CIENCIA 6(1), 31-38, 1998
Maracaibo, Venezuela

Copper, chromium and zinc content in human semen and its possible effect on sperm quality of fertile and infertile men in the city of Caracas, Venezuela

Luz Marina Foglietta[1], *Marma Gladys Muñoz*[1] *and José Alvarado*[2]*
[1]*Departamento de Biología de Organismos.* [2]*Departamento de Química*
Universidad Simón Bolívar, Apdo. 89000. Caracas, Venezuela

Recibido: 23-09-96 Aceptado: 17-12-97

Abstract

Copper, chromium and zinc concentrations were determined by atomic absorption spectrometry in semen samples. The concentration of these metals was determined in 43 infertile men and 27 fertile men. Semen quality was evaluated based on the norms established by the World Health Organization. Statistical analysis of the seminal parameters showed significant differences among fertile and infertile men regarding sperm concentration, progressive linear motility and normal morphology. Heavy metal concentrations showed no significant correlation when comparing the results obtained for semen from fertile and infertile men. Overall, the comparison between semen quality and heavy metal concentration showed no correlation; the copper, chromium and zinc concentrations found in the semen samples do not seem to affect sperm quality. This is the first report in Venezuela about the semen concentration of heavy metals.

Key words: Chromium; copper; human semen; infertility; seminal parameters; zinc.

Contenido de cobre, cromo y cinc en semen humano y su posible efecto sobre la calidad espermática de hombres fértiles e infértiles en la ciudad de Caracas, Venezuela

Resumen

Se determinaron las concentraciones de cobre, cromo y cinc en muestras de semen mediante espectrometría de absorción atómica. Se determinaron las concentraciones de estos metales en 43 hombres infértiles y 27 fértiles. La calidad del semen fue evaluada según las normas establecidas por la Organización Mundial de la Salud. El análisis estadístico de los parámetros seminales mostró diferencias significativas entre los hombres fértiles e infértiles, considerando la concentración espermática, movilidad progresiva lineal y morfología normal. Las concentraciones de los metales pesados no mostraron una correlación significativa al comparar los resultados obtenidos para el semen de hombres fértiles e infértiles. Además, la comparación entre la calidad del semen y la concentración de metales pesados no mostró una

* Laboratorio de Espectrometría de Absorción Atómica. Departamento de Química. Universidad Simón Bolívar. Apartado 89000. Caracas 1080A. Venezuela. Fax: 02-9063981. E-mail: lfogliet@usb.ve

correlación; las concentraciones de cobre, cromo y cinc encontradas en las muestras de semen no parecen afectar la calidad espermática. Este es el primer estudio de la concentración de metales pesados en semen y su correlación con la calidad seminal realizado en Venezuela.

Palabras clave: Cobre; cromo; infertilidad; parámetros seminales; semen humano; zinc.

Introduction

In the last decades a decreasing quality of semen (1,2) without defined cause, has been reported (3,4). Probably the decline in semen quality observed in those studies could be attributed to behavioral and/or environmental factors. An increase in the heavy metal concentrations in the environment could be related to a decrease in semen quality since it has been observed that some heavy metals can be very effectively accumulated in body tissues, specially in those of the reproductive system (5).

The detrimental effects of heavy metals on male reproductive functions have been consistently documented. The effects of copper on the percentage of progressive motility and its probable action on the hypophysiary receptors controlling the release of luteotropic hormone, have been clearly demonstrated (6). It has also been shown that chromium has a toxic effect on the human sperm and on the testicular functions in rodents (7,8). On the other hand zinc is abundant in the sperm (9) and is present mainly in the prostate gland (10). Zinc could act as a stabilizer of biological membranes (11) and of sperm chromatin (12). Zinc is directly related to the sperm capacitation and acrosome reaction (13,14) and also to the sperm motility (15,16).

The levels of heavy metals in men, exposed and non exposed to toxic environments, has been studied in several countries (17-19). In Venezuela, the rate of infertility in industrial workers has been associated with the possibility of toxic environmental conditions due to the increase in sources of particulate emissions. However, a study to determine the concentrations of heavy metals in semen of unexposed men, has never been done. Due to the lack of scientific information regarding the reasons for some infertility cases, this is an open topic for debate and research. The possible effect of the presence or absence of some metals in semen samples of fertile and infertile individuals could be related to their particular situation in this aspect. The purpose of this work was to measure copper, chromium and zinc content in semen samples of fertile and infertile men and to correlate them with sperm quality.

