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Fluoride-induced disruption of reproductive hormones in men

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Abstract

Fluoride-induced reproductive effects have been reported in experimental models and in humans. However, these effects were found in heavily exposed scenarios. Therefore, in this work our objective was to study reproductive parameters in a population exposed to fluoride at doses of 3–27 mg/day (high-fluoride-exposed group—HFEG). Urinary fluoride levels, semen parameters, and reproductive hormones in serum (LH, FSH, estradiol, prolactin, inhibin-B, free and total testosterone) were measured. Results were compared with a group of individuals exposed to fluoride at lower doses: 2–13 mg/day (low-fluoride-exposed group—LFEG). A significant increase in FSH ($P<0.05$) and a reduction of inhibin-B, free testosterone, and prolactin in serum ($P<0.05$) were noticed in the HFEG. When HFEG was compared to LFEG, a decreased sensitivity was found in the FSH response to inhibin-B ($P<0.05$). A significant negative partial correlation was observed between urinary fluoride and serum levels of inhibin-B ($r=-0.333$, $P=0.028$) in LFEG. Furthermore, a significant partial correlation was observed between a chronic exposure index for fluoride and the serum concentrations of inhibin-B ($r=-0.163$, $P=0.037$) in HFEG. No abnormalities were found in the semen parameters studied in the present work, neither in the HFEG, nor in the LFEG. The results obtained indicate that a fluoride exposure of 3–27 mg/day induces a subclinical reproductive effect that can be explained by a fluoride-induced toxic effect in both Sertoli cells and gonadotrophs.
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1. Introduction

In various countries, such as India (Jolly et al., 1969), China (Wang and Huang, 1995), and Mexico (Díaz-Barriga et al., 1997), human exposure to fluoride is a public health issue, and the main pathway of exposure is the ingestion of tap water from contaminated groundwater sources. Different biological effects have been related to chronic exposure to fluoride above 1.5 mg/L in drinking water. Among them, dental fluorosis (Besten, 1994), skeletal fluorosis (Cooper et al., 1991; Riggs et al., 1990), neurological effects (Zhao et al., 1996; Mullenix et al., 1995), and reproductive effects (Narayana and Chinoy, 1994; Susheela and Kumar, 1991; Neelam et al., 1987; Tokar and Savchenko, 1977; Freni, 1994) have been reported in humans and in experimental animals. However, reproductive effects in males have not been fully characterized, and contradictory data exist in the literature.

In sodium fluoride-treated male rats (10 mg/kg/day, for 30–50 days), decreased sperm motility and a reduction in serum testosterone levels have been reported (Chinoy et al., 1994, Narayana and Chinoy,

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1994). Furthermore, structural defects were observed in the flagellum, the acrosome, and the nucleus of the spermatids and epididymal spermatozoa of fluoride-treated rabbits (10 mg NaF/kg/day, for 18 months) (Kumar and Susheela, 1994); when the exposure time was extended to 29 months, spermatogenesis ceased in these rabbits (Susheela and Kumar, 1991). In contrast to the above findings, adverse effects were not found in sperm morphology in mice exposed to sodium fluoride by stomach intubation at different doses (0.1–70 mg/kg/day for 5 weeks or 0.3–23 mg/kg/day for 21 weeks) (Li et al., 1987; Dunipace et al., 1989). Also, no structural defects were found in testes of F-1 generation Sprague–Dawley male rats exposed to 25–250 mg/L fluoride in utero and until 14 weeks post weaning (Sprando et al., 1998). When compared to a control group of rats, testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) serum concentrations from these fluoride-treated F-1 rats were not found to be significantly different (Sprando et al., 1997). More recently, the effects of sodium fluoride ingestion at concentrations between 25 and 250 ppm in drinking water measured in rats throughout three generations have been reported (Collins et al., 2001). In this work, mating, fertility and survival indices were not affected in the treated animals.

In humans, it has been reported that fluoride decreases circulating levels of testosterone; however, these changes were not statistically significant to indicate an effect on reproductive functions. The study was performed in areas of India with a fluoride content in water from 1.0 to 6.5 mg/L (Michael et al., 1996). At a higher dose of exposure (1.5–14.5 mg fluoride/L in drinking water), a significant reduction of serum testosterone was reported among Indians with skeletal fluorosis (Susheela and Jethanandani, 1996). Similar results were observed in Russia, in workers exposed to cryolite (Na_3AlF_6) (Tokar and Savchenko, 1977). In this group, when compared to a control population, testosterone concentration was decreased and FSH concentration was elevated in those workers with skeletal fluorosis, whereas LH concentration increased only in those individuals with fluorosis who had long contact with fluorine compounds (over 15 years). Finally, in another Indian study, infertility was reported among married men in a highly endemic area with fluoride concentrations up to 38.5 mg/L (Neelam et al., 1987).

The above human studies show some limitations. For example, they included only populations heavily exposed to fluoride, and in some of them, exposure was not followed by the use of biomarkers, such as urinary fluoride. Furthermore, an integral study including analysis of reproductive hormone levels and direct semen analysis was not performed in any of the reports. Thus, in the present work, we studied the reproductive effects of populations environmentally and/or occupationally exposed to fluoride. The study comprises the exposure assessment to fluoride, the analysis of different reproductive hormones, and the assessment of semen parameters.

