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Associations between age at puberty, growth traits, plasma IGF-I and leptin concentrations with reproductive performance of Rahmani and Barki male Lambs

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SUMMARY

The present study aimed to investigate the effect of breed type and puberty status on growth performance, blood IGF-I, leptin concentrations, and the subsequent reproductive performance of Rahmani and Barki male lambs. After weaning at week 14th, two equal groups of Rahmani and Barki male lambs (n = 20 each) were used in the present study till 40 week of age. All lambs followed to tracking the growth traits and puberty development. Blood samples were collected from each lamb every two weeks for IGF-I, leptin, and testosterone examination. Results indicated that Rahmani and Barki lambs had a similar value of growth traits during the experimental period. Also, the average of age at puberty was (37.48 weeks) in Rahmani lambs without significant differences with Barki lambs (36.72 weeks). Besides, the concentration of plasma IGF-I in Barki lambs was higher ($P < 0.05$) than Rahmani lambs starting from week 26 onwards. However, the concentration of plasma leptin in Rahmani lambs was higher ($P < 0.05$) than Barki lambs starting from week 28 onwards. Thus, there was a linear increase in plasma testosterone levels during the pre-pubertal up to puberty stage without any significant differences between both breeds. Also, values of semen quality and quantity recorded for lambs of both breeds were almost comparable. Accordingly, the current study results suggest that body mass represented by muscle and fat accumulation were equal for both breeds. These might modify the circulating of IGF-1, and leptin, respectively, exerting an equally positive influence on the reproductive performance of Rahmani and Barki lambs at puberty.

Keywords: *Rahmani sheep, Barki sheep, IGF-1, Leptin, Testosterone*.

INTRODUCTION

Sheep considered as a vital part of the animals' total number in Egypt. The total number of Egyptian sheep breeds has increased rapidly throughout the last five years, reaching about 5.7 million animals in 2017. They are raised commonly for mutton production, while wool and milk considered as secondary products **(FAO, 2017)**. The most common local sheep breeds in Egypt are Ossimi, Rahmani, and Barki **(Galal et al., 2005)**. Rahmani sheep produce good quality of meat and respond well to genetic improvement through selection **(El-Khalifa et al., 2013)**. They are also known for their ability to survive with harsh environmental conditions such as long periods of drought and high temperatures **(El-Khalifa et al., 2013; El-Shahat et al., 2014)**. Moreover, Barki sheep are well adapted to the harsh desert conditions in the Mediterranean region and can produce a considerable amount of meat, wool, and milk under these conditions **(Abousoliman et al., 2020; El-Wakil et al., 2008)**. Therefore, Rahmani and Barki sheep are raised in several Egyptian regions as a significant source of meat and a valuable source of income for farmers.

Description of puberty and early sexual development is an essential tool for selection within the males of a given breed. The onset of puberty is the outcome of a sequence of complex events that occur within the reproductive endocrine axis **(Hafez and Hafez, 2000)**. Also, the inception of puberty is influenced by genetic and environmental factors such as breed, climate, and nutrition **(El-Khalifa et al., 2013)**. Furthermore, the onset of puberty is dependent on the achievement of sufficient body mass **(Nieto et al., 2013)**. Thus, in sheep, genetic selection can improve growth rates and muscle development and permit reproduction at a

younger age **(Nieto et al., 2013)**. Besides, fertility is under the signaling control of sex hormones, gonadotropic hormones, and some metabolic hormones such as GH, IGF-I, insulin, and leptin **(Chandrashekar and Bartke, 2003; Robinson et al. 2006; Abadjieva et al. 2011; El-Shahat et al. 2014)**. Also, metabolic hormones, IGF-I, and leptin might act as a physiological link between the growing tissues and the reproductive axis **(Nieto et al., 2013)**. Besides, evaluate concentrations of metabolic hormones and leptin in the blood are significant to recognize changes in the metabolism and nutrient supply of growing lambs **(Antunović et al., 2010)**. Also, the growth, fatness, and the onset of puberty are all associated with concentrations circulate of IGF-I and leptin **(Roberts et al., 1990; Chilliard et al., 2005)**. In addition, reproductive development might explain by the secretory patterns of these two hormones **(Nieto et al., 2013)**. Thus, male lambs with great signs for early puberty and characteristic sexual behavior can improve the flock fertility during breeding and ultimately improve the genetics **(Ibarra et al., 2000)**. Accordingly, the objective of this study was to evaluate the effect of breed type and puberty status on growth performance, blood IGF-I, leptin concentrations, and the subsequent reproductive performance of Rahmani and Barki growing lambs.

