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ORIGINAL ARTICLE

Semen Analysis

A decade of WHO 2010: total sperm number temporal trend and role of lifestyle factors

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After a huge decline in sperm concentration between 1938 and 1991 was reported, many researchers investigated the possibility of a worsening of human sperm quality. Despite massive efforts, published evidence is still controversial. Similarly, the role of lifestyle factors on semen parameters is debated. We conducted a monocentric Italian study to evaluate the total sperm number trend over the last 10 years (from 2010 to 2019). Additionally, we evaluated the association between lifestyle factors and total sperm number in order to identify possible damaging factors. We performed a retrospective study analyzing subjects aged 18–55 years who had their semen analyzed between 2010 and 2019. A total of 3329 subjects were included: 1655 subjects referred to our department (Department of Experimental Medicine, Sapienza University of Rome, Roma, Italy) for idiopathic infertility and 1674 subjects referred for preconceptional or andrological screening with no confirmed andrological diseases. Semen samples were examined according to World Health Organization (WHO) 2010 criteria by two seminologists with the same training and the same equipment. For statistical evaluations, only total sperm number ($\times 10^6$ per ejaculate) was taken into consideration. We detected no significant changes in mean total sperm number during the last decade, in either the entire population or the two subgroups (infertile group and control group). In a multivariate analysis total sperm number was significantly associated with the history of infertility, body mass index (BMI) and cigarette smoking. Our results suggest that infertile men are "vulnerable" subjects, particularly susceptible to several negative factors, many of which still remain unknown. Our study highlights the need for studies addressing men's lifestyle in order to find and reduce deleterious agents.

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Keywords: aging; male infertility; obesity; smoking; sperm decline

INTRODUCTION

Since 1992, when Carlsen *et al.*[1] reported evidence of a huge decline of sperm concentration between 1938 and 1991, many papers fuelled the discussion about the possibility of a human sperm quality decline. Despite massive efforts, published evidence is still controversial. It may be argued that trends may differ from country to country, as evidence of a sperm decline has come from reports from different countries, but ultimately, it is difficult to demonstrate worldwide sperm parameters fluctuations owing to difficulty in systematically comparing these reports.[2] Most studies evaluated sperm parameters trend over long periods of time, usually 15 years or more. Consequently, methodologies and technicians' experience, quality control, and different semen analysis evaluation criteria may have a role in explaining variability of reported results. In a meta-analysis, Levine *et al.*[3] reported a significant decline in sperm count between 1973 and 2011 among men unselected by fertility from North America, Europe, Australia, and New Zealand. Considering European men, Sengupta *et al.*[4] observed a significant decline in sperm concentration over the past 50 years. Few studies analyzed sperm characteristics in a time frame of 10 years. Furthermore, variability of results can be explained by different study designs (multi- or monocentric study, subject randomization, retrospective or observational, subject selection, *etc.*). In addition, decline in sperm quality may be triggered by several possibly coexisting factors, rarely investigated in these studies. Testicular alterations, including sperm chromatin damage, epigenetic remodeling, and stem cell exhaustion, may be the consequence of aging-related proinflammatory environment.[5] The same proinflammatory environment and testicular alteration had been reported in smokers[6] and obese patients.[7] Oxidative stress has been proposed as the main cause of poor semen quality in smokers,[8] whereas in obese subjects, several mechanisms may be involved, such as oxidative stress, increase in testicular temperature, preferential accumulation of liposoluble toxic substances, decreased Sertoli cell function, chronic inflammation, and secondary hypogonadism.[9] Other possible concurrent factors may be related to eating habits,[10] occupational toxicants[11] and environmental exposure.[12] Therefore, in order to limit the impact of these factors, we conducted a monocentric Italian study to evaluate total sperm number over the last ten 10 years (from 2010 to 2019), corresponding to the introduction of World Health Organization (WHO) 2010 criteria.[13] Additionally, our study evaluated the association between lifestyle factors and total sperm number in order to identify possible damaging factors.

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PARTICIPANTS AND METHODS

Study population

The present study protocol was reviewed and approved by the institutional review board of Policlinico Umberto I University Hospital (Roma, Italy; Approval No. 182/11). Informed consent was submitted by all subjects when they were enrolled. Data collection followed the principles outlined in the Declaration of Helsinki. We searched the database of the Laboratory of Seminology-Department of Experimental Medicine (Sapienza University of Rome - Policlinico Umberto I University Hospital, Roma, Italy) for subjects aged 18–55 years who had had their semen analyzed between 2010 and 2019. Medical history and information as age, sexual abstinence, body mass index (BMI), smoking habit, job, and area of origin and birthplace had been recorded at the moment of the examination. Exclusion criteria were nationality other than Italian, testicular cancer or other tumors, previous chemo- or radiotherapy or other gonadotoxic treatments, Klinefelter syndrome and other chromosomal abnormalities and genetic syndromes, azoospermia, cryptorchidism, clinical varicocele, and any other endocrinological/andrological condition known to affect semen quality (drugs, fever, urinary tract infections, urinary tract surgery, *etc.*). The patient selection process is summarized in **Figure 1**. In brief, of 33 743 subjects who had at least one semen analysis performed between 2010 and 2019, a total of 3329 subjects were included in the retrospective analysis.

