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The high incidence of meiotic errors increases with decreased sperm count in severe male factor infertilities

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BACKGROUND:The high frequency of aneuploidy sperm raises concerns that there may be an increased incidence of aneuploid offspring in ICSI programmes. In order to assess the role that chromosome complement plays in normal and abnormal fertility, detailed molecular cytogenetic studies must be done on sperm samples from men with normal and abnormal fertility. METHODS: To understand more clearly the cytogenetic make-up of sperm from oligoasthenoteratozoospermic (OAT) patients, multi-colour fluorescence *in situ* hybridization was used to determine numerical chromosome abnormalities. RESULTS: Increased aneuploidy frequencies for chromosomes 13, 18, 21, X and Y were detected in sperm from OAT patients. The frequencies of diploidy also increased. There were no differences in non-disjunction at meiosis I compared to meiosis II. Sperm count inversely correlated with the frequencies of diploidy, aneuploidies for chromosomes 13 and 21 in OAT patients. Twenty-two cycles of ICSI and 18 embryo transfers were performed in 20 couples. Only three cases achieved successful pregnancies. CONCLUSIONS: A higher incidence of meiotic errors and lower sperm counts was found in sperm from OAT patients.

Key words: aneuploidy/FISH/male infertility/meiosis/sperm

Introduction

Worldwide, infertility affects ~15% of couples. Male factor infertility is the primary problem in about half of these couples. Elucidation of the biological, including genetic, factors required for normal sperm function *in vivo* is a prerequisite for the full understanding of the ontogeny of male infertility. One aspect of infertility which has received little attention is the potential role of aneuploid sperm.

The successful implementation of ICSI has provided a unique means to allow couples suffering from severe male factor infertility to achieve their reproductive goals. If the infertile man has a germ cell in the ejaculate or if one can be retrieved from the reproductive tract, ICSI can be carried out with remarkable fertilization results. However, despite the great therapeutic advantages of the technique, ICSI provides solutions to clinicians often in the absence of an aetiological or pathophysiological diagnosis. Therefore, it is imperative that we continue our efforts toward the identification of the specific sperm defects involved as well as their origin (genetic, developmental, environmental or others).

To evaluate the cytogenetic make-up of sperm from oligoasthenoteratozoospermic (OAT) males, several centres have reported the incidence of aneuploidy and structural abnormalities in sperm from infertile men. Pang *et al.*, (1994) performed a preliminary study on 15 OAT patients using fluorescence *in situ* hybridization (FISH) to determine aneuploidy for chromosomes 1, X and Y. There were significant increases in aneuploidy for all chromosomes analysed in OAT patients, compared with controls. Similar results were obtained and extended to other chromosomes (Moosani *et al.*, 1995; Aran *et al.*, 1999; Pang *et al.*, 1999; Pfeffer *et al.*, 1999; Ushijima *et al.*, 2000; Vegetti *et al.*, 2000). Recently, Schmid *et al.*, (2004) showed a significant increase in the average frequencies of sperm with duplications and deletions in oligozoospermic patients.

In order to assess the role that chromosome complement plays in normal and abnormal fertility, detailed molecular cytogenetic studies must be done on sperm samples from men with normal fertility, and also from men with abnormal fertility. Care should be taken to fully characterize each patient's clinical picture so that data on subjects with identical presentations can later be pooled. Correlations of meiotic cytology and FISH studies on sperm from the same individuals may also prove highly informative.

To understand more clearly the cytogenetic make-up of sperm from OAT patients, patients with low sperm count, poor motility and low levels of normal forms, FISH was used to determine numerical chromosome abnormalities.

Frequencies of aneuploidy for chromosomes 13, 18, 21, X and Y, frequency of non-disjunction at meiosis I or II in sperm from normal men and OAT patients were examined. We also analysed the effect of semen parameters on the frequency of aneuploidy in OAT patients.

