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Research Article

Correlation of Uric Acid, Urea, Ammonia and Creatinine of Seminal Plasma With Semen Parameters and Fertilization Rate in Infertile Couples

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Abstract

Background: Ammonia, urea, uric acid, and creatinine are the major non-protein nitrogenous compounds (NPNs). It is reported that the concentration of NPNs in the seminal plasma of normal and infertile individuals is different and sperm is affected by NPNs.

Objectives: The aim of this study was to determine the quantities of ammonia, urea, uric acid, and creatinine in seminal plasma and the correlation of these compounds with the fertilization rate after an intracytoplasmic sperm injection (ICSI).

Methods: The levels of ammonia, urea, uric acid, and creatinine were determined in seminal plasma collected from 50 men. The ammonia and urea were determined by L-glutamate dehydrogenase and diacetyl monoxime method, respectively. Uric acid and creatinine were detected by enzymatic method and Jaffe reaction, respectively. The fertilization was evaluated around 16–18 hours post-ICSI on the appearance of 2 pronuclei and 2 polar bodies. The fertilization rate was calculated by the number of fertilized oocytes per the number of oocytes injected.

Results: There was a significant negative correlation between seminal ammonia and sperm motility ($P<0.05$). Urea and creatinine had a negative correlation with respect to the sperm count ($P<0.05$). In addition, there was an inverse correlation between urea and uric acid of seminal plasma and sperm morphology ($P<0.05$). A significant negative correlation was found between seminal uric acid and urea with respect to the percentage of the fertilization rate ($P<0.05$).

Conclusions: The findings of the present study showed that urea and uric acid in seminal plasma have a negative impact on the fertilization rate.

Keywords: Ammonia, Fertilization, Non-protein nitrogenous compounds, Semen, Urea, Uric acid

Background

The catabolism of proteins and nucleic acids results in the formation of non-protein nitrogenous compounds (NPNs) (1). Ammonia, urea, uric acid, and creatinine are NPNs (1,2). NPNs are metabolic products originating from biochemical pathways. They have a significant clinical utility in various diseases and organ functions. Urea is the major nitrogen-containing metabolic product of protein catabolism (1). Uric acid, on the other hand, is a major compound of purine nucleosides catabolism (3). Adenosine and guanosine are 2 purine nucleosides that have an essential role in the DNA structure. Their catabolism results in the formation of uric acid (3). Ammonia is associated with amino acid metabolism. The catabolism of various amino acids is associated with ammonia production (2).

NPNs exist in various biological fluids of our body and our body cells are in contact with it. Sperm is affected by some biochemical compounds found in seminal plasma (4). Seminal plasma consists of many biochemical ingredients, such as proteins, enzymes, different cations, urea, creatinine, uric acid, ammonia, and other compounds (5,6). Urea, ammonia, uric acid, and creatinine are reported to have been found in seminal plasma (7). The source and function of NPN compounds are not clear. They may have originated from local production in the male reproductive tract (8) and transudation from blood circulation (9). The relation between some NPN compounds and semen parameters have been shown (10). It is reported that the concentration of uric acid in the seminal plasma of normal and infertile individuals is different (7,11). In addition, the direct correlation

between seminal uric acid and semen parameters has been revealed (11).

According to the World Health Organization (WHO), infertility affects about 15% of couples around the world (12). The intracytoplasmic sperm injection (ICSI) is an efficient treatment for infertility (13). Classical assessments for semen analysis, which focus on sperm count, motility, morphology, and percentage of viable cells, did not provide enough information for predicting the fertility outcome (14), and some men with normal semen features would be classified as infertile (15).

Analysing the seminal plasma ingredients may be helpful to evaluate male infertility and predict the assisted reproductive technology (ART) outcome. We hypothesize that the NPNs of seminal plasma may have an impact on the result of this technique. Most previous works only focused on sperm for evaluating infertility and predicting the ART outcome. The NPN metabolites of seminal plasma have not been studied extensively.

Objectives

The aim of this study was to determine the amount of ammonia, urea, uric acid, and creatinine in seminal plasma and the correlation of these compounds with the fertilization rate after ICSI.