Semen analysis

Between 2010 and 2019 in our center (Laboratory of Seminology-Department of Experimental Medicine), two seminologists (DP and FF) with the same training and the same equipment performed all the semen analyses. Standardization of analyses was achieved by the participation in an international external quality control program (UKNeqas andrology scheme for semen analysis - sperm motility, concentration and morphology) and routine execution of an internal quality control with coefficient of variation of seminologists for sperm counts around 3.5%–5.0%. Internal and external quality controls were performed according to the WHO 2010 criteria.[13] Semen samples were all collected by masturbation into a sterile plastic container after 2–7 days of sexual abstinence. They were examined by light microscopy according to the WHO 2010 criteria.[13] Semen volume, sperm concentration ($\times 10^6$ ml^{-1}), and total sperm number (TSN; $\times 10^6$ per ejaculate) were the only variables taken into consideration, as TSN, in particular, provides a measure of the capability of the testes to produce spermatozoa and the patency of the male tract.[13]

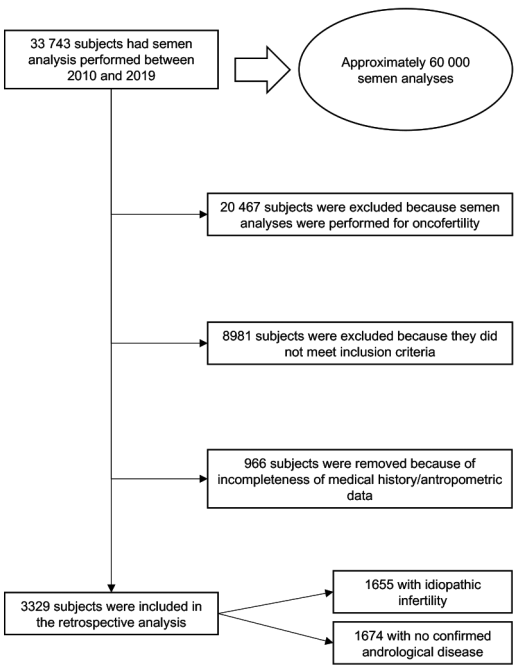

Figure 1: Patient selection process performed within the database of the Laboratory of Seminology-Department of Experimental Medicine (Policlinico Umberto I University Hospital).

Statistical analyses

Descriptive statistics were performed to summarize the data. Frequencies and percentages were calculated for categorical data. Numerical data were represented as mean and standard deviation (s.d.) or median and interquartile range (IQR), as appropriate. Chi-squared tests were used to compare categorical variables; the independent samples *t*-test or Wilcoxon rank sum test (also named Mann–Whitney U test) were used for continuous variables of different distribution types. Longitudinal box plots were used to represent the distribution of the total sperm number over years. Univariable and multivariable linear regression were used to assess the relationship between total sperm number and the other factors (time, history of infertility, age, BMI, smoking habit, seasonality, job, and area of origin and birthplace). In order to test stability in the point and interval estimates of our model, a sensitivity analysis was performed by considering a square root transformation of the response variable or by omitting the potential outliers. All the analyses were performed based on R version 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

A total of 3329 subjects were included in the retrospective analysis: 1655 subjects referred to our department for idiopathic infertility (infertile group) and 1674 subjects referred for preconceptional or andrological screening with no confirmed andrological diseases (control group). General characteristics of both groups are summarized in **Table 1**. Considering the entire population, 63.6% of our caseload came from a metropolitan area, whereas 21.4% and 15.0% from rural and industrial areas, respectively. However, most subjects were from Roma and provinces of Lazio (Italy). Among the subjects, 63.8% performed a sedentary job, in particular, 38.5% were office workers, 11.2% were students, 10.5% were unemployed, and 3.6% were free-lance professionals; whereas, in heavy workers, 21.7% were factory or manual workers, 8.5% belonged to police or military forces, and 5.9% were health-care professionals. Considering control and infertile groups, controls were significantly younger than infertile (mean age: 32.3 years *vs* 38.3 years, $P < 0.001$). Moreover, mean BMI (24.6 kg m^{-2} *vs* 26.1 kg m^{-2}, $P < 0.001$) and percentage of smokers (29.2% *vs* 33.8%, $P = 0.005$) were significantly lower in controls. **Supplementary Table 1** shows all relevant demographic characteristics of the recruited population. Regarding spermatogenesis, the median TSN was significantly higher in the control group (192.0 [IQR: 96.0–320.0] $\times 10^6$ per ejaculate *vs* 138.0 [IQR: 60.0–249.0] $\times$ 10^6 per ejaculate, $P < 0.001$). Considering year of semen examination, there was no significant changes in mean total sperm number during the last decade, in either the entire population or the two subgroups, as shown in **Figure 2**. No significant difference was detected in the other analyzed semen parameters. **Supplementary Table 2** shows their stratification by year. In a multivariate analysis, total sperm number was significantly associated with infertility ($\beta = -50.931$, $P < 0.001$), BMI ($\beta = -2.476$, $P = 0.009$), and smoking ($\beta = -27.155$, $P < 0.001$). The sensitivity analysis confirmed the stability of our results.

Table 1: General characteristics of the studied population

Characteristic	*All*	*Control group*	*Infertile group*	*P*
Participants (*n*)	3329	1674	1655	
Age (year), mean (s.d.)	35.3 (8.0)	32.3 (8.6)	38.3 (5.8)	<0.001
BMI (kg m^{-2}), mean (s.d.)	25.3 (3.6)	24.6 (3.5)	26.1 (3.5)	<0.001
Smoking habit, *n* (%)				0.005
Nonsmokers	2280 (68.5)	1185 (70.8)	1095 (66.2)	
Current smokers	1049 (31.5)	489 (29.2)	560 (33.8)	
Job, *n* (%)				<0.001
Freelance professionals	121 (3.6)	59 (3.5)	62 (3.7)	
Health-care workers	198 (5.9)	117 (7.0)	81 (4.9)	
Manual/factory workers/heavy activity	724 (21.7)	268 (16.0)	456 (27.6)	
Office workers/sedentary jobs	1282 (38.5)	579 (34.6)	703 (42.5)	
Police/military/pilot	282 (8.5)	109 (6.5)	173 (10.5)	
Students	372 (11.2)	368 (22.0)	4 (0.2)	
Others/unemployed	350 (10.5)	174 (10.4)	176 (10.6)	
Seasonality, *n* (%)				<0.001
Autumn	889 (26.7)	400 (23.9)	489 (29.5)	
Spring	926 (27.8)	459 (27.4)	467 (28.2)	
Summer	734 (22.0)	459 (27.4)	275 (16.6)	
Winter	780 (23.4)	356 (21.3)	424 (25.6)	
Area of origin and birthplace, *n* (%)				0.042
Rural area	713 (21.4)	345 (20.6)	368 (22.2)	
Metropolitan area	2117 (63.6)	1098 (65.6)	1019 (61.6)	
Industrial area	499 (15.0)	231 (13.8)	268 (16.2)	
Total sperm number (×10^6 per ejaculate), median (IQR)	160.0 (76.0–288.0)	192.0 (96.0–320.0)	138.0 (60.0–249.0)	<0.001