Materials and methods

Normal donors and OAT patients

Semen samples, obtained from 12 fertile donors and 30 OAT patients, were immediately analysed after liquefaction. The sperm count and percentage motility were analysed by a computer-aided sperm analysis system, CellTrak/S (Motion Analysis Corp., USA). The normal morphology was evaluated by strict criteria (Kruger *et al.*, 1986). The normal subjects used to determine control aneuploidy frequencies ranged in age from 26 to 41 years (mean: 33.3 years). Their sperm counts varied from 38 to 190 $\times$ 10^6/ml (mean = 93.3 $\times$ 10^6/ml). Between 59 and 80% (mean: 71.0%) of sperm were motile and normal morphology was seen in 14–34% (mean: 22.1%). All donors abstained from sexual activities and alcohol consumption for 3 days. All had at least one child. The OAT patients ranged in age from 25 to 46 years (mean: 35.4 years). Sperm counts were between 0.05 and 9.6 $\times$ 10^6/ml (mean: 4.5 $\times$ 10^6/ml). Motility ranged between 3 and 40% (mean: 24.0%) and between 0 and 9% sperm showed normal forms (mean: 3.4%). All subjects had normal somatic karyotypes.

Sperm decondensation and sperm heading swelling

Semen samples were immediately washed after liquefaction and decondensed as previously described (Pang *et al.*, 1999). Briefly, semen samples were washed once in phosphate-buffered saline (PBS). Following centrifugation, the pellet was suspended with 1 ml PBS containing 6 mmol/l EDTA and centrifuged. The pellet was resuspended with 1 ml PBS containing 2 mmol/l dithiothreitol, incubated for 45 min at 37 °C and centrifuged. Following centrifugation, the supernatant was discarded and the pellet was resuspended in fresh fixative (3 parts methanol:1 part glacial acetic acid). Slide preparation was by the smear method and short-term slide storage was at 4 °C.

FISH

To investigate both the autosomes and the sex chromosomes, simultaneous three-probe three-colour FISH was performed using probe sets for chromosomes 13, 18 and 21 (Set I) and X, Y and 18 (Set II). Probes used for FISH were from loci 13q22 contig (RB1), D18Z1, 21q22.1 contig (D21S259-D21S341-D21S342), DXZ1 and DYZ3. All probes were directly labelled with fluorescent dyes, chromosome 13 with Spectrum Green, chromosome 21 and the X chromosome with Spectrum Orange, the Y chromosome with Spectrum Aqua, and chromosome 18 with Spectrum Aqua (Set I) or Spectrum Green (Set II). Alpha satellite and contig DNA probes were obtained from Vysis, Inc. (USA). All hybridizations for patients' and control sperm were performed at the same time. Approximately 3000 sperm per subject were scored with each autosome probe set and ~2000 sperm per subject were scored using the X, Y, 18 probe set. Three-probe FISH was used to differentiate disomy due to non-disjunction from diploidy due to non-reduction. Simultaneous scoring of three chromosomes also provided an internal control to differentiate nullisomy from lack of hybridization. The hybridization mixture contained 1 μl of three probes each, 6 μl of formamide, and 1 μl of 20 $\times$ standard saline citrate (SSC) (total volume = 10 μl). Hybridization mixes were added to pre-warmed slides (42 °C) and covered with 22 $\times$ 22 mm coverslips which were sealed with rubber cement. Slides were denatured at 80 °C for 5 min. All slides were hybridized in a moist chamber for 6 h at 42 °C. The slides were then washed three times (5 min each) in 50% formamide, 2 $\times$ SSC, pH 7.0, followed by 5 min 2 $\times$ SSC, 0.1% NP-40, pH 7.0, all at 37 °C. Transition to antifade was accomplished by a 5 min PBS wash at room temperature. Coverslips were added over 13 μl antifade with or without counterstain. Microscopy was performed using a Nikon E600 epifluorescent microscope. Multiple fluorescent signal detection was accomplished using an automatic filter wheel with four different excitation filters. Images were captured using ChIPS Genetic Workstations and Image Software (GenDix, Inc., Korea).

