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Relationship between seminal plasma arginase activity and semen quality in Saanen bucks

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ABSTRACT

This study was conducted to determine the relationship between seminal plasma arginase activity and spermatological parameters in bucks. In this study, 5 ejaculates were collected by artificial vagina from each of 5 Saanen bucks of proven fertility. Spermatological parameters (semen volume, semen pH, mass sperm activity, sperm motility and concentration and percentage abnormal sperm) were evaluated immediately after collection in each ejaculate. After semen collection, samples were centrifuged and stored at −20 °C for analysis of the arginase activity. The mean level seminal plasma arginase activity recorded was 0.87 ± 0.12 units/mg protein. There existed a positive correlation between the seminal plasma arginase activity and sperm mass activity ($r = 0.548$, $p < 0.01$), sperm motility ($r = 0.408$, $p < 0.05$) and sperm concentration ($r = 0.793$, $p < 0.01$); However, a negative correlation was recorded between seminal plasma arginase activity and the percentage abnormal sperm ($r = -0.427$, $p < 0.05$). This study suggests that a significant correlation exists between seminal plasma arginase activity and certain spermatological parameters. Therefore, seminal plasma arginase activity may be used as a biochemical criterion to determine sperm quality in bucks.

1. Introduction

Arginase (EC 3.5.3.1) is an enzyme of the urea cycle that catalyzes the hydrolysis of L-arginine to urea and ornithine (Keskinege et al., 2001). At least two isoforms of arginase exist in mammals. Type I arginase (cytosolic form), is highly expressed in the liver and is thought to be primarily involved in ureagenesis. Type II arginase (mitochondrial form), is thought to be more widely expressed and to be involved in the biosynthesis of polyamines, the amino acids, ornithine, proline, and glutamate, and in the inflammatory process (Stephen et al., 2004).

The function of type II arginase very likely differs between the different types of tissue. Type II arginase may also play a role in regulating the synthesis of nitric oxide (NO) (Jenkinson et al., 1996). Polyamines are ubiquitous organic polycationnic substances, usually involved in cell replication and differentiation (Thomas and Thomas, 2001). Polyamines have been found in spermatozoa (Melendrez et al., 1992; Rubinstein and Breitbart, 1994), seminal plasma (Oefner et al., 1992) and the epididymis (Purvis and Egdetveit, 1993), as well as in other parts of the male reproductive tract (Calandra et al., 1996). In sperm, polyamines also seem to play a role in motility (Melendrez et al., 1992). Polyamines may also mediate the action of the androgens (Thomas and Thomas, 2001).

Arginase is an enzyme which synthesizes nitric oxide (NO), the principal mediator of penile erection (Bivalacqua et al., 2001; Wilson, 2003). The effect of nitric oxide on spermatogenesis and sperm function has been studied by

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Table 1
The mean (±SE), semen volume, semen pH, semen mass activity, sperm motility and concentration and percentage abnormal sperm, seminal plasma arginase activity in Saanen bucks.

Buck	Spermatological parameters						
	Semen volume (ml)	Semen pH	Semen mass activity (0–5)	Sperm concentration ($\times 10^9$/ml)	Sperm motility (%)	Percentage abnormal sperm (%)	Seminal plasma arginase activity (units/mg protein)
1	0.9 ± 0.1	7.1 ± 0.2	2.2 ± 0.6	1.5 ± 0.5	61.3 ± 8.6	9.8 ± 1.7	1.1 ± 0.3
2	0.8 ± 0.1	7.4 ± 0.2	3.6 ± 0.2	2.8 ± 0.5	82.0 ± 2.0	9.6 ± 1.7	0.9 ± 0.1
3	1.0 ± 0.1	7.4 ± 0.4	2.6 ± 0.5	1.9 ± 0.8	75.3 ± 4.6	8.5 ± 1.5	0.9 ± 0.4
4	0.9 ± 0.1	7.3 ± 0.5	1.7 ± 0.5	1.1 ± 0.4	56.0 ± 9.9	11.1 ± 1.8	0.7 ± 0.1
5	1.0 ± 0.0	7.9 ± 0.0	2.0 ± 0.3	0.9 ± 0.3	70.7 ± 4.3	11.9 ± 1.3	0.7 ± 0.1
Total mean	0.9 ± 0.0	7.4 ± 0.1	2.4 ± 0.2	1.6 ± 0.3	69.1 ± 3.3	10.2 ± 0.7	0.9 ± 0.1

Data are presented as mean ± SEM.

several research groups and nitric oxide was found to reduce or inhibit sperm motility (Weinberg et al., 1995; Herrero et al., 1996), while nitric oxide synthatese (NOS) inhibitor, which inhibits the formation of nitric oxide, was shown to prevent sperm motility decline – indicating a role for the arginase–nitric oxide pathway in the modulation of sperm motility (Perera et al., 1996).

