Relationships between metabolic status, seminal adipokines, and reproductive functions in men from infertile couples

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Abstract

Objective: Adipokines could be a link between metabolic syndrome (MS) and infertility. While the association between circulating adipokines and fertility has been extensively studied in females, this relationship in males was less investigated, although some adipokines are detectable in seminal plasma (SP). The aim of this study was to determine adipokine levels in blood and SP and to assess the relationships between adipokines, MS and semen parameters in men from infertile couples.

Design: Male partners of infertile couples referred to four medical French centers were enrolled in years 2013–2016.

Methods: Subjects ($n = 160$) aged 18–45 years were assessed for anthropometric, biochemical, sperm, and circulating hormonal parameters. Leptin, adiponectin, resistin, chemerin, visfatin, and IL-6 were measured in serum and SP.

Results: Infertility duration was higher in men with than without MS. Adipokine concentrations were higher in blood than in SP, except for IL-6 and visfatin. The most striking result was the significant correlation observed between seminal IL-6 and spermatozoid concentration, progressive motility, and sperm vitality. Moreover, while men with MS exhibited an expected lower adiponectinemia, they displayed 2.1-fold higher adiponectin levels in SP than men without MS. Finally, logistic regression analysis showed that BMI, infertility duration, and adiponectin serum/SP ratio were independently associated with MS.

Conclusions: These results suggest an involvement of seminal adipokines to modulate fertility in men with MS and that seminal IL-6 could play a beneficial role on sperm functionality. Further mechanistic studies are necessary to investigate the precise roles of these adipokines in male reproduction.

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Introduction

Increased body weight is a worldwide concern (1): in 2016, 39% of adults over 18 years were overweight, and 13% obese (2, 3). Metabolic syndrome (MS), defined by the

presence of three out of five features including abdominal adiposity and a cluster of cardiometabolic risk factors, is associated with an increased risk of multiple chronic

diseases, including diabetes, cancer and cardiovascular diseases (4). MS prevalence is also increasing worldwide with a rise of 35% from 1988–1994 to 2007–2012 in the United States (4). Infertility is also a global public health problem as it affects one out of four couples in high and low-medium income countries (<http://www.who.int/reproductivehealth/topics/infertility/burden/en/>). This could result from factors related only to women, in one-third of cases mostly ovulatory disorders or only to men in one-fifth of cases, with a decrease in semen parameters or both to women and men in 40–50% of cases (5, 6, 7, 8). A secular decrease of sperm concentration has been ongoing for the last decades in Western Europe (9, 10). While obesity has been clearly associated with reduced fertility in men (11), metabolic disorders, as seen in MS, are rarely considered in the current treatment of infertility. Even if low testosterone levels have been reported in subjects with MS (12, 13, 14, 15), studies that evaluated semen parameters in these subjects (2, 13, 16, 17, 18, 19) reported contradictory results. Therefore, the mechanisms underlying the relationships between MS and disturbed fertility remain unclear and multiple and complex interactions between some metabolic pathways and reproductive functions could exist.

The reproductive endocrine aspects of white adipose are now well accepted (20). In rats, the removal of the epididymal adipose tissue causes a significant decrease in sperm count (21). Adipose tissue-secreted hormones, known as adipokines, play an important role in the regulation of several major hormonal pathways such as the pituitary and reproductive axis. The levels of several circulating adipokines are related to BMI and also to semen parameters (22). Thus, obesity is associated with hyperleptinemia and leptin resistance (23), whereas adiponectinemia is negatively associated with BMI (24). Several adipokines are involved in the regulation of the reproductive axis, with correlations between the levels of circulating adipokines and semen parameters. It has been shown that leptinemia was positively related to abnormal sperm morphology, and negatively to both sperm concentration and motility (25, 26). Therefore, reduced fertility in MS men could be partly related to the high levels of circulating leptin, leading to a decreased testosterone production by Leydig cells, which interferes with the normal cycle of spermatogenesis (27). Otherwise, studies performed in bulls showed that circulating adiponectin levels were related to sire conception rates (28).

In addition, adipokines are present in the seminal plasma (SP), and also inside germ cells (29), raising the possibility that they can also directly act on gonadic cells

to modulate their functions, and in turn fertility. However, relationships between adipokines in the SP and fertility have been poorly studied. More investigation is required to decipher their pathophysiological roles and to identify new potential targets to improve male fertility. Thus, the aim of this study was to determine adipokine levels in blood and semen and to assess the relationships between adipokines, MS and semen parameters in a population of men issued from infertile couples.

