LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

UCRL-JRNL-200885

Detection of structural and numerical chomosomal abnormalities by ACM-FISH analysis in sperm of oligozoospermic infertility patients

T. E. Schmid, M. H. Brinkworth, F. Hill, E.D. Sloter, A. Kamischke, F. Marchetti, E. Nieschlag, A. J. Wyrobek

November 11, 2003

Human Reproduction

Human Reproduction

Detection of Structural and Numerical Chromosomal Abnormalities by ACM-FISH Analysis in Sperm of Oligozoospermic Infertility Patients

T.E. Schmid[1,2], M.H. Brinkworth[1]*, F. Hill[2], E.D. Sloter[2], A. Kamischke[3], F. Marchetti[2], E. Nieschlag[3], A.J. Wyrobek[2]

[1]Department of Biomedical Sciences, University of Bradford, UK.

[2]Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, USA.

[3]Institute of Reproductive Medicine of the University, Münster, Germany.

*To whom correspondence should be addressed

email: m.h.brinkworth@bradford.ac.uk

Key words: ACM, FISH, oligozoospermic, infertility, sperm

Abstract

Modern reproductive technologies are enabling the treatment of infertile men with severe disturbances of spermatogenesis. The possibility of elevated frequencies of genetically and chromosomally defective sperm has become an issue of concern with the increased usage of intracytoplasmic sperm injection (ICSI), which can enable men with severely impaired sperm production to father children. Several papers have been published about aneuploidy in oligozoospermic patients, but relatively little is known about chromosome structural aberrations in the sperm of these patients. We examined sperm from infertile, oligozoospermic individuals for structural and numerical chromosomal abnormalities using a multicolor ACM FISH assay that utilizes DNA probes specific for three regions of chromosome 1 to detect human sperm that carry numerical chromosomal abnormalities plus two categories of structural aberrations: duplications and deletions of 1pter and 1cen, and chromosomal breaks within the 1cen-1q12 region. There was a significant increase in the average frequencies of sperm with duplications and deletions in the infertility patients compared with the healthy concurrent controls. There was also a significantly elevated level of breaks within the 1cen-1q12 region. There was no evidence for an increase in chromosome-1 disomy, or in diploidy. Our data reveal that oligozoospermia is associated with chromosomal structural abnormalities suggesting that, oligozoospermic men carry a higher burden of transmissible, chromosome damage. The findings raise the possibility of elevated levels of transmissible chromosomal defects following ICSI treatment.

Introduction

Abnormal reproductive outcomes have been associated with paternally transmitted cytogenetic abnormalities since the introduction of human sperm chromosome karyotyping (Rudak et al., 1978; Moosani et al., 1995). This concern has become more relevant as ICSI and IVF have gained more acceptance for the treatment of male infertility (Palermo et al., 1992). Using ICSI, even a single sperm retrieved from seminal, epididymal or testicular samples can give a chance of fathering a child. Assisted conception techniques have a live birth rate per treatment cycle of around 17% for IVF and 22% for ICSI (data from the UK for 1998/99). The reasons why ~4 out of 5 treatment cycles fail to result in a live birth are largely unknown,. When the cause of the defective spermatogenesis has a genetic basis, there may also be a risk that male offspring could inherit that defect, as has been shown for microdeletions in the Y-chromosome (Kamischke et al., 1999; Oates et al., 2002). This means that male ICSI-derived children can inherit defects causing genetic infertility. It is not yet known whether severely oligozoospermic men have increased frequencies of sperm with structural chromosomal aberrations involving autosomes.