BMI: body mass index; IQR: interquartile range; s.d.: standard deviation

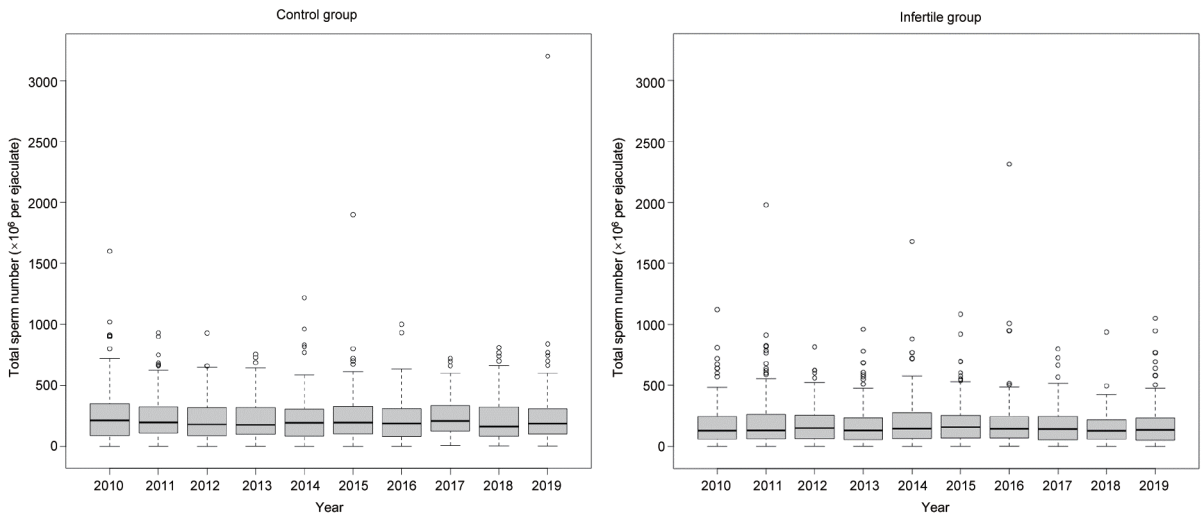

Figure 2: Total sperm number temporal trend between 2010 and 2019 in control group and infertile group.

Results are summarized in **Table 2**. BMI and prevalence of smoking habit, as well as the other considered variables, did not differ between years, as shown in **Figure 3**, **Table 3**, and **Supplementary Figure 1** and **2**.

DISCUSSION

Temporal sperm number trend

Our monocentric study showed no significant change in total sperm number during the last decade in our Italian population. We did not observe any decline in either control or idiopathic infertile groups. Many studies have suggested a continuous decline in semen quality and quantity over time, since the meta-analysis by Carlsen *et al.*[1] showed a significant decrease in sperm concentration and semen volume with time. However, these results have been much criticized by many authors. Changes were considered to be a global phenomenon, although the geographical distribution of included studies was wide and irregular. More recently, two recent meta-analyses showed similar results.[3,4] However, geographical differences in human semen quality may even be regional, within the same country.[14] Few studies have investigated sperm temporal trends in Italy. Menchini-Fabris *et al.*[15] reported a decline in sperm count and motility between 1975 and

1994 in a seemingly healthy population. In the same way, Bilotta *et al*.[16] observed a reduction in all semen parameter values in semen donors in a period of 15 years (1981–1995). However, in neither study was statistical analysis performed. Afterward, our group compared semen characteristics of patients in the period of 1982–1994. A worsening was observed considering all patients, but in sperm bank donors, no decline was observed, suggesting a real seminal decay only in patients with lower levels of semen quality.[17] On the contrary, Vicari *et al*.[18] showed a temporal decline of sperm quality in fertile men and healthy subjects or donors in a South-East area of Sicily, from 1982 to 1999. More recently, the same group reported the presence of a not significant declining trend in total sperm count in a cohort of 1409 semen tests randomly selected during the last decade.[19] Similarly, we did not observe any significant change in total sperm number in a healthy Italian population, though a decade might be considered a short range of time. A longer study period could be useful indeed to understand any change in spermatogenesis process but forces the researcher to deal with several methodological issues. First, the evaluation of extended periods of time involves different semen analysis methodologies. The WHO criteria for semen analysis were introduced in 1980 and then revised in 1987, 1992, 1999, 2010, and 2021, with differences especially in sperm morphology evaluation. In addition, the important technological breakthroughs, with many advances in the last decades, should be considered as components of light microscopes have improved. Therefore, we decided to limit analyses to the last decade, a narrower time frame than most studies, roughly corresponding to the period of the introduction of WHO 2010 criteria. This allows reduction of the previously cited potential biases. Moreover, in this period in our center, two seminologists with the same training and the same equipment performed all the semen analyses. Quality control was performed according to WHO 2010 criteria. This should be considered a strength of our study. Furthermore, investigated populations are different between studies, with most of them investigating sperm donors or infertile men. For this reason, we decided to evaluate two subgroups: idiopathic infertile men and subjects referring for preconceptional or andrological screening. In both cases, medical history was accurately recorded, and only healthy subjects were included. Finally, discrepancies observed in literature may result from several limitations that are inherent in human studies. Indeed, data suggest that sperm quality can be impaired by many factors, which are often related. For this reason, we performed a multivariate analysis to investigate an association between total sperm number and some known risk factors.

