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Efficacy of Gonadotropin Releasing Hormone on Puberty in Iraqi Awassi Cross Breed Ewe Lambs

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Abstract

THIS STUDY investigate how gonadotropin injection affects the growth and onset of puberty in Awassi crossbred ewe lambs. *Methods*: Fourteen Awassi cross breed ewes–lambs of 5±0.5months of age and average body weight (23.7±1.48) kg were divided into two equal groups at random. One group was used as a control, and the other received Gonadotropin Releasing Hormone (GnRH) treatment. Weighing was done on the ewe-lambs both before and after the experiment, Furthermore, measuring the body condition score, number of ewes expressing first estrus after treatment. Samples of blood were taken biweekly until the observation of pubertal signs (ten weeks). Progesterone and insulin-like growth factor-1 (IGF-1) were measured in sera samples. The results revealed that the group receiving gonadotropin reached puberty 17 days ahead of the control group.The body weight and body condition score of the GnRH treated Awassi ewe lambs were found to be higher compared to those of the control group. Gonadotropin administration had a beneficial effect on increasing progesterone and IGF-1. *Conclusion* Gonadotropin administration has accelerated puberty in Awassi ewe lambs and there are more trophic signals available, as seen by higher serum progesterone and IGF-1 secretions.

Key words: Puberty, Gonadotropic releasing hormone, progesterone, IGF-1, ewe lambs

Introduction

The domesticating of sheep, which took place some 11,000–9,000 BC, was a major turning point in the history of interactions between humans and animals. Sheep were among the first animals to be domesticated for their meat, milk, and wool [1]. Sheep and goats were some of the first animals that humans domesticated, mostly for their diverse uses as meat, milk, fleece, dairy products, and skin materials [2] specially Awassi sheep in Iraq [3,4]

In Iraq, small ruminants are crucial to the food security of both the rural populace and the sheep population, which numbers 13.025 million heads [5]. In spite of they are semi seasonal [6,7].

Sheep are commonly classified as short-day breeders and seasonal polyestrous animals [8-10] variations in the reproductive activity of sheep breeds occur in subtropical, temperate, and high-latitude environments. The breeding season usually starts in the fall and ends in the winter, with anoestrus taking place in the spring and summer [7, 11, 12] .The beginning and conclusion of the breeding season are regulated by an endogenous circannual rhythm, which is impacted by the annual photoperiod cycle for regulation and synchronization [12].

The majority of farm income from the sale of lambs goes to them as well as the most significant farm animals. Due to the lower investments needed, shorter production cycles, higher development rates, and more environmental adaptation compared to large ruminants [13].

The ease with which meat could be preserved—as well as the ease with which livestock could be raised and slaughtered whenever there was a market for meat was a key argument in favor of keeping these animals on farms [14,15]. The estimation of reproductive efficiency is influenced by a wide range of parameters [16]. Genetic factors [17] can be indirectly improved and flock fertility can be increased during breeding by ewe lambs with high early puberty scores [18]. *El-Shahat et al*. (2014) state that characterization of puberty as well as

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early sexual development is useful tool for selection within the breed (19). Early puberty is linked to the time of birth and the nutritional planes [20]. According to [21] fast-growing ewe lambs reached puberty earlier than low-growing ewe lambs

However, interactions between the photoperiod and elements like breed, geographic origin, and diet can affect the time and length of the breeding season [22-24].

According to modeling conducted in Australia [25, 26], producing ewe lambs has the potential to increase farm profitability as a whole. According to Young and others [26], if research was directed at improving the reproductive performance of ewe lambs, there would be a significant return on investment.

Awassi ewes also have the ability to graze over long distances across pastures and have highly desirable traits including resilience to various illnesses, endurance to dietary changes, and resistance to parasites bred on farms shaped like scorching deserts [27, 28].

Many developmental processes take place inside the reproductive axis when puberty first appears.