Patients and methods

Patients

The multicentric and transversal Metasperme study (Biomedical Research AOM 10020 - NI 10033 – ID-RCB 2011-A01052-3), was performed from July 2013 to January 2016 by consecutive inclusions of male partners of infertile couples. The present work on blood and semen adipokines corresponds to an ancillary study from this cohort. Four medical French centers included male partners aged 18–45 years from infertile couples, with primary or secondary infertility for at least 12 months. Exclusion criteria were difficulties to understand, seropositivity for HIV, HBV or HCV, smoking more than 40 cigarettes a day as well as male explained infertility (for example after chemotherapy, varicocele or genital surgeries). Participants gave full informed written consent to participate in the study and ethical approval was obtained from the Ethics Committee of the University of Paris Ile-De-France IV.

Clinical and biological measurements

We measured weight, height, waist circumference (WC), hip circumference according to WHO guidelines (30), and blood pressure (BP). Infertility duration was defined by the time between the beginning of regular unprotected sexual intercourse and the day of inclusion. Ejaculated semen samples were collected after 3–5 days of sexual abstinence. Sperm parameters namely volume, pH of the ejaculate, sperm count, motility and vitality were analyzed according to bioforma (31) adapted from WHO guidelines (32) (Supplementary Table 1, see section on [supplementary materials](#) given at the end of this article). Semen was kept for 20–60 min for liquefaction. Then, semen volume and pH were measured and recorded. Semen samples were evaluated for motility in duplicates according to standard WHO guidelines (32). Sperm numeration was obtained manually in duplicates by Kova chamber counting.

Sperm vitality was assessed by eosin-nigrosin staining on 100 sperm. Sperm morphology was evaluated after Harris–Schorr staining using modified David's score (33). Remaining semen was centrifuged at 600 *g* for 10 min. Aliquots were stored at -80°C until assay analysis. Fasting blood samples were centrifuged at 1900 *g* for 10 min, serum was aliquoted and kept at -80°C until use.

Sex hormone-binding globulin (SHBG) was measured on Access analyzer (Beckman Coulter). Total testosterone was measured by RIA (Beckman Coulter – Immunotech, Marseille, France). Bioavailable testosterone was measured after ammonium sulfate precipitation as previously described (34, 35). Follicle-stimulating hormone (FSH), luteinizing hormone (LH), inhibin B, estradiol, glucose, triglycerides, total and HDL cholesterol, insulin, and C-reactive protein (CRP) were routinely measured on Cobas analyzer (Roche Diagnostics). The insulin resistance HOMA-IR index was calculated as (glycemia (mmol/L) $\times$ insulinemia (mU/L)/22.5) (36).

Adipokine measurements from frozen samples were performed in both semen plasma and serum according to pre-analytical conditions previously described (37). Leptin (blood, SP), adiponectin (SP), resistin (blood, SP), chemerin (blood, SP) and IL-6 (blood, SP) were measured by ELISA (Quantikine R&D Systems). Biovendor (Brno, Czech Republic) and ALPCO were used for ELISA measurements of visfatin (blood, SP) and adiponectin (blood), respectively. In serum, intra-assay and inter-assay coefficients of variations were under 8% and in SP, intra-assay coefficients of variations were under 6% and inter-assay coefficients of variations under 10% (37).

Metabolic syndrome classification

MS was defined according to the harmonized classification of 2009 (38) by the presence of at least three of the five following criteria: WC ≥ 94 cm, systolic BP ≥ 130 mmHg and/or diastolic blood pressure ≥ 85 mmHg and/or antihypertensive treatment, triglyceride levels ≥ 1.7 mmol/L and/or taking a specific treatment, glycemia ≥ 5.6 mmol/L and/or taking anti-diabetic treatment, HDL cholesterol level < 1.03 mmol/L. One hundred seventy patients were selected for the study, whilst seven were excluded. Of the 134 patients for whom all the required criteria were available, 42 patients were diagnosed with MS and 92 without. Of the 29 subjects with missing data, those who had at least three positive criteria were considered with MS ($n=5$) and those who had at least three negative criteria were classified without MS ($n=24$). Thus, only three patients could not be included regarding their MS status (Fig. 1).

Figure 1
Flow chart.

