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Paternal lifestyle factors in relation to semen quality and in vitro reproductive outcomes

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Abstract

This prospective-cohort study aimed at investigating the influence of paternal lifestyle factors on semen parameters and intracytoplasmic sperm injection (ICSI) outcomes. The influence of paternal lifestyle factors on seminal quality and ICSI outcomes was investigated in male patients undergoing conventional semen analysis. Cigarette smoking negatively influenced semen volume (B: −0.417, slope: 1.570, $p = 0.047$), sperm count/ml (B: −7.363, slope: 52.298, $p = 0.014$), total sperm count (B: −4.43, slope: 178.165, $p = 0.023$), total motile sperm count (B: −1.38, slope: 100.276, $p = 0.045$) and SDF (B: 0.014, slope: 9.767, $p = 0.033$). Alcohol consumption negatively influenced sperm count/ml (B: −12.527, slope: 42.255, $p = 0.040$) and sperm DNA fragmentation (B: 5.833, slope: 9.680, $p = 0.002$). There were no significant influences of other paternal lifestyle factors. Cigarette smoking negatively influenced the fertilisation rate (B: −1.349, slope: 21.950, $p = 0.039$) and the blastocyst formation rate (B: −14.244, slope: 28.851, $p = 0.025$). Alcohol consumption negatively influenced fertilisation rate (B: −3.617, slope: 20.138, $p = 0.041$) and blastocyst formation rate (B: −34.801, slope: 30.044, $p = 0.042$). Cigarette smoking and alcohol consumption appear to reduce semen quality, fertilisation and blastocyst formation rates; thus, it would be wise to recommend that male partners reconsider their lifestyle during in vitro reproduction treatment.

KEYWORDS

ICSI, lifestyle, semen analysis, semen quality

1 | INTRODUCTION

Infertility challenges nearly 15% of couples trying to conceive, and poor semen quality is observed in about half of them (Ricci et al., 2017).

According to two meta-analyses, seminal parameters, which are predictors of male fertility potential, have been declining over time worldwide (Carlsen, Giwercman, Keiding, & Skakkebaek, 1992; Swan & Elkin, 1999), and recent studies have corroborated this finding (Borges, Setti, Braga, Figueira Rde, & Iaconelli, 2015; Fernandez et al., 2012; Sengupta, Dutta, & Krajewska-Kulak, 2017; Splingart et al., 2012). This decline in semen quality is likely multifactorial, and a variety of lifestyle factors have been proposed to influence spermatogenesis and reproductive function, either positively (Gaskins, Colaci, Mendiola, Swan, & Chavarro, 2012; Minguez-Alarcon et al., 2012) or negatively (Braga et al., 2012; Gaskins et al., 2014; Joo, Kwon, Myung, & Kim, 2012; Silva et al., 2017).

The role of reactive oxygen species (ROS) and oxidative stress (OS) in semen quality decline has also been investigated (Agarwal, Virk, Ong, & Plessis, 2014; Hamada, Esteves, Nizza, & Agarwal, 2012).

Seminal levels of ROS (Barazani, Katz, Nagler, & Stember, 2014), semen parameters (La Vignera, Condorelli, Balercia, Vicari, & Calogero, 2013) and testicular and pituitary–gonadal axis dysfunction (Pizent, Tariba, & Zivkovic, 2012) are known to be influenced by lifestyle.

Fertilisation and pre-implantation embryo development are influenced by sperm-derived factors that may impact ICSI outcomes (Tesarik, 2005; Tesarik, Mendoza-Tesarik, & Mendoza, 2006). Lifestyle factors are under one's own control and could be modified to improve general health; therefore, adjusting for their influence may yield valuable information for counselling couples submitted to ICSI. The aim of this study was to investigate the influence of alcohol consumption, cigarette smoking, environmental and occupation exposure, medications and physical activity on (a) basic and advanced semen parameters, and (b) the results of ICSI in patients submitted to semen analysis for infertility investigation followed by ICSI.

2 | MATERIAL AND METHODS

2.1 | Experimental design

This prospective-cohort study included 965 male patients submitted to seminal analysis, in a private university-affiliated IVF centre, between October 2015 and December 2016. Regression analyses were conducted to investigate the relation between of alcohol consumption, cigarette smoking, environmental and occupation exposure, medications and physical activity, and semen quality for all 965 men.

