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Relationship between the leptin, progesterone, body weight, and onset of puberty in ewe lambs

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Abstract

The current study aimed to determine the relationship between serum level of leptin, progesterone hormones, bodyweight with the onset of puberty in the ewe lambs. Eight healthy weaned local breeds of ewe lambs at the age of 90 days were introduced in the study. Body weights were recorded, blood samples serum was collected and stored at -20°C until hormonal assays. Ovaries and ovarian follicles were checked by ultrasound to measure follicular diameter until estrus was detected every two weeks. The result showed that the earliest estrus behaviour was noticed at age 202. Significantly increased body weight was recorded during the trial times at $P<0.05$. Moreover, serum leptin levels elevated significantly coinciding with puberty at $P<0.05$. Also, progesterone level was elevated significantly as the age of puberty at $P<0.05$. In addition to that, the follicular diameter recorded a significant increase from day 160 till the end of the trial. Studying the correlation among leptin, progesterone, and follicle diameter revealed a direct correlation with the age of puberty and total weight body at prepuberty days. In addition, a close positive direct correlation was observed between the progesterone hormone level and the follicle diameter with the level of the leptin hormone as progressing age of puberty, and the increase in the diameter of the follicle with a rise in the level of serum progesterone hormone. The study concludes that puberty in ewe lambs was found a strong positive correlation among the studied parameters with a time of puberty of sexually mature lambs.

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Introduction

The appearance of puberty in the female sheep depends on both age at first estrus behavior and accumulation of adipose tissues in muscles of body mass (1). Therefore, ewe lamb's puberty starts when they reach 55-70% of the bodyweight of mature ewe (2). Recently the 'body weight has been related to puberty and enhanced fertility in ewe lambs (3). Insulin-like growth factor 1 (IGF-1), insulin, growth hormone (GH), follistatin, and leptin hormone play a vital influence role in the accumulation of adipose tissues (4). Leptin is released from adipose tissue, which is a protein in nature with molecular weight 16-kDa composed of 167-amino acid made (5). The importance of leptin hormone play as cellular signaling to regulate the energy metabolisms, body mass, and reproduction activity, Leptin level in ewes is correlate highly with body mass. Therefore, its level decreases during fasting or feed restrictions (6). The occurrence of puberty was found to be linked with "body fat mass which considered as a biomarker for puberty at a critical point of the body mass in ewes (7). Accumulation of adipose tissues and leptin secretion is associated with the onset of puberty and changes in the blood circulating of leptin (2). A different physiological change happens at the beginning of puberty in estrus cycle (8). Monitoring the reproductive system in ewe lambs using ultrasound with trans rectal probes 5.5-7.5MHz is necessary for evaluating ovarian status in terms of anatomical and functional aspects (9). Rectal ultrasonography is used to measure the diameter of the antral follicle and growing follicles with 1-2 mm (10).

While some publishers showed a rise in the number of small follicles diameter with 2- is considered as onset of puberty (11).

So, the study aims to determine the relationships between the onset of puberty and body weight with circulating concentrations of leptin and progesterone in prepubertal until the onset of puberty in ewe lambs.

Materials and methods

Animals

The study was conducted on the farm of the College of Agriculture, the University of Mosul, eight ewe lamb's health weaned of local breed at the age of 90 days were used. The project was started in January 2021. They were mixed with sexually male lambs and were kept under the same breeding conditions of light, temperature, and nutrition. Body weights were recorded by electric balance every two weeks periodically from 90 days until the first estrus was detected.

Blood sampling and hormonal assay

Samples were collected from the jugular vein and placed in plain sterile tubes (gel tubes). Every two weeks and continuously from the age of 90 days. Blood serum was obtained by separating the blood by a centrifuge for 10 min (3000 rpm) and placed in an Eppendorf tube and stored at -20°C until assays were performed (12).

Sheep leptin assay was estimated by radioimmunoassay (MY BioSource, Competitive ELISA, Antibody-Protein ELISA kit, catalog number: MBS742106, Southern California, San Diego, USA) in duplicate 100 L, as described by Blache *et al*. (13). The limit value for detection of leptin was 0.06 ng/mL and the intra-assay CVs were 7.3% at0.73 ng/mL, 4.4% at 0.84 ng/ml, and 2.4% at 1.61 ng/mL. Progesterone concentrations were determined by direct solid-phase radioimmunoassay (RIA) (Elecsys Progesterone III; Cobas e411, Roche Diagnostics GmbH, D-68298 Mannheim, Germany) (14).

Ultrasound examination

The study was carried out on local ewe lambs at age weaning, scanning ovaries and follicles periodically every two weeks until the appearance of signs of the first estrus, which is considered signs of sexual puberty. An ultrasound device of the type xianfeng XF30B was used to check the ovarian status in measuring the diameter of the ovaries and the presence of follicles via using transrectal probe 5.5-7.5 MHz with dimensions of 10 cm length, 1.5 cm height, and 1.5 cm width. The probe was inserted into the rectum carefully, about 5-7 cm, where the urinary bladder appeared well, which is the point of access to the animal's ovary. Once identifying the bladder, the probe was slowly inserted, directing the wave beam at the ventral direction and rotated sideways about 45˚ degrees in both directions until the appearance of the ovary and follicle.