2. Methods

2.1. Population

One hundred and sixty men aged 20–50 years were studied. Twenty-seven of them were only exposed to fluoride in drinking water; therefore, they constituted the low-fluoride-exposed group (LFEG). The remaining 133 individuals were considered the high-fluoride-exposed group (HFEG) because they were occupationally exposed to fluoride in addition to drinking water. Drinking water was an exposure pathway because all the men included in this work are residents of San Luis Potosí, Mexico, where the annual mean fluoride level in tap water is 3.0 mg/L (Grimaldo et al., 1995, 1997). The HFEG individuals, in addition, worked in a fluoride industry that produces fluorhydric acid and aluminum fluoride. All of them had worked there for at least 1 year. The HFEG was divided into four working area subgroups: administration, maintenance (individuals working in all industry areas), fluorhydric plant (producing HF and AlF_3), and sulfuric plant (in this area the fluorspar is dehydrated). The lowest exposure to fluoride was in the administration area subgroup. None of the individuals in the LFEG worked in the fluoride industry.

An exposure to fluoride from 3.4 to 27.4 mg F/day was estimated for HFEG, considering (a) that the range of urinary fluoride among the workers went from 1.0 to 8.07 mg F/g of creatinine (b) that the mean daily urinary creatinine excretion is about 1.7 g/day and (c) that urinary fluoride represents about 50% of the exposure dose (DHHS, 1991). In contrast, in the LFEG with a urinary fluoride range of 0.5–3.7 mg F/g of creatinine, an exposure dose of 2–13 mg/day was estimated.

After informed signed consent was obtained, a questionnaire was applied and samples were taken. The questionnaire registered exposure to different fluoride sources, sociodemographic characteristics, occupation, reproductive history, alcohol consumption, and tobacco exposure. The samples were obtained between April and August 1998.

2.2. Urinary fluoride

The method of the National Institute for Occupational Safety and Health (NIOSH) for "fluoride in urine" (NIOSH, 1984) was followed as previously described (Grimaldo et al., 1995). In brief, first void

urine samples were collected in polyethylene bottles containing 0.2 g EDTA; they were refrigerated until analysis was performed. Prior to this, samples were mixed with TISAB buffer prepared as described by NIOSH. Then fluoride levels were quantified with a selective ion electrode. As an internal quality control program, reference standards from the National Institute of Standards and Technology were assessed (NIST 2671a). The fluoride recovery was $93 \pm 6\%$. Furthermore, our laboratory participated in the Interlaboratory Comparison Program for urinary fluoride, organized by the Centre de Toxicologie du Quèbec, with a mean accuracy of 97.3%.

2.3. Hormonal analyses

Blood samples were collected between 7:00 and 9:00 a.m. Free and total testosterone and estradiol were quantified by solid phase RIA, with detection limits and within-assay variation of 10 pg/mL and 7.0% for estradiol, 0.0005 ng/mL and 5.7% for free testosterone, and 0.04 ng/mL and 7.9% for total testosterone. FSH, LH, and prolactin were analyzed by RIA using a double antibody technique (Diagnostic Products Corporation), with detection limits and within-assay variations as follows: 2.0 IU/L and 6% for FSH, 3.0 IU/L and 8% for LH, and 6.0 ng/mL and 6% for prolactin. Inhibin-B was quantified by ELISA with a specific kit for the α–βB dimer, with a detection limit of 15 pg/mL (Serotec) (Groome et al., 1996). The within-assay variation coefficient was 4.6%.

2.4. Semen analysis

All 27 individuals from the LFEG and 54 workers from the HFEG participated in the semen analysis. The semen samples were collected in plastic bottles by masturbation, after at least 2 days of abstinence. All semen samples were analyzed within 1 h from sample production. Sperm count, morphology, motility, and viability were examined (a) manually, in accordance with the World Health Organization guidelines for the examination of human semen (OMS, 1992), and (b) with the Sperm Quality Analyzer IIB (SQA IIB) (Johnston et al., 1995). Both methods were assessed for agreement and showed proportionality in the measurements. The statistical analysis was done with the results of the SQA IIB because its within-assay variation coefficient (5%) was lower than that of manual analysis.

2.5. Lead analyses

Blood was obtained by venous puncture using lead-free tubes containing EDTA as anticoagulant. Lead in blood was analyzed with matrix modifier (diammonium hydrogen-phosphate-Triton X-100 in the presence of 0.2% HNO_3) (Subramanian, 1987). The analyses were done with an atomic absorption spectrophotometer (Perkin–Elmer 3110), and a graphite furnace was used. Our laboratory participates in the blood lead proficiency-testing program of the Centers for Disease Control (CDC).