Aneuploidy and chromosomal aberrations can cause major morphological and biochemical defects in offspring. Most aneuploidy is attributed to errors in meiotic chromosome segregation during meiosis resulting in aneuploid gametes (Robbins et al., 1995). In the last years several papers reported significantly elevated levels of aneuploidy in oligozoospermic patients (Bernadini et al., 1997; Aran et al., 1999; Pang et al., 1999; Vegetti et al., 2000; Schmid et al., 2003), but relatively little is known about chromosome structural abnormalities in the sperm of these patients. Chromosome structural abnormalities are less common than aneuploidy at birth (0.25% vs. 0.33%, Hassold, 1998), but it is estimated that about 80% are paternally

derived (Chandley, 1991). Hitherto, most data on chromosome structural abnormalities in sperm have been derived from studies of human sperm-hamster oocyte hybrids that allow analysis of the paternal chromosome complement (Rudek et al., 1978). This is a time consuming, though thorough, technique by which it has been estimated that such aberrations occur at a rate of 5-7% in normozoospermic, fertile males. Since only about a third of human conceptions are thought to result in a live birth, it has been assumed that chromosome aberrations may account for much of this failure.

In this study a multicolor FISH assay (ACM) for the simultaneous detection of sperm carrying numerical chromosomal abnormalities (disomy and diploidy) as well as structural abnormalities (partial chromosomal duplications and deletions and chromosomal breaks) (Sloter et al., 2000) was used. This methodology utilizes multiple fluorescent colors to locate chromosomal domains directly in human sperm and thus provides a direct approach to quantify abnormality levels at the loci studied. DNA probes specific for three regions of chromosome 1 are used to detect human sperm that carry numerical abnormalities and structural aberrations (duplications, deletions and breaks).

Material and Methods

Study Participants

The samples were obtained from 9 oligozoospermic patients attending the Institute of Reproductive Medicine of the University (IRM), Münster, Germany because of infertility (Table 1). They were mixed non-smokers and smokers aged 27-48 years (mean 35 years) and had no known medical or occupational exposure to genotoxic

agents. All of them were diagnosed to have idiopathic infertility. The actual mean duration of infertility was 3.5 years (range 1.5 to 11 years). Sperm samples were also obtained from the IRM from 11 healthy normal subjects participating in a contraceptive trial. The samples used in this study were from those obtained before the start of any treatment. They were mixed non-smokers and smokers aged 27-42 years (mean 32 years), reported no chronic health or fertility problems, and had no known medical or occupational exposure to genotoxic agents.. Ethical permission to use the samples in Bradford was also obtained by the local (University of Bradford) ethics committee and by the Lawrence Livermore National Laboratory (LLNL) Institutional Review Board.

Samples from the patients and volunteers investigated in the present study have previously been analyzed for aneuploidy and for genetic damage measured in the Comet, Sperm Chromatin Structure (SCSA) and inverse, Restriction Site Mutation (iRSM) assays (Schmid et al., in press).

Semen analysis

All patients and volunteers underwent the full diagnostic work-up routinely performed at the Institute of Reproductive Medicine, Münster, as described (Behre et al. 2000.) Semen analysis was performed according to the WHO guidelines (WHO, 2000). After removal of aliquots for examination the ejaculates were frozen at –20°C.

ACM assay

Semen specimens were aliquoted and shipped without preservative to Livermore on dry ice. The aliquots were thawed at room temperature (RT) and a volume of 10µl was smeared onto an ethanol-cleaned microscope slide and air-dried for one day.

Decondensation of sperm nuclei was performed using DTT and LIS according to the method of Sloter et al. (2000).

The sperm ACM assay utilized DNA probes for three repetitive-sequence regions on chromosome 1 (D1Z5 (alpha satellite or A), pUC1.77 (classical satellite or C), and D1Z2 (midisatellite or M). In situ hybridization was performed according to the method of Sloter et al. (2000). Briefly, the probe mix for each slide contained 20 ng each of D1Z2 and pUC1.77 probe, 30 ng of D1Z5 probe, and 10 ng of herring sperm DNA (carrier molecule) in a final concentration of 55% formamide/2x SSC and 10% dextran sulfate. Hybridization was carried out over 2 nights. Prior to washing, the coverslips were removed in 2x SSC at RT. Slides were washed in 60% formamide/2x SSC at 45°C for 5 min, followed by two 10 min washes in 2x SSC (pH 7.0) at RT. The biotinylated and digoxigenin-labeled probes were detected using a 1:100 dilution (in PNM buffer) of streptavidin Pacific Blue (stock concentration 2.5 mg/ml; Molecular Probes) and sheep anti-digoxigenin-fluorescein isothiocyanate (FITC; stock concentration 0.2 mg/ml; Boehringer Mannheim). Immunofluorescence was carried out for 30 min at RT in a humidity chamber, followed by two washes in 2x SSC for 3 min each. 4,6-diamidino-2-phenylindole (DAPI), diluted to 10 ng/ml in Vectashield antifade medium (Vector), was used as counterstain. All hybridizations were performed at LLNL.