Table 2: Univariate and multivariate analysis considering total sperm number and year of semen analysis, infertility, age, body mass index, smoking habit, seasonality, job, and area of origin and birthplace

Variable	*Univariate analysis*		*Multivariate analysis*	
	β	*P*	*β*	*P*
Year	−1.177	0.295	−1.593	0.153
Infertile group	−49.326	<0.001	−50.931	<0.001
Age	−0.643	0.125	0.713	0.117
BMI	−3.478	<0.001	−2.476	0.009
Current smoker	−30.557	<0.001	−27.155	<0.001
Spring	9.599	0.288	6.069	0.498
Summer	−6.862	0.475	−15.463	0.106
Winter	1.615	0.864	1.480	0.874
Sedentary job	10.237	0.140	−0.934	0.894
Metropolitan area	0.509	0.951	−1.975	0.811
Industrial area	3.242	0.773	2.491	0.823

BMI: body mass index

Sperm parameter values and risk factors

Our multivariate analysis showed a significant correlation between total sperm number and infertility, BMI, and smoking habits. Consequently, lifestyle habits should not be overlooked in couples trying to conceive. For the infertile group, the median total sperm number was significantly lower than that in the control group, though within WHO 2010 5th centile. It is well-known that female age is a key factor in couple infertility, with a fertility decline already starting at 25–30 years of age.[20] Scant data are available for men. In our study, the mean age of the infertile group was significantly higher than that of the control group, suggesting that couples are trying to conceive naturally at a later age. We found no correlation between age and total sperm number, though aging may have a role in reducing sperm quality rather than total sperm number.[21] Regarding lifestyle changes, the number of smokers has increased globally over the years,[22] as has the prevalence of obesity.[23] Smoking is considered a risk factor for reproductive health, with much evidence suggesting a negative effect on spermatogenesis

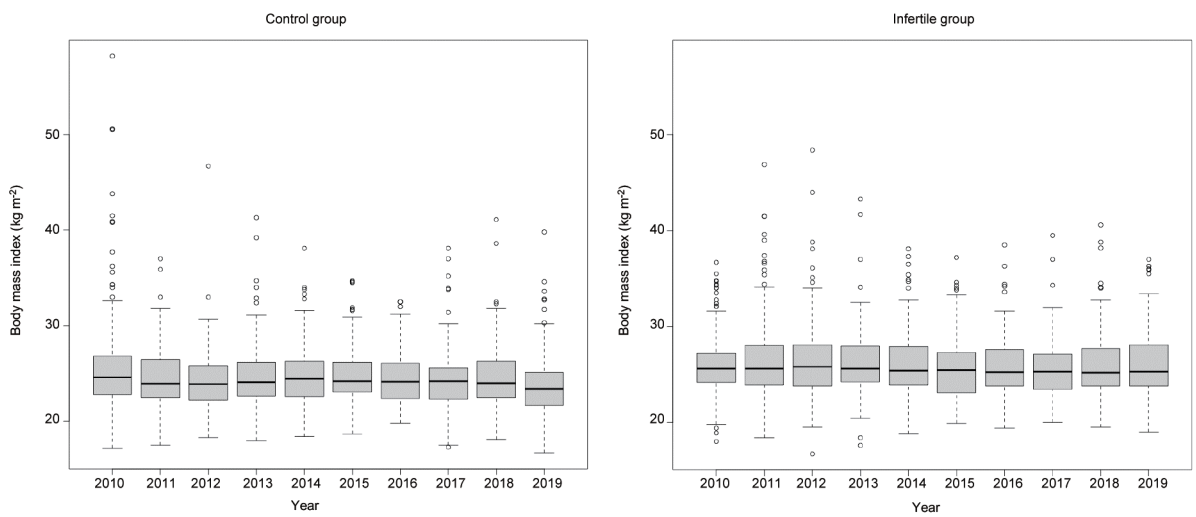

Figure 3: Body mass index temporal trend during the last ten years in control group and infertile group.

Table 3: Characteristics of the studied population according to year of semen analysis examination

