Evaluation of semen parameters in semen donors in a ten-year period in the city of São Paulo

Avaliação dos parâmetros seminais em doadores de sêmen no período de dez anos na cidade de São Paulo

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ABSTRACT

Objective: To evaluate sperm concentration, morphology and motility of Brazilian semen donors from 1992 to 2003, in the city of São Paulo. **Methods:** Retrospective study analyzing 182 donor semen samples from 1992 to 2003. The first and the second donated sample were analyzed for each donor. Donor average age was 30.8 years. Means with standard errors, medians with minimum and maximum values, and interquartile ranges were calculated for age, sperm concentration, semen volume, oval morphology and motility. The relation between each characteristic of the semen samples and the year of donation, as well as donor age and season of the year were studied by linear and multiple regression analysis. **Results:** Linear regression analysis showed that the sperm concentration ($R^2 = 19.1\%$, $R^2 = 20.2\%$, $p < 0.0001$ respectively) and the oval morphology ($R^2 = 13\%$; $R^2 = 13.5\%$; $p < 0.0001$, respectively) decreased significantly, even when the first or the second sperm collection is considered. The ejaculated volume showed slight increase during the period for both samples ($R^2 = 2.2\%$, $p = 0.048$; R-sq = 2.4%. $p = 0.038$, respectively). All characteristics did not depend on the donors' age or season of the year when the samples were obtained. **Conclusions:** There was a decrease in spermatic concentration and percentage of oval sperm of semen donors samples from 1992 to 2003, in the city of São Paulo.

Keywords: Semen; Spermatozoa; Semen analysis

RESUMO

Objetivo: Avaliar as características seminais dos doadores de sêmen na cidade de São Paulo, no período de 1992 a 2003. **Métodos:** Análise retrospectiva das amostras seminais de 182 doadores de um único Banco de Sêmen, na cidade de São Paulo, no período de 1992 to 2003, que tinham a idade média de 30,8 anos. Foram analisadas a primeira e a segunda amostra de cada doador. Médias com desvios padrões, medianas com valores máximos e mínimos e intervalo interquartil foram calculados para idade, volume seminal, concentração, motilidade e morfologia espermática. As relações entre cada característica das amostras seminais e o ano da doação foram estudadas por análise de regressão linear simples. Modelos de regressão linear múltipla foram aplicados para examinar a relação do ano de doação com cada característica seminal, controlando para potencias fatores de confusão, como idade dos doadores e estação do ano em que a coleta foi realizada. **Resultados:** Análise de regressão linear mostrou que a concentração espermática ($R^2 = 19,1\%$, $R^2 = 20,2\%$, $p < 0,0001$, respectivamente) e a morfologia oval dos espermatozoides ($R^2 = 13\%$; $R^2 = 13,5\%$; $p < 0,0001$, respectivamente) diminuíram significativamente, na primeira e na segunda coleta seminal. O volume do ejaculado mostrou um discreto, porém significativo, aumento nas duas coletas ($R^2 = 2,2\%$, $p = 0,048$; R- sq = 2,4%, $p = 0,038$, respectivamente). Todas as alterações não se correlacionaram com a idade do doador nem com a estação do ano em que as coletas de sêmen foram realizadas. **Conclusões:** Houve diminuição na concentração espermática e na percentagem de espermatozoides nas amostras seminais dos doadores de sêmen, no período de 1992 a 2003, na cidade de São Paulo.

Descritores: Sêmen; Espermatozoides; Análise do sêmen

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INTRODUCTION

The question of whether male fertility is really decreasing is very controversial in the field of human reproduction. Many different authors have showed either decreasing or maintenance of the semen quality in the last 20 years[1]. Macomber and Sanders conducted the first study about normal sperm concentration in 1929, based on the sample counts of 294 individuals. Those authors reported the normal sperm concentration to be 100 x 10^6 sperm/ml[2].