For the investigation of the influence of lifestyle factors on ICSI outcomes, only couples presenting with isolated male infertility, as a result of abnormal semen parameters, such as oligozoospermia, astenozoospermia, teratozoospermia or a combination of those, undergoing their first ICSI cycle, in which female partner was ≤36 year old, were included in the analysis (n = 233).

Written informed consent forms were obtained from all patients.

The local Institutional Review Board approved this study (Medical College of Jundiai Ethics Committee, #411/2012).

2.2 | Controlled ovarian stimulation

Recombinant follicle-stimulating hormone (r-FSH, Gonal-F®, Serono, Geneve, Switzerland) and gonadotropin-releasing hormone (GnRH) antagonist, cetrorelix acetate (Cetrotide; Serono Laboratories) were administered for ovarian stimulation, as previously described (Setti et al., 2018). Oocyte maturation was triggered with recombinant human chorionic gonadotrophin (hCG, Ovidrel™, Serono). Oocyte retrieval was performed 35 hr later.

2.3 | Paternal lifestyle habits questionnaire

Prior to semen sample collection, men were asked to fill a detailed nonvalidated questionnaire containing the following questions:

1. How many cigarettes do you smoke per day?
2. What is your weekly frequency of alcohol consumption?
3. What was your weekly exercise frequency over the past 3 months?
4. Did you take any medications in the past 3 months? Which one?
5. Are you exposed to any hazardous agents, such as pesticides, radiation and, etc., in your workplace? Which one?

The data collection was supervised by trained nurses.

The period of 3 months is a parameter used in our centre, taking into account that it takes about 72 days for a sperm cell to be created, mature and get ejaculated. Therefore, conventional wisdom states that it takes about 2–3 months to see effects of any kind in sperm quality.

2.4 | Seminal analysis

After liquefaction for 30 min, sperm concentration, motility and morphology were evaluated, according to the WHO criteria (World Health Organization, 2010). Prewash total motile sperm count (TMSC) was calculated by multiplying the ejaculate volume by the sperm concentration/ml by the percentage of motile spermatozoa (a + b) in the neat sample (Hamilton et al., 2015).

Sperm samples were prepared using the density gradient centrifugation technique. Briefly, the lower layer (90% Isolate, Irvine Scientific, Santa Ana, CA, USA) was transferred into a conical centrifuge tube, and the "upper layer" (50% Isolate, Irvine Scientific) was gently dispensed on top of the lower layer. A liquefied 2.0 ml semen sample was placed on top of the upper layer and the tube was centrifuged (20 min at 330 × g), and this process was repeated using additional tubes until the whole semen sample was processed. The gradient layers were aspirated without disturbing the pellet, which was re-suspended in 1.0 ml of HEPES-buffered human tubal fluid medium (mHTF, Global, LifeGlobal, CT, USA) and then centrifuged (7 min at 330 × g). The washing procedure was repeated. The supernatant was then removed, and the pellet finally suspended in a volume of 0.5 ml of mHTF. Both media and semen samples were maintained under 37.0°C at all times.

2.5 | Sperm DNA fragmentation (SDF)

Two hundred sperm cells (Lim et al., 2013) were examined for SDF with the sperm chromatin dispersion test (Halosperm®, Halotech, Madrid, Spain). Briefly, a semen aliquot of 60 µl, previously diluted to 10 million/ml, was added to the agarose containing Eppendorf tube, provided in the kit and mixed. A 20 µl aliquot of the mix was transferred to the agarose pre-coated slide, provided in the kit and covered with a coverslip. The slide was refrigerated for 5 min. The coverslip was removed, and the slide immersed in an acid solution and incubated for 7 min. The slide was immersed in 10 ml of the lysing solution for 25 min, washed with distilled water, dehydrated in ethanol baths and air-dried. The slide was stained using the rapid panoptic, and examined under light microscopy for enumeration of spermatozoa with and without halos. Results were interpreted as SDF index and considered normal when ≤20%.

2.6 | ICSI procedures

Mature oocytes were injected with spermatozoa selected at 400× magnification (Palermo, Joris, Devroey, & Van Steirteghem, 1992). The injection was performed in a micro-injection dish prepared with 4-IL droplets of buffered medium (Global w/HEPES, LifeGlobal) and covered with paraffin oil on a heated stage at 37.0°C on an inverted microscope. Fertilisation was evaluated 16 hr post-ICSI. Embryos were cultured in a 50-IL drop of single-step culture medium (Global, LifeGlobal) supplemented with 10% protein supplement and covered with paraffin oil in a bench top incubator (K-Systems G185, Kivex Biotec Ltd, Denmark) under 8% $CO_{2\%}$ and 6% O_2 at 37°C for 5 days. No medium refresh was performed (Hardarson et al., 2015).

