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Effect of L-Arginine Supplementation in Ossimi Rams' Diets on Biometry of Testes and Epididymis, Immune-Histochemical Expression and Characteristics of Epididymal Spermatozoa

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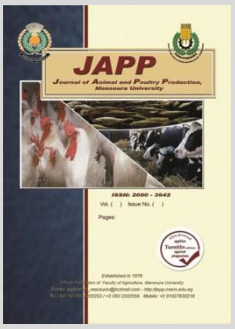

ABSTRACT

This experiment was conducted to evaluate the impact of dietary L-arginine supplementation on some epididymal spermatozoa characteristics, differential White blood cells count, some reproductive organs'metrics. Fifteen adult Ossimi rams averaging 44.9± 0.57 kg were utilized, divided into three comparable groups (5 rams each) , were fed a basal ration supplemented with L- arginine at concentration of 0.0 (G1) or 0.5(G2) or 1.0%(G3) of body weight/day for two months. At the end of the study rams were slaughtered for conducting biometric studies on tests and epididymis and testicular Immunohistochemical NOS analysis. Epididymis Semen was collected and evaluated for motility, viability, abnormality and acrosome integrity.L-arginine Supplementation at 0.5(G2) or 1.0%(G3) significantly (P<0.05) improved epididymal spermatozoa characteristics, as % of motility, livability, acrosome integrity while, % of dead and abnormal spermatozoa decreased in supplemented groups as compared with G1. This improving was greatest in G2 than that happened in G3. Measurements of testes and epididymis increased in G2 and G3 compared with G1 . In addition, there was a significant increase of immunoexpression of testicular Nitric oxide synthase (NOS) in rams received L-arginine at 1% or 0.5% of body weight/day as compared to G1, and this expression was clearly found within the spermatid layer and few sertoli cells in both treated groups, while in G1, it was limited to spermatid layer only. . It could be concluded that dietary L- arginine supplementation to Ossimi ram ration has positive effects on epididymal spermatozoa characteristics, Sexual organs anthropometries and immunohistochemical expression of testicular NOS.

Keywords: L-arginine, rams, sexual organs metrics, semen, immune expression.

INTRODUCTION

The use of highly fertile rams is critical for improving sheep production, as ram fertility has an impact on flock performance and reproductive efficiency. The number of good qualities spermatogenic cells produced by a testicle and its ability to store produced spermatozoa are the two most important factors that breeders consider when selecting breeding males (Ewuola, 2013). Morris *et al.* (1999) proposed that within a species of animals, there is a positive association between spermatozoa development, and size and length of the testes.

Nutrition is one of the factors that influence the production of sperm and their ability to fertilize (Rurangwa *et al.*, 2004; Canyurt and Akhan 2008). Amino acids are required for the synthesis of a wide range of proteins and for the regulation of key metabolic pathways involved in the immune response to infectious pathogens (Kelly and Pearce, 2020). The best prototypes are arginine, glutamine, and cysteine precursors, which have well-defined functions and a wide range of applications in livestock production and nutrition (Holecek and Sispera, 2016). Arginine must be provided orally during reproductive age of adult males. It is an important amino acid that plays a role in sperm formation (Küçükbay *et al.*, 2008). It is a basic component of the nucleoprotein that is necessary during both mitosis and meiosis in spermatogenesis (Altınısık, 2016). This amino acid improves immune function (Kudsk, 2006) and sperm motility (Patel *et al.*, 1998) by raising the rate of glycolysis, which raises the rate of adenosine triphosphate (ATP, Patel *et al.*, 1998 and 1999). L-arginine forms are more appropriate for body use than D-arginine forms (Gokcek, 2016). Furthermore, it has been suggested that L-arginine aids sperm motility, capacitation, and acrosome reaction in cattle (O'Flaherty *et al*., 2004; Leal *et al*., 2009; da Silva *et al*., 2014) as well as maintaining the structural and functional integrity of spermatozoa (Srivastava *et al*., 2000; O'Flaherty *et al*., 2004). The sperm cell's production of nitric oxide (NO) from L-arginine has been related to such effects (O'Flaherty *et al.*, 2004; Leal *et al.*, 2009). Moreover, the successful fertilization requires physiological level of NO (Kim *et al.*, 2004). The contraction and relaxation of tunica albuginea which are responsible for transport of spermatozoa to the epididymis are regulated by PKG, a downstream effector of NOS/NO (Middendorff *et al.*, 2002). Through the regulation of germ cell apoptosis and the maintenance of the optimal ratio between Sertoli and germ cells, this factor contributes to the control of germ cell differentiation and growth, resulting in the production of viable and fertile spermatozoa (Lee and Cheng 2008). In animals, the size of the testes is a strong predictor of current and potential sperm development (Gage and Freckleton,

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2003). In this respect, El-Neweshy *et al.* (2013) linked testicular volume reduction to a drop in testosterone levels, which leads to low sperm production.