In 1992, Carlsen et al. carried out a systematic review of the literature published since 1930. They analyzed semen data from 14,947 men in 61 papers. Linear regression analysis showed a significant decrease of the median sperm concentration from 113 million/ml in 1940 to 66 million/ml in 1990 and of seminal volume from 3.4 to 2.75 ml[3]. In 1997, Swan, Elkin and Fenster reanalyzed data of 56 papers that had been reviewed by Carlsen et al.[3]. They claimed that the alterations found by them could be due to a mistaken statistical analysis. However, they confirmed that there was indeed a decrease of sperm concentration in the United States and Europe, but not in the Non-Western countries, after a sophisticated statistical analysis[4].

Van Waeleghem et al., in 1996, reported a deterioration of sperm quality in young healthy Belgian men. Sperm concentration, motility and morphology, evaluated in 416 consecutive healthy young men selected as potential sperm bank donors for 19 years, showed a significant decrease[5]. However, in the same year, Paulsen, Berman and Wang reported no downward trend in semen quality from 1,283 men in the greater Seattle area and raised the concept that deterioration of semen quality is not geographically uniform[6].

More recent studies have suggested a worldwide trend of semen quality decline, but it seems clear that there are large geographic differences in sperm counts[7,8]. Auger and Jouannet, in 1997, evaluated seminal samples of 4,710 semen donors from 1973 to 1993, collected in eight different regions in France and found statistical differences among the samples of different areas. They stated that regional discrepancies should be considered on the evaluation of papers showing a decrease of semen quality over time[9].

The possible causes for the negative impact on semen characteristics and for the large geographic discrepancies could be industrialization, environmental pollution, the use of chemicals, repeated exposure to hazardous compounds at work and variations related to laboratory techniques and different technicians. Other more speculative reasons have been raised; Sheiner et al. reported a possible association between male infertility and psychological job stress[8]. According to Storgaard et al., sons of mothers who smoked more than ten cigarettes per day during pregnancy had a significant decrease in sperm density, total sperm count and inhibin-B plasma levels[7].

Furthermore, the effect of psychological stress on semen quality cannot be ignored. Several authors have suggested that male psychological stress can affect semen quality and couple fertility[10]. Hammond et al. showed a negative impact of the anxiety on semen quality comparing semen samples collected for diagnosis and at the moment of treatment (assisted reproduction techniques)[11].

Any man's first semen collection can occur under psychological pressure and it is recommended that any seminal abnormality be confirmed on a second sample before any diagnosis is made[10]. Apparently, the need to obtain a semen sample at a specific time for an infertility treatment procedure or for sperm donation has the potential to produce considerable performance anxiety[12], and possibly jeopardize the quality of the first semen sample. Thus, it is important to compare the data of the first and second collection from semen donors and evaluate if there is any difference in quality between them.

Furthermore, for many mammals fertility status can change according to the season of the year. Gyllenborg et al., in 1999, found a significant increase of sperm concentration and a decrease of sperm motility when semen samples of 1,927 potential semen donors, from 1977 to 1995, in Copenhagen, Denmark. They also found that the sperm concentration was higher in spring and lower in summer[13].

OBJECTIVE

To evaluate sperm concentration, morphology and motility from semen donors in the city of São Paulo from 1992 to 2003, comparing data of the first and second sperm collections and analyzing donor ages and the season of the year when the samples were obtained.

METHODS

Study design

Retrospective study analyzing semen samples from 182 donors in the last ten years (1992 to 2003), at the Sperm Bank from Hospital Israelita Albert Einstein. All the analyses were independently performed for the first and second collections.

Sperm donors' selection parameters

The mean age of semen donors was 30.8 years (ranging from 18 to 40). All donors lived in São Paulo metropolitan area. Before being accepted in the donation program, all

the candidates were submitted to a medical screening with assessment of sexual habits, general health, serological tests (HIV-1, HIV-2, HIV-P24 antigen, HTLV-I, HTLV-II, syphilis, HBsAg, HBc, hepatitis C antibody and Chagas disease), chromosome analysis, and sperm culture (*Chlamydia trachomatis, Mycoplasma sp, Ureaplasma urealyticum* and aerobic culture).