Scoring criteria

Nuclei were scored only if they were not over-decondensed, did not overlap and were intact with clearly defined borders. A sperm was scored as disomic for a particular chromosome if it showed two signals for that chromosome and one each for the simultaneously probed chromosomes. For disomy to be counted, the distance between the two signals had to equal or be greater than the diameter of one fluorescent domain. Two spots separated by less than the diameter on one domain were scored as a single signal. The absence of signal for a single chromosome was scored as nullisomy for that chromosome. Sperm showing signal for none of the chromosomes of a probe set were scored as such but were not included in the calculation of nullisomy frequencies as this outcome may be an artefact resulting from unsuccessful hybridization due to inadequate nuclear decondensation. A cell was scored as diploid if there were two signals for each probed autosome and two sex chromosomes present.

Statistical analyses

Using χ^2-analyses, we compared the frequencies of aneuploidy for individual chromosomes both within patients and between patients. Due to the heterogeneity of aneuploidy frequencies both within and between patients, these data were not pooled. However, data from controls were homogeneous and were therefore pooled. Linear regression analysis was used to correlate total aneuploidy frequencies and sperm parameters. All data analyses were done by SAS system (version 8.0).

Results

Meiotic errors in OAT patients and controls

A total of 216 438 sperm nuclei was scored from 12 normal donors and 30 OAT patients, with 61 067 for the normal donors and 155 371 for the OAT patients. On average, 0.13% of sperm nuclei were found to be disomic for chromosome 13, 0.1% for chromosome 18, 0.12% for chromosome 21 and 0.22% for sex chromosomes in donors. In patients, 0.99% (range: 0.2–3.07) of sperm nuclei were found to be disomic for chromosome 13, 1.19% (0.2–2.66) for chromosome 18, 0.97% (0.2–2.74) for chromosome 21 and 2.06% (0.63–5.17) for sex chromosomes. The average frequencies of diploid sperm nuclei in donors and OAT patients were 0.22 and 2.04% (0.52–6.93) respectively. All the patients had significantly more sperm with autosomal disomy, gonosomal disomy and diploidy than normal controls. Diploid sperm are formed by a failure of the first or second meiotic division so that the sperm nucleus contains either 46,XX, 46,YY or 46,XY. Consistent elevations of nullisomic sperm nuclei for

Table I. Frequencies (%) of haploidy, disomy and nullisomy for chromosomes 13, 18 and 21, and diploidy in controls and oligoasthenoteratozoospermic (OAT) patients