MATERIALS AND METHODS

Animals

The present study has been conducted with the guidelines of the ethical principles of animal experimentation and approved by the Ethical Animal Care and Use Committee of Damanhour University. Experimental fieldwork carried in Agriculture Experimental Station, Faculty of Agriculture, Damanhour University, Al-Behera governorate, Egypt. After weaning at 14 weeks, two equal groups of Rahmani and Barki male lambs (n = 20 each) were used in the present experiment with similar management condition. At the beginning of the study, the average live body weight was 17.25 ± 2.42 kg and 17.78 ± 2.62 kg for Rahmani and Barki lambs, respectively. The lambs were fed according to **NRC, (2007)**. The freshwater and salt blocks were available continuously during the experimental period, which started from post-weaning until week 40th of age.

Puberty assay

All lambs were observed two times weekly to observe puberty development from post-weaning till the noticing of puberty (first ejaculate with motile sperm). Sexual behavior for each lamb was detected according to the method of **El-Khalifa et al. (2013)**. Briefly, male lambs were observed within 25 min using estrus female, considering the following criteria: mounting without an erection (stage I), the mounting within 1st penile protrusion (stage II), and puberty as age at first collected ejaculate containing motile sperm (stage III). Age of lambs was registered at each stage of puberty. Moreover, testicular parameters (scrotal circumference, and testes volume) were determined at each stage of puberty. Scrotal circumference was evaluated using a flexible tape at the point of the maximum circumference of paired testes. Testes volume was estimated according to the equation assumed by **El-Zelaky et al. (2011)**. Testes volume (cm^3) = 0.0396 x testicular length x (scrotal circumference) ^{2}. The measurement of testis length was taken at the point of top and bottom dimensions of the testis with calipers.

Growth traits examine

The live body weight was measured every two weeks for each lamb during the experimental period. Moreover, body length, rump height, and heart girth were registered at each stage of puberty. Body length was considered as the space between the point of the shoulder and pin bone **(Afolayan et al., 2006)**. Heart girth was the perimeter measurement taken behind the four legs **(Ibrahim, 2015)**. Rump height was measured from the hips up to the end of the hoof **(Afolayan et al., 2006)**.

Blood sampling and hormones examination

Blood samples were collected from the jugular vein in a heparinized tube every two weeks for each lamb during the experimental period. Plasma was separated immediately after blood collection by centrifugation at 1500 x g for 15 min at 4 °C. Plasma was harvested, aliquot, and stored at −20°C until hormones analysis. Plasma concentrations of IGF-I were determined by radioimmunoassay (RIA) in duplicate 100 µl

aliquots using a commercial kit (Diagnostic Product Company, LOS Angeles, CA). Assay sensitivity was 0.2 ng/ml with a coefficient of variation of < 7 %. Likewise, plasma testosterone was analyzed using an RIA kit (Diagnostic Product Company, LOS Angeles, CA). All samples were run in a single assay having a CV of 5%. While, plasma leptin concentrations were assayed by ELISA technique using sheep leptin kit (Sino Gene Clon Biotech Co., Ltd) with a sensitivity of 0.1 ng/ml.

Semen evaluation

After puberty, semen samples were collected by artificial vagina in two ejaculates weekly for up to two weeks. The semen ejaculates were immediately transported to the laboratory and immersed in a water bath at 37°C to evaluate semen characteristics as described by **Marco-Jimenez et al. (2005)**. Briefly, semen samples were evaluated for volume, initial motility, live spermatozoa, abnormal sperm percentage, and sperm cells concentration $x10^9$.