Semen analysis

All donors were instructed to collect their first two semen samples in the same month, at least one week apart. The samples were collected by masturbation, in special plastic containers after two to five days of sexual abstinence. The sample was kept at 37 ºC, until complete liquefaction (maximum of 30 minutes), and an initial macroscopic examination of the following parameters was performed: viscosity (or consistency), pH, appearance (homogeneity and coloration) and volume. Then, a microscopic examination was performed to evaluate the sperm count and motility (Makler Counting Chamber). Sperm morphology was evaluated according to Kruger's strict criteria[14]. Semen smears were stained by Papanicolau method and at least 100 spermatozoa were microscopically examined at X1,000 magnification. Semen analyses were performed by the same three laboratory technicians during the whole period. During this ten-year period, the same laboratory methods were used to perform the semen analysis.

Statistical analysis

Minitab statistical software was used for statistical analysis. Means with standard errors, medians with minimum and maximum values, and interquartile ranges were calculated for age, sperm concentration, semen volume, oval morphology and motility. Sperm motility and oval morphology had normal distribution, which did not happen for semen volume and sperm concentration, and the square root transformation was used to obtain a normal distribution. Anderson-Darling test was used to test the normal values of quantitative variables.

The relation between each semen sample feature and the year of donation were studied by linear regression analysis. Multiple linear regression was used to evaluate the relationship between the year of semen collection and each seminal parameter; potential confounders, as donor ages and season when the semen was collected, were controlled. The p values < 0.005 were considered as significant. Seasons of the year were defined with an equinox-solstice table from 1992 to 2005, found at http//astro.if.ufrgs.br/estacoes.html.

This project was analyzed and approved by the Ethics Committee of Hospital Israelita Albert Einstein – HIAE (CEP/Einstein 10-1374).

RESULTS

One hundred and eighty-two males between 18 and 40 years of age volunteered for semen donation in the period from 1992 to 2003.

Values of the donors' first semen sample during this period were: median volume of ejaculated sperm was 3 ml (ranging from 0.3 to 10 ml). Median concentration was 110 million/ml (ranging from 8 to 400 million/ml), median percentage of motile sperm was 63.5% (ranging from 25 to 95%), and the median percentage of oval morphology was 20% (ranging from 7 to 43%) (Table 1). Values of the donors' second sample were: median volume of ejaculated sperm was 3 ml (ranging from 1.0 to 11.3 ml). Median concentration was 120 million/ml (ranging from 6 to 300 million/ml), median percentage of motile sperm was 65% (ranging from 26 to 91%) and the median percentage of oval morphology was 20% (ranging from 7 to 43%) (Table 2).

Table 1. Values of the first semen donor's samples from 1992 to 2003

Statistical values	Volume (ml)	Concentration (millions sp/ml)	Oval sp (%)	Motile sp (%)
Mean ± sd	3.2 ± 1.8	120.7 ± 64.6	21.3 ± 5.0	63.6 ± 13.5
Median (min-max)	3 (0.3 - 10)	110 (8 - 400)	20 (7 - 43)	63.5 (25 - 95)
Interquartile	2.0 - 4.2	72 - 160	18 - 23	54.7 - 73

sp: sperm; ml: mililiter; min: minimum; max: maximum; sd: standard deviation.

Table 2. Values of the second semen donor's samples from 1992 to 2003

Statistical values	Volume (ml)	Concentration (millions sp/ml)	Oval sp (%)	Motile sp (%)
Mean ± sd	3.4 ± 1.8	126.6 ± 66.0	21.3 ± 5.0	62.6 ± 12.7
Median (min-max)	3 (1.0 - 11.3)	120 (6 - 300)	20 (7 - 43)	65 (26 - 91)
Interquartile	2.20 - 4.30	76.2 - 163.7	18 - 23	55 - 71

ml: mililiter; sp: sperm; min: minimum; max: maximum; sd: standard deviation.

