

# The effect of L-arginine on erection time, sperm quality and the seminal plasma arginase activity in rams

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## SUMMARY

The purpose of this study is to investigate the effects of L-arginine on sperm quality in the ram. Twelve Akkaraman fertile rams, 2-3 years old and weighing 50-60 kg, were divided into 2 lots: 6 treated and 6 controls. The treated animals were injected intramuscularly every 48 hours with 5 mg/kg L-arginine; for 7 weeks; the witnesses an injection of isotonic saline of sodium chloride at the same rate. Semen was collected once a week in the presence of a sheep in heat (caused by an injection of prostaglandin). While intramuscular administration of L-arginine caused significant reduction in erection time (in 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> weeks) and abnormal spermatozoon ratio (only in 2<sup>nd</sup> week) when compared to control group, mass activity, motility, concentration (in 3<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> week), membrane integrity (in 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> weeks) were significantly increased. Consequently, intramuscular administration of L-arginine provides improvement in sperm quality parameters while reduces erection time in rams.

**Keywords:** L-arginine, sperm, arginase, ram

## RÉSUMÉ

**L'effet de la L-arginine sur le temps d'érection, la qualité des spermatozoïdes et l'activité de l'arginase séminale plasmique chez les béliers**

Le but de cette étude est de rechercher les effets de la L-arginine sur la qualité du sperme chez le bélier. Douze béliers de race Akkaraman, fertiles, âgés de 2 à 3 ans et pesant de 50 à 60 kg, ont été divisés en 2 lots : 6 traités et 6 témoins. Les animaux traités ont reçu une injection par la voie intramusculaire, toutes les 48h, de 5 mg/kg de L-arginine; pendant 7 semaines; les témoins une injection de solution isototonique de chlorure de sodium selon le même rythme. Le sperme a été récolté une fois par semaine en présence d'une brebis en chaleurs (provoquée par une injection de prostaglandine). Alors que l'administration intramusculaire de L-arginine a provoqué une réduction significative du temps d'érection (dans les 4<sup>ème</sup>, 5<sup>ème</sup>, 6<sup>ème</sup> et 7<sup>ème</sup> semaines) et un rapport anormal des spermatozoïdes (seulement en 2<sup>ème</sup> semaine) par rapport au groupe témoin, la motilité de masse, la motilité, la densité (en 3<sup>ème</sup>, 5<sup>ème</sup> et 6<sup>ème</sup> semaine), l'intégrité de la membrane (dans les 4<sup>ème</sup>, 5<sup>ème</sup>, 6<sup>ème</sup> et 7<sup>ème</sup> semaines) ont été significativement augmentées. Par conséquent, l'administration intramusculaire de L-arginine améliore les paramètres de qualité du sperme tout en réduisant le temps d'érection chez les béliers.

**Mots-clés :** L-arginine, sperme, arginase, bélier

## Introduction

L-arginine (2-Amino-5-guanidinopentanoic acid) is synthesized in different chemical pathways in the body. L-arginine participates different metabolic pathways in various tissues in animal cells and involves in the structure of the significant compounds. It participates both protein synthesis and formation of glucose and glycogen, synthesis of nitric oxide (NO), ornithine and urea, synthesis of some hormones (insulin, prolactin, glucagon, growth hormone) as well as biosynthesis of creatine, agmatine, glutamate, proline and polyamines and in the structure of antidiuretic hormone (ADH). L-arginine catabolizes into ornithine and urea with arginase enzyme [13, 18, 21].

L-arginine additives have also excessive effects on urogenital system. It is effective in both sexual functions of males and females. It promotes orgasm by increasing vaginal and clitoral blood flow through NO in females. In males, it causes increased libido and erection by increasing blood flow in genital area [12, 15].

*In vivo* and *in vitro* studies have proved that penis erection formed after activation of L-arginine/NO pathway. In patients with erectile dysfunction, it has been shown that the addition of L-arginine improves sexual function. Today, the addition of L-arginine has begun to be used promisingly in the treatment of erectile dysfunctions, male infertility, low spermatozoon number (oligozoospermia) and the sperm motility is normally low (asthenospermia) [1, 4].