Statistical analysis

We used StatView software (SAS Institute, Inc, copyright 1992–1998). Qualitative variables were analyzed using chi-square test or when appropriate, Fisher's exact test. Quantitative variables were analyzed using the nonparametric Mann–Whitney test, the Wilcoxon rank test and the Spearman correlation coefficient. Descending stepwise logistic regression analyses were used to identify the independent variables associated to MS. This analysis was performed in 126 subjects for whom all logistic regression parameters (age, infertility time, sperm concentration, BMI, SHBG, total testosterone, inhibin B, seminal leptin, adiponectin serum/SP ratio) were available. The significance level was 0.25 for the univariate phase and 0.05 for the multivariate phase. For group comparisons, *P* values < 0.05 were considered statistically significant (two sides).

Results

Clinical, metabolic and sperm characteristics of subjects with or without MS

One hundred sixty men were included in the study (Fig. 1). Clinical characteristics, hormonal and metabolic status of the population studied are presented in Table 1. As expected we observed that subjects presenting MS had

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higher BMI, waist circumference, systolic and diastolic blood pressure, HOMA-IR, triglycerides, and lower HDL cholesterol.

Importantly infertility duration was higher in MS subjects (Table 1). Regarding sperm parameters, they were not different between subjects with or without MS (except pH), suggesting that parameters other than those classically evaluated in the sperm can play a role.

Adipokine levels in blood versus seminal plasma

We observed that, in the entire population, the concentrations of leptin ($P<0.0001$), adiponectin ($P<0.0001$), resistin ($P=0.01$), chemerin ($P<0.0001$) and insulin ($P<0.05$) were significantly higher in serum

than in SP (Fig. 2 and Table 2). Besides, glucose levels were similar between the serum and semen. Contrarily, circulating levels of IL-6 ($P<0.0001$) and visfatin ($P<0.0001$) were lower in serum than in SP. These results were also found when we separated the samples into two groups, with or without MS, except for resistin, levels of which were not different in the MS group, and insulin, for which the results were not statistically significant but had a tendency to resemble the previous results.

There was a positive correlation between serum and SP for resistin ($\rho=0.23$, $P<0.05$) and insulin ($\rho=0.52$, $P<0.0001$) and no correlation for leptin, IL-6, chemerin and visfatin. Interestingly, we observed a negative correlation between serum and SP for adiponectin ($\rho=-0.20$, $P<0.05$).

Table 1 Characteristics of included population. Table shows clinical, biological and spermatic characteristics of the whole population included, the metabolic syndrome group (with MS) and the non-metabolic syndrome (no MS) group. P value corresponds to the difference between the metabolic and non-metabolic syndrome groups.