2.6. Chronic exposure index

This parameter was calculated for both HFEG and LFEG, using the product of the urinary fluoride of each individual multiplied by a time factor. This factor was calculated considering that in LFEG fluoride exposure was the result of environmental exposure only, whereas, in HFEG, it was the result of both environmental and occupational exposures. The latter implicated 8 h per day, 5 days per week and 50 weeks per year of time exposed in the industry $(8/24 \times 5/7 \times 50/52 = 0.229)$. Thus, for the HFEG the time factor was calculated as [(time worked at working area $\times$ 0.229) + (time living at present home address $\times$ 0.771)], whereas for the LFEG the time factor was calculated as (time living at present home address $\times$ 1.0). The chronic exposure index was generated assuming a stable exposure during the period of time considered. Evidence in support of that assumption was fulfilled in the present study, and it came from comparing fluoride levels in urinary samples of 99 workers in the present study, collected first during 1993 and again in 1998; the mean concentrations in these samples were (mean $\pm$ SEM) 5.9 ± 0.40 vs. 6.5 ± 0.35 mg/L, respectively. No significant differences were found between these results. It should be noticed that the time living at home address is important because it has been demonstrated that fluoride concentration in drinking water varies among the different areas of San Luis Potosí (Grimaldo et al., 1997).

2.7. Statistical analysis

Concentrations of serum hormones were transformed logarithmically to adjust to a normal distribution, and their values are presented as geometric means $\pm$95% confidence intervals. Urinary fluoride underwent square-root transformation, and its value is presented as quadratic mean $\pm$ 95% confidence intervals. The parameters of semen analysis adjusted to the normal. To compare low fluoride (LFEG) and high fluoride (HFEG) exposed groups a *t*-test was performed. Comparison of LFEG vs. HFEG subgroups and administration subgroup vs. remaining HFEG subgroups was done by a one-way analysis of variance followed by Dunnett test. The regression and correlation analyses were done with log-transformed data (Altman et al., 1983). Parallelism was tested by comparing two straight lines using separate regression fits (Kleinbaum et al., 1988). In order to assess the influence of age,

alcohol consumption, tobacco, lead in blood, and years working in the exposure area adjust over urinary fluoride, hormone values, and semen characteristics in the different subgroups of study, an analysis of covariance was performed. These confounders were included in the analysis because they had a bivariate correlation to $P<0.2$ with urinary fluoride and/or serum hormones or semen parameters. To determine which of the several independent variables were important for predicting adjust serum inhibin-B or FSH concentrations in the two groups studied, a multiple regression analysis was performed using the smallest AIC value as criterion for selecting the best model (Akaike, 1974). A $P<0.05$ value was considered statistically significant. The statistical analysis was conducted using SPSS 8.0, STATA 5.0, and/or JMP V4.04.

3. Results

When high- and low-fluoride-exposure groups (HFEG and LFEG, respectively) were compared, urinary fluoride was higher in the HFEG than in the LFEG (Table 1). The magnitude of this parameter was independent of either the time worked at working area or the time lived at present home address. Within the industry, individuals in fluorhydric, maintenance, and sulfuric subgroups had urinary fluoride significantly higher than those working in the administration area (Table 1 and Fig. 1a). In relation to reproductive hormones, HFEG showed higher FSH serum concentration than LFEG, due to levels significantly higher in all of the working areas (Table 1 and Fig. 1b). In the groups as a whole, inhibin-B, prolactin, and free testosterone serum concentrations were significantly lower in HFEG than in LFEG, although all of them fell within the ranges considered normal (Table 1 and Fig. 1c). With respect to the last three hormones, the mean values in each work area subgroup trended to be lower that the mean value observed in LFEG, although in the case of inhibin-B, administration, fluorhydric and maintenance subgroups were statistically significant. In the case of prolactin, only fluorhydric and maintenance subgroups were significant, whereas in the case of free testoterone, all subgroups were significantly lower than in LFEG (Table 1). When it was corrected by potential confounders such as smoking habits, alcohol consumption, age, lead in blood, and working time in the area, the statistical differences among subgroups persisted. No differences were found between LFEG and HFEG for total testosterone, estradiol, or LH (Table 1).

Multiple regression analysis allowed us to document that FSH serum levels primarily, strongly, and negatively predict the inhibin-B serum concentration in both LFEG ($r=-0.63$, $P<0.0001$) and HFEG ($r=-0.48$, $P<0.00001$). On the other hand, serum inhibin-B primarily, strongly and negatively predicts the FSH serum concentrations in the low- and high-fluoride-exposure groups ($r=-0.63$, $P<0.0001$ and $r=-0.48$, $P<0.00001$, respectively). This analysis also showed that age is a good positive predictor of FSH serum concentrations in HEFG ($r=0.21$, $P<0.007$) but not in

Table 1
Urinary fluoride and reproductive hormone levels in individuals exposed to fluoride

