24.16 ± 1.05 kg and an age of 5±0.5 months, were randomly assigned. One group was used as a control, whereas the other received treatment with GH and GnRH. Weighing was done on the ewe-lambs both before and after the experiment. Furthermore, the number of ewes exhibiting their first estrus at the end of therapy, as well as the body condition scores. Blood samples were taken until the onset of pubertal symptoms also progesterone and insulin-like growth factor-1 (IGF-1) levels in sera samples were measured. Findings: It was shown that the group administered with Growth and GnRH reached puberty 3.5 weeks (24 days) sooner than the control group. The body weight (38.22±1.21b) kg , body condition scores (4.14±0.14 b) and pregnancy rate (71.43%) of the Iraqi local ewe lambs was higher than those of the control group. Progesterone and IGF-1 levels in Iraqi local ewe lambs were higher in treated group compared to control group. Conclusion: GH. and GnRH injection accelerate puberty.

Keywords: Growth hormone, Gonadotropic Releasing Hormone, Puberty, Ewe lambs, Progesterone, IGF-1.

Introduction

Sheep's numerous uses as supplies of meat, milk, dairy products, wool, and skin made them one of the first animals that humans tamed [1, 2, and 3]. Awassi sheep from Iraq are raised for their meat, milk, and wool [2-6]. Awassi ewes also have the ability to graze over large distances across pastures and have highly desirable traits like resilience to various illnesses, endurance to dietary changes, and resistance to parasites that are produced on farms designed to resemble scorching deserts [7, 8] Although lambs in flocks are semi-seasonal, they symbolize the herd's future [9] Compared to large ruminants, they have a higher rate of development, a lower rate of infection at a distance, palatable meat, a shorter production cycle, and more environmental adaption [10] For small farmers, it serves a unique and crucial role[11]. Birth timing and dietary levels are related to early puberty [12]. Nevertheless, the duration and timing of the breeding season can be impacted by interactions between the photoperiod and factors like as breed, place of origin, and nutrition [13 – 15].

The onset of puberty revealed that the reproductive axis, which runs from the brain to the gonads, undergoes multiple developmental processes. Baseline luteinizing hormone, or LH, secretion has been gradually rising, and gonadotropin releasing hormone (GnRH) secretion shows a high frequency pattern just prior to the beginning [16].

The pituitary gonadotropins' response to growth hormone (GH) which affects important metabolic pathways of intermediate metabolism, which serves as a significant regulator of growth and development throughout the postnatal period. as well as insulin-like growth factor 1 (IGF 1) might also be affected by changes in GnRH secretion [17].

The impact of growth hormone, also known as the Growth axis, on ruminant puberty has been researched, Its function is to explain the

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immunoneutralization action of the growth hormone releasing factor, which can occasionally induce growth hormone (GH) deficit in some animals, such as heifers, delaying the start of puberty [18, 19].

Puberty in animals is the time between the first estrus and ovulation [18]. The animal eventually acquires the ability to reproduce [19]. It is believed that the activity of the hypothalamic-pituitary-ovarian axis (HPO) is significantly influenced by puberty [20].

The hypothalamic gonadotropin-releasing hormone, or GnRH, neurons are the main regulators of puberty and fertility [21]. In the early stages of puberty, GnRH can activate the established gonad axis [22]. During puberty, the release of GnRH in a pulsating manner from the hypothalamus toward the anterior pituitary gland facilitates the synthesis and production of luteinizing hormone and follicle-stimulating hormone (FSH) more easily [23].

Through blood circulation, FSH and LH enter the ovary to stimulate ovarian growth and trigger the ovary's production of estrogen [24]. Exogenous GnRH injection affects not just the hypothalamic-pituitary function but also a number of physiological processes related to reproduction [25, 26]. Many non-hypthalamic reproductive organs express GnRH and its receptors, such as the ovary, uterus, and fallopian tube [27, 28]. During the onset of puberty, the ovary serves as a vital reproductive organ. GnRH has the power to affect ovarian function and participate in related reproductive processes, such as follicle expansion, steroid synthesis, and ovarian epithelial cell proliferation [29]. As can be seen from the above, a number of studies examined how the growth hormone axis affects puberty in cattle. There are few researches examining how growth hormone affects sheep puberty. Based on the research done before and the results of Rami et al., which showed that cross Awassi ewe lambs reach puberty at ages between 232 and 255 days [30]. This middle east native breed of ewe lambs was used in the current study to examine the effects of GH and GnRH which administered 70 days prior to puberty [31] on the attainment of puberty (based on serum progesterone level and serum IGF-1 levels).

