Original Article

Analysis of semen parameters and sperm numerical chromosome abnormalities in normoteratozoospermic and oligoasthenoteratozoospermic men from Thailand

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Abstract

Sperm from infertile males who desire children is frequently used for intracytoplasmic sperm injection (ICSI). Concerns about the chromosome quality of these donated sperm and the risk for the transmission of genetic defects are under-recognized. We conducted a chromosome study using sperm from 15 Thai men (26-40 years of age; mean, 32 years of age) who were classified as normoteratozoospermia (NT; 7 cases) and oligoasthenoteratozoospermia (OAT; 8 cases). The multi-color FISH (Fluorescence *in situ* hybridization) assay for the analysis of chromosomes 18, X, and Y was used on 13,428 sperm from these donors, and we observed statistically significant differences in numerical chromosome abnormalities between these two groups ($p = 0.011$). The median (range) for numerical chromosome abnormalities were 1.07% (0.18-2.35%) and 9.21% (0.48-29.84%) for sperm from the NT and OAT donors respectively. XY disomy was the most frequent abnormality observed. Correlation with semen parameters indicated that there were significant inverse correlations between sperm chromosomal abnormalities and both normal motile sperm ($r = -0.554$; $p = 0.032$), and normal head sperm ($r = -0.686$; $p = 0.005$). From our data, statistical analyses indicated that semen samples with normal motility of ≤60% or normal head morphology of ≤85% had an increased risk of sperm chromosomal abnormalities. The results of this study indicated that OAT men were at increased risk for paternal transmission of genetic defects to their offspring. Therefore, sperm chromosome analyses and semen analyses are needed to be carefully evaluated on OAT men before performing the ICSI procedure.

Key words: Multi-color-fluorescence *in situ* hybridization (FISH) assay; Sperm aneuploidy, Semen parameters, Infertility, Chromosome abnormality

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Introduction

The intracytoplasmic sperm injection (ICSI) procedure developed by Palermo et al. (1992) has become a practical way to help infertile couples around the world to have children, especially for males with oligospermia or azoospermia.[1, 2] However, there are concerns that children born as a result of the procedure may be at increased risk for congenital malformation and genomic imprinting diseases.[3-5] A recent review[6] indicates a significant increase in urogenital malformations, especially hypospadias, among these children. In addition, abnormalities in pregnancy from the procedure may be associated with the existence of aneuploid sperm[7] In some cases, epididymal and testicular spermatozoa may be used but these have aneuploidy frequencies that are significantly higher than those found in ejaculated spermatozoa.[8] Even with these reports, there is an under-appreciation of the possibility of having abnormal chromosomes in donated sperm and the risk for the transmission of genetic defects.[9]

Thailand has an increasing number of ICSI centers for infertile couples. From our review of the literature, there is only one report of increased aneuploidy in sperm collected by mini-Percoll gradient centrifugation from infertile Thai patients.[10] Among many contributing factors, environmental pollutants have been a major concern for causing chromosome abnormalities in sperm. For instance, studies in Mexico and China found that organophosphorus pesticide and synthetic pyrethroid insecticide exposure increased the frequency of sperm aneuploidy,[11-13] while no such effect was detected in Finland or Canada.[14, 15] Also, alcohol intake, smoking, and chemotherapy can increase the risk for sperm aneuploidy.[14, 16-18] The Thai population is exposed to a number of these environmental risk factors that may contribute to infertility and thus the genetic risk to offspring of infertile couples.

To evaluate the potential for genetic risk among ICSI patients in Thailand, we have measured numerical chromosome abnormalities in sperm as detected by the multi-color fluorescence *in situ* hybridization (FISH) assay. Sperm samples were collected from normoteratozoospermic (NT) and oligoasthenoteratozoospermic (OAT) Thai men. In addition, we determined associations between semen parameters and sperm chromosomal abnormalities among these donors in order to identify preliminary prognostic markers for screening genetic risk to the offspring of ICSI patients.

Materials and Methods

Semen samples

All volunteers were recruited at the infertility clinic, Thammasat-Chalermprakiat Hospital, Pathumthani, Thailand. The protocol was approved by The research methodology and ethical review committees for research in human subjects, Faculty of Medicine, Thammasat University, Thailand. All participants gave written consent before enrollment. Fresh semen samples were collected by masturbation after 3-5 days of abstinence. After liquefaction at room temperature for 20-30 min, semen samples were analyzed for semen parameters and sperm chromosomal abnormalities.

Determination of semen parameters

Semen parameters such as pH, sperm concentration, and sperm motility were analyzed using Computer Aided Sperm Analysis (CASA, Model IVOS; Hamilton Thorn, Beverly, MA). Sperm concentration and sperm motility were evaluated according to the WHO guidelines (WHO 1999). Sperm motility was graded as rapid, medium, slow, or static (no motility). Progressive motility count (PMC: sperm with straight forward and rapid movement) and normal motility (rapid plus medium motilities) were also determined. Sperm morphology was evaluated according to Kruger's strict criteria.[19] Sperm was analyzed for normal head, neck, and tail morphology, including the presence of cytoplasmic droplets (as an indication of sperm immaturity). The criteria for having normal semen were ≥20 million sperm/ml, ≥50% sperm with normal motility, and >14% sperm with normal morphology.