Group by area	*n*	Urinary fluoride (mg F/g creatinine)	FSH (IU/L)	Inhibin-B (pg/mL)	Prolactin (ng/mL)	Free testosterone (ng/mL)	Total testosterone (ng/mL)	Estradiol (pg/mL)	LH (IU/L)
LFEG	27	1.6 (1.3–1.9)	4.7 (3.7–5.9)	200 (172–233)	11.6 (9.9–13.6)	0.027 (0.024–0.030)	5.6 (4.9–6.3)	38.4 (34.2–43.1)	5.1 (4.2–6.2)
HFEG	133	3.2* (2.9–3.4)	8.4* (7.8–9.0)	155* (144–166)	9.2* (8.5–9.7)	0.016* (0.014–0.017)	5.2 (4.9–5.5)	39.1 (37.0–41.2)	4.8 (4.5–5.2)
Administration	13	2.2 (1.5–2.9)	7.2a (5.6–9.2)	143a (109–187)	10.8 (9.0–13.0)	0.014a (0.011–0.017)	4.3 (3.5–5.3)	39.7 (33.2–47.4)	4.0 (3.4–4.7)
Fluorhydric	65	3.3a,b (2.9–3.6)	8.6a (7.7–9.5)	157a (140.–176)	8.9a (8.1–9.6)	0.016a (0.015–0.018)	5.5 (5.0–6.0)	44.8 (37.6–44.6)	5.1 (4.6–5.6)
Maintenance	35	3.3a,b (2.9–3.7)	8.8a (7.7–10.2)	144a (129–161)	8.8a (7.5–10.3)	0.017a (0.016–0.019)	5.6 (5.0–6.3)	37.2 (33.8–40.9)	4.9 (4.2–5.8)
Sulfuric	20	3.3a,b (2.5–4.2)	7.6a (6.5–9.0)	177 (142–220)	9.6 (7.9–11.7)	0.012a (0.010–0.015)	4.4 (3.7–5.2)	36.2 (31.5–41.6)	4.4 (3.6–5.4)
Normal values		<3.0[c]	2.5–7.0[d]	140–225[e]	7.0–30.0[d]	0.012–0.040[d]	2.6–15.9[d]	ND–44.0[d]	4.0–10.0[d]

Urinary fluoride is quadratic mean and the hormones are geometric means (CI 95%). LFEG: low-fluoride-exposed group. HFEG: high-fluoride-exposed group. LFEG vs. HFEG (*t*-test, * = $P<0.005$). Using one-way ANOVA followed by Dunnett test for posthoc analysis, we analyzed LFEG vs. HFEG subgroups (a = $P<0.05$) and HFEG subgroups vs. HFEG administration (b = $P<0.05$). No influence of age, tobacco, alcohol consumption, blood lead, or years of working was observed in the covariance analysis.

[c] ACGIH-BEI (1994).

[d] Diagnostic Products Corporation,

[e] Pierik et al. (1998).

