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ORIGINAL ARTICLE

Machine learning approach to assess the association between anthropometric, metabolic, and nutritional status and semen parameters

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Many lifestyle factors, such as nutritional imbalance leading to obesity, metabolic disorders, and nutritional deficiency, have been identified as potential risk factors for male infertility. The aim of this study was to evaluate the relationship between semen parameters and anthropometric, metabolic and nutritional parameters. Relationship was first assessed individually, then after the application of a previously constructed and validated machine learning score that allows their combination. Anthropometric, metabolic, antioxidant, micronutrient, and sperm parameters from 75 men suffering from idiopathic infertility from four infertility centers in France (Jean-Verdier ART Center Hospital, Bondy; North Hospital ART Center, Saint-Étienne; Navarre Polyclinic ART Center, Pau; and Cochin Hospital ART Center, Paris) between September 2009 and December 2013 were collected. After assessing standard correlation analysis, a previously built machine learning model, providing a score ranging from 0 (the poorest) to 1 (the most favorable), was calculated for each man in the study cohort. This machine learning model, which separates infertile/fertile men with unexplained infertility on the basis of their bioclinical signature, provides a more holistic evaluation of the influence of the considered markers (anthropometric, metabolic, and oxidative status). We observed a significant correlation of some anthropometric, metabolic, and nutritional disorders with some sperm characteristics. Moreover, an unfavorable machine learning score was associated with a high level of sperm DNA fragmentation. Favorable anthropometric, metabolic, and oxidative patterns, which may reflect an appropriate lifestyle, appear to positively impact overall health, in particular reproductive function. This study, consistent with previous publications, suggests that beyond semen quality parameters, in an essential assessment of male fertility, other key factors should be taken into account. In this regard, the application of emerging artificial intelligence techniques may provide a unique opportunity to integrate all these parameters and deliver personalized care.

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Keywords: lifestyle; machine learning; metabolism; nutrition; sperm DNA fragmentation

INTRODUCTION

As recently published by Levine *et al*.,[1] fertility has been declining worldwide for the past half century, especially sperm quality. Many lifestyle factors have been identified as potential risk factors for infertility in men. Among them, overweight and obesity have been particularly studied.[2] A global increase in the prevalence of obesity has been observed over the last three decades, and now, more than half of men in reproductive age are currently considered to be overweight or obese. It has been well established that overweight and obesity are associated with male infertility[3] and have a negative impact on semen parameters, including sperm count[2,4] and sperm DNA fragmentation.[5,6] In addition, metabolic disorders that are often the consequence of an unbalanced diet and/or insufficient physical activity also contribute to impaired reproductive function.[7]

Recently, our team has highlighted that not only metabolic syndrome but also metabolic disorders (increased fasting blood glucose) and anthropometric disorders (increased body mass index [BMI] and abdominal obesity) may be risk factors for idiopathic infertility.[8] We also observed that male partners of infertile couples were less physically active than fertile men.[9] They also had lower plasma levels of antioxidant vitamins.[10]

There are many complex mechanisms involved in these phenomena. Overweight, obesity, and metabolic disorders can lead to impaired functioning of the hypothalamic–pituitary–gonadal (HPG) axis. The process of converting steroids into estrogens in peripheral adipose tissue leads to an elevation in estradiol levels, which exerts a negative feedback loop at the hypothalamic–pituitary level. In these men, secondary hypogonadism is therefore observed, characterized by a decrease in serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone.[11,12] Hyperinsulinemia leads to a decrease in hepatic production of sex

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hormone-binding globulin (SHBG).[13] Consequences are increasing levels of free testosterone available for conversion to estradiol. Chronic systemic inflammation, which plays a critical role in the development of metabolic syndrome, is associated with obesity.[14] Levels of inflammatory cytokines (interleukin 1-alpha [IL-1A], interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α], activin A, *etc.*), which are essential for normal spermatogenesis, are disturbed.[15] Systemic oxidative stress is also associated with increased body weight and obesity.[16] Oxidative stress and lipid peroxidation are increased in the testicular microenvironment following the accumulation of adipose tissue.[17] Furthermore, high blood glucose levels have been shown to increase oxidative stress through mitochondrial oxidation of glucose, which releases a substantial amount of free radicals into the cytosol.[18] In addition, oxidative stress leads to the production of nitric oxide (NO) which oxidizes sperm membrane lipids. Oxidative sperm impairs spermatogenesis and sperm motility.[19] Germ cells are thought to be more sensitive to free radical oxidation than somatic cells because their plasma membranes contain a greater quantity of polyunsaturated fatty acids leading to lipid peroxidation.[20] Oxidative stress increases sperm damage and results in increased sperm DNA fragmentation, which affects early embryo development and future child health, and increases the risk of miscarriage.[21,22]

