



The Relationship Between Leptin, Testosterone Hormonal Assay Linked with Scrotal Circumference and Ultrasound Investigation of Testicular Measurements During Onset of Puberty in Awassi Male Lambs

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Abstract

The aim of this study was to find out the correlation between serum leptin, testosterone hormones, and body weight with puberty beginning in male lambs. Eighteen weaned 90day old male lambs, Blood collected to get serum and kept at -20°C for hormonal assay. An ultrasound was used to Get the testicular measurements and measure their echogenicity every 14 days. The results founded that testosterone levels were raised and associated with the age of puberty, and this increase was statistically dissimilar between the following period and each period. The circumference of the testis clearly and significantly increased from day 161 till the examination period finished. Studying the relationship between leptin, testosterone, and testicular size revealed a clear connection between total body weight in pre-puberty and the onset of puberty. Additionally, the testicular circumference and testosterone level were found to have a significant direct association with the Leptin hormone level as the advancing age of puberty. By 174 days of age, the first indications of sexual performance were seen throughout the experimental periods. Moreover, increasing serum leptin levels were seen as puberty progressed. The study finding of male lambs' puberty In pre-pubertal lambs, there was a significant positive connection between the testis parenchyma imaging, moderate echogenicity. and elevated echogenicity in rams that were mature or close to puberty. The study concludes that testosterone levels were statistically raised and associated with the age of puberty with a significant positive association between the pubertal age of Iraqi male lambs at 6 months and the studied leptin, testosterone, and testicular size revealed a clear connection between total body weight which may be useful in determining the pubertal age.

Keywords: Male lambs, Puberty, Body weight, Sheep leptin, Testosterone

Introduction

One of the key elements that affects production efficiency is reproductive efficacy, particularly in regions where the sheep industry, and specifically meat production, is significant. [1]. Improvements in the productive and reproductive qualities of sheep can be made through a variety of techniques, including crossbreeding, introducing new sires, and selection or cooperate with them. Typically, they are grown to produce mutton, with milk and wool serving as supplemental commodities. [2]. The Awassi sheep breed is the most prevalent in the

Middle East, which serves three goals (mutton, milk and wool) [3]. Improving these Productive properties became a crucial aim for sheep breeding since the competence of sheep production mostly depended on genetic enhancements for reproduction, meat production, and growth. [4].

This study has supposed that different physiological outcomes may appear due species-specific secretion profiles during sexual development .Because ultrasound was simple to use and had no negative side effects; it was selected as the diagnostic tool for the initial measuring of the scrotal sac and its contents. Saaed and Zaid, 2018 [5] showed that there

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were significant differences in between pre pubertal age and mature rams at three to four months in length and width by ultrasound [6]. A reliable indicator of puberty is the change to increase the scrotal circumference with age. The reproductive endocrine axis is the site of a complex series of processes that lead to the beginning of puberty [7]. The metabolic hormones that may act as a physiological linkage between the reproductive axis and proliferating tissues are Leptin and IGF-I. [8]. A relation between scrotal circumference and sexual maturation was reported in British breeds and crossbred of male goats (9). Male Shiba goats have showed alteration in plasma concentrations of androgens (androstenedione, testosterone) as well as scrotal circumference within prepubertal age [10].

Anyway, the correlation among testicular size along with peripheral levels of Leptin, Testosterone and ultrasound evaluation of testicular changes during pubertal advancement as yet to be explained in any local male species as well as male lambs, elevated level of testosterone increase sexual activity during puberty, spermatogenesis and its maintenance, also required for accomplishment of meiosis and differentiation of spermatids [11].

The purpose of this study was to examine the relationship between leptine, testosterone hormonal assay linked with scrotal circumference and ultrasound investigation of testicular measurements s during onset of puberty in male lambs

Material and Methods

Animals

The work was accomplished in the pens of the Animals Production Department, Agriculture College in Mosul University, eighteen ram lambs' well-being weaned (13 weeks) of Awassi domesticated animals were used. The project implemented in January 2021, Male lambs were relatively mixed among ewe lambs, and were Underwent same status of accommodation dietary, lighting, and temperature. Digital balance was used for weighting Body weights Every 14 days consecutive from the week 13th until successfully mate ewes was detected. During the experimental period, salt blocks and fresh water were continuously available.

Hormonal Assay and Blood sampling

Blood samples were collective with highly strict sterilization every 14 days and continuously from 13 weeks old. By centrifuging the blood (3000 rpm) for ten minutes, blood serum was obtained, located in

Eppendorf tube and then kept at -20°C till analyses were achieved.