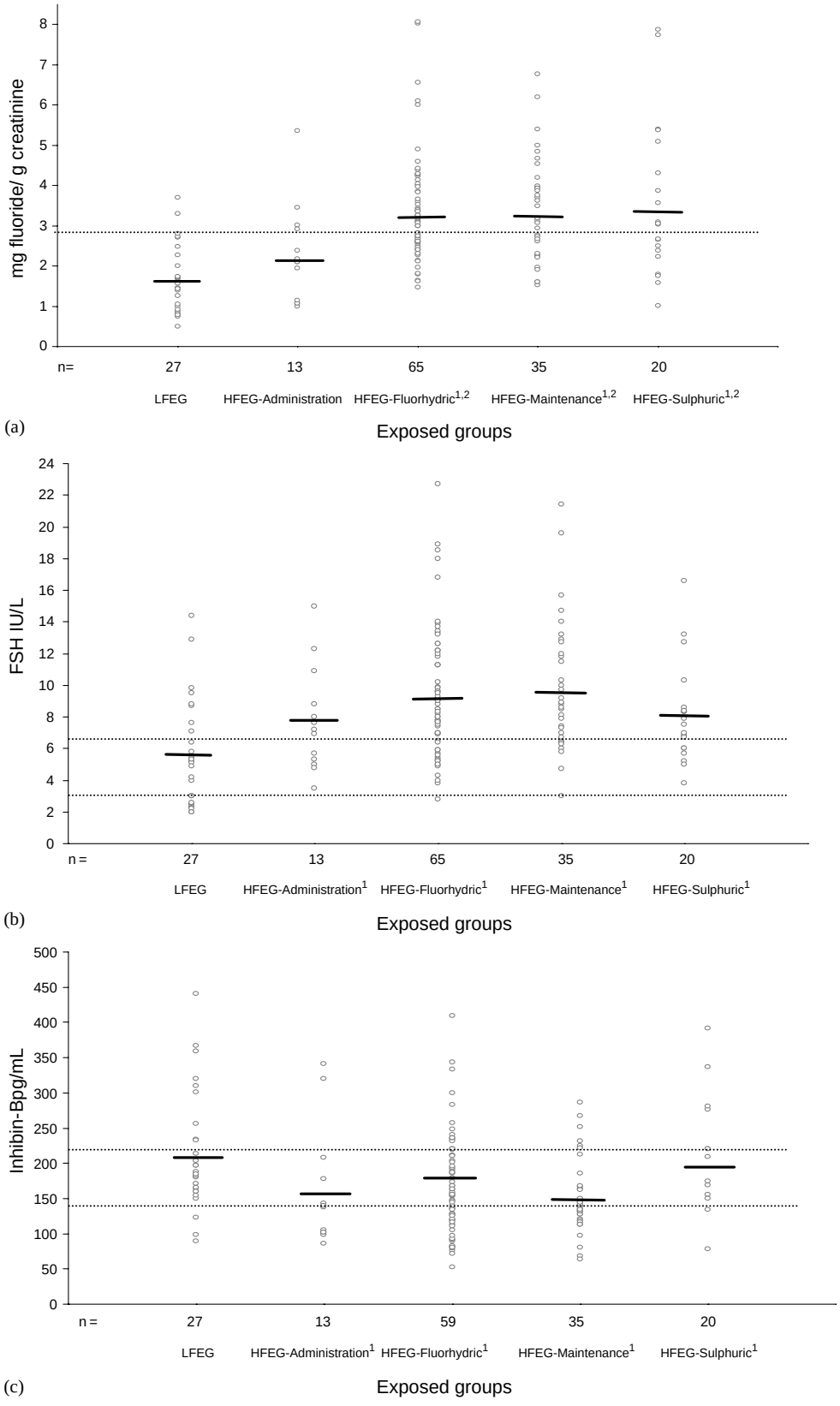

Fig. 1. Urinary fluoride and serum levels of FSH and inhibin-B in LFEG and HFEG: (a) urinary fluoride, (b) FSH, and (c) inhibin-B serum levels assessed in the LFEG and the HFEG. The occupationally exposed groups were divided into the different work area subgroups. Dashed lines represent normal values. $^{1}P<0.05$ vs. LFEG. $^{2}P<0.05$ vs. administration.

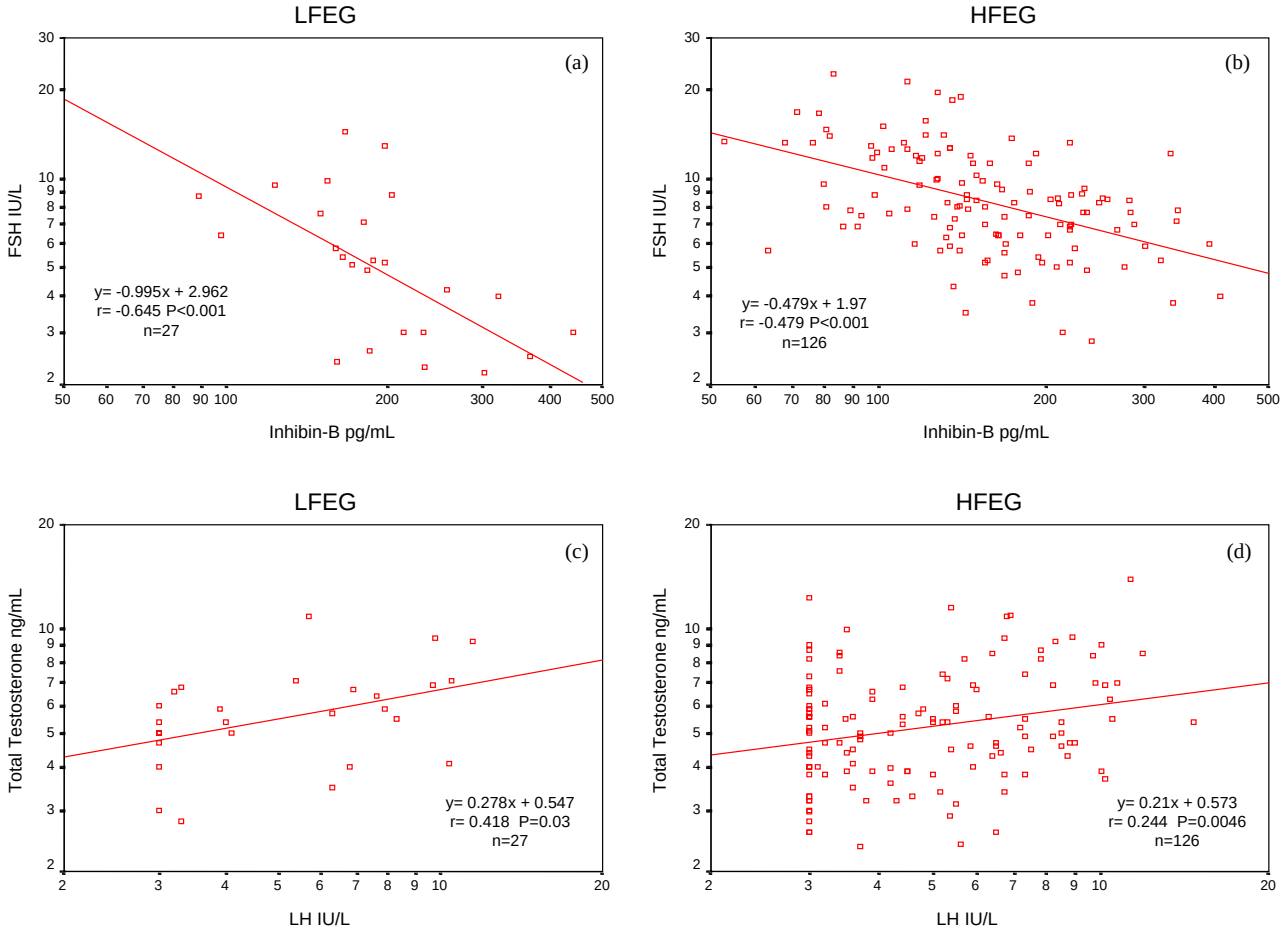

Fig. 2. Regression analysis between reproductive hormones. The regression analysis between FSH and inhibin-B (a and b) and between total testosterone and LH (c and d) for LFEG and HFEG are shown. The slopes of FSH vs. inhibin-B relationships were significantly different between groups ($P<0.05$).