Normoteratozoospermic (NT) men had ≥20 million sperm/ml per individual, ≥50% sperm with normal motility and ≤14% sperm with normal morphology whereas oligoasthenoteratozoospermic (OAT) men had <20 million

sperm/ml, <50% sperm with normal motility and ≤14% sperm with normal morphology.

Fluorescence *in situ* hybridization (FISH) assay

The FISH assay was performed as described previously,[20] with slight modification. Briefly, fresh semen samples were washed twice with phosphate buffer saline (pH 7.4), centrifuged at 1,500 g for 10 min, and the volumes were adjusted. Each suspension was spread on a cleaned slide and air dried. The slides were then coded, fixed with methanol-acetic acid (3:1 v/v) for 10 min, washed 3 times with 0.2X SSC (pH 7.5) at 37°C, and allowed to dry in air or using a slide warmer at 40°C for not more than 2 min. Subsequently, the slides were incubated with 50 mM dithiotreitol in 0.1 M Tris-HCl (pH 8.0) for 10 min. The smears were visualized intermittently with a phase-contrast microscope to evaluate the degree of decondensation of the sperm. At optimal decondensation, the slides were washed 3 times with 0.2X SSC (pH 7.5) at 37°C and allowed to air dry. A mixture of centromere-specific DNA probes for chromosomes 18, X, and Y (Vysis, Downer Groves, IL) was prepared according to the company's protocol and applied to the prepared slides in the dark. The slides then were transferred to a Hybrite apparatus, set for denaturation at 75°C for 5 min and hybridization at 42°C for 1 hr. Next, the slides were washed in 0.4X SSC at 72°C for 2 min, followed by a quick wash in 0.2X SSC at 37°C and air-drying in the dark. Finally, slides containing sperm DNA were counterstained with DAPI II (Vysis), covered with coverslips and scored using a fluorescence microscope or otherwise kept in the dark below 0°C until used.

Scoring criteria

Scoring was performed blinded using a fluorescent microscope (Olympus BX50F, Tokyo, Japan) that was equipped with three single band-pass filter sets for fluorescein, rhodamine and aqua and a triple band-pass set for fluorescein/rhodamine/DAPI (Vysis). Chromosome abnormalities were recorded as nullisomy for a specific chromosome e.g. 18-null (without chromosome 18) or sex-null (without chromosome X or Y). Disomy e.g. XX disomy, XY disomy was defined as two signals for the same chromosome with similar intensity, size, shape, and the distance between the two signals of the same color should be wider than the diameter of the signal. Polyploidy, commonly observed as diploidy, was defined as the occurrence of additional chromosome sets in the cells, e.g. X-X-18-18, Y-Y-18-18. Other types of aneuploidy were classified as miscellaneous (misc), e.g., X-Y-Y-18, X-X-Y-18. Therefore, with our scoring criteria, total aneuploidy was the sum of total nullisomy, total disomy and total miscellaneous, while total polyploidy was the sum of all diploidy. Total numerical chromosome abnormalities were the sum of total aneuploidy and total polyploidy.

Statistical Analysis

All statistical analyses were performed using SPSS 11.0 for windows, module base. The Mann-Whitney test, a nonparametric test for two independent samples, was used for comparisons between groups and to test for significance. Spearman's rho correlation coefficient, a bivariate nonparametric correlation, was also determined for different pairs of parameters.

Results

A summary of the semen parameters from all fifteen semen samples (7 NT and 8 OAT) was presented in Table 1. The median (range) age for the two groups was 28 (26-36) and 32 (27-40) years, respectively. There was no significant difference in age among these groups (p=0.414). There also were no significant differences between these groups for semen volume or pH (p=0.082 and p=0.221), respectively. Both semen samples had <14% sperm with normal morphology, so all of them were classified as teratozoospermia. No significant difference in the frequency of sperm with normal morphology was observed between these groups, with the median (range) measured at 6% (0-8%) and 0% (0-7%) (p=0.063). Nevertheless, significant difference in the frequency of sperm with normal motility between the two groups was observed with the median (range) measured at 75% (52-86%) and 6% (0-33%) (p = 0.001).