	Haploid	Disomy 13	Nullisomy 13	Disomy 18	Nullisomy 18	Disomy 21	Nullisomy 21	Diploidy
Controls	99.00	0.13	0.14	0.10	0.13	0.12	0.16	0.22
OAT1	88.64	1.59	1.15	1.50	1.68	1.68	1.32	2.44
OAT2	76.91	2.17	2.83	2.55	2.77	2.74	3.09	6.93
OAT3	89.42	1.02	0.66	2.66	2.53	1.31	0.95	1.45
OAT4	85.06	1.29	2.48	1.63	2.68	1.66	1.94	3.26
OAT5	84.96	1.53	1.74	1.53	2.96	1.67	1.63	3.98
OAT6	87.40	1.17	1.53	2.34	2.14	1.56	1.66	2.21
OAT7	84.20	1.95	1.95	2.60	1.78	2.09	2.02	3.39
OAT8	81.97	3.07	2.07	2.10	2.30	2.04	1.67	4.77
OAT9	87.09	1.52	1.25	2.08	2.32	1.76	1.52	2.46
OAT10	97.01	0.35	0.29	0.35	0.58	0.26	0.32	0.86
OAT11	91.76	1.05	1.21	1.34	0.73	1.18	1.14	1.59
OAT12	96.06	0.33	0.33	0.77	1.22	0.33	0.29	0.66
OAT13	94.08	0.69	1.16	0.30	0.79	0.23	0.99	1.75
OAT14	93.97	0.51	0.58	0.96	0.77	0.39	0.51	2.31
OAT15	90.95	0.52	1.11	1.27	1.43	1.66	1.53	1.53
OAT16	97.43	0.20	0.28	0.31	0.66	0.25	0.23	0.64
OAT17	88.70	1.04	1.76	1.20	2.17	0.94	1.48	2.71
OAT18	95.73	0.50	0.41	0.93	0.75	0.28	0.56	0.84
OAT19	93.62	0.68	0.48	1.23	1.22	0.68	0.52	1.48
OAT20	95.58	0.40	0.31	0.93	0.56	0.53	0.56	1.12
OAT21	93.51	1.07	0.62	1.20	1.04	0.49	0.68	1.40
OAT22	89.79	1.21	1.71	1.12	1.18	1.40	1.58	2.02
OAT23	91.10	1.47	1.08	1.17	0.98	1.01	0.68	2.51
OAT24	95.36	0.35	0.56	0.71	0.86	0.35	0.56	1.24
OAT25	90.74	1.45	1.09	0.48	0.87	1.06	1.77	2.54
OAT26	96.41	0.39	0.36	0.58	1.00	0.29	0.36	0.61
OAT27	97.74	0.50	0.20	0.20	0.20	0.20	0.27	0.70
OAT28	97.22	0.31	0.21	0.62	0.72	0.21	0.21	0.52
OAT29	92.71	1.08	1.47	0.49	0.59	0.42	0.36	2.87
OAT30	96.02	0.30	0.30	0.60	0.93	0.33	0.38	1.13

Table II. Frequencies (%) of haploidy, disomy and nullisomy for sex chromosomes, and diploidy in controls and oligoasthenoteratozoospermic (OAT) patients

	23X	23Y	24XY	24XX	24YY	22–	46XY	46XX	46YY
Controls	49.68	49.72	0.11	0.06	0.05	0.18	0.11	0.05	0.05
OAT1	44.93	45.19	2.09	1.38	0.92	3.01	1.27	0.66	0.56
OAT2	44.13	41.11	1.69	0.77	1.49	3.59	4.56	1.69	0.97
OAT3	47.68	44.79	1.70	0.80	0.75	2.89	0.75	0.25	0.40
OAT4	47.51	43.50	1.32	0.49	0.73	3.08	1.81	0.83	0.73
OAT5	45.15	42.62	2.44	0.81	1.31	3.70	2.12	0.99	0.86
OAT6	44.81	43.56	2.41	1.20	1.56	4.54	0.85	0.49	0.58
OAT7	44.98	44.16	1.71	0.78	1.09	3.98	1.71	0.72	0.88
OAT8	44.09	42.23	2.20	1.37	0.98	4.59	2.20	1.12	1.12
OAT9	47.16	44.75	1.32	0.75	0.80	2.58	1.43	0.63	0.57
OAT10	49.69	48.14	0.31	0.16	0.21	0.62	0.47	0.16	0.26
OAT11	45.72	49.85	0.50	0.20	0.35	1.71	0.86	0.35	0.45
OAT12	47.65	49.66	0.53	0.22	0.16	0.95	0.32	0.26	0.16
OAT13	46.63	47.94	0.86	0.30	0.40	2.11	0.86	0.35	0.55
OAT14	47.31	48.30	0.44	0.35	0.15	1.14	1.14	0.64	0.54
OAT15	45.64	49.41	0.99	0.40	0.30	1.68	0.84	0.45	0.30
OAT16	47.44	50.82	0.26	0.21	0.16	0.47	0.26	0.21	0.16
OAT17	45.64	47.52	1.04	0.50	0.89	1.98	1.14	0.40	0.89
OAT18	49.60	47.91	0.55	0.30	0.25	0.60	0.45	0.15	0.20
OAT19	45.78	48.78	0.85	0.55	0.45	2.05	0.80	0.40	0.35
OAT20	49.10	47.38	0.56	0.26	0.34	1.24	0.52	0.30	0.30
OAT21	48.78	46.00	0.56	0.72	0.56	2.11	0.61	0.39	0.28
OAT22	48.68	45.89	0.85	0.50	0.30	1.75	1.00	0.40	0.65
OAT23	50.46	44.80	0.53	0.35	0.27	1.15	1.24	0.53	0.66
OAT24	49.18	47.38	0.45	0.30	0.35	1.05	0.65	0.35	0.30
OAT25	47.60	46.86	0.83	0.42	0.37	1.38	1.25	0.69	0.60
OAT26	49.65	47.05	0.70	0.35	0.40	1.20	0.35	0.15	0.15
OAT27	49.68	47.51	0.54	0.25	0.05	1.33	0.30	0.30	0.05
OAT28	49.06	48.86	0.40	0.30	0.10	0.74	0.25	0.15	0.15
OAT29	47.49	46.98	0.66	0.46	0.36	1.17	1.37	0.66	0.86
OAT30	48.96	48.29	0.38	0.33	0.17	0.54	0.63	0.33	0.38

