ANEUPLOIDY DETECTION IN SPERM NUCLEI USING FLUORESCENCE *IN SITU* HYBRIDIZATION

DETECCIÓN DE ANEUPLOIDÍA, EN NÚCLEO ESPERMÁTICO EMPLEANDO HIBRIDACIÓN *IN SITU* FLUORESCENTE

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Palabras clave adicionales

Aneuploidía. Núcleo espermático. FISH.

SUMMARY

Tobacco smoking has been shown to have detrimental effects on sperm density, motility, and structure but little was know about genetic defects in sperm. Semen samples were obtained from fifteen heavy smokers (more than 20 cigarettes per day) and 15 nonsmokers who lived in the heavily industrialized Teplice district in the Czech Republic. Smokers had significantly elevated cotinine levels in their urine and consumed more alcohol and caffeine than did nonsmokers.

Three-chromosome simultaneous fluorescence *in situ* hyhridization (FISH) was used to characterize the aneuploidy and diploidy levels in sperm of smokers and nonsmokers. The smokers had higher frequencies than nonsmokers for all classes of aneuploidy. Significant differences were found for YY8 ($p=0.003$), 88.(XorY) ($p=0.04$) and total hyperhaploid frequencies ($p=0.02$). The two groups of men did not differ in their frequencies of XY8, XX8 or diploid sperm. When compared to a group of nonsmokers from California, the nonsmokers from the Teplice district had higher frequencies of XX8, XY8, and XY88 aneuploidy.

Other measurements of semen quality included standard semen analysis, computeraided sperm analysis (CASA), and a sperm chromatin structure assay (SCSA). The smokers and the nonsmokers did not differ significantly in any of these parameters except for percent of sperm with a round head which was elevated in smokers (4.0 ± 0.6 vs. 2.4 ± 0.2 $p=0.01$).

Our results indicate that tobacco consumption is associated with an increase in the production of

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certain classes of aneuploidy sperm in men who show few other semen changes. Further studies are needed to determine whether tobacco products or associated lifestyle factor(s) are responsible for these effects.

RESUMEN

Se ha demostrado que fumar tabaco va en detrimento de la densidad espermática y de la motilidad y la estructura de los espermatozoides, pero se sabe poco acerca de defectos genéticos en los espermatozoides. Se obtuvieron muestras de semen de quince grandes fumadores (más de 20 cigarrillos por día) y quince no fumadores habitantes del altamente industrializado distrito de Teplice, en la República Checa. Los fumadores presentaban niveles significativamente elevados de cotinina en orina y consumían más alcohol y cafeína que los no fumadores.

Se utilizó la hibridación *in situ* fluorescente (FISH) simultánea para tres cromosomas con objeto de caracterizar los niveles de aneuploidía y diploidía en los espermatozoides de fumadores y no fumadores. Los fumadores presentaron mayores frecuencias que los no fumadores para todas las clases de aneuploidía. Se encontraron diferencias significativas para YY8 (p= 0,003), 88.(XóY) (p= 0,04) y frecuencia total de hiperhaploides (p= 0,02). Los dos grupos de hombres no diferían en sus frecuencias de XY8, XX8 o espermatozoides diploides. En comparación con un grupo de no fumadores de California, los no fumadores del distrito de Teplice presentaban mayor frecuencia de aneuploidías del tipo XX8, XY8 y XY88.

Otras medidas de la calidad del semen incluyeron análisis estándar del semen, análisis de espermatozoides asistido por computador (CASA) y pruebas de la estructura de la cromatina de los espermatozoides (SCSA). Los fumadores y los no fumadores no diferían significativamente en ninguno de estos parámetros, excepto en el porcentaje de espermatozoides con cabeza redondeada, que era elevado en los fumadores (4,0±0,6 frente a 2,4±0,2, p= 0,01).

Nuestros resultados indican que el consumo de tabaco se asocia a un incremento en la aparición de ciertos tipos de espermatozoides aneuploides en los hombres, que muestran pocos cambios más en su semen. Son necesarios más estudios para determinar si son los productos derivados del tabaco los responsables de estos cambios, o bien si los causantes son otros factores asociados al estilo de vida.

SPERM ANEUPLOIDY IN SMOKERS AND NONSMOKERS LIVING IN THE TEPLICE DISTRICT OF THE CZECH REPUBLIC

INTRODUCTION

Tobacco smoking has been shown to have detrimental effects on sperm density, motility, and structure but little was know about genetic defects in sperm.

OBJECTIVE

Three-chromosome simultaneous fluorescence *in situ* hybridization (FISH) was used to characterize the aneuploidy and diploidy levels in sperm of smokers and non-smokers.

Other measurements of semen quality included standard semen analysis, computer-aided sperm analysis (CASA), and a sperm chromatin structure assay (SCSA).

MATERIAL AND METHODS

Semen samples were obtained from fifteen heavy smokers (more than 20 cigarettes per day) and 15 nonsmokers who lived in the heavily industrialized Teplice district in the Czech Republic.

Smokers had significantly elevated cotinine levels in their urine and consumed more alcohol and caffeine than did nonsmokers.

Multi-chromosome fluorescence *in*

Table I. *Comparison of sperm aneuploidy levels between Teplice and California nonsmokers.* (Comparación de los niveles de aneuploidía espermática entre no fumadores de Teplice y California).