Thus, the mechanisms involved in the impact of obesity and metabolic disorders on male reproductive functions are numerous but strongly interlinked. Until recently, most of the studies have independently investigated the relationship between infertility and anthropometric, metabolic, and dietary elements. Therefore, identifying the parameter(s) involved in male or female infertility is challenging. Artificial intelligence, especially machine learning, has been adopted in scientific and medical research in the last few years, providing access to innovative and more powerful tools. We previously built and evaluated a machine learning (ML) model to identify and stratify fertile couples as well as those with idiopathic infertility. This score was based on a panel of lifestyle-influenced parameters, including the anthropometric, metabolic, and antioxidant status of both partners. This tool combines several easily measurable parameters and generates a score to assess the impact of anthropometric, metabolic, and antioxidant factors on the couple's risk of infertility. Although less efficient, we have also shown that this score, in its version built on male parameters alone, could identify almost 70% of men who were at risk of infertility.[10] However, a score combining different elements influenced by lifestyle has never been used to identify the risk of impaired sperm parameters.

The objective of this study was to evaluate the relationship between semen parameters and anthropometric, metabolic, and nutritional parameters. First, we analyzed each parameter individually, and then, we applied a previously constructed and validated machine learning score to combine them.

MATERIALS AND METHODS

Subjects

"ALImentation et FERTilité" (ALIFERT) is a cross-sectional case–control study enrolling infertile men between September 2009 and December 2013. This study is registered and approved by National Biomedical Research (Paris, France; Approval No. P071224), Ethics Committee of 'Protection of Persons Committee' (Paris, France; Approval No. AOM 2009-A00256–51), NEudra CT (Paris, France; Approval No. 08180), and ClinicalTrials.gov (No. NCT01093378). The design of ALIFERT was multicenter. The study cohort included 75 infertile men recruited from four infertility centers in France (Jean-Verdier ART Center Hospital, Bondy [JV]; North Hospital ART Center, Saint-Étienne [SE]; Navarre Polyclinic ART Center, Pau [PAU]; and Cochin Hospital ART Center, Paris [CCH]).

Men were between 18 years and 45 years old and exhibiting idiopathic infertility for over 12 months. Eligibility criteria have been described previously and include the provision of written informed consent.[10]

Eligibility criteria for patients were as follows: (1) primary idiopathic infertility >12 months; (2) age between 18 years and 45 years; (3) absence of severe oligozoospermia ($<5 \times 10^6$ ml^{-1}); (4) absence of male reproductive tract abnormalities such as undescended testis, varicocele, or infection; and (5) written informed consent. Patients with any current known or previous metabolic or digestive disease and smokers were excluded.

Assessments

Anthropometric, metabolic, antioxidant, and micronutrient parameters were assessed as described below.

Height, weight, visceral fat (Tanita BC-420MA analyzer; TANITA USA, Issaquah, WA, USA), and waist circumference measured at the narrowest point between the lower edge of the ribs and the iliac crest were measured by the investigator. The measurements were carried out by a trained investigator in the morning under fasting conditions.

Blood samples were obtained after 12 h of fasting for measurement of total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and glucose in fresh plasma. Both serum and plasma were frozen at −80°C until further analysis.

After 5 min of rest, systolic and diastolic blood pressures were assessed with a sphygmomanometer cuff around the patient's forearm while lying down. The values for systolic and diastolic pressures were calculated as the mean of the right and left measurements.