Sheep leptin assay were valued through radioimmunoassay by using sheep Leptin ELISA kit, Southern California, San Diego, USA, catalog number: MBS742106 according to some authors [12;13]. Commercial kit (Immulite Total Testosterone, Siemens Healthcare Diagnostic Inc., UK) is used considering serum samples of testosterone by radioimmunoassay method for animal use

Testicular circumference and ultrasound evaluation

Measurements of the testicular circumference was estimated linked with weight Progressing and ultrasound examinations of the tests were done every 14 days, from weaning (around 13 weeks old) at intervals of 2 weeks till the study end, The evaluate Scrotal circumference (SC) was estimated in centimeters (cm) using a millimeter tape on the part of the greatest diameter of the scrotum [14;15]. Testicular ultrasonographic investigations were done. By using ultrasound device of the xianfeng XF30B type using linear probe 5.5-7.5 MHz for image documentation

Statistical Analysis

The data that statistically analyzed were measured by using the statistical SPSS program v.23 software (SPSS In. Chicago, IL., USA). Standard error (S.E.) and $\text{mean} \pm$ was used to express all findings . LSD test and One-way ANOVA was established to determine the significant between groups and P values of under 0.05 were estimated as significant.

Results

The earliest sexual behavior was documented at age 174 days (≈ 5.8 months).The body weight data showed that there were significant variations among the days. There is a significant differentiation as in table 1 at $P \leq 0.05$ represented by augmented weight increment, additionally the results about the leptin level study showed a considerable increase according to age of puberty, in a straight and clear manner among the intentional days at $P \leq 0.05$. The results showed a clear statistical augment correlated with puberty and age, that increase was statistically dissimilar among the following duration at $P \leq 0.05$. In addition to, the measuring the circumference of the testis, it was detected that there was a definite rise in the testis' circumference since day 160 till day 174 (finishing time of examination) and its thoroughly associated toward the time of puberty (Table 1, Fig. 1, 2).

TABLE 1. Show the value of Heaviness, Testicular Circumference, Hormonal assay (leptin and Testosterone correlated including pre-puberty related by time in male sheep.

Days	Heaviness (B.W)Kg	Testicular Circumference	Leptin	Testosterone
90	20 ± 1.231 ^a	12.25±.211 ^a	0.002±.001 ^a	0.817±.057 ^a
104	21.375±1.218 ^a	13.225±.389 ^b	0.0022±.001 ^b	1.2±.059 ^b
118	22.35±.978 ^{ab}	14.2±.477 ^c	0.0023±.001 ^b	1.645±.052 ^c
132	24.225±.501 ^{bc}	15.325±.433 ^d	0.0027±.001 ^c	2.352±.046 ^d
146	26.375±.557 ^d	16.75±.559 ^e	0.0029±.001 ^d	2.672±.61 ^d
160	29.75±.411 ^e	18.5±.866 ^f	0.0033±.001 ^e	3.1875±.059 ^e
174	31.575±.339 ^f	20.25±.619 ^g	0.0037±.001 ^f	3.76±.063 ^f
188	32.125±.228 ^f	20.75±.490 ^{gh}	0.004±.001 ^g	4.415±.088 ^g
202	33±.267 ^f	21.25±.411 ^h	0.0042±.001 ^h	4.85±.0831 ^h

Different letter in the same column mean that there are significant differences at $p \leq 0.05$ between 90 to advancing 202 days, among the period of study.