Seminal plasma improves motility of ram sperms and their viability. It decreases the risk of exposure to cold shock of spermatozoids at a definitive temperature and prevents membrane damages [2]. L-arginine is found in the seminal plasma [4]. Medeiros et al. [14] have reported that they exposed to hypertonic environment during freezing and diluting process and seminal plasma could be removed from membranes and protective molecules in seminal plasma were mainly proteins. On the other hand, it has also been reported that the arginase enzyme responsible for the metabolism of L-arginine has a negative correlation between the levels of seminal plasma of rams and male goats and abnormal spermatozoa ratios [6, 26].

A variety of factors play a role in spermatogenesis. However, all these factors, and especially those that have adverse effects on spermatogenesis, are hard to identify and often unfeasible, so treatment remains a major problem. In the idiopathic infertility cases, hormones, androgens, drugs and vitamins are used but the results obtained from them remain often insufficient. In addition to these, L-arginine supplement has recently been started to use in alternative practices and publications have been started to show that very positive results are obtained from L-arginine applications in humans.

L-arginine is necessary for continuation of spermatogenesis and has been reported that it participates the structure of nucleoproteins and forms an important nucleoprotein component in spermatozooids. Spermatogenesis requires a great quantity of nucleoproteins in both mitosis and meiosis. Spermatozoid nucleoprotein is rich in L-arginine [22, 23, 25].

L-arginine is known to increase sperm motility due to adenosine triphosphate (ATP) synthesis [8, 16, 19]. The lack of it causes defects in spermatogenesis and decrease in motility. It has been suggested that there is a relationship between this situation and the lack of L-arginine [22].

L-arginine, in the form of phosphoric acid, plays a role in the re-synthesis of ATP, helping to provide energy for the spermatozoon motility. However, it has been suggested that high concentrations of L-arginine have adverse effects on motility [11]. It has been reported that L-arginine-administered rabbits and male goats have significant increases in spermatozoon motility, which is said to be associated with an increase in ATP levels of spermatozoa due to L-arginine and consequently an increase in the rate of glycolysis [17, 30]. Schachter et al. [19] have reported that L-arginine administration not only caused an increase in spermatogenesis but also L-arginine, acting like a phosphoric acid, was necessary energy source for spermatozoon motility.

This study aimed to investigate the effect of intramuscular administration of L-arginine to rams on erection time, spermatological parameters and seminal plasma arginase enzyme activity.

## Materials and Methods

### ANIMALS AND EXPERIMENTAL DESIGN

In this study, twelve Akkaraman rams and one sheep, which are 2-3 years old and weighing 50-60 kg, clinically healthy, without any pathological findings as a result of genital organ examination and with known fertility were used. Before starting the study, the approval of the Animal Experiments Local Ethics Committee of the Firat University was obtained (2013/10). The concentrated and high quality coarse fodder were given to animals during the experimental applications. Drinking water was supplied ad libitum. Two

months before study, the rams were treated with anti-parasitic therapy.

Research and application was carried out at the Faculty of Veterinary Medicine at Firat University. Sperm retrieval was carried out between September 2014 and January 2015 within the breeding season. In the study, the spermatological characteristics and rams' erection times of taken ejaculates were examined to separate the animals into the control and experimental groups, and the animals were divided into 2 groups as balanced.

### COLLECTION OF SEMEN AND DETERMINATION OF RAMS' ERECTION TIME

Before starting to work, the rams were libido stimulated for 2 months using a separate place from the rams and using the sheep brought to the oestrus with hormonal treatments (PGF<sub>2α</sub> Dinoprost, Dinolytic, Pfizer, 4ml). It was provided that sperm of rams were delivered to artificial vagina. Sperm samples taken from each rams were examined separately throughout the study period after the adaptation period.

Erection time was determined as the time from the arrival of the ram to the side of the sheep until the erection occurred and recorded as «seconds».