Finally, concentrations of serum vitamin D (ng ml^{-1}), vitamin B9 (folic acid-erytho; nmol l^{-1}), vitamin B9 (nmol l^{-1}), vitamin B12 (pmol l^{-1}), alpha-tocopherol (vitamin E; μmol l^{-1}), zinc (mmol l^{-1}), selenium (mmol l^{-1}), vitamin C (mg ml^{-1}), alpha-carotene (μmol l^{-1}), beta-carotene (μmol l^{-1}), lycopene (μmol l^{-1}), lutein (μmol l^{-1}), beta-cryptoxanthin (μmol l^{-1}), and retinol (vitamin A; μmol l^{-1}) levels were determined at the Department of Integrated Biology – Nutritional Biology and Oxidative Stress (Grenoble Hospital, Grenoble, France). Ascorbic acid (vitamin C) in serum was assessed by an automated continuous-flow method. The concentrations of retinol, tocopherol, and carotenoids (lutein, beta-cryptoxanthin, lycopene, alpha-carotene, and beta-carotene) in serum were obtained by high-performance liquid chromatographic (HPLC; Biotek-Kontron, Montigny-le-Bretonneux, France). Concentrations of serum zinc were measured by flame atomic absorption spectrometry (model 3110; PerkinElmer, Norwalk, CT, USA) and selenium by atomic absorption spectrometry (4100 ZL; PerkinElmer).

Semen quality parameters

The semen specimens were collected in the laboratory by masturbation into a sterile plastic cup after an abstinence period of 2 days to 5 days. The samples were liquefied at room temperature for 30 min, and conventional semen quality (semen volume, sperm concentration, vitality, and motility) was assessed according to the 2010 World Health Organization (WHO) guidelines.[23] David's criteria were used to evaluate sperm morphology.[24,25]

Sperm DNA fragmentation

The terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) technique with an *In Situ* Cell Death Detection Kit (Fluorescein; Roche Applied Science, Meylan, France) was used to determine sperm DNA fragmentation.

Machine learning model

In previous work, our team developed and evaluated three ML models, capable of separating infertile/fertile couples, men and women through their bioclinical signature, to facilitate the management of couples with unexplained infertility.[10] This signature was the result of a precise selection of biomarkers among hundreds of parameters investigated. It was based on the findings of a large-scale clinical study evaluating the impact of lifestyle and nutrition on couples' infertility. A major contribution of lifestyle parameters to the discriminatory power of the model had been demonstrated. The result generated and calculated by the model produces a score ranging from 0 (the highest risk of infertility with involvement of nutritional and metabolic factors) to 1 (the lowest risk, therefore rather healthy nutritional and metabolic profile). We calculated the model score for each man in the study cohort, using the formula obtained previously.[10] A score between 0 and 1 was calculated for each man in the cohort, with the aim of assessing more globally the nutritional and metabolic status on fertility (**Figure 1**) and comparing its results with sperm parameters.

Statistical analyses

Python 3.9.12 (Python Software Foundation) was used to carry out the statistical analysis, and the SciPy package version 1.9.3 was used. Data were shown as mean and standard deviation (s.d.). Correlation analysis of metabolic and sperm parameters was investigated by the Pearson's parametric test. $P < 0.05$ was considered statistically significant.

RESULTS

Data were summarized as mean and s.d. and shown in **Table 1**. The association between anthropometric, metabolic, and dietary parameters and semen parameters is presented in **Table 2**.

In this population of idiopathic infertile men, we observed a correlation between some anthropometric characteristics and semen parameters. A decrease in sperm motility was observed with high BMI ($r = -0.233$, $P < 0.05$). In addition, high visceral fat was associated with a decrease in sperm vitality and motility ($r = -0.285$, $P < 0.01$ and $r = -0.270$, $P < 0.05$, respectively).

Regarding the correlation between metabolic characteristics and sperm parameters, high levels of total cholesterol and serum LDL were associated with lower sperm count ($r = -0.272$ and $r = -0.249$, both $P < 0.05$). In addition, lower sperm vitality was observed in patients with high total cholesterol and serum LDL ($r = -0.311$ and $r = -0.326$, both $P < 0.01$). Finally, sperm DNA fragmentation was negatively correlated with serum HDL concentration ($r = -0.364$, $P < 0.01$).

A high concentration of certain antioxidants in the serum is associated with better sperm parameters. Correlation between sperm count and plasma glutathione peroxidase (GPX) activity ($r = 0.230$, $P < 0.05$), as well as between sperm motility and vitamin B12 (cobalamin) concentration, was observed ($r = 0.275$, $P < 0.05$).

An unfavorable machine learning score, based on a combination of anthropometric, metabolic, and oxidative status, was associated with a high level of sperm DNA fragmentation ($r = -0.263$, $P < 0.05$).

DISCUSSION

The aim of this study was to explore the impact of factors such as anthropometric and metabolic status along with the nutritional environment on semen parameters in a population of patients with unexplained infertility. This study explored the relationship between a circulating metabolic and nutritional signature and sperm DNA fragmentation, a qualitative marker of spermatogenesis.

