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Seminal arginase activity in infertility

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Abstract Arginase (Arg) activity in seminal plasma and sperm cells from infertile men and healthy fertile donors was measured. There were no statistically meaningful differences in seminal plasma Arg activity between the two groups whereas sperm cells from oligospermic infertile men had a higher Arg activity compared with the controls. Some important correlations were established between sperm count and Arg activity (negative values) and sperm motility and Arg activity (positive values) in both sperm cells and plasma samples from infertile men. Results suggest that the arginine-nitric oxide pathway within sperm cells from oligospermic infertile men is disturbed by enhanced Arg activity. We think that this may play a part in sperm dysfunction and male infertility.

Key words Arginase · Seminal plasma · Sperm cell · Infertility

Introduction

Arginase (Arg) (EC 3.5.3.1) is an enzyme of the urea cycle catalyzing the hydrolysis of L-arginine to urea and ornithine in liver. Extrahepatic Arg may play a role in reactions other than those of the urea cycle. In mammalians, it is also found in prostate [3, 21] testis [13], epididymis [17], seminal plasma vesicles [18] and human sperm cells [15].

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Since Arg is an arginine-depleting enzyme it is an important part of the cellular arginine regulatory system affecting nitric oxide synthase (NOS) activity [12, 21]. NOS catalyzes the synthesis of nitric oxide (NO) from L-arginine. Nitric oxide synthase activity has been found in several mammalian reproductive tissues including testis and epididymis [26] and it has been suggested that a possible role for NO could be the modulation of spermatogenesis, sperm motility and maturation [16].

NO has also been shown to possess an ability to inactivate superoxide anion [11], which causes lipid peroxidative damage to sperm membrane lipids, leading to impaired sperm function [1, 4, 6, 24]. However, some researchers claim that hydrogen peroxide or superoxide anion can promote the capacitation process in human spermatozoa [7, 8, 19] in a dose-dependent manner while excess NO causes oxidation of sperm membrane lipids resulting in sperm dysfunction and toxicity [19, 25, 20]. On the other hand, it has been shown that NO reduced sperm motility [22] and thus, NOS inhibition might have potential protective effects on motile sperm [16].

There are relatively few reports about the role of Arg in reproduction or fertility. However, it has been shown that arginine content is low in abnormal sperm suggesting that this might result from an increased Arg activity [15].

As can be seen, there are limited and conflicting data concerning the involvement of NO in sperm motility and function. Besides, there are no recent reports about the role of Arg in sperm function and fertility. Therefore, the present study was devised to measure Arg activity in seminal plasma and spermatozoa from infertile men, especially to evaluate the role of the arginine-NO pathway in infertility through Arg activity.

Materials and methods

Human semen samples were obtained from 12 volunteer fertile male donors (mean age ± SD = 29.13 ± 4.43 years) and from 18 infertile men with oligospermia (mean age ± SD = 31.56 ± 5.67 years) who were admitted to the Fertility Section of Urology

Department of Gazi University, Medical Faculty. Samples were collected by masturbation following abstinence for at least 48 h. After liquefaction, samples were prepared for routine analyses according to WHO guidelines [23]. Seminal plasma and sperm cells were prepared as described previously [9]. Arg activity was measured according to the spectrophotometric method described by Chinard [2]. One unit of Arg activity was defined as one micromole of liberated ornithine per minute at 37 °C. Protein content of the samples was measured according to the method of Lowry et al. [10]. Arg activity was expressed as specific activity in U/mg protein.

Statistical analyses of data were performed using Student's *t*-test and Pearson correlation analysis. For the evaluation of the Arg activity assay, within and between batch analyses and linearity tests were performed.

Results

There were no statistically meaningful differences between seminal plasma Arg activities of patients and controls (Table 1). However, Arg activity was higher in sperm cells from infertile males compared with those from fertile men. Scatterplots displaying Arg activity in controls and patients are given in Figs. 1 and 2. Sperm motility and sperm count values are significantly higher in the control group compared with the infertile group (Table 2). Correlation analyses revealed inverse correlations between sperm count and Arg activity in the infertile group in both plasma ($r = -0.23$, ns) and sperm cells ($r = -0.53$, $P < 0.05$) which indicate that increases in cell count are accompanied by decreases in Arg activity. Positive correlations were established between sperm motility and Arg activity in the infertile group in both plasma ($r = 0.34$, ns) and sperm cells ($r = 0.56$, $P < 0.05$) which indicate that Arg activity increases in parallel with sperm motility.

Table 1 Arginase acitivity (U/mg protein) in seminal plasma and cells from fertile and infertile men. Student's *t*-test (*P ns* and <0.0005). *ns* Not significant ($P > 0.05$)

Groups	Plasma Arg	Cell Arg
Controls ($n = 12$)	0.020 ± 0.005	0.016 ± 0.005
Oligospermia ($n = 18$)	0.021 ± 0.008	0.029 ± 0.009

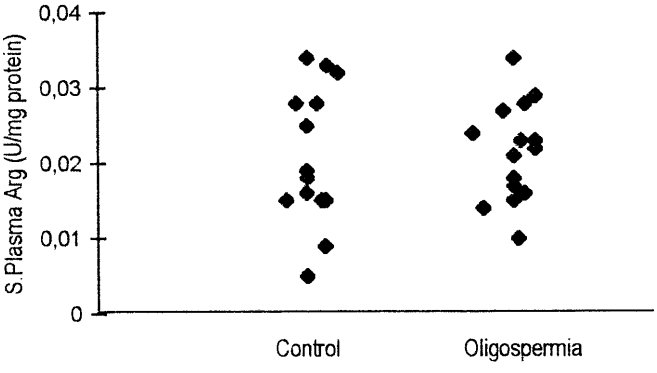

Fig. 1 Distribution of seminal plasma arginase activities (U/mg protein) in the control and oligospermia groups