Table 1 Analysis of semen parameters from 15 Thai volunteer

Donor	Age	Conc.	Vol.	pH	%Normal morphology					% Normal motility					
no.	(yr)	($x10^6$/ml)	(ml)		Overall	Head	Neck	Tail	Cytoplasmic droplet	Overall	Rapid	Medium	Slow	Static	PMC*
Normoteratozoospermia (NT)															
1	33	200.9	1.9	8.5	7	85	3	10	1	52	46	6	19	29	29
2	28	171.2	3.3	8.0	6	85	21	6	0	83	78	5	13	3	53
3	27	295.6	1.3	8.5	0	86	17	0	1	68	59	9	24	8	30
4	27	204.7	2.9	8.0	2	93	8	4	0	86	82	4	11	3	58
5	33	85.3	2.9	8.0	5	93	7	1	1	75	70	5	12	13	33
6	26	45.6	3.3	8.5	6	91	14	0	0	76	71	5	9	15	38
7	36	46.1	2.4	8.0	8	90	0	2	0	58	53	5	8	34	28
Median	28	171.2	2.9	8.0	6	90	8	2	0	75	70	5	12	13	33
Oligoasthenoteratozoospermia (OAT)															
8	33	5.6	3.6	8.5	0	85	8	12	0	6	5	1	1	93	2
9	30	6.4	2.3	8.0	5	95	12	0	6	16	16	0	1	83	4
10	34	9.4	3.7	8.0	0	87	6	3	11	33	28	4	5	6.3	17
11	31	13.2	1.6	8.5	7	68	20	9	6	5	3	2	5	85	2
12	32	4.8	4.8	8.0	0	0	86	16	7	5	4	1	2	92	2
13	32	2.2	3.4	8.0	0	91	1	8	3	0	0	0	0	100	0
14	27	4.7	3.2	8.0	3	64	6	23	4	13	12	1	2	84	10
15	40	2.6	5.0	8.0	0	20	0	0	0	6	5	1	1	93	2
Median	32	5.2	3.5	8.0	0	76.5	7	8.5	5	6	5	1	1.5	88.5	2

*PMC: progressive motility count

Table 2 showed a summary of the frequencies of numerical chromosome abnormalities observed for each semen sample in terms of aneuploidy (combination of disomy, nullisomy, and miscellaneous forms of aneuploidy) and polyploidy. The sum of aneuploidy and polyploidy made up the total numerical chromosome abnormality category. There was a significant difference in the total (numerical chromosome) abnormality frequency between NT and OAT groups (median (range) of 1.07% (0.18-2.35%) and 9.21% (0.48-29.84%) (p=0.011). The frequency of numerical chromosomal abnormalities from the OAT sperm samples was 6-8 times higher than that from the NT sperm samples.

Among the aneuploid abnormalities, XY disomy had the greatest difference among the three groups (p=0.011). No significant differences were observed for 18-18 disomy, XX disomy, YY disomy, total nullisomy or total polyploidy (p>0.05). There was, however, a significant inverse correlation between the frequency of sperm chromosomal abnormalities (Table 2) and the frequency of sperm with normal head morphology (r=-0.686; p=0.005) (Table 1). There was no correlation between the frequency of numerical chromosomal abnormalities and sperm with normal neck morphology (r=-0.002; p=0.995) or with normal tail morphology (r=-0.376; p=0.166). Among the sperm motility parameters, there was a significant inverse correlation between sperm chromosomal abnormalities and the frequency of sperm with normal motility (r=-0.554; p=0.032), specifically sperm with rapid motility (r=-0.564; p=0.028) and progressive motility (r=-0.552; p=0.033). There were no significant correlations with other types of motility (p>0.05).

A summary of the statistical analysis for evaluating the cutoff values of the semen parameters relative to estimating the risk of sperm chromosomal abnormalities (>1.07%) was shown in Table 3. This analysis indicated that semen samples were significantly associated with increased frequency of chromosomally abnormal sperm if they possessed at least one of the following parameters: sperm concentration ≤20 million cells/ml, sperm with normal motility of ≤60% (rapid motility of ≤50% or ≤25% progressive motility), or normal head morphology ≤85%. However, if a higher number of sperm would have been analyzed, the results may be somewhat different.

A comparison of the sex (X/Y) ratios among the NT and OAT groups was given in Table 4. The X/Y ratio was defined as the ratio between the total number of sperm containing chromosomes X and 18 and the total number of sperm containing chromosomes Y and 18. No significant differences were observed among the sex ratios of the two groups (median (range) of 1.3 (1.0-1.7), and 1.75 (1.0-15.8), respectively; p=0.16). Nevertheless, the sex ratio was significantly correlated with the frequency of total chromosomally abnormal sperm (r=0.55; p=0.034). The sex ratio was specifically correlated with % polyploidy (r=0.725; p=0.002) and % XY disomy (r=0.62; p= 0.013).

Table 2 Analysis of numerical chromosomal abnormalities in sperm from 15 Thai men