Successful ewe lamb breeding enhanced earnings and decreased business risk.

Material and Methods

Experimental animals

At a private farm in the Nineveh governorate—whose latitude is 36.340000 and its longitude is 43.130001—the current study was conducted last year. Using fourteen Awassi ewe lambs from the local area that were paired with adult Rams that had demonstrated fertility between the ages of two and four years, the study's ewe lambs are healthy and free of any external or internal parasite symptoms. The animals were housed in partially open shade and fed a concentrated fodder three times a day in natural daylight circumstances, supplemented with straw and green forage.

Study design

Fourteen Iraqi cross female lambs (n=14) aged between 150 ±15days and average body weight was 20-24.16 ± 1.05 kg were used in present study. Animals were randomly divided into two groups (n=7, for each one) and kept together in same conditions [32].

The first group was kept as a control group (n=7) injected with normal saline each week. The second group injected subcutaneously, with Somatotropin (Growth Hormone) (Sandoz GmbH Schaftenaou, Long kampfen, Austria) in a dose of 0.1 mg/kg BW biweekly in same dose used by [33; 34] and GnRH Argentina commercially called Gistar intramuscularly, weekly (0.00042g/10ml) (0.1mg/animal) until approaching puberty ,ten weeks is the treatment period(35). Body weight changes were taken into account when calculate the dose in Kg in age of 150±15depending on previous report (36) who consider this end of weaning period and nutritional support estrus detection were observed by using aproned rams and consider as guide for pubertal time and pregnancy ratio of both groups were estimated after estrus synchronization.

Animal feeding

Balanced grain diet (wheat 51%, barley 40%, soy 5%, limestone 2%, sodium 1%, minerals and vitamins 1%). (37-40). Supplying water on demand, a daily concentrated diet, and green forage [41] Fresh water was also constantly available, and the ewe-lambs had unrestricted access to grain (weighing what was left over each night). For every group, estimates were given for the beginning and ending weights as well as the daily increase in body weight. In addition, the trial's body condition score was established at its conclusion [42, 43].

Blood samples, insulin-like growth factor-1) and progesterone detection

Blood samples were drawn on the 0 day of the trial and subsequently once a week until the end (expressing estrus). Centrifugation was used to extract sera from blood samples for 15 minutes at 3000 rpm after samples of blood were given time to coagulate. Until the levels of progesterone and insulin-like growth factor-1 were measured, sera were maintained at -20 degrees Celsius.

Hormonal assay

In the same manner as described by Miles LM, a two-site immunoradiometric method was utilized to quantify insulin-like growth factor-1 (IRMA). (Miles et al.,), the inspection was conducted every two weeks until the investigation's conclusion. Sheep

Insulin-Like Growth Factor-1 (ELISA Kit) from Sun Long Biotech Co., Ltd. (China) was used for the analysis. Progesterone level was assessed by competitive ELISA using kits purchased from (Elecsys Progesterone III;Cobas e411, D-68298 Mannheim, Roche Diagnostics GmbH, Germany,2023every week in blood samples used to gauge the onset of puberty [17; 44].

Estrus detecting and mating

Lambs' puberty is taken into account when determining estrus time or evidence [9, 44]. Using fertile rams (two used for natural breeding and two for detecting estrous with an apron), the time of estrus and accepting the ram were noted.

Estrus Synchronization

Applying vaginal sponges (.06 grams of Medroxy acetate P4/MAP) plus 500 IU of pregnant mare serum gonadotropin (PMSG), estrus synchronization was achieved for a precise mating timing. [45], On day 12, PMSG was utilized to synchronize ewe lambs following sponges with drawl. For this reason, the time of estrus and accepting ram were recorded by four fertile rams (two for natural breeding and the other two for using an apron to detect estrous) [44, 46-48].

Time of estrus and accepting ram were recorded. Pregnancy detection was conducted by using ultrasound Linear Probe at early period and curved probe at mid pregnancy period [48-50].

Statical analysis

Statistical Analysis System - version 9.1 (SAS) was used to do the statistical analysis of the data. Significant differences between means were evaluated using a one-way, two-way ANOVA, and a least significant differences (LSD) post hoc test. The significant differences between the percentages were tested using the Chi-square method. It is deemed statistically significant when $P < 0.05$. The relationship between age of ewes and level of IGF-1 and progesterone was represented by several equations and the best equation was chosen according to value of the coefficient of determination (R^2) [51].

Results

The group treated with GH and GnRH showed a substantial improvement in both dry matter intake and average daily gain(weight)when compared to the control group (1.53± 0.02, 170.1 ± 3.53 g vs. 1.16 ± 0.02, 69.1 ± 11.1g, respectively). As indicated by (Table1), animals in the control group had significantly lower body weight as well as condition scores than animals in the GH +GnRH treatment group (Table1).