Scoring

TES was trained at LLNL by EDS according to the method described in Sloter et al. (2000). Scoring was performed in Bradford using a Leica DM photofluorescence microscope with a triple-band filter set for simultaneous visualization of Pacific blue, FITC and Texas Red. The following criteria for abnormal sperm phenotypes were

used: Sperm carrying an abnormal number of same-color domains were scored as abnormal only if the domains were of similar size and intensity (except for breaks within 1q12) and clearly separated. Each fluorescence domain had to be located within the boundary of an intact sperm nucleus. Overlapping sperm nuclei were not scored. Only cells with a flagellum (or tail-attachment site) under bright-field microscopy were scored. Cells outside the normal size limits for decondensed sperm, as assessed by a microscope-eyepiece graticule were not scored.

The following describes the nomenclature and scoring criteria developed for the ACM assay (Sloter et al., 2000). One-letter abbreviations were used to denote the presence of each fluorescence domain: A (alpha satellite, 1cen); C (classical satellite, 1q12); and M (midisatellite, 1p36.3). The A and C regions are contiguous on chromosome 1, and the fluorescent domains are adjoined in normal sperm. A normal sperm was scored as ACM; addition or absence of letters denoted duplications or deletions. An "O" was used to indicate the absence of an expected domain. Thus, for example, sperm containing a duplication or deletion of M were represented by ACMM and ACO, respectively. The two M domains had to be separated by the distance of at least one normal M domain. ACACM and OOM represented centromeric duplications and deletions, respectively, of only the AC region.

Sperm carrying breaks within the 1cen-1q12 (AC) region were divided into two groups based on their fluorescence phenotype. The first group included sperm containing a separation directly between the A and C regions and were denoted by A-CM The A and C domains had to be separated by at least half the diameter of the C domain. Sperm classified into the second group carried two C domains and were represented by ACCM.

Sperm containing two copies of each domain represented chromosome 1 disomy or sperm diploidy. Each same-color domain had to be separated by the distance of at least one full domain width. The C domain, which is larger than the others, required a half-domain separation. The absence of all three fluorescent domains was denoted by "OOO" (i.e., nullisomy 1). This phenotype could also represent lack of hybridization for technical reasons. In this study no sperm with “OOO” were found.

Coding and statistical analysis

All slides were hybridized and encoded by a person in LLNL (FH) not involved in the scoring and scoring was performed in Bradford by TES. At least 5,000 sperm were scored from the left half of each slide. The slides were then recoded, and a second set of 5,000 sperm analyzed from the right half of each slide, for a total of 10,000 sperm per slide. The CytoScore© software program developed at LLNL was used for the scoring.

The slides were decoded by the original encoder, and the 1st and 2nd scoring analyses of each slide were compared using Chi-square analysis. Inter- and intradonor variation in the frequencies of abnormal sperm was evaluated using contingency table analysis.

Comparisons between the oligozoospermic and normozoospermic groups were carried out with the Mann–Whitney *U*-test.

Results

Semen analysis

The results of the semen analysis has been published elsewhere (Schmid et al., 2003). Briefly, the oligozoospermic patients had mean sperm concentrations of 9.9 $x10^6$ /ml (range: 4.3 - 17.0 $x10^6$ /ml), while among the normal volunteers the mean was 57 $x10^6$ /ml (range: 35 - 102 $x10^6$ /ml). Mean sperm motility in the patient group was slightly decreased with 7 out of 9 patients showing abnormal sperm motility. According to WHO criteria, 8/9 of the patients showed less than 15% normal morphology. In contrast, in the control group only 4 volunteers had moderate asthenozoospermia and all showed normal morphology.