Puberty in animals is the time between the first estrus and ovulation [29], the animal eventually acquires the ability to reproduce [30]. Puberty is thought to be a significant indicator of the hypothalamic-pituitary-ovarian axis' activity (HPO) [31]. The primary regulators of puberty and fertility are gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus [32]. GnRH can trigger the established gonad axis during the onset of puberty [33]. During puberty, follicle-stimulating hormone (FSH) and luteinizing hormone are synthesized and produced more readily when GnRH is released in a pulsing fashion from the hypothalamus toward the anterior pituitary gland (LH) [34, 35].

Through blood circulation, FSH and LH enter the ovary to stimulate ovarian growth and trigger the ovary's production of estrogen [36].The administration of exogenous GnRH will impact various physiological processes associated with reproduction in addition to the regulation of hypothalamic-pituitary functions [37].Numerous reproductive organs that are not hypothalamic, including the ovary, uterus, and fallopian tube, express GnRH and its receptors [38, 39].

At the beginning of puberty, the ovary plays a significant function as a reproductive organ. GnRH has the ability to influence ovarian function and take part in associated reproductive processes, including controlling the growth of follicles, the production of steroids, and the proliferation of ovarian epithelial cells [40]. A variety of nonhypothalamic reproductive organs, including the ovary, uterus, and fallopian tube, express GnRH and its receptors.

Material and Methods

Experimental animals:

The current investigation was carried out at private farm in Nineveh governorate(The latitude is 36.340000, and the longitude is 43.130001). Fourteen local Awassi ewe lambs with (4) adult Rams of proven fertility of (2-4) years old used in the study, all ewe lambs are in good health and free from any clinical signs of internal or external parasites. The animals were accommodated in partially opened shade, receiving a concentrated diet three times a day along with supplemental straw and green fodder, all under natural daylight conditions.

Study design

The study design included dividing ewe lambs borne at the end of January in two groups first group (treated group) received GnRH treatment, and the second was used as a control group in age of 150±15days and average body weight (23.7±1.48) kilogram) and kept together in same condition depending on previous report [41] who considers this end of weaning period and nutritional support. Serum levels of insulin-like growth factor-1(IGF-1) and progesterone were measured in both groups. Estrus synchronization and fertilization rate were calculated to show the differences between groups and recorded effect of gonadotropic releasing hormone (GnRH) in treated group if present [42].

Treated group injected intramuscularly weekly, with GnRH Argentina commercially called Gistar (0.00042g/10ml) (0.1mg/animal) until approaching puberty, consider as treatment group (n=7),

The second group was kept as a control group and injected intramuscularly with normal saline each week for ten weeks. 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(Poly urethane sponges soaked with Medroxyprogesterone acetate, remained for13days then removed ,after that injected intramuscularly with 500 IU PMSG **)**

Animal feeding

Balanced diet of grains (barley 40%, wheat 51%, soya been 5%, limestone 2%, Nacl 1%, minerals and vitamins 1%) [43-46], providing with green fodder, and concentrated diet daily and water ad libidum [47]. In addition, the ewe-lambs had unlimited access to feed (weighing the remaining each night), and fresh water was always accessible. Estimates were made of the starting and ending weights as well as the overall daily increment in body weight for each group. Furthermore, the body

condition score was determined at the conclusion of the trial [48].

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

Blood samples were taken 0 days then every week until the study's conclusion (expressing estrus). Sera were separated from blood samples using centrifugation at 3000 rpm for 15 minutes. Sera were preserved at -20 centigrade until the levels of progesterone and insulin-like growth factor-1 were assessed.

Hormonal assay

A two-site immunoradiometric technique was used to quantify insulin-like growth factor-1 (IRMA) in same way described by Miles LM's (Miles et al, 1974) examination was repeated every two weeks until the end of the study. The examination was done by using commercial Sheep Insulin –Like Growth Factor -1, ELISA Kit purchased from Sun long Biotech Co., Ltd (China).