### IN VIVO APPLICATION OF L-ARGININE

Six rams were applied with 1 ml of placebo saline intramuscular for every other day during the spermatogenesis (7 weeks) to be used in the control group. Six rams in the experimental group were injected with 5 mg/kg dosage L-arginine (Burlington, MA, USA, CAS No: 1119-34-2, Mol. Weight: 210.66 g/mol (C<sub>6</sub>H<sub>14</sub>N<sub>4</sub>O<sub>2</sub>HCl)) intramuscular for every other day for 7 weeks. Stock L-arginine solution (1 Molar) was prepared fresh by dissolving in saline. Stock L-arginine solution was injected about 1.2 – 1.45 mL in proportion to each ram body weight (weighing 50-60 kg). Sperm samples were taken once a week from each ram as the injections continued. The erection times of each ram in the control and experimental groups were determined prior to sperm collection. Plasma membrane integrity and arginase enzyme activity were determined by routine spermatological characteristics (sperm mass, mass motility, spermatozoon motility and density, abnormal spermatozoa ratio, sperm pH) of each ejaculate.

### ROUTINE SEMEN EXAMINATION

Sperm volume was recorded as «ml» by reading the dimension lines on the grade sperm collection cup. Mass activity was determined by giving a score between 0 and 5 by examining three different areas of the spermatozoa with strong motions, taking into account the degree of movement in the form of boiling or wave intervention [3, 7].

In determining the sperm motility, firstly 0.5 ml of tris liquidizer was added on the slide that is on the heating tray in the microscope with a temperature of 37 °C and then 3µl was diluted by putting onto it. The coverslip was closed on it and the motility was determined as 3 different region at 10x40 magnification under the phase-contrast microscope [3, 7].

Sperm concentration was determined using the hemocytometric method [3, 7]. To determine the abnormal spermatozoon ratio in sperm samples, a tusche was used to prepare the smear. The rate of abnormally shaped sperms were expressed as % by counting 400 sperms under the 40x objective of microscope [3, 7].

The pH values of the sperm samples were measured using a previously calibrated digital pH meter. The hypoosmotic swelling (HOS) test was used to determine the integrity of the sperm plasma membrane. The percentage of healthy spermatozoa with swelled and curled tail was expressed as percentage (%) [20].

#### DETERMINATION OF ARGINASE ENZYME ACTIVITY

Semen samples were centrifuged at 15000 g for 13 min in refrigerated centrifuge (at +4 °C) to obtain seminal plasma. The obtained seminal plasma was stored at -20 °C until the arginase activity was determined. Seminal plasma arginase activity was determined spectrophotometrically using a modified version of Thiosemicarbazide-Diacetyl-Monoxime-Urea (TDMU) method as described by Gayer and Dabich [5]. The protein concentration was determined by method of Lowry et al. [10]. Then, results were given as U/mg protein.

#### STATISTICAL ANALYSIS

Shapiro-Wilk normality test was applied to data that were obtained from the study. The data obtained in the study were presented as mean and standard deviation ( $\pm$  SD) values. Two-way analysis of variance for repeated measures was used for determining the differences between the time points and groups. The SPSS statistical program (22.0 Chicago, IL, USA) was used to compare data statistically.  $P < 0.05$  value was taken as statistically significant.

## Results

It had been observed that L-arginine injections at 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> weeks reduced significantly compared to control group in erection times ( $P < 0.05$ ) (Table I).

A significant difference in the amount of semen was found between all weeks and 7<sup>th</sup> week in terms of the weekly semen amount in control group ( $P < 0.05$ ). There were a significant difference within the L-arginine group between all weeks and 2<sup>th</sup> week in terms of the amount of semen ( $P < 0.05$ ) (Table I).

It has been found that the application of L-arginine decreases the semen pH values compared to the control

group, and this decrease is statistically insignificant. There was also no significant difference between control and L-arginine groups and times (Table I).

Mass activity of sperm at all weeks in experimental group was found to be statistically significant when compared with control group. Significant differences were not found in the control group between 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 6<sup>th</sup> weeks and mass activity of sperm was increased in the 5<sup>th</sup> and 7<sup>th</sup> weeks. Statistically significant differences were not found in the experimental group between 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> weeks and mass motility of sperm was increased in the 6<sup>th</sup> and 7<sup>th</sup> weeks (Table II).

Sperm motility increased significantly at all weeks when compared to the control group with L-arginine administration group ( $P < 0.05$ ). No statistically significant difference was observed in both control and arginine groups at 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> weeks and a significant increase was observed in the 7<sup>th</sup> week compared to the other weeks. (Table II).