	TEPLICE n=15 Frequency per 10K[1]	CALIFORNIA n=14 Frequency per 10K[1]	Statistic Analysis ANOVA p-Value	Mann Whitney p-Value
Hyperhaploidy				
XX8	5.7±0.6	3.1±0.5	0.012	0.022
YY8	2.3±0.3	3.1±0.7	0.236	0.255
XY8	14.4±1.4	9.5±0.7	0.01	0.021
88(XorY)	4.9±0.6	6.6±0.7	0.046	0.029
Total	27.2±1.8	22.3±1.0	-	-
Diploidy				
XX88	2.4±0.6	2.2±0.4	0.87	0.585
YY88	2.6±0.6	1.7±0.4	0.248	0.692
XY88	22.1±5.3	10.6±1.4	0.145	0.727
Total	27.1±5.9	14.5±1.4	-	-

[1]±SE

situ hybridization was applied to samples using DNA probes specific for chromosomes X, Y and 8.

RESULTS

Sperm aneuploidy

The smokers had higher frequencies than nonsmokers for all classes of aneuploidy **(table I** and **II)**. Significant differences were found for W8 (p=0.003), 88(XorY) (p=0.04) and total hyperhaploid frequencies (p=0.02). The two groups of men did not differ in their frequencies of XY8, XX8 or diploid sperm.

When compared to a group of nonsmokers from California, the nonsmokers from the Teplice district had higher frequencies of XX8, XY8, and XY88 aneuploidy.

Other semen parameters

Smokers and the nonsmokers did not differ significantly in any of these parameters except for percent of sperm with a round head which was elevated in smokers.

CONCLUSION

Our results indicate that tobacco consumption is associated with an increase in the production of certain classes of aneuploidy sperm in men who show few other semen changes.

Further studies are needed to determine whether tobacco products or associated lifestyle factor(s) are responsible for these effects.

Table II. *Sperm aneuploidy and diploidy in smokers and non-smokers.* (Aneuploidía y diploidía espermática en fumadores y no fumadores).

	NONSMOKERS n=15 Frequency per 10K[1]	SMOKERS n=15 Frequency per 10K[1]	Statistical Analysis ANOVA p-Value	Mann Whitney p-Value
Hyperhaploidy				
XX8	5.7±0.6	6.6±0.7	0.46	0.37
YY8	2.3±0.3	4.0±0.5	0.002	0.003
XY8	14.4±1.4	19.0±3.2	0.14	0.39
88(XorY)	4.9±0.6	7.6±1.1	0.02	0.04
Total	27.2±1.8	37.2±3.8	-	-
Diploidy				
XX88	2.4±0.6	4.8±1.4	0.08	0.06
YY88	2.6±0.6	3.0±1.0	0.66	0.65
XY88	22.1±5.3	23.3±5.4	0.54	0.22
Total	27.1±5.9	31.1±7.4	-	-

[1]±SE

CONFIRMATION OF DIPLOID SPERM IN HUMAN SEMEN USING MULTI-PROBE FLUORESCENCE *IN SITU* HYBRIDIZATION AND PHASE CONTRAST MICROSCOPY

INTRODUCTION

Three chromosome FISH has been used to detect aneuploidy in human sperm. Diploid cells have been detected using DNA probes specific for chromosomes X and Y and more recently for the sex chromosomes in combination with autosome specific probes.

Many authors believe that the XY88 phenotype represents either a somatic cell or a diploid sperm.

Diploid sperm have been implicated in the origin of some hydatiform moles and their frequencies in semen may be associated with reduced fertility.

OBJECTIVE

Quantitatively characterize the occurrence of diploid sperm and somatic cells in human semen of healthy men.

Determine the exact difference in size between diploid and haploid sperm.

MATERIAL AND METHODS

Semen samples were obtained from 13 healthy men ranging in age from 18 to 40 who were participating in a larger study to determine the impact of lifestyle on sperm aneuploidy levels.

Multi-chromosome fluorescence in situ hybridization was applied to samples using DNA probes specific for chromosomes X, Y and 8.

Phase contrast microscopy was used to determine the presence of tails.

Cell size was measured using an eyepiece reticule under phase microscopy.

RESULTS

The occurrence of tails in diploid cells was only 10 p. cent. lower than in haploid cells. The average area of diploid sperm was 2.2 times larger then normal haploid sperm. There was no significant difference between the areas of diploid (XY88) and auto-diplod (XX88 and YY88) sperm.

CONCLUSION

Our work provides direct evidence that the majority of diploid cells in semen found by FISH are diploid sperm rather than somatic cells.

Seven of our donors had between 0.5 and 1 p. cent. diploid sperm and the other six had fewer. Assuming an average sperm concentration of 50×10^{-6}/ml these donors had up to 5×10^{-6} diploid sperm per ml of semen.

Further studies are needed to determine whether diploid sperm have significant impact on reproductive outcome and to determine how certain chemical exposures or physiological conditions may affect the levels of diploid sperm or somatic cells in semen.

STARTING OF ANEUPLOIDY DETECTION IN DOMESTIC ANIMAL SPERM NUCLEI USING FLUORESCENCE *IN SITU* HYBRIDIZATION

We are trying to apply the experience obtained at the human sperm aneuploidy study to domestic animals.

At present we are at the stage of searching and preparation of suitable probes.