Group	*Year of semen analysis examination*									
	2010	*2011*	*2012*	*2013*	*2014*	*2015*	*2016*	*2017*	*2018*	*2019*
Age (year), mean (s.d.)										
All	34.2 (8.4)	35.4 (7.2)	35.1 (7.6)	34.6 (8.2)	35.7 (8.0)	37.2 (7.6)	36.2 (7.8)	36.6 (7.8)	35.3 (6.8)	34.0 (8.8)
Control group	32.1 (9.5)	32.4 (7.9)	31.4 (8.3)	32.1 (9.1)	31.82 (8.5)	33.9 (7.8)	33.9 (9.1)	34.1 (8.1)	33.2 (7.8)	30.0 (8.5)
Infertile group	36.7 (6.0)	37.9 (5.4)	38.3 (5.1)	37.6 (5.7)	38.7 (6.1)	39.9 (6.3)	38.4 (5.6)	39.7 (6.1)	37.0 (5.1)	39.2 (5.9)
BMI (kg m^{-2}), mean (s.d.)										
All	25.8 (4.3)	25.6 (3.8)	25.5 (3.9)	25.3 (3.5)	25.5 (3.3)	25.2 (3.1)	25.2 (3.0)	24.9 (3.4)	25.4 (3.7)	24.8 (3.5)
Control group	25.5 (5.0)	24.5 (3.1)	24.3 (3.3)	24.6 (3.4)	24.8 (3.3)	24.8 (3.0)	24.5 (2.7)	24.3 (3.4)	24.7 (3.5)	23.7 (3.1)
Infertile group	26.1 (3.2)	26.5 (4.1)	26.4 (4.1)	26.1 (3.4)	26.0 (3.2)	25.6 (3.2)	25.8 (3.2)	25.6 (3.2)	26.1 (3.7)	26.2 (3.6)
Nonsmokers, *n* (%)										
All	317 (71.2)	237 (66.9)	248 (71.7)	260 (68.1)	222 (63.6)	188 (63.7)	174 (70.7)	206 (72.3)	156 (66.4)	272 (69.4)
Control group	187 (76.3)	104 (65.0)	114 (71.2)	147 (69.7)	103 (66.9)	83 (61.5)	87 (73.7)	118 (74.7)	80 (72.7)	162 (72.6)
Infertile group	130 (65.0)	133 (68.6)	134 (72.0)	113 (66.1)	119 (61.0)	105 (65.6)	87 (68.0)	88 (69.3)	76 (60.8)	110 (65.1)
Current smokers, *n* (%)										
All	128 (28.8)	117 (33.1)	98 (28.3)	122 (31.9)	127 (36.4)	107 (36.3)	72 (29.3)	79 (27.7)	79 (33.6)	120 (30.6)
Control group	58 (23.7)	56 (35.0)	46 (28.7)	64 (30.3)	51 (33.1)	52 (38.5)	31 (26.3)	40 (25.3)	30 (27.3)	61 (27.4)
Infertile group	70 (35.0)	61 (31.4)	52 (28.0)	58 (33.9)	76 (39.0)	55 (34.4)	41 (32.0)	39 (30.7)	49 (39.2)	59 (34.9)
Total number of participants (*n*)	445	354	338	382	349	295	246	285	235	392

BMI: body mass index; s.d.: standard deviation

both in infertile and fertile men.[6] On the contrary, the impact of obesity on sperm parameters is rather controversial, with many conflicting studies.[9] Meta-analyses on this topic are also conflicting.[24] Different results may be due to related confounding factors, not investigated in most studies. In fact, dietary patterns may alter sperm parameters[10] as can alcohol consumption.[25] In our population, percentage of smokers and mean BMI were significantly higher in infertile group. We found that total sperm number was negatively correlated with both BMI and smoking habits. Therefore, our results suggest that they may act as concurrent factors in impairing spermatogenesis. We also observed an association between total sperm number and history of infertility in the multivariate analysis. This suggests that infertile men are "vulnerable" subjects, particularly susceptible to numerous potential negative factors. BMI and smoking habit, as well as the other considered variables, did not significantly differ between years in our caseload. Therefore, a cause of the lack of variability in this decade could be the stability of some confounding factors.

Regarding seasonality, our samples were collected throughout the 12 months in each year, with a similar frequency of sperm collection in winter/spring. We did not observe any significant change, in contrast to a study by Kabukçu *et al*.[26] where ambient temperature has been proposed as a possible determinant of sperm parameters' seasonal variability. This aspect can also be strictly related to seasonal variations in daylight exposure, as well as to variation in circadian rhythms by various causes (*i.e*., sleep-wake cycle) which is known to affect hormone secretion and several physiologic functions, including reproduction. Ultimately, sperm physiology may vary according to ultradian and infradian rhythms, also suggesting a role of melatonin circannual cycle.[27]

In our study, we also evaluated a possible correlation between subjects' jobs and total sperm number. Most of the existing literature focused on the role of chemical exposure, because of the endocrine-disrupting activity of numerous chemicals, but study results are often discordant, and the effects are still largely debated.[28] Limited data on physical exertions and sedentary positions in an occupational context are reported. In the Longitudinal Investigation of Fertility and the Environment (LIFE) Study, Eisenberg *et al*.[29] reported an association between work-related heavy exertion and spermatogenesis impairment, while no correlation between semen parameter values and prolonged sitting was observed. Although sitting position has been associated with testicular overheating,[30] other studies failed to demonstrate any correlation between sedentary work and poor semen quality.[31] However, a recent study observed higher sperm DNA fragmentation despite normal semen parameters in sedentary workers, suggesting a possible negative role in male fertility.[32] Our study showed no differences in total sperm number between sedentary and factory/heavy manuals workers, which were about 63.8% and 36.2% of our caseload, respectively. Regarding a possible role of environmental pollution, in our analysis, we considered geographical area of origin. Use of pesticides in rural areas has been associated with poorer sperm quality,[33] as well as urban air pollution.[34] Most of our subjects (about 63%) came from a metropolitan area, and no significant differences were observed between them and subjects from rural or industrial areas. However, we could not identify every shift made by all subjects over the years before the examination. Some of these substances have a long half-life, even years, and their effects may be revealed after a long time.[28]

Therefore, our results confirm that studies in literature focusing on sperm decline over the years can be confounded by age and lifestyle factors. Traditional confounders such as smoking, BMI, alcohol consumption and eating habits, occupational and environmental toxicants, but also emerging factors as recreational drugs,[35] stress,[36] and use of mobile phones[37] should be taken in consideration. Many confounding factors might be responsible for an apparent decline in male fertility, but their stability in the latest years might be reassuring. Additionally, there is increasing evidence of a transgenerational effect, through which paternal lifestyle can influence offspring health.[38] For all these numerous reasons, at the current time, it is extremely difficult to reach a definitive conclusion on this topic and further studies are needed to confirm whether semen quality is really declining or not.

CONCLUSIONS

In our monocentric study, the main result was a substantial stability of total sperm number during the last decade in healthy subjects. We also

found a significant negative effect of smoking habits and BMI on semen quality. Our study remarks that several negative factors, of which many still remain unknown, may potentially impact on semen parameters. As in presence of infertility and other andrological diseases, these negative effects might have a greater impact, it is necessary to focus on subjects' lifestyle in order to find and eliminate potential deleterious agents.