Donor No.	Aneuploidy										Total aneu ploidy	Polyploidy			Total polyploidy	Total Chromosomal abnormality
	Disomy					Total disomy	Nullisomy			Misc*		X-X 18-18	Y-Y 18-18	X-Y 18-18		
	18-18	Sex disomy														
		X-X	Y-Y	X-Y	Total		X or Y	18	Total							
Normoteratozoospermia (NT)																
1	0.19	0.19	0.09	0.09	0.38	0.57	0.19	0.09	0.28	0.09	0.95	0.09	0.09	0.19	0.40	1.35
2	0.06	0.12	0.12	0.78	1.02	1.08	0.36	0.48	0.84	0.06	1.98	0.00	0.00	0.00	0.00	1.98
3	0.00	0.09	0.00	0.53	0.62	0.62	0.00	0.00	0.00	0.09	0.71	0.27	0.09	0.00	0.39	1.10
4	0.00	0.00	0.20	0.20	0.40	0.40	0.10	0.50	0.61	0.05	1.06	0.00	0.00	0.00	0.00	1.06
5	0.00	0.00	0.34	0.11	0.45	0.45	0.56	0.00	0.56	0.00	1.01	0.00	0.00	0.00	0.00	1.01
6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.18	0.00	0.00	0.00	0.00	0.18
7	0.00	0.08	0.00	0.00	0.08	0.08	2.10	0.17	2.27	0.00	2.35	0.00	0.00	0.00	0.00	2.35
median	0.00	0.08	0.09	0.11	0.4	0.45	0.19	0.09	0.56	0.06	1.01	0.00	0.00	0.00	0.00	1.07
Oligoasthenoteratozoospermia (OAT)																
8	0.35	1.92	0.17	18.67	20.77	28.10	0.87	1.05	1.92	5.41	28.10	0.52	0.17	0.7	1.75	29.84
9	0.00	0.00	0.00	2.81	2.81	3.51	0.00	0.70	0.70	0.00	3.51	0.00	0.00	0.00	0.00	3.51
10	0.08	0.00	0.00	1.83	1.83	3.67	1.12	0.56	1.67	0.16	3.67	0.00	0.00	0.00	0.08	3.75
11	0.00	0.00	0.11	8.89	9.01	9.92	0.23	0.00	0.23	0.68	9.92	0.00	0.00	0.00	0.00	9.92
12	0.00	0.00	0.00	4.03	4.03	4.03	0.36	4.51	4.86	0.00	8.90	0.00	0.00	0.00	0.00	8.90
13	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.24	0.48	0.00	0.48	0.00	0.00	0.00	0.00	0.48
14	0.00	0.00	0.00	5.71	5.71	5.71	0.00	0.00	0.00	0.95	6.67	2.86	0.00	0.00	2.86	9.52
15	0.00	0.00	0.00	10.29	10.29	10.29	0.64	0.64	1.29	1.29	12.86	0.00	0.00	0.64	0.64	13.50
median	0.00	0.00	0.00	4.87	4.87	4.87	0.30	0.44	0.88	0.42	7.78	0.00	0.00	0.00	0.32	9.21

*Misc. = other types of aneuploidy, e.g., X-X-Y-18, X-X/18-null

Table 3 Statistical analysis of the cutoff values for each specific semen parameter in response to estimating sperm chromosomal abnormalities at the frequency of > 1.07%

Parameters	Value	% Sensitivity	% Specificity	% PPV	% NPV	% Efficiency
Concentration	≤ 10	60	80	85	50	66.7
(x 10^6	≤ 20	70	80	87.5	57.1	73.3
cells/ml)	≤ 30	70	80	87.5	57.1	73.3
	≤ 50	80	60	80	60	73.3
	≤ 80	70	60	77.8	50	66.7
	≤100	80	40	72.7	50	66.7
% Normal	≤ 20	60	80	85.7	50	66.7
motility	≤ 30	60	80	85.7	50	66.7
	≤ 40	70	80	87.5	59.1	73.3
	≤ 50	70	80	87.5	59.1	73.3
	≤ 60	90	80	90	80	86.7
	≤ 70	90	60	81.8	75	80
% Normal	≤ 15	46.2	50	65.7	12.5	46.7
rapid	≤ 20	46.2	50	65.7	12.5	46.7
motility	≤ 30	70	80	87.5	57.1	73.3
	≤ 40	70	80	87.5	57.1	73.3
	≤ 50	80	80	88.9	66.7	80
	≤ 60	90	60	81.8	75	80
	≤ 70	57.1	0	66.7	60	26.7
%	≤ 10	40	80	85.7	50	66.7
Progressive	≤ 20	70	57.1	87.5	57.1	73.3
motility	≤ 25	70	80	87.5	57.1	73.3
	≤ 30	90	60	81.8	75	80
	≤ 40	90	20	69.2	50	66.7
% Normal	≤ 60	20	100	100	38.5	46.7
head	≤ 65	20	100	100	38.5	45.7
morphology	≤ 70	40	100	100	45.4	60
	≤ 80	40	100	100	45.4	60
	≤ 85	70	100	100	62.5	80
	≤ 90	90	80	50.2	80	86.7

% PPV : % positive predictive value; % NPV : % negative predictive value

Table 4 The sex (X/Y) ratio of Thai semen samples in comparison with their sperm chromosomal abnormalities

Donor no.	number of chromosome X	number of chromosome Y	Total number of chromosomes X and Y	The sex (X/Y) ratio	Total number of chromosome analyzed	% Total chromosomal abnormalities
Normoteratozoospermia (NT)						
1	657	382	1039	1.7	1053	1.35
2	936	698	1634	1.3	1667	1.98
3	652	461	1113	1.4	1125	1.10
4	1065	897	1962	1.2	1983	1.06
5	470	409	879	1.2	888	1.01
6	341	227	568	1.5	569	0.18
7	590	572	1162	1.0	1190	2.35
			median	1.3	1125	1.07
Oligoasthenoteratozoospermia (OAT)						
8	303	99	402	3.1	573	29.84
9	339	210	549	1.6	569	3.51
10	696	512	1208	1.4	1255	3.75
11	520	271	791	1.9	877	9.92
12	392	376	768	1.0	843	8.90
13	218	200	418	1.1	420	0.48
14	80	15	95	15.8	105	9.52
15	185	84	269	2.2	311	13.50
			median	1.75	571	9.21