all tested chromosomes were seen among all the patients (Tables I and II).

When the mean frequencies of aneuploidy per chromosome, defined as the sum disomy per chromosome plus nullisomy per chromosome, were compared, only the sex chromosomes had significantly higher frequencies of aneuploidy than any other autosome tested in the two groups. No differences were detectable between each chromosome in normal controls. However, in OAT patients, significant differences were seen for each of the chromosomes studied (Table III).

Errors in meiosis I and II

Consistent with theoretical expectation, the proportion of X- and Y-bearing sperm was 49.67 and 49.73% respectively and was close to the 1:1 ratio. The combined frequency of 24,XX and 24,YY sperm resulting from non-disjunction during the second meiotic division of spermatogenesis, and 24,XY sperm resulting from non-disjunction during the first meiotic division was estimated to be 0.11 and 0.11% respectively in controls ($P = 0.89$), and 1.07% and 0.99 respectively in patients ($P = 0.20$) (Table IV). The mean frequency of XX- + YY- and XY-bearing sperm was close to the 1:1 ratio expected in both groups.

The effect of semen parameters on the frequency of aneuploidy

We also analysed the effect of semen parameters (sperm count, % motility and % normal morphology) on the frequency of aneuploidy using regression analysis. There was no correlation between the frequency of aneuploidy and each of three semen parameters in controls. However, sperm count inversely correlated with aneuploidies for chromosomes 13 ($r = -0.47$, $P = 0.009$; Figure 1A) and 21 ($r = -0.49$, $P = 0.006$; Figure 1C), frequencies of diploidy ($r = -0.49$, $P = 0.005$; Figure 1E). There was, however, no significant correlation between sperm count and the frequencies of aneuploidy for chromosome 18 ($r = -0.34$, $P = 0.066$; Figure 1B) and sex chromosomes ($r = -0.32$, $P = 0.089$; Figure 1D). Moreover, there was no significant correlation between other sperm parameters and the frequencies of cytogenetically abnormal sperm.

Table III. Differences of aneuploid frequency between chromosomes

	Chromosome 13	Chromosome 18	Chromosome 21
Control			
Chromosome 18	0.2756		
Chromosome 21	1.0000	0.2575	
Sex chromosomes	0.0140	0.0000	0.0169
OAT patient			
Chromosome 18	0.0000		
Chromosome 21	0.0010	0.0000	
Sex chromosomes	0.0000	0.0430	0.0000

OAT = oligoasthenoteratozoospermic.