The studies concerning the impact of L-arginine as an essential amino acid on the ram's reproduction efficiency are scarce. Thus, the aim of this work was to see if adding L-arginine to rams' diets affects reproductive performance, epididymal semen quality, and measurements of some male reproductive organs (testes and epididymis). The testes of Ossimi rams were studied for anthropometries and immunohistochemical expression.

MATERIALS AND METHODS

Animals and treatments

From the sheep herd belonged to the Experimental Station of the Animal Production Department, Faculty of Agriculture, Menoufia University, Shibin El-Kom, fifteen adult and healthy Ossimi rams, aged 12-14 months and weighing 24.9+0.57 kg live body weight, were randomly used in this experiment. These rams were divided into three comparable groups (5 rams each).

For two months, rams were given a basal diet with no additives (control group; G1), a basal diet supplemented with L-arginine at concentrations of 0.5% (0.225 g / ram/ day) (G2), and 1.0% (0.445 g / ram/ day) (G3) of live body weight/day for two monthes, respectively.

Metric evaluation of sexual organs

The experimental rams were slaughtered at the end of the feeding period according to the Islamic procedure, and the genital tracts were isolated, cleaned from the extraneous tissues, and examined thoroughly. According to the methods of Thakur and Dixit (2006) and Amini and Kamkar (2005), the biometry of the testes and epididymis of each ram was assessed as follows: The testes with epididymis of each experimental ram were separated free of adhering connective tissues and fats, collected, and placed in plastic bags labelled with animal specificities and transported in an ice pack to the laboratory, where the epididymis were separated from testes and then testicular and epididymal measurements were determined. The testicular length and width as well as testicular weight of each animal's paired testes (left and right) were evaluated and recorded. The two testes measurements were averaged. The width of the testicles was measured in the center of the testicular poles, at its widest point. Testicular length was also measured from one pole of the testis to the other pole along the longitudinal axis of the testis. Every animal's left and right epididymis was then divided into caput, corpus, and caudal parts, which were then weighed, measured, and recorded.

Collection and preparation of epididymal spermatozoa

In the laboratory, the right and left epididymis were trimmed of the body of the testes, various incisions were performed in the epididymal caudal segment with a scalpel, the incisions were also flushed with 2-3 drops of 2.9% buffered sodium citrate kept at body temperature, then the caudal epididymal spermatozoa were obtained and sucked into a Pasteur pipette, and the suspended or recovered semen samples were placed in a 5 ml tube and examined for Initial motility, and percentage of live and abnormal spermatozoa and acrosomal Integrity.

Spermatozoa evaluation

The samples were tested for the following characteristics immediately after collection of epididymal spermatozoa.

Initial motility of spermatozoa

A drop of the obtained epididymal spermatozoa was examined under a microscope at 200X magnification and the percentages of initial motility evaluated according to the method of Amann and Hammerstedt (1980).

Percentage of live and abnormal spermatozoa

According to Hackett and Macpherson (1965) procedure, a smear of the freshly collected epididymal sample was made on a clean glass slide and stained with 1% water soluble eosin and 5% nigrosine dissolved. A total of 200 epididymal spermatozoa were counted on each slide to determine the percentage of live and abnormal spermatozoa.

Acrosomal Integrity

The integrity of the acrosome was determined using the Giemsa staining technique (Watson, 1975). Glass slides with semen smeared on them were immersed in a 5% formaldehyde solution and fixed at 37°C for 30 minutes. After washing and air drying, smeared slides of spermatozoa were put into the working solution (Giemsa's stock-3 ml, Sorenson's phosphate buffer-2 ml and dist. water- 45 ml) and kept at 37ºC for 3 hours. At 1000X magnification (Olympus optical,made in japan), count of sperm with intact, partially damaged, and completely damaged acrosomes were determined.