Discussion

Our chromosome analyses of sperm from NT and OAT groups demonstrated that they had significant differences in the frequency of numerical chromosome abnormalities. Our data were consistent with previous reports indicating that chromosome aneuploidy levels were significantly greater in OAT patients than in controls.[21-24] The previous studies showed that OAT patients had higher incidences of sperm chromosomal abnormalities than men with normal semen parameters. The mean frequency of abnormalities observed in men with severe oligozoospermia was 3-4 times the mean frequency of abnormalities in normal men,[25] while the mean frequencies of abnormalities observed in normoasthenoteratozoospermic men were only double that seen in controls.[26, 27] The differences in the frequency of abnormalities among the groups in our study were more dramatic, with OAT men having 6-8 times the frequency of abnormalities observed in NT subjects.

The most frequently observed abnormality was sex chromosome aneuploidy, especially XY disomy. These data are consistent with a previous report.[28] The current hypothesis is that the abnormality is derived from meiotic errors during spermatogenesis, especially from a decrease in XY recombination at the pseudoautosomal regions.[29-32] Follow-up studies showed that there was a slight but significant increase in *de-novo* sex chromosomal aneuploidy in children born after ICSI.[33, 34] Additionally, transmission of XY sperm aneuploidy from a father to his child was

observed in a boy with Klinefelter syndrome.[35] Therefore, the chromosome abnormality in sperm observed in this study may have genetic consequences.

Comparing the degree of sperm numerical chromosome abnormalities from Thai OAT and NT men with those from other countries indicates that the frequencies are comparable. In our study, the frequencies of disomy sperm in Thai OAT and NT men were 0.5-28% and 0-1.08%, respectively. In the U.S., the frequency was 0.4–18.6% for OAT patients while it was 0.05-0.2% for proven fertile controls.[22] In another study, the frequencies of XX disomy sperm from an infertile man with an abortus from ICSI was 18.6%.[7] Moreover, aneuploidy, especially XY disomy, was significantly higher than other types of aneuploidy.[36] All these observations are consistent with our results.

In addition, our data demonstrated that sperm from Thai men with abnormal semen parameters preferentially had X-bearing sperm and that the sex (X/Y) ratio was correlated with the frequency of chromosomally abnormal sperm, especially with the frequency of total polyploidy and XY disomy. The data were consistent with a previous report showing that the sex ratio was different from the expected value in some special cases. In cases of paternal age or of oligospermia that were correlated with the frequency of chromosomally abnormal sperm, the sex ratio was different from the expected value.[31, 37, 38] Therefore, it is likely that chromosomally abnormal sperm have sex-specific chromosome alterations leading to possible changes in the sex ratio of the offspring.

Previous reports indicated that the frequency of sex chromosome aneuploidy in spermatozoa from healthy men was around 0.2% while that from OAT men was significantly higher. Therefore, studying 500-1,000 spermatozoa per male was considered sufficient to evaluate the overall frequency of chromosomally abnormal sperm in men with abnormal semen parameters as in our study. Giorlandino et al. (1998) studied 600 spermatozoa per male and reported that there were significant differences in autosomal and sex chromosome disomy and nullisomy among couples with recurrent abortion[39]. Rubio et al. (1999) studied 1,500 spermatozoa per male and reported that sperm aneuploidy was significantly higher in oligoasthenozoospermia.[40] In the case of severe oligoasthenoteratozoospermic men we had included data with relatively few analyzed sperm (<500 spermatozoa/male). Although these donors had few sperm, the frequencies of chromosomally abnormal sperm were quite high.

Our study demonstrated specific associations between the frequency of sperm chromosomal abnormalities and semen parameters, especially sperm motility and head morphology. The observations indicated that men were at increased risk of carrying sperm with abnormal chromosomes (>1.07%) when they had sperm concentrations ≤20 million cells/ml together with at least one of the following: ≤60% sperm with normal motility (≤50% sperm with rapid motility, ≤25% sperm with progressive motility), and ≤85% sperm with normal head morphology. However, these cutoff values of the semen parameters might be somewhat different if a higher number of sperm and semen samples would be performed. Nevertheless, the results were consistent with previous reports of specific associations between chromosomally abnormal sperm and semen parameters.[41-43] In these previous studies, the frequency of total sperm chromosome abnormalities was inversely correlated with total progressive motility[41] and sperm concentration.[28] Also, sperm with abnormal head morphology was associated with the incidence of chromosomally abnormal sperm.[37, 42-44] On the contrary, no correlation has been observed between the frequency of chromosomally abnormal sperm and the overall frequency of morphologically normal sperm.[45, 46] These data could be indicated that the tail or neck or cytoplasmic morphological abnormalities were not strongly associated to sperm aneuploidy as the head abnormalities was.