Materials and Methods

Sample collection

Semen samples were obtained from 43 men of infertile couples whose infertility is attributable to male partner and 27 fertile men who had lived for at least 8 years in Caracas. Infertile men were defined here as those who could not procreate after two years of unprotected sexual relations. Fertile men were those who had had at least one child without resorting to any artificial conception method within the last 3 years. All men were between 22 to 35 years of age and randomly selected. The subjects were not occupationally exposed to heavy metals. Other personal characteristics such as weight, previous clinical history, physical condition, special diets, etc. were also considered in the selection of the donors.

The semen samples were obtained by masturbation after 2 to 5 days of sexual and alcoholic abstinence. All donors were instructed on the negative effects of sample contamination and on the best way to eliminate or minimize it. They were collected in polypropilene containers. A part of each sample was taken for sperm quality analysis which was performed 60 minutes after sam-

ple collection. The rest of the sample was stored frozen at -20 °C for heavy metal determination.

Sperm quality analysis

For the sperm quality test a variable number of sample was considered according to Table 3.

These analyses were carried out following the criteria stablished by WHO (20).

The semen samples studied were distributed in the following groups:

a) Men with 20 x 10^6 or more spermatozoa/mL and

b) men with less than that sperm concentration.

c) Men with 25% or more progressive linear sperm motility and

d) men with less than that percentage.

e) Men with 50% or more normal sperm morphology and

f) men with less than that percentage.

Heavy metal determination

Frozen samples were liquefied at room temperature and digested in covered beakers in a fume cupboard with a 1:1 solution of ultrapure HNO_3 under moderate heating conditions (~ 85°C).

All labware used was previously treated with 10% nitric acid for 48 hours and copiously rinsed with distilled-deionized water to eliminate possible traces of heavy metals.

For copper and chromium determinations, semen samples were diluted 1:20 with distilled-deionized water. Semen samples for zinc determination were diluted 1:250.

Copper and chromium were determined by graphite furnace atomic absorption using L'vov platforms. Zinc was determined after atomization in an acetylene-air flame. A Perkin-Elmer 2100 Spectrometer with deuterium background correction was used for the atomic absorption measurements. For the graphite furnace atomization an AS-70 autosampler was used for sample injection.

Concentrations were determined by comparison with standard addition curves covering the concentration ranges of 0 - 20 μg/L for copper and 0 - 8 μg/L for chromium. Zinc concentrations were obtained by comparison with aqueous standard calibration curves covering between 0.2 and 1.5 μg/L. The instrumental conditions used are shown in Tables 1 and 2. The graphite furnace parameters were chosen so to work under stabilized temperature platform furnace, STPF, conditions (21). Drying, charring and atomization temperatures, hold times and ramps were optimized in order to obtain the best possible sensitivity of the measurements. A cleaning and a cooling stages were introduced in the heating program to avoid possible memory effects and injections on a hot platform. Volumes of standards and sample solutions of 10 μL and 20 μL were injected for cooper and chromium, respectively. For flame atomization of zinc the wavelength at 213.8 nm, a band width of 0.7 nm and a lamp current of 10 mA were used. An acetylene flow of 2.5 L/min and an air flow of 8 L/min, were used to obtain the best zinc atomic absorption signals. Aqueous standards for plotting calibration graphs were obtained by serial dilution of stock solutions containing 1000 μg/mL of the analyte as nitrates. Blanks were prepared in a similar fashion as samples. Absorbance values for blanks were negligible.

Statistical analysis

All correlations performed were determined by means of the Mann-Whitney non-parametric analysis using $\alpha \leq 0.05$ (22).

