Contribution of environmental factors to the risk of male infertility

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BACKGROUND: An increasing number of reports suggest that chemical and physical agents in the environment, introduced and spread by human activity, may affect male fertility in humans. We investigated the relationships between exposure to environmental agents and seminal characteristics, and the concentrations of reproductive hormones in the serum of men seeking infertility treatment. METHODS: We studied 225 male partners from consecutively recruited couples, who had their first infertility consultation between 1995 and 1998, in the Litoral Sur region of Argentina, one of the most productive farming regions in the world. RESULTS: A multivariate logistic regression model showed that exposure to pesticides and solvents is significantly associated with sperm threshold values well below the limit for male fertility. We also found that men exposed to pesticides had higher serum oestradiol concentrations, and that men exposed to solvents had lower LH concentrations than non-exposed men. All of these effects were greater in men with primary infertility than in men with secondary infertility. CONCLUSION: We have shown that environmental factors contribute to the severity of infertility, and that this may worsen the effects of pre-existing genetic or medical risk factors.

Key words: environment/hormones/male infertility/spermatozoa

Introduction

Humans are exposed to many environmental agents that may be hazardous to their reproductive capacity. Male reproductive function is known to be highly sensitive to many chemicals and physical agents generated by industrial or agricultural activities (Bonde, 1996; Spira and Multigner, 1998). Such agents are commonly present in some occupational activities and in the general environment.

Environmental hazards to male reproductive function were revealed 30 years ago, when pesticide manufacturers and agricultural workers in contact with the nematocide, 1,2-dibromo-3-chloropropane (DBCP), suffered from severely impaired spermatogenesis, leading to infertility (Whorton *et al.*, 1977; Slutsky *et al.*, 1999). Since then, different chemical classes of pesticides and solvents have been demonstrated to be male reproductive toxicants in animal models (Sundaram and Witorsch, 1995). However, the number of substances that have been proven to have a deleterious effect on human spermatogenesis is very small, and these observations are limited to a few cross-sectional studies done on occupational populations that were exposed to these substances at very high concentrations (Cohn *et al.*, 1978; Wyrobek *et al.*, 1981; Ratcliffe *et al.*, 1987; Schrader *et al.*, 1988; Ratcliffe *et al.*, 1989). Due to the widespread use of such chemicals, and their potential for leakage into the environment, they constitute a putative hazard to male fertility.

Male reproductive function in the general population has attracted increasing attention due to reports suggesting that the occurrence of several biological problems affecting the male genital tract have increased during the last 50 years (Toppari *et al.*, 1996). These include an increased incidence of testicular cancer and some congenital anomalies, such as cryptorchidism or hypospadias, and an apparent decline of sperm production in the overall male population. Such events have been tentatively linked to the widespread use of chemicals with hormonal properties, also called endocrine disruptors (Sharpe and Skakkebaek, 1993).

An obvious undesirable consequence of reproductive toxicants is infertility. Infertility, defined as the inability to conceive after 1 year of unprotected intercourse, concerns ~15% of couples in Western countries (Thonneau and Spira, 1991; Irvine, 1998). A male contributory factor is involved in approximately half of these cases (Irvine, 1998). However, in a considerable proportion of men with semen anomalies, no medical or surgical factors are implicated, and the aetiology remains unclear. Nowadays, there is an increasing awareness of the potential risk of genetic, chemical, and physical environmental agents on male infertility.

The involvement of environmental factors in male infertility and the suspected increased incidence of male-related infertility induced by such agents are of great concern. It is not known if the reported decline of semen quality, or the increased

incidence in genital anomalies, is linked to the prevalence of infertility. It has been stressed that the percentage of men whose sperm count has fallen below the level associated with optimal fertility has increased (Carlsen *et al*., 1992; Jouannet and Auger, 1996; Bonde *et al*., 1999).

Toxic damage to the testes can result in many effects, namely, reduced sperm production, the production of defective spermatozoa, and impaired androgen production. In man the non-invasive method for assessing male fertility involves semen analysis, measurement of reproductive hormones in the blood, and evaluation of previous success in impregnating a partner. Semen analysis allows the male reproductive function to be evaluated directly and the relationship between exposure to environmental agents and fertility to be investigated. However, semen samples are difficult to obtain in general population studies and the participation rate, which is usually <20%, may impair conclusions (Bonde, 1996). Studies of populations in which men are seeking infertility treatment avoid this problem, because sperm analysis is a key part of their fertility evaluation. If the different bias and confounding factors are taken into account, this population provides the opportunity to study the associations between risk factors and outcomes. However, environmental or occupational exposures may be low in the population that the consulting men come from, and thus the statistical power may be insufficient to obtain a significant conclusion. We studied a population of men consulting infertility clinics in the Litoral Sur region of Argentina. This area is characterized by intensive agricultural and industrial activity, and thus favours an elevated prevalence of environmental exposures. We analysed the effect of environmental exposures on semen characteristics and reproductive hormone concentrations in men having their first infertility consultation in one of three health centres in the Litoral Sur.