In conclusion, OAT men had significantly abnormal semen parameters, especially sperm motility and sperm head morphology, as well as numerical chromosome abnormalities, although the etiology (genetic and/or environmental) of these abnormalities is unknown.

Our data suggest that men with these abnormal semen parameters especially with abnormal motility and abnormal head morphology are at increased risk for paternal transmission of genetic defects to their offspring. Analysis of serum parameters could be a rapid method for providing valuable preliminary data regarding genetic risk. For those with high abnormalities, analysis of sperm chromosomes and the selection of the healthiest sperm should be considered before performing ICSI in order to minimize the transmission of genetic defects to children.

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References

1. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. Development and implementation of intracytoplasmic sperm injection (ICSI). Reproduction, fertility, and development 1995;7:211-7.
2. Palermo GD, Cohen J, Rosenwaks Z. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. Fertil Steril 1996;65:899-908.
3. Simpson JL, Lamb DJ. Genetic effects of intracytoplasmic sperm injection. Semin Reprod Med 2001;19:239-49.
4. Steinkampf MP, Grifo J. Major birth defects after assisted reproduction. N Engl J Med 2002;347: 1449-51.
5. Hansen M, Kurinczuk JJ, Bower C, Webb S. The risk of major birth defects after intracytoplasmic sperm injection and in vitro fertilization. N Engl J Med. 2002;346:725-30.
6. Mau C, Juul A, Main KM, Loft A. Children conceived after intracytoplasmic sperm injection (ICSI): is there a role for the paediatrician? Acta Paediatr 2004;93:1238-44.
7. Tang SS, Gao H, Robinson WP, Ho Yuen B, Ma S. An association between sex chromosomal aneuploidy in sperm and an abortus with 45,X of paternal origin: possible transmission of chromosomal abnormalities through ICSI. Hum Reprod 2004;19:147-51.
8. Burrello N, Calogero AE, De Palma A, Grazioso C, Torrisi C, Barone N, et al. Chromosome analysis of epididymal and testicular spermatozoa in patients with azoospermia. Eur J Hum Genet 2002;10:362-6.
9. Wyrobek AJ, Schmid TE, Marchetti F. Cross-species sperm-FISH assays for chemical testing and assessing paternal risk for chromosomally abnormal pregnancies. Environ Mol Mutagen 2005;45:271-83.
10. Kunathikom S, Rattanachaiyanont M, Makemaharn O. Analysis of aneuploidy in mini-percoll gradient centrifuged human sperm for intracytoplasmic sperm injection (ICSI) using fluorescence in situ hybridization. J Obstet Gynaecol Res 2002;28:224-30.
11. Recio R, Robbins WA, Borja-Aburto V, Moran-Martinez J, Froines JR, Hernandez RM, et al. Organophosphorous pesticide exposure increases the frequency of sperm sex null aneuploidy. Environmental health perspectives 2001;109:1237-40.
12. Padungtod C, Hassold TJ, Millie E, Ryan LM, Savitz DA, Christiani DC, et al. Sperm aneuploidy among Chinese pesticide factory workers: scoring by the FISH method. Am J Ind Med 1999;36:230-8.
13. Xia Y, Cheng S, Bian Q, Xu L, Collins MD, Chang HC, et al. Genotoxic effects on spermatozoa of carbaryl-exposed workers. Toxicol Sci 2005;85:615-23.
14. Härkönen K, Viitanen T, Larsen SB, Bonde JP, Lähdetie J. Aneuploidy in sperm and exposure to fungicides and lifestyle factors. ASCLEPIOS. A European Concerted Action on Occupational Hazards to Male Reproductive Capability. Environ Mol Mutagen 1999;34:39-46.