Statistical analysis

All data were analyzed using the general linear model procedure of Stat View 5.0 (SAS Institute Inc., Cary, NC, USA) and JMP 6 (SAS Institute Inc., Cary, NC, USA). The Student's t-test and 2-way ANOVA were used to compare the proportions and mean values, respectively, between the two groups. Absolute values were combined in the model including time and breed as fixed effects. All values were presented as mean ± SEM and the differences were considered to be statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Growth traits and puberty development

The live body weight of Rahmani and Barki male lambs from week 14 till week 40 of age is displayed in Figure 1. As shown, there was a linear increase in lamb's live body weights toward advanced ages. However, no differences ($P > 0.05$) were found in live body weight between Rahmani and Barki lambs during the experimental period (Fig. 1). Likewise, no differences ($P > 0.05$) were detected in live body weight between both breeds during the prepubertal up to puberty stage (Table 1). Furthermore, no differences ($P > 0.05$) were identified in body length, rump height, and heart girth between Rahmani and Barki lambs during the prepubertal up to puberty stages (Table 1). Also, no significant variances were found in the mean ages between both breeds during the prepubertal up to puberty stages (Table 1). Moreover, no differences ($P > 0.05$) were detected in the testes volume between Rahmani and Barki lambs during the prepubertal up to puberty stages. However, the scrotal circumference in Barki lambs was bigger ($P < 0.05$) compared with Rahmani lambs at stage II and III of puberty (22.18 ± 4.75 vs. 18.32 ± 6.23 cm, and 26.76 ± 7.73 vs. 23.42 ± 6.42 cm, respectively, Table 1).

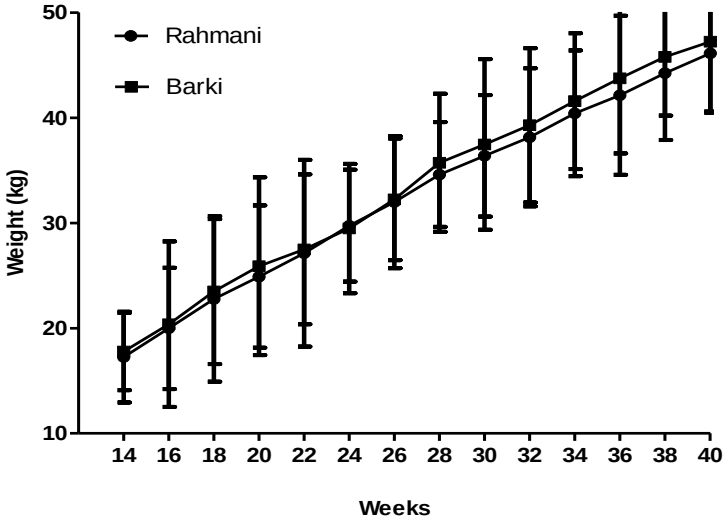

Figure 1. Average live body weight of Rahmani and Barki lambs from week 14 till week 40 of age.

Concentration of IGF-I and leptin

The concentration of plasma IGF-I in Barki lambs was higher ($P < 0.05$) than that in Rahmani lambs starting from week 26 onward till the end of the experimental period (Fig. 2). Furthermore, during the pre-pubertal up to puberty stage, IGF-I levels were higher ($P < 0.05$) in Barki lambs compared with Rahmani lambs (Table 1). Contrariwise, the concentration of plasma leptin in Rahmani lambs was higher ($P < 0.05$) than that in Barki lambs starting from week 28 onwards (Fig. 3). Besides, leptin levels in Rahmani lambs were greater ($P < 0.05$) compared with Barki lambs at stage II and III of puberty (3.68 ± 0.23 vs. 2.74 ± 0.34 ng/ml, and 3.74 ± 0.17 vs. 2.66 ± 0.34 ng/ml, respectively, Table 1).

Table 1. Puberty parameters in Rahmani and Barki lambs.

