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Male infertility and the impact of lifestyle in the Greek population: A case–control study

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Abstract

Background and Aims: Collection of epidemiological data has become a crucial step in every fertility evaluation, especially regarding idiopathic male infertility. Information on data such as tobacco smoking, alcohol intake, and body mass index can provide crucial information regarding the dynamics between fertility status and everyday practices. We aim to set the base for epidemiological studies on male infertility in the Greek population.

Methods: Four hundred and fourteen Greek volunteers were asked to fill in a questionnaire regarding their characteristics and lifestyle preferences, followed by a seminogram. Depending on their answers, they were divided into groups and data were analyzed for correlation with seminogram parameters using Spearman's rank correlation test.

Results: Our results indicate that a high body mass index (BMI) is negatively correlated with all three seminogram parameters (number, motility, and morphology) and exposure to radiation or chemicals is negatively correlated with sperm motility, with a $p < 0.01$.

Conclusions: These findings indicate negative correlations of BMI and exposure to radiation/chemicals with semen parameters in the Greek population. Such information can be used to plan a diagnostic approach or even therapeutic interventions.

KEYWORDS
correlation, epidemiology, Greek population, male infertility

1 | INTRODUCTION

Infertility, described as the inability to achieve pregnancy after a year of unprotected intercourse, affects 10%–15% of couples worldwide and has become one of the most explored conditions analyzed in the recent years. It has been found that the male factor contributes up to 50% of all cases.[1] Male infertility can include cases of oligospermia ($\leq 15 \times 10^6 \, \text{ml}^{-1}$, total sperm count), asthenozoospermia ($\leq 39 \times 10^6$, total motility), teratozoospermia ($\leq 4\%$ spermatozoa with normal morphology), a combination of the aforementioned, and azoospermia (absence of living spermatozoa in the ejaculate), which can be divided into two categories: obstructive (due to injuries, surgery, or

ejaculatory duct pathology) and nonobstructive azoospermia (due to intrinsic testicular impairment and hormonal imbalances).[2] The basic diagnostic approach specialists use for the infertile male includes a seminogram, ultrasound of the testes, physical examination, a full history of the patient's possible injuries or surgeries, family history of infertility, and hormonal testing.[3]

These methods, albeit useful, are not always conclusive regarding the patient's cause of infertility, a finding that proposes the importance of genetics in the pathogenesis of the disease. Advances in research methods and the development of techniques such as transcriptome sequencing (RNA-seq), exome sequencing, DNA genotyping applied in genome-wide association studies, and subsequent bioinformatics analyses have aided researchers to pinpoint polymorphisms, pathways, and genes that play an important role in male infertility,[4–8] especially for idiopathic or unexplained cases.

Apart from the genetic landscape of the disease, which should be thoroughly analyzed, one should not ignore the information collected from epidemiological and lifestyle data. There have been numerous studies[9–12] that pose the question whether lifestyle choices can affect sperm quality and cause infertility and it is slowly becoming a trend in the field to obtain additional patient data in the form of questionnaires and surveys. The implementation of epidemiological data is of great importance as it proposes that exposure to environmental factors can lead to male infertility and these. These factors include increasing age, cigarette smoking, alcohol, exposure to chemical agents or radiation, obesity, and drug usage among others.

The other important and useful perspective of utilizing questionnaires and epidemiological data is the creation of databases. Major foreign countries such as Finland (Finngen), China,[13] England,[14] and Austria (Graz Biobank) have created databases for their population, with data related to serious diseases such as cancer, brain diseases, obesity, and infertility of both gender. The creation of such a database should be done with a careful planning and accuracy regarding the data that will be collected from either public hospitals, private clinics, or private microbiological clinics.

In this study, epidemiological data from 414 Greek individuals were collected via questionnaire, to construct a database for the Greek population containing useful information regarding lifestyle factors. These data were utilized to perform correlations for each parameter examined and elucidate a potential role in the pathogenesis of male infertility.

Epidemiological studies regarding the effects of lifestyle choices are sparse and this is due to the inability to obtain useful and valid information from patients. This endeavor sets the base for the epidemiological studies on the Greek population in the future, since it is a very minimally examined population, both epidemiologically but also genetically, for the trait of male infertility.