Linear regression analysis showed that sperm concentration decreased significantly, both on the first and second sperm collection ($R^2 = 19.1\%$, $p < 0.0001$; $R^2 = 20.2\%$, $p < 0.0001$, respectively) (Figures 1A and 1B). The same finding was observed on the percentage of oval sperm ($R^2 = 13\%$, $p < 0.0001$; $R^2 = 13.5\%$, $p < 0.0001$, respectively) (Figures 2A and 2B). The ejaculated volume showed little increase during the period for both samples ($R^2 = 2.2\%$, $p = 0.048$; R-sq = 2.4%, $p = 0.038$, respectively) (Figure 3A and 3B). The only parameter that showed no changes was the sperm

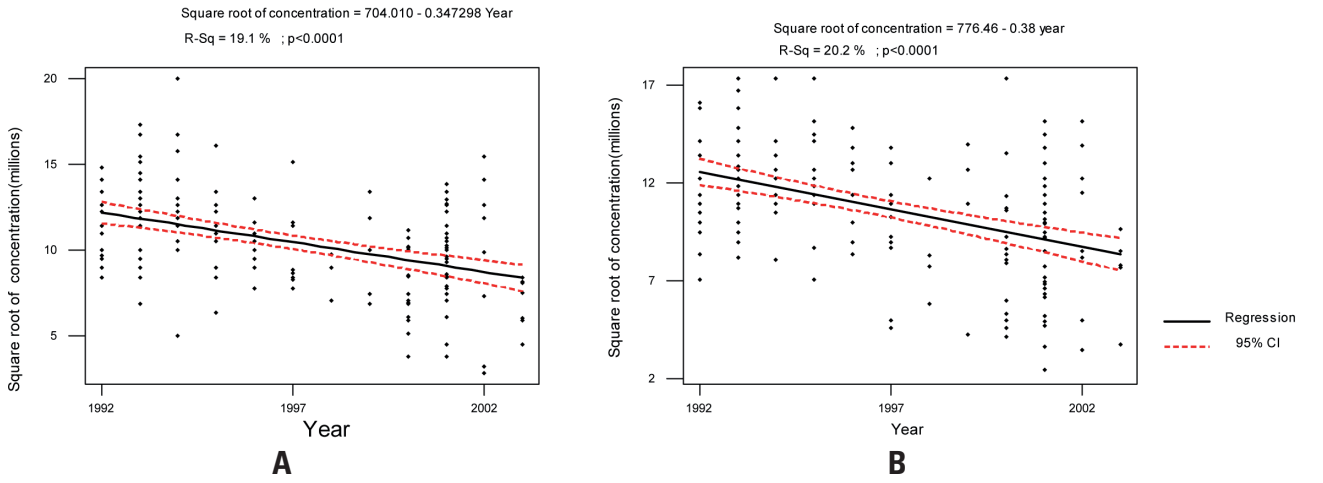

Figure 1. Evaluation of sperm concentration of donors from 1992 to 2003. A: first collection and B: second collection.