	All subjects		No MS		With MS		P value without vs with MS
	n	Mean $\pm$ S.E.	n	Mean $\pm$ S.E.	n	Mean $\pm$ S.E.	
Age (years)	160	37.1 $\pm$ 0.4	113	36.2 $\pm$ 0.4	47	39.1 $\pm$ 0.6	<0.001
BMI (kg/m ²)	159	26.4 $\pm$ 0.3	112	25.2 $\pm$ 1.1	47	29.3 $\pm$ 0.6	<0.0001
WC (cm)	157	92.9 $\pm$ 0.9	110	89.3 $\pm$ 1.0	47	101.3 $\pm$ 1.4	<0.0001
SBP (mmHg)	137	125.6 $\pm$ 1.0	95	123.4 $\pm$ 0.9	42	130.7 $\pm$ 2.0	<0.001
DBP (mmHg)	137	79.7 $\pm$ 0.8	95	78.3 $\pm$ 1	42	83.0 $\pm$ 1.7	<0.01
Infertility duration (months)	148	39.8 $\pm$ 2.2	102	36.4 $\pm$ 2.5	46	47.4 $\pm$ 4.0	<0.01
CO expired (%)	145	6.8 $\pm$ 0.52	102	6.66 $\pm$ 0.59	43	7.39 $\pm$ 1.07	0.62
HOMA-IR	158	2.6 $\pm$ 0.2	111	1.8 $\pm$ 0.1	47	4.3 $\pm$ 0.5	0.001
Triglycerides (mmol/L)	159	1.7 $\pm$ 0.1	112	1.3 $\pm$ 0.1	47	2.5 $\pm$ 0.2	<0.0001
Total cholesterol (mmol/L)	159	5.2 $\pm$ 0.1	112	5.1 $\pm$ 0.1	47	5.4 $\pm$ 0.2	0.05
HDL cholesterol (mmol/L)	159	1.23 $\pm$ 0.03	112	1.32 $\pm$ 0.03	47	1.02 $\pm$ 0.04	<0.0001
LDL cholesterol (mmol/L)	157	3.28 $\pm$ 0.07	112	3.23 $\pm$ 0.09	45	3.41 $\pm$ 0.14	0.18
SHBG (nmol/L)	159	31.2 $\pm$ 1.1	112	33.5 $\pm$ 1.3	47	25.8 $\pm$ 1.7	0.0005
Total testosterone (nmol/L)	159	17.7 $\pm$ 0.5	112	19.1 $\pm$ 0.7	47	14.5 $\pm$ 0.8	<0.0001
Bioavailable testosterone (nmol/L)	147	5.5 $\pm$ 0.2	102	5.8 $\pm$ 0.2	45	4.9 $\pm$ 0.3	<0.01
Inhibin B (pg/mL)	159	193.7 $\pm$ 6.4	112	205.4 $\pm$ 7.9	47	167.7 $\pm$ 9.8	<0.05
FSH (UI/L)	159	4.8 $\pm$ 0.2	112	4.6 $\pm$ 0.3	47	5.2 $\pm$ 0.6	0.40
LH (UI/L)	159	5.3 $\pm$ 0.2	112	5.2 $\pm$ 0.3	47	5.4 $\pm$ 0.3	0.38
Estradiol (pmol/L)	159	97.7 $\pm$ 2.3	112	98.9 $\pm$ 2.9	47	95.5 $\pm$ 3.9	0.78
CRP (mg/L)	159	2.4 $\pm$ 0.3	112	2.1 $\pm$ 0.3	47	2.9 $\pm$ 0.4	<0.005
Ejaculate volume (mL)	154	3.6 $\pm$ 0.1	109	3.6 $\pm$ 0.2	45	3.4 $\pm$ 0.2	0.49
pH	154	7.85 $\pm$ 0.03	109	7.82 $\pm$ 0.03	45	7.91 $\pm$ 0.05	<0.05
Sperm concentration ($\times 10^6$ /mL)	154	50.3 $\pm$ 3.8	109	47.1 $\pm$ 4.3	45	59.2 $\pm$ 7.8	0.09
Total sperm count ($\times 10^6$ /ejaculate)	154	179 $\pm$ 17	109	173 $\pm$ 22	45	192 $\pm$ 29	0.16
Progressive motility (%)	154	39.9 $\pm$ 1.4	109	40.1 $\pm$ 1.6	45	39.5 $\pm$ 2.7	0.89
Immobility (%)	154	54.4 $\pm$ 1.4	109	54.0 $\pm$ 1.6	45	55.3 $\pm$ 2.8	0.58
Vitality (%)	148	71.6 $\pm$ 1.3	104	72.0 $\pm$ 1.5	44	70.7 $\pm$ 2.5	0.72
Normal morphology (%)	148	16.0 $\pm$ 1.1	106	15.5 $\pm$ 1.4	42	15.3 $\pm$ 2.0	0.58
DNA spermatozoa fragmentation (%)	147	8.4 $\pm$ 0.5	105	8.3 $\pm$ 0.6	42	8.7 $\pm$ 0.8	0.33

CRP, C-reactive protein; DBP, diastolic blood pressure; FSH, follicle-stimulating hormone; HOMA-IR, homeostasis assessment of insulin resistance; LH, luteinizing hormone; SBP, systolic blood pressure; SHBG, sex hormone-binding globulin; WC, waist circumference.

Figure 2

Ratios of the blood to seminal plasma concentration of metabolic parameters and adipokines in the entire group and in patients with and without MS. Logarithmic value of the ratio of blood/SP levels for metabolic parameters and adipokines. **** $P < 0.0001$: MS versus without MS.

Correlations between adipokines and semen parameters

We searched for correlations between semen parameters and adipokine values in serum and SP. Semen volume was negatively correlated with seminal visfatin ($\rho = -0.29$, $P < 0.005$) and seminal IL-6 ($\rho = -0.24$, $P < 0.005$) (Table 3). Semen volume was also correlated with seminal glucose ($\rho = -0.38$, $P < 0.0005$) and glucose serum/SP ratio ($\rho = 0.36$, $P < 0.0005$).

