Article

# Exposure to environmental toxins in males seeking infertility treatment: a case-controlled study

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## Abstract

This case–control study explored the role of environmental toxins in male infertility in patients attending an assisted reproduction clinic in southeastern Spain. Exposures were compared by questionnaire for 30 infertile oligoasthenoteratozoospermic males (cases) and 31 normozoospermic controls residing in the area. Odds ratios and 95% confidence intervals (CI) were used to estimate differences in lifestyle and chemical occupational exposures. More than two-thirds of the cases (23/30), compared with less than one-third of controls (10/31), had been exposed occupationally to at least one toxin or pollutant (OR = 6.9; 95% CI: 2.2–21.4) and were also more exposed to them currently (OR = 5.2; 95% CI: 1.6–17.2). Exposure to glues, solvents or silicones (OR = 22.9; 95% CI: 2.8–190.9), metals (OR = 8.8; 95% CI: 1.4–54.2) and physical agents (OR = 7.3; 95% CI: 1.4–36.7) in the past, as well as current exposure to glues, solvents or silicones (OR = 10.4; 95% CI: 2.6–42.5) and physical agents (OR = 4.7; 95% CI: 1.1–19.2), were significantly higher in cases than in controls. Average duration of exposure was also significantly higher in cases ($P < 0.001$). This study suggests that male infertility in patients attending infertility clinics may often be the result of occupational exposure.

***Keywords***: *chemicals, environmental toxins, male infertility, occupational exposures, semen quality*

## Introduction

Several studies have suggested that human semen quality and fecundity have been declining during recent decades (Carlsen *et al*., 1992; Auger *et al*., 1995; Adamopoulus *et al*., 1996; Irvine *et al*., 1996; Becker and Berhane, 1997; Swan *et al*., 1997, 2000; Skakkebæk *et al*., 2001, 2006; Aitken *et al*., 2004; Hauser, 2006; Jørgensen *et al*., 2006; Swan, 2006), although other studies (Bujan *et al*., 1996; Fisch *et al*., 1996a; Paulsen *et al*., 1996) have reported results indicating that these changes have not developed equally. In fact, geographical differences in semen quality support the idea that it has decreased only in some areas (Fisch and Goluboff, 1996; Fisch *et al*., 1996b; Jørgensen *et al*., 2001; Swan *et al*., 2003). Changes in seminal samples might be related to environmental pollutants, occupational and lifestyles characteristics (Figà-Talamanca *et al*., 1996; Tielemans *et al*., 1999; Jung *et al*., 2005; Homan *et al*., 2007; Lopez-Teijón *et al*., 2007).

Volatile organic compounds (VOC) (Wagner *et al*., 1990), certain halogenated compounds (Whorton and Foliart, 1983), several heavy metals (Robins *et al*., 1997; Irgens *et al*., 1999; Benoff *et al*., 2000) or xenoestrogens such as

some polychlorinated biphenyls (PCB) (Wassermann *et al*., 1979; Rozati *et al*., 2002; Spano *et al*., 2005), organochlorine compounds (pesticides) (Swan, 2005; Carreño *et al*., 2007) and phthalate esters (PE) (Duty *et al*., 2003) may all compromise reproductive male function. Xenoestrogens have also been identified as endocrine disruptors that might not only cause testicular dysgenesis syndrome, but also disturb meiosis in developmental germinal cells (Sharpe and Skakkebaek, 1993; Skakkebaek *et al*., 2001). Chemicals and toxins could adversely affect the male reproductive system, either by causing gonadal endocrine disruption (Skakkebæk *et al*., 2001; Sharpe and Irvine, 2004), or by affecting spermatogenesis, resulting in poor semen quality (Assennato *et al*., 1987; Robins *et al*., 1997; Wyrobek *et al*., 1997). Occupational activities involving exposure to chemicals or toxins could impair male reproductive health and cause infertility in humans (Mortensen, 1988; Ng *et al*., 1991; Lerda, 1992; Bonde, 1993; Fredricsson *et al*., 1993; Lähdetie, 1995; Alexander *et al*., 1996, 1998; Wyrobek *et al*., 1997; Bigelow *et al*., 1998; Irgens *et al*., 1999; Viskum *et al*., 1999; Zenzes *et al*., 1999; Sallmen *et al*., 2000; Figà-Talamanca *et al*., 2001; Sheiner *et al*., 2003; Chang *et al*., 2004; Jensen *et al*., 2006; Queiroz and Waissmann, 2006).