Blood cell analysis

On the morning of the slaughtering day, a 3 ml blood sample was drawn from each ram's jugular vein with a disposable syringe, placed in sterile capped tubes with EDTA to avoid coagulation, and transported to the laboratory for hematological examination. For counting the different types of white blood cells (neutrophils, basophils, eosinophils, monocytes, macrolemphs and microlemphs), thin blood smears were fixed in alcohol and stained with Giemsa stain. The percentage of every type was calculated (REFERENCE).

Immunohistochemical analysis of NOS

Every ram's testicular segments were fixed in neutral buffered saline. According to Saber *et al.* (2019) dewaxed serial parts were hydrated and soaked in an antigen retrieval solution (EDTA solution, PH 8). After that, they were treated with 0.3% hydrogen peroxide and protein block before being incubated with a rabbit NOS / Nitric Oxide Synthase polyclonal antibody (MyBioSource, Inc. San Diego, CA 92195-3308, USA; 1:200 dilution). The slides were rinsed three times with phosphate buffer saline (PBS) before being incubated for 30 minutes at room temperature with anti-mouse IgG secondary antibodies (EnVision + System HRP; Dako), visualised with di-aminobenzidine commercial kits (Liquid DAB +Substrate Chromogen System; Dako), and finally counterstained with Mayer's haematoxylin. The primary antibody was swapped out for normal mouse serum as a negative control procedure. The percentage of positive cells per total 1000 counted cells in about 8 to 10 high power fields was used to calculate the caspase 3 labelling index.

Statistical analysis

Data were examined for normality prior to the analysis and all percentages and data on semen traits and

white blood cell differentiation were subject to arcsine transformation.

According to the following statistical model, data were analyzed using one-way analysis of variance:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where: Y_{ij} is the observation; μ is the overall mean; G_i is the treatment fixed effect (T_1 to T_3); and e_{ij} is the residual of the model.

Duncan's Multiple Range Test (Duncan, 1955) checked the significance ($P < 0.05$) of the differences among treatment means. Statistical analyses were performed by using SAS software (SAS, Version 9.1.3. SAS Institute Inc., Cary, NC, 2007).

RESULTS AND DISCUSSION

Physical characteristics of epididymal spermatozoa

Table 1 shows the effect of L-arginine supplementation on the physical properties of epididymal spermatozoa in Ossimi rams. The addition of L-arginine to the experimental rams' ration had a marked effect on all physical characteristics of sperm examined in this study (percentage of sperm motility, livability, dead, abnormal, and acrosome integrity). The percentage of motility, livability, and acrosome integrity increased significantly ($P < 0.5$). In contrast, the percentage of abnormal and dead epididymal spermatozoa of rams fed L-arginine-containing rations was significantly lower than that of rams fed L-arginine-free rations. In this regard, the motility percentage of epididymal spermatozoa collected from rams fed a diet containing L-arginine at level of 0.5% of LBW was (71.66±0.35) significantly ($P < 0.5$) higher than that recorded for rams fed diet free of L-arginine (57.50± 3.54). However, the differences between the two treated groups for the motility percentage of epididymal spermatozoa were not significant. Furthermore, the results showed that the percentage of sperm livability and acrosome integrity for the L-arginine supplemented group at level of 0.5% was 86.66±2.47% and 86.66±1.07%, respectively. These figures were higher than those observed for the L-arginine supplemented group at a level of 1.0% (81.83±0.6% and 80.50±1.4%, respectively) and higher than those observed for the control group (76.0±0.71% and 77.83±3.54%, respectively). These positive improvements in the physical characteristics of epididymal spermatozoa as a result of adding L-arginine to the diet of Ossimi rams at a level of 0.5 or 1.0% of LBW (Table 1) are in accordance of previous results reported by Aydin and Alagöl (1995); Patel *at al.* (1998); O'Flaherty *et al.* (2004); Srivastava *et al.* (2006) and Silva *et al.* (2012), who found that L-arginine plays a key role in improving sperm motility in humans, rabbits, goats, and cattle, respectively. Furthermore, Ahangar *et al.* (2017) found that adding L-arginine to the diet improved all reproductive traits in male broilers, as well as the ability of sperm to penetrate the perivitelline membrane. Aydin and Alagöl (1995) demonstrated that L-arginine administration increased sperm concentration and motility without causing any side effects. The observed improvement in the physical characteristics of epididymal spermatozoa in Ossimi rams as a consequence of L-arginine supplementation may be attributed to improved glycolysis, which results in higher rates of ATP and lactate development in spermatozoa (Patel *et al.*, 1998 and 1999), enhancing defense and cellular immunity (Srivastava *et al.,* 2006), and protecting the spermatozoa against lipid peroxidation effect (Srivastava *et al.,* 2000). On the other hand, Doshi *et al.* (2012) proposed that L-impact arginine's on sperms may be due to cells synthesizing NO endogenously (via oxidation of L-arginine), which has a regulatory effect on sperm motility and viability, and thus sperm fertilization capacity.