In conclusion, it is clear that assessing the fertility of each individual should be a holistic approach for health professionals, including all possible factors. Examining each patient individually by taking a detailed history, seminogram, and a lifestyle survey, as well as various questionnaire responses, can provide new insights into infertility management.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 414 males aged 18–40 years old were recruited for this study by attending the IVF unit Embryolab in Thessaloniki Greece by volunteering for the Spermogene program. Patients were part of the general population with no exclusion criteria. Semen and blood samples were collected from individuals (cases group and control group) under written consent and the study was approved by the Ethics Committee of the University of Thessaly, Greece. All volunteers who were recruited for the study underwent an andrological examination including recording their medical history and a semen analysis. Semen samples were collected via masturbation after a 2–3-day abstinence and were left to liquify at 37°C for 30 min. The main analysis (seminogram) was performed using the Cell vision counting slides (Tek-Event) for cell counting and observation on Nikon Eclipse TS100, Nikon Eclipse E200, and Nikon Eclipse Ts2 microscopes. All the semen parameters were determined based on the criteria by World Health Organization guidelines.[15]

TABLE 1 Phenotypes, ages, and seminogram parameters of the groups analyzed

Phenotypic group	Age (mean ± SD)	Sperm count (mean ± SD)	Total motility (mean ± SD)	Sperm morphology (mean ± SD)
Normozoospermia (n = 237)	34.4 ± 7	42 ± 28.8	62.4 ± 8.7	7.8 ± 2.84
Asthenozoospermia (n = 4)	27.5 ± 4.38	25.7 ± 10.8	38.2 ± 5.53	4.7 ± 0.8
Asthenoteratozoospermia (n = 8)	36.35 ± 6.23	30.25 ± 14.7	31.1 ± 10.8	2.5 ± 0.5
Teratospermia (n = 29)	38.7 ± 5.5	25.5 ± 10.4	55.6 ± 9.8	2.41 ± 0.6
Oligoasthenozoospermia (n = 2)	36 ± 3	13.5 ± 0.5	39 ± 1	6 ± 1
Oligoasthenoteratozoospermia (n = 83)	38.5 ± 5.79	2.22 ± 3.66	11 ± 15	0.55 ± 0.83
Oligospermia (n = 22)	35.5 ± 6.74	9.22 ± 4	56.9 ± 10.4	6.63 ± 3.61
Oligoteratozoospermia (n = 29)	35.1 ± 5.2	8.24 ± 4.7	52.4 ± 9	2.24 ± 0.7

Among the participants, 237 were found to be normospermic regarding their semen parameters (sperm count $\geq 15 \times 10^6\,ml^{-1}$, total sperm count $> 39 \times 10^6$, total motility >40% motile sperm, vitality >58% living sperm, sperm with normal morphology >4%). Remaining individuals were found to have abnormal seminogram parameters and were asthenozoospermic ($n = 4$), asthenoteratozoospermic ($n = 8$), teratozoospermic ($n = 29$), oligoasthenozoospermic ($n = 2$), oligoasthenoteratozoospermic ($n = 83$), oligozoospermic ($n = 22$), and oligoteratozoospermic ($n = 29$). All patients had been screened and found negative for Y-chromosome microdeletions and had no history of varicocele. All information regarding individuals and their phenotypes is presented in Table 1.

Then, all volunteers were given a questionnaire to fill in, containing questions about their lifestyle and everyday activities. Additionally, information regarding their age, previous successful pregnancy outcomes, employment, and medical data regarding medications were gathered.

Volunteers had to choose between different answers to summarize findings in an orderly way.

The questions posed and the numbers of the groups assigned to each answer are presented in Figure 1 as a conceptual framework and in Table 2.

Measurements of age, height (cm), and weight (kg) were made accurate to one decimal and body mass index (BMI) was calculated using the formula BMI $= kg/m^2$, where kg is a person's weight in kilograms and m^2 is their height in meters squared. Regarding BMI, the classification of individuals was based on Centers for Disease Control and Prevention criteria, and they were divided into four groups: underweight ($\leq 18.50\,kg/m^2$), normal BMI and weight ($18.50\,kg/m^2 \leq BMI < 25.00\,kg/m^2$), overweight ($25.00\,kg/m^2 \leq BMI$

< $30.00\,kg/m^2$), and obese (BMI $\geq 30\,kg/m^2$). Details are presented in Table 3.