LFEG. Age is also a primary and negative predictor of free testosterone serum concentration in both groups (LFEG: $r=-0.62$, $P<0.006$; HFEG: $r=-0.28$, $P<0.0009$). The ages in the two groups were (mean ± SEM) LFEG = 30.6 ± 1.3 and HFEG = 35.3 ± 0.72 years ($P=0.006$).

Among reproductive hormones and semen analysis performed, only serum inhibin-B significantly and negatively correlated with urinary fluoride in LFEG after controlling for serum FSH ($r=-0.333$, $P=0.028$) and after controlling for FSH and age ($r=-0.304$, $P=0.043$). In the HFEG, a negative and significant correlation was found between serum inhibin-B and chronic exposure index after controlling for serum FSH ($r=-0.163$, $P=0.037$) and after controlling for FSH and age ($r=-0.170$, $P=0.030$). Serum FSH concentrations did not correlate with urinary fluoride nor with chronic exposure index in any of the two groups. With respect to chronic exposure index and its two components (urinary fluoride and time factor; see methods), both were significantly ($P<0.0001$) greater in HFEG (3.3 ± 0.2 mg/g of creatinine and 12.1 ± 0.7 years) than in LFEG (1.6 ± 0.2 mg/g of creatinine and 6.1 ± 0.9 years).

In both groups, FSH and inhibin-B serum concentrations showed an inverse correlation (Figs. 2a and b), whereas total testosterone and LH levels showed a positive correlation (Figs. 2c and d). When FSH vs. inhibin-B regression slopes from each group were compared, they showed a statistically significant difference ($P<0.05$). In contrast, there was no difference in total testosterone vs. LH regression slopes between groups (Figs. 2c and d).

Semen parameters were within normal ranges, in both LFEG and HFEG (Table 2). Also, blood lead levels were quantified, considering that this metal has been reported as a reproductive toxicant (ATSDR, 1999a). The geometric mean for lead in blood concentrations found in the studied population was 5.6 μg/dL, with range between 2.0 and 24.0 μg/dL. Even the highest range value (24 μg/dL) was lower than the concentration at which reproductive effects have been reported (40 μg/dL) (ATSDR, 1999a).

Table 2
Semen analysis of individuals exposed to fluoride

Group by area	*n*		Sperm concentration (Mill/mL)	Sperm motility (%)	Normal morphology (%)	Sperm viability (%)
LFEG	27	Mean	74.63	49.63	33.19	93.96
		SD	38.44	12.55	8.74	4.54
		Range	19–194	24–82	18–52	80–98
HFEG-administration	7	Mean	75.43	48.00	33.29	92.57
		SD	38.44	18.75	11.97	6.02
		Range	10–126	10–66	10–44	80–98
HFEG-fluorhydric	21	Mean	82.71	51.57	34.62	93.24
		SD	43.06	13.88	9.54	6.25
		Range	18–164	23–73	18–48	75–98
HFEG-maintenance	18	Mean	66.72	47.61	32.22	90.61
		SD	26.80	10.72	7.88	8.13
		Range	17–101	22–60	18–42	75–98
HFEG-sulfuric	8	Mean	91.75	56.75	38.75	94.00
		SD	28.46	10.35	7.91	3.30
		Range	29–126	33–66	20–44	90–98
Normal values		Good	>60	>50	>30	>75
		Medium	20–60	30–50	20–30	
		Low	0–20	0–30	0–20	

The results were obtained with Sperm Quality Analyzer IIB. Differences were not significant among means (ANOVA $P<0.05$).

4. Discussion

Groundwater contamination with fluoride is a public health issue in some countries, including Mexico, where more than six million inhabitants are exposed to high fluoride levels. These levels are higher than the national guideline of 1.5 mg/L (NOM-127-SSA1, 1996). The concentration of fluoride in the majority of wells analyzed in Mexico is lower than 10 mg/L (Díaz-Barriga et al., 1997). Thus, the exposure to fluoride is lower than the exposure reported in other nations, such as India, where fluoride levels in groundwater up to 38.5 mg/L have been detected (Neelam et al., 1987). In India, reproductive effects were described in various reports among individuals living in areas with hydrofluorosis (Jolly et al., 1969; Michael et al., 1996; Susheela and Jethanandani, 1996). Evidence of fluoride-induced reproductive effects at concentrations lower than those found in India was obtained in an ecological study done in the United States (Freni, 1994). In this study, fluoride concentrations higher than 3.0 mg/L in drinking water were associated with decreased birth rate, but due to the ecological nature of the study design, the results must be considered with caution.

Considering that fluoride is a public health issue in Mexico and that the weight of the evidence in human beings and animals associates fluoride exposure with reproductive effects, we decided to assess these effects in male workers occupationally exposed to fluoride. The present study was performed in a population exposed to fluoride levels lower than those previously reported in experimental or epidemiological investigations.

It has been documented that fluoride levels in spot urine samples adjusted to creatinine levels correlate linearly and significantly with both 24-h excreted urinary fluoride and with the measured fluoride concentration of drinking water (Kertesz et al., 1989). In the present study, HFEG showed urinary fluoride levels significantly higher than LFEG, but their magnitude was not related with the time of exposure in any of the two groups. This suggests that when fluoride occupational exposure is superposed to an environmental exposure of this extent, it propitiates a higher urinary fluoride excretion whose magnitude is independent of the time spent in the working area.