Table 1. Epididymal sperm characteristics in Ossimi rams as influenced by dietary supplementation with L-arginine.

Semen characteristics	L-Arginine supplementation		
	G1 (control)	G2 (0.5% L-arginine)	G3 (1% L-arginine)
Sperm motility (%)	57.50^{b}±3.54	71.66^{a}±0.35	65.83^{a}±1.41
Live sperm (%)	76.00^{c}±0.71	86.66^{a}±2.47	81.83^{b}±0.60
Dead sperm %	24.00^{a}±0.71	14.16^{c}±2.47	18.16^{b} ±1.20
Abnormal sperm (%)	19.83^{a}±3.18	11.33^{b}±1.41	13.66^{b}±1.20
Acrosome integrity (%)	77.83^{b}±3.54	86.66^{a}±1.07	80.50^{b}±1.40

Means in the same row superscripted by different alphabetic letters are significantly ($P < 0.05$) different.

Biometry of testes and epididymis

The biometrics of testes and epididymis organs obtained from slaughtered Ossimi rams in different experimental groups are shown in Tables 2 and 3. In contrast to control rams L-arginine treatment at a rate of 0.5 or 1.0% of LBW increased different biometrics (weight, width, and length) for the testes. In this regard, the weight and width of testes of rams treated with a L-arginine level of 0.5% of LBW (166.5±6.8 gm, and 17.0±0.25 cm, respectively) were substantially ($P < 0.05$) higher than those treated with L-arginine level of 1.0% of LBW (130.6±10.89 gm and 16.46±0.22 cm, respectively) and control rams (108.9±6.0 gm and 15.21±0.28 cm, respectively). However, the differences between rams fed diet containing 1.0% L-arginine and the control rams for weight and width of the testicle were not significant.

Concerning the testicle length, rams treated with 0.5% L-arginine of LBW had longer testes (11.6±0.64 cm, $P < 0.05$) than the control rams (8.7±0.18 cm). However, the differences between treated groups for the testicle length were not significant.

Regarding to the effect of L-arginine supplementation on the biometry of epididymis. In general, supplementing the Ossimi ration with L-arginine increased the total epididymis weight and length of rams treated with L-arginine at level 0.5% (23.69 gm, and 17.44 cm, respectively) as compared to those treated with L-arginine1.0% of LBW (23.0 gm, and 16.16 cm, respectively) or untreated rams (21.96 gm and 16.41 cm). The effect of supplementing Ossimi rams with L-arginine on various epididymis segments (caput, body, and caudal) on different measurements (weight, length, and width) varied from one segment to the next. Dietary supplementation of L-arginine resulted in heavier cauda for rams fed diets containing 0.5% of LBW (10.67±0.84 gm) and 10.50±0.61 gm for rams fed diets containing 1.0% of LBW as compared to the control group (7.06±1.63 gm). Contrarily, dietary supplementation of L-arginine resulted in epididymis head and body becoming marginally lighter. The control group had heavier head and body of the epididymis (9.13±0.39 and 5.50±1.0 gm, respectively) than the 0.5% L-arginine treated group (8.84± 0.33 and 4.18±0.3 gm, respectively) and the 1.0% L-arginine supplemented group (8.95±0.25 and 3.55±0.40 gm, respectively). Based on these findings, dietary L-arginine

supplementation in the Ossimi ration increased the weight and length of epididymis segments (caput, body, and caudal) as well as the weight and length of the testes (weight, width, and length) when compared to rams fed a ration free of L-arginine (Tables 2 and 3).