Variables	Breed	
	Rahmani	**Barki**
Stage I *(first mounting without an erection)*		
Age (weeks)	26.72 ± 5.34	26.83 ± 7.63
Live body weight (kg)	32.98 ± 5.48	33.18 ± 5.22
Length (cm)	89.54 ± 1.56	87.93 ± 1.74
Rump height (cm)	62.67 ± 0.98	64.87 ± 1.12
Heart girth (cm)	70.97 ± 1.86	73.23 ± 2.13
Scrotal circumference (cm)	16.18 ± 3.32	17.76 ± 2.98
Testes volume (cm3)	152.47 ± 5.67	153.12 ± 8.46
IGF-I concentration (ng/ml)	294.87 ± 0.78[a]	388.95 ± 0.54[b]
Leptin concentration (ng/ml)	3.18 ± 0.23	2.78 ± 0.18
Testosterone concentration (ng/ml)	1.93 ± 0.19	2.82 ± 0.17
Stage II (first mounting with an erection)		
Age (weeks)	31.28 ± 7.54	30.79 ± 8.12
Live body weight (kg)	37.54 ± 7.52	38.12 ± 8.63
Length (cm)	95.47 ± 3.18	92.98 ± 2.45
Rump height (cm)	67.35 ± 0.87	69.94 ± 1.13
Heart girth (cm)	74.97 ± 2.75	75.89 ± 2.27
Scrotal circumference (cm)	18.32 ± 6.23[a]	22.18 ± 4.75[b]
Testes volume (cm3)	216.22 ± 8.20	218.61 ± 6.34
IGF-I concentration (ng/ml)	337.68 ± 0.56[a]	438.89 ± 0.72[b]
Leptin concentration (ng/ml)	3.68 ± 0.23[a]	2.74 ± 0.34[b]
Testosterone concentration (ng/ml)	2.74 ± 0.42	3.07 ± 0.36
Stage III (first mounting with erection and ejaculation)		
Age (weeks)	37.48 ± 5.17	36.72 ± 7.34
Live body weight (kg)	43.47 ± 3.98	44.20 ± 3.68
Length (cm)	96.10 ± 2.38	93.59 ± 2.78
Rump height (cm)	69.65 ± 1.18	71.87 ± 1.78
Heart girth (cm)	78.94 ± 1.76	77.86 ± 1.23
Scrotal circumference (cm)	23.42 ± 6.42[a]	26.76 ± 7.73[b]
Testes volume (cm3)	338.19 ± 7.56	341.67 ± 8.12
IGF-I concentration (ng/ml)	500.17 ± 0.78[a]	598.73 ± 0.65[b]
Leptin concentration (ng/ml)	3.74 ± 0.17[a]	2.66 ± 0.34[b]
Testosterone concentration (ng/ml)	3.32 ± 0.22	3.86 ± 0.35

[a, b] Means within the same row with different superscripts are significantly different at ($P < 0.05$).

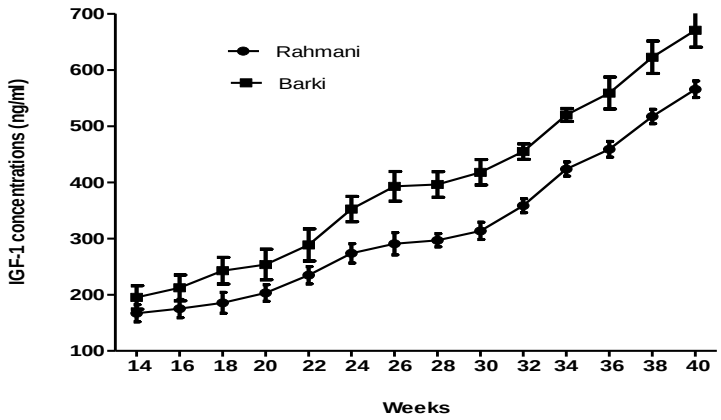

Figure 2. IGF-1 concentration (ng/ml) in the blood plasma of Rahmani and Barki lambs from week 14 till week 40 of age.

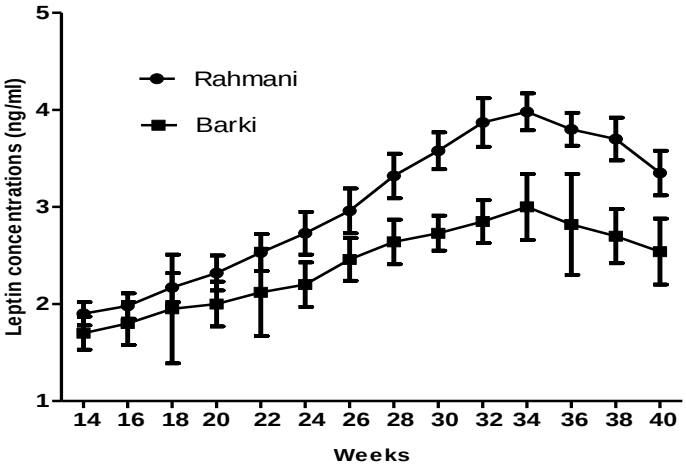

Figure 3. Leptin concentration (ng/ml) in the blood plasma of Rahmani and Barki lambs from week 14 till week 40 of age.