Sperm concentration was correlated with seminal IL-6 ($\rho = 0.19$, $P < 0.05$), seminal chemerin ($\rho = 0.26$, $P < 0.01$) (Table 3) and their serum/SP ratios ($\rho = -0.25$, $P < 0.005$ and $\rho = -0.29$, $P < 0.005$, respectively). Sperm concentration was also correlated with seminal glucose ($\rho = 0.22$, $P < 0.05$) and glucose serum/SP ratio ($\rho = -0.20$, $P < 0.05$). However, when we compared subjects according to the WHO guidelines threshold²³ ($15 \times 10^6/\text{mL}$ of spermatozoa) (32) that classifies subjects as normo- or oligo-zoospermic, we did not find any significant difference of adipokine concentration in blood or in semen (Supplementary Table 2a and b).

Progressive motility was negatively correlated with circulating IL-6 ($\rho = -0.17$, $P < 0.05$, Table 3) and blood/SP ratio of IL-6 ($\rho = -0.29$, $P < 0.001$), whereas it was positively related to seminal IL-6 ($\rho = 0.23$, $P < 0.01$, Table 3). By comparing patients according to normal progressive motility (higher than 32% according to WHO guidelines (32)), we confirmed the increase of circulating IL-6 ($P < 0.05$) (Supplementary Table 2a) and of IL-6 serum/SP ratio (0.091 (0.037–0.139) vs 0.045 (0.023–0.102), $P = 0.01$) in asthenospermic subjects.

Sperm vitality was negatively correlated to IL-6 blood/SP ratio ($\rho = -0.23$, $P < 0.01$) and positively related to seminal IL-6 ($\rho = 0.17$, $P < 0.05$) (Table 3). When we

compared patients according to normal vitality threshold (threshold 58%, WHO guidelines (32)), we confirmed the increase of IL-6 blood/SP ratio (0.100 (0.044–0.168) vs 0.051 (0.024–0.105), $P = 0.01$) and the decrease of seminal IL-6 ($P < 0.05$) in subjects with necrospemia (Supplementary Table 2a).

Altogether, these results emphasize the relationship between IL-6 in SP and semen parameters and supports a favorable effect of the locally present IL-6. Conversely, circulating IL-6 is negatively related to sperm quality. Likewise, sperm concentration, total count and motility are correlated with CRP ($\rho = -0.17$, $P < 0.05$; $\rho = -0.22$, $P < 0.01$ and $\rho = -0.24$, $P < 0.005$ respectively), further stressing a negative impact of circulating low-grade inflammation on fertility in men.

Sperm morphology did not correlate with any adipokine levels neither in blood, nor in SP (Table 3).

Relationships between blood and seminal adipokines according to the MS status

In our population, 29.4% of subjects presented a MS (Table 1), with 47 patients with MS and 113 without MS.

As expected, we detected higher circulating levels of insulin, leptin and IL-6 and by contrast lower adiponectin levels in individuals with MS compared to those without MS (Table 2). In SP, we observed higher seminal concentrations of leptin ($P < 0.005$), resistin ($P < 0.005$) and insulin ($P < 0.0001$) in the MS group than in the non-MS group (Table 2). Interestingly, while serum adiponectin levels were lower in the MS group (3.1 vs 4.0 mg/L, $P < 0.0001$), the opposite was seen in the SP, with adiponectin levels being 2.1-fold higher ($P < 0.0001$) in patients with MS (Table 2). The specificity of the adiponectin pattern was also seen when the results were expressed as blood/SP adipokine

Table 2 Metabolic and adipokine profile of the studied population. Concentrations in blood and in seminal plasma for each adipokine measured in the whole population, in the group without metabolic syndrome and in the group with a metabolic syndrome.