To test for correlations, all parameters were classified into groups represented by numbers (1, 2, 3) as seen in Table 2, depending on the volunteers' answers to the questionnaire. To test for correlation, one should apply the Shapiro–Wilk test for normality at first to determine if the data are normally distributed. Our data exhibited abnormal distribution, so a nonparametric test was applied.

The Spearman's rank correlation coefficient was applied to test for statistical significance between the groups analyzed through the R working interface. This test is a measure of the power of a linear correlation between two variables. The strength of the correlation is expressed by the value of r, a variable with values ranging from −1 to 1. Values greater than zero indicate a positive correlation, while values less than zero indicate a negative correlation. Nevertheless, the values of r, which are very close to zero, indicate a low or noncorrelation. For all tests, a p value of 0.01 was considered as statistically significant. Tables 3–7 present the sample numbers and the phenotypic distribution of each group created for correlation testing.

It should be noted here that six individuals have not answered whether they have used drugs and were excluded.

3 | RESULTS

3.1 | Age

Ages of 414 men were tested for correlation against semen parameters such as number, motility, and morphology. The statistical tests mentioned above were applied, and in terms of aging, the following were found as shown in Figure 2.

It is evident that increasing age of the male negatively effects sperm number via a slight negative correlation, but also greatly affects sperm motility and morphology as described by the correlation plot.

3.2 | Smoking

In the sample of our study of 414 people, 57.2% answered that they do not smoke at all or have quit years ago, 16.42% smoke 1 pack a

FIGURE 1 Conceptual framework for utilizing questionnaires

TABLE 2 Questions included in the volunteer questionnaire, grouping for correlation testing

Question	Possible answers
Do you smoke? If yes, how often?	No (1), Occasionally (2), Yes a pack/day (3), Yes more than a pack/day (4), Yes (not stating frequency) (5)
Have you used drugs?	No (1), Yes in the last 6 months (2), Yes in the last 1 year (3)
Have you been exposed to any chemicals or radiation?	Yes (1), No (2)

TABLE 3 Sample numbers and phenotypic distribution for the trait of BMI

BMI	Underweight	Normal weight	Overweight	Obese
Sample number	1	139	182	91
Phenotype distribution	1 Normal	84 Normal, 1 NAN, 4 NAT, 9 NNT, 1 OAN, 28 OAT, 5 ONN, 6 ONT	88 Normal, 1 NAN, 5 NAT, 10 NNT, 50 OAT, 12 ONN, 6 ONT	30 Normal, 1 NAT, 7 NNT, 33 OAT, 4 ONN, 16 ONT
Parameters range (number/motility/morphology)	41.5×10^{6}/ 71%/9%	$0\text{–}209 \times 10^{6}$/0%–78%/0%–16%	$0\text{–}161 \times 10^{6}$/0%–86%/0%–20%	$0\text{–}130 \times 10^{6}$/0%–77%/0%–15%

Abbreviations: NAN, asthenozoospermic; NAT, asthenoteratozoospermic; NNT, teratozoospermic; OAN, oligoasthenozoospermic; OAT, oligoasthenoteratozoospermic; ONN, oligozoospermic; ONT, oligoteratozoospermic.