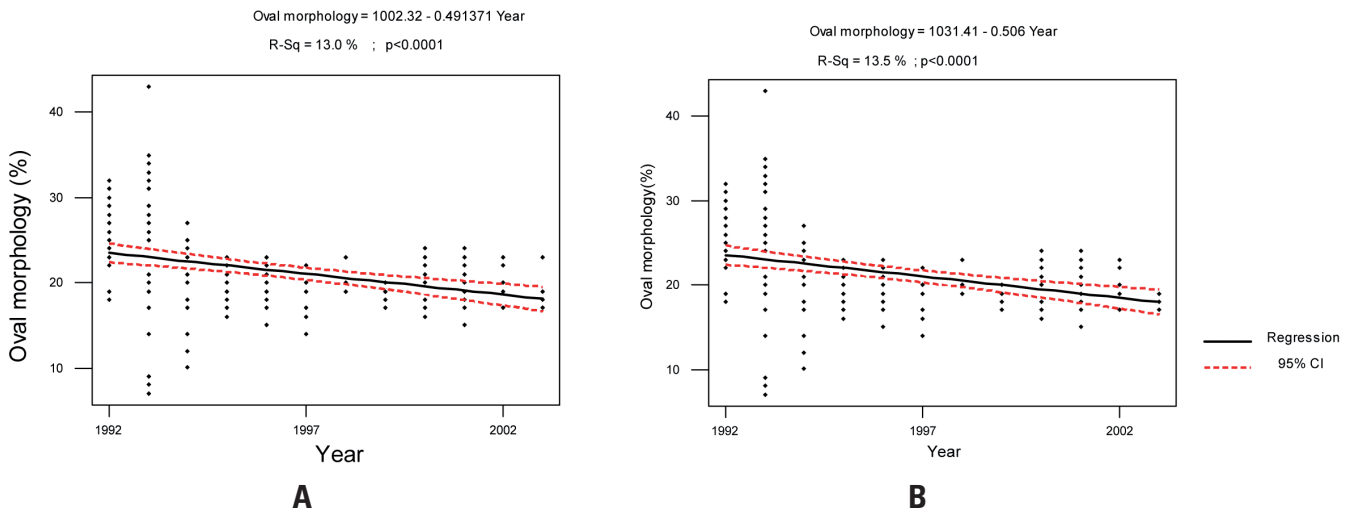

Figure 2. Evaluation of sperm morphology of sperm donors from 1992 to 2003. A: first collection and B: second collection.

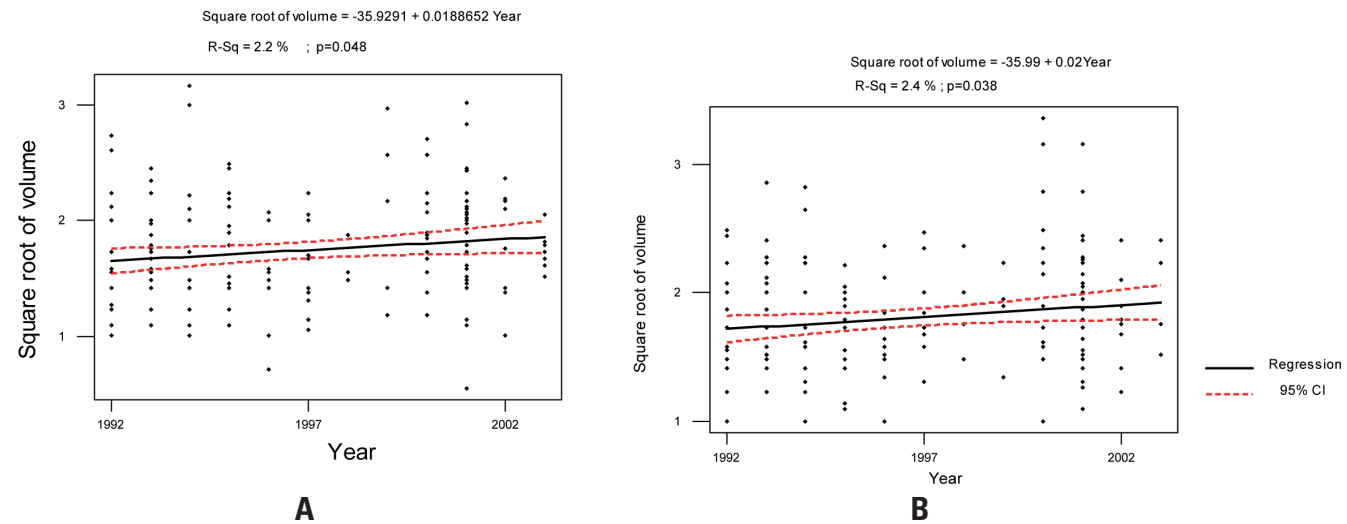

Figure 3. Evaluation of semen volume of sperm donors from 1992 to 2003. A: first collection and B: second collection.

motility ($R^2 = 0.1\%$, p = 0.653; R-sq = 0.4%, p = 0.376, respectively) (Figures 4A and 4B).