Materials and methods

Study area

This study took place in the Santa Fe and Entre Rios provinces in Argentina. These provinces constitute the Litoral Sur region, which is one of the most fertile farmland zones in the world. These neighbouring provinces are separated by the Parana river, and occupy 78 781 km^2 and 133 007 km^2, respectively. The combined population of these provinces was estimated at 4 173 601 inhabitants, in 1991. The Litoral Sur is a rich agricultural and cattle ranching area. There is also much industrial development, centred mainly around the three largest cities, Santa Fe capital (449 000 inhabitants), Rosario (1 200 000 inhabitants) and Parana (320 000 inhabitants).

Subjects

The study sample comprised 253 consecutively recruited male partners from couples having their first infertility consultation at the Andrology Unit of one of three private institutions (Hospital Italiano Garibaldi, Rosario; Centro de Urologia, Santa Fe; Sanatorio Adventista del Plata, Libertador General San Martin, Entre Rios), between January 1995 and December 1998. They represented all causes of male infertility, except testicular cancer. Approval for this study was obtained from the Institutional review board.

Data sources

Information was collected at several stages. A structured interview was conducted, during the first visit, to obtain information on basic demographic, medical, surgical, and reproductive history, recent illnesses and treatment, familial history of infertility, sexuality, occupational history, and lifestyle. During the second visit, which took place 2–4 weeks later, a complete physical and andrological examination was carried out and men gave a semen specimen and a blood sample.

Exposure assessment

The degree of exposure was assessed from the initial interview, by studying the detailed history of past and present jobs and lifestyle habits. Men were asked about their contact with chemical substances or physical agents during the previous 10 years. An industrial hygienist verified the correlation between jobs and the declared exposures. Patients were divided into five groups: non-exposed (men who did not report any exposure and whose occupation did not expose them to any of the agents); and three groups of men who were only exposed to one kind of agent: pesticides (herbicides, fungicides, insecticides, fumigants, and rodenticides), solvents (paints, varnish, lacquers, thinners, degreasers, and inks), and heat (prolonged sitting position or radiant heat). Finally, due to the small number of individuals, we regrouped men who were in contact with more than one of these exposures and/or exposed to miscellaneous agents, including heavy metals, oils, acids, explosives, and low temperatures (mixed-group). We excluded any individual who could not provide clear information on their job circumstances or exposure risks.

Semen analysis

Semen samples were obtained by masturbation after a recommended period of 3–5 days sexual abstinence. Seminal volume was measured in a graduated pipette, accurate to within 0.1 ml. Sperm concentration was determined by haemocytometer (improved Neubauer counting chamber), after an appropriate dilution. Sperm motility was assessed by direct observation under a microscope (×400). Sperm morphology was assessed by staining slides (May–Grunwald–Giemsa) and direct observation under a microscope (×1000). All procedures followed WHO guidelines (World Health Organization, 1992). The three laboratories were examined every month by the same quality control officer. Measurements were evaluated in each laboratory by the same person during the study period. Between- and within-assay coefficients of variation were 7 and 6% for sperm concentration, 17 and 9% for sperm motility, and 14 and 8% for sperm morphology respectively.

Reproductive hormones

Blood samples were collected in a 10 ml plastic syringe, and kept at room temperature until serum was separated. After centrifugation, the serum was transferred to a new tube, kept at –20°C, and assayed within 4 weeks. FSH and LH were measured by an immunoradiometric assay, using the Coat-a-Count kit (Diagnostic Products Corporation, Los Angeles, CA, USA). The dose-response curve of FSH was calibrated according to the WHO 2nd International Reference Preparation (IRP) 78/549, and the LH dose-response curve according to the WHO 2nd IRP 80/552 and WHO 1st IRP 68/40. Prolactin was measured by a double-antibody radioimmunoassay (Diagnostic Products Corporation) and calibrated according to the WHO 1st IRP 75/504 and the 3rd International Standard 84/500. Total testosterone and oestradiol were measured by radioimmunoassay, using the Coat-a-Count kit (Diagnostic Products Corporation). Between- and within-assay coefficients of variation were 9.2 and 8.5% for FSH, 10.8 and 12.1% for LH, 9.8 and 9.5% for prolactin, 12.5 and 8.5% for testosterone, and 16.1 and 12.2% for oestradiol respectively.