ACM assay

A total of 201,137 sperm were evaluated by the ACM FISH assay. A significant increase in the frequency of sperm carrying either partial chromosomal duplications or deletions of chromosome 1 was found in the infertility patients compared with the normozoospermic controls, (14.7 ± 3.6 vs. 8.7 ± 2.9 per 10^4 sperm, $p<0.01$) (Table 2). Sperm carrying duplications or deletions of 1p occurred more frequently in the infertility patients than in the control group, (8.2 ± 2.2 vs. 5.7 ± 1.9 per 10^4 sperm, $p<0.01$). There was a two-fold higher frequency of sperm with duplications and deletions of 1pter in the infertile group (6.5 ± 1.6 vs. 3.0 ± 1.0 per 10^4, $p<0.01$). In both groups, the frequencies of sperm carrying duplications versus deletions did not differ significantly from a 1:1 ratio, suggesting symmetrical mechanisms of formation. Sperm with duplications and deletions of 1cen were detected at average frequencies of 3.0 ± 1.0 in the control group and 6.5 ± 1.6 per 10^4 sperm in the infertility group. There was no significant inter-donor variation for these types of sperm defects. Two

types of breaks were detected by ACM sperm FISH. The average frequency of sperm carrying a break between 1cen and 1q12 region was significantly higher in the infertility group compared to the control group (4.0 ± 1.6 vs. 3.0 ± 1.3 per 10^4 sperm, $p<0.05$). Breaks within 1q12 occurred in 12.4 ± 3.8 per 10^4 sperm in the oligozoospermic patients which was significantly higher than in the control group (10.4 ±1.2, $p<0.05$). There was also no significant inter-donor variation in the total frequency of breaks among the individuals in each group studied.

Sperm carrying two copies of all three target regions indicated the presence of an extra copy of chromosome1, which can be due to disomy 1 or q form of sperm diploidy. However, the frequencies of these sperm did not differ between the infertile and control groups (22.1 ± 3.4 vs. 20.3 ± 4.2 per 10^4 sperm).

Discussion

Chromosome structural damage in sperm may be associated with pregnancy loss as well as specific heritable syndromes. Both numerical and structural chromosomal abnormalities in sperm can lead to seriously detrimental effects and loss of human embryos. For example, chromosomal structural aberrations are observed in about 6% of spontaneous abortions (Shelby et al., 1993) and 0.25% of live births (Hassold, 1998). Chromosomal aberrations can also be detected in normal human sperm (e.g., Baumgartner et al., 1999) and many patients with low sperm counts appeared to have a high prevalence of abnormal karyotypes (Bourrouillou et al., 1985). However, the methodology used in the latter work (the zona-free, hamster oocyte cytogenetic assay) does not lend itself to the routine screening of chromosomal defects in human sperm samples. The ACM assay used in this present study is a FISH-based method that allows the rapid detection of chromosomal breaks and rearrangements in sperm

and provides an important new approach for assessing such damage in infertility patients.

Our data represent the first demonstration with sperm FISH that oligozoospermic infertility patients show higher frequencies of chromosomal structural aberrations of chromosome 1 in their sperm than normozoospermic men. The ACM assay detects damage in specific regions of just one chromosome and it is possible that similar aberrations or rates of damage will not be seen on all chromosomes. However, extrapolation of the ACM data for the control group (244 sperm with chromosomal structural aberrations/110,619 sperm analyzed) to the haploid genome yields an estimate of 4.4%-6.1% sperm carrying structural aberrations. This is in line with was has been reported using the hamster-egg assay for healthy donors (Sloter et al., 2000). These results suggest that structural aberrations may be a widespread phenomenon in the sperm of infertile, oligozoospermic men and may provide an explanation for some of the failures of assisted conception cycles in infertile patients and highlight the need for investigations of heritable chromosome damage in the offspring that are produced by assisted reproductive techniques.