As a group, HFEG, in contrast with LFEG, had a significantly lower serum concentration of inhibin-B. Whereas this hormone correlated significantly and negatively with urinary fluoride in LFEG, the same hormone correlated significantly and negatively with chronic exposure index in HFEG. This suggests that relatively prolonged environmental fluoride exposure is able to induce subtle decreases in serum inhibin-B, reflecting a testicular fluoride clearance to these levels and durations of exposure, but that only with prolonged fluoride occupational exposure at higher doses (i.e., as those observed in HFEG: 3–27 mg/day) is inhibin-B metabolism openly affected.

FSH serum levels of individuals working in all areas of the industry were above normal values and significantly higher than in LFEG (Table 1 and Figs. 1a and b). In both groups serum inhibin-B concentrations primarily, strongly and negatively predicted serum FSH levels, whereas only in HFEG did age lightly and positively predicted the serum FSH levels, suggesting that observed FSH increased levels in HFEG are the result of at least two factors: a greater reduction in inhibin-B levels in HFEG than in LFEG and a discretely higher age of occupationally exposed individuals. Thus, all the above appears to indicate that a long-term fluoride expose, at a dose similar to the one described for the HFEG in this study, is able to propitiate, in addition to the reduction of inhibin-B serum levels, an abnormal increase in serum FSH.

Recapitulating, in the HEFG group, inhibin-B correlated significantly and negatively with the chronic exposure index, a criterion that in our thinking better reflects the long-term effects of high fluoride exposure. Moreover, inhibin-B and FSH serum levels were significantly different between groups, beside the fact that both hormones had a reciprocal behavior in each group. Thus, all of this evidence suggests the existence of a fluoride-induced endocrine disruption over the hypothalamic–pituitary–testis axis. A relatively low inhibin-B serum concentration in HFEG could indicate that fluoride targets Sertoli cells, as inhibin-B is selectively secreted from these cells (Anawalt et al., 1996). Normally, the Sertoli cells would respond to an increase in FSH concentration by *secreting* more inhibin-B (Wallace and Healy, 1996). In turn, inhibin-B acts as a negative feedback signal for FSH at the pituitary level (Anawalt et al., 1996). In this work we found greater FSH serum concentrations in HFEG than in LFEG. In spite of that, inhibin-B serum levels in more exposed individuals were lower than in LFEG. A low concentration of inhibin-B in the presence of high FSH levels has been considered a biomarker of cellular damage for Sertoli cells (Anawalt et al., 1996; Pierik et al., 1998; Nachtigall et al., 1996). A decreased concentration of inhibin-B has been linked to a reduction in the number of Sertoli cells (Childs et al., 1997), but it has also been reported that mercury and platinum at doses that reduced Sertoli cell viability only moderately markedly decreased inhibin-B levels (Monsees et al., 2000). Thus, whether our results may be explained by a fluoride-induced cell function effect or by an inhibitory effect of fluoride over the synthesis or secretion of inhibin-B, the matter deserves further research.

When FSH vs. inhibin-B regressions slopes were compared between groups, a statistically significant decrease in the slope of the HFEG was found (Figs. 2a and b). Assuming a similar decrease in inhibin-B serum concentrations in both groups, the corresponding increase of FSH serum levels was lower in HFEG than in LFEG. This strongly suggests that fluoride exposure above the threshold level reduces the sensitivity of hypothalamic–pituitary axis to negative feedback action of inhibin-B. Without a reduction in this sensitivity, the increase in FSH serum levels, as consequence of a fluoride-induced serum inhibin-B reduction, would have been much greater. These effects of fluoride could be mediated by an increment in the hypothalamic–pituitary receptor affinity by inhibin-B and/or by a gonadotroph postreceptor attenuation of the response to reduced levels of inhibin-B.

Furthermore, we need to consider that the carboxylic end of the inhibin-B molecule is rich in basic amino acids (Seidah et al., 1984; Ramasharma et al., 1984); it has been demonstrated that the FSH inhibiting activity of inhibin-B resides within this region of the molecule (Sewani et al., 1998). Interestingly, fluoride has a great affinity for electropositive basic amino acids, and in fact, the binding of fluoride to this type of amino acids, has been shown in other proteins (Neri et al., 1997; Edwards et al., 1984). Thus, a direct interaction of fluoride with the inhibin-B molecule is possible and could explain the observed and discussed effects in both Sertoli cells and gonadotrophs.

A significant reduction in free testosterone serum concentrations in HFEG was also found (Table 1) and appears to progress with age. In this regard, it is important to consider that approximately 97–98% of testosterone that circulates in human blood is bound to the sex-hormone-binding globulin (SHBG) and only 2–3% is free and thus biologically active (Moore and Bulbrook, 1988; Rosner, 1990). An indirect pathway for regulating testosterone concentration becomes evident; an increase in SHBG content, or an increase in its affinity toward testosterone, would decrease the free testosterone concentrations in serum. The latter mechanism has been described in individuals with hypogonadism (Vermeulen et al., 1971). The effect of fluoride exposure on free testosterone levels deserves more studies, as we did not find a significant correlation with the chronic exposure index or with urinary fluoride. However, as stated in the introduction section, at high doses of exposure significant reduction of serum testosterone has been reported in humans (Susheela and Jethanandani, 1996; Tokar and Savchenko, 1977).