Compared with unexposed workers, semen samples from males occupationally exposed to hydrocarbons such as toluene, benzene and xylene (BTX) present anomalies, including alterations in viscosity, liquefaction capacity, sperm count, sperm motility, and the proportion of spermatozoa with normal morphology (Xiao *et al*., 1999; De Celis *et al*., 2000; De Rosa *et al*., 2003). Similarly, exposure to solvents may affect human seminal quality (Tielemans *et al*., 1999; Jensen *et al*., 2006) proportionally with the range of exposure (Cherry *et al*., 2001).

Exposure to metals (mainly lead and cadmium) has long been associated with low sperm motility and density, increased morphological anomalies and male infertility (Wagner *et al*., 1990). Males employed in metal industries had decreased fertility when compared with other males as shown by delayed pregnancy and reduced semen quality (Umeyama *et al*., 1986; Assennato *et al*., 1987; Xuezhi *et al*., 1992; Gennart *et al*., 1992; Bonde, 1993; Chia *et al*., 1994; Robins *et al*., 1997; Spinelli *et al*., 1997; Danadevi *et al*., 2003; Akinloye *et al*., 2006; Naha and Chowdhury, 2006). Finally, different reports have shown a significant inverse correlation between blood lead concentration and semen quality in human males (Benoff *et al*., 2000, 2003; Telisman *et al*., 2000; Eibensteiner *et al*., 2005). Recently, Telisman *et al*. (2007) reported reproductive toxicity of low-level lead exposure in men with no occupational exposure to metals. However, other articles are less conclusive and show no apparent adverse effects of lead or cadmium exposure on semen quality or decreased fertility (Abou-Shakra *et al*., 1989; Coste *et al*., 1991; Hovatta *et al*., 1998).

Smoking is the best-studied environmental exposure affecting male and female fertility (Curtis *et al*., 1997). Moreover, smoking is an important confounding variable when considering the effects of lead and cadmium exposure on human health (Sram *et al*., 1996).

Although there is a growing body of literature relating to the effect of specific substances on semen quality, the relationship between chemical exposures and male infertility is more contradictory and less well documented. Only a few studies have explored differences between infertile males attending fertility clinics and controls (Bonde, 1993; Bigelow *et al*., 1998; Irgens *et al*., 1999; Chia *et al*., 2000; Chia and Tay, 2001; Cherry *et al*., 2001), but findings are inconclusive. While differences in occupational activities (welding, white collar professionals, exposure to metals), chemicals (solvents) or physical exposures (electromagnetic fields, heat) are found in some studies, the same or similar exposures (welding fumes, metals, and pesticides) were not associated with infertility in others.

The aim of the study was to explore the role of exposure to environmental toxicants in male infertility by comparing normozoospermic and oligoasthenoteratozoospermic patients attending an assisted reproduction clinic for infertility treatment.