TABLE 4 Sample numbers and phenotypic distribution for the trait of smoking

Smoking	No	Occasionally	Yes, a pack/day	Yes, more than pack/day	Yes, not stating frequency
Sample number	237	65	68	16	28
Phenotype distribution	115 Normal, 2 NAN, 4 NAT, 12 NNT, 1 OAN, 67 OAT, 14 ONN, 20 ONT	37 Normal, 1 NAT, 3 NNT, 16 OAT, 4 ONN, 4 ONT	29 Normal, 3 NAT, 7 NNT, 17 OAT, 2 ONN, 10 ONT	5 Normal, 1 NNT, 9 OAT, 1 ONN	17 Normal, 3 NNT, 2 OAT, 1 ONN, 5 ONT
Parameters range (number/ motility/morphology)	$0\text{–}209 \times 10^{6}$/0%–84%/0%–20%	$0\text{–}137.5 \times 10^{6}$/0%–79%/0%–16%	$0\text{–}160 \times 10^{6}$/0%–86%/0%–14%	$0\text{–}56 \times 10^{6}$/ 0%–69%/0%–12%	$1.4\text{–}78 \times 10^{6}$/ 39%–75%/2%–13%

Abbreviations: NAN, asthenozoospermic; NAT, asthenoteratozoospermic; NNT, teratozoospermic; OAN, oligoasthenozoospermic; OAT, oligoasthenoteratozoospermic; ONN, oligozoospermic; ONT, oligoteratozoospermic.

TABLE 5 Sample numbers and phenotypic distribution for the trait of drug usage

Drugs	No	Yes, in the last 6 months	Yes, in the last year
Sample number	331	34	43
Phenotype distribution	155 Normal, 2 NAN, 8 NAT, 18 NNT, 1 OAN, 95 OAT, 17 ONN, 35 ONT	24 Normal, 2 NAT, 3 NNT, 4 OAT, 1 ONT	22 Normal, 4 NNT, 9 OAT, 5 ONN, 3 ONT
Parameters range (number/motility/morphology)	$0–209 \times 10^6$/0%–86%/0%–20%	$0–137.5 \times 10^6$/0%–79%/0%–15%	$0–161 \times 10^6$/0%–84%/0%–13%

Note: Six individuals have not answered whether they have used drugs and were excluded.

Abbreviations: NAN, asthenozoospermic; NAT, asthenoteratozoospermic; NNT, teratozoospermic; OAN, oligoasthenozoospermic; OAT, oligoasthenoteratozoospermic; ONN, oligozoospermic; ONT, oligoteratozoospermic.

**TABLE 6** Sample numbers and phenotypic distribution for the trait of alcohol intake

Alcohol	None	2 drinks/week	Less than 2 drinks/week	More than 2 drinks/week
Sample number	1	101	250	62
Phenotype distribution	1 OAT	49 Normal, 3 NAT, 9 NNT, 22 OAT, 7 ONN, 11 ONT	117 Normal, 2 NAN, 6 NAT, 13 NNT, 1 OAN, 76 OAT, 12 ONN, 23 ONT	37 Normal, 1 NAT, 4 NNT, 12 OAT, 3 ONN, 5 ONT
Parameters range (number/motility/morphology)	0/0/0	$0–161 \times 10^6$/0%–86%/0%–14%	$0–209 \times 10^6$/0%–80%/0%–20%	$0–115 \times 10^6$/0%–84%/0%–16%

Abbreviations: NAN, asthenozoospermic; NAT, asthenoteratozoospermic; NNT, teratozoospermic; OAN, oligoasthenozoospermic; OAT, oligoasthenoteratozoospermic; ONN, oligozoospermic; ONT, oligoteratozoospermic.

TABLE 7 Sample numbers and phenotypic distribution for the trait of radiation and chemicals exposure

Radiation/chemicals	No	Yes
Sample number	342	72
Phenotype distribution	173 Normal, 2 NAN, 8 NAT, 23 NNT, 84 OAT, 18 ONN, 34 ONT	30 Normal, 2 NAT, 3 NNT, 27 OAT, 4 ONN, 5 ONT
Parameters range (number/motility/morphology)	$0–209 \times 10^6$/0%–86%/0%–20%	$0–130 \times 10^6$/0%–79%/0%–15%