Embryos quality was evaluated according to the criteria determined in an Expert Meeting on Assisted Reproduction, which defined a globally accepted consensus (Medicine & Embryology, 2011). Cleavage-stage embryos presenting 8 to 10 cells on day 3, less than 15% fragmentation, symmetric blastomeres, no multinucleation, colourless cytoplasm, no perivitelline space granularity and ZP dimorphisms were considered high-quality embryos.

Up to three embryos were transferred per patient on day five of embryo development.

2.7 | Data analysis and statistics

Data are presented as the mean ± standard deviation for numerical variables and percentages for dichotomous variables.

For statistical purposes, paternal lifestyle habits were treated as independent variables, while seminal quality and the outcomes of ICSI were treated as dependent variables.

In the first analysis, the association between paternal lifestyle factors and semen quality was assessed by linear regression models, after adjustments for male age.

In the second analysis, logistic and linear regression models, controlled for male and female ages, and number of retrieved and injected oocytes, were used to assess the association between paternal lifestyle factors and ICSI results. Implantation rates were calculated per patient. Clinical pregnancy and live birth rates were calculated per transfer. Miscarriage was defined as pregnancy loss before 20 weeks.

Firstly, associations were made by comparing subjects exposed to with those not exposed to each independent variable. Daily medication was considered the intake of any kind of medicine. We did not take medicine type or quantity into account (e.g., 1 medicine/day was treated as equally as >1 medicine/day). For environmental exposure, we used the same approach as with medications. This information is now provided in text. For cigarette smoking, alcohol consumption and physical activity, further associations were estimated by taking into account the subjects' reported frequencies. For cigarette smoking, subjects were subdivided according to the number of cigarettes smoked per day: 0, nonsmokers; 1, 1–20 cigarettes, smokers; >20 cigarettes, heavy smokers. For alcohol consumption, stratification was made according to the weekly frequency of alcohol consumption: never, nonconsumers; once or twice a week, occasional consumers; >twice a week, frequent consumers. For physical activity, subjects were stratified according to their weekly exercise frequency over the past 3 months: never, sedentary; once or twice a week, lightly active; three times a week, moderately active; and >3 times a week, highly active.

For each continuous parameter, unstandardised linear regression coefficients (B) and slopes were used to describe the association between the parameter's mean value and paternal lifestyle factors. Odds ratios (OR) and 95% confidence intervals (CI) were used to describe the association between lifestyle factors and the outcomes of pregnancy. A $p < 0.05$ was considered statistically significant. Data analyses were performed with the Minitab® version 17 statistical program (2010).

3 | RESULTS

3.1 | Paternal lifestyle factors

Of the 965 subjects included in this study, 811 were nonsmokers (84.0%) and 154 (16.0%) were cigarette smokers. After stratification, 84 subjects (54.5%) were considered smokers and 70 (45.4%) heavy smokers (range: 2–30 cigarettes/day).

A total of 167 (17.3%) subjects reported alcohol consumption. One hundred and fifty-one (90.4%) were considered occasional consumers, and 16 (9.6%) heavy consumers (range: 1–5 times/week).

A total of 181 (18.8%) were under use of medication, such as anticoagulant (1, 0.5%), antifungal (1, 0.5%), gout medication (2, 1.1%), antihistaminic (3, 1.7%), antiasthmatic (3, 1.7%), analgesic (4, 2.2%), antibiotic (4, 2.2%), corticoids (5, 2.8%), anticonvulsant (6, 3.3%), antidiabetic (8, 4.4%), antidepressant (14, 7.7%), hormone (14, 7.7%), cholesterol lowering medication (15, 8.3%), gastrointestinal protectants (16, 8.8%), antihypertensive (26, 14.4%) or a combination of them (59, 32.6%).

Occupation exposure was reported by 8 (0.8%) men, which were all exposed to x-radiation in workplace.

Two hundred and one (20.8%) men were physically active. A total of 25 men (12.4%) exercised once or twice a week, 103 (51.2%) three times a week and 73 (36.3%) > 3 times a week (range: 1–7 times/week).