This improvement in genital organ weight may be attributed to an increase in serum testosterone concentration as a result of L-arginine supplementation, where testosterone promotes the development and function of the reproductive organs (Ahangar *et al.*, 2017). The increment in serum testosterone levels may be associated with more development in circumference of the testis scrotum as well as semen volume (Sajjad *et al.*, 2007;Mohamed *et al.*, 2020). Moreover, arginine stimulates the release of pancreatic and anterior pituitary hormones, which regulates protein, amino acid, fatty acid, and glucose metabolism (Ren *et al.*, 2015).

Table 2. Biometry of testes in Ossimi rams as influenced by dietary supplementation with L-arginine.

Testicular measurement	L-Arginine supplementation		
	G1 (control)	G2 (0.5% L-arginine)	G3 (1% L-arginine)
Weight (g)	108.90^{b}±6.00	166.50^{a}±6.80	130.60^{b}±10.89
Length (cm)	8.70^{b}±0.18	11.60^{a}±0.64	11.10^{ab}±0.64
Width (cm)	15.21^{b}±0.28	17.00^{a}±0.25	16.46^{b} ±0.22

Means in the same row superscripted by different alphabetic letters are significantly ($P < 0.05$) different.

Table 3. Biometry of epididymis (caput, corpus, cauda) in Ossimi rams as influenced by dietary supplementation with L-arginine.

Item	Measure	L-Arginine supplementation		
		G1 (control)	G2 (0.5% L-arginine)	G3 (1% L-arginine)
Total epididymis	Weight, gm	21.70^{c}±1.42	23.70^{a}±0.87	23.00^{b}±0.71
	Length, cm	16.42^{b}±0.83	17.45^{a}±0.83	16.17^{c}±0.49
	Width, cm	6.22^{b}±0.32	5.73^{c}±0.31	7.25^{a}±1.54
Epididymal caput	Weight, gm	9.13±0.30	8.84±0.33	8.95±0.25
	Length, cm	4.50±0.31	4.75±0.17	4.58±0.39
	Width, cm	2.78^{b}±0.23	2.36^{b}±0.11	4.51^{a}±1.48
Epididymal corpus	Weight, gm	5.50^{a}±1.08	4.18^{ab}±0.31	3.55^{b}±0.40
	Length, cm	5.83^{b}±0.58	8.53^{a}±0.41	7.08^{b}±0.15
	Width, cm	1.20±0.35	0.75±0.08	0.43±0.08
Epididymal cauda	Weight, gm	7.06^{b}±1.63	10.67^{a}±0.84	10.50^{a}±0.61
	Length, cm	6.08±0.98	4.16±0.42	4.50±0.27
	Width, cm	2.23±0.32	2.61±0.20	2.30±0.23

Means in the same row superscripted by different alphabetic letters are significantly ($P < 0.05$) different.

White blood cells differentiation

The percentages of monocytes, lymphocytes (micro and macro), eosinophils, and neutrophils were not substantially affected by administering L- arginine to Ossimi rams at 0.5 or 1.0% of LBW (Table 4).

Table 4. White blood cells differentiation in Ossimi rams as influenced by dietary supplementation with L-arginine.

Types of white blood cells (%)	L-Arginine supplementation		
	G1 (control)	G2 (0.5% L-arginine)	G3 (1% L-arginine)
Neutrophil	17.60±5.37	12.60±2.84	15.80±2.84
Eosinophil	11.00±1.26	8.60±0.32	11.20±0.95
Basophils	6.40^{c}±0.32	13.80^{a}±0.95	10.80^{b}±1.26
Macrolemphocytes	31.00±5.06	26.60±0.95	23.00±0.32
Microlemphocytes	24.80±2.21	21.60±1.26	25.40±6.01
Monocytes	10.40±3.16	11.60±0.63	15.60±1.26

Means in the same row superscripted by different alphabetic letters are significantly ($P < 0.05$) different.

However, the percentage of basophils was significantly ($P < 0.05$) affected in rams fed diets containing L-arginine at levels of 0.5%, being higher (13.8±0.95) than in rams fed diets containing L-arginine at levels of 1.0% (10.8±1.26) and control rams (6.4±0.32). In agreement with the insignificant reduction in lymphocytes percentage by L-arginine treatment Gonçalves *et al.* (2012) found an increase in lymphocytes is simultaneously reduced by arginine supplementation compared to the control group. Given that white blood cells are a vital predictor of an animal's immune state, arginine supplementation may be considered as an immune system stimulator, boosting T output, rising antibody production, speeding wound healing mediated by immune cells, or lowering infection cells (Tur *et al.*, 2006).