Table IV. Proportion of 24,XY and 24,XX +24,YY in controls and oligoasthenoteratozoospermic (OAT) patients

	24,XY	24,XX + 24,YY	*P*
Control	0.11 ± 0.05	0.11 ± 0.06	0.89
OAT patient	0.99 ± 0.66	1.07 ± 0.71	0.20

Results of ICSI using sperm from OAT patients

Finally, 22 cycles of ICSI and 18 embryo transfers were performed in 20 couples. Four cycles had no embryo transfer due to fertilization failure. Two patients had pre-clinical abortions and one a first trimester spontaneous abortion. Only three cases achieved successful pregnancies. No cytogenetic data were available on the aborted fetuses to demonstrate the role of aneuploidy. The overall fertilization rate was 60% (range: 0–100%). Because of the small number of patients studied, statistical significance cannot be ascertained.

Discussion

The data from Skakkebaek *et al.* (1973) showed that the relative frequency of spermatogonial metaphases was higher in infertile men (19.7%) than in normal controls (9.4%). They suggested that the absolute number of spermatogonia may be the same in both groups. The elevated frequency seen in the infertile subjects may have resulted from the reduction in their number of spermatocytes. Also they evaluated pairing of sex chromosomes and autosomes at metaphase I. The failure of the X and Y chromosomes to be paired, seen in 15% of 1159 cells, was the most common finding noted. Their data showed only a few small autosomes separated in a rare cell. In these regards, no significant differences existed between infertile patients and normal control donors. Unpaired homologous chromosomes at metaphase I could lead to non-disjunction and the production of aneuploid sperm. Such abnormalities could give rise to zygotes showing for example, 47,XXY (Klinefelter syndrome), 45,X (Turner syndrome), and 47,XY, + 21 (Down syndrome).

Our data showed that individual patients had significantly higher proportions of disomic, nullisomic and diploid sperm nuclei compared to normal control group. Also between-patient heterogeneity exists for all OAT patients. The factor(s) responsible for the heterogeneity present both within and between samples from OAT patients is obscure. Cytogenetic heterogeneity in the OAT population might account for some of the cytogenetic variability seen between patients but would be implausible to generate the observed heterogeneity seen between chromosomes within patients. It is possible that mutations within a gene or genes associated with meiosis may predispose a particular chromosome to non-disjoin at a higher frequency than other chromosomes within the same gamete. The results of our data provide suggestive evidence for the existence of chromosome-specific patterns of non-disjunction in OAT patients.

Our data suggest that the frequency of aneuploid sperm seems to be influenced by sperm count. Frequencies of sperm chromosome abnormalities increased with decreased sperm count in OAT patients. Our findings in OAT

patients are in agreement with other previous reports which show that the frequency of meiotic non-disjunction increases when the sperm count decreases (Rives *et al.*, 1998; Vegetti *et al.*, 2000; Martin *et al.*, 2003). Also, our results are in accordance with a study done in somatic cells from infertile males. Data suggest that the frequency of chromosome abnormalities in somatic cells in males selected for infertility is inversely proportional to the sperm concentration (Vendrell *et al.*, 1999). Therefore our studies confirm the direct correlation between the sperm count and the frequency of aneuploidy, i.e. meiotic errors.

Vegetti *et al.* (2000) reported that the risk of a chromosomal aneuploidy in sperm seems to be inversely correlated with sperm count and total progressive motility in patients with abnormal semen parameters. Moreover, Calogero *et al.* (2001) reported that sperm aneuploidy rate was negatively