The frequencies of sperm with duplications, deletions and breaks in our normal reference were similar to those reported by Sloter et al. (2000), demonstrating that the assay is robust for inter-laboratory comparisons. Our results also confirm the findings of Sloter et al., (2000) that the spontaneous frequencies of sperm with structural chromosomal abnormalities are higher than those of numerical abnormalities and that chromosome breaks are more prevalent than partial duplications and deletions

The formation of chromosome aberrations requires a DNA double strand break, which may be followed by rearrangement onto another chromosome. When DNA

strand breakage occurs before or during male meiosis, it can lead to sperm carrying partial duplications and deletions of chromosomal regions (Van Hummelen et al., 1996). Our study showed significantly higher frequencies of sperm with duplications, deletions and breaks in the infertility patients compared with the controls. This can be associated with the recent finding that oligozoospermic patients have also significantly higher levels of chromatin disturbances and DNA strand-breaks using SCSA and the Comet assay respectively, than normozoospermic controls (Schmid et al., 2003). It was previously assumed that Comet and SCSA damage in sperm arise during spermiogenesis. However it was recently shown that X-irradiation of spermatogenic stem cells, proliferating spermatogonia and spermatocytes can induce Comet assay damage in resulting sperm (Haines et al., 2002). This suggests that the Comet sperm assay could also be an indicator of pre-chromosomal lesions in pre-meiotic and meiotic male germ cells. Thus, it is possible that our group of infertile men had DNA damage in their meiotic or pre-meiotic germ cells that lead to the induction of chromosome aberrations. In support of this suggestion, the Comet assay data (Schmid et al., 2003) showed highest levels of damage in the sperm of the same patients in which the highest rates of structural aberrations were found in the present study. There was a slight correlation between the sperm concentration of the oligozoospermic patients and the number of breaks in the 1cen - 1q12 region of chromosome 1, suggesting that the mechanism that leads to a low sperm count could also lead to a higher rate of breaks (Figure 1). Interestingly, there was no correlation of aberrations with motility or morphology.

It is interesting that an increase in autosomal disomy of chromosome 1 or diploidy was not found in this study. This is consistent with our previous finding that aneuploidy among infertile, oligozoospermic men occurred in the gonosomes and not

chromosome 18 (Schmid et al., 2003). This difference in chromosomal susceptibility may be related to the differing size of the X and Y chromosomes, which may lead to a greater chance of non-disjunction than in homologous autosomes (Qinghua and Martin, 2001; Schmid et al., 2003).

Finally, our study indicates that oligozoospermia is associated with chromosomal structural abnormalities and that the ACM assay is a robust approach for assessing the genetic integrity of the male germ-line

Acknowledgements

Dr. Schmid is a Wellcome Trust Travelling Research Fellow, grant reference number: 062288. Work undertaken at the Institute of Reproductive Medicine was supported by the German Federal Health Ministry. TES was trained in the ACM assay in the Wyrobek laboratory at the Lawrence Livermore National Laboratory (LLNL). The work at LLNL was performed under the auspices of the U.S. Department of Energy by LLNL, contract W-7405-ENG-48, with funding support from NIEHS Superfund P4ZES04705.

Literature

Aran, B., Blanco, J., Vidal, F., Vendrell, J.M., Egozcue, S., Barri, P.N., Egozcue, J., Veiga, A. (1999). Screening for abnormalities of chromosomes X,Y, and 18 and for diploidy in spermatozoa from infertile men participating in an in vitro fertilization-intracytoplasmic sperm injection program. Fertil Steril 72(4): 696-701.

Baumgartner A., Van Hummelen P., Lowe X.R., Adler I.-D., Wyrobek A.J. (1999). Numerical and structural chromosomal abnormalities detected in human sperm with a combination of multicolor FISH assays. *Environ Mol Mutagen*, 33:49-58

Behre, H.M., Yeung, C.H., Holstein, A.F., Weinbauer, G.F., Gassner, P. and Nieschlag, E. Diagnosis of male infertility and hypogonadism. *In:* Nieschlag, E. and Behre, H.M., eds. *Andrology: male reproductive health and dysfunction*, 2nd edition, Heidelberg: Springer, 2000:90-124.

Bernadini, L