Outcomes

Continuous outcomes (as evaluated by the laboratory) were seminal volume (ml), sperm concentration (×10^6/ml), total sperm output

($\times 10^6$; the product of seminal volume $\times$ sperm concentration), percentage of total motile spermatozoa, percentage of spermatozoa with normal morphology, and concentration of reproductive hormones in the serum. To study the effect of exposure on these outcomes, seminal characteristics were categorized as dichotomous variables. Seminal volume, sperm concentration and sperm output were categorized according to the 25th, 50th or 75th percentiles, determined from the distribution in the study sample. To determine threshold values which gave the maximum likelihood ratio, we successively compared the exposure association below and above the 25th, 50th or 75th percentile limits of distribution. We used thresholds of 50 and 30%, for sperm motility and sperm morphology respectively, based on the WHO (1992) guidelines for defining reference limits. We compared concentrations of hormones in the serum as continuous variables between groups. As serum hormone values are highly skewed and do not follow a normal distribution, we normalized the distributions by applying a $\log_{10}$ transformation (confirmed by Kolmogorov–Smirnov test for normality).

Data classification

We recorded and classified the following information as continuous variables: age, weight, height, time trying to conceive with the present partner with unprotected intercourse before consultation (waiting time), average monthly frequency of intercourse, duration of the length of abstinence before the semen analysis, and testicular volume. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). We also recorded the following data: season of sperm analysis (spring, summer, autumn, winter); annual income (<$12 000, between $12 000 and $36 000, >$36 000); smoking (current smoker versus non-smoker), alcohol consumption ($\leqslant$20 g/alcohol per day versus >20 g/alcohol per day); fertility status of men whether they had fathered a child or impregnated the present partner, regardless of the outcome, before consultation (secondary infertility), or not (primary infertility). All retrospective health information and data obtained from the physical examination and the laboratory were used to define the potential risk factors for male infertility, in accordance with WHO recommendations (1993). Medical or surgical male infertility risk factors were categorized as follows (yes or no): reported family history of infertility, cryptorchidism (treated or not), varicocele (treated or not), testicular torsion, testicular trauma, genital infections, orchitic mumps, orchido-epididymitis, sexual dysfunction, systemic diseases (tuberculosis, diabetes mellitus), allergies, inguinal hernia (treated), urinary infections and treatments which may interfere with testicular function.

Statistical analysis

Comparisons between groups were made using the χ^2-test for categorized variables, and the *t*-test and analysis of variance/co-variance for continuous variables. In the analysis involving a categorized outcome variable, we used multiple logistic regression analysis to produce odds ratio (OR) and the 95% confidence intervals (95% CI) for the association between seminal characteristics and exposure variables, adjusted for confounding factors. Univariate logistic regression analysis was used to evaluate potential confounding factors in each exposed group, separately. Factors were considered confounding if their inclusion in the model modified the estimate of the OR by >10% (Greenland and Rothman, 1998). Age, length of abstinence, income, health center, BMI, and smoking were always included as confounding factors. All analyses were carried out using the Statview software package (SAS Institute Inc., Cary, USA). All *P*-values were two-sided, and considered to be significant if $P < 0.05$.

Table I. Occupational circumstances of exposure

Groups	Activity	n
Non-exposed (n = 80)	Professionals	23
	Administrative	21
	Technicians	20
	Sales workers	10
	Others	6
Pesticides (n = 40)	Farmers	23
	Animal husbandry	5
	Fumigators	5
	Pesticide factory workers	4
	Others	3
Solvents (n = 22)	Mechanics	12
	Painters	5
	Printers	3
	Woodworkers	2
Heat (n = 21)	Drivers	13
	Bakers	3
	Cooks	2
	Others	3
Mixed (n = 14)	Farmers	6
	Chemical factory workers	2
	Welders	2
	Others	4

Results

Characteristics of the population

Of the initial 253 consulting men, 189 attended the second consultation and gave a sperm sample. Of these 189 men, 80 were classified as non-exposed, 40 as exposed to pesticides, 22 as exposed to solvents, 21 as exposed to heat, 14 as exposed to a mixture of the above. Twelve men were unclassified, and excluded, as described in Materials and methods. The age, infertility status and exposure characteristics of the 64 men who did not supply sperm samples did not differ from the 189 men who did supply a sperm sample (data not shown). The occupational exposures of the 177 men included in this analysis are shown in Table I. The median exposure times were 7, 4, 3.5, and 4 years for the pesticide, solvent, heat, and mixed exposure groups respectively.