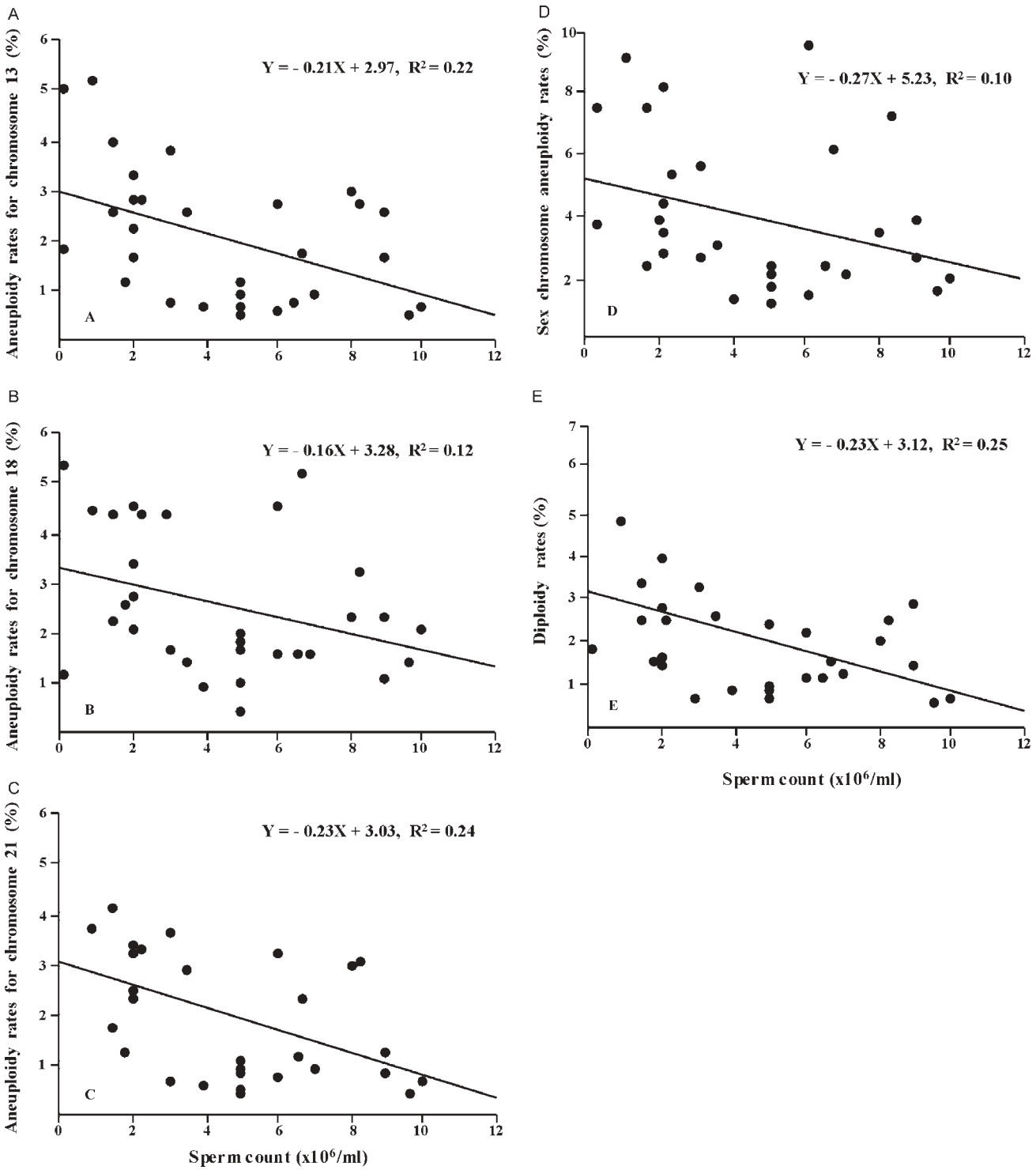

Figure 1. Regression equations and R^2 values between sperm count and frequencies of aneuploidy for chromosomes 13 (**A**), 18 (**B**), 21 (**C**), sex chromosomes (**D**), or frequencies of diploidy (**E**) in oligoasthenoteratozoospermic patients.

correlated with sperm concentration and particularly with the percentage of normal forms. Interestingly, we did not find a significant correlation between aneuploidy rates and abnormal motility and abnormal morphology of sperm.

However, we can assume that there is a significant correlation between the higher frequencies of aneuploidy or diploidy and the lower sperm number. This assumption is well supported by the findings in our patients. However, it is likely that some of the sperm with defective meiosis gradually reduced during the stages of maturation and fertilization. Which suggests meiotic quality control system is relatively strong but not perfect in man. Vegetti *et al.* (2000) suggested that this higher meiotic error in sperm, which is inversely correlated with sperm count and motility, should not be ignored, since data from Pang *et al.* (1999) indicate that if each chromosome shows this slight increase in aneuploidy rate, it is possible to speculate that the total rate of aneuploidy is ~33–74% in sperm from OAT patients.