Multiple regression analyses for each semen feature, controlling age and seasons, showed that concentration and oval morphology still decreased significantly from 1992 to 2003 (p < 0.0001; p < 0.0001), whereas motility and volume did not change over the same studied period (Tables 3 to 6). Therefore, no correlation was observed between donors' ages and season's of the year.

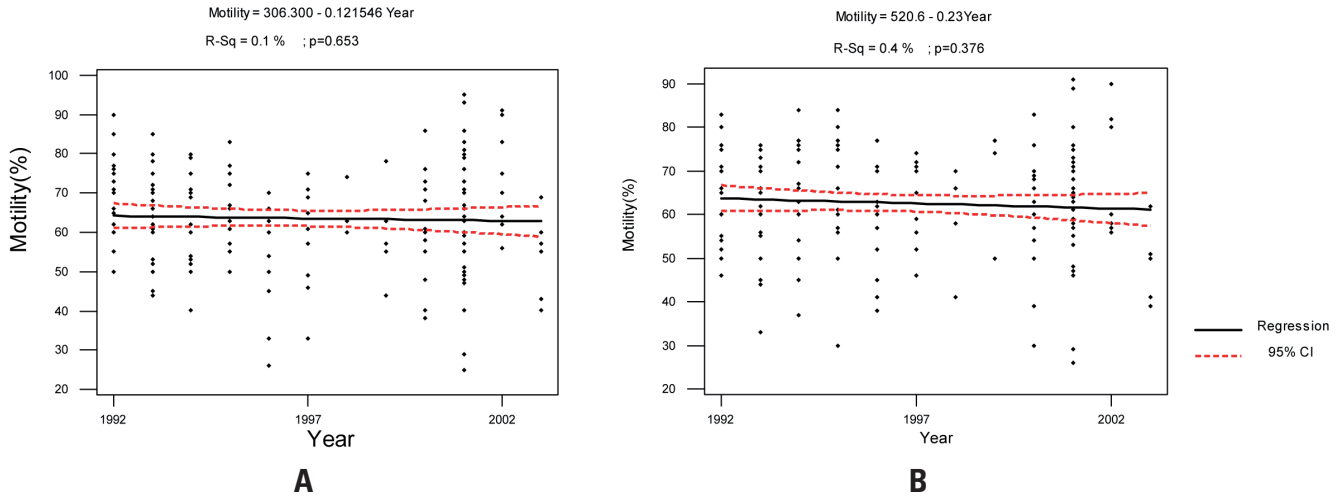

Figure 4. Evaluation of sperm motility of sperm donors from 1992 to 2003. A: first collection and B: second collection.

Table 3. Multiple linear regression of square root of sperm concentration of sperm donors from 1992 to 2003

Variable	Reference	β coefficient	p value
Year of sample collection	Unit change	-0.343	< 0.0001
Age	Unit change	-0.0132	0.722
Season			
Summer	Autumn	-0.731	0.227
Spring	Autumn	-0.7525	0.225
Winter	Autumn	0.0653	0.911

R^2: 20.9%.

Table 4. Multiple linear regression of oval morphology of sperm donors from 1992 to 2003

Variable	Reference	β coefficient	p value
Year of sample collection	Unit change	-0.491	< 0.0001
Age	Unit change	0.10399	0.121
Season			
Summer	Autumn	-1.534	0.153
Spring	Autumn	-0.009	0.994
Winter	Autumn	-1.179	0.255

R^2: 15.9%.

Table 5. Multiple linear regression of sperm motility of sperm donors from 1992 to 2003

Variable	Reference	β coefficient	p value
Year of sample collection	Unit change	-0.0719	0.797
Age	Unit change	0.0242	0.897
Season			
Summer	Autumn	0.179	0.953
Spring	Autumn	4.227	0.179
Winter	Autumn	-0.083	0.977

R^2: 1.8%.