3.2 | Semen quality

Mean male age was 38.1 ± 6.4 years. Nine hundred and eight men (94.1%) had abnormal semen parameters, defined as having at least one of the following: sperm count <15 million spermatozoa/ml, sperm motility <40%, or normal sperm morphology <4%. Oligozoospermia was manifested in 169 patients (17.5%), asthenozoospermia in 133 patients (13.8%) and teratozoospermia in 908 patients (94.1%). Considering rapid progressive motility, asthenoteratozoospermia was manifested in 766 patients (79.4%). Mean SDF index was 17.8% ± 9.6%, 687 (71.2%) men had normal SDF index (SDF ≤ 20%), 193 (20%) had inconclusive results (20 > SDF ≤ 30) and

TABLE 1 General seminal characteristics of men undergoing conventional semen analysis for infertility investigation (n = 965)

Variable	Reference values[a]	Mean	Standard deviation
Male age (years)	—	38.1	6.4
Semen volume (ml)	≥1.5	3.1	1.7
Sperm count (×10^6/ml)	≥15	60.6	50.6
Total sperm count (×10^6)	≥39	176.6	169.4
Total sperm motility (%)	≥40	57.1	18.8
Progressive sperm motility (%)	≥32	49.1	18.8
Rapid sperm motility (%)	—	8.1	5.3
Total motile sperm count (×10^6)	—	97.6	101.2
Sperm normal morphology (%)	≥4	1.3	1.3
Sperm DNA fragmentation (%)	≤15	17.8	9.6

[a]World Health Organization (2010).

TABLE 2 Linear regression analyses' results for the influence of paternal lifestyle factors on semen quality (n = 965)

	Cigarette smoking		Alcohol consumption		Occupation exposure		Physical activity		Medication use	
Lifestyle factors	***B***	***p***	***B***	***p***	***B***	***p***	***B***	***p***	***B***	***p***
Semen quality										
Semen volume	−0.417	0.047	−0.1363	0.592	−0.2611	0.702	0.1146	0.436	0.0219	0.880
Sperm count/ml	−7.363	0.014	−12.527	0.040	−31.10	0.169	−3.329	0.494	0.984	0.838
Total sperm count	−4.43	0.023	−34.91	0.156	−80.79	0.299	5.85	0.728	−2.75	0.868
Total sperm motility	2.316	0.347	0.342	0.895	−7.362	0.285	−0.728	0.617	−0.595	0.684
Progressive sperm motility	−0.369	0.887	2.547	0.240	−7.660	0.297	−0.983	0.528	−0.225	0.885
TMSC	−1.38	0.045	−16.33	0.278	−43.23	0.330	0.094	0.992	−1.319	0.889
Sperm morphology	−0.0563	0.779	0.3751	0.180	0.2071	0.713	−0.1977	0.098	−0.0633	0.598
SDF	0.014	0.033	5.833	0.002	−2.334	0.586	−1.1684	0.221	0.6005	0.521

Note. B: unstandardised regression coefficient; SDF: sperm DNA fragmentation; TMSC: total motile sperm count.

85 (8.8%) had abnormal index (SDF > 30%). General seminal profile is shown in Table 1.

Results from linear model analysis of the influence of paternal lifestyle factors on semen quality are shown in Table 2.

Cigarette smoking negatively influenced semen volume (B: −0.417, slope: 1.5700, p = 0.047), sperm count/ml (B: −7.363, slope: 52.2981, p = 0.014), total sperm count (B: −4.43, slope: 178.165, p = 0.023), TMSC (B: −1.38, slope: 100.276, p = 0.045) and SDF (B: 0.014, slope: 9.76741, p = 0.033). When patients were subdivided in light, moderate and heavy smokers, no significant differences were observed (data not shown).

Alcohol consumption negatively influenced sperm count/ml (B: −12.527, slope: 42.2553, p = 0.040) and SDF (B: 5.833, slope: 9.68068, p = 0.002). The other investigated semen parameters were not significantly influenced by alcohol consumption. When patients were subdivided into occasional and frequent alcohol consumers, no significant differences were observed among them (data not shown).

There were no significant influences of other investigated paternal lifestyle factors on semen quality (Table 2).

3.3 | ICSI outcomes

After the application of inclusion criteria for the association between paternal lifestyle factors on ICSI outcomes, 233 couples were included in the analysis. Mean male age was 35.6 ± 4.4 years, and mean female age was 32.7 ± 2.8 years.