# Materials and methods

## Design and patients

This was designed as an analytical observational case-controlled study. The patients were males in infertile couples (without female infertility factor) attending the IVF clinics of the Bernabeu Institute (IB) Cartagena, Elche and Alicante (southeast region of Spain), between 2005 and 2007. The patients were divided into two groups on the basis of seminal quality according to World Health Organization (WHO) criteria (World Health Organization, 1999): (i) cases ($n$ = 30): patients with severe or moderate oligozoospermia (<5 $\times 10^6$ sperm/ml or between 5 and 20 sperm/ml respectively) and severe teratozoospermia [<6% normal forms according to strict criteria (Kruger *et al*., 1986)]; and (ii) controls ($n$ = 31) comprising normozoospermic patients (≥20 $\times 10^6$ spermatozoa/ml, ≥50% motile spermatozoa and ≥14% normal forms strict criteria). Only males from infertile couples with no known female infertility factor (such as blocked tubes, endometriosis, polycystic ovarian syndrome or hormonal abnormalities) were eligible to be included as cases in this study. Subjects provided at least two semen samples after an abstinence period of between 3 and 5 days. Analyses of samples were performed following WHO criteria (World Health Organization, 1999). Semen analyses were performed by the same technician. Semen parameters evaluated included: ejaculate volume, sperm concentration, percentage of motile spermatozoa (grades a and b, according to WHO criteria), and percentage of normal forms following Kruger's strict criteria (Kruger *et al*., 1986). Semen pH was read on pH paper (samples ranged between pH 7.0 and pH 8.1). Semen volume was measured using a calibrated pipette. To assess motility, a 5 $\mu$l aliquot was added to a Makler chamber (Sefi-Medical Instruments Ltd, Haifa, Israel) and spermatozoa were visualized by phase contrast microscopy (Olympus CX21; Olympus, Tokio, Japan) at ×200 magnification. A 20 $\mu$l semen aliquot smear was dried at room temperature and stained with Papanicolau stain (Vázquez-Levin *et al*., 1998).

Patients who showed a history of varicocoele, cryptorchidism or endocrine hypogonadism (abnormal hormonal

concentrations), chemo- and/or radiotherapy, and who presented anomalies in the karyotype or Y chromosome microdeletions were excluded from the study. Patients were evaluated based on illnesses and medication being taken (e.g. therapeutic drugs and chemotherapy). No differences were found between the two groups in this respect. This study was approved by the Institutional Review Board of the clinics and patients were included in the study after giving informed written consent.

## Questionnaire

All patients were interviewed face-to-face by the same interviewer before or after the first semen sample was obtained. The questionnaire was adapted from the one developed by the Agency for Toxic Substances and Disease Registry (ATSDR) in co-operation with NIOSH (1992). A detailed work history containing current and past occupational exposures was obtained and patients were also asked about their current occupation and daily activities. Past exposures directly related to the jobs and exposures detailed in the occupational history were named as 'frequent past exposures'. An additional question was included to cover infrequent past exposures: 'Have you ever worked or been in direct contact (touching, inhaling or ingesting), at your work or as a consequence of a hobby, with any of the following substances?' Exposures identified by that question were named as 'general past exposures'. The list read out contained 52 chemicals, including specific metals, acids, bases or alcohols, pesticides, insecticides, glues, solvents or silicones, gasoline, exhaust fumes, paintings or dyes, dust particles, fibres, physical agents, fuel-oil or greases and welding fumes or gases. Lifetime exposure to chemicals was estimated from the occupational history and the information provided by the patients. Information on current environmental and lifestyle exposures were also recorded (e.g. toxic habits, house environment, hobbies, daily clothes, underwear). Administration of the questionnaire took 1 h on average. All questionnaires were completed by the same interviewer.

## Statistical analysis

Odds ratios and 95% confidence intervals were used to explore differences in occupational and lifestyle exposures. Significance level for all tests was set at $P \leq 0.05$. Analyses were performed using the Statistics Package for Social Sciences, SPSS 13.0. When one of the cells contained a '0' in the contingency tables, odds ratios were estimated using the null hypothesis method. Non-parametric methods were used to compare the mean number of years of exposure between cases and controls.

# Results

**Table 1** shows the semen parameters and hormonal concentrations of the cases and controls. Hormonal values were normal and similar between two groups. There were no significant differences between the two semen samples within groups. As expected, due to the study design, sperm concentration, motility and morphology were significantly lower in cases than in controls ($P \leq 0.001$). Seminal volume did not differ between the two groups. **Table 2** provides a summary of lifestyle and home or recreational exposures to toxins and pollutants in the two groups. No statistically significant differences were found between cases and controls.