We also observed a significant reduction in prolactin in some subgroups of the HFEG (Table 1), although the levels were within normal ranges. It would be important to study in experimental models the effects of higher exposure doses to fluoride in dopamine concentrations, because it is well known that dopamine inhibits prolactin production (Freeman et al., 2000).

Considering the sperm defects and the increased infertility rates reported in animals (Chinoy et al., 1994; Narayana and Chinoy, 1994; Kumar and Susheela, 1994; Susheela and Kumar, 1991) and humans

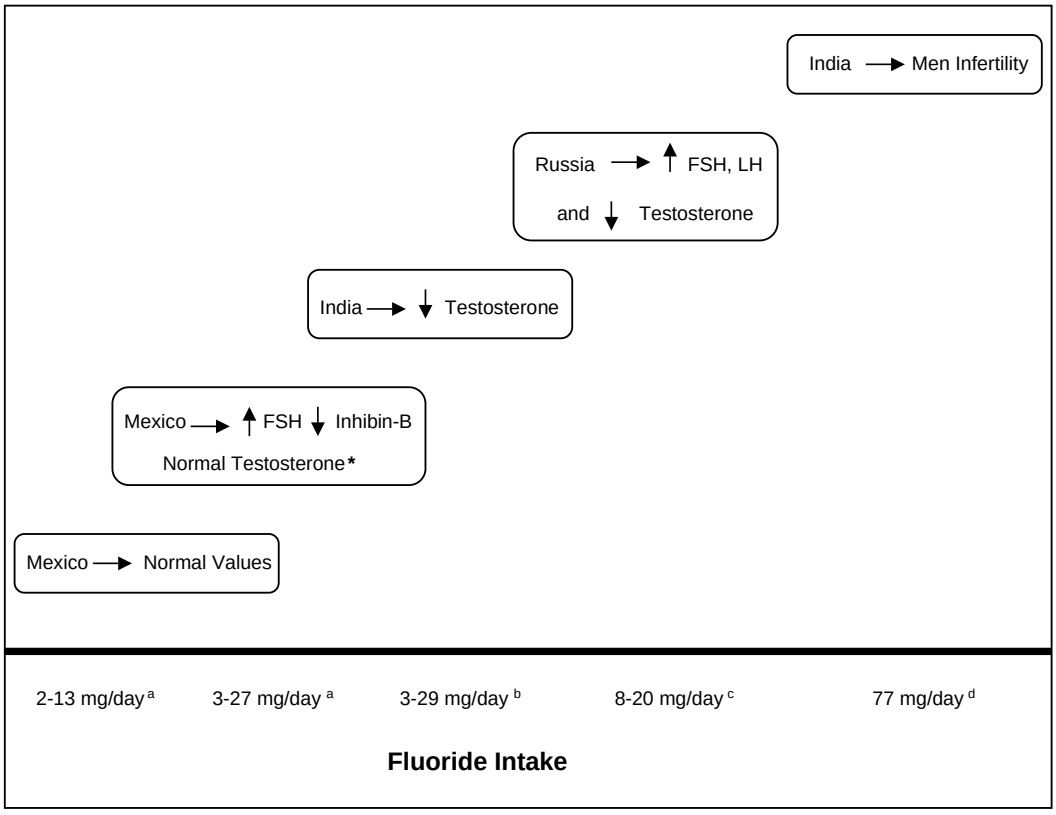

Fig. 3. Comparison of reproductive effects reported in humans exposed to several fluoride levels. [a]Present study in San Luis Potosi, Mexico, [b]Michael et al. (1996), [c]Tokar and Savchenko (1977), [d]Neelam et al. (1987). *In the present study a reduction in free testosterone was found (Table 1). Normal values in Mexico were found at doses higher than those reported in other countries, e.g., United States and Canada.

(Michael et al., 1996; Susheela and Jethanandani, 1996; Tokar and Savchenko, 1977; Neelam et al., 1987) exposed to fluoride, we decided in this work to study sperm parameters between LFEG and HFEG. Results of the sperm analysis of these individuals were normal.

Finally, it is important to consider that besides fluoride, the workers in this study were simultaneously exposed to aluminum and sulfur dioxide. Although reproductive effects have not been reported in animals or humans exposed to these compounds (Petruzzi et al., 1996; ATSDR, 1998, 1999b), we cannot rule out their partial participation in the effects observed in the present study.

The only abnormal effect that we found associated with long-term fluoride exposure was a decrease in inhibin-B and a secondary increase in FSH. However, our results are in agreement with the evidence that has been reported to date. As it is shown in Fig. 3, fluoride-induced reproductive effects appear to increase with increasing doses of exposure to this element. Therefore, fluoride might be a reproductive toxicant for humans. Nevertheless, it is important to perform more complete studies in those communities heavily exposed to fluoride, e.g., to analyze the hormone levels in those individuals showing infertility. In conclusion, our study supports the hypothesis that fluoride may be a reproductive toxicant, even though at the doses reported in this work only subclinical effects were found. Also, it should be noted that the doses in this study are higher than those reported in the United States (ATSDR, 2001) and Canada (ATSDR, 2001).

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