Statistical analysis

Statistical analyses of the data were evaluated using the statistical SPSS program v.23 software (SPSS In. Chicago, IL., USA). All results were expressed as mean ± standard error (mean ± SE). One-way ANOVA and LSD test evaluated the significance between groups, and P values of less than 0.05 were considered significant (15,16).

Results

As shown in Table 1, the average age of puberty onset significantly differs between days. The earliest estrus behavior was recorded at 202 days (≈6.73 months). The body weight data revealed significant variations among the days at $P<0.05$, represented by increased body weight. Moreover, the data of leptin level analysis recorded a significant elevation coinciding with the age of puberty, directly and straightforwardly among the studied days at $P<0.05$. Statistical analysis of progesterone hormone data showed an apparent significant increase associated with puberty and that this rise was significantly different between each period and the following period at $P<0.05$.

Table 1: Effect of time on some physiological parameters associated with pre-puberty in lambs

Days	Body Weight Kg	Leptin level	Progesterone level	Diameter of mature follicle
90	14.37±0.46 f	0.0015±0.001 a	0.05±0.00 f	0.00±0.00 e
104	17.51±0.76 e	0.0016±0.001 a	0.05±0.00 f	0.00±0.00 e
118	20.55±0.81 d	0.0019±0.001 d	0.05±0.00 f	0.00±0.00 e
132	21.39±0.56 d	0.0021±0.001 c	0.054±0.004 f	0.00±0.00 e
146	24.11±0.51 c	0.0023±0.001 bc	0.08±0.004 e	0.00±0.00 e
160	25.35±0.82 bc	0.0024±0.001 abc	0.11±0.002 d	0.67±0.036 d
174	26.98±0.47 ab	0.0025±0.001 ab	0.12±0.001 c	0.86±0.032 c
188	28.67±0.72 a	0.0026±0.001 a	0.14±0.003 b	1.12±0.045 b
202	29.08±1.24 a	0.0026±0.001 a	0.16±0.003 a	1.34±0.041 a

Different letters in the same column mean significant differences at $P<0.05$.

Additionally, measuring the follicle diameter by ultrasonography, it was observed that there was an apparent increase in the diameter of the follicle from day 160 until the end of the examination period. It is closely related to the age of puberty (Table 1). (Figures 1 and 2).

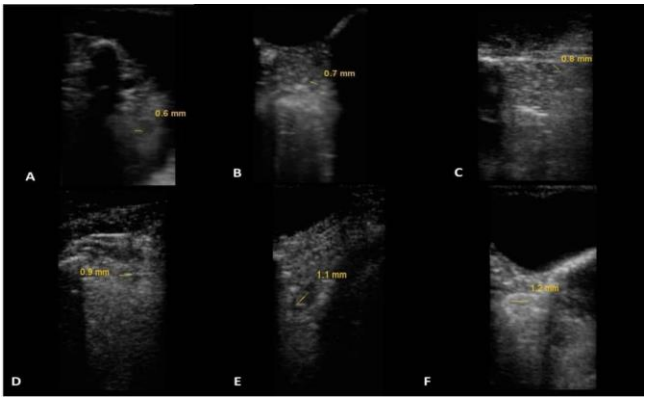

Figure 1: Ultrasonographic images show the ovary of ewe lambs with the increase in diameter of the follicle at pre-puberty and puberty days (A, B) at day 160 (C, D) at day 174 (E, F) at day 188.

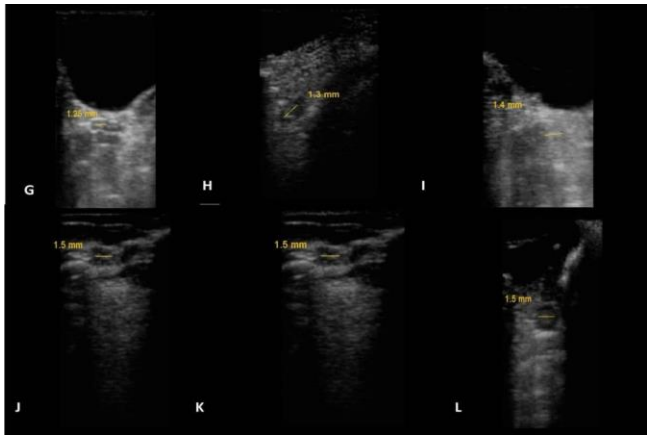

Figure 2: Ultrasonographic images show the ovary of ewe lambs with the increase in diameter of the follicle at puberty days (G, H, I, J, K, and L) at day 202.