By competitive ELISA using kits purchased from Elecsys Progesterone III;Cobas e411, D-68298 Mannheim, Roche Diagnostics GmbH, Germany,2023, progesterone level was assessed weekly in serum samples taken as a measure of puberty attainment[49,50].

Estrus detection and mating

Estrus time or evidence considers time of puberty for ewe lambs [51, 52]. Fertile rams (two for natural breeding and two to use an apron to detect estrous) used for this purpose, time of estrus and accepting ram were recorded.

Estrus Synchronization

Estrus synchronization was done for accurate mating time by using Vaginal sponges (60 milligrams of Medroxy acetate P4/MAP) + 500 IU of Pregnant Mare Serum Gonadotropin[53] were used after sponges with drawl to synchronize ewe lambs in day 11[52 54- 56]. 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 [57].

Statistical 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) [58]

Results

As indicated by table: 1, animals in the control group had significantly lower body weight as well as body condition scores in comparison to the GnRH treatment group. Furthermore, compared to the control group, the dry matter intake and average daily gain of the GnRH-treated group were significantly higher (Table 1).

The experiment started at the beginning of June**,** there was no significant difference between the groups' serum progesterone levels. Serum progesterone levels were observed to respond time-dependently to GnRH administration as in (Table2).

The group administered with GnRH experienced puberty for the first time after seven weeks of research. Most of the ewe lambs in the GnRH treated group achieved puberty seven to nine weeks after the experiment started respectively, based on detection and hormonal analysis (Table 3), while the control group didn't reach puberty and the season ended.

As during the sixth to eighth week of the trial, the progesterone level in GnRH -treated group was considerably higher ($P<0.05$) than the group under control as in (Table 3). In table 3 significant differences exist between means in the same row that have different capital letters ($P<0.05$).

All synchronized ewes had a reduction in progesterone levels in the final days prior to the removal of the sponges [59].

Overall, the GnRH group had higher levels of IGF-1 than the control group furthermore the trial's weeks interacted with the GnRH growth effect in the weeks two, four, and ten of the investigation.

IGF-1 levels were recrded to be gradually rising in both groups, and in the last few weeks, both groups have significantly increased. The GnRH group's level of IGF-1 was significantly greater ($P<0.05$) than the control group (Table 4). Group treated with GnRH was a head in time, 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 and given Gonadotropic releasing hormone attained puberty 17 days sooner than control ewe lambs, this agreed with Shirley *et al.* and Salem *et al.*[60,41].

As evidenced by two weeks in a row where serum progesterone levels increased to 1 ng/mL [41, 60- 63]. Additionally, they noted that the

treated group's live body weight at puberty was considerably higher than that of the control group [65, 40], and this result was found to be consistent with our findings in accelerating puberty. Lactating dams were dosed with the same drug throughout the post-weaning phase, and the resulting lambs that born in winter displayed an increase in LBW in comparison with that born in winter, which was also noted during the suckling period [60].

Throughout the trial, GnRH therapy had an effect on progesterone levels of treated group, particularly in the final stages of the study (0.705±0.10 ng/L), in comparison with ewe lambs that were not given medication (0.557.02 ng/L). This progesterone spike suggests that ovulation has taken place [64] ,ovulation occurs earlier than control group and this result is in accord with information provided by [65] .Number of ewe lambs became pregnant agreed along with findings of [65].

The gonads produce sex hormones in response to the generation of GnRH, which is produced in the hypothalamus' neurons. In the end, this hormone controls the onset of puberty, sexual maturation, and female ovulatory cycles.

Conclusion

The present study elucidated that GnRH administration accelerated puberty in Awassi ewe lambs.

Conflict of Interest

There are no conflicts of interest to be declared.

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TABLE 1. Revealed Animals in the control group and treated groups exhibited substantially food conversion body weight and body condition.