At the start of the experiment, there was no significant difference between the groups' serum progesterone levels. Serum progesterone levels were observed to respond time-dependently to GH and GnRH administration as in (Table 2).

The group administered with GH and GnRH experienced puberty for the first time after seven weeks of research. Most of the ewe lambs in the GH and GnRH treated group achieved puberty seven and nine weeks after the experiment started, respectively, based on detection and hormonal analysis (Table 3).

As during the sixth and eighth week of the trial, the progesterone level in GH and GnRH -treated group was considerably higher ($P<0.05$) than the group under control as in (Table 3).

All synchronized ewes had a reduction in progesterone levels in the final days prior to the removal of the sponges [52]. Overall, the GH and GnRH group had higher levels of IGF-1 than the control group furthermore the trial's weeks interacted with the GH and GnRH growth effect in all weeks of the investigation.

IGF-1 levels are gradually rising in each group, especially in the last few weeks, they have significantly increased. The GH and GnRH group's level of IGF-1 was significantly greater ($P<0.05$) than the group under control (Table 4).

Group treated with GH and GnRH was advanced in time (3.5weeks), number of ewe lambs reaching puberty and number of pregnant ewes comparatively with control group (Table 5).

Discussion

The results of this investigation demonstrated that ewe lambs which are borne in winter given Growth Hormone and Gonadotropic releasing hormone attained puberty about (3.5 weeks ~ 24 days) sooner than control ewe lambs, this agreed with[53- 55]who injected GH only and [28,56] who injected GnRH only, as evidenced by two weeks in a row where serum progesterone levels increased to 1 ng/mL [57-61]. Likewise, a 60-day reduction in age ($P<0.05$) at puberty was observed after injecting GH.

Additionally, they noted that the treated group's live body weight at puberty was considerably higher than that of the control group [36, 62] which gave GH and [62, 63] who gave GnRH alone, and this result consistent with our findings in accelerating puberty. Lactating dams were dosed with the same drug throughout the post-weaning phase, and the resulting lambs displayed an increase in LBW, which was also noted during the suckling period [53].

Throughout the trial, GH and GnRH therapy had an effect on progesterone levels, particularly in the final stages of the study (0.906±0.13) and in ewe lambs that were not given medication (0.557±0.02 ng/L). This progesterone spike suggests that ovulation has taken place and this agree with [59]. Ovulation occurs more greater than untreated control

group and this result is in accord with information provided by [60]. Number of ewe lambs which became pregnant agreed along with findings of [60]. One of the possible signals that GH and GnRH used to trigger early puberty could be IGF-1 who increases in circulation as puberty approaches, is able to induce the release of prepubertal GnRH, and can advance the timing of puberty.

Sakurai and his group Found at (2003) that the plasma IGF-1 concentration in Shiba goats did not significantly alter until five weeks before to puberty [64] and this agree with our findings. The current study found that Iraqi cross local ewe lambs given GH and GnRH had higher levels of IGF-1. The data of present study showed that ewe lambs treated with Growth Hormone and GnRH reached puberty 3.5 weeks (24 days) earlier than control ewe lambs.

Conclusion

The current study demonstrated that giving Growth Hormone (GH) and Gonadotropin Releasing Hormone (GnRH) to Awassi ewe lambs increased their rate of rapid puberty.

TABLE 1. Reveled Animals in the control group and treated groups exhibited substantially

Groups	Dry Matter in take (kg)	Average daily gain(gm.)	Body weight (kg)	Body score
Control	1.16± 0.02 [a]	69.1± 11.1[a]	31.40±0.99 [a]	2.79±0.18 [a]
GH&GnRH treated	1.53± 0.02[b]	170.1± 3.53[b]	38.22±1.21[b]	4.14±0.14 [b]

In the same column, means that have a little letter are significantly different (P<0.05).

TABLE 2. Progesterone levels (ng/mL) in serum during the first four weeks of the trial in Awassi ewe lambs treated with GH+GnRH and control groups.

Groups	Weeks of the study			Over all mean of treatment effect
	0 week	2nd week	4th week	
Control	A0.065±0.003[a]	A0.068±0.001[a]	A0.073±0.003 [a]	A0.068±0.002
GH+GnRH treated	A0.050±0.008[a]	A0.061±0.005[a]	A 0.140±0.004 [a]	A0.083±0.006

Duration interaction x L.S.D. of treatment =0. 14, *P<0.05 control vs. GH and **GnRH** dealed with group. In the same column, means that have a distinct little letter are significantly different (P<0.05). Significant differences exist between means in the same row that have different capital letters (P<0.05).