Comparisons between some general characteristics in each of the exposed groups and the non-exposed group are shown in Table II. The mean age of the study population was 33.6 years, this value did not differ between groups. Nearly 40% of the men were overweight (BMI between 25 and 30), obesity (BMI $\geqslant$30) was more frequent in the solvent-exposed group (40% of cases compared with 20% in other groups). Alcohol consumption was similar in each group, but there was a higher proportion of smokers in the solvent- and heat-exposed groups. The main risk factors identified for infertility were: varicocele (37% of the overall population), orchido-epididymitis (28%), cryptorchidism (8.5%) and orchitic mumps (6.9%). The proportion of medical and surgical infertility risk factors was equal in groups (data not shown). Men with primary infertility were younger, and had lower seminal characteristics to men with secondary sterility (Table III).

Table II. General characteristics of exposure groups

	Non-exposed (n = 80)	Exposed to (n = 83)								
		Pesticides (n = 40)	P	Solvents (n = 22)	P	Heat (n = 21)	P	Mixed (n = 14)	P
Age (years)[a]	33.7 (5.3)	33.2 (4.2)	ns	33.7 (7.0)	ns	33.6 (6.5)	ns	33.8 (6.4)	ns
Body mass index[a]	25.8 (3.3)	26.7 (3.8)	ns	28.9 (4.0)	0.003	26.0 (3.6)	ns	28.1 (5.6)	ns
Testicular volume (ml)[a]	43.5 (9.2)	44.4 (10.0)	ns	47.3 (8.2)	ns	42.4 (9.8)	ns	41.9 (11.9)	ns
Waiting time before consultation (months)[b]	24	24	ns	36	ns	24	ns	24	ns
Monthly frequency of intercourse[a]	11.6 (5.9)	11.9 (7.5)	ns	10.5 (6.0)	ns	11.3 (6.5)	ns	10.6 (4.4)	ns
Sexual abstinence (days) [a]	4.1 (0.6)	4.3 (0.6)	ns	4.2 (0.6)	ns	4.2 (0.6)	ns	4.1 (0.5)	ns
Infertility status[c]									
Secondary	32 (40.0)	10 (25.0)	ns	11 (50.0)	ns	9 (42.9)	ns	5 (38.5)	ns
Primary	48 (60.0)	30 (75.0)		11 (50.0)		12 (57.1)		8 (61.5)	
Seasons of sperm analysis[c]									
Spring	22 (27.5)	8 (20.0)	ns	6 (27.3)	ns	5 (23.8)	ns	3 (21.4)	ns
Summer	14 (17.5)	9 (22.5)		1 (4.5)		7 (33.3)		5 (35.7)	
Autumn	4 (5.0)	3 (7.5)		1 (4.5)		0 (0.0)		2 (14.3)	
Winter	40 (50.0)	20 (50.0)		14 (63.6)		9 (42.9)		4 (28.6)	
Tobacco[c]									
Non-smokers	63 (78.8)	33 (82.5)	ns	7 (31.8)	0.0001	49 (42.9)	0.0012	10 (71.4)	ns
Current smokers	17 (21.2)	7 (17.5)		15 (68.2)		12 (57.1)		4 (28.6)	
Alcohol[c]									
≤20 g/day	62 (77.5)	27 (67.5)	ns	16 (72.7)	ns	17 (81.0)	ns	12 (85.7)	ns
>20 g/day	18 (22.5)	13 (32.5)		6 (27.3)		4 (19.0)		2 (14.3)	

[a]Mean (SD).
[b]Median.
[c]n (%).

Table III. Age and seminal characteristics according to infertility status

	All (n = 177)		Primary infertility (n = 110)		Secondary infertility (n = 67)	
	Mean (SD)	Median	Mean (SD)	Median	Mean (SD)	Median
Age (years)	33.6 (5.5)	33.0	31.9 (4.3)	31.6	36.4 (6.1)	35.3
Seminal volume (ml)	3.1 (1.6)	2.8	3.0 (1.7)	2.5	3.1 (1.4)	3.0
Sperm concentration ($\times 10^6$/ml)	28.6 (46.0)	14.2	21.9 (29.6)	10.9	39.6 (63.3)	21.6
Sperm output ($\times 10^6$)	82.8 (110.7)	41.6	70.3 (106.1)	23.8	103.4 (115.8)	65.0
Sperm motility[a] (%)	36.6 (24.4)	41.0	33.5 (24.5)	38.5	41.7 (23.6)	50.0
Sperm morphology[a] (%)	29.8 (15.7)	28.5	27.3 (15.7)	26.0	33.6 (14.9)	36.0

[a]Seven azoospermic men were not included (five primary infertility and two secondary infertility).