	All subjects			Without MS			With MS			P value without vs with MS
	n	Median (IQR)	P value (blood vs semen)	n	Median (IQR)	P value (blood vs semen)	n	Median (IQR)	P value (blood vs semen)	
Glucose, mmol/L	159	5.2 (4.8-5.6)	0.61	112	5.0 (4.7-5.3)	0.17	47	5.7 (5.0-6.2)	0.29	<0.0001
Blood	102	5.3 (3.5-7.5)		76	5.5 (3.6-7.6)		26	4.6 (3.4-6.3)		0.69
Insulin, U/L	158	7.3 (5.3-12.5)	<0.05	111	6.6 (5.0-10.4)	0.16	47	12.6 (7.8-20.3)	0.10	<0.0001
Blood	107	6.8 (4.1-11.8)		79	5.3 (3.8-8.8)		28	10.5 (9.0-14.8)		<0.0001
Leptin, µg/L	158	4.8 (3.0-8.3)	<0.0001	111	4.0 (2.4-6.7)	<0.0001	47	7.3 (4.2-12.8)	<0.0001	<0.0001
Blood	137	7.8 × 10 ⁻³ (4.3-20.0 × 10 ⁻³)		98	6.9 × 10 ⁻³ (3.1-10.3 × 10 ⁻³)		39	9.6 × 10 ⁻³ (7.1-26.3 × 10 ⁻³)	<0.0001	<0.005
Adiponectin, mg/L	158	4.0 (3.0-5.5)	<0.0001	111	4.4 (3.3-5.6)	<0.0001	47	3.1 (2.4-4.1)	<0.0001	<0.0001
Blood	142	62.2 × 10 ⁻³ (33.6-131.4 × 10 ⁻³)		102	51.5 × 10 ⁻³ (30.9-86.9 × 10 ⁻³)		40	130.8 × 10 ⁻³ (56.1-252.2 × 10 ⁻³)	0.85	<0.0001
Resistin, µg/L	103	8.8 (5.2-13.6)	0.01	71	7.7 (5.0-11.8)	0.001	32	10.0 (5.5-14.1)		0.16
Blood	100	3.2 (1.1-10.7)		69	1.8 (1.0-7.8)		31	5.3 (2.4-19.9)	<0.0005	<0.005
Chemerin, µg/L	104	1.2 (0.9-1.4)	<0.0001	72	1.1 (0.9-1.4)	<0.0001	32	1.2 (0.9-1.5)		0.228
Blood	104	0.4 (0.3-0.8)		72	0.4 (0.3-0.7)		32	0.5 (0.3-1.0)	<0.0001	0.195
Visfatin, µg/L	104	0.8 (0.5-1.4)	<0.0001	72	0.7 (0.5-1.3)	<0.0001	32	1.1 (0.6-1.7)		0.072
Blood	104	152.4 (91.9-220.6)		72	150.7 (90.8-212.1)		32	149.0 (89.3-211.6)	<0.0001	0.88
IL-6, ng/L	158	0.8 (0.5-1.3)	<0.0001	111	0.7 (0.5-1.1)	<0.0001	47	0.9 (0.6-1.6)		0.019
Blood	142	13.3 (8.6-25.8)		102	12.9 (8.6-23.7)		40	20.2 (8.3-30.4)		0.56

MS, metabolic syndrome.

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Table 3 Correlations between adipokines and semen parameters.

Sperm parameters	Leptin		Adiponectin		IL-6		Resistin		Chemerin		Visfatin	
	Blood	SP	Blood	SP	Blood	SP	Blood	SP	Blood	SP	Blood	SP
Volume												
rho	−0.04	0.06	0.01	−0.08	−0.10	−0.24	0.02	−0.11	0.01	−0.18	−0.05	−0.29
P	0.64	0.52	0.91	0.33	0.21	<0.005	0.86	0.25	0.94	0.06	0.60	<0.005
Sperm concentration												
rho	−0.05	0.08	−0.04	0.01	−0.15	0.19	0.04	−0.08	−0.11	0.26	−0.10	0.14
P	0.53	0.33	0.59	0.90	0.06	<0.05	0.65	0.42	0.25	<0.01	0.33	0.14
Sperm count												
rho	−0.04	0.11	−0.02	0.01	−0.17	0.08	0.04	−0.12	−0.09	0.14	−0.12	−0.01
P	0.57	0.19	0.80	0.95	<0.05	0.36	0.71	0.25	0.36	0.14	0.22	0.92
Progressive mobility												
rho	−0.12	−0.05	0.08	0.04	−0.17	0.23	−0.14	0.02	−0.12	0.13	−0.04	0.05
P	0.12	0.50	0.37	0.69	<0.05	<0.01	0.15	0.85	0.22	0.20	0.67	0.65
Vitality												
rho	0.01	−0.05	0.13	0.08	−0.08	0.17	−0.18	0.03	−0.05	0.09	0.02	0.08
P	0.96	0.54	0.13	0.38	0.31	<0.05	0.07	0.80	0.62	0.39	0.82	0.43
Typical forms												
rho	−0.02	0.02	0.04	−0.03	−0.07	0.10	−0.17	0.06	−0.08	−0.01	−0.19	0.11
P	0.79	0.81	0.61	0.74	0.36	0.22	0.09	0.53	0.42	0.97	0.06	0.28

Statistically significant values are indicated in bold characters.

ratio (Fig. 2), blood/SP adiponectin ratio being higher in individuals without MS compared to those with MS.