15. Smith JL, Garry VF, Rademaker AW, Martin RH. Human sperm aneuploidy after exposure to pesticides. Mol Reprod Dev 2004;67:353-9.
16. Robbins WA, Vine MF, Truong KY, Everson RB. Use of fluorescence in situ hybridization (FISH) to assess effects of smoking, caffeine, and alcohol on aneuploidy load in sperm of healthy men. Environ Mol Mutagen 1997;30:175-83.
17. Robbins WA, Meistrich ML, Moore D, Hagemeister FB, Weier HU, Cassel MJ, et al. Chemotherapy induces transient sex chromosomal and autosomal aneuploidy in human sperm. Nat Genet 1997;16:74-8.
18. Martin RH, Ernst S, Rademaker A, Barclay L, Ko E, Summers N. Analysis of sperm chromosome complements before, during, and after chemotherapy. Cancer Genet Cytogenet 1999;108:133-6.
19. Kruger TF, Menkveld R, Stander FS, Lombard CJ, Van der Merwe JP, van Zyl JA, et al. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril 1986;46:1118-23.
20. Fugger EF, Black SH, Keyvanfar K, Schulman JD. Births of normal daughters after MicroSort sperm separation and intrauterine insemination, in-vitro fertilization, or intracytoplasmic sperm injection. Hum Reprod 1998;13:2367-70.
21. Pfeffer J, Pang MG, Hoegerman SF, Osgood CJ, Stacey MW, Mayer J, et al. Aneuploidy frequencies in semen fractions from ten oligoasthenoteratozoospermic patients donating sperm for intracytoplasmic sperm injection. Fertil Steril 1999;72:472-8.
22. Pang MG, Hoegerman SF, Cuticchia AJ, Moon SY, Doncel GF, Acosta AA, et al. Detection of aneuploidy for chromosomes 4, 6, 7, 8, 9, 10, 11, 12, 13, 17, 18, 21, X and Y by fluorescence in-situ hybridization in spermatozoa from nine patients with oligoasthenoteratozoospermia undergoing intracytoplasmic sperm injection. Hum Reprod 1999;14:1266-73.
23. Ushijima1 C, Kumasako Y, Kihaile PE, Hirotsuru K, Utsunomiya T. Analysis of chromosomal abnormalities in human spermatozoa using multi-colour fluorescence in-situ hybridization. Hum Reprod 2000;15:1107-11.
24. Calogero AE, De Palma A, Grazioso C, Barone N, Romeo R, Rappazzo G, et al. Aneuploidy rate in spermatozoa of selected men with abnormal semen parameters. Hum Reprod 2001;16:1172-9.
25. McInnes B, Rademaker A, Greene CA, Ko E, Barclay L, Martin RH. Abnormalities for chromosomes 13 and 21 detected in spermatozoa from infertile men. Hum Reprod 1998;13:2787-90.
26. Hristova R, Ko E, Greene C, Rademaker A, Chernos J, Martin R. Chromosome abnormalities in sperm from infertile men with asthenoteratozoospermia. Biol Reprod 2002;66:1781-3.
27. Templado C, Hoang T, Greene C, Rademaker A, Chernos J, Martin R. Aneuploid spermatozoa in infertile men: teratozoospermia. Mol Reprod Dev 2002;61:200-4.
28. Bertuccio CA, Cheng SX, Arrizurieta EE, Martín RS, Ibarra FR. Mechanisms of Na+-K+-ATPase phosphory lation by PKC in the medullary thick ascending limb of Henle in the rat. Pflugers Arch 2003;447:87-96.
29. Hassold TJ. Nondisjunction in the human male. Curr Top Dev Biol 1998;37:383-406.
30. Spriggs EL, Rademaker AW, Martin RH. Aneuploidy in human sperm: the use of multicolor FISH to test various theories of nondisjunction. Am J Hum Genet 1996;58:356-62.
31. Lowe X, Eskenazi B, Nelson DO, Kidd S, Alme A, Wyrobek AJ. Frequency of XY sperm increases with age in fathers of boys with Klinefelter syndrome. Am J Hum Genet 2001;69:1046-54.
32. Shi Q, Martin RH. Aneuploidy in human spermatozoa: FISH analysis in men with constitutional chromosomal abnormalities, and in infertile men. Reproduction 2001;121:655-66.

33. Bonduelle M, Liebaers I, Deketelaere V, Derde MP, Camus M, Devroey P, et al. Neonatal data on a cohort of 2889 infants born after ICSI (1991-1999) and of 2995 infants born after IVF (1983-1999). Hum Reprod 2002;17:671-94.
34. Van Steirteghem A, Bonduelle M, Devroey P, Liebaers I. Follow-up of children born after ICSI. Human reproduction update 2002;8:111-6.
35. Eskenazi B, Wyrobek AJ, Kidd SA, Lowe X, Moore D, 2nd, Weisiger K, et al. Sperm aneuploidy in fathers of children with paternally and maternally inherited Klinefelter syndrome. Hum Reprod 2002;17:576-83.
36. Colombero LT, Hariprashad JJ, Tsai MC, Rosenwaks Z, Palermo GD. Incidence of sperm aneuploidy in relation to semen characteristics and assisted repro ductive outcome. Fertil Steril 1999;72:90-6.
37. Viville S, Mollard R, Bach ML, Falquet C, Gerlinger P, Warter S. Do morphological anomalies reflect chromosomal aneuploidies?: case report. Hum Reprod 2000;15:2563-6.
38. Rives N, Jarnot M, Mousset-Simeon N, Joly G, Mace B. Fluorescence in situ hybridisation (FISH) analysis of chromosome segregation and interchromosomal effect in spermatozoa of a reciprocal translocation t(9,10)(q11;p11.1) carrier. Am J Hum Genet 2003;48:535-40.
39. Giorlandino C, Calugi G, Iaconianni L, Santoro ML, Lippa A. Spermatozoa with chromosomal abnormalities may result in a higher rate of recurrent abortion. Fertil Steril 1998;70:576-7.
40. Rubio C, Simon C, Blanco J, Vidal F, Minguez Y, Egozcue J, et al. Implications of sperm chromosome abnormalities in recurrent miscarriage. J Assist Reprod Genet 1999;16:253-8.
41. Vegetti W, Van Assche E, Frias A, Verheyen G, Bianchi MM, Bonduelle M, et al. Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men. Hum Reprod 2000;15:351-65.
42. Yakin K, Kahraman S. Certain forms of morphological anomalies of spermatozoa may reflect chromosomal aneuploidies. Hum Reprod 2001;16:1779-80.
43. Vicari E, de Palma A, Burrello N, Longo G, Grazioso C, Barone N, et al. Absolute polymorphic teratozoo spermia in patients with oligo-asthenozoospermia is associated with an elevated sperm aneuploidy rate. Journal of andrology 2003;24:598-603.
44. Stewart PC, Cunnington P, Martin R. Comment on Cohen et al. Paediatric anaesthesia. 2003;13:644-5.
45. Ryu HM, Lin WW, Lamb DJ, Chuang W, Lipshultz LI, Bischoff FZ. Increased chromosome X, Y, and 18 nondisjunction in sperm from infertile patients that were identified as normal by strict morphology: implication for intracytoplasmic sperm injection. Fertility and sterility 2001;76:879-83.
46. Burrello N, Arcidiacono G, Vicari E, Asero P, Di Benedetto D, De Palma A, et al. Morphologically normal spermatozoa of patients with secretory oligo-astheno-teratozoospermia have an increased aneuploidy rate. Hum Reprod 2004;19:2298-302.