In the L-arginine administration group, the lowest density was observed in the 1<sup>st</sup> week and the highest density was observed in the 6<sup>th</sup> week. There was no significant difference between all weeks within the control group. Significant differences were found between week 1<sup>st</sup> and 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> weeks, 2<sup>nd</sup> and 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> weeks and 3<sup>rd</sup> and 6<sup>th</sup> weeks in the L-arginine group ( $P < 0.05$ ) (Table II).

In the L-arginine group, it was observed a statistically significant decrease in the rate of abnormal sperm in the 2<sup>nd</sup> week compared to the control group. Significant differences were found between the values at 4<sup>th</sup> and 6<sup>th</sup> weeks and the values of other weeks in terms of abnormal spermatozoon ratio in the control group ( $P < 0.05$ ). The ratio of abnormal sperm in the group of L-arginine was found to be significantly different between 1<sup>st</sup> and 6<sup>th</sup>, 7<sup>th</sup> weeks and between the 4<sup>th</sup> and 7<sup>th</sup> weeks (Table III).

HOS test showed that L-arginine administrations increases sperm membrane integrity (except for the first week), this increase was found to be statistically significant except for the 2<sup>nd</sup> and 3<sup>rd</sup> weeks ( $P < 0.05$ ). Within the control group, significant differences were found between 1<sup>st</sup> and 4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup> weeks and also 2<sup>nd</sup> and 6<sup>th</sup> weeks and 3<sup>rd</sup> and 6<sup>th</sup>, 7<sup>th</sup> weeks and 4<sup>th</sup> and 6<sup>th</sup> week and 5<sup>th</sup> and 6<sup>th</sup> weeks ( $P < 0.05$ ) in terms of sperm HOS test. Within the L-arginine administration group, significant differences were found between 7<sup>th</sup> week and other all weeks ( $P < 0.05$ ) (Table III).

It was observed that L-arginine administration reduced seminal plasma arginase activity compared to control group as shown in the table III, this reduction was determined as statistically insignificant. Similarly, there were no significant differences between weeks in both control and experimental groups (Table III).

## Discussion

L-arginine and NO are found in various physiological systems and play a decisive role in the regulation of multiple functions within male and female reproductive systems, either directly or indirectly. In this study which rams were administrated 5 mg/kg L-arginine for seven weeks, erection

time was significantly reduced in intramuscular injected L-arginine group compared with placebo 1 ml of normal saline injected control group. Chen et al. [4] observed that erectile problems were resolved in nine of 29 patients given 5 g daily oral L-arginine per day in two of 17 patients who underwent placebo in 4 ml saline for 6 weeks in the study of 50 men with erectile dysfunction. Klotz et al. [9] found that erectile dysfunction improved in 40% of patients who used

	Erection Time (Seconds)		Amount of Sperm (ml)		Sperm pH	
	Control	L-Arginine	Control	L-Arginine	Control	L-Arginine
Weak 1	17.5 ± 5.7	12.8 ± 9.9	0.70 ± 0.2 <sup>a</sup>	0.93 ± 0.3 <sup>b</sup>	7.0 ± 0.3	7.0 ± 0.5
Weak 2	13.7 ± 6.9	10.8 ± 5.9	0.68 ± 0.4 <sup>a</sup>	0.66 ± 0.2 <sup>a</sup>	6.9 ± 0.2	6.8 ± 0.6
Weak 3	16.8 ± 7.2	11.2 ± 5.5	0.78 ± 0.3 <sup>a</sup>	0.91 ± 0.2 <sup>b</sup>	6.5 ± 0.3	6.4 ± 0.6
Weak 4	14.8 ± 4.4 <sup>B</sup>	9.7 ± 4.2 <sup>A</sup>	0.55 ± 0.2 <sup>a</sup>	0.80 ± 0.4 <sup>b</sup>	6.9 ± 0.3	6.7 ± 0.4
Weak 5	16.5 ± 6.6 <sup>B</sup>	8.3 ± 2.2 <sup>A</sup>	0.73 ± 0.3 <sup>a</sup>	0.95 ± 0.3 <sup>b</sup>	6.7 ± 0.3	6.5 ± 0.4
Weak 6	17.5 ± 9.8 <sup>B</sup>	6.7 ± 1.5 <sup>A</sup>	0.63 ± 0.2 <sup>a</sup>	0.83 ± 0.4 <sup>b</sup>	6.7 ± 0.4	6.6 ± 0.6
Weak 7	18.7 ± 24.1 <sup>B</sup>	6.7 ± 2.3 <sup>A</sup>	1.08 ± 0.4 <sup>b</sup>	1.33 ± 0.7 <sup>b</sup>	6.7 ± 0.5	6.7 ± 0.5

a,b,c, d and e: Different letters within a column showed significant differences between weeks.