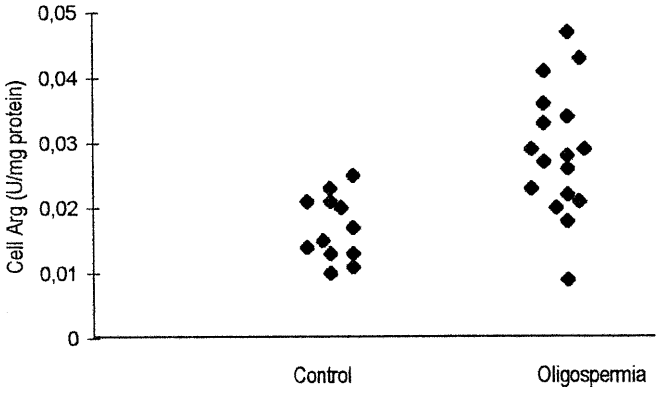

Fig. 2 Distribution of sperm cell arginase activities (U/mg protein) in the control and oligospermia groups

Within and between batch analyses results (Table 3) show that our Arg activity method is quite reliable.

Discussion

The effect of NO on spermatogenesis and sperm function has been studied by several research groups. NO was found to reduce or inhibit sperm motility [5, 22] while NOS inhibitor, which inhibits the formation of NO, was shown to prevent sperm motility decline indicating a role for the Arg-NO pathway in modulation of sperm motility and motility survival [16]. It was also indicated that a low concentration of NO may have some physiological role in fertilization through the enhancement of capacitation and zona pellucida binding [19]. However, excessive NO, reacting with superoxide or hydrogen peroxide and forming peroxynitrite, hydroxyl radical, NO_2 or singlet oxygen can cause oxidation of sperm membrane lipids resulting in sperm dysfunction and toxicity [19, 20, 25]. In contrast, owing to its ability to inactivate superoxide anion [11], NO was found to be beneficial to sperm viability and motility in both fertile and infertile

Table 2 Mean ± SD values of sperm count (million/milliliter) and motility (percent) of controls and infertile men. Student's *t*-test ($P < 0.0005$ and <0.0005)

Groups	Count	Motility
Controls ($n = 12$)	40.45 ± 8.30	60.3 ± 12.23
Oligospermia ($n = 18$)	5.29 ± 2.14	33.62 ± 6.42

Table 3 Within and between batch analyses of results show that our Arg activity method is quite reliable. Linearity was also very good ($r = 0.99$ and $Y = 0.24X + 0.013$, where Y = Arg activity, IU/ml and X = sample amount in milliliters)

CV%	Plasma	Sperm cell
Within batch analysis	5.1	7.2
Between batch analysis	5.4	5.9

individuals by a mechanism involving the reduction of lipid peroxidative damage to sperm membranes [4, 24].

We observed that sperm cells from infertile men with oligospermia had a significantly higher Arg activity than the controls while there was no difference between seminal plasma samples of fertile and infertile men. This finding was in accordance with a previous study suggesting that a low arginine content in pathological sperm from infertile males might be due to increased Arg activity not the precursor metabolism. This meant that the arginine supply was not impaired [15]. Negative correlations established between sperm count and Arg activity in the oligospermic group also indicated that increases in cell count were accompanied by decreases in Arg activity. This was in a good agreement with higher Arg activity measured in the oligospermic group compared to healthy controls. Since Arg is an important part of the cellular arginine-regulating system affecting NO generation and arginase-induced depletion of arginine may lead to inhibition of NO synthesis [3, 12, 21], our findings suggest a possible involvement of increased Arg activity and NO metabolism in infertility. The results of this study may indicate that arginine content is lowered in sperm cells of infertile men due to enhanced Arg activity, which leads to diminished production of NO. The supposed decrease in NO synthesis due to as yet unknown reason(s) in the oligospermic group may be responsible for the impairment of spermatogenesis, sperm viability and motility. Positive correlations established between sperm motility and Arg activity values demonstrate that increased Arg activity is in accordance with increased sperm motility. This is also in good agreement with a previous evaluation that NO reduces or inhibits sperm motility [5, 22] while NOS inhibitors prevent sperm motility decline [16].

Furthermore, no significant difference was found between infertile and fertile seminal plasma Arg activity, while sperm Arg activity was higher in infertile men with oligospermia as mentioned above. Although the cellular source of NO in semen is not clear yet, the presence of neuronal NOS has been demonstrated in spermatozoa [5]. So, it is likely that the arginine-NO pathway including Arg and NOS, is involved in sperm viability and motility, but not in the plasma fraction. This probably indicates that local environmental conditions for spermatozoa provided by the seminal plasma, are suitable for motility and action at least regarding Arg activity. It may also indicate that the pathway is disturbed within the sperm cells but not in the surrounding extracellular medium.

In the light of these evaluations, we suggest that the arginine-NO pathway together with Arg and NOS, may be involved in male infertility. If this is really the case, then Arg inhibitors might be beneficial to infertile men. We think that further studies using a larger series should be carried out to clarify the mechanisms underlying the disease process.

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