We observed a significant correlation of some anthropometric, metabolic, and nutritional disorders with some sperm characteristics.

Table 1: Baseline characteristics from clinical, biological, and sperm analysis of patients (*n*=75)

Parameter	*Values, mean±s.d.*
Baseline, metabolic, and biological parameters	
Age (year)	33.6±5.3
BMI (kg m^{-2})	26.1±4.3
Waist measurement (cm)	92.6±11.3
Visceral fat (%)	7.0±4.2
Systolic blood pressure (mmHg)[a]	126.8±12.2
Diastolic blood pressure (mmHg)[a]	80.8±10.0
Glycemia (mmol l^{-1})	5.0±0.7
Total cholesterol (mmol l^{-1})	5.2±1.0
LDL (mmol l^{-1})	3.3±1.0
HDL (mmol l^{-1})	1.3±0.4
Triglyceride (mmol l^{-1})	1.4±0.9
Micronutrients and vitamins	
Vitamin D (ng ml^{-1})	23.2±11.3
Folate (nmol l^{-1})	12.7±7.2
Cobalamin (pmol l^{-1})	300.0±111.0
Retinol (µmol l^{-1})	2.1±0.5
Alpha-tocopherol (µmol l^{-1})	25.0±6.1
Zinc (µmol l^{-1})	12.8±1.9
Selenium (mmol l^{-1})	1.2±0.2
GPX (mUI ml^{-1})	391.6±61.0
Ascorbic acid (mg ml^{-1}) [a]	42.3±18.8
Alpha-carotene (µmol l^{-1})	0.1±0.1
Lycopene (µmol l^{-1})	0.6±0.3
Lutein (µmol l^{-1})	0.3±0.2
Beta-cryptoxanthin (µmol l^{-1})	0.2±0.2
Glutathione (µmol l^{-1})	829.6±200.0
Beta-carotene (µmol l^{-1})	0.4±0.3
ML model score	0.5±0.1
Semen volume (ml)	3.4±1.6
Sperm concentration (×10^6 ml^{-1})	44.2±29.8
Total sperm count (×10^6)	143.7±109.4
Progressive motility (a + b), %	37.5±11.6
Vitality (%)	63.4±15.4
Normal morphology (%)	20.7±9.9
DNA fragmentation rate (%)	28.6±18.4

[a]Four patients with unknown systolic blood pressure (mmHg) and diastolic blood pressure (mmHg), and 6 patients with unknown ascorbic acid. s.d.: standard deviation; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ML: machine learning; GPX: glutathione peroxidase

These associations were not as pronounced as those described in other publications.[2,4–6] This difference may be related to the fact that men in our study did not have major alterations in conventional semen parameters. Moreover, traditional sperm parameters are known to be highly variable between ejaculates, meaning intraindividual variability. DNA fragmentation, on the other hand, appears to be more stable across ejaculates. Traditional univariate analyses, particularly simple pair-wise correlations, may have limitations in this work, given this particular population, and may lack the power to capture more complex associations. The contribution and integration of new methods from artificial intelligence may provide an interesting way to better process available clinical and biological data.

The fertility score previously developed and published by our team was built on anthropometric, metabolic, and antioxidative factors, which were parameters close to those studied in this work. As shown in **Figure 1**, the result of the score was put into perspective with

Table 2: Correlations (Pearson's parametric test) between the studied parameters and sperm quality