A and B: Different letters within a line showed significant differences between control and L-arginine groups.

TABLE I: The effect of L-arginine injection on the rams' erection time, amount of sperm and sperm pH.

	Mass activity of Sperm (0-5)		Sperm Motility (%)		Spermatozoon Concentration (...X10 <sup>9</sup> /ml)	
	Control	L-Arginine	Control	L-Arginine	Control	L-Arginine
Weak 1	3.2 ± 0.2 <sup>a,B</sup>	4.2 ± 0.3 <sup>a,A</sup>	69.2 ± 2.0 <sup>a,B</sup>	79.7 ± 2.0 <sup>a,A</sup>	3.0 ± 0.6	3.7 ± 0.9 <sup>a</sup>
Weak 2	3.0 ± 0.5 <sup>a,B</sup>	3.9 ± 0.4 <sup>a,A</sup>	65.0 ± 5.5 <sup>a,B</sup>	76.7 ± 8.2 <sup>a,A</sup>	3.1 ± 1.0	3.9 ± 0.6 <sup>ab</sup>
Weak 3	3.5 ± 0.4 <sup>a,B</sup>	4.2 ± 0.5 <sup>a,A</sup>	68.3 ± 2.6 <sup>a,B</sup>	78.3 ± 7.5 <sup>ab,A</sup>	3.7 ± 0.3 <sup>B</sup>	4.8 ± 0.9 <sup>bcd,A</sup>
Weak 4	2.8 ± 0.5 <sup>a,B</sup>	4.1 ± 0.4 <sup>a,A</sup>	61.7 ± 11.7 <sup>a,B</sup>	75.0 ± 10.5 <sup>a,A</sup>	3.5 ± 1.0	5.1 ± 2.0 <sup>bcde</sup>
Weak 5	3.6 ± 0.4 <sup>b,B</sup>	4.0 ± 0.3 <sup>a,A</sup>	63.3 ± 12.1 <sup>ab,B</sup>	75.0 ± 10.5 <sup>ab,A</sup>	3.3 ± 1.5 <sup>B</sup>	5.3 ± 0.8 <sup>de,A</sup>
Weak 6	3.1 ± 1.1 <sup>a,B</sup>	4.5 ± 0.4 <sup>b,A</sup>	63.3 ± 20.6 <sup>ab,B</sup>	80.0 ± 12.6 <sup>ab,A</sup>	3.4 ± 1.2 <sup>B</sup>	5.4 ± 0.9 <sup>ce,A</sup>
Weak 7	3.8 ± 0.5 <sup>c,B</sup>	4.6 ± 0.4 <sup>c,A</sup>	78.3 ± 7.5 <sup>b,B</sup>	86.7 ± 5.2 <sup>b,A</sup>	3.7 ± 1.5	5.3 ± 1.8 <sup>de</sup>

a,b,c, d and e: Different letters within a column showed significant differences between weeks.

A and B: Different letters within a line showed significant differences between control and L-arginine groups.

TABLE II: The effect of L-arginine injection on the rams' mass motility of sperm, sperm motility and spermatozoon density.

