THE CHROMOSOMAL CONSTITUTION OF SPERMATOZOA FROM EIGHT NORMAL, HEALTHY BRAZILIAN MEN

A CONSTITUIÇÃO CROMOSSÔMICA DE ESPERMATOZÓIDES DE OITO HOMENS BRASILEIROS NORMAIS

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ABSTRACT: We describe the chromosomal analyses of sperm from eight normal brazilian men using the human sperm-hamster egg fusion technique. The frequency of total chromosomal aberrations was 5.4% (range, 1.0-13.5%), of structural aberrations 1.4% (range, 0.0-3.3%), of hyperhaploidy 2.0% (range, 0.0-5.1%) and of hipohaploidy 5.6% (range,0.0-11.3%). The proportion of X-bearing (54.1%) and Y-bearing (45.9%) spermatozoa differed significantly at 5% level. The results obtained in this work are similar to those reported in the literature.

UNITERMS: Chromosome Aberrations. Human. Cytogenetics. Spermatozoa. Chromosomes, Human.

1. INTRODUCTION

Since the first publication by Rudak et al. (1978)[(1)] of a method for the direct investigation of human sperm chromosomes after a sperm has penetrated a golden hamster egg some groups have used this approach to study human spermatozoa[(2/9)].

This technique has been used to evaluate the sperm complement of healthy normal men, to analyze meiotic segregation patterns in males heterozygous for constitutional chromosomal aberrations, to screen cancer patients submitted to chemotherapy and in men with idiopathic infertility (see Guttenbach et al., 1997[(10)] for a review).

In the present paper, we present the results obtained from eight normal, healthy brazilian donors using this technique and also describe the cytogenetic characteristics of the sperm chromosomal complement for these men.

2. SUBJECTS AND METHODS

Semen samples were obtained from eight normal, healthy donors, 21-45 years old, with no history of exposure to known mutagens or clastogens, and no current treatment for any illness. Six of the men were of proven fertility and two were of unproved fertility. Only one of the subjects was a cigarette smoker.

The technique for obtaining human sperm complements after penetration in zona-free hamster oocytes has been described by Martin et al.(1983)[(11)] and adapted in our laboratory using a similar protocol to that of Templado et al. (1988)[(7)].

3. RESULTS

Six hundred and forty six sperm chromosomal complements were analyzed, with a mean number of 80 metaphases per donor (range, 25-142). The proportion of X-(54,1%) and Y-bearing (45,9%) sperm is

significantly different at 5% level from the expected 1:1 ratio (Table I).

The mean frequency of structural aberrations was 1.4% (0.0% - 3.4%) and the mean frequency of aneuploidy was 7.6% (3.1% - 14.1%) with a hypohaploidy rate of 5.6% (0.0% - 11.3%) and a hyperhaploidy rate of 2.0% (0.0% - 5.1%) (Table I). The loss of chromosomes can be due to methodological reasons, and considering each hyperhaploid cell produced a complementary hypohaploid cell, the international consensus is to consider the aneuploidy rate as being twice the frequency of hyperhaploidy. Using this criterion the total frequency of chromosomal aberrations (structural aberrations plus 2X hyperhaploidy) was 5.4% (1% - 13.5%) (Table II).Of the nine structural aberrations detected four were chromosomal breaks, three were chromatid breaks and two were acentric fragments (Table II). If one does not consider the chromatid breaks, which result from rearrangements during DNA replication in the hamster egg, then the mean frequency of structural aberrations was 0.9% and the total frequency of chromosomal aberrations was 4.9%.

In the hyperhaploid complements there was an involvement of acrocentric (D and G) and C chromosomal groups only, and a more general chromosomal involvement (chromosomal groups B, C, D, E, F, and G) in the hypohaploid complements; the structural aberrations included breaks in some chromosomes (chromosome 2, B and C groups) (Table III).

4. DISCUSSION

The excess of X-bearing spermatozoa observed in this work, which is significant at 5% level ($X^2 = 4.34$), was observed by other researchers (Balkan & Martin, 1983[12]; Martin, 1983[11]; Burns et al,1986[13]; Jenderny, 1992[14]). In pertinent literature it was also observed an excess of Y-bearing spermatozoa too, which can be attributed to a small number of complements analyzed (Guttenbach et al,1997)[10].