Table 2: Correlation among the studied parameters

	Pre-puberty	Body weight		Leptin	Progesterone days			Diameter of follicle	
	r	*P*	*r*	*P*	*r*	*P*	*r P*	*r P*	
Pre-puberty days	1	0.909	0.000	0.835	0.000	0.946	0.000	0.905	0.000
Body weight		1		0.777	0.000	0.814	0.000	0.764	0.000
Leptin				1		0.742	0.000	0.682	0.000
Progesterone						1		0.956	0.000
Diameter of follicle								1	

Discussion

The observed and recorded data in the current work were consistent with Lozano *et al*. (17), who found that the onset of puberty and prevalence of estrus behavior started at 5.4 to 6.9 months. In addition to that, the bodyweight data agreed with Ehtesham and Vakili, Nieto *et al*. (18,19) that recorded an increase in body weight at the time of puberty 210-240 days, reaching 35 Kg.

Body mass plays a crucial role via changes in growth hormone during aging, especially at the early stages of life till puberty, with the consequence to fat deposition in the body. Fat deposition plays a vital role in puberty and the onset of estrus via increased leptin formation and its release in the body (20). This is logically accepted and supports our finding, which records an increase in leptin level as age progresses and increases body weight (21,22). Therefore, body weight strongly correlates with reproductive health system function and activity in terms of interference with sexual hormones.

This result agrees with Ehtesham and Vakili (18) revealed a positive correlation between increased leptine hormone associated with higher body fat, accumulation of fatty tissue, and increased growth, conversely the increased leptin hormone release (3). Although its role in critical leptin hormone in the early post-natal period processes affects the body and regulation of puberty (21). Leptin hormone concentration was related to age at first estrus and positively correlated with earlier puberty onset was depending on the time of ovarian activity and BW at first estrus in ewe lambs (3).

The present findings found that the progesterone hormone level was significantly increased in age progress toward puberty in ewe lambs. This result agrees with Corner *et al*. (23), who revealed that during pregnancy, the ewe lambs are not only supporting a developing fetus but are also likely to be still growing themselves. Da Silva *et al*. (24) have previously shown that lamb's ewe can partition available resources towards themselves rather than the growing fetus. Progesterone hormone can be used to detect

puberty in post-weaning ewe lambs. In addition, the progesterone level was more significant in high BW lambs than low BW in ewe lambs. The release of progesterone hormone played an essential role in the prepubertal period that leads to early puberty onset in ewe lambs (25).

Conclusion

The study concludes that puberty in ewe lambs will be early onset with higher breeding values and influenced by an increase in the body weight, concentration of leptin, and progesterone hormone—the positive relationship between them. High leptin and progesterone concentrations were recorded to increase adipose tissue accumulation and muscle, so we must inspect sound body weight effects in puberty in ewe lambs.

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Conflict of interest

The author has no conflict of interest.

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العلاقة بين اللبتين، البروجسترون، وزن الجسم وظهور البلوغ في فطائم النعاج

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الخلاصة

الفطائم والوزن الكلي في أيام فترة ما قبل البلوغ وصولا إلى سن البلوغ وظهور علامات الشبق، إضافة لذلك وجد علاقة موجبة مباشرة بين مستوى هرمون البروجستيرون وقطر الجريب مع هرمون اللبتين مع تقدم عمر البلوغ، وان الزيادة في مستوى هرمون البروجستيرون في مصل الدم تزداد مع ارتفاع قطر الجريب. الدراسة استنتجت أن بلوغ فطائم النعاج له ارتباط قو ي موجب في القيم المدروسة مع وقت البلوغ في فطائم النعاج.

هدفت الدراسة الحالية على معرفة علاقة مستوى هرموني اللبتين والبروجستيرون ووزن الجسم مع وقت حدوث البلوغ والفعالية التناسلية في فطائم النعاج، شملت الدراسة ثمان من الفطائم المحلية ومفطومة بعمر تسعين يوما، عند بدا الدراسة سجل وزن جسم الفطائم بالميزان الإلكتروني، سحبت عينات الدم منها وتم فصل مصل الدم ووضعت في أنابيب وخزنت عند درجة -٢٠ حتى إجراء تقدير مستوى الهرمونين، والتحري عن المبايض والجريبات المبيضية باستخدام جهاز الأمواج فوق الصوتية وقياس قطر الجريب كل أسبوعين حتى ظهور الشبق فيه. أظهرت نتائج الدراسة ظهور سلوك الشبق مبكرا بعمر ٢٠٢ يوم، كما سجلت زيادة معنوية في إوزان الجسم باختلاف الأوقات عند مستوى معنوية (٠,٠٥) إضافة لذلك فقد س

جل تحليل مستوى هرمون اللبتين ارتفاعا معنويا متزامنًا مع سن البلوغ عند مستوى معنوية (٠,٠٥)، وكذلك وجد ارتفاع معنويا في مستوى هرمون البروجستيرون مع تقدم عمر الفطائم عند مستوى معنوية (٠,٠٥) إضافة لذلك سجل ارتفاعا معنويا في قطر الجريب مرتبطة بعمر البلوغ عند مستوى معنوية (٠,٠٥) من اليوم ١٦٠ حتى نهاية فترة الفحص، الدراسة وجدت ارتباط مباشرة بين مستوى هرمون البروجستيرون، اللبتين و قطر الجريب وبشكل مباشر مع تقدم عمر