	Abnormal Spermatozoon Ratio (%)		HOS (%)		Seminal Plasma Arginase Activity (U/mg protein)	
	Control	L-Arginine	Control	L-Arginine	Control	L-Arginine
Weak 1	9.2 ± 5.8 <sup>a</sup>	6.7 ± 2.2 <sup>a</sup>	85.5 ± 3.0 <sup>a,B</sup>	78.0 ± 5.3 <sup>a,A</sup>	0.77 ± 0.6	0.57 ± 0.1
Weak 2	8.8 ± 1.2 <sup>a,B</sup>	5.7 ± 2.2 <sup>abc,A</sup>	80.3 ± 10.1 <sup>abcd</sup>	80.5 ± 2.7 <sup>a</sup>	0.61 ± 0.5	0.49 ± 0.1
Weak 3	7.5 ± 5.5 <sup>a</sup>	4.2 ± 1.2 <sup>abc</sup>	74.3 ± 11.4 <sup>abc</sup>	81.0 ± 3.7 <sup>a</sup>	0.86 ± 0.7	0.48 ± 0.1
Weak 4	5.8 ± 1.5 <sup>b</sup>	4.8 ± 1.2 <sup>ab</sup>	71.0 ± 9.0 <sup>bcd,B</sup>	82.2 ± 2.0 <sup>a,A</sup>	0.63 ± 0.4	0.52 ± 0.1
Weak 5	6.5 ± 4.4 <sup>a</sup>	4.5 ± 1.3 <sup>abc</sup>	69.3 ± 8.9 <sup>cd,B</sup>	78.3 ± 3.7 <sup>a,A</sup>	0.59 ± 0.4	0.49 ± 0.2
Weak 6	5.0 ± 2.2 <sup>b</sup>	3.7 ± 0.5 <sup>bc</sup>	55.2 ± 4.9 <sup>ce,B</sup>	81.3 ± 2.4 <sup>a,A</sup>	0.67 ± 0.5	0.47 ± 0.2
Weak 7	5.8 ± 3.5 <sup>a</sup>	3.2 ± 1.0 <sup>c</sup>	64.0 ± 11.6 <sup>de,B</sup>	88.3 ± 3.0 <sup>b,A</sup>	0.78 ± 0.6	0.72 ± 0.4

a,b,c, d and e: Different letters within a column showed significant differences between weeks.

A and B: Different letters within a line showed significant differences between control and L-arginine groups.

TABLE III: The effect of L-arginine injection on the rams' abnormal spermatozoon ratio, HOS and seminal plasma arginase activity.

500 mg of L-arginine three times a day for 2 weeks in the study to determine the effect of L-arginine on erectile dysfunction and when the rest of the patients continued to use it for 6 weeks, they stated that erectile dysfunction of 31% of the patients was improved. In this study, the results of previous studies on L-arginine, which shortens the time for the penis to be erect in healthy rams, are supported [4, 9]. The reason of the early erection of the penis in L-arginine administrated rams compared the control group may be explained by the fact that L-arginine relaxes the penis muscles over the NO levels and causes more blood flow to flow.

In the present study, the amount of sperm obtained by applying L-arginine for 7 weeks was found to be higher than the amount of sperm obtained in the control group, but this increase was considered statistically insignificant. Wu et al. [27] reported that pigs that received 1% L-arginine-HCl for 30 days did not have a statistically significant effect on the amount of semen, but increased the motility to 8% and the density to 18%. This result overlaps with the findings obtained in the present study.

Another measurement of mass activity is that spermatozoon motility and concentration are good. The low level of each of these characteristics, or both, directly affects mass activity. In this study, it was found that the mass activity of sperm obtained from the L-arginine-administrated experimental group increased compared the control group, and the statistical analysis of this increase was found to be significant  $P < 0.05$  level. In terms of being the data obtained in this study first is important due to lack of any scientific study on how L-arginine affects sperm mass activity. This is likely to be explained by the fact that the result of the application of L-arginine probably reflects an identified increase in the motility and concentration of the spermatozoon.

Tanimura [24] have reported increased spermatozoon motility in infertile humans who were orally given 0.5 g/day L-arginine for 6-8 weeks. Increase in spermatozoon motility of L-arginine administrated rats with oligospermia and asthenospermia have been reported [17]. In another study, it was suggested that a dose of 0.004 M from different L-arginine ratios added in the diets of 12 participating men with low spermatozoon motility resulted in an increase in spermatozoon motility ( $81.6 \pm 9.96\%$ ) [8]. Schachter et al. [19] found that advanced increase in 111 cases and mild increase in 21 cases in spermatozoon motility when they administrated L-arginine to the 178 people with oligospermia for 2 months, and again Schachter et al. [19] found that when a dose of 4 mg L-arginine/day was administrated daily to 141 patients with oligospermia and asthenospermia, there was advanced increase in spermatozoon motility and intensity in 83 patients, moderate increase in 24 patients, and no improvement in 34 patients. Ratnasooriya and Dharmasiri [17] reported that they gave different doses of L-arginine via gavage to rats, leading to a significant increase in the epididymal spermatozoon motility.