AUTHOR CONTRIBUTIONS

DP carried out semen analysis, and conceived and designed the study. FC wrote the article. FC, FP, TC, and FF performed database search. FP, ARV, and DAF performed statistical analyses. AL and FL contributed to data interpretation and manuscript revision. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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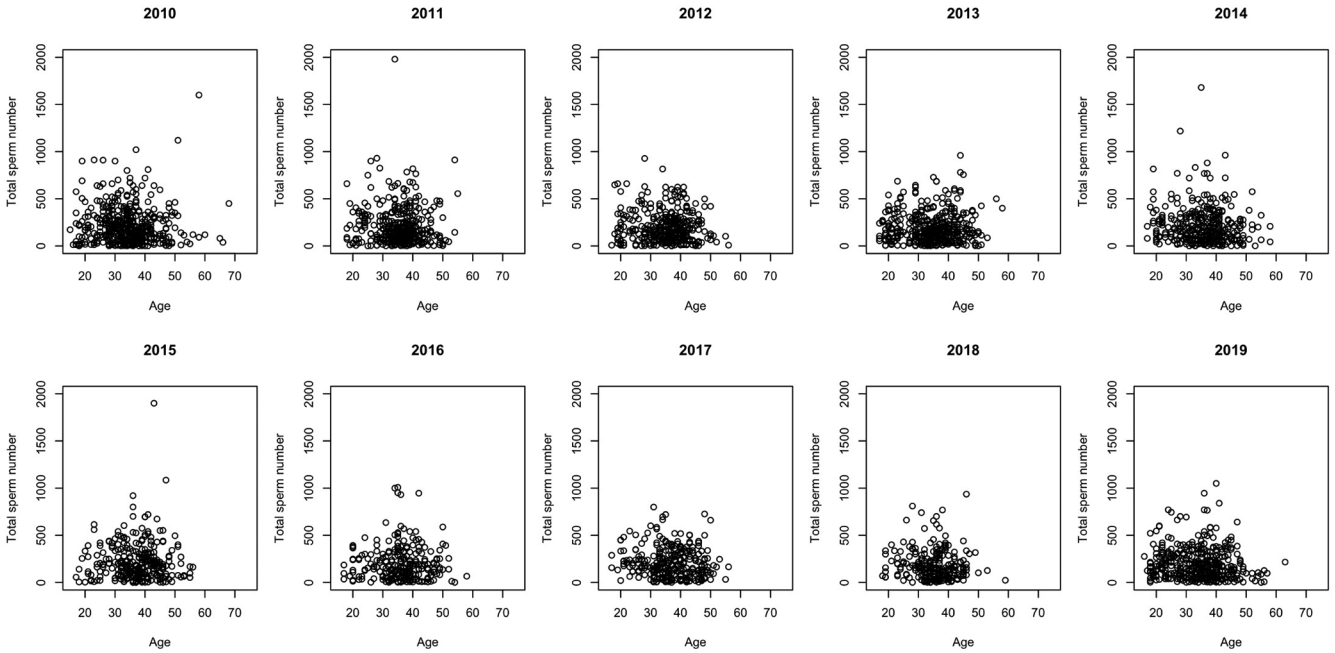
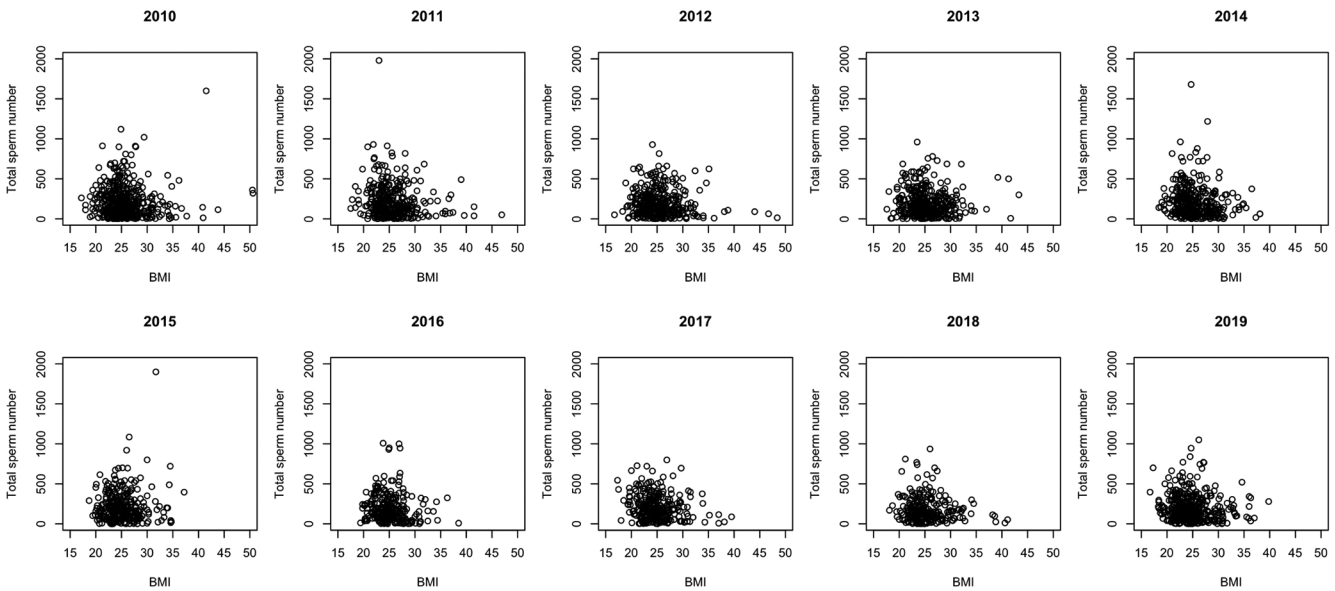


Supplementary Figure 1: Scatterplots for age and total sperm number, each stratified by year.