The mean frequencies of hypohaploidy and hyperhaploidy detected in our sample (5.6% and 2.0%, respectively) were compatible with the range reported by others (0.5% - 9.1% for hypohaploid complements and 0.5% - 5.4% for hyperhaploid complements). The predominance of hypohaploid sperm complements relative to hyperhaploid complements agrees with other studies. However, in contrast to the preferential loss of smaller chromosomes reported in the literature, we observed an equal loss in hypohaploid complements involving 17 of the largest chromosomes (B and C groups) and 17 of the smallest ones (D, E, F and G groups). This equilibrium was also observed for the hyperhaploid complements studied: 6 extra chromosomes from C group and 7 extra chromosomes from D and G groups. No hyperhaploid complement was observed to involve chromosomes with a large heterochromatic region.

Table I - Results of sperm chromosomal analysis

					Aberrations							
Donor	Age	Sperm analysed	Sex Chromosome		Aneuploid		Hyperhaploid		Hypohaploid		Structural	
		n°	X (%)	Y (%)	n°	(%)	n°	(%)	n°	(%)	n°	(%)
1	21	59	(54.2)	(45.8)	8	13.6	3	5.1	5	8.5	2	3.4
2	25	88	(48.9)	(51.1)	9	10.2	2	2.3	7	7.9	2	2.3
3	25	25	(56.0)	(44.0)	1	4.0	1	4.0	0	0.0	0	0.0
4	27	95	(54.7)	(45.3)	3	3.1	0	0.0	3	3.1	1	1.0
5	27	64	(53.1)	(46.9)	4	6.2	1	1.5	3	4.7	0	0.0
6	35	142	(59.1)	(40.9)	8	5.6	3	2.1	5	3.5	1	0.7
7	42	102	(56.9)	(43.1)	6	5.9	1	1.0	5	4.9	2	2.0
8	45	71	(49.3)	(50.7)	10	14.1	2	2.8	8	11.3	1	1.4
	TOTAL	646	(54.1)	(45.9)	49	7.6	13	2.0	36	5.6	9	1.4

Table II – Global frequency of chromosomal aberrations in sperm chromosomes

Donor	Age	Sperm analysed	Aneuploidy (2X Hyperhaploidy)		Structural		Total chromosomal aberrations	
			n°	(%)	n°	(%)	n°	(%)
1	21	59	6	10.2	2	3.3	8	13.5
2	25	88	4	4.5	2	2.3	6	6.8
3	25	25	2	8.0	0	0.0	2	8.0
4	27	95	0	0.0	1	1.0	1	1.0
5	27	64	2	3.1	0	0.0	2	3.1
6	35	142	6	4.2	1	0.7	7	4.9
7	42	102	2	2.0	2	2.0	4	4.0
8	45	71	4	5.6	1	1.4	5	7.0
	TOTAL	646	26	4.0	9	1.4	35	54.0

Table III – Frequency and type of chromosomal aberrations according to chromosomal groups

Type of chromosomal aberration	Sperm karyotype	Total number of chromosomal aberrations
Hyperhaploid	24,X, + C	3
	24,X, + D	2
	24,X, + G	3
	24,Y, + C	3
	24,Y, + D	1
	24,Y, + G	1
Hypohaploid	22,X, - B	2
	22,X, - C	9
	22,X, - D	2
	22,X, - E	1
	22,X, - F	1
	22,X, - G	2
	22,Y, - B	1
	22,Y, - C	5
	22,Y, - D	2
	22,Y, - F	2
	22,Y, - G	7
	22,?, -C or –G or –Y	2
Structural	23,X, crb B (q)	1
	23,X, crb C(q)	2
	23,X, ctb C (q)	2
	23,Y, crb 2 (p)	1
	23,Y, ctb B(q)	1
	23,Y, + ace	2
	TOTAL	58

The mean frequency of structural aberrations (1.4%) was also within the range of other studies (0.0% - 13%), but differed from the results of studies that analyzed higher numbers of sperm complements (4-9%). Our lower value was more similar to that reported by Martin et al. (1982)[2] (1.7% for structural chromosomal aberrations). The types of structural aberrations detected were similar to other studies, with a predominance of chromosomal breaks, followed by chromatid breaks and acentric fragments. No chromosomal rearrangments were observed. The structural aberrations were preferentially concentrated in the largest chromosomes (chromosome 2 and B and C chromosomal groups), as also reported by others (Brandriff et al., 1984[3]; Martin et al., 1982[2]; Benet et al., 1982)[15].