Semen properties at puberty:

As shown in Table 2. no differences (P > 0.05) were detected in the seminal characteristics (volume, initial motility, abnormal spermatozoa, and sperm cell concentration) between both breeds at puberty. However, the live spermatozoa percentage in Barki lambs was higher (P < 0.05) compared with that of Rahmani lambs (81.55% vs. 69.32%).

Testosterone concentrations

Plasma testosterone concentrations during the experimental period are displayed in figure (4). As shown, there was a linear increase in plasma testosterone levels during the pre-pubertal up to puberty stage in both breeds. However, no differences (P > 0.05) were identified in testosterone levels between Rahmani and Barki lambs during the experimental period (Fig. 4).

Table 2. Semen characteristics at puberty in Rahmani and Barki lambs.

Items	Breed	
	Rahmani	Barki
Ejaculate volume (ml)	0.63 ± 0.12	0.68 ± 0.14
Initial motility (%)	65.77 ± 2.67	68.36 ± 3.18
Live sperm (%)	69.32 ± 2.51[a]	81.55 ± 3.46[b]
Abnormal sperm (%)	17.53 ± 3.49	15.83 ± 2.78
Sperm cell concentration (x109/ml)	1.28 ± 0.27	1.34 ± 0.37

[a, b] Means within the same row with different superscripts are significantly different at (P < 0.05).

Growth traits are vital aspects in sheep breeding, as they, among other factors, affect the breeder's income. Also, the identification of associations between body weight and breed is very beneficial for selecting faster-growing lambs, which regulate incomes from meat production. Consequently, the present study results indicate that the live body weights of Rahmani and Barki lambs have significantly increased with age. However, there weren't any differences in live body weight between both breeds. These results are in agreement with the previous report stated by **Ashour et al. (2020)**, which indicated that no significant differences were found in live body weights between Rahmani and Barki lambs at different age categories (7 – 9, 10 – 12, and 13 – 16 months). Also, they found a positive effect of advanced age for both breeds in live body weight till the second age category. Moreover, the results of the current study showed no differences in physical measurements which, include body length, rump

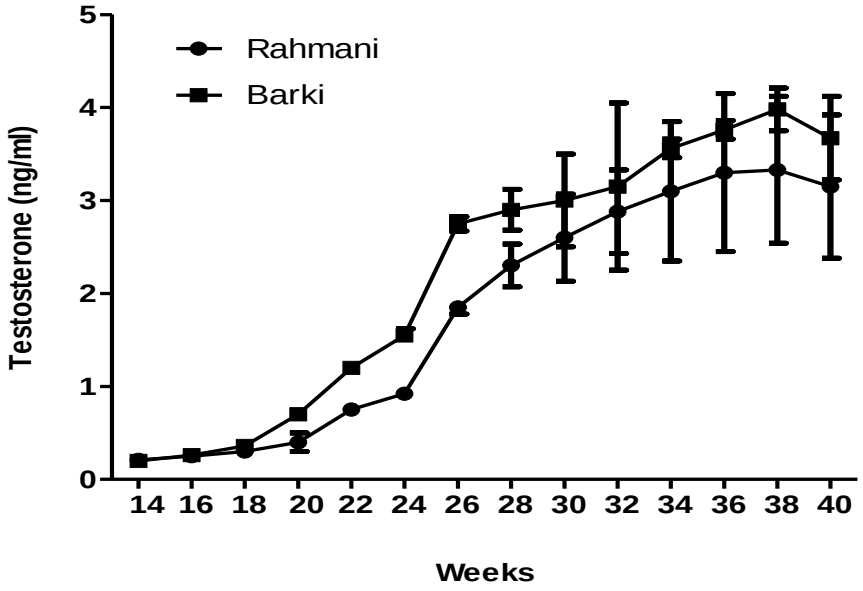

Figure 4. **Testosterone concentration (ng/ml) in the blood plasma of Rahmani and Barki lambs from week 14 till week 40 of age.**

height, and heart girth between both breeds during the pre-pubertal up to puberty stage.

These results are in parallel harmony with previous reports obtained by **Abdel-Moneim (2009)** and **Shirzeyli et al. (2013)**, which showed that no clear effect of sheep breed was established on sheep's body length, hip height, and heart girth. Also, **Ashour et al. (2020)** revealed no effect of sheep breed on sheep's body length at different age categories (7 – 9, 10 – 12, and 13 – 16 months). Moreover, no significant differences in rump height and heart girth were found between Rahmani and Barki lambs at 7 – 9 months of age **(Ashour et al., 2020)**.