Mean values regarding response to controlled ovarian stimulation were as follows: total dose of FSH administered 2,521.4 ± 616.5 UI, oestradiol peak on the day of hCG trigger 2,317.1 ± 2,146.1 pg/ml, number of follicles 14.0 ± 10.9, number of retrieved oocytes 10.1 ± 8.1, number of mature oocytes 7.6 ± 6.4, mature oocyte rate 74.7%.

TABLE 3 Linear regression analyses' results for the association between paternal lifestyle factors and ICSI outcomes (n = 233)

	Cigarette smoking		Alcohol consumption		Occupation exposure		Physical activity		Medication use	
Lifestyle factors	***B***	***p***	***B***	***p***	***B***	***p***	***B***	***p***	***B***	***p***
ICSI outcomes										
Fertilisation rate	−1.349	0.039	−3.617	0.041	3.71	0.759	1.600	0.473	−2.236	0.406
High-quality embryos rate on day 3	4.383	0.450	9.559	0.166	−11.24	0.619	1.359	0.704	6.925	0.182
Blastocyst formation rate on day 5	−14.244	0.025	−34.801	0.042	0.13	0.996	−6.411	0.111	−3.691	0.548
Implantation rate	5.384	0.451	−0.770	0.190	−23.94	0.475	−2.913	0.469	9.502	0.142

Note. B: unstandardised regression coefficient; ICSI: intracytoplasmic sperm injection

Mean values regarding ICSI outcomes were as follows: fertilisation rate 85.1%, high-quality embryos rate on day 3 44.1% ± 31.3%, blastocyst formation rate 44.2% ± 28.8%, number of transferred embryos 2.0 ± 0.7, implantation rate 25.0% ± 34.3%, clinical pregnancy rate 46.5% (88/189), ongoing pregnancy rate 89.8% (79/88), miscarriage rate 10.2% (9/88), live birth rate 41.3% (78/189).

The association between ICSI outcomes and paternal lifestyle factors is shown in Tables 3 and 4 respectively. Cigarette smoking and alcohol consumption negatively influenced fertilisation rate (B: −1.349, slope: 21.9506, p-value: 0.039 and B: −3.617, slope: 20.1380, p-value: 0.041, respectively) and blastocyst formation on day 5 (B: −14.244, slope: 28.8513, p-value: 0.025 and B: −34.801, slope: 30.0446, p-value: 0.042 respectively). There were no significant influences of other investigated paternal lifestyle factors on ICSI outcomes. When patients were subdivided in light, moderate and heavy smokers or occasional and frequent alcohol consumers, no significant differences were observed (data not shown).

4 | DISCUSSION

Previous studies assessing the influence of paternal lifestyle habits on semen quality have yielded conflicting results. Our results showed that cigarette smoking negatively influences semen volume, sperm count/ml, total sperm count, TMSC and SDF, whereas alcohol consumption negatively influences sperm count/ml and SDF. Additionally, cigarette smoking and alcohol consumption negatively influenced fertilisation rate and blastocyst formation on day 5, post-ICSI.

Negative effects of smoking on several semen parameters, such as volume (Zhang et al., 2000), sperm density (Kunzle et al., 2003; Vine, Tse, Hu, & Truong, 1996; Zhang et al., 2000), total sperm count (Joo et al., 2012; Rubes et al., 1998; Vine et al., 1996), sperm motility (Kunzle et al., 2003; Rubes et al., 1998; Vine et al., 1996; Zhang et al., 2000), sperm morphology (Rubes et al., 1998) and sperm viability (Zhang et al., 2000), have been previously reported. Moreover, smoking has been associated with sperm aneuploidy (DeMarini, 2004; Shi et al., 2001). On the contrary, some studies have found no association between cigarette smoking and semen quality (Li et al., 2009; Swan et al., 2003; Trummer, Habermann, Haas, & Pummer, 2002).

It has been demonstrated that smoking is associated with a reduction of approximately 20% in sperm count (Vine, Margolin, Morrison, & Hulka, 1994). A more recent meta-analysis found that smoking reduces sperm count and motility, in a dose-dependent manner (Sharma, Harlev, Agarwal, & Esteves, 2016).