Donor age had no influence on the structural and numerical aberrations observed.

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RESUMO: Apresenta-se a análise citogenética de cromossomos de espermatozóides de oito homens brasileiros normais, utilizando-se a técnica de fertilização heteróloga homem-hamster. Os resultados obtidos são semelhantes aos descritos em outros laboratórios que dominam esta técnica. Obteve-se freqüência de 5.4% (variação de 1.0-13.5%) de aberrações cromossômicas, sendo 1.4% de aberrações estruturais (variação de 0.0-3.3%), freqüência de hiper-haploidia de 2.0% (variação entre 0.0-5.1%) e freqüência de hipo-haploidia de 5.6% (variação de 0.0-11.3%). A diferença entre as proporções de espermatozóides X (54.1%) e Y (45.9%) foi significativa, ao nível de 5%.

UNITERMOS: Aberrações Cromossômicas. Humano. Citogenética. Espermatozóide. Cromossomos Humanos.

REFERENCES

1 - RUDAK E; JACOBS PA & YANAGIMACHI R. Direct analysis of the chromosome constitution of human spermatozoa. **Nature 274**: 911-913, 1978.

2 - MARTIN RH; LIN CC; BALKAN W & BURNS K. Direct chromosome analysis of human spermatozoa: preliminary results from 18 normal men. **Am J Hum Genet 34**: 459-468, 1982.

3 - BRANDRIFF B; GORDON L; ASHWORTH L; WATCHMAKER G & CARRANO AV. Chromosomal abnormalities in human sperm: comparisons among four healthy men. **Hum Genet 66**: 193-201, 1984.

4 - KAMIGUCHI Y & MIKAMO K. An improved, efficient method for analyzing human sperm chromosomes using zona-free hamster ova. **Am J Hum Genet 38**: 724-740, 1986.

5 - JENDERNY J & RÖHRBORN G. Chromosome analysis of human sperm. I. First results with a modified method. **Hum Genet 76**: 385-388, 1987.

6 - PELLESTOR F; SELE B & JALBERT H. Chromosome analysis of spermatozoa from a male heterozygous for a 13;14 Robertsonian translocation. **Hum Genet 76**: 116-120, 1987.

7 - TEMPLADO C; NAVARRO J; BENET J; GENESCÀ A; PÉREZ MM & EGOZCUE J. Human sperm chromosomes studies in a reciprocal translocation t(2,5). **Hum Genet 79:** 24-28, 1988.

8 - ESTOP AM; CIEPLY K; VANKIRK V; MUNNÉ S & GARVER K. Cytogenetic studies in human sperm. **Hum Genet 87**: 447-451, 1991.

9 - ROSENBUSCH B & STERZIK K. Sperm chromosome and habitual abortion. **Fert Steril 56**: 370-372, 1991.

10 - GUTTENBACH M; ENGEL W & SCHMID M. Analysis of structural and numerical chromosome abnormalities in sperm of normal men and carriers of constitutional chromosome aberrations. A review. **Hum Genet 100**: 1-21, 1997.

11 - MARTIN RH; BALKAN W; BURNS K; RADEMAKER AW; LINN CC & RUDD NL. The chromosome constitution of 1000 human spermatozoa. **Hum Genet 63**: 305-309, 1983.

12 - BALKAN W & MARTIN RH. Segregation of chromosomes into the spermatozoa of a man heterozygous for a 14;21 Robertsonian translocation. **Am J Med Genet 16:** 169-172, 1983.

13 - BURNS JP; KODURU PRK; ALONSO ML & CHAGANTI RSK. Analysis of meiotic segregation in a man heterozygous for two reciprocal translocations using the hamster in vitro penetration system. **Am J Hum Genet 38:** 954-964, 1986.

14 - JENDERNY J; JACOBI ML; RÜGER A & RÖHRBORN G. Chromosome aberrations in 450 sperm complements from eight controls and lack of increase after chemotherapy in two patients. **Hum Genet 10:** 150-154, 1992.

15 - BENET J; GENESCÁ A; NARARRO J; EGOZCUE J & TEMPLADO C. Cytogenetic studies in motile sperm from nornal men. **Hum Genet 89:** 176-180, 1992.

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