Abbreviations: NAN, asthenozoospermic; NAT, asthenoteratozoospermic; NNT, teratozoospermic; OAN, oligoasthenozoospermic; OAT, oligoasthenoteratozoospermic; ONN, oligozoospermic; ONT, oligoteratozoospermic.
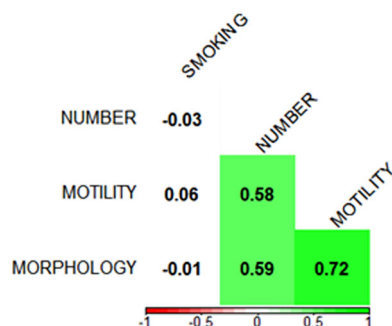
FIGURE 2 Correlation plot for increasing age and sperm parameters
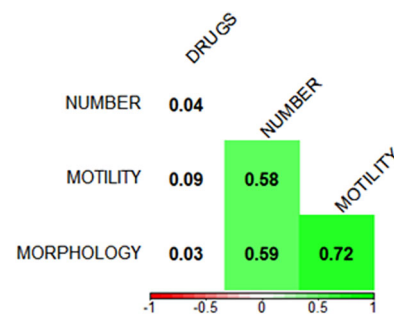
FIGURE 3 Correlation plot for smoking frequency and sperm parameters

day, 3.86% smoke more than 1 pack a day, 15.7% smoke occasionally, while 6.76% smoke but do not determine the frequency.

The statistical tests mentioned above were applied, and in terms of smoking, the following were found as shown in Figure 3.

According to the analysis, the smoking frequency does not show any correlation with the sperm parameters as for each of them (number, motility, and morphology) the value of the *r* coefficient is very close to zero.
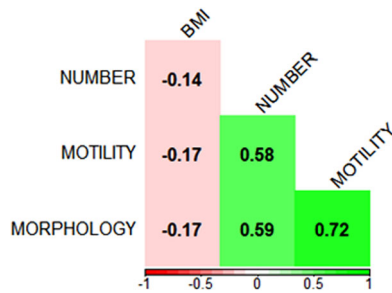
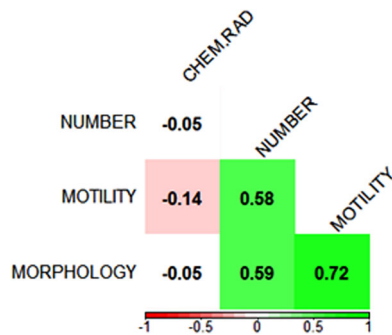**FIGURE 4** Correlation plot for drug/substance use and sperm parameters

FIGURE 5 Correlation plot for body mass index and sperm parameters

3.3 | Drug use

The biggest percentage of volunteers in the study (79.7%) have not used drugs, while 10.62% have used them more than a year ago, with 7.97% having used them in the last 6 months. Drug usage concerns mainly recreational drugs and not intravenous drugs. Figure 4 represents the correlations.

According to the analysis, drug use has no correlation with sperm parameters as for each of them (number, motility and morphology) the value of the r coefficient is very close to zero.

3.4 | BMI

The BMI is obtained through an equation that includes the height and weight of the person. It is a parameter that can be divided into groups that indicate the relationship of the individual with the level of obesity. People with a BMI below 18.5 are considered to be underweight, BMI of 18.5–25 as normal weight, and BMI of 25–30 as overweight, while BMI values above 30 indicate obesity. In our sample, only 0.24% were underweight, 33.57% had normal BMI, 43.9% were overweight, and 21.9% were obese.

The statistical analysis of our sample is presented in Figure 5.

A slightly negative correlation between BMI level and sperm parameters is observed in the results of the correlation analysis. Mobility and morphology show a slightly more negative correlation. It

is concluded that the higher the BMI, the more negatively the sperm parameters are affected, confirming the studies in which fertility is affected in obese men.