Seminal characteristics

Dichotomized dependent variables were used to assess the association between exposure and seminal characteristics by logistic regression. Table IV shows the adjusted OR; the reference group (OR = 1) is always the non-exposed group. Exposure to pesticides significantly increased the risk of a seminal volume of >3.8 ml (corresponding to the 75th percentile). However, after stratification, this increase was only significant in men with secondary infertility. Moreover, we observed a higher risk in men who were frequently exposed to pesticides [OR 8.5 (95% CI 1.2–58.8)], than that in those who were only exposed occasionally 4.4 (0.5–38.3). Exposure to pesticides was associated with <1×10⁶ spermatozoa per ml (corresponding to the 25th percentile), a sperm output of <3×10⁶ (corresponding to the 25th percentile), and <50%

motile sperm cells. Exposure to pesticides was also associated, but not significantly, with <30% of sperm cells being morphologically normal. After stratification, men with primary infertility were found to have an increased risk for all these anomalies, and men with secondary infertility were found to have an increased risk of low sperm motility. We observed a dose-related response in men with primary infertility exposed to pesticides. These associations were higher for sperm concentration [4.4 (1.2–15.7)], sperm output [3.8 (1.1–14.3)], sperm motility [7.8 (1.5–41.0)], and sperm morphology [3.6 (1.1–11.5)], in men who were frequently exposed than in men who reported occasional exposure [3.1 (0.2–43.3) for sperm concentration, 3.4 (0.2–48.4) for sperm output, 0.7 (0.1–9.6) for sperm motility, and 1.2 (06–11.5) for sperm morphology]. Men exposed to solvents showed elevated OR for inferior

Table IV. Odds ratios (OR) and 95% confidence intervals (95% CI) for the relationship between seminal characteristics and exposure

	Seminal volume >3.8 ml		Sperm concentration <1×10⁶/ml		Sperm output <3×10⁶		Sperm motility[a] <50%		Sperm morphology[a] <30%	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
All										
Non-exposed	1.0		1.0		1.0		1.0		1.0	
Pesticides	2.8 (1.2–6.6)	0.019	3.0 (1.2–7.4)	0.015	2.7 (1.1–6.7)	0.031	4.5 (1.8–11.5)	0.002	2.1 (1.0–4.8)	0.064
Solvents	2.4 (0.8–7.6)	0.130	2.7 (0.9–8.3)	0.088	2.5 (0.8–7.9)	0.105	3.1 (1–9.5)	0.050	3.0 (1.0–9.0)	0.055
Heat	1.6 (0.5–5.2)	0.459	1.9 (0.6–6.3)	0.265	2.3 (0.7–7.3)	0.141	1.3 (0.5–3.6)	0.612	0.9 (0.3–2.4)	0.811
Mixed	0.8 (0.1–6.8)	0.794	1.8 (0.3–11.1)	0.529	3.2 (0.9–11.1)	0.073	2.6 (0.6–12.0)	0.221	0.8 (0.2–3.7)	0.822
Primary infertility										
Non-exposed	1.0		1.0		1.0		1.0		1.0	
Pesticides	2.2 (0.7–6.5)	0.164	3.4 (1.2–9.7)	0.024	3.0 (1.0–8.5)	0.048	3.6 (1.1–11.4)	0.031	4.1 (1.4–12.0)	0.011
Solvents	2.3 (0.4–12.6)	0.318	3.9 (0.9–17.3)	0.078	3.6 (0.8–16.4)	0.092	10.6 (1.1–105.6)	0.044	4.4 (0.7–26.2)	0.103
Heat	1.1 (0.2–6.7)	0.875	1.7 (0.4–7.8)	0.470	2.4 (0.6–10.4)	0.236	2.2 (0.5–9.6)	0.300	1.0 (0.3–3.9)	0.982
Mixed	nc[b]		1.4 (0.1–16.6)	0.773	3.9 (0.9–13.7)	0.080	2.0 (0.3–15.3)	0.512	1.5 (0.2–10.8)	0.712
Secondary infertility										
Non-exposed	1.0		1.0		1.0		1.0		1.0	
Pesticides	6.6 (1.4–31.4)	0.019	1.8 (0.3–12.0)	0.534	1.8 (0.3–12.0)	0.534	5.8 (1.0–32.7)	0.046	0.4 (0.1–2.1)	0.265
Solvents	2.3 (0.5–11.7)	0.310	2.3 (0.3–18.3)	0.426	2.3 (0.3–18.3)	0.426	2.1 (0.5–9.3)	0.321	2.7 (0.6–13.2)	0.208
Heat	2.3 (0.4–12.6)	0.352	2.7 (0.3–21.6)	0.338	2.7 (0.3–21.6)	0.338	0.9 (0.2–4.4)	0.874	0.6 (0.1–3.2)	0.534
Mixed	1.8 (0.1–23.7)	0.649	1.7 (0.1–49.2)	0.752	1.4 (0.1–17.1)	0.783	9.1 (0.6–131.9)	0.103	0.3 (0.1–4.2)	0.377

[a]Seven azoospermic men were not included (two non-exposed, two pesticide-exposed, one solvent-exposed and one mixed-exposed.
[b]Non-calculable.