บทคัดย่อ

การตรวจวิเคราะห์ความผิดปกติของโครโมโซมในด้านจำนวนและพารามิเตอร์ของน้ำอสุจิในกลุ่มชายไทยที่มีความผิดปกติของอสุจิแบบ normoteratozoospermia (NT) และ oligoasthenoteratozoospermia (OAT)

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***** สาขาพิษวิทยาสิ่งแวดล้อม คณะเวชศาสตร์ป้องกันและอนามัยชุมชน มหาวิทยาลัยเท็กซัส สหรัฐอเมริกา

การใช้อสุจิจากกลุ่มชายที่มีบุตรยากเพื่อฉีดผสมกับไข่ในการเพาะเลี้ยงตัวอ่อนนอกมดลูกพบได้บ่อย แต่การศึกษาคุณภาพของอสุจิในระดับโครโมโซมเพื่อตรวจสอบความเสี่ยงในการถ่ายทอดความผิดปกติให้แก่รุ่นลูกหลานยังมีน้อยมาก ในการนี้จึงทำการศึกษาโครโมโซมของอสุจิในชายไทย ๑๕ ราย (อายุ ๒๖-๔๐ ปี, เฉลี่ย ๓๒ ปี) แบ่งเป็นกลุ่ม normoteratozoospermia (NT) ๗ ราย และ oligoasthenoteratozoospermia (OAT) ๘ ราย การตรวจวิเคราะห์โครโมโซม ๑๘, X, และ Y ด้วยวิธี multi-color FISH (fluorescence *in situ* hybridization) ครั้งนี้ทำการศึกษาด้วยจำนวนอสุจิทั้งหมด ๑๓,๔๒๘ ตัว ผลการศึกษาพบว่ามีความแตกต่างของความผิดปกติของโครโมโซมในด้านจำนวนระหว่าง ๒ กลุ่มที่ศึกษาอย่างมีนัยสำคัญ (ค่าพี = ๐.๐๑๑) ค่าเฉลี่ยมัธยฐานร้อยละ ๑.๐๗ (ร้อยละ ๐.๑๘-๒.๓๕) และร้อยละ ๙.๒๑ (ร้อยละ ๐.๔๘-๒๙.๘๔) สำหรับกลุ่ม NT และ กลุ่ม OAT ตามลำดับ โดยพบความผิดปกติแบบ XY disomy มากที่สุด กลุ่มอสุจิที่มีความผิดปกติของโครโมโซมมีความสัมพันธ์เป็นส่วนกลับกับกลุ่มอสุจิที่ไม่มีความผิดปกติในการเคลื่อนไหว ($r = -0.๕๕๔$; ค่าพี = ๐.๐๓๒) และเป็นส่วนกลับกับกลุ่มอสุจิที่มีส่วนหัวปรกติ ($r = -0.๖๘๖$; ค่าพี = ๐.๐๐๕). โดยผลการศึกษาพบว่า น้ำอสุจิที่มีค่า motility ≤ร้อยละ ๖๐ หรือ มีรูปร่างส่วนหัวปรกติ ≤ร้อยละ ๘๕ จะมีความเสี่ยงในการที่จะมีจำนวนโครโมโซมผิดปกติด้วย ผลการศึกษานี้แสดงให้เห็นว่ากลุ่ม OAT มีความเสี่ยงที่จะถ่ายทอดความผิดปกติของบิดาไปยังรุ่นลูกหลาน ดังนั้นการตรวจวิเคราะห์ความผิดปกติในระดับโครโมโซมของอสุจิและการตรวจพารามิเตอร์ต่างๆ ของน้ำอสุจิโดยเฉพาะกลุ่ม OAT เป็นสิ่งสำคัญที่ควรพิจารณาให้ถี่ถ้วนก่อนการทำ ICSI

คำสำคัญ: ฟลูออเรสเซนท์ อินไซทู ไฮบริไดเซชัน แอสเสย์, ความผิดปกติของโครโมโซมด้านจำนวนของอสุจิ, พารามิเตอร์ของน้ำอสุจิ, ผู้มีบุตรยาก, ความผิดปกติของโครโมโซม