Materials and Methods

The study was performed at Infertility Unit and Endometrium and Endometriosis Research Center of Fatemyeh hospital, affiliated to Hamadan University of Medical Sciences, Hamadan, Iran. All patients were from Hamadan province. Hamadan province is a cold and mountainous region that is located in the west of Iran. From April 2013 to August 2013, 50 infertile couples who were candidate for ICSI were accepted in Fatemyeh hospital and enrolled in this study. Infertile couples had no children after 1 year (or more) of unprotected sexual intercourse. The study plan was explained to all the couples. Written consent for using the semen for investigation was obtained from all the patients. This study was approved by the Ethics Committee of Hamadan University of Medical Sciences. For male individuals, inclusion criteria were unexplained infertility. The mean duration of infertility of infertile couples was 4.4 years. The age range of men was 25–40 years. Infertile men with certain infertility causes (e.g. abnormal karyotype, infectious diseases, and varicocele, cigarette smoking, or chronic diseases) were excluded from our study. Women were excluded from the study if one of them had disorders like endometriosis, diabetes, polycystic ovary syndrome, breast cancer, cigarette smoking, or chronic diseases. In addition, women with less than 3 mature oocytes after ovarian stimulation were excluded from study. The age range of women was 23–35 years.

Primary outcome of the present study was relationship of NPN compounds of seminal plasma with outcome of ICSI. In addition, for secondary outcome, we determined the correlation of NPN compounds with semen parameters from infertile males.

Semen samples were obtained by masturbation after at least 72 hours of sexual abstinence on the day of oocyte retrieval. After approximately 30 minutes of liquefaction at room temperature, the specimens were evaluated for semen volume, appearance, pH, and viscosity. Semen parameters, such as sperm count, motility and viability were assessed according to the WHO criteria for semen analysis (16). The morphology of the sperm was determined after Diff-Quik staining. We used the Eosin staining method to assess sperm viability (16).

Levels of NPN in seminal plasma were measured using laboratory kits (Pars Azmun Company, Iran). The ammonia was determined by L-glutamate dehydrogenase with α-ketoglutaric acid and reduced nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. Urea measurement was done by diacetyl monoxime method. Uric acid and creatinine were determined by enzymatic method (uricase) and Jaffe reaction, respectively.

Ovarian Stimulation and Oocyte Retrieval

All patients carried out controlled ovarian hyperstimulation (COH) with recombinant FSH in accordance with established GnRH agonist or antagonist protocol. After that at least 3 follicles with 18 mm diameter were detected, a 10000 IU of human chorionic gonadotropin (hCG) (Choriomon, IBSA, Lugano, Switzerland) was administered intramuscularly. Then, 34–38 hours after the hCG injection, under ultrasound guidance, follicles that were larger than 15 mm in diameter were aspirated with a 17-gauge Cook needle and oocytes were retrieved. The cumulus cells of the cumulus oocyte complexes were removed from the oocytes by gently pipetting with 0.1% hyaluronidase. Then, the ICSI was carried out. The fertilization was evaluated around 16–18 hours post-ICSI on the appearance of 2 pronuclei and 2 polar bodies. We calculated the fertilization rate by the number of fertilized oocytes per the number of oocytes injected.

Statistical Analysis

Data were analyzed using SPSS software version 16.0 and the results were presented as mean ± standard deviation (SD), median, and minimum-maximum levels. Using Kolmogorov–Smirnov test, the distribution of sperm motility, morphology, viability and count, were not normal, and fertilization rate, uric acid, urea, creatinine and ammonia had normal distribution. For correlation assessment, Pearson coefficient (r) and Spearman

coefficient (r) were used for variables with normal and non-normal distribution, respectively. Statistical significance was set at $P<0.05$.

Results

A number of 50 couples were screened for this study. All patients were Iranian, and men and women were in the age ranges of 25–40 and 23–35 years, respectively. Table 1 represents basic semen parameters and the fertilization rate. The seminal plasma concentration of ammonia, urea, uric acid, and creatinine are shown in Table 2. The relationship between NPN compounds and sperm parameters is given in Table 3. There was a significant negative correlation between seminal ammonia and sperm motility ($P<0.05$). Urea and creatinine had negative correlations with the sperm count ($P<0.05$). In addition, there was an inverse correlation between urea and uric acid of the seminal plasma and the sperm morphology ($P<0.05$).