Table V. Serum reproductive hormones in exposed groups

| | Non-exposed | Exposed to: | | | | | | | | |
| | | Pesticides | | Solvents | | Heat | | Mixed | |
	Mean (SD)	Mean (SD)	P	Mean (SD)	P	Mean (SD)	P	Mean (SD)	P
All									
FSH (IU/l)	10.6 (15.3)	8.2 (6.9)	0.965	5.8 (4.3)	0.225	10.5 (12.6)	0.945	9.9 (12.8)	0.200
LH (IU/l)	5.1 (4.3)	6.0 (5.0)	0.441	3.3 (2.1)	0.073	3.8 (2.5)	0.333	5.2 (4)	0.884
Testosterone (ng/ml)	5.3 (2.3)	5.5 (2.8)	0.927	5.2 (5.5)	0.419	4.6 (1.8)	0.292	4.5 (3.6)	0.107
Oestradiol (pg/ml)	23.0 (15.8)	36.8 (25.7)	0.002	23.1 (17.2)	0.647	27.3 (22.9)	0.428	25.8 (18.4)	0.416
Prolactin (ng/ml)	12.3 (7.8)	11.5 (7.3)	0.910	11.2 (6.4)	0.705	14.4 (9.1)	0.221	14.8 (11.4)	0.290
Oestradiol/testosterone ratio	5.2 (4.8)	8.6 (10.2)	0.005	5.0 (4.6)	0.763	6.5 (7.0)	0.267	6.3 (7.0)	0.415
Primary infertility									
FSH (IU/l)	10.2 (12.2)	8.7 (7.0)	0.907	7.0 (5.5)	0.608	9.4 (9.1)	0.863	10.9 (15.6)	0.393
LH (IU/l)	5.4 (3.8)	6.7 (5.4)	0.509	2.6 (0.9)	0.004	4.2 (3.2)	0.185	5.0 (4.7)	0.210
Testosterone (ng/ml)	5.3 (2.3)	5.6 (2.5)	0.611	4.4 (3.5)	0.062	4.5 (1.4)	0.374	5.4 (4.1)	0.606
Oestradiol (pg/ml)	24.0 (16.7)	34.1 (20.7)	0.019	20.7 (8.9)	0.882	27.3 (21.7)	0.606	31.0 (16.2)	0.161
Prolactin (ng/ml)	13.3 (8.9)	11.7 (7.9)	0.598	10.5 (5.5)	0.553	13.4 (7.2)	0.621	14.7 (13.3)	0.899
Oestradiol/testosterone ratio	5.1 (4.4)	6.9 (4.7)	0.041	5.1 (2.3)	0.527	6.0 (5.0)	0.432	6.5. (7.7)	0.347
Secondary infertility									
FSH (IU/l)	11.4 (19.4)	6.8 (6.7)	0.786	4.6 (2.6)	0.300	11.9 (16.8)	0.773	8.0 (6.6)	0.659
LH (IU/l)	4.6 (5.1)	3.8 (2.9)	0.889	3.9 (2.6)	0.956	3.2 (0.9)	0.921	5.4 (2.3)	0.436
Testosterone (ng/ml)	5.3 (2.4)	5.3 (3.8)	0.361	6.0 (3.5)	0.522	4.6 (2.3)	0.579	4.8 (1.8)	0.847
Oestradiol (pg/ml)	21.5 (14.4)	45.9 (38.8)	0.061	24.9 (22.0)	0.587	27.2 (25.4)	0.522	19.9 (20.9)	0.692
Prolactin (ng/ml)	10.8 (5.4)	10.6 (5.0)	0.755	12.0 (7.5)	0.865	15.9 (11.5)	0.211	15.0 (8.3)	0.326
Oestradiol/testosterone ratio	5.5 (5.4)	14.6 (19.2)	0.043	4.9 (5.8)	0.705	7.0 (8.9)	0.276	5.8 (6.0)	0.652

sperm characteristics, but this was only significant for sperm motility. After stratification, the OR were higher in men with primary infertility. We did not find any significant association between categorized seminal outcomes, and heat exposure, before or after stratification in either infertility status. Finally, for the mixed exposure group, an increased risk for low sperm output was observed, although the 95% CI includes one, and this was still present in men with primary infertility.