Table 1
Graphite furnace atomic absorption instrumental conditions for copper and chromium determination in human semen samples. Pyrolytic graphite coated graphite tubes and pure pyrolytic graphite L'vov platforms were used for atomization

Parameters	Elements	
	Copper	Chromium
Wave lenght (nm)	324.7	357.8
Band width (nm)	0.7	0.7
Lamp current (mA)	15	18
Argon flow (mL/min) during atomization	Interrupted	Interrupted
Injection volume (L)	10	20
Pre-dry temperature (C)	90	90
Pre-dry ramp time (s)	5	5
Pre-dry hold time (s)	10	10
Dry temperature (°C)	120	120
Dry ramp time (s)	10	10
Dry hold time (s)	10	10
Ashing temperature (°C)	1100	1700
Ashing ramp (s)	5	15
Ashing hold time (s)	20	15
Atomizing temperature (°C)	2600	2600
Atomizing ramp (s)	0	0
Atomizing hold time (s)	5	7
Clearing temperature (°C)	2650	2650
Clearing ramp (s)	1	1
Clearing hold time (s)	3	3
Cooling temperature (°C)	20	20
Cooling ramp (s)	1	1
Cooling hold time (s)	10	10

Results

Sperm quality

Comparison of sperm quality parameters of fertile and infertile men showed high significant differences in ejaculate volume, sperm concentration ($P < 0.0001$), progressive linear motility ($P < 0.0002$) and normal morphology ($P < 0.0006$). Sperm quality was fairly superior in fertile men (Table 3).

Table 2
Operating instrumental conditions for flame atomic absorption determination of zinc in human semen samples

Parameters	
Wave length (nm)	213.8
Band width (nm)	0.7
Lamp current (mA)	10
Acetylene flow (L/min)	2.5
Air flow (L/min)	8

Table 3
Means and standard deviations of sperm quality parameters of fertile and infertile men

Quality Parameter	Infertile Donors	Fertile Donors	p <
Volume (mL)	2.6 ± 1.4 (n = 32)	3.4 ± 1.8 (n = 27)	0.0485*
Sperm Concentration (10^6/mL)	70.4 ± 66.4 (n = 32)	143.4 ± 77.5 (n = 27)	0.0002*
Total Count (10^6 spermatozoa)	159.6 ± 127.0 (n = 32)	503.4 ± 386.9 (n = 27)	0.0001*
Total Motility (% spermatozoa)	54.0 ± 16.6 (n = 27)	62.0 ± 12.3 (n = 27)	0.0217*
Progressive linear motility (% spermatozoa)	28.9 ± 9.9 (n = 18)	43.2 ± 10.2 (n = 25)	0.0002*
Normal Morphology (% spermatozoa)	38.6 ± 10.8 (n = 27)	49.8 ± 11.5 (n = 27)	0.0006*
Round Cells (10^6/mL)	13.0 ± 9.4 (n = 23)	11.1 ± 8.0 (n = 27)	0.2703
Fructose (mg/mL)	3.0 ± 1.6 (n = 31)	2.2 ± 1.0 (n = 27)	0.0743

* Statistically significant (p< 0.05).

Table 4
Copper, chromium and zinc concentration in semen samples from infertile and fertile men

Concentration	Infertile Donors (n=43)	p<	Fertile Donors (n=27)
Copper (µg/L)	196.0 ± 80.9 (82.3 - 450.8)	0.2670	223.9 ± 92.8 (92.0 - 420.0)
Chromium (µg/L)	83.3 ± 78.0 (13.2 - 294.9)	0.9663	68.4 ± 48.8 (13.0 - 218.0)
Zinc (mg/L)	164.0 ± 98.2 (10.5 - 407.9)	0.8659	151.5 ± 75.5 (47.6 - 388.0)

* Statistically significant (p< 0.05).

Copper, chromium and zinc determinations

Results obtained in the determination of these heavy metals in the semen samples analyzed showed considerable variability, specially for chromium and zinc (Table 4). The comparison of copper, chromium and zinc concentration in semen from fertile and infertile men did not show any significant difference (Table 4).