**Table 3** shows the occupational exposures of the two groups. More than two-thirds of the cases (23/30) had been exposed occupationally in the past to at least one toxin or pollutant (OR = 6.9; 95% CI: 2.2–21.4). Half of the cases were also currently exposed occupationally to toxins or pollutants (OR = 5.2; 95% CI: 1.6–17.2). Cases had been more frequently exposed to glues, solvents or silicones in the past (OR = 22.9; 95% CI: 2.8–190.9) and continued to be more frequently exposed at the time of the interview (OR = 10.4; 95% CI: 2.6–42.5). Cases had been more exposed to metals in the past (OR = 8.8; 95% CI: 1.4–154.2), but not at the time of the study. Current and past exposures to physical agents (heat or cold) were also more frequent in cases

**Table 1.** Sperm quality and hormone concentrations in infertile oligoasthenoteratozoospermic men (cases) and in infertile normozoospermic controls.

| *Variable* | *Cases (n = 30)* | | | *Controls (n = 31)* | | | *P-value* |
|---|---|---|---|---|---|---|---|
| | *1st sample* | *2nd sample* | *Average*[a] | *1st sample* | *2nd sample* | *Average*[a] | |
| *Semen sample* | | | | | | | |
| Volume (ml) | 3.9 ± 1.3 | 3.7 ± 1.1 | 3.8 ± 1.2 | 3.6 ± 1.0 | 3.4 ± 1.7 | 3.5 ± 1.4 | NS |
| Concentration ($\times 10^6$/ml) | 3.2 ± 2.3 | 3.7 ± 1.8 | 3.3 ± 4.1 | 41.7 ± 15.6 | 37.4 ± 13.6 | 39.5 ± 14.6 | <0.001 |
| Sperm motility (grade a + b) | 29.2 ± 19.8 | 25.5 ± 17.4 | 27.4 ± 18.6 | 51.1 ± 10.3 | 53.3 ± 14.3 | 52.2 ± 12.3 | <0.001 |
| % Normal morphology[b] | 3.6 ± 1.3 | 3.8 ± 1.7 | 3.7 ± 1.5 | 23.4 ± 4.9 | 21.1 ± 4.1 | 22.3 ± 4.5 | <0.001 |
| *Hormone concentration* | | | | | | | |
| FSH (mUI/ml) | 6.4 ± 2.1 | – | – | 6.5 ± 1.4 | – | – | NS |
| LH (mUI/ml) | 4.2 ± 1.2 | – | – | 4.1 ± 1.4 | – | – | NS |
| Testosterone (ng/ml) | 5.3 ± 1.6 | – | – | 5.4 ± 1.3 | – | – | NS |

Values are mean ± SD. NS = not statistically significant.
[a]No significant differences were found between the first and second samples in cases or controls.
[b]Strict criteria: Kruger *et al.* (1986).

**Table 2.** Comparison of general characteristics of infertile oligoasthenoteratozoospermic men (cases) and infertile normozoospermic controls.

| *Variable* | *Cases* (n = 30) | *Controls* (n = 31) | *OR* | *95% CI* |
|---|---|---|---|---|
| Age in years (mean ± SD) | 34.2 ± 3.7 | 32.8 ± 3.9 | NS | – |
| Clinic attended | | | | |
| Alicante | 14 (46.7) | 18 (58.1) | – | – |
| Cartagena | 13 (43.3) | 13 (41.9) | – | – |
| Elche | 3 (10.0) | – | – | – |
| Current smoker | 8 (26.7) | 11 (35.5) | 0.66 | 0.22–1.97 |
| Number of years smoking (mean ± SD) | 18.8 ± 4.1 | 15.8 ± 3.5 | NS | – |
| Ever smoked | 11 (36.7) | 11 (35.5) | 1.06 | 0.37–2.99 |
| Passive smoking at home | 1 (3.3) | 0 (0.0) | – | – |
| Passive smoking at work | 5 (16.7) | 4 (12.9) | 1.35 | 0.33–5.60 |
| Current alcohol drinking | 14 (46.7) | 18 (58.1) | 0.63 | 0.23–1.74 |
| Nail biting | 7 (23.3) | 11 (35.5) | 0.55 | 0.18–1.70 |
| Sedentary occupation | 9 (30.0) | 13 (41.9) | 0.59 | 0.21–1.71 |
| Coach/lorry driver | 2 (6.7) | 2 (6.5) | 1.03 | 0.13–7.87 |
| Self car repair | 5 (16.7) | 4 (12.9) | 1.35 | 0.33–5.60 |
| Hobbies or handicrafts with toxic products | 6 | 2 (6.5) | 3.62 | 0.67–19.63 |
| Recently refurbished home | 6 (20.0) | 4 (12.9) | 1.47 | 0.47–6.60 |
| Lead pipeline at home | 1 (3.3) | 4 (12.9) | 0.23 | 0.02–2.21 |
| Living near pollutant areas | 3 (10) | 2 (6.5) | 1.61 | 0.25–10.40 |
| Heavy traffic near home | 13 (43.3) | 12 (38.7) | 1.21 | 0.44–3.36 |
| Use of synthetic clothes | 12 (40.0) | 15 (48.4) | 1.41 | 0.51–3.88 |
| Fitted trousers | 14 (46.7) | 16 (51.6) | 0.82 | 0.30–2.24 |
| Fitted underwear | 26 (86.7) | 25 (80.6) | 1.56 | 0.39–6.19 |
| Cell phone in pocket | 26 (86.7) | 29 (93.5) | 0.45 | 0.08–2.65 |

