

Seminal Plasma Visfatin Levels Negatively Correlate with Sperm Concentration

Kalliopi Anagnostopoulou¹, Despina Tzanakaki², Alexandros Gryparis³, Stella Demeridou⁴, Stavroula Baka⁴

¹ Gemima IVF Clinic, Chalandri, Athens, Greece

² Second Department of Obstetrics and Gynecology, Assisted Reproduction Unit, Aretaieion Hospital, National and Kapodistrian University of Athens, Athens, Greece

³ Unit of Endocrinology, Diabetes Mellitus, and Metabolism, Aretaieion Hospital, National and Kapodistrian University of Athens, Athens, Greece

⁴ Department of Biopathology - Microbiology and Biochemistry, Aretaieion Hospital, National and Kapodistrian University of Athens, Athens, Greece

Corresponding author: Stavroula Baka, Department of Biopathology - Microbiology and Biochemistry, Aretaieion Hospital, National and Kapodistrian University of Athens, 76 Vas Sofias Ave, 11528 Athens, Greece; Email: sbaka@aretaieio.uoa.gr; Tel.: +30 210 7286238

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Abstract

Introduction: Visfatin is involved in nicotinamide adenine dinucleotide biosynthesis, with a possible role in spermatogenesis. We investigated seminal plasma visfatin levels and its possible correlations with sperm parameters (concentration, motility, morphology) and BMI.

Materials and methods: We included 79 semen samples obtained from men from infertile couples presenting for sperm analysis. The samples were divided into 2 groups: a group of 35 samples with normal sperm parameters and another group of 44 samples with at least one abnormal sperm parameter. Seminal plasma visfatin levels were determined using commercially available enzyme-linked immunosorbent assay kits.

Results: Demographic data and body mass index (BMI) were similar in our subjects. As expected, the sperm parameters were significantly different between the 2 groups we studied. Visfatin levels did not differ between groups (66.6 ng/ml in normal samples and 72.7 ng/ml in abnormal samples, $p=0.114$) and did not correlate with sperm motility, sperm morphology, and BMI. However, a negative correlation between visfatin levels and sperm concentration ($r=-0.28$; $p=0.014$) and sperm count ($r=-0.3$; $p=0.009$), respectively, was detected.

Conclusions: Visfatin was detected in all human seminal plasma samples. Although its levels were similar in subjects with and without normal sperm parameters, a role for visfatin in sperm physiology cannot be ruled out at this point and further research is required.

Keywords

infertility, semen parameters, seminal plasma, visfatin

INTRODUCTION

Infertility has increased worldwide over the past few decades and the medical community is always in search of possible causes and better therapeutic management. Since infertility has been linked to obesity, the adipokines, which

are secreted by the adipose tissue, could represent the link between obesity and infertility. In contrast to female infertility, where possible relationships to different adipokines have been extensively investigated, the male counterpart has been less studied.^[1] Thus, although some adipokines have been isolated in human seminal plasma, their rela-

tionship with sperm parameters has not been fully elucidated.

Visfatin, also known as nicotinamide phosphoribosyltransferase (NAMPT), is an adipocytokine with potential role in different metabolic and immune disorders.^[2] NAMPT regulates nicotinamide adenine dinucleotide (NAD) levels, an essential coenzyme in cellular metabolism^[3], with a significant effect on the Sertoli cells^[4]. Its reduced form, NADH, is implicated in the capacitation of human spermatozoa, a critical event for male fertilization potential.^[4]

Ocón-Grove et al.^[4] detected visfatin in chicken seminal plasma and testis, consistent with previous reports on the detection of this protein in pig^[5] and mouse testis^[3]. They provided novel evidence that visfatin expression increased during sexual maturation since visfatin was found significantly elevated in adult chicken testis compared to the prepubertal one, and was accompanied by a 28-fold increase in plasma levels, concluding that visfatin is important for energy metabolism in testicular function. Furthermore, visfatin has been detected in human seminal plasma in almost 100-fold higher concentrations compared to serum.^[6]

Interestingly, although visfatin expression was demonstrated in a variety of tissues including testes^[3], its role in the testicles has not been elucidated. Very few studies have focused on the presence of visfatin in the seminal plasma and a possible relation with the main functional parameters of spermatozoa.^[6,7] It is possible that since this protein is present in human testes, spermatozoa, and seminal plasma, it might have an influence on sperm parameters (concentration, motility, and morphology).