The relationship between the percentage of the fertilization rate and NPN compounds is shown in Table 3. A significant negative correlation of seminal uric acid and urea with the percentage of the fertilization rate of the oocyte was found ($P<0.05$), but the correlation between ammonia and creatinine was not significant. The correlation between uric acid concentration of seminal plasma and fertilization rate of oocyte after ICSI is presented in Figure 1.

Discussion

The current study tried to determine the level of NPN compounds in the seminal plasma of infertile males. The infertile males and their partners were candidates for ICSI. We focused on ammonia, urea, uric acid, and creatinine as important NPN compounds in seminal plasma and the correlation of these compounds with semen parameters. In addition, the fertilization rate after ICSI was investigated. The results of the present study showed a significant negative correlation of seminal uric acid and urea with the fertilization rate of the oocyte. However, the correlations of ammonia and creatinine of seminal plasma with the fertilization rate of the oocyte were not significant.

The level of NPN in seminal plasma detected in the present study is in agreement with the reported findings (5,7). The results showed that seminal plasma ammonia was correlated with sperm motility. This correlation was inverse and with the increase of ammonia in seminal plasma, sperm motility decreased. Ammonia in seminal plasma can be produced by the oxidative deamination of various amino acids, spermidine, and spermine by spermatozoa (17). Kim and Kim (10) reported that when semen mixed with urine was supplemented with ammonia, sperm motility was decreased. They used various concentrations of ammonia and showed that even a low ammonia level can affect sperm motility. Other NPN compounds including creatinine, urea, and uric acid had no effect on sperm motility, which is in agreement with the findings of the current study. It seems that an increased level of ammonia in seminal plasma is toxic for spermatozoa and the decrease of motility was due to this toxic effect.

In this study, the urea content of seminal plasma showed a negative correlation with sperm count, sperm morphology, and fertilization rate. Urea is the main nitrogen-containing metabolic product of protein catabolism in humans. Urea synthesis is carried out by hepatic enzymes of the urea cycle (1). Urea originates in semen from blood circulation (9). The rate of urea synthesis not only depends on endogenous protein

Table 1. Basic Semen Parameters Including Sperm Motility, Count, Morphology, Viability of Infertile Males and Percentage of Fertilization Rate of Oocyte After an ICSI (n = 50)

	Sperm Viability (%)	Sperm Normal Morphology (%)	Sperm Motility (%)	Sperm Count (10^6/Ml)	Fertilization Rate (%) [a]
Mean ± SD	83.2±1.4	78.8±5.9	45±5.9	49.1±18	59±19
Median	85	80	45	50	60
Minimum-Maximum levels	40-95	40-95	25-65	10-90	20-100

Abbreviation: intracytoplasmic sperm injection.
Results are presented as mean ± standard deviation (SD), median and minimum-maximum levels.
[a] Appearance of 2 pronuclei and 2 polar bodies and calculated by the number of fertilized oocytes per the number of oocytes injected.

Table 2. Levels of Ammonia, Urea, Uric Acid and Creatinine in Seminal Plasma of Infertile Males (n = 50)

Parameters	Ammonia (μM)	Urea (mg/dL)	Uric acid (mg/dL)	Creatinine (mg/dL)
Mean ± SD	91.2±24.7	46.5±24.4	3.8±1.5	3.58±1.13
Median	85	35.6	3.3	3.59
Minimum-Maximum levels	58-155	17.6-88.9	1.6-6.8	1.6-6

Results are presented as mean ± standard deviation (SD), median and minimum-maximum levels.

Table 3. Correlation of Seminal Plasma Ammonia, Urea, Uric Acid and Creatinine With Sperm Parameters of Infertile Males and Percentage of Fertilization Rate After an ICSI (n = 50)

Parameters	Ammonia (μM)	Urea (mg/dL)	Uric Acid (mg/dL)	Creatinine (mg/dL)
Sperm count (10^6/mL)	NS	r=-0.283 [a] *P*=0.046	NS	r=-0.282 [a] *P*=0.048
Sperm motility (%)	r=-0.318 [a] *P*=0.024	NS	NS	NS
Sperm normal morphology (%)	NS	r=-0.330 [a] *P*=0.019	r=-0.351 [a] *P*=0.012	NS
Sperm viability (%)	NS	NS	NS	NS
Fertilization rate (%) [c]	NS	r=-0.287 [b] *P*=0.043	r=-0.317 [b] *P*=0.03	NS