Immunoexpression of NOS

Both treated groups showed a strong expression of NOS protein, which was localized in the spermatid layer and a few Sertoli cells, as shown in Figure 1 (B and C). In the control group, NOS protein expression was limited only to the spermatid layer (Figure 1A). NOS immunoexpression was higher in G2 and G3 (received arginine at level of 0.5 or 1% of LBW/day, respectively) than in the control untreated community (Figure 1D). Our findings are consistent with those of Javanmard *et al.* (2009) and Zhang *et al.* (2006), who found that supplementing L-arginine increased eNOS expression. The desired effects of L-arginine may be due to an increase in eNOS expression, as arginine regulates iNOS expression through translational control of iNOS mRNA (Lee *et al.*, 2003). Previous research had established the importance of certain amino acids, such as leucine and arginine, in the early stages of mRNA translation (Sattlegger and Hinnebusch 2000). Furthermore, according to Lee and Cheng (2004), the involvement of NOS in all cell types within the testis suggests that NO/NOS plays an important role in spermatogenesis. Within the seminiferous epithelium, the three NOS isoforms have been discovered in both germ and Sertoli cells (Sattlegger and Hinnebusch 2000).

Globally, the current research found that rams fed a diet containing L-arginine had improved in most of the studied traits, including physical characteristics of epididymal spermatozoa and testes, epididymis biometrics, and a reduction in white blood cells count, as compared to

those fed a diet without L-arginine. Furthermore, when comparing rams fed a diet containing a lower level (0.5%) or a higher level (1.0%) of L-arginine, this increase was relatively higher for rams fed a lower level (0.5%) of L-arginine. In this way, Hassanpour *et al.* (2007) stated that the effects of L-arginine on the motility of ejaculated sperm *in vitro* are dose-dependent, with low concentrations of L-arginine increasing sperm motility and high concentrations of L-arginine decreasing sperm motility. Moreover, Ratnasooriya and Dharmasiri (2001) found that high levels of L-arginine reduce animal fertility. The effect of L-arginine on sperm cells may be attributable to endogenous cells producing nitric oxide (NO) via the oxidation of L-arginine, which has a regulatory effect on sperm motility and viability, and thus sperm fertilizing capacity. Nitric oxide has a bimodal effect on sperm motility; low concentrations of NO increase sperm motility, whereas high concentrations of NO decrease it (Doshi *et al.*, 2012).