Values are *n* (%), unless otherwise stated.
No significant differences were found between the two groups for any of the variables.
CI = confidence interval; NS = not statistically significant; OR = odds ratio.

than in controls (OR = 4.7; 95% CI: 1.1–19.2, and OR = 7.3; 95% CI: 1.4–36.7 respectively).

**Table 4** shows the mean duration of lifetime occupational exposures in years in cases and controls. Infertile males had had a significantly higher duration of exposure to overall toxins and pollutants ($P < 0.001$), to metals ($P < 0.02$), and to glues, solvents or silicones ($P < 0.001$). **Table 5** compares the main types of activities in cases and controls. Infertile males were more likely to be occupied in industrial activities (shoe and leather industry, shipyard workers, welders, plumbers or other industries) and significantly less frequently occupied as white collar professionals or clerical workers (OR = 0.4; 95% CI: 0.13–1).

**Table 3.** Profile of occupational and environmental exposures in infertile oligoasthenoteratozoospermic men (cases) and normozoospermic controls.

| *Variable* | *Cases (n = 30)* | *Controls (n = 31)* | *OR* | *95% CI* |
|---|---|---|---|---|
| *Frequent past exposure to:* | | | | |
| Overall toxics or pollutants | 23 | 10 | **6.9** | **2.2–21.4** |
| Metals | 5 | 0 | **8.8**[a] | **1.4–54.2** |
| Acids, bases or alcohols | 2 | 2 | 1.1 | 0.1–7.9 |
| Pesticides or insecticides | 4 | 2 | 2.2 | 0.4–13.2 |
| Glues, solvents or silicones | 13 | 1 | **22.9**[a] | **2.8–190.9** |
| Gasoline or exhaust fumes | 8 | 5 | 1.9 | 0.6–6.6 |
| Paintings or dyes | 3 | 0 | 8.2 | 0.8–81.9 |
| Dust particles or fibres | 5 | 1 | 6.0 | 0.7–54.8 |
| Physical agents (heat, cold) | 7 | 2 | 4.4 | 0.8–23.3 |
| Fuel-oil or greases | 2 | 3 | 0.7 | 0.1–4.3 |
| Welding fumes or gases | 2 | 0 | 7.9[a] | 0.5–129.5 |
| *General past exposure to:* | | | | |
| Overall toxics or pollutants | 23 | 10 | **6.9** | **2.2–21.4** |
| Metals | 7 | 0 | **9.6**[a] | **2.1–45.7** |
| Acids, bases or alcohols | 5 | 4 | 1.4 | 0.3–5.6 |
| Pesticides or insecticides | 4 | 2 | 4.6 | 0.5–43.9 |
| Glues, solvents or silicones | 15 | 3 | **9.3** | **2.3–37.4** |
| Gasoline or exhaust fumes | 8 | 5 | 1.9 | 0.6–6.6 |
| Paints or dyes | 3 | 0 | 8.2[a] | 0.8–81.9 |
| Dust particles or fibres | 9 | 1 | **12.9** | **1.5–109.3** |
| Physical agents (heat, cold) | 10 | 2 | **7.3** | **1.4–36.7** |
| Fuel-oil or greases | 4 | 2 | 2.2 | 0.4–13.2 |
| Welding fumes or gases | 5 | 1 | 6 | 0.7–54.8 |
| *Current exposures to:* | | | | |
| Overall toxics or pollutants | 15 | 5 | **5.2** | **1.6–17.2** |
| Metals | 4 | 1 | 4.6 | 0.5–43.9 |
| Acids, bases or alcohols | 1 | 1 | 1.1 | 0.1–17.3 |
| Pesticides or insecticides | 1 | 1 | 1.1 | 0.1–17.3 |
| Glues, solvents or silicones | 9 | 0 | **10.4**[a] | **2.6–42.5** |
| Gasolines or exhaust fumes | 3 | 4 | 0.8 | 0.2–3.7 |
| Paintings or dyes | 2 | 0 | 7.9[a] | 0.5–129.5 |
| Dust particles or fibres | 7 | 2 | 4.4 | 0.8–23.3 |
| Physical agents (heat, cold) | 10 | 3 | **4.7** | **1.1–19.2** |
| Fuel-oil or greases | 4 | 2 | 2.2 | 0.4–13.2 |
| Welding fumes or gases | 3 | 0 | 8.2[a] | 0.8–81.9 |
| Professional colleagues with health problems | 5 | 0 | **8.8**[a] | **1.4–54.2** |
| Physical discomfort due to occupational expositions | 3 | 0 | 8.2[a] | 0.8–81.9 |