Parameter	*Semen volume (ml), r (P)*	*Sperm concentration ($\times 10^6$ ml^{-1}), r (P)*	*Total sperm count ($\times 10^6$), r (P)*	*Progressive motility (a + b, %), r (P)*	*Vitality (%), r (P)*	*Normal morphology (%), r (P)*	*DNA fragmentation rate (%), r (P)*
Baseline, metabolic, and biological parameters							
Age (year)	−0.142 (NS)	0.243 (NS)	0.199 (NS)	−0.066 (NS)	−0.119 (NS)	0.045 (NS)	0.107 (NS)
BMI (kg m^{-2})	−0.307*	−0.080 (NS)	−0.136 (NS)	−0.233*	−0.217 (NS)	−0.068 (NS)	0.078 (NS)
Waist measurement (cm)	−0.286*	−0.102 (NS)	−0.157 (NS)	−0.191 (NS)	−0.169 (NS)	0.015 (NS)	0.152 (NS)
Visceral fat (%)	−0.221 (NS)	−0.060 (NS)	−0.062 (NS)	−0.270*	−0.285**	−0.067 (NS)	0.152 (NS)
Blood pressure systolic (mmHg)	−0.194 (NS)	0.129 (NS)	0.053 (NS)	0.087 (NS)	−0.045 (NS)	0.073 (NS)	−0.120 (NS)
Blood pressure diastolic (mmHg)	−0.23 (NS)	0.187 (NS)	0.078 (NS)	−0.011 (NS)	−0.107 (NS)	0 (NS)	−0.088 (NS)
Glycemia (mmol l^{-1})	0.059 (NS)	0.127 (NS)	0.132 (NS)	−0.147 (NS)	−0.166 (NS)	0.018 (NS)	−0.064 (NS)
Total cholesterol (mmol l^{-1})	−0.189 (NS)	−0.155 (NS)	−0.272*	−0.211 (NS)	−0.311**	−0.117 (NS)	−0.034 (NS)
LDL (mmol l^{-1})	−0.130 (NS)	−0.201 (NS)	−0.249*	−0.208 (NS)	−0.326**	−0.022 (NS)	0.053 (NS)
HDL (mmol l^{-1})	0.031 (NS)	−0.045 (NS)	−0.054 (NS)	−0.003 (NS)	0.011 (NS)	−0.186 (NS)	−0.364**
Triglycerides (mmol l^{-1})	−0.152 (NS)	0.088 (NS)	−0.040 (NS)	−0.016 (NS)	−0.041 (NS)	−0.066 (NS)	0.100 (NS)
Micronutrients and vitamins							
Vitamin D (ng ml^{-1})	0.208 (NS)	−0.028 (NS)	0.095 (NS)	−0.077 (NS)	0.002 (NS)	0.120 (NS)	0.149 (NS)
Folate (nmol l^{-1})	0.090 (NS)	−0.121 (NS)	0 (NS)	0.197 (NS)	0.027 (NS)	0.155 (NS)	0.161 (NS)
Cobalamin (pmol l^{-1})	−0.173 (NS)	0.057 (NS)	−0.080 (NS)	0.275*	−0.063 (NS)	0.106 (NS)	0.074 (NS)
Retinol (µmol l^{-1})	−0.049 (NS)	−0.046 (NS)	−0.076 (NS)	0.025 (NS)	0.159 (NS)	−0.037 (NS)	0.126 (NS)
Alpha-tocopherol (µmol l^{-1})	−0.042 (NS)	−0.194 (NS)	−0.226 (NS)	0.050 (NS)	−0.035 (NS)	−0.105 (NS)	−0.043 (NS)
Zinc (µmol l^{-1})	−0.039 (NS)	−0.001 (NS)	0.008 (NS)	−0.122 (NS)	0.124 (NS)	0.043 (NS)	0.027 (NS)
Selenium (mmol l^{-1})	−0.031 (NS)	0.022 (NS)	0.015 (NS)	0.051 (NS)	−0.105 (NS)	0.030 (NS)	0.070 (NS)
GPX (mUI ml^{-1})	0.055 (NS)	0.204 (NS)	0.230*	0.017 (NS)	−0.182 (NS)	−0.053 (NS)	0.002 (NS)
Ascorbic acid (mg ml^{-1})	−0.114 (NS)	−0.077 (NS)	−0.145 (NS)	0.141 (NS)	0.067 (NS)	0.091 (NS)	0.129 (NS)
Alpha-carotene (µmol l^{-1})	−0.082 (NS)	−0.003 (NS)	−0.050 (NS)	−0.042 (NS)	0.041 (NS)	−0.05 (NS)	−0.041 (NS)
Lycopene (µmol l^{-1})	0.130 (NS)	−0.111 (NS)	−0.074 (NS)	−0.032 (NS)	−0.221 (NS)	−0.117 (NS)	−0.077 (NS)
Lutein (µmol l^{-1})	−0.086 (NS)	−0.148 (NS)	−0.872 (NS)	0.149 (NS)	−0.050 (NS)	0.009 (NS)	0.073 (NS)
Beta-cryptoxanthin	−0.146 (NS)	−0.028 (NS)	−0.112 (NS)	0.069 (NS)	−0.018 (NS)	0.045 (NS)	−0.086 (NS)
Glutathione (µmol l^{-1})	0.137 (NS)	−0.041 (NS)	0.067 (NS)	−0.003 (NS)	−0.078 (NS)	−0.035 (NS)	0.135 (NS)
Beta-carotene (µmol l^{-1})	0.022 (NS)	−0.078 (NS)	−0.111 (NS)	−0.104 (NS)	−0.140 (NS)	0.044 (NS)	−0.059 (NS)
ML model score	0.091 (NS)	0.008 (NS)	−0.008 (NS)	0.132 (NS)	0.167 (NS)	−0.060 (NS)	−0.263*