It has further been reported that the arginase enzyme is found in the ram epididymal spermatozoa (Mendez and Martinez, 1995). In mammals, it is also found in the prostate, testis, seminal plasma, human sperm cells (Keskinege et al., 2001) and in different parts of the reproductive systems of bulls (Razmi et al., 2005) and rams (Razmi et al., 2004). Although arginase activity has usually been detected in different tissues, including the reproductive tract, no data relating to the activity of arginase in ejaculated buck semen has been reported. Therefore, this study was designed to determine seminal plasma arginase activity in ejaculated buck semen and to investigate the relationship between seminal plasma arginase activity and certain spermatological parameters in bucks.

2. Materials and methods

2.1. Animals

In this study, 5 fertility proven Saanen bucks, 2–4 years of age, with body weights of between 50 and 60 kg were used. The study was carried out in Elazığ province of Turkey, located at latitude of 38°40′N in the Centre of Education, Research and Application at the Faculty of Veterinary Medicine, at the Fırat University. The bucks were fed grass, supplemented with alfalfa hay. Drinking water was provided *ad libitum*.

2.2. Semen collection and evaluation

Semen was collected 5 times from each buck with the aid of an artificial vagina (5 samples per buck), at 2 days intervals. After collection of the semen, spermatological parameters were immediately determined in each ejaculate. Semen volume was measured by using graded test tubes and for the determination of mass activity, a drop of semen was placed on a warm slide (37 °C), under a light microscope with a heated stage at 100× magnification. The following criteria were used to assess mass sperm activity, (5) rapid dark swirls; (4) slower dark swirls and eddies; (3) slightly slower swirls; (2) no swirls, but prominent individual cell motion; (1) little individual sperm cell motion; and (0) no individual cell motion (Bozkurt et al., 2007).

The sperm motility rate was evaluated using a light microscope fitted with a heated stage (Bearden et al., 2004). A microscope slide was placed under the light microscope with the heated stage (37 °C), and then several droplets of Tris buffer solution (0.3 M Tris (hyroxymethyl) aminomethane, 0.027 M glucose, 0.1 M citric acid in 100 ml distilled water) pipetted onto the slide. A very small drop of semen was then added to the Tris buffer solution, and mixed with the aid of a cover-slip. The sperm motility rate was evaluated by rating the motile spermatozoa, to other spermatozoa with circular, reverse, vibrating and rocking movements. Sperm motility estimates were performed in three different microscopic fields in each sample, visually at 400× magnification. The mean of the three successive estimates was taken as the final motility score.

Sperm concentration was determined with the aid of the haemocytometric method, using the standard haemocytometer (Improved Neubauer, Deep 1/10 mm, Labart, Germany) slide and the dilution pipette designed for the counting of red blood cells (Bearden et al., 2004; Gür et al., 2005). The percentage of morphologically abnormal sperm was determined from slides prepared using Indian ink. A total of 300 spermatozoa were counted on each slide, under a phase contrast microscope at 400× magnification (Evans and Maxwell, 1987).

Finally, semen samples were centrifuged at 15,000 × *g* in a refrigerated centrifuge (+4 °C) for 13 min, and the seminal plasma that was obtained stored at −20 °C, until the plasma was analyzed for the enzyme arginase.

2.3. Enzyme assay

Seminal plasma arginase activity was spectrophotometrically determined using a modification of the Thiosemicarbazide–Diacetylmonoxime urea (TDMU) method, as described by Geyer and Dabich (1971). Measurements were performed in duplicate. Briefly, 0.1 ml seminal plasma was diluted with 1 mM $MnCl_2$ at the rate of 1:40 (v/v), and used as an enzyme source, by preincubating it for 12 min, at 55 °C. Tubes containing 0.3 ml of the enzyme source, 0.3 ml L-arginine (120 mM, pH 9.5) and 0.4 ml carbonate buffer (200 mM, pH 9.5) were incubated for 10 min at 37 °C. The reaction was stopped by adding 3 ml acid reagent to the tubes at the end of the incubation period. Thereafter 2 ml color reagent was added to the tubes which were kept in a boiling water bath for 10 min. The tubes were then removed from the boiling water bath, and cooled and the absorbance recorded at 520 nm. The protein concentration was determined using the method of Lowry et al. (1951), with bovine serum albumin as standard. Briefly, tubes containing 1 ml alkaline copper reagent and 0.1 ml supernatant samples were mixed, and incubated for 10 min at room temperature. After this, 4 ml folin and Ciocalteu's phenol reagent were added to the tubes, and mixed and incubated for 5 min, at 55 °C. The absorbance of the samples was recorded at 650 nm, using a Shidmadzu UV 240 spectrophotometer.