TABLE 3. Serum progesterone levels (ng/mL) in control as well as GnRH treated Awassi ewe lambs throughout the final six weeks of the trial.

Groups	Weeks of the study			Over all mean of treatment effect
	6th week	8th week	10th week	
Control	A0.110±0.05 [a]	B0.558±0.04[a]	B0.557±0.02[a]	0.408±.003[a]
GH+GnRH treated	A0.173±0.04 [a]	B0.688±0.08 [a]	C 0.906±0.13[b]	0.589±0.08[a]

Interaction between treatment and duration L.S.D. of treatment x duration interaction = 0.14, *P<0.05, control group versus GH and GnRH treated group. . In the same column, means that have a distinct little letter are significantly different (P<0.05). Significant differences exist between means in the same row that have different capital letters (P<0.05).

TABLE 4.Effect of ten weeks of GnRH and Growth Hormone administration on serum insulin-like growth factor 1(ng/ml).

Weeks of the study	Groups	
	Control	GH +GnRH
0 week	A 2.88±0.11[b]	B2.54±0.03[c]
2ed week	A 2.94±0.13[b]	A2.88±0.03 [c]
4th week	B 2.95±0.09[b]	**A3.57±0.17 [b]**
6th week	B 3.01±0.13[b]	A3.59±0.14[b]
8th week	B 3.09±0.15[ab]	A3.62±0.16 [ab]
10th week	B 3.38±0.13[a]	A3.94±0.19 [a]

In the same column, means that have a distinct little letter are substantially different (P<0.05).
Significant differences exist between means in the same row that have different capital letters (P<0.05). LSD of overall treatment effect: 0.33

TABLE 5. Shows time reaching puberty, number of ewe lambs reaching puberty and number of pregnant ewes:

Groups	Time Reaching puberty (days)	No. of ewe lambs reaching puberty	No. of Pregnant ewes
Control	0^{b}	0^{b}	0^{b}
GH+GnRH treated	207 ± 10^{a}	6^{a}	5^{a}

In the same column, means that have a distinct little letter are substantially different (P<0.05).

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تاثير هرمون النمو وهرمون المطلق لموجهة الغدد التناسليه (GnRH) على البلوغ مستندا الى مصل البروجستيرون ومستوى عامل النمو الشبيه بالأنسولين -1 (IGF-1) في حملان النعاج العراقيه المحليه (العواسيه المهجنه)

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المستخلص

دراسة كيفية تأثير حقن هرمون النمو(GH) وهرمون المطلق لموجهة الغدد التناسلية (GnRH) على النمو وبداية البلوغ في حملان النعاج العراقية العواسيه المهجنة.الطرق: تم تقسيم أربعة عشر نعجة من سلالة العواسي العراقيه المهجنه بعمر 5 ± 0,5 شهر ومتوسط وزن الجسم24,16 ± 1,05 كغم الى مجموعتين متساويتين. تم استخدام المجموعة الاولى كمجموعة ضابطة، في حين حقنت المجموعة الثانيه ب GH و GnRH. تم وزن الحملان قبل وبعد التجربة علاوة على ذلك، عدد النعاج التي تظهر أول شبق لها في نهاية العلاج، وكذلك درجات حالة الجسم. تم أخذ عينات الدم كل أسبوعين لمدة عشرة أسابيع حتى ظهور علامات البلوغ. تم قياس مستويات هرمون البروجسترون وعامل النمو الشبيه بالأنسولين -1 (IGF-1) في عينات الأمصال. وفقًا للنتائج، وصلت المجموعة التي تلقت هرمون النمومع هرمون المطلق لموجهة الغدد التناسلية إلى سن البلوغ قبل 3.5 أسبوع (24 يومًا) من المجموعة الضابطة. وزن الجسم (38,22±1,21) كغم درجة حالة الجسم للحملان العراقية (0,14 ±4,14) في مجموعة المعامله كانت أعلى من تلك الخاصة بالمجموعة الضابطة. كانت مستويات البروجسترون و IGF-1 في حملان النعاج العراقية المهجنه أعلى بعدحقن هرمون النموو وهرمون المطلق لموجهة الغدد التناسلية مقارنة بحملان السيطرة ،حيث ان اعطاءالهرمونين كلاهماعجل من الضهور المبكر لعلامات البلوغ

الكلمات الدالة: هرمون النمو، الهرمون المطلق لموجهة الغدد التناسلية، البلوغ،حملان النعاج، البروجسترون، IGF-1