To discriminate major parameters independently correlated to MS, we performed statistical logistic regression analysis including nine parameters (age, infertility time, sperm concentration, BMI, SHBG, total testosterone, inhibin B, seminal leptin, adiponectin serum/SP ratio) statistically different between the subjects with and without MS (Supplementary Table 3). The analysis shows that three parameters were independently associated to MS ($r^2=0.27$): BMI (OR=1.2, 95% CI=1.046–1.377, $P<0.01$), infertility duration (OR=1.017, 95% CI=1.0–1.034, $P<0.05$), and adiponectin serum/SP ratio (OR=0.986, 95% CI=0.976–0.996, $P<0.01$) (Table 4).

Discussion

Our study aimed to identify the potential relationships between circulating or SP adipokines and semen

parameters, and whether they were influenced by the metabolic status of male partners from infertile couples. To perform this work, we carried out a preliminary study to optimize the pre-analytical conditions for adipokine assays in SP, since this is a challenging and critical point. We validated a method that allowed us to obtain an homogeneous seminal fluid leading to excellent repeatability and reproducibility of the essays, comparable to those described by the manufacturers for blood samples (37).

Our study shows that all adipokines studied were present in SP. Most of them were higher in the blood than in the semen in the whole population (Fig. 2). This observation suggests that these adipokines could diffuse from the blood into the SP compartment, but we could not eliminate the possibility of a local production by the genital tracts. The opposite situation was observed for IL-6 and visfatin with higher concentrations in the SP than in the circulation, suggesting a local production. Accordingly, some studies revealed that Sertoli cells, Leydig cells and even germ cells produce IL-6 (39, 40). Furthermore, visfatin (nicotinamide phosphoribosyltransferase or NAMPT) is present and also released from human spermatozoa (41).

Regarding the MS, it can be pointed that 29.4% of the included men presented a MS, whereas this is the case for only 17.5% of French men aged from 18 to 50 years (42). This difference was also reported in a recent study showing that MS was more prevalent in male partners of infertile couples than in documented fertile men (17). Strikingly, we showed that men from infertile couples with MS had

Table 4 Logistic regression for multivariate analysis demonstrating factors independently correlated to MS ($n=132$, $r^2=0.27$).

	OR	P value	95% CI
Infertility duration	1.017	0.0465	1.0–1.034
BMI	1.200	0.0094	1.046–1.377
Adiponectin blood/SP ratio	0.986	0.0074	0.976–0.996

MS, metabolic syndrome; SP, seminal plasma.

a particularly long infertility period compared to those without MS (Tables 1 and 4). This strengthens the concept that the MS could impact male fertility. Thus, it could be useful to detect these patients early on in order to target a specific therapeutic management of their MS to improve their fertility. Indeed, some studies have shown that men exercising moderate physical activity had better sperm parameters than sedentary men (43, 44). Other studies have shown better sperm parameters in cases of strong adherence to a healthy diet (45, 46). In addition, a recent study showed that coaching a couple on lifestyle and diet during the pre-pregnancy period increased the pregnancy rate (47). Thus, it is tempting to speculate that improving MS through dietary and physical interventions will likely have a beneficial effect on sperm parameters and fertility.

The differences obtained in the concentrations of the different adipokines between SP and blood (Fig. 2) agree with the scarce data previously published (24, 48, 49). As reported, we didn't find any correlations between the two matrices for leptin, chemerin and visfatin (24). The blood/SP adipokine ratios were not different between individuals with or without MS, except for the blood/SP adiponectin ratio that was three-fold lower in subjects with MS as compared to those without MS. As expected, we found lower circulating adiponectin levels in the MS group than in the non-MS group (50). Unexpectedly, we observed an increase of SP adiponectin and subsequently a decrease of the adiponectin blood/SP ratio in the MS group compared to the non-MS group. Several hypotheses could be proposed to explain this increase of adiponectin in SP in patients with MS. First, there could be a local production of seminal adiponectin. Indeed, adiponectin receptors are expressed in human testes including the Leydig cells but also in the epididymis, and spermatozoa, and adiponectin is expressed in Leydig cells (51, 52, 53) even if, to the best of our knowledge, there is no indication of adiponectin production by the genital male tract up to now. Secondly, there could be an active or facilitated uptake of adiponectin from the blood to the seminal compartment. A recent study showed a decrease in tight junctions at the blood–testis barrier in mice infused intra-peritoneally by recombinant leptin (54). Considering the increased circulating leptin concentrations in patients with MS, it is conceivable that this leptin-induced disturbance in blood–testis barrier permeability could contribute to increased adiponectin transfer toward the SP. Finally, another possibility is that adiponectin could be protected from degradation in the SP compartment, with a slower turn-over. Our results indicate that increased adiponectin