Sperm quality and copper, chromium and zinc concentration

According to their respective values of sperm concentration, progressive linear motility and normal morphology, the donors

Table 5
Copper, chromium and zinc concentration values classified according to the sperm quality parameters of the semen samples analyzed

		Copper (µg/L)	Chromium (µg/L)	Zinc (mg/L)
Sperm Concentration (10^6 /mL)	< 20 n=8	205.7 ± 109.4 (82.3 - 450.8)	70.4 ± 47.6 (26.3 - 162.2)	123.9 ± 88.0 (62.8 - 321.1)
	≥20 n=51	209.7 ± 89.0 (92.0 - 429.2)	88.9 ± 80.0 (13.0 - 294.9)	163.3 ± 91.7 (10.5 - 407.9)
	p<	0.8162	0.7973	0.1212
Progressive Linear Motility (%)	<25 n=12	193.6 ± 73.6 (114.0 - 386.4)	84.3 ± 84.5 (13.2 - 257.9)	136.8 ± 82.2 (10.5 - 407.9)
	≥25 n=32	214.3 ± 91.8 (92.0 - 420.0)	79.9 ± 66.1 (13.0 - 274.0)	165.5 ± 74.9 (47.6 - 388.0)
	p<	0.6259	0.2571	0.0721
Normal Morphology (%)	<50 n=35	215.1 ± 98.0 (108.0 - 450.8)	83.4 ± 72.4 (13.2 - 274.0)	158.3 ± 94.6 (10.5 - 407.9)
	≥50 n=20	206.9 ± 84.1 (92.0 - 367.6)	80.1 ± 74.7 (13.0 - 294.9)	161.7 ± 81.8 (47.6 - 388.0)
	p<	0.9651	0.6618	0.6555

* Statistically significant (p 0.05).

were classified as already described in the sperm quality analysis section. This classification allowed for comparison of the heavy metal concentrations between samples with different sperm quality (Table 5). Copper, chromium and zinc concentrations of the samples analyzed have no correlation with sperm quality, as shown by the p values obtained. Although the statistical analysis do not show a significant correlation between zinc concentration and progressive linear motility, there is a tendency towards higher zinc concentration in samples with higher percentage of progressive linear motility.

Discussion

As already stated, comparison of the parameters which usually define sperm quality showed significant differences in the semen samples of fertile and infertile men. In general, the results show that those parameters normally used as indicators to predict fertility or infertility were altered in infertile men. However, it is important to mention that among semen samples analyzed there were some cases in which those indicators showed normal values for infertile men and abnormal or altered values in some fertile men. Therefore, it is shown that these indicators alone would not be enough evidence for a sound prediction of fertility or infertility conditions. It is well known that although evaluation of sperm quality is useful in predicting fertility, there remain some infertility cases for which the sperm quality is not consistent or enough for making predictions (3,4). As it is known infertility is a multifactorial problem, which has to take into consideration the effects of infectious dis-

eases and the presence of antispermatic antibodies caused by the infection itself (23,24), therefore implications based only on alteration of sperm quality parameters should be considered with caution.

The determination of copper, chromium and zinc concentration in semen of fertile and infertile men showed that the average concentration levels of those metals do not correlate with infertility in the samples studied. Our results regarding copper concentration in semen contradict those of Umeyama *et al.* (19), who found higher concentrations of this metal in infertile men than in fertile men; however, we agree with those authors and with Arver (25) regarding their findings related to chromium and zinc concentrations.

The wide variability of the results obtained for copper, chromium and zinc concentrations in semen samples from fertile and infertile men could possibly be related to differences in the metabolism, the capacity for accumulation of heavy metals and/or dietary habits of the donors. Other authors have also observed similar variability (6,18,19,26,27). Due to the wide range of concentration values found for copper, chromium and zinc in the semen samples analyzed, it was considered interesting to check out the possibility that the extremes, lowest and highest, values of said ranges could bear a correlation with the sperm quality of the donors. No such correlation was found, thus leading to the conclusion that, although the variability of the concentrations of copper, chromium and zinc is considerably wide, the level of these metals existing in the semen samples analyzed apparently do not exert any effects on sperm quality.

The present study has allowed, for the first time in Venezuela, to determine the copper, chromium and zinc concentrations in semen samples of fertile and infertile men, not exposed to heavily contaminated environmental conditions. It has also permitted evaluating the possible correlation between the concentrations of those three metals and the sperm quality of the donors. The concentration levels found for copper, chromium and zinc in the semen samples analyzed are similar to those found in other parts of the World (18,19,27-29). The analytical methodology used in this work will be useful in a similar study aimed at the evaluation of semen samples from men exposed to heavily contaminated working environments, such as the mining and welding workers from the southeastern part of Venezuela, where studies show that male infertility cause have increased in the last years. The results of the present work will be useful to set a baseline for comparison with other unpolluted or polluted regions of the country.