The present study results showed that the average age at puberty was (37.48 weeks) in Rahmani male lambs without significant differences with Barki lambs (36.72 weeks). These results are in parallel agreement with the earlier reported by **El-Ashry et al. (2000)** and **El-Khalifa et al. (2013)**. They mentioned that Rahmani male lambs reached puberty at an age ranged between 32 to 40 weeks. Furthermore, the onset of puberty is related to reaching sufficient body mass **(Nieto et al., 2013)**. Hence, present study results showed that growth traits at puberty age were similar in both breeds, so the similarity of age at puberty could be expected. Also, puberty occurrence is related to the nutritional levels, and especially the protein ratio that plays a vital role in many aspects of male properties include the attainment of body weight, sexual maturity, and onset of puberty **(Foruie et al., 2004)**. Therefore, the similarity of puberty age

for both breeds in the current study may be due to the equal feeding level.

The obtained concentration of IGF-I during the present study (167–570 ng/ml) in the two breeds is close to that reported by **Barkawi et al. (2009)**, which showed that IGF-I concentration noticeably increased from birth to 360 days. They found that IGF-I levels have a range of < 500 ng/ml from birth to 240 days of age and improved to > 500 ng/ml from 240 to 360 days of age with a temporary drop at day 300. Also, the current study results are consistent with previous studies reporting results for IGF-1 concentrations **(Roberts et al., 1990)** as days of the experiment, age and size of lambs increased the concentrations of IGF-1. These findings most probably relate to increasing muscle mass **(Montelli et al., 2021)**. Besides, the determination of plasma IGF-I concentration in growing lambs may provide a physiological explanation of growth features **(Medrano and Bradford, 1991; Gatford et al., 1996, 1997; Whisnant et al., 1997).** Furthermore, muscle development is a yield of protein synthesis and degradation controlled by the action of IGF-I **(Gatford et al., 1996; Oksbjerg et al., 2004)**. So, the higher concentration of IGF-I in Barki compared with Rahmani lambs from week 26 to 40 during the present study suggest that Barki lambs tend to increase their body mass in muscle tissues more than Rahmani lambs during the previous period. On the other hand, the obtained concentration of plasma leptin during the present study is close to the earlier studies reporting results for leptin concentrations **(Altmann, Sauerwein, & von Borell, 2006; Radwanska & Kosior-Korzecka, 2016)**. The blood leptin concentration increased with the growth of lambs **(Tokuda et al., 2001; Cestnik et al., 2004; Čebulj-Kadunc and Cestnik, 2005)**. In these studies, the increase of leptin concentration after weaning and during the fattening period could have been due to the rise in the live weight and subsequently increase adipose cells volume **(Tokuda et al., 2001)**. Also, these findings may be associated with an increase in the size of adipocytes as leptin is secreted and fat proportion **(Antunović et al., 2010)**. Thus, in the current study, the concentration of plasma leptin in Rahmani lambs was greater than Barki lambs starting from week 28 onwards suggested that Rahmani lambs tend to increase their body mass in adipose tissues more than Barki lambs during the previous period. Also, this might refer to the bigger size of the fat tail in Rahmani sheep compared to Barki sheep. In the present study, the onset of puberty and growth traits improvement in both breeds are related to the increase of IGF-I, and leptin concentrations, which are consistent with the results of **Nieto et al. (2013)**. Thus, this supports the suggestion that these hormones inform the central nervous system of the metabolic status of the body, perhaps specifically the accumulation of muscle (IGF-I) and fat (leptin), and thus permit the initiation of puberty **(Nieto et al., 2013)**.