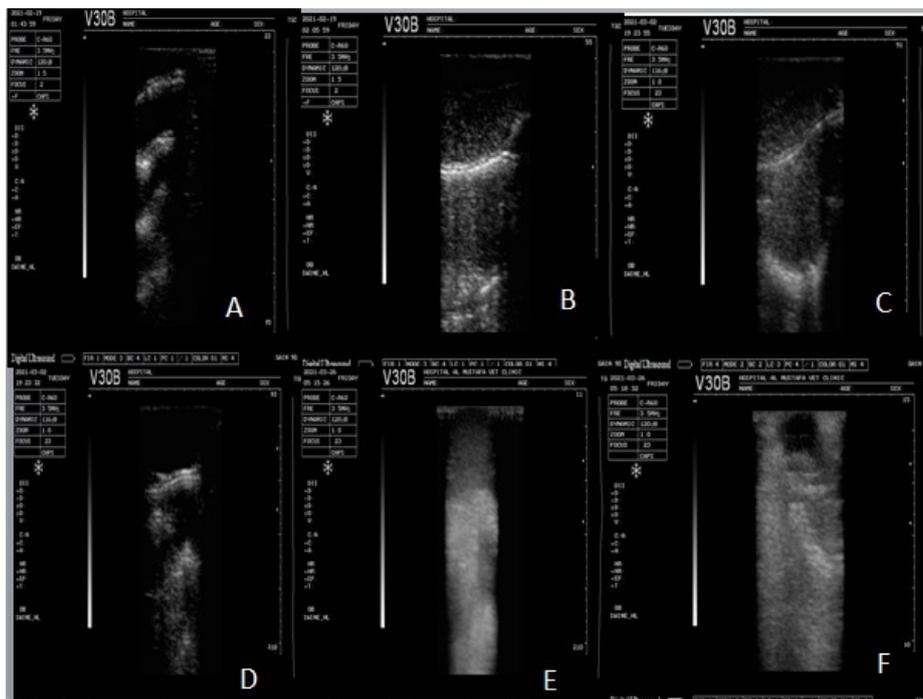


Fig. 1. Ultrasonographic pictures show the testis of male lambs including moderate echogenicity of the testicular parenchyma; mediastinum testicular highly echogenic. of testis before puberty(A,B) concerning day 118 (C,D) concerning day 132 while (E,F) concerning day 160.

Ultrasound pictures demonstrate moderate echogenicity of the testicular parenchyma is similar (118-132day) but the mediastinum testicular diffusely echogenic.

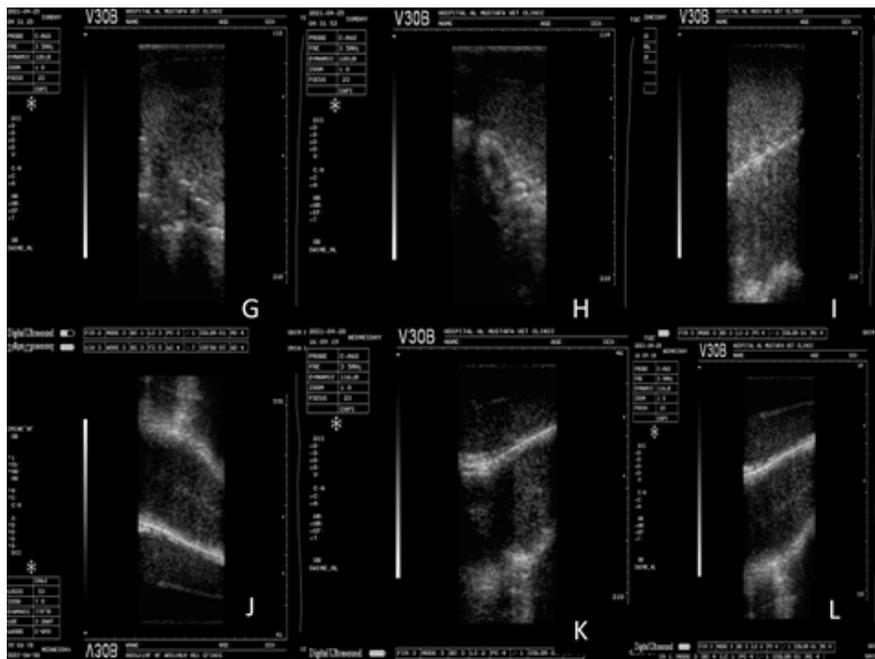


Fig. 2. The testicular parenchyma of male lambs is highly echogenic on ultrasound pictures, and the mediastinum is diffusely echogenic.. (G, H, I, J, K and L) concerning day 188-190 (5.5-7MHz).

Regardless of the testis or as the scan plane that was used, the testis parenchyma appeared homogeneous at ultrasonography, with moderate echogenicity before puberty A&B concerning day 118 (Figure 1) to high C,D and E,F concerning day 132 and 160 respectively. In contrast, all animals had imaging of the testicular mediastinum, which was characterized as a hyper-echoic line with

varying thickness in the middle of the testis parenchyma and was categorized as having diffuse echogenicity (figure 1). (A,B,C&D), and lower in (E,F). At age (188-190) the testicular parenchyma of male lambs is highly echogenic on ultrasound pictures, and the mediastinum is diffusely echogenic.. (G, H, I, J, K and L) as in (figure 2).

TABLE 2. Correlation between the parameters under study.

	Body weight		Leptin		Testosterone		Circumference Testis			
	P	r	P	r	P	r	P	r		
Pre-puberty days	0.00	1	0.00	0.9866	0.00	0.9909	0.00	0.9981	0.00	0.9912
Body weight			0.00	1	0.00	0.9895	0.00	0.9821	0.00	0.9981
Leptin					0.00	0.9945			0.00	0.9916
Testosterone					0.00	1			0.00	0.9882
Testis Circumference									0.00	1

** . Correlation is significant at the 0.01 level (2-tailed).

Discussion

Puberty occurred in male lambs at different ages but with similar weights. This demonstrates that the beginning of puberty is more strongly influenced by body weight than by age, this finding was corresponding by some authors [16].