In our study, it was found that the sperm motility significantly increased significantly when L-arginine was administrated intramuscular at 5 mg/kg for 7 weeks compared to control group. As seen in the studies, it seems that L-arginine deficiency causes a decrease in spermatozoon motility and that L-arginine (as in our study) causes an increase in spermatozoon motility. The response of polyamines as result of L-arginine administration can be suggested as the mechanism of motility increases in this study. L-arginine is known to increase sperm motility due to ATP synthesis [8, 14, 16].

Sukardi et al. [23] 100, 200 and 300 mg/kg of different doses of L-arginine were administered for 4 weeks in rabbits. It has been suggested that 200 mg/kg L-arginine administration to rabbits increased NO concentration and this increase resulted in a 25.5% increase in spermatozoid and there was a positive relationship between NO concentration and L-arginine consumption ( $r=0.624$ ). It has been reported that NO concentration had a positive relationship with number of spermatozoid ( $r=0.584$ ) and negative relationship with motility ( $r=-0.775$ ). Oligospermia and asthenospermia rats [17] and humans [19] were reported to increase in number of spermatozoa following administration of L-arginine. Tanimura [24] showed that orally administrated L-arginine for 6-8 weeks in infertile people caused an increase in the number of spermatozoa. Ratnasooriya and Dharmasiri [17] reported that L-arginine, administered orally at different doses in rats, caused a significant increase in the number of epididymal spermatozoa.

In our study, it was found that the spermatozoon concentration obtained from the L-arginine administrated ram group increased compared to the concentration of spermatozoa from the control group rams, but it was found statistically significant at 3<sup>rd</sup>, 5<sup>th</sup>, 6<sup>th</sup> weeks between control and L-arginine groups. This increase was observed to be similar to the increase in spermatozoon concentration that other researchers have achieved. However, in our study, the application material is ram, the application method is parenteral (intramuscular), and in other studies, the difference is that the material is human or rat and the application route is oral. In this study, induction of L-arginine-induced polyamine synthesis and stimulation of spermatogenesis may be shown as a possible cause of increases in spermatozoid concentration by parenteral administration of L-arginine.

Ratnasooriya and Dharmasiri [17] showed that oral L-arginine doses at 100 and 200 mg/kg doses did not cause any changes in spermatozoid morphology of rats, whereas Schachter et al. [19] suggested that the lack of L-arginine in the diet caused anomalies in the morphological structure of spermatozoa. In our study, it was seen that the application of L-arginine reduced the rate of abnormal spermatozoa and the decrease in the 2<sup>nd</sup> week was significant. It has been seen that the results of the studies by other researcher are similar to our study results. Decreases in abnormal spermatozoid rates in the present study, although at a reduced level, may

be explained by normal formation of robust morphologically structured spermatozoa formed during spermatogenesis by promoting nucleoproteins in the spermatozoid core by exogenous L-arginine.

When sperm samples obtained from the result of L-arginine intramuscular injected rams were evaluated in terms of spermatozoa membrane integrity, L-arginine was found to increase and this increase was statistically significant ( $P < 0.05$ ) except 2 and 3 weeks in 7 week period. No previous work on this type of parameter has been found. However, L-arginine has been reported to have a protective effect against lipid peroxidation and to maintain membrane integrity by inhibition of NOS enzyme [22].

No significant difference was found in terms of pH values and arginase activity when we compared the sperm samples obtained from the control and L-arginine administrated rams in our study. Therefore, according to the results of this study, It has been suggested that L-arginine i.m. injections have no positive or negative effect on sperm pH and seminal plasma arginase activity.

In conclusion, weekly parenteral administration of 5 mg/kg L-arginine for 7 weeks decreased the erection time of rams, increased sperm mass activity, increased spermatozoid motility and density, lowered abnormal spermatozoid ratio and preserved spermatozoa membrane integrity, and did not affect seminal plasma arginase enzyme activity.

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