The principle of this arginase activity determination relies on the spectrophotometric measurement of urea, produced by the hydrolysis of L-arginine, by arginase. One unit of arginase activity was expressed as the amount of enzyme catalyzing the formation of 1 μmol of urea/h, at 37 °C. The results were then expressed as units/mg of protein (specific activity).

2.4. Statistical analyses

All data are presented as the mean ± S.E.M. The Pearson's correlation test was applied to determine the relationship between the seminal plasma arginase activity and the spermatological parameters. To determine differences between rams with respect to spermatological parameters and seminal plasma arginase activity, a one-way analysis of variance and post hoc Tukey-HSD test was used. All data were analyzed using the SPSS software package program (Windows 10.0).

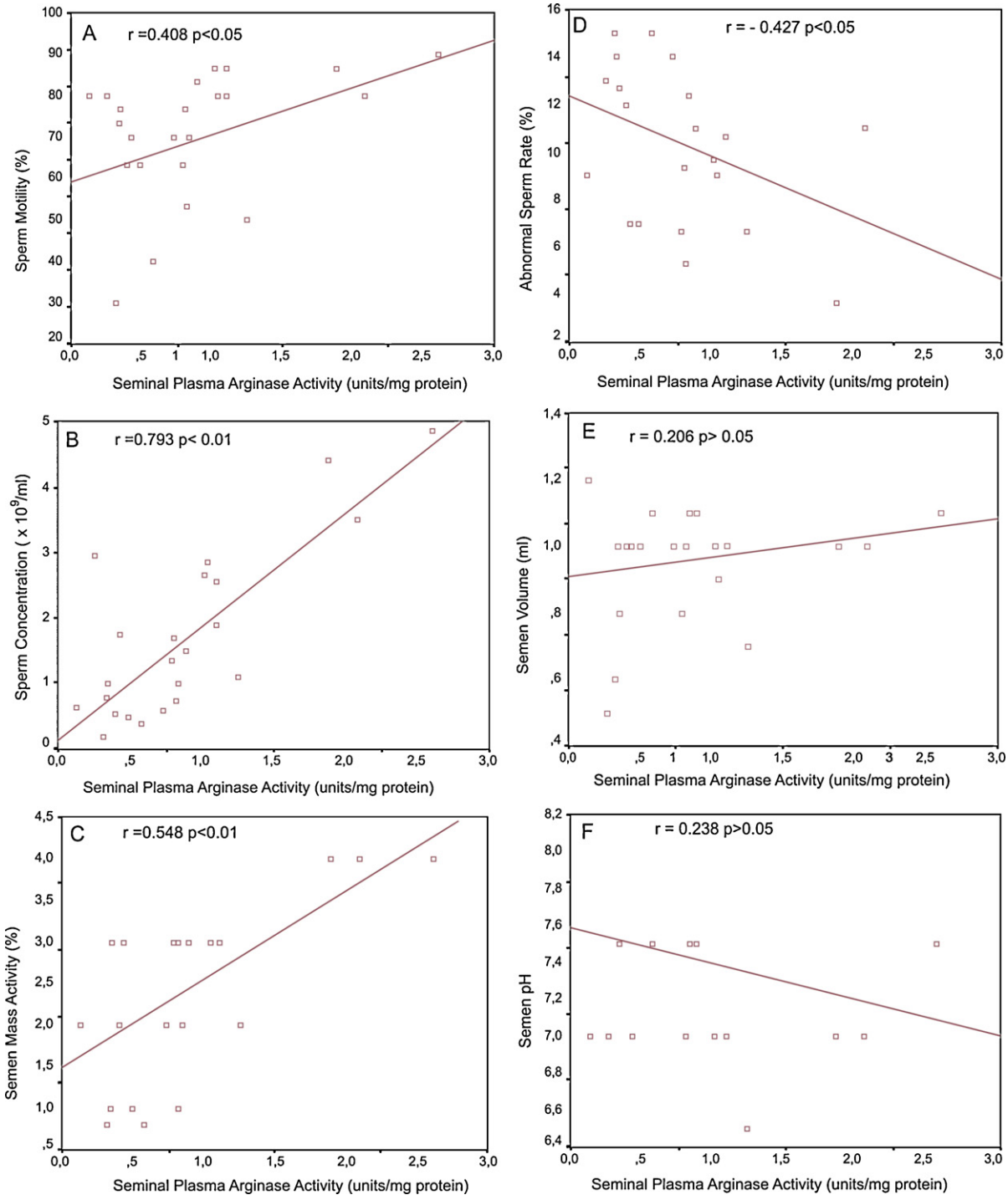

Fig. 1. The correlation between seminal plasma arginase activity and (A) sperm motility (B) sperm concentration, (C) semen mass activity, (D) percentage abnormal sperm (E) semen volume and (F) semen pH in Saanen bucks.

3. Results

The spermatological parameters and seminal plasma arginase activity in the Saanen bucks are set out in Table 1. The correlation between the spermatological parameters and seminal plasma arginase activity in the Saanen bucks are also illustrated in Fig. 1. A positive correlation between seminal plasma arginase activity and semen mass activity ($r = 0.548$, $p < 0.01$); sperm motility ($r = 0.408$, $p < 0.05$) and sperm concentration ($r = 0.793$, $p < 0.01$) was recorded. However, a negative correlation between seminal plasma arginase activity and percentage abnormal

sperm ($r = -0.427$, $p < 0.05$) was recorded. Additionally, no significant correlation between the seminal plasma arginase activity and semen volume ($r = 0.206$) and semen pH ($r = -0.238$) respectively, was found.

No significant differences were recorded between different ejaculates of each Saanen buck and between bucks, with respect to the semen parameters (semen volume, semen pH, semen mass activity, sperm concentration and motility, percentage abnormal sperm) and seminal plasma arginase activity.

4. Discussion

There are relatively few reports regarding the role of arginase in reproduction or fertility. It has been reported that the arginase enzyme is found in ram epididymal spermatozoa (Mendez and Martinez, 1995), prostate and other parts of the reproductive system of rams (Razmi et al., 2004). In this study, the mean seminal plasma arginase activity recorded was 0.87 ± 0.12 units/mg protein. This arginase activity in rams was higher