AIM

Thus, our objective was to detect seminal plasma visfatin levels and to investigate the association, if any, between visfatin and the sperm parameters.

MATERIALS AND METHODS

Our sample comprised 79 men from infertile couples presenting for sperm analysis at our hospital. The sample was divided into two groups: 35 with normal sperm parameters, and 44 with at least one abnormal sperm parameter. The study was approved by the Ethics Committee of our teaching hospital, and informed consent was obtained from each participant.

Sperm analysis was performed manually according to the WHO criteria.^[8] Sperm samples were collected by masturbation into a sterile plastic container after a period of sexual abstinence of 3-5 days. After liquefaction, semen samples were evaluated for sperm concentration, motility, and morphology according to WHO guidelines by a single examiner. The remaining part of the samples was centrifuged for 10 minutes at 2500 g, and the supernatant (seminal plasma) was collected and immediately stored at

-80°C until further analysis. Seminal plasma visfatin levels were determined in duplicate using Visfatin C-Terminal (Human) ELISA assay (Phoenix Pharmaceuticals, Inc, Belmont, CA, USA) according to manufacturer's instructions. The minimum detectable visfatin concentration was 0.1 ng/mL, while intra-assay and interassay coefficients of variation were 5% and 12%, respectively.

For the statistical analysis, normality was examined by Kolmogorov-Smirnov test. Data regarding seminal plasma visfatin were not normally distributed; therefore, non-parametric procedures (Wilcoxon signed rank test) were used. Variables not normally distributed are presented as median (range). Spearman correlation coefficient was applied to detect any positive or negative correlations between visfatin and other variables, while multiple regression analysis was performed to examine the effect of different confounding factors on visfatin. *P* values less than 0.05 were considered statistically significant, while values $\geq 0.05 \leq 0.10$ were considered as indicative. Statistical analysis was performed using SPSS v. 17.0 (Chicago, IL, USA).

RESULTS

Demographic data, sperm analysis results, and visfatin levels in the study population are presented in **Table 1**. Age, BMI, and visfatin levels did not differ between groups. However, as expected, sperm parameters were significantly different.

By means of Wilcoxon test, no significant correlation was detected between visfatin and any of the sperm parameters except for normal morphology where only an indicative correlation was observed ($p=0.084$).

Using Spearman correlation coefficient, visfatin levels were significantly negatively correlated with sperm count ($r=-0.3$; $p=0.009$) and sperm concentration ($r=-0.28$, $p=0.014$).

Multiple regression analysis revealed only sperm count ($p=0.021$) and sperm concentration ($p=0.011$) as confounding factors.

DISCUSSION

In the last decades, it has been proven that white adipose tissue is more than a fat storing organ capable of secreting significant proteins that regulate metabolic homeostasis such as the adipokines. The purpose of this study was to detect visfatin, one of the adipokines, in the seminal plasma of men from infertile couples presenting for sperm analysis, and correlate its levels with the investigated semen parameters.

Sperm analysis is usually the first diagnostic test carried out for infertility assessment and, as a result, multiple sperm parameters are investigated to assess sperm quality as well as probable fertilization potential.^[8,9] In fact, the concentration of spermatozoa in a semen sample has been correlated to the fertilization and the pregnancy rates.^[9] Studies based

Table 1. Demographic and biochemical data in subjects with normal and abnormal sperm parameters