*P<0.05; **P<0.01. NS: not significant; BMI: body mass index; LDL: low-density lipoprotein; HDL: high-density lipoprotein; ML: machine learning; GPX: glutathione peroxidase

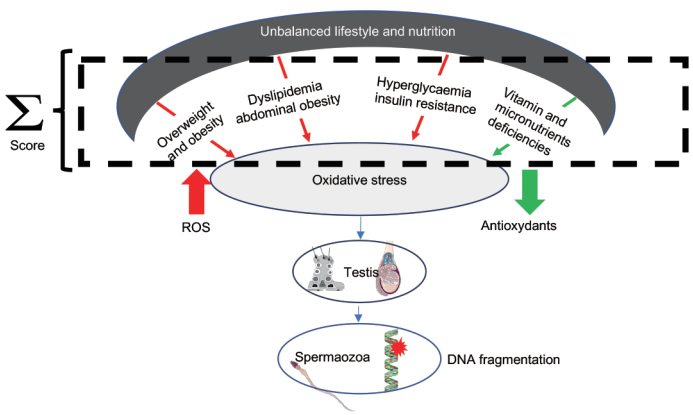

Figure 1: A complex relationship between lifestyle, diet, antioxidants, and sperm DNA fragmentation. An unbalanced lifestyle and diet lead to anthropometric, metabolic, and nutritional changes, which can lead to an imbalance in oxidative stress. These consequences are multiple and interrelated, and the use of artificial intelligence represents a unique opportunity to provide a more holistic evaluation of the influence of the considered marker. This oxidative stress could be one of the main drivers leading to a decline in male reproductive functions and consequently in sperm parameters, in particular sperm DNA fragmentation. ROS: reactive oxygen species.

sperm parameters. Interestingly, this score is the only feature, along with HDL level, that correlates significantly with DNA fragmentation level. Notwithstanding controversial findings in the literature, DNA fragmentation level is widely recognized as a marker of intrinsic semen quality. Its levels seem to affect embryo quality, implantation, and miscarriage risk.[26] Many factors can increase the level of sperm DNA fragmentation, including obesity, high local temperature, drug treatment, exposure to environmental pollutants, and smoking.

Using the strength of multivariate statistical modeling, the score may reveal underlying phenomena that are difficult to identify using conventional univariate methods alone. By correlating significantly with DNA fragmentation, the score may provide a more comprehensive way of assessing male semen quality, particularly in patients with normal or subnormal semen. This assumption must be demonstrated by further studies. Furthermore, from an analytical and technical point of view, DNA fragmentation is a more challenging and less standardized biomarker than other semen analysis parameters.

Although only men with no severe sperm alteration were recruited, a negative correlation between BMI, visceral fat, and sperm motility was observed. Obesity in males has been found to be a risk factor for infertility and increased time to conception.[3] In men, many studies, supported by a meta-analysis,[27] have reported an alteration of sperm parameters in case of overweight and obesity, more specifically a decrease in sperm concentration and sperm count.[28–30] Sperm motility also appears to be affected by overweight or obesity.[31]

Dyslipidemia has been reported to negatively affect testicular and epididymal function, sperm maturation and quality, and ejaculatory function.[32] Consistent with these findings, in this population of patients with unexplained infertility, we observed an association between lipid levels and some semen parameters. Indeed, despite the absence of patients with dyslipidemia, unfavorable lipid composition (high total and LDL cholesterol and/or low HDL cholesterol) may be negatively associated with semen parameters. However, while blood glucose was a strongly discriminating factor between fertile and infertile couples (as studied previously[10]), it does not appear to be correlated with sperm parameters in this population of infertile men. The molecular mechanisms involved in this phenomenon are unclear, but hypotheses have been proposed. Impairment of steroidogenesis has been reported. Cholesterol may modulate the renin–angiotensin system in the testes, leading to the inhibition of steroidogenesis. This, in turn, leads to a decrease in testosterone production.[33] Posttesticular sperm maturation may also be affected. Hypercholesterolemia would induce changes in the epididymal epithelium like an accumulation of cholesterol ester lipid droplets in the smooth muscle of the epididymal tract. Epididymal peristalsis contractions would be impaired, compromising sperm progression inside the epididymal lumen, and thus, the maturation process.[34] Eventually, an increase of oxidative stress and an excess of free radical production could also be the consequence of dyslipidemia.[35] This last hypothesis is consistent with our findings. Indeed, in our cohort, the HDL level (the "good" cholesterol level) was correlated with the DNA fragmentation level, directly related to oxidative stress.