The recorded and observed results in this study were compatible with the findings of Saeed & Zaid [17], who discovered that sexual behavior was

prevalent at (6 to 7 months) also puberty began at this age .As well as the body weight data was agree with Moulla *et al.* [18], that First ejaculation (puberty) began at an average age of 224 ± 7 days, with average weight of 36.3 ± 3 kg (43% of male adult weight), average scrotal circumference of 4.3 ± 0.5 cm, comparable to average testosterone concentration of 3.5 ng/ml . By an increase in leptin production and release, fat deposition contributes significantly to the

onset of puberty and estrus, and this is consistent with Lomas *et al.* [19]. With variations in growth hormone with aging, particularly during early life till puberty, body mass plays a crucial role with importance of body fat deposition [20]. This makes sense and supports our findings, which show that Leptin levels rise as age advance and weight increased. Very significant relationships ($p \leq 0.001$) between body weight and scrotal circumference and between testicular measures, body weight, and puberty age were both found.

Conclusion

The research proves such early puberty at male lambs will increase breeding value and positively impacts by increasing body weight, testosterone and leptin hormone concentrations. The supportive connection among them, elevated testosterone and leptin concentration was documented to an augmented muscle and adipose tissue aggregation, so we essential to advance inspection in male lambs good body weight affects in puberty.

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Authors Contribution

The authors each contributed equally.

Conflict of interest:

The author declares that there is no conflict of interest.

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دراسة العلاقة بين مستوى هرمون اللبتين والتستوستيرون وارتباطه مع قياس محيط الصفن تقويم التغيرات لنسيج الخصيه بالامواج فوق الصوتية اثناء حدوث البلوغ الجنسي في ذكور الحملان العواسية

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الخلاصة

هدفت الدراسة الحالية على معرفة علاقة مستوى هرموني اللبتين والتستوستيرون ووزن الجسم مع وقت حدوث البلوغ والفعالية التناسلية في ذكور الحملان ، شملت الدراسة ثمان عشرة من حملان المحلية ومقطومة بعمر تسعين يوماً، عند بدا الدراسة سجل وزن جسم ذكور الحملان بالميزان الالكتروني، سحبت عينات الدم منها وتم فصل مصل الدم ووضعت في انابيب وخزنت عند درجة -20 حتى اجراء تقدير مستوى الهرموني، معرفة عمر البلوغ، والتحرري عن الصدى وشدة انعكاس الامواج فوق الصوتية للخصي باستخدام جهاز الامواج فوق الصوتية وقياس محيط الخصيه كل اسبوعين. أظهرت نتائج الدراسة وجود ارتفاع معنوي في مستوى هرمون التستوستيرون مع تقدم عمر الحملان كما سجلت زيادة معنوية في محيط الخصية حتى يوم 161، اضافة لذلك فقد سجل علاقة بين مستوى هرمون اللبتين والتستوستيرون مع حجم الخصية ووزن الجسم ارتباطا واضحا ارتفاعا معنوياً متزامناً مع فترة ما قبل البلوغ والبلوغ ، كذلك سجل ارتفاعاً معنوياً في محيط الخصيه بعمر البلوغ عند مستوى معنوية (0.05) من اليوم 174 حتى نهاية فترة الفحص التجريبي. الدراسة وجدت ارتباط مباشرة بين مستوى هرمون اللبتين و محيط الخصيه وبشكل مباشر مع تقدم عمر الحملان والوزن الكلي في أيام فترة ما قبل البلوغ وصولاً إلى سن البلوغ وظهور علامات الرغبة الجنسية، إضافة لذلك وجد علاقة موجبة مباشرة بين مستوى هرمون التستوستيرون ،محيط الخصيه و هرمون اللبتين مع تقدم عمر البلوغ، كذلك وجدت الدراسة علاقة موجبة مباشرة في شدة انعكاس معتدله للامواج فوق الصوتية لمتن الخصي بعمر ما قبل البلوغ الجنسي مع شدة انعكاس عاليه للامواج بعمرالبلوغ . استنتجت الدراسة ارتفاع معنوي لمستوى هرمون التستوستيرون مع عمر البلوغ وأن بلوغ ذكور حملان بعمر 6 اشهر له ارتباط قوي موجب مع مستوى اللبتين و التستوستيرون ومحيط الخصية ومع وزن الجسم والتي تكون مفيدة في تحديد وقت البلوغ ذكور لحملان