Variables	Total samples	Normal samples	Abnormal samples	P
Valid N	79	35	44	
Age (years)	40 (28-52)	40 (28-51)	40 (33-52)	0.420
Weight (kg)	85 (57-140)	82 (62-139)	89 (57-140)	0.161
Height (cm)	170 (165-194)	170 (165-190)	180 (170-194)	0.055
BMI (kg/m ²)	27.4 (19.3-47.0)	26.8 (19.4-47.0)	28.1 (19.3-43.2)	0.646
Sperm volume (ml)	3.4 (1.0-8.1)	3.6 (1.6-6.3)	3.1 (1.0-8.1)	0.140
Sperm concentration (×10 ⁶ /ml)	30 (<0.1-190)	57 (15-190)	10 (<0.1-170)	<0.001
Sperm count (×10 ⁶)	85.0 (<0.1-858.6)	224.0 (52.5-858.6)	35.5 (<0.1-231.0)	<0.001
Total motility (%)	58 (0-90)	69 (37-90)	51 (0-82)	<0.001
Normal morphology (%)	6 (1-19)	8 (4-19)	3 (1-19)	<0.001
Visfatin (ng/mL)	71.3 (8.1-442.4)	66.6 (8.1-96.5)	72.7 (10.0-442.4)	0.114

Note: Values are median (range); BMI: body mass index

on correlations between semen adipokine levels and sperm motility are scarce; thus, functional studies will be necessary to identify their role for sperm parameters.

In agreement with previous data^[6], we detected visfatin in all seminal plasma samples studied. Interestingly, higher visfatin levels have been reported in human seminal plasma compared to those in serum.^[6,7] The high visfatin levels found in the seminal plasma point to special physiologic functions of this protein in the male reproductive tract although its source remains uncertain. In fact, any cell or tissue (testis, epididymal adipocytes, accessory glands, and spermatozoa) might be involved in producing and releasing this protein. Alternatively, although the blood-testis barrier restricts the passage of serum proteins, it is possible that visfatin might be retained into the testis through different mechanisms. Furthermore, it is possible that the regulation of visfatin in the reproductive tract and peripheral blood might be completely different.

BMI and visfatin levels did not differ between the groups under investigation. Previous work demonstrated the association between increased BMI and abnormal sperm parameters, in particular increased prevalence of azoospermia or oligozoospermia.^[10,11] Obesity and diabetes have negative effects on sperm parameters, triggering degenerative changes in the testes.^[12] Visfatin is an insulin-mimicking agent^[2]; however, its role in male fertility is still unclear. Recently, visfatin levels were found significantly elevated in rats with diabetes and obesity, alone or combined, and their levels negatively correlated to sperm parameters suggesting that visfatin has a role in male fertility.^[12] The observation of a negative correlation between visfatin levels and semen parameters in our group supports this hypothesis. However, in contrast to our results, others failed to find any effect of visfatin levels on human sperm parameters.^[6,7]

Visfatin is an important enzyme for energy metabolism and apoptosis, with regulatory effect on NAD, a co-sub-

strate for dehydrogenases which have been implicated in spermatogenesis. Ocón-Grove et al.^[4] hypothesized that during sexual maturation, NAD would be a vital molecule in spermatogenesis by producing the required energy for sperm survival and motility. Therefore, testicular visfatin expression would increase during sexual maturation. In their immunohistochemical study, Gurusubramanian and Kumar Roy^[13] showed that, in the rat testis, visfatin was found in Leydig cells and seminiferous tubules in the early stages of round spermatids and spermatozoa, as previously demonstrated in mice^[3], chicken^[4], and pigs^[5]. Furthermore, Riammer et al.^[14] reported for the first time the presence of visfatin in human spermatozoa. In particular, higher protein amounts were found in immature compared to mature spermatozoa, making the authors conclude that visfatin levels decrease during sperm maturation. Although the reason for this difference is unknown, it is possible that visfatin may activate a number of major cellular signalling pathways implicated in the regulation of sperm function. Probably, these findings are indicative and may identify visfatin as a marker of impaired fertility. On the other hand, Tu et al.^[15] reported that visfatin increased testosterone levels in cultured Leydig cells, suggesting a possible role in steroidogenesis, while Jeremy et al.^[16] correlated visfatin with testicular aging, since decreased visfatin levels in Leydig cells inhibited testosterone synthesis, increased germ cell death, and caused regressive changes in spermatogenesis.