Supplementary Figure 2: Scatterplots for BMI and total sperm number, each stratified by year. BMI: body mass index.

Supplementary Table 1: Characteristics of the studied population stratified by year of semen analysis examination

					Controls					
Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
n	245	160	160	211	154	135	118	158	110	223
Age	32.1 (9.5)	32.4 (7.9)	31.4 (8.3)	32.1 (9.1)	31.8 (8.5)	33.9 (7.8)	33.9 (9.1)	34.1 (8.1)	33.2 (7.8)	30 (8.5)
	32 (26–37)	33 (26–38)	31 (25–38)	32 (24–38)	32 (25–38)	35 (28–39.5)	35 (27–40)	34 (28–40)	34 (27–38)	29 (23–35)
BMI	25.5 (5.0)	24.5 (3.1)	24.3 (3.3)	24.6 (3.4)	24.8 (3.3)	24.8 (3.0)	24.5 (2.7)	24.3 (3.4)	24.7 (3.5)	23.7 (3.1)
	24.6 (22.8–26.8)	24.0 (22.5–26.4)	23.9 (22.2–25.8)	24.1 (22.6–26.2)	24.4 (22.6–26.3)	24.2 (23.0–26.2)	24.1 (22.4–26.1)	24.2 (22.3–25.6)	24.0 (22.5–26.3)	23.4 (21.7–25.1)
Season										
Winter	52 (21.2)	28 (17.5)	42 (26.2)	49 (23.2)	1 (0.6)	37 (27.4)	34 (28.8)	39 (24.7)	23 (20.9)	51 (22.9)
Spring	83 (33.9)	43 (26.9)	50 (31.2)	57 (27.0)	0 (0.0)	47 (34.8)	28 (23.7)	54 (34.2)	34 (30.9)	63 (28.3)
Summer	35 (14.3)	35 (21.9)	30 (18.8)	105 (49.8)	123 (79.9)	17 (12.6)	17 (14.4)	21 (13.3)	22 (20.0)	54 (24.2)
Autumn	75 (30.6)	54 (33.8)	38 (23.8)	0 (0.0)	30 (19.5)	34 (25.2)	39 (33.1)	44 (27.8)	31 (28.2)	55 (24.7)
Job										
Heavy	76 (31.0)	53 (33.1)	48 (30.0)	63 (29.9)	37 (24.0)	39 (28.9)	32 (27.1)	46 (29.1)	38 (34.5)	62 (27.8)
Sedentary	169 (69.0)	107 (66.9)	112 (70.0)	148 (70.1)	117 (76)	96 (71.1)	86 (72.9)	112 (70.9)	72 (65.5)	161 (72.2)
Origin										
Industrial	31 (12.7)	26 (16.2)	16 (10.0)	23 (10.9)	23 (14.9)	19 (14.1)	23 (19.5)	32 (20.3)	14 (12.7)	24 (10.8)
Metropolitan	166 (67.8)	107 (66.9)	112 (70)	145 (68.7)	91 (59.1)	93 (68.9)	74 (62.7)	93 (58.9)	65 (59.1)	152 (68.2)
Rural	48 (19.6)	27 (16.9)	32 (20.0)	43 (20.4)	40 (26.0)	23 (17.0)	21 (17.8)	33 (20.9)	31 (28.2)	47 (21.1)
Smoke										
Yes	58 (23.7)	56 (35.0)	46 (28.7)	64 (30.3)	51 (33.1)	52 (38.5)	31 (26.3)	40 (25.3)	30 (27.3)	61 (27.4)
No	187 (76.3)	104 (65.0)	114 (71.2)	147 (69.7)	103 (66.9)	83 (61.5)	87 (73.7)	118 (74.7)	80 (72.7)	162 (72.6)
					Infertile					
n	200	194	186	171	195	160	128	127	125	169
Age (year)	36.7 (6.0)	37.9 (5.4)	38.3 (5.1)	37.6 (5.7)	38.7 (6.0)	40.0 (6.3)	38.4 (5.6)	39.7 (6.1)	37 (5.1)	39.2 (5.9)
	36 (33–40)	37 (35–41)	38 (35–42)	37 (33–42)	38 (35–42)	39 (36–45)	38 (35–42)	39 (35–44)	37 (33–40)	39 (35–42)
BMI	26.1 (3.2)	26.5 (4.1)	26.4 (4.1)	26.1 (3.5)	26.0 (3.3)	25.6 (3.2)	25.8 (3.2)	25.6 (3.2)	26.2 (3.7)	26.2 (3.6)
	25.6 (24.2–27.2)	25.6 (23.9–28.0)	25.8 (23.8–28.1)	25.6 (24.2–28.0)	25.4 (23.9–27.9)	25.4 (23.1–27.2)	25.2 (23.8–27.6)	25.3 (23.4–27.1)	25.2 (23.8–27.7)	25.3 (23.8–28.1)
Season										
Winter	58 (29.0)	30 (15.5)	56 (30.1)	44 (25.7)	56 (28.7)	50 (31.2)	31 (24.2)	28 (22.0)	38 (30.4)	33 (19.5)
Spring	51 (25.5)	68 (35.1)	48 (25.8)	44 (25.7)	45 (23.1)	35 (21.9)	50 (39.1)	39 (30.7)	31 (24.8)	56 (33.1)
Summer	30 (15.0)	27 (13.9)	32 (17.2)	25 (14.6)	38 (19.5)	30 (18.8)	16 (12.5)	25 (19.7)	18 (14.4)	34 (20.1)
Autumn	61 (30.5)	69 (35.6)	50 (26.9)	58 (33.9)	56 (28.7)	45 (28.1)	31 (24.2)	35 (27.6)	38 (30.4)	46 (27.2)
Job										
Heavy	91 (45.5)	81 (41.8)	88 (47.3)	73 (42.7)	80 (41.0)	60 (37.5)	60 (46.9)	51 (40.2)	46 (36.8)	80 (47.3)
Sedentary	109 (54.5)	113 (58.2)	98 (52.7)	98 (57.3)	115 (59.0)	100 (62.5)	68 (53.1)	76 (59.8)	79 (63.2)	89 (52.7)
Origin										
Industrial	30 (15.0)	30 (15.5)	32 (17.2)	16 (9.4)	33 (16.9)	25 (15.6)	26 (20.3)	20 (15.7)	31 (24.8)	25 (14.8)
Metropolitan	125 (62.5)	120 (61.9)	99 (53.2)	116 (67.8)	123 (63.1)	100 (62.5)	77 (60.2)	77 (60.6)	79 (63.2)	103 (60.9)
Rural	45 (22.5)	44 (22.7)	55 (29.6)	39 (22.8)	39 (20.0)	35 (21.9)	25 (19.5)	30 (23.6)	15 (12.0)	41 (24.3)
Smoker										
Yes	70 (35.0)	61 (31.4)	52 (28.0)	58 (33.9)	76 (39.0)	55 (34.4)	41 (32.0)	39 (30.7)	49 (39.2)	59 (34.9)
No	130 (65.0)	133 (68.6)	134 (72.0)	113 (66.1)	119 (61.0)	105 (65.6)	87 (68.0)	88 (69.3)	76 (60.8)	110 (65.1)