3.5 | Radiation and chemicals

A slightly negative correlation is observed between the exposure of a patient to chemicals and their sperm motility in Figure 6.
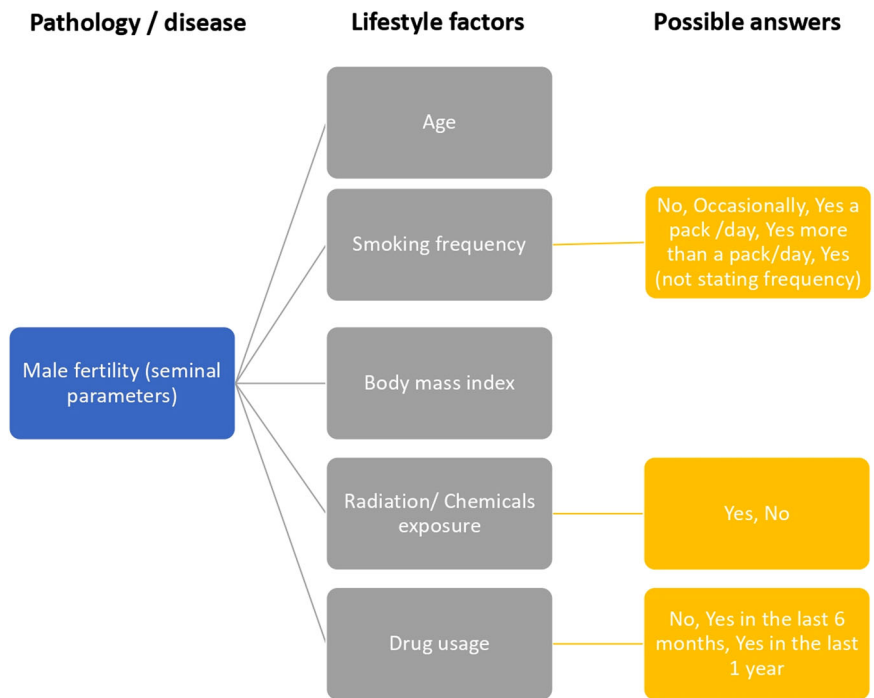
4 | DISCUSSION

For the first time, male individuals from Greece participated in a case–control study like this, to elucidate whether lifestyle factors can affect the seminogram parameters and help construct an online database with epidemiological data as well as genetic data for Greek men.

It is becoming more evident than ever that infertility diagnosis should not be limited to checking seminal parameters, ultrasounds, and physical exams, but should be thoroughly examined via the collection of detailed epidemiological data for each patient. This way, it is easier to produce effective therapies and follow certain routines that can alleviate an existing pathology. The factors examined in this study were age of the individual, smoking frequency, drug usage, body mass index, and potential exposure to radiation and/or chemicals. These factors constitute the basis of any questionnaire used in this type of study.

Among the factors examined, increasing age, BMI, and exposure to radiation and/or chemicals were shown to exhibit a negative correlation with male infertility in the Greek population. Regarding increasing age, its effects on semen quality have been studied over the years, for different populations and different patient numbers. It is widely accepted that even though spermatogenesis is a process that can be completed even in old age in men, a debate on how increasing age affects sperm still exists. Aging affects testosterone production negatively[16] and causes decreased Leydig cell function. Hormonal regulation is of great importance in spermatogenesis, a process that requires smooth and organized functioning of the prostate and epididymis,[17] and results from several studies[16,18–20] propose that increasing age causes oxidative stress, which, in turn, leads to cell apoptosis in the delicate testicular environment.

Additionally, obesity has also been extensively studied for its effects on male fertility indirectly or directly. Mainly, obesity negatively affects the hypothalamic–pituitary–gonadal axis, deregulating the individual's hormonal activity. Increased insulin levels prevent the normal binding of testosterone and estradiol by stopping the function of sex hormone-binding globulin (SHBG). The presence of elevated adipose tissue levels also leads to an imbalance in estrogen levels compared to testosterone leading to infertility.[21] Obesity also leads to increased temperature in the genital area due to increased adipose tissue volume and this in combination with a

FIGURE 6 Correlation plot for radiation/chemical substances and sperm parameters

Pathology / disease	Lifestyle factors	Possible answers
	Age	
	Smoking frequency	No, Occasionally, Yes a pack /day, Yes more than a pack/day, Yes (not stating frequency)
Male fertility (seminal parameters)	Body mass index	
	Radiation/ Chemicals exposure	Yes, No
	Drug usage	No, Yes in the last 6 months, Yes in the last 1 year

sedentary life causes a decrease in sperm density.[22] Another way that fertility is affected through obesity is sleep apnea caused in obese people due to obstruction of the upper airways, which leads to interrupted sleep. Thus, patients with sleep apnea have a disturbed nocturnal increase in testosterone levels leading to infertility.[23]