than in cat testicular specific activity (0.012 U/mg protein) (Mahmoud et al., 2007), normal men (0.016 ± 0.005 U/mg protein) and men with oligospermia (0.029 ± 0.009 U/mg protein) (Elgun et al., 2000). A positive correlation was reported between sperm motility and arginase activity in both the seminal plasma and spermatozoa (Elgun et al., 2000). Similarly, Eskiocak et al. (2006) reported a positive correlation between seminal plasma arginase activity and rapid progressive sperm motility. In the current study, a positive correlation existed between the seminal plasma arginase activity and sperm motility. The main role of arginase in the testes, is however said to be the regulation of nitric oxide (NO) concentration (Nathan, 1997). Low concentrations of NO have been shown to enhance sperm motility (Herrero et al., 2001), while the overproduction results in the impairment of spermatogenesis in the gonads (Taneli et al., 2005). In vitro studies have also shown that low concentrations of NO enhance the motility of mouse and human sperm, while high concentrations decrease sperm motility (Rosselli et al., 1998; Herrero et al., 2003). Increased arginase activity generally results in a lower NO concentration and, thereafter subsequently leads to increased sperm motility (Elgun et al., 2000). Furthermore, Mendez and Haro (2006) have reported that arginase in ram spermatozoa is positively correlated with the degree of maturation of the sperm cell, and arginase could possibly be used by spermatozoa in promoting motility and preventing a premature acrosome reaction.

A negative correlation was recorded between the sperm count and arginase activity, which also indicated that increases in cell counts were accompanied by decreases in arginase activity in oligospermic men (Elgun et al., 2000). Seminal plasma arginase activity was positively correlated with sperm concentration (Eskiocak et al., 2006). Arginine content is then also generally high in the normal sperm nucleoprotein (Yılmaz, 2005) and arginase is an arginine-depleting enzyme that catalyzes the hydrolysis of L-arginine to urea and ornitine (Kandemir and Özdemir, 2009). Ornitine may then support the synthesis of polyamines such as spermidine, spermine and putrescine in the seminal fluid (Kocna et al., 1996). These polyamines play an important role in cell growth, cell proliferation and differentiation (Razmi et al., 2005). The results of this present study thus indicated that a positive correlation exists between seminal plasma arginase activity and the sperm concentration. The reason for the positive correlation may then be attributed to the hypothesis mentioned above.