Values in bold indicate a statistically significant association. CI = confidence interval.
[a]Odds ratio (OR) was calculated using the null hypothesis.

**Table 4.** Duration of lifetime occupational exposure in infertile oligoasthenoteratozoospermic men (cases) and in infertile normozoospermic controls.

| *Frequent past exposure to:* | *Cases (n = 30)* | *Controls (n = 31)* | P-*value*[a] |
|---|---|---|---|
| Overall toxins or pollutants | 7.8 ± 7.5 | 2.6 ± 5.1 | <0.001 |
| Metals | 1.2 ± 3.2 | 0[b] | 0.02 |
| Acids, bases or alcohols | 0.8 ± 3.2 | 0.2 ± 0.8 | NS |
| Pesticides or insecticides | 0.7 ± 2.7 | 0.3 ± 1.3 | NS |
| Glues, solvents or silicones | 4.4 ± 7.0 | 0.1 ± 0.2 | <0.001 |
| Gasoline or exhaust fumes | 1.3 ± 2.9 | 2.1 ± 5.1 | NS |
| Paints or dyes | 1.0 ± 3.5 | 0[b] | NS |
| Dust particles or fibres | 0.9 ± 2.8 | 0.2 ± 1.3 | NS |
| Physical agents (heat, cold) | 2.4 ± 5.2 | 0.7 ± 2.8 | NS |
| Fuel-oil or greases | 0.4 ± 1.5 | 1.4 ± 4.5 | NS |
| Welding fumes or gases | 1.0 ± 3.9 | 0[b] | NS |

Values are mean ± SD years exposure. NS = not statistically significant.
[a]Non-parametric methods were used to compare mean years of exposure in cases and controls.
[b]No exposure.

**Table 5.** Occupational activities in infertile oligoasthenoteratozoospermic men (cases) and infertile normozoospermic controls.

| *Occupation* | *Total* | *Cases* | *Controls* | *OR* | *95% CI* |
|---|---|---|---|---|---|
| Professionals and clerical workers | 32 | 12 | 20 | 0.4 | 0.13–1.0 |
| Construction, transport and electricians | 16 | 8 | 8 | 1.1 | 0.3–3.3 |
| Industrial workers[a] | 13 | 8 | 3 | 4.7 | 1.1–19.2 |

CI = confidence interval; OR = odds ratio.
[a]Includes shoe and leather industry, shipyard workers, welders, plumbers other industrial workers.

## Discussion

Infertile oligoasthenoteratozoospermic patients, but not normozoospermic controls, had a high frequency of exposure to chemicals, and marked associations between some well known hazards for semen quality and infertility in patients attending infertility clinics. Some occupational activities were clearly overrepresented in infertile patients while others appeared to be protective of semen quality in this setting. More specifically, the study strongly suggests that exposure to solvents, to metals, and to some physical agents (extreme heat or cold) may play a large role in male infertility.