Acknowledgments

The authors thank to Decanato de Investigaciones de la Universidad Simón Bolívar for financial support (Grant # DI-CB-206-94) and to Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICIT) for grant MPS-RP-VII 260076 and S1-950000713.

References

1. AUGER J., KUNSTMANN J.M., CZYCLIK F., JOVANNET P. ***New Engl J Med*** 332:281-285, 1995.
2. CARLSEN E., GIWERGMAN A., KEIDING N., SKAKKEBACK N. ***Br Med J*** 305:609-613, 1992.
3. INSLER V., LUNENFELD B. ***Infertility: male and female.*** Churchill Livingstone (UK), pp.740, 1986.
4. MOGHISSI K.S., WALLACH E.E. ***Fertil Steril*** 39:5-21, 1983.
5. STACHEL B., DOUGHERTY R.C., LAHL U., SCHLOSSER M., ZESCHMAR B. ***Andrologia*** 21:282-291, 1989.
6. SKANDHAN K.P. ***Rev Fr Gynecol Obst*** 87:594-598, 1992.

7. DANIELSSON B.R.G., DENCKER L., LINDGREN A., TJÄLVE H. ***Arch Toxicol*** 7:177-180, 1984.
8. ERNST E., BONDE J.P. ***Hum Exp Toxicol*** 11:255-258, 1992.
9. BERTRAND G., VLADESCO R. ***CR Acad Sci*** Paris 173:176, 1921.
10. MARMAR J.L., KATZ S., PRAISS D.E., DE BENEDICTS J.J. ***Fertil Steril*** 26:1057, 1975.
11. BETTGER W., O'Dell B. ***Life Sci*** 28:1425-1438, 1981.
12. KVIST U. ***Acta Physiol Scand*** 109:79-84, 1980.
13. ANDREWS J.C., NOLAN J.P., HAMMERSTEDT R.H., BAVISTER B.D. ***Biol Reprod*** 51:1238-1247, 1994.
14. RIFFO M., LEIVA S., ASTUDILLO J. **Int J Androl** 15: 229-237, 1992.
15. CARPINO A., SISCI D., AQUILA S., SALERNO M., SICILIANO L., SESSA M., ANDO S. ***Arch Androl*** 32:37-43, 1994.
16. CARRERAS A., MENDOZA C. ***Andrologia*** 22:279-283, 1990.
17. BONDE J.P.E. ***Dan Med Bull*** 37:105-108, 1990.
18. NOACK G., DE BEER C., SEIBERT H. ***Andrologia*** 25:7-12, 1993.
19. UMEYAMA T., ISHIKAWA H., TAKESHIMA H., YOSHII S., KOISO K. ***Fertil Steril*** 46:494-499, 1986.
20. WORLD HEALTH ORGANIZATION: ***WHO laboratory manual for the examination of human semen and semen-cervical mucus interaction***. Cambridge University Press, Cambridge (England), pp. 67, 1990.
21. SLAVIN W., MANNING D.C., CARNRICK G.R. ***At Spectrosc*** 137, 1981.
22. SOKAL R.R., ROHLF F.J. ***Biometry***. W.H. Freeman Co. (USA), pp. 776, 1969.
23. MUÑOZ M. G., JEREMIAS J., WITKIN S. S. ***Hum Reprod*** 11:101-104, 1996.
24. OLIVIERI M.T., VERA O., ROSENBERG E., BRONFENMAYER S., MUÑOZ M. G. ***Rev Clin Med H.C.C.*** 1:33-42, 1996.
25. ARVER S. ***J Trace Elem Electr Health Dis*** 1:45-48, 1987.
26. BEHNE D., GEBNER H., WOLTERS G., BROTHERTON J. ***Int J Androl*** 2: 415-423, 1988.
27. JOCKENHÖVEL F., BALS M., BERTRAM H.P., NIESCHLAG E. ***Andrologia*** 22:503-511, 1990.
28. SKANDHAN K.P., MAZUMDAR B.N. ***Experientia*** 35:877-878, 1979.
29. STANWELL R., THOMPSON S.G., HAINES A.P., WARD R.J., CASHMORE G., STEDRONSKA J., HENDRY W.F. ***Fertil Steril*** 40:670-677, 1983.