Semen mass activity is generally an indicator for predetermining sperm motility and concentration. Therefore, there generally exists a positive correlation between semen mass activity and sperm motility and between mass activity and sperm concentration (Bearden et al., 2004). In the present study, it was also found that a positive correlation exists between seminal plasma arginase activity and mass activity. This could be attributed to the positive correlations found between sperm motility and arginase activity and between sperm concentration and semen mass activity.

In this study, it was also observed that a negative correlation was recorded between seminal plasma arginase activity and the percentage abnormal sperm. The reason for this correlation is unknown. However, arginase activity regulates the NO concentration (Nathan, 1997), which decreases as a result of increased arginase activity (Elgun et al., 2000). Aksoy et al. (2000) also reported a significant positive correlation to exist between the nitric oxide concentration in the seminal plasma and the occurrence of abnormal sperm. Therefore, if nitric oxide concentration in seminal plasma decreases, arginase activity in the seminal plasma increases and this increase may be the reason for the decrease in the proportion of abnormal sperm.

5. Conclusion

In conclusion it can be said that this study clearly suggests that a correlation exists between seminal plasma arginase activity and certain semen parameters in bucks. Therefore, seminal plasma arginase activity may be a biochemical criterion also to be used to determine sperm quality in bucks.

References

Aksoy, H., Aksoy, Y., Özbey, I., Altuntaş, I., Akçay, F., 2000. The relationship between variococele and semen nitric oxide concentrations. Urol. Res. 28, 357–359.

Bearden, H.J., Fuquay, J.W., Willard, S.T., 2004. Applied Animal Reproduction. Prentice-Hall International, London, UK.

Bivalacqua, T.J., Hellstrom, W.J., Kadowttz, P.J., Champion, H.C., 2001. Increased expression of arginase II in human diabetic corpua cavernosum: in diabetic-associated erectile dysfunction. Biochem. Biophys. Res. Commun. 283, 923–927.

Bozkurt, T., Turk, G., Gur, S., 2007. Effects of exogenous oxytocin on serologic and seminal steroids and semen characteristics in rams. Turk. J. Vet. Anim. Sci. 31 (5), 303–309.

Calandra, R.S., Rulli, S.B., Frungieri, M.O., Suescun, C., Gonzalez-Calvar, S.I., 1996. Polyamines in the male reproductive system. Acta Physiol. Pharmacol. Ther. Latinoam. 46, 209–222.

Elgun, S., Kaçmaz, M., Şen, I., Durak, I., 2000. Seminal arginase activity in infertility. Urol. Res. 28, 20–23.

Eskiocak, S., Gozen, A.S., Taskiran, A., Kilic, A.S., Eskiocak, M., Gulen, S., 2006. Effect of psychological stress on the L-arginine–nitric oxide pathway and semen quality. Braz. J. Med. Biol. Res. 39, 581–588.

Evans, G., Maxwell, W.M.C., 1987. Handling and examination of semen. In: Maxwell, W.M.C. (Ed.), Salamon's Artificial Insemination of Sheep and Goats. Butterworths, Sydney, pp. 93–106.