in the SP was observed in MS subjects with poorer fertility. As well, discordant data regarding the beneficial effect of adiponectin was reported in the cardiometabolic field. Indeed, it has been shown that, while low levels of circulating adiponectin are generally considered as a cardiovascular risk factor, a pejorative cardiovascular outcome was reported in subjects with high adiponectin plasma value (55). Whatever the explanations of the increased SP adiponectin concentration in subjects with MS, there was no correlation between the levels of this adipokine and semen parameters, raising the possibility that adiponectin could act beyond these parameters on male fertility.

In contrast with other adipokines, we observed significant and inverse relationships between SP and plasma IL-6 levels and sperm parameters. Circulating IL-6 is usually considered as a biomarker of inflammation that would be rather deleterious and associated with infertility (56). Consistent with this concept, we found a negative correlation between circulating IL-6 and the number and motility of spermatozoa. Accordingly, CRP level was also negatively associated with sperm quality parameters stressing for a deleterious impact of low-grade systemic inflammation.

By contrast, we found a positive and perhaps a beneficial association between seminal IL-6, and functional parameters of sperm that could be linked to the non-inflammatory effect of IL-6, previously described for the skeletal muscle (57). Indeed, IL-6 was shown as a beneficial factor on metabolism when secreted by muscle under exercise and fasting, whereas it was deleterious when secreted by adipose tissue during inflammatory processes as observed in the context of obesity (58, 59). IL-6 effects on muscle cells have been reported to be mediated by activation of AMPK (5'-adenosine monophosphate-activated protein kinase) (58). The active phosphorylated form of AMPK (phospho-AMPK-Thr172) has been identified in pig, horse, mouse, rooster and human sperm, and particularly on the whole flagellum and acrosome for roosters, which suggests an important role in motility and acrosomal reaction (60, 61). AMPK is involved in the modulation of the nutritional function of the germ line by increasing the lactate production of Sertoli cells in response to an increase of glucose uptake (62), which could explain the positive association between IL-6 and sperm concentration. In addition, mice KO for AMPK α 1 become asthenospermic (63), an observation in agreement with the positive correlation we observed between IL-6 and sperm motility. AMPK would act by phosphorylation of the protein substrates involved in

the functioning of the central apparatus of the axoneme, which are essential for flagellar motility (64).

The findings of our study should be interpreted within certain limitations. The volume of the ejaculate is a factor limiting the number of assays that could be performed, which explains the disparity between adipokines in the number of both assays and analyses. Otherwise, there are frequent intra-individual variations between semen samples. However, to make the link between the blood and seminal parameters, it was important to collect blood and semen concomitantly. Another limitation is the absence of proven fertile control men. However, when comparing men with a previous fertility ($n=35$ for subjects without MS and $n=17$ for subjects with MS), we retrieved our main results i.e. an increase of SP adiponectin in patients with MS ($P<0.005$) and a positive trend between seminal IL-6 levels and sperm vitality ($P=0.05$). In addition, men with MS had lower testosterone levels as expected, but hormonal measurements were not performed by mass spectrometry and may thus be inaccurate at low concentrations. This result needs, therefore, to be interpreted with caution. Finally, we present an observational study, which does not allow mechanistic insights. However, despite these limitations, this study included a well-defined cohort of 160 subjects.

We found an independent association between MS in male partners of infertile couples and BMI, adiponectin serum/SP ratio and infertility time (Table 4) suggesting the importance of MS in male fertility. The data found on SP IL-6 suggest a potentially beneficial role of seminal IL-6 on progressive motility, sperm vitality, and sperm concentration. However, mechanistic studies are now needed to further investigate the local effects of IL-6 and adiponectin on male reproductive function in MS.

Supplementary materials

This is linked to the online version of the paper at <https://doi.org/10.1530/EJE-19-0615>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

B F, R L, and J-P B conceived and designed the study. Y E, A B, S F, C F, C M, and J-P B performed most of the experiments. P L, F F, and J-P B performed the statistical analysis. J C, J D, and R L critically revised the manuscript. B F and J-P B wrote the manuscript.

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