Besides overweight and lipid and carbohydrate profile, some microelements and vitamins were positively correlated with sperm count and motility. Vitamin B12 levels and GPX activity were correlated with sperm motility and total sperm count, respectively, in this cohort. A meta-analysis by Banihani[36] highlighted the importance of vitamin B12 in sperm physiology and quality, particularly on total sperm count.[37] Indeed, lower vitamin B12 levels have been reported in infertile men.[38] Several beneficial mechanisms of vitamin B12 have been described, including its link to homocysteine metabolism and its antioxidant properties.[39]

Numerous studies have demonstrated the importance of the GPX during spermatogenesis and its relationship with male fertility.[40,41] Similarly, previous studies have reported higher GPX activity in seminal plasma in patients with better sperm quality.[42,43] GPX plays a key function as reactive oxygen species (ROS) scavengers for spermatozoa, to maintain the balance between ROS production and recycling.[44]

A limitation of this study was the lack of measurement of antioxidant status in seminal plasma for the entire cohort of patients. In addition, in accordance with international recommendations,[45–47] exploratory andrological assessments (hormone tests, ultrasound, *etc.*) were not carried out in our patients.

This study focused on a particular population of men from couples with unexplained infertility, and a limited population size of 75 patients. However, measurement of sperm parameters alone, which were normal or subnormal in this population, does not appear to be sufficient to evaluate and stratify these patients. These findings suggest that despite normal or subnormal semen parameters, the metabolic and oxidative status factors considered in this study, which are probably in part linked to the lifestyle and behavior of these patients, should be integrated into a holistic and comprehensive diagnostic and therapeutic strategy. Unlike other factors or causes of infertility (genetic or hormonal abnormalities, chemotherapy, *etc.*), in this case, patients can be involved in the management of their health, especially given the reversible character of these indicators. Indeed, an improvement in metabolic and oxidative status could be associated with a reduced risk of infertility, and even a better chance of success when *in vitro* fertilization (IVF) treatment is still required.

Improving the lifestyle of these patients therefore appears to be essential, and even beyond reproductive health alone, for overall physical and mental well-being. Such improvement should be advised by health-care professionals, using dietary and physical activity coaching or guidance properly designed and validated by international guidelines. Interventional studies are needed to confirm the positive impact of lifestyle improvement.[48]

CONCLUSION

Favorable anthropometric, metabolic, and oxidative patterns, which may reflect an appropriate lifestyle, appear to positively impact overall health, in particular reproductive function. Despite normal or subnormal semen parameters, findings reported in this work using patients from a cohort of idiopathic infertile couples show that altered body composition, as well as unfavorable lipid balance and vitamin and microelement deficiency, was associated with worse semen parameters.

This study, consistent with previous publications in this area, suggests that beyond semen quality parameters, in an essential assessment of male fertility, other key factors should be taken into account. A more comprehensive evaluation of the patients' health, affecting their nutritional, metabolic, and oxidative status, seems to be an indispensable element to consider for more holistic care. In this regard, the application of emerging artificial intelligence techniques may provide a unique opportunity to integrate all these parameters and deliver personalized care.

AUTHOR CONTRIBUTIONS

GB participated in the study's conception and design, performed statistical analysis, and drafted the manuscript. RL supervised the study, participated in patient recruitment, interpreted the data, and critically revised the manuscript for intellectual content. CF was involved in study conception and design, patient recruitment, interpretation of data, and critical revision of the manuscript for intellectual content. SC and AL both worked on the study conception and design and critically revised the manuscript for intellectual content. CD contributed to the study conception and design, patient recruitment, interpretation of data, and drafting of the manuscript. The ALIFERT collaborative group members contributed to the study design and were involved in patient recruitment or analysis. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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