Our study has some limitations. As adipose tissue is an important factor in relation to the visfatin levels, its effect could not be investigated since our groups did not differ regarding their BMI. Sperm analysis did not include some other parameters such as DNA fragmentation test or ROS levels, which might have offered important correlations. We presented only statistical relationships and speculations as for the role visfatin might play in male fertility, and did not present any proof for the physiologic effects on

the spermatozoa. Although it has been demonstrated that adipokines have a significant role in different physiological processes, and some of them specifically on male reproduction, there are still many aspects that need to be elucidated. In this direction, experimental studies could shed light on the specific mechanisms visfatin uses to influence sperm function, if any.

CONCLUSIONS

We demonstrated that visfatin was present in seminal plasma and was negatively correlated with sperm concentration and sperm count in men from infertile couples. Since a definitive functional role in human male reproduction has not yet been attributed to visfatin, the mechanisms that govern its role in male fertility must be revealed through further experiments and studies.

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Уровни висфатина в семенной плазме отрицательно коррелируют с концентрацией сперматозоидов

Калиопи Анагностопулу¹, Деспина Тсанакаки², Александрос Грипарис³, Стела Демериду⁴, Ставрула Бака⁴

¹ Репродуктивная клиника „Генима“, Халандри, Афины, Греция

² Второе отделение акушерства и гинекологии, Отделение вспомогательных репродуктивных технологий, Больница „Аретаеион“, Афинский национальный университет им. Каподистрии – Афины, Греция

³ Отделение эндокринологии, сахарного диабета и метаболических заболеваний, Больница „Аретаеион“, Афинский национальный университет им. Каподистрии – Афины, Греция

⁴ Отделение биопатологии-микробиология и биохимия, Больница „Аретаеион“, Афинский национальный университет им. Каподистрии – Афины, Греция

Адрес для корреспонденции: Ставрула Бака, Отделение биопатологии-микробиология и биохимия, Больница „Аретаеион“, Афинский национальный университет им. Каподистрии – Афины, Авеню „Вас Софияс“ № 76, 11528 Афины, Греция; Email: sbaka@aretaieio.uoa.gr; Тел.: +30 210 7286238

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Резюме

Введение: Висфатин участвует в биосинтезе никотинамидадениндинуклеотида, возможно, в сперматогенезе. Мы исследовали уровни висфатина в семенной плазме и его возможную корреляцию с параметрами сперматозоидов (концентрация, подвижность, морфология) и ИМТ.

Материалы и методы: Мы включили 79 образцов спермы, полученных от мужчин от бесплодных пар, представленных для анализа спермы. Образцы были разделены на 2 группы: группа из 35 образцов с нормальными параметрами спермы и другая группа из 44 образцов с хотя бы одним аномальным параметром спермы. Уровни висфатина в семенной плазме определяли с использованием имеющихся в продаже наборов для твердофазного иммуноферментного анализа.

Результаты: Демографические данные и индекс массы тела (ИМТ) у наших испытуемых были схожими. Как и ожидалось, параметры спермы значительно различались между двумя изученными нами группами. Уровни висфатина не различались между группами (66.6 ng/ml в нормальных образцах и 72.7 ng/ml в аномальных образцах, $p=0.114$) и не коррелировали с подвижностью сперматозоидов, морфологией сперматозоидов и ИМТ. Однако была обнаружена отрицательная корреляция между уровнями висфатина и концентрацией сперматозоидов ($r=-0.28$; $p=0.014$) и количеством сперматозоидов ($r=-0.3$; $p=0.009$) соответственно.

Заключение: Висфатин был обнаружен во всех образцах семенной плазмы человека. Хотя его уровни были одинаковыми у субъектов с нормальными параметрами спермы и без них, на данный момент нельзя исключить роль висфатина в физиологии сперматозоидов, и требуются дальнейшие исследования.

Ключевые слова

бесплодие, параметры спермы, семенная плазма, висфатин