The results of the present study showed that Rahmani and Barki lambs produced a similar semen quality and quantity. However, the live spermatozoa percentage in Barki lambs was more than that in Rahmani lambs. Also, in the current study, the semen characteristics were related to testicular parameters, which support that number of spermatozoa per ejaculate is related to testis volume and testis weight **(Bernardini et al., 2011)**. This effect has been correlated to an increase in testicular size since it is due to a rise in the capacity of the seminiferous epithelium and the width of seminiferous tubules **(El-Khalifa et al., 2013)**. Moreover, semen quality and quantity were affected by final testis size, a pattern of circulating testosterone, and diet **(El-Zelaky et al., 2011)**. Besides, testosterone plays a vital role in developing male reproductive organs and promoting other sexual characteristics such as increased bone and muscle mass **(Mooradian et al., 1987)**. In agreement, the current study results show that Rahmani and Barki lambs have a similar plasma testosterone concentration and a similar body weight. Also, it has been showing that testis parameters increased by the progress of age till puberty in both breeds. Besides, age and body weight had a significant influence on testicular length, testicular diameter, scrotal circumference, and scrotal length **(Karakus, 2010)**. The present trend of increase in plasma testosterone concentration during the pre-pubertal up to puberty stage is consistent with previous results of **El-Khalifa et al. (2013)**,

which showed that testosterone levels were low at early stages of sexual development and progressively increased with advanced age.

CONCLUSIONS

Based on the results obtained in this study, it could indicate that Rahmani and Barki male lambs had a similar value of growth traits from post-weaning till week 40 of age. Also, age, body length, rump height, and heart girth values for both breeds were very similar during the pre-pubertal up to puberty. Besides, the recorded values of both breeds for testicular characteristics, production of testosterone, and semen quality, and quantity were almost comparable. Thus, the current study results suggest that body mass represented by muscle and fat accumulation for both breeds were almost equal. These might modify the circulating of IGF-1 and leptin, respectively, exerting an equally positive influence on the testosterone concentration and subsequently the reproductive performance of Rahmani and Barki lambs at puberty.

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Arabic summary

الارتباط بين سن البلوغ، وخصائص النمو، وتركيزات عامل النمو المشابه للأنسولين واللبتين في البلازما مع الأداء التناسلي لذكور الحملان الرحماني والبرقي.

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هدفت الدراسة الحالية إلى معرفة تأثير نوع السلالة وحالة البلوغ على مقاييس النمو وتركيزات هرموني عامل النمو المشابه للأنسولين واللبتين في الدم والأداء التناسلي اللاحق لذكور الحملان الرحماني والبرقي. تم استخدام مجموعتين متساويتين من الحملان الرحماني والبرقي (٢٠ حمل لكل مجموعة) في الدراسة الحالية من بعد الفطام حتى الأسبوع ٤٠ من العمر. حيث تمت متابعة جميع الحملان لتسجيل خصائص البلوغ ومقاييس النمو ووصول الحملان لعمر البلوغ كما تم جمع عينات الدم من كل حمل مرة كل أسبوعين لتقدير نسبة هرمونات عامل النمو المشابه للأنسولين واللبتين والتستوستيرون في البلازما. أشارت النتائج إلى أن الحملان الرحماني والبرقي كان لهما نفس القيم لمقاييس النمو خلال فترة التجربة. كما بلغ سن البلوغ (٣٧،٤٨ أسبوعًا) في الحملان الرحمانيّ دون وجود فروق معنويّة مع الحملان البرقيّ (٣٦،٧٢ أسبوعًا). إلى جانب ذلك، كان تركيز عامل النمو المشابه للأنسولين في البلازما أعلى بصورة معنويه في الحملان البرقيّ مقارنة بالحملان الرحماني بدءًا من الأسبوع ٢٦ فصاعدًا. ومع ذلك، كان تركيز اللبتين في البلازما في الحملان الرحماني أعلى بصورة معنويه مقارنة بالحملان البرقيّ بدءًا من الأسبوع 28 فصاعدًا. وبالتالي، كانت هناك زيادة خطية في مستويات هرمون التستوستيرون في البلازما خلال فترة ما قبل البلوغ وحتى مرحلة البلوغ دون أي فروق ذات دلالة إحصائية بين السلالتين. كما أن جودة وكمية السائل المنوي المسجلة لكلا السلالتين كانت متقاربة تقريبًا. وبناءً على ذلك، تشير نتائج الدراسة الحالية إلى أن كتلة الجسم المتمثلة في تراكم العضلات والدهون كانت متساوية لكلا السلالتين مما يوثر على معدلات هرموني عامل النمو المشابه للأنسولين واللبتين في الدم على التوالي، مما يؤدي إلى تأثير إيجابي مماثل على الأداء التناسلي للحملان الرحماني والبرقي عند البلوغ.

الكلمات المفتاحية: الاغنام الرحماني، الاغنام البرقي، عامل النمو المشابه للأنسولين، اللبتين، التستوستيرون.