Groups	Dry Matter in take (kg)	average daily gain (g) vs	Body weight	Body score
Control	1.16± 0.02 [a]	69.1± 11.1[a]	31.40±0.99 [a]	2.79±0.18[a]
GnRH treated	1.24± 0.02 [a]	104.1± 3.23[b]	32.50±1.16 [a]	3.21±0.21[a]

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 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
GnRH treated	A0.054±0.008[a]	A0.056±0.005[a]	A0.062±0.004[a]	A0.0573±0.006

Duration interaction x L.S.D. of treatment =0. 14, *P<0.05 control vs. **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[b]	A0.558±0.04[a]	B0.557±0.02[a]	A0.408±.003
GnRH-treated	A0.101±0.003b	A0.510±0.04a	A0.705±0.10a	A0.438±0.1

Interaction between treatment and duration L.S.D. of treatment x duration interaction = 0.14, *P<0.05, control group versus 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 administration on serum insulin-like growth factor 1(IGF-1) **(ng/ml).**

Weeks of the study	Groups	
	Control	GnRH
0 week	A 2.88±0.11[b]	A 2.92±0.06[c]
2ed week	A 2.94±0.13[b]	A 3.04±0.03[b c]
4th week	B 2.95±0.09[b]	B 3.16±0.08[abc]
6th week	B 3.01±0.13[b]	B3.11±0.05[abc]
8th week	B 3.09±0.15[ab]	B 3.30±0.07[ab]
10th week	B 3.38±0.13[a]	B 3.40±0.09[a]

In the same column, means that have a distinct little letter are substantially different (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	0b	0b
GnRH treated	**214**±10[a]	5[a]	4[a]

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

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تأثير هرمون المطلق لموجهة الغدد التناسلية (GnRH) على البلوغ مستندا الى مصل البروجستيرون ومستوى عامل النمو الشبيه بالأنسولين -1 (1-IGF) في حملان النعاج العراقية المحلية (العواسيه المهجنة)

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الخلاصة

تبحث دراسته في كيفية تأثير حقن الهرمون المطلق لموجهة الغدد التناسليه(GnRH)على النمو وبداية البلوغ في حملان النعاج العواسية المهجنة. الطرق: تم تقسيم أربعة عشر نعجة من سلالة العواسي بعمر 5 ± 0.5 شهر ومتوسط وزن الجسم (23.7 ± 1.48) كجم إلى مجموعتين متساويتين عشوائياً. تم استخدام مجموعة واحدة كمجموعة تحكم، بينما تلقت المجموعة الأخرى حقن الهرمون المطلق لموجهة الغدد التناسليه. تم وزن النعاج قبل وبعد التجربة، علاوة على قياس درجة حالة الجسم، عدد النعاج التي تظهر أول شبق بعد العلاج. تم أخذ عينات من الدم كل أسبوعين حتى ملاحظة علامات البلوغ (عشرة أسابيع). تم قياس هرمون البروجسترون وعامل النمو الشبيه بالأنسولين -1 (1-IGF) في عينات الأمصال. وفقًا للنتائج، وصلت المجموعة التي تلقت الهرمون المحرر للقند إلى سن البلوغ قبل 17 يومًا (17 أسبوعًا) من المجموعة الضابطة. مجموعة حملان النعاج العواسية المعالجة كانت درجة وزن الجسم وحالة الجسم للنعاج أعلى مقارنة بالمجموعة الضابطة. كان لإعطاء الهرمون المطلق لموجهة الغدد التناسلية تأثير مفيد في زيادة هرمون البروجسترون وعامل النمو الشبيه بالأنسولين-1 في حملان النعاج العواسية على عكس مجموعة السيطرة. الاستنتاج: إعطاء موجهة الغدد التناسلية أدى إلى زيادة وتسريع البلوغ في الحملان العواسية. وذلك لأن هناك المزيد من الإشارات الغذائية المتاحة، كما يتضح من ارتفاع هرمون البروجسترون في الدم وإفرازات IGF-1.

الكلمات المفتاحية: البلوغ، الهرمون المطلق لموجهة الغدد التناسلية، البروجسترون، IGF-1، الحملان