Age and BMI are expressed as mean (s.d.) in the upper line, median (IQR) in the lower line. Season, job, origin and smoke are expressed as frequency, numbers in brackets represent percentages. IQR: interquartile range; BMI: body mass index; s.d.: standard deviation

Supplementary Table 2: Abstinence, semen volume, concentration and total sperm number of the studied population stratified by year of semen analysis examination

					Controls					
Year	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
n	245	160	160	211	154	135	118	158	110	223
Abstinence (days)	4 (1.0)	3.7 (1.0)	4.9 (11.8)	3.8 (0.9)	4.0 (1.0)	3.9 (1.0)	3.8 (0.8)	3.8 (0.9)	3.8 (1.0)	3.9 (1.0)
	4 (3–5)	3 (3–4)	4 (3–4)	4 (3–4)	4 (3–5)	4 (3–5)	4 (3–4)	4 (3–4)	4 (3–4)	4 (3–4)
Volume (ml)	3.2 (1.7)	3.0 (1.4)	3.3 (1.6)	3.2 (1.4)	3.4 (1.6)	3.2 (1.5)	3.3 (1.6)	3.3 (1.4)	3.3 (1.8)	3.1 (1.5)
	3.0 (2.0–4.0)	3.0 (2.0–3.8)	3.2 (2.0–4.2)	3.0 (2.1–4.0)	3.2 (2.3–4.2)	3.0 (2.0–4.1)	3.0 (2.3–4.0)	3.0 (2.0–4.3)	2.9 (2.0–4.2)	3.0 (2.0–4.0)
Sperm concentration (million/ml)	90.8 (79.7)	85.4 (67.8)	72.9 (54.9)	74.8 (56.1)	77.9 (78.7)	79.2 (64.3)	67.4 (52.7)	78.2 (56.6)	75 (59.9)	78.8 (69.5)
	75 (35–130)	70 (40–120)	61 (37–95)	62 (35–100)	63 (28–97)	62 (40–96)	64 (30–91)	68 (46–97)	63 (32–90)	70 (40–98)
Total sperm number (million/ejaculate)	253.5 (220.3)	236.1 (181.4)	225.0 (175.5)	216.9 (158.1)	229.5 (195.5)	238.6 (220.2)	218.1 (181.7)	235.7 (152.8)	219.5 (181.3)	230.9 (254.6)
	213 (88–350)	197 (108–324)	180 (87–315)	176 (97–316)	194 (84–304)	196 (101–329)	188 (81–307)	208 (127–336)	161 (83–322)	187 (99–307)
					Infertile					
n	200	194	186	171	195	160	128	127	125	169
Abstinence (days)	3.8 (1.0)	3.8 (1.0)	6.9 (44.3)	3.7 (0.8)	3.8 (0.9)	4.1 (2.5)	3.5 (0.8)	4.0 (1.0)	3.7 (0.8)	3.8 (1.0)
	4 (3–5)	4 (3–4)	3 (3–4)	4 (3–4)	4 (3–4)	4 (3–4)	3 (3–4)	4 (3–5)	4 (3–4)	4 (3–4)
Volume (ml)	3.3 (1.6)	3.2 (1.5)	3.1 (1.4)	3.0 (1.4)	3.2 (1.4)	3.3 (1.5)	3.2 (1.4)	3.2 (1.6)	3.2 (1.8)	3.0 (1.6)
	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)	3 (2–4)
Sperm concentration (million/ml)	59.1 (59.7)	65.0 (68.0)	61.7 (50.2)	61.7 (57.2)	65.2 (61.1)	62.8 (58.1)	65.5 (73.6)	63.5 (58.8)	60.4 (62.1)	60.6 (54.2)
	42 (20–80)	45 (24–84)	49 (28–85)	48 (20–85)	50 (25–88)	48 (27–80)	52 (22–78)	54 (18–84)	52 (20–80)	50 (20–85)
Total sperm number (million/ejaculate)	172.4 (165.2)	199.6 (224.8)	180.8 (151.0)	168.8 (161.6)	192 (197.8)	199 (181.8)	198.5 (258.5)	177.8 (156.5)	153.7 (131.5)	175.1 (177.9)
	127 (60–242)	129 (63–262)	149 (65–256)	129 (53–231)	144 (66–275)	157 (71–251)	142 (70–241)	140 (52–245)	126 (60–216)	133 (49–230)

Variables are expressed as mean (s.d.) in the upper line, median (IQR) in the lower line. s.d.: standard deviation; IQR: interquartile range