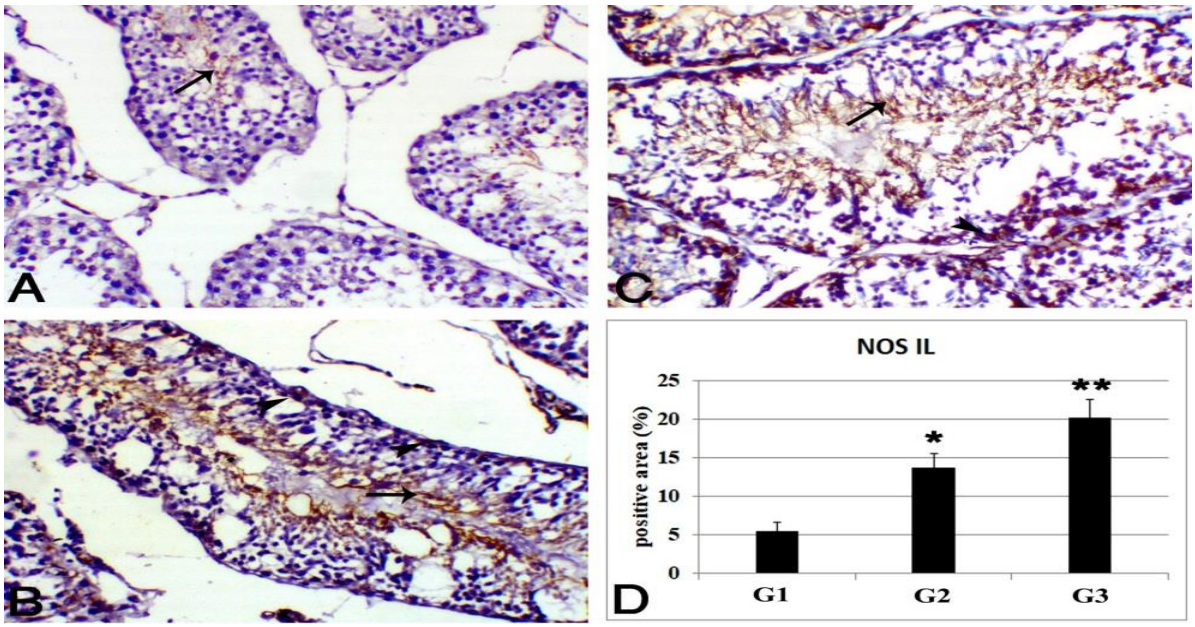

Figure 1. Immunohistochemical expression of NOS.

A) Testis of control ram showing immunoexpression within the spermatid layer only (arrow).

B) Testis of ram supplemented with arginine at a concentration of 0.5% of body weight showing increased expression of NOS within the spermatid layer (arrow) and few Sertoli cells (arrowhead).

C) Testis of ram supplemented with arginine at a concentration of 1% of body weight showing increased expression of NOS within the spermatid layer (arrow) and few Sertoli cells (arrowhead), NOS IHC, X200.

D) Area positive percentage of the treated groups showed significant increase of NOS immunoexpression in comparison with control group.

CONCLUSION

The supplementation of Ossimi rams' diets with L-arginine at level 0.5 or 1.0% of the live body weight has beneficial effects on the physical characteristics of epidiymal spermatozoa and the evaluated biometrics (weight, width, length) for the testes and epididymal segments, white blood cells and expression of NOS protein in spermatid layer and few Sertoli cells. However, the best recommended level in this study was 0.5% of live body weight/day L-arginine.

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Conflict of Interests

The authors declare that they have no competing interests.

Animal Welfare Statement

This study was carried out according to the suggestions and guidelines of the Committee of Animal Care and Welfare, Menoufia University, Egypt. The ethical approval No 2020-3.

Data Availability Statement

The datasets of the current study are available from the corresponding author upon reasonable request.

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تأثير إضافة الأرجنين الى علائق كباش الأوسيمى على مقاييس الخصية والمبيض والتعبير الهستولوجى المناعى وخصائص السائل المنوي

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أجريت هذه الدراسة لتقدير تأثير إضافة الأرجنين الى عليقة كباش الأوسيمى على خصائص السائل المنوي المستخرج من البربخ، كرات الدم البيضاء بأنواعها المختلفة، مقاييس بعض الأعضاء الجنسية، معدلات تخليق أكسيد النيتريك (NO) كمؤشر للتعبير المناعي في نسيج الخصية.تم إستخدام عدد ١٥ كبش أوسيمى بمتوسط وزن (٤٤,٩+ ٠,٥٧ جم) وعمر (١٢-١٤ شهر). وتم تقسيمهم عشوائيا الى ٣ مجاميع (٥ في كل مجموعة)، ثم قمنا بتغذيتهم لمدة شهرين كالتالي: - المجموعة الأولى (المجموعة الكنترول): عليقة أساسية بدون أي إضافات. المجموعة الثانية: عليقة أساسية + ٠,٥ % من وزن الكبش أرجنين (٠,٢٢٥ جم/ اليوم). المجموعة الثالثة: عليقة أساسية + ١% من وزن الكبش أرجنين (٠,٤٤٥ جم / اليوم). وتم ذبح الحيوانات في نهاية فترة التغذية وم أخذ عينات الدم، كما تم اخذ بعض المقاييس (طول، عرض، قطر) لكلا من الخصية وأجزاء البربخ ، كما تم جمع السائل المنوي المخزن في البربخ وتقييم بعض خصائصه مثل الحركة ، الحيوية ، نسبة الحيوانات المنوية الشاذة والطبيعية ، سلامة الأكر وسوم ، كما تم عمل قطاعات هستولوجية للخصية والبربخ . وقد أظهرت النتائج أن إضافة الأرجنين لعليقة كباش الأوسيمى بنسبة ٠,٥ ، ١% من وزن الكبش أدت إلى تحسين خصائص السائل المنوي وزيادة مقاييس الخصية والبربخ بينما لم يحدث تغيير معنوي في نسبة كرات الدم البيضاء ، كما أنها أدت الى انتشار أكبر لأكسيد النيتريك (NO) في الخصية كمؤشر للتعبير المناعي عن المجموعة الكنترول.