Table 6. Multiple linear regression of square root of sperm donors' seminal volume from 1992 to 2003

Variable	Reference	β coefficient	p value
Year of sample collection	Unit change	0.017352	0.079
Age	Unit change	0.003543	0.593
Season			
Summer	Autumn	0.0604	0.574
Spring	Autumn	0.0093	0.933
Winter	Autumn	-0.0665	0.519

R^2: 3.5%.

DISCUSSION

The studied data showed that the semen quality in a group of healthy young donors, at a Semen Bank in the city of São Paulo, had a statistically significant decrease from 1992 to 2003. That finding seems to be a biologic phenomenon, once methodological and laboratorial personnel had been the same over the whole period, which reduces the technical variables. Although the period of sexual abstinence had ranged from two to five days, this cannot be considered as a bias, since sperm concentration and seminal volume start to increase after the fifth day of abstinence in normal men[15]. The Semen Bank of Hospital Israelita Albert Einstein initiated in 1989, and the period from 1992 to 2003 was chosen because the same technical standards had been kept in that period.

These findings confirmed other studies that had shown a decrease in the seminal quality over time in many regions of the world[3,5,9,16]. However, this finding has not been universal and has presented a large geographic variation[9].

The possibility that semen alterations are different in the first or second sample because of a possible negative psychological impact, as noted by Clarke et al.[12], did not happen in this study. The decrease observed in sperm concentration and the percentage of oval sperm, the increase in semen volume and the non-abnormal sperm motility happened in both donor samples.

The semen's abnormalities found in this paper did not show correlation with donors ages and with the season when the semen was collected, contrarily to what was shown by Gandini et al., in 2000[1], Gyllenborg et al., in 1999[13] and Yogev et al., in 2004[17] .

Environmental deterioration is the main candidate as a possible cause of a decline in semen quality and geographic discrepancies. In particular, the pollution caused by xenobiotics with estrogen-like activity (endocrine disruptors) is suspected of determining various andrological pathologies, such as testicular cancer, hypospadia, cryptorchidism and reduction in spermatogenesis through a reduction of Sertoli cells during fetal life[1]. Therefore, prenatal exposure to tobacco smoke can be hypothesized to be an alternative explanation for the possible decline in sperm counts in some countries[7]. Reduced fecundability has been found in women prenatally exposed to their mothers' cigarette smoking, and delayed time to pregnancy has been observed in both men and women whose mothers smoked during pregnancy[8].

A study performed with 202 consecutive male patients, attending a fertility clinic, found male infertility to be associated with industry and construction jobs and in workers who suffer of work burnout[8]. However, in the same study, the authors found no significant association between male infertility and potential physical or chemical exposures[8]. However, in a recent study, it was found that continuous exposure to traffic pollutants impairs sperm quality in young/middle-aged men. The comparative evaluation of sperm parameters, absorption markers and environmental concentrations indicate that lead (Pb) was probably the cause of the impaired spermatogenesis[18]. Sokol et al., in 2006, analyzed 5,134 semen samples in Los Angeles, California, and found a negative correlation between the atmospheric concentration of ozone and seminal quality. They argued that exposure to ozone could induce an inflammatory response, which could lead to an abnormal oxygen reactive-species concentration in the seminal tract, with consequent deterioration of semen quality[19].

It is important to state that fertility status is not based only on the semen features. The ideal way to evaluate if fertility is declining would be to study the pregnancy rate obtained with these donors' samples, which will be the aim for the future, although, female factors would also be included in the study. Though a significant decrease in the quality of semen was found during the studied period, one could question the clinical meaning of those findings, since the figures obtained were still in the range of normal values according to the World Health Organization[20].

São Paulo is an 11-million city with moderate to high environmental pollution rates, and this could be one of the main reasons of the present findings. Further studies are being developed to correlate air conditions with the semen's quality.

CONCLUSIONS

There was a decrease in sperm concentration and in the percentage of oval sperm in samples of semen donors, from 1992 to 2003, in the city of São Paulo.

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