The association between chemical solvents and low semen quality has been reported previously, although the strengths of the associations, ranging from 1.4 to 7.7, were lower than the ones reported here (Tielemans *et al.,* 1999; Cherry *et al.,* 2001; Jensen *et al.,* 2006). In experimental models with male rats (Yamada, 1993), inhaling organic solvent vapours decreased plasma testosterone concentrations as well as the weight of testes and accessory reproductive organs. Sperm concentrations in the epididymis also decreased following inhalation of solvent vapour. In this study population, although job rotation was limited, there had been changes in occupational exposures over time. Surprisingly, the strength of the association was more than double for past than for current exposures, suggesting that the effect on the male reproductive system may be cumulative and not easily reversible. The finding that exposure to solvents is associated with low sperm quality in clinically infertile males is important, because the widespread use of solvents implies that an important proportion of males attending fertility clinics may have had that exposure. Moreover, there is a great potential for prevention through health education and reduction of exposures at work (Cherry *et al.,* 2001).

The association between metal exposure and infertility in observational studies is more ambiguous (Irgens *et al.,*

1999), though the majority of studies have found a significant association between lead, cadmium and other metals and male infertility (Benoff *et al.*, 2000). It was also found that males with reduced semen quality had been more often subjected to extreme physical exposures (heat, cold), as described in other reports (Figà-Talamanca *et al.*, 1996; Sheiner *et al.*, 2003; Jensen *et al.*, 2006).

No association was found with mobile phones, as found by Agarwal and co-workers (Agarwal *et al.* 2007; Deepinder *et al.*, 2007) but the wording of the questions may not have been able to capture correctly the level of exposure in the study patients. No differences were found in exposure to tobacco or pesticides. The deleterious effect of tobacco on seminal parameters is clearly established (Hassa *et al.*, 2006). In the present study, cases had been similarly exposed to tobacco compared with controls (past smokers), although they smoked less than controls at the time of the interview.

Some studies have reported a relationship between semen quality and different professional groups (Chia *et al.*, 2000; Chia and Tay, 2001). In the present study, industrial blue collar workers had a higher risk clearly related to occupational exposures, while clerical workers and professionals appeared to be protected.

Some possible limitations of this study design should be discussed. The case-controlled study, as an observational design, does not allow one to infer causality in the associations. The main concern with the present study refers to sample size that would particularly affect the power of the study to detect differences between the two groups. Although the sample size should not affect the validity of the associations observed, there may have been a failure to observe other true differences between the groups. Selection of controls is an important concern in case-controlled studies. The main criteria for selecting appropriate controls are to ensure comparability between the two groups. The controls would have been cases if they had had poor semen quality, as they were both recruited in the clinics. The other major concerns with cases–control studies are information bias, especially recall bias, and confounding factors. Recall bias is certainly one of the most serious concerns in case-controlled studies, and this study may not be an exception. Recall bias would be a concern if recall of occupational exposures were differential between cases and controls. Although all interviews were made under similar circumstances and by the same trained interviewer, it cannot entirely be ruled out that patient knowledge of his semen quality might have influenced the intensity or meticulousness of the recall of occupational exposures. Direct measures of toxins in blood or seminal plasma of the patients was desirable but could not be performed in this study. Therefore, as specific occupational exposures were derived from the information reported by patients, a certain degree of misclassification cannot be entirely dismissed.

Recent studies have suggested that some environmental toxicants and chemicals might alter sperm DNA integrity (Stronati *et al.*, 2006; Aitken and De Iuliis, 2007). DNA fragmentation may be an excellent marker for exposure to potential reproductive toxicants and a diagnostic tool for potential male infertility (Evenson and Wixon, 2005; Ozmen *et al.*, 2007).

In conclusion, male infertility in patients attending infertility clinics may be largely the result of occupational exposures, at least in this study. More research is needed to better understand the underlying mechanisms, additive or multiplicative effects of concomitant exposures, and different individual's susceptibility on the male reproductive system to the influence of chemical occupational exposures. Research focused on antioxidant and/or exercise therapies might be useful in order to revert the deleterious effects on male infertility in exposed patients.

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