In this study, we failed to determine a dose–response relationship between cigarette smoking and semen quality. However, previous studies suggested a decline in semen parameters with an increase in the smoking quantity (Pasqualotto, Sobreiro, Hallak, Pasqualotto, & Lucon, 2006; Ramlau-Hansen et al., 2007; Zhang et al., 2000).

It is well known that spermatozoa are sensitive to ROS (Maneesh & Jayalekshmi, 2006), and tobacco contains several dangerous substances that are known to generate them (Traber, Vliet, Reznick, & Cross, 2000). Saleh, Agarwal, Sharma, Nelson, and Thomas (2002) found that smoking was associated with increased ROS concentrations. Excessive ROS production related to smoking could increase the antioxidant capacity, leading to aerobic injury of seminal plasma, OS (Lavranos, Balla, Tzortzopoulou, Syriou, & Angelopoulou, 2012) and sperm DNA damage (Cocuzza, Sikka, Athayde, & Agarwal, 2007). Direct correlations with DNA damage have been shown in many studies (Anifandis et al., 2014; Fariello et al., 2012; Linschooten et al., 2011; Taha, Ez-Aldin, Sayed, Ghandour, & Mostafa, 2012).

Other cigarette compounds, such as cadmium and lead, can cause DNA damage (Hengstler et al., 2003) and are detected in seminal fluid presenting oxidative stress (Kiziler et al., 2007). Nicotine induces sperm DNA breaks (Arabi, 2004), and cotinine, which is its major metabolite, is detected in smokers' seminal plasma (Wong et al., 2000).

Moreover, Ramlau-Hansen et al. (2007) observed that cigarette smoking disrupts the hypothalamic–pituitary–gonadal axis' function, represented by an increased LH and testosterone levels. Moreover, dose-dependent rises in FSH and inhibin B levels were also observed.

We observed that semen quality is also affected by alcohol consumption, represented by lower semen volume, sperm concentration, motility and normal morphology. Previous studies have suggested a negative association between alcohol consumption and semen

TABLE 4 Binary regression analyses' results for the association between paternal lifestyle factors and ICSI outcomes (n = 233)

ICSI outcomes	Pregnancy rate	Miscarriage rate
Lifestyle factors		
Cigarette smoking		
OR	1.22	1.19
CI	0.49–3.06	0.15–2.11
p	0.673	0.178
Alcohol consumption		
OR	3.00	0.43
CI	0.19–7.58	0.05–4.10
p	0.122	0.634
Occupation exposure		
OR	1.42	1.48
CI	0.73–2.78	0.33–1.65
p	0.308	0.632
Physical activity		
OR	0.82	1.51
CI	0.48–1.41	0.37–6.10
p	0.474	0.568
Medication use		
OR	1.70	3.96
CI	0.78–3.66	0.60–26.29
p	0.180	0.149

Note. Values are OR: odds ratio; CI: 95% confidence interval; ICSI: intracytoplasmic sperm injection.

quality (Gaur, Talekar, & Pathak, 2010; Joo et al., 2012; Martini et al., 2004; Muthusami & Chinnaswamy, 2005; Silva et al., 2017; Stutz et al., 2004), although others did not confirm these findings (de Jong, Menkveld, Lens, Nienhuis, & Rhemrev, 2014; Hansen et al., 2012; Jensen et al., 2014; Lopez Teijon et al., 2007). Decreased semen quality and occasional azoospermia have also been found in heavy alcohol consumers (Guthauser, Boitrelle, Plat, Thiercelin, & Vialard, 2014).

Adverse effects on both testosterone metabolism and spermatogenesis have been proposed as mechanisms through which alcohol consumption compromises semen quality. Alcohol intake was found to modify free oestradiol and free testosterone ratio (Hansen et al., 2012), and to be consistently associated with spermatogenetic arrest and Sertoli-cell-only syndrome (Pajarinen et al., 1996). Recently, Silva et al. (2017) showed that cigarette and alcohol are associated with increased oxidative stress, and compromised epididymal and accessory sex glands functions. Additionally, ethanol has been shown to compromise sperm nuclear maturity and DNA integrity in rats (Talebi, Sarcheshmeh, Khalili, & Tabibnejad, 2011). It has been suggested that semen alterations caused by etilism may be reversible if patient quits consumption (La Vignera et al., 2013).