Abbreviation: ICSI, intracytoplasmic sperm injection; NS, not significant.
Results are presented as mean ± standard deviation (SD), median and minimum-maximum levels.
[c] Spearman's coefficient (r) was used.
[b] Pearson's coefficient (r) was used.
[c] Appearance of 2 pronuclei and 2 polar bodies and calculated by the number of fertilized oocytes per the number of oocytes injected.

catabolism but also on an exogenous intake of nitrogen (1). In this study, we did not control the diet or protein consumption of the patients. Consistent with the result of the present study, a negative correlation between urea and sperm count has been previously reported (18). In addition, the current study showed an inverse correlation between the urea level of seminal plasma and fertilized oocytes, which is clinically important. It seems that the effect of urea from seminal plasma on the fertilization rate is indirect and mediated by sperm cells that were used for injection to oocyte.

In the present study, the uric acid of seminal plasma showed a negative correlation with sperm morphology and fertilization rate. Uric acid is an important product related to purine nucleotide metabolism, which is the backbone of DNA molecules (3). It is reported that uric acid in the semen of bulls is probably the result of the metabolism of xanthine and hypoxanthine in the seminal vesicle (19). As uric acid is an end product of purine base metabolism, seminal plasma uric acid appeared to be of particular importance and high levels indicate an increased nucleotide catabolism in the male reproductive tract (5). Thus, an inverse correlation between the uric acid of seminal plasma and the fertilization rate is justified.

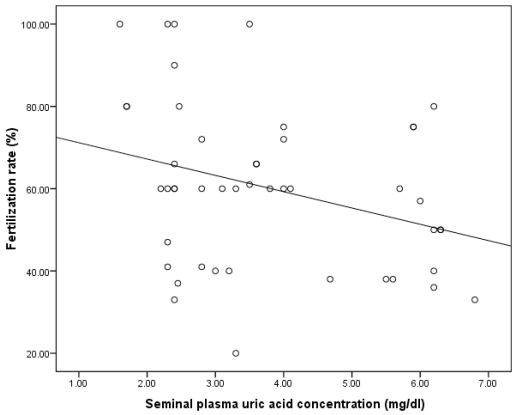

Figure 1. Correlation Between Uric Acid Concentration (mg/dL) of Seminal Plasma From Infertile Male (n = 50) With Percentage of Fertilization Rate After an Intracytoplasmic Sperm Injection (Pearson's coefficient, r =-0.317, *P* = 0.03).

Another result of the current study was the inverse correlation between creatinine and sperm count. The free creatinine is a waste product of creatine metabolism (20). There is little information about the importance of creatinine or creatine in human semen. Srivastava et al (5) reported that the levels of seminal creatine in the normal control subjects were different from azoospermic patients and vasectomized individuals. The source of creatine in the male reproductive tract is not well understood. It is suggested that creatine is synthesized in the testes and epididymides, and transported during circulation to the seminal vesicles (21).

To our knowledge, this is the first study on the relationship between NPN compounds of seminal plasma and the fertilization rate of the oocyte after ICSI. The limitation of this study was lack of lifestyle data including diet or protein consumption from patients. Since the amount of urea and uric acid in the body are influenced by dietary intake, diet of participants should be controlled. In addition, the amount of some amino acids such as glutamine in semen fluid is also important and can affect the ammonia levels of seminal plasma.

In conclusion, ammonia, urea, and uric acid in seminal plasma seem to be important factors in sperm motility, sperm morphology, and fertilization rate. The present findings showed that urea and uric acid in seminal plasma have a negative impact on fertilization rate. Because of the observed association of NPN compounds with semen parameters and fertilization rate, the determination of these compounds in the clinic can be important in the management of infertility in the couples. The findings of the present study suggested that controlling the urea and uric acid levels in seminal plasma may improve fertilization

rate and ICSI outcome. Although, further studies with dietary control and determination of amino acids in semen would help clarify the functional importance of NPN and ICSI outcome.

Authors' Contributions

SA, MA, HT and MB designed the study and contributed to the critical revision. MM, HG and MB wrote the article. SA and MA contributed to sample collection and performing experiments.

Conflict of Interest Disclosures

None.

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