It is also very important to note that since the developed world is now highly dependent on technological discoveries and uses them extensively in their daily lives, people are exposed daily to nonionizing electromagnetic radiation, which is harmful not only to many systems in the body but also to fertility. Testicular tissue is one of the most sensitive tissues to radiation and is affected due to the increase of free radicals which cause oxidative stress and lipid peroxidation, which, in turn, leads to membrane disruption and reduced spermatozoa mobility.[24] The level of damage to tissue and cells is proportional to the radiation they receive. It has been found that long-term exposure to the radio frequency of 4G mobile phones and computers with Wi-Fi affects the sperm and the testicular environment, with Leydig cells being the most sensitive. In the case of laptops, exposure to Wi-Fi radiation significantly affected sperm motility due to reoxidation of phospholipids, an important component of sperm mitochondria.[25] Exposure to chemicals is also an important factor in causing infertility in men. Harmful chemicals are mainly defined as chemicals that affect the endocrine system (endocrine-disrupting chemicals). Such chemicals are found in a variety of everyday products, from plastic bottles to pesticides (phthalates, bisphenols, etc.) and have been found in large proportions (about 90%) in the urine of people abroad. Phthalate metabolites cause a huge reduction in murine testosterone production, but with no ability to replicate in humans.[26] Bisphenol A has been found to cause an earlier onset of prostate cancer, which affects

fertility as the prostate provides the prostate fluid needed to complete the ejaculation process.[27,28] According to studies, exposure to certain chemicals that disrupt the endocrine system and nonionizing radiation affect spermatozoa and more specifically spermatozoa motility[29] as it is affected by oxidative stress, without them having special endogenous antioxidant protection.

Smoking and drug usage, albeit harmful for the individual in numerous ways, have not been correlated with male fertility in the Greek population. Smoking and more specifically the smoke produced by its combustion contains over 4000 harmful chemicals that are released. Tobacco contains carbon monoxide along with nicotine and tar.[30] Among the chemicals that affect sperm are mainly lead and cadmium. Smoking and inhalation of chemicals cause an increase in oxidative stress in the body leading to problems in spermatogenesis as smokers do not have an adequate defense against free radicals.[31] In addition, smoking causes epididymitis and varicose veins mainly due to reduced blood flow to the testicular environment. However, further research is needed on the effects of smoking on male fertility and sperm quality.

Additionally, drug use and its effect on fertility have not been thoroughly investigated. However, there have been studies linking marijuana use to reduced levels of luteinizing hormone (LH)[32] and testosterone, with both hormones being responsible for normal sperm production. Opioids also cause hormonal deregulation due to decreased production of gonadotropin-releasing hormone with consequent reductions in LH and testosterone.[33] Cocaine and its influence have been studied in animal models and highly elevated levels of apoptosis in the epithelial cells of spermatocytes and spermatogonia were found.[34]

5 | CONCLUSION

Conclusively, this study presents certain factors that influence the seminal parameters in the Greek population. This database will continue to grow and expand with more individuals and detailed information and also with genetic data to enhance the power of future case–control analyses for the trait of male infertility. The addition of more individuals can also enhance weak existing correlations or highlight new ones, a fact that will eventually help scientists understand the pathogenesis of diseases, but also offer the ability to personalize treatments for different patients.

AUTHOR CONTRIBUTIONS

Maria Markantoni: Conceptualization; formal analysis; writing—original draft; writing—review and editing. **Theologia Sarafidou**: Conceptualization; writing—review and editing. **Alexia Chatziparasidou**: funding acquisition; resources. **Nicolas Christoforidis**: funding acquisition; resources. **Zissis Mamuris**: conceptualization; funding acquisition; writing—review and editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

TRANSPARENCY STATEMENT

The manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and any discrepancies from the study as planned (and, if relevant, registered) have been explained.

ETHICS STATEMENT

All the participants were informed about the study and they gave their consent in order to participate by filling out a questionnaire along with the consent form. Both the study and the consent procedure were approved by the ethics committee of the Medical Faculty of the University of Thessaly. All authors have read and approved the final version of the manuscript. Zissis Mamuris had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

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