The association between semen quality and physical activity remains unclear, and we failed to demonstrate such an association. There is evidence that exercising may improve general semen quality (Gaskins et al., 2014, 2015; Hajizadeh Maleki, Tartibian, Eghbali, & Asri-Rezaei, 2013; Vaamonde, Da Silva-Grigoletto, Garcia-Manso, Barrera, & Vaamonde-Lemos, 2012), or not (Eisenberg, Kim, et al., 2015; Minguez-Alarcon, Chavarro, Mendiola, Gaskins, & Torres-Cantero, 2014). Despite the numerous health benefits obtained from physical activity, previous studies have shown that vigorous exercise, such as long-distance running and endurance cycling, is detrimental to semen quality and male fertility (Safarinejad, Azma, & Kolahi, 2009; Vaamonde et al., 2009).

Male occupation may be a potential source of adverse exposure to chemical, physical and psychological factors (Eisenberg, Chen, Ye, & Buck Louis, 2015). In this study, we failed to demonstrate an association between occupational exposure and semen quality. However, it is important to highlight that only eight men reported occupational exposure and were all exposed to x-radiation, which is an ionising radiation, in workplace. It is known that testicular function is extremely sensitive to ionising radiation (Jensen, Bonde, & Joffe, 2006). Transitory reduced sperm count and even long-lasting or permanent azoospermia could result from radiation exposure, depending on the dose (Rowley, Leach, Warner, & Heller, 1974). Occupational exposure limits have been implemented in many countries, and if not surpassed, testicular effects are improbable (Jensen et al., 2006).

In this study, the use of medications and semen parameters was not associated. However, a recent study found a negative association between the quantity of medications and sperm count (Eisenberg, Chen, et al., 2015). The same study demonstrated that hypertensive men have lower percentage of normal sperm forms compared with normotensive men. As possible links between somatic health and semen quality have been suggested (Eisenberg, Li, Behr, Pera, & Cullen, 2015), it is not possible to determine if those associations are a proxy for health status or indicative of pharmacotoxic effects.

As for ICSI outcomes, we found that cigarette smoking and alcohol consumption are negatively associated with fertilisation rate and blastocyst formation on day 5. We could suggest that the same mechanisms responsible for the negative effects of those habits on seminal quality are behind those associations.

Although studies assessing the effects of cigarette smoking and alcohol consumption on in vitro reproduction results are scarce, reduced odds of live birth were observed post-recent male partner alcohol consumption (Klonoff-Cohen, Lam-Kruglick, & Gonzalez, 2003) and cigarette smoking (Fuentes et al., 2010). It was demonstrated that fertilisation, cleavage and blastocyst formation rates after IVF were significantly reduced in animals exposed to cigarette smoke for 10 weeks, compared to animals exposed to incense stick smoke and to control group animals. Additionally, the live offspring rate was smaller in animals exposed to cigarette smoke (Kapawa et al., 2004).

It is important to highlight that not only environmental influences, but also some sexually transmitted diseases (e.g., gonorrhoea, chlamydia), which are closely related to lifestyle, may affect spermatogenesis, leading to male infertility (Kim et al., 2017). Infection and inflammation of the male reproductive tract result in an intense release of cytokines and other inflammatory mediators that have

marked effects on the regulation of spermatogenesis (Azenabor, Ekun, & Akinloye, 2015). Additionally, inflammatory damage on the male genital tract leads to the increased generation of ROS (Agarwal, Saleh, & Bedaiwy, 2003).

This relation between inflammation and semen quality points to a drawback in this study, which is the lack of evaluation of white blood cells in semen samples. In addition, other drawbacks are as follows: (a) information on paternal lifestyle factors is collected through self-completed nonvalidated questionnaires, (b) we only assessed lifestyle factors through a few questions, which may have introduced under-reporting and underestimation of the true association, and (c) female lifestyle were not taken into account, which may have biased the associations with both semen quality and ICSI outcomes. The strengths and differentials of this study are (a) the large number of participants, (b) its homogenised population, as the effects of the independent variables such as alcohol consumption, cigarette smoking, environmental and occupation exposure, medications and physical activity on semen quality were analysed within the same population, while previous studies deal with one or two independent variables only, and (c) we went beyond the effects on sperm quality and evaluated the association between male lifestyle factors and ICSI outcomes, and such studies are scarce.

In conclusion, smoking and alcohol drinking habits seem to reduce semen quality, fertilisation and blastocyst formation rates. Thus, it would be wise to recommend that male partners reconsider their lifestyle during in vitro reproduction treatment.

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