Geyer, J.W., Dabich, D., 1971. Rapid method for determination of arginase activity in tissue homogenates. Anal. Biochem. 39, 412–417.
Gür, S., Bozkurt, T., Türk, G., 2005. Short term effects of dexamethasone on hyaluronidase activity and sperm characteristics in rams. Anim. Reprod. Sci. 90, 255–263.
Herrero, M.B., Martinez, S.P., Viggiano, J.M., Polak, J.M., de Gimeno, M.F., 1996. Localization by indirect immunofluorescence of nitric oxide synthase in mouse and human spermatozoa. Reprod. Fertil. Dev. 8, 931.
Herrero, M.B., de Lamirande, E., Gagnon, C., 2001. Tyrosine nitration in human spermatozoa: a physiological function of peroxynitrite, the reaction product of nitric oxide and superoxide. Mol. Hum. Reprod. 7, 913–921.
Herrero, M.B., de Lamirande, E., Gagnon, C., 2003. Nitric oxide is a signaling molecule in spermatozoa. Curr. Pharm. Des. 9, 419–425.
Jenkinson, C.P., Grody, W.W., Cederbaum, S.D., 1996. Comparative properties of arginases. Comp. Biochem. Physiol. 114B, 107–132.
Kandemir, F.M., Özdemir, N., 2009. Some kinetic properties of arginase in sheep spleen tissue. J. Fac. Vet. Med. Unv. Kafkas. 15 (4), 553–559.
Keskinege, A., Elgun, S., Yilmaz, E., 2001. Possible implications of arginase and diamine oxidase in prostatic carcinoma. Cancer Detect. Prev. 25, 76–79.
Kocna, P., Fric, P., Zavoral, M., Pelech, T., 1996. Arginase activity determination. A marker of large bowel mucosal proliferation. Eur. J. Clin. Biochem. 34, 619–623.
Lowry, O.H., Rosenbroug, N.J., Farr, A., Randall, L.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.
Mahmoud, A., Homayoon, R.S., Azadeh, E., 2007. Distribution of arginase in tissues of cat (Felis catus). J. Feline Med. Surg. 9, 133–139.
Melendrez, C.S., Ruttle, J.L., Hallford, D.M., Chaudhry, P.S., Casillas, E.R., 1992. Polyamines in ejaculated ram spermatozoa and their relationship with sperm motility. J. Androl. 13, 293–296.
Mendez, J.D., Martinez, I., 1995. Arginase activity in ram epididymal/ejaculated spermatozoa. Arta 7, 131–136.
Mendez, J.D., Haro, R.D., 2006. Arginase distribution in ram spermatozoa during epididymal maturation. World Appl. Sci. J. 1 (2), 107–114.
Nathan, C., 1997. Inducible nitric oxide synthase: what differences does it make? J. Clin. Invest. 100, 2417–2423.
Oefner, P.J., Wongyai, S., Bonn, G., 1992. High-performance liquid chromatographic determination of free polyamines in human seminal plasma. Clin. Chim. Acta 205, 11–18.
Perera, D., Katz, M., Heenbanda, S.R., Marchant, S., 1996. Nitric oxide synthase inhibitor NG-monomethyl-L-arginine preserves sperm motility after swim-up. Fertil. Steril. 66, 830.
Purvis, K., Egdetveit, I., 1993. Segmental distribution of alpha-glucosidase, ornithine decarboxylase and polyamines in the human epididymis. J. Reprod. Fertil. 97, 575–580.
Razmi, N., Jelodar, G.A., Nazifi, S., Dehghani, A., 2004. Arginase status ram reproductive system. J. Appl. Anim. Res. 26, 57–59.
Razmi, N., Jelodar, G.A., Nazifi, S., Dehghani, A., 2005. Arginase status cattle reproductive system. Vet. Arhiv. 75, 31–38.
Rosselli, M., Keller, R.J., Dubey, R.K., 1998. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Hum. Reprod. Update 4, 3–24.
Rubinstein, S., Breitbart, H., 1994. Cellular localization of polyamines: cytochemical and ultrastructural methods providing new clues to polyamine function in ram spermatozoa. Biol. Cell 81, 177–183.
Stephen, D.C., Hong, Y., Wayne, W.G., Rita, M.K., Paul, Y., Ramaswamy, K.I., 2004. Arginases I and II: do their functions overlap? Mol. Gen. Metab. 81, 38–44.
Taneli, F., Vatansever, S., Ulman, C., Yilmaz, O., Giray, G., Genc, A., Taneli, C., 2005. The effect of spermatic vessel ligation on testicular nitric oxide levels and germ cell specific apoptosis in rat testis. Acta Histochem. 106, 459–466.
Thomas, T., Thomas, T.J., 2001. Polyamines in cell growth and cell death: molecular mechanisms and therapeutic applications. Cell. Mol. Life Sci. 58, 244–258.
Weinberg, J.B., Doty, E., Bonaventura, J., Haney, A.F., 1995. Nitric oxide inhibition of human sperm motility. Fertil. Steril. 64, 408.
Wilson, L., 2003. Arginase inhibitors touted as potential drug target for sexual dysfunction. Biochemistry 81, 9.
Yılmaz, M., 2005. Arginine metabolism. Adnan Menderes University, Faculty of Medicine (ADUFT) Department of Biochemistry. Lecture Notes Aydın, Turkey, p. 13.