Hormonal characteristics

Table V shows the mean concentration of reproductive hormones in the serum in each group. It also shows the statistical comparison, as continuous variables, between each exposure group and the non-exposed group, after adjustment for potential confounding factors (age, BMI, and smoking). The pesticide-exposed group showed significantly higher oestradiol values than the non-exposed group, and after stratification this remained significant in men with primary infertility, and was nearly significant in men with secondary infertility. Moreover, there was a significantly higher oestradiol/testosterone ratio in the pesticide-exposed group than in the non-exposed group, in men with both primary and secondary infertility. The solvent-exposed group had a lower LH concentration than the non-exposed group, and after stratification this difference was highly significant in men with primary infertility. No differences were observed for any reproductive hormones, before or after stratification, in the heat-exposed group or in the mixed-exposed group, compared with the non-exposed group.

Discussion

This study aimed to evaluate the effect of environmental agents on seminal characteristics and reproductive hormone concentrations in the serum of male partners of infertile couples. We studied a population of men seeking infertility treatment, which allowed us to investigate the association between high prevalent risk factors and the outcomes related to infertility. Nevertheless, these men constitute a selected population, thus conclusions derived from such studies should be interpreted with caution.

Over half the men included in this study were exposed to either chemical or physical factors that may be associated with infertility. This proportion suggests a relationship between these factors and male infertility, but may also express a selection bias. The exposure prevalence may be artificially increased by the chosen geographic site which was an industrial and agricultural area. Moreover, not all couples with an infertility problem seek medical help. If the decision to seek medical care is related to occupational exposure, this may result in increased prevalence of such factors. Patients with primary infertility are known to seek medical help more promptly than those who have already conceived. The proportion of primary infertility was higher in the pesticide-exposed group, but we do not know how one factor may influence the other. Therefore, we cannot exclude a selection bias, with people exposed to certain agents consulting more frequently. To counteract this potential bias we stratified our analysis, according to the infertility status.

Another source of bias is the misclassification of the type of exposure. We evaluated the type of exposure based on detailed questionnaires, mostly consisting of occupational questions, which provided a qualitative assessment. We are aware that many different active compounds are combined in each of the exposure groups and that the exposure conditions differ between individuals (i.e. intensity and frequency).

However, further sub-grouping and quantitative evaluation were impaired by the limited size of our sample. Biological assessment of exposure would have been more precise indicators, but this was limited by the cost and the large number of suspected chemicals that individuals were exposed to. Despite these limitations, questionnaires have provided good estimates of exposures (Tielemans et al., 1999a).

This study showed that, in an infertility-consulting population, environmental exposure, particularly to pesticides and solvents, is associated with dramatic changes in seminal characteristics. Our results suggest that toxicants act on the testes and post-testicular sites, including the accessory sex glands. Our observations are consistent with those found in previous cross-sectional studies on workers exposed to specific pesticides, such as DBCP (Lipshultz et al., 1980), chlordecone (Cohn et al., 1978), carbaryl (Wyrobek et al., 1981), ethylene dibromide (Ratcliffe et al., 1987; Schrader et al., 1988) or 2-4D (Lerda and Rizzi, 1991), and to solvents, such as glycol ethers (Welch et al., 1988; Ratcliffe et al., 1989), carbon disulphide (Lancranjan, 1972), perchloroethylene (Eskenazi et al., 1991) or 2-bromopropane (Kim et al., 1996). A study of men attending a sperm bank (Bigelow et al., 1998) found higher seminal volumes and lower sperm concentrations and motility, in farm workers than in non-farm workers. Previous studies on patients from infertility clinics showed that glycol ethers (Veulemans et al., 1993) and aromatic solvents (Tielemans et al., 1999b) are associated with reduced semen quality. Although scrotal thermal irradiation and sitting for prolonged periods of time are strongly suspected to be infertility risk factors (Thonneau et al., 1998), we did not observe an increased risk of poor sperm characteristics in the heat-exposed group. This discrepancy may be due to low exposure times or intensities. Most of the men in this group spend prolonged periods in the sitting position (e.g. taxi drivers), which may be not sufficient to cause dramatic seminal modifications. Despite the small number of men in the mixed-exposure group, an increased risk of low sperm production was observed. This group comprised individuals exposed to heavy metals, which are known testicular toxicants, and individuals exposed to multiple agents which may increase the risk of seminal anomalies.