All the chromosomes studies had an increased frequency of aneuploidy in the OAT patients. Interestingly, sperm count significantly and inversely correlated with the frequencies of diploidy, and aneuploidies for chromosomes 13 and 21 in our OAT patients. Martin *et al.* (2003) reported an inverse correlation between the frequency of sperm chromosome abnormalities and the sperm count for sex chromosome disomy and diploidy. Warburton and Kinney (1996) hypothesized that chromosome differential susceptibility to aneuploidy is related to chromosomal differences in structures and behaviours. However, further studies are required.

The much higher incidences of chromosomal abnormalities after ICSI reported by In't Veld *et al.* (1995) and Van Opstal *et al.* (1997) are consistent with our data. Previously, Martin (1996) suggested that there may be a risk of transmitting chromosomal abnormalities to offspring following ICSI. Rosenbusch and Sterzik (1996) discussed the possibility of irregular chromosome segregation following ICSI. The elevated frequency of autosomal non-disjunction, which we observed, may not markedly impact the frequencies of autosomal abnormalities in newborns because all resulting autosomal monosomies and most trisomies are lethal and autosomal aneuploidy in oocytes is common. It is unclear what the contribution of aneuploid sperm is to the frequency of implantation and the incidence of 'early' spontaneous abortions containing aneuploid cells of paternal origin. However, the elevated frequencies of aneuploidy observed in the sperm from this population of OAT males suggest that they may be at increased risk for transmitting genetic abnormalities to their offspring. This low pregnancy rate and high implantation failure may be associated with aneuploidy in the male gametes. Aneuploidy in sperm may be associated with lack of implantation and/or the ability to carry a fetus to term. Further studies are required.

Fortunately, sperm cells from these OAT patients are unlikely to fertilize an ovum successfully by themselves. Also, there is an earlier and a stronger selection against autosomal trisomic and monosomic embryos in the human body. The only full numerical autosomal anomalies surviving to birth are trisomy 13 (probability of survival to birth of 2.8%), trisomy 18 (probability of 5.4%) and trisomy 21 (probability of 22.1%), but only trisomy 21 allows survival into puberty and adulthood (Jacobs and Hassold, 1995). Most numerical autosomal anomalies originate during maternal meiosis I (trisomies 8, 13, 15, 16, 18 and 21) although some cases (up to 10%) are paternal in origin (Nicolaidis and Peterson, 1998). However, most embryos exhibiting sex chromosome abnormalities cross the pregnancy–birth barrier and have a probability of survival to birth of 55.3% for XXY, 70% for XXX, 100% for XYY and 3% for XO. Triploids survive to birth only exceptionally (Jacobs and Hassold, 1995). As a result, most infertile males with meiotic disorders are sterile or have a history of low reproductive efficiencies. Our data with in accordance with those of Aran *et al.* (1999), which showed a lower implantation rate (16%) and a higher abortion rate (33%) in infertile males.

In conclusion, we confirm that OAT patients had significantly higher frequencies of disomic, nullisomic and diploid sperm nuclei compared to a normal control group. Also between-patient heterogeneity was observed in all OAT patients. Sperm count inversely correlated with the frequencies of diploidy and aneuploidies for all chromosomes tested in OAT patients. Twenty-two series of ICSI and 18 embryo transfers were performed in 20 couples. Only three cases achieved successful pregnancies.

IVF protocols incorporating ICSI are being performed and this technique is now the treatment of choice for OAT males. Therefore, the high frequency of aneuploidy raises concerns that there may be an increased incidence of aneuploid babies, especially sex chromosome abnormalities. The risk of chromosome abnormalities in the offspring of patients undergoing ICSI must be carefully evaluated.

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