We observed higher values of oestradiol in the pesticide-exposed group, and lower LH values in the solvent-exposed group, than in the non-exposed group. Few studies have investigated the impact of pesticide exposure on blood reproductive hormones. These studies only investigated the effect of DBCP (Whorton et al., 1977; Egnatz et al., 1980; Lipshultz et al., 1980; Lantz et al., 1981; Eaton et al., 1986) and para-tertiary butyl benzoic acid (Whorton et al., 1981) on FSH, LH and testosterone production. These studies gave contradictory results, and interpretation was rendered difficult because hormonal values vary according to the time between the last toxicant exposure and the time of hormonal evaluation (Lipshultz et al., 1980). Moreover, serum concentrations of reproductive hormones fluctuate considerably over time, and conclusions obtained from one sample should be interpreted with caution. The high serum concentrations of oestradiol in the group exposed to pesticides, in both primary and secondary infertility, is surprising. The oestrogen/androgen imbalance was evaluated by calculating the oestradiol/testosterone ratio. This ratio was significantly increased in the pesticide-exposed group and may reflect the quality of the endocrinological milieu of the testis (Itoh et al., 1994). An increased oestradiol/testosterone ratio may be a marker of infertility in obese men (Jarow et al., 1993). Nevertheless, we found that the oestradiol/testosterone ratio was significantly increased in pesticide-exposed group, even after adjustment for the BMI. Many pesticides act as direct testicular toxicants (Schrader and Kesner, 1992; Bonde, 1996), but some of them are now believed to exert toxicity due to their similarity to reproductive steroid hormones (Cheek and McLachlan, 1998). Pesticides can therefore bind to endocrine receptors, and may act as hormonal antagonists or agonists, disrupting biological responses (Johnson et al., 2000). Further studies are required to determine whether these mechanisms cause the observed seminal modifications in the pesticide-exposed group.

Little is known about the effects of solvents on reproductive hormones. It is noteworthy that exposure to carbon disulphide, an industrial organic solvent, is accompanied by reduced circulating concentrations of LH (Lancranjan, 1972). Most solvents seem to be direct testicular toxicants (Schrader and Kesner, 1992; Bonde, 1996). The low concentrations of LH that accompany exposure to solvents suggests a feedback mechanism induced by a gonadal action; however, an effect on the pituitary gland or the hypothalamus cannot be excluded.

Only values under a certain limit are considered to contribute to infertility and reduce the likelihood to pregnancy. However, these limits are variable and are not strictly defined. Seminal volume, except for very low volumes, does not seem to be significantly related to fertility. However, very high volumes may reduce the sperm concentration considerably and prove critical when sperm output is already low. Sperm concentrations below 5, 10 or 20 $\times 10^6$/ml have been associated with increased infertility, and 5×10^6/ml is considered to be the clinically significant threshold of male infertility (Jouannet et al., 1988). The proportion of sperm with normal morphology was strongly correlated to the likelihood of pregnancy, and was independent of sperm concentration. Thus, the probability of pregnancy was reduced when the proportion of spermatozoa with normal morphology was <40% (Bonde et al., 1998). The likelihood of pregnancy was also decreased if <60% of sperm cells were motile, but after adjustment for sperm concentration and morphology this decrease was not significant (Bonde et al., 1998). In this study, we showed that exposure to pesticides and solvents is significantly associated with threshold sperm values, much lower than the considered limits for male fertility.

We noted that the significant associations between exposure to agents and all seminal characteristics, except seminal volume, were more frequent in men with primary infertility than in men with secondary infertility. This may be because environmental factors potentiate the adverse effects of

predisposing genetic, medical or surgical factors for infertility frequently found in men with primary infertility.

In conclusion, we believe that environmental factors, particularly exposure to pesticides and solvents, may contribute to the severity of sperm parameters, and that infertile patients constitute a highly susceptible group. The testicles are one of the most vulnerable organs to environmental physical and chemical agents. The use of these agents has increased substantially since the 1940s, due to industrial and agricultural activities, and Argentina has become a major user of pesticides. The main agricultural activities are cereal culture (mainly wheat and soya beans) and fruit trees, which require large quantities of insecticides, fungicides and herbicides. The recent development of transgenic plants that are resistant to herbicides has also led to extended use of these products. In the absence of reliable data on the quantitative distribution of the various active compounds used, only the economic data can be compared with other countries. Thus, between 1990 and 1998, the turnover represented by the import and export of pesticides by Argentina increased by 270%. In comparison, during the same period, this increase was 49 and 62% for France and the USA respectively (Food and Agriculture Organization of the United Nations, 2001).

There has been increased concern in many Western countries regarding the deleterious effects of environmental chemical agents on male reproduction. Attention to this issue should now be given by developing countries. Finally, our conclusions should promote further evaluation of male reproductive toxicity of commonly used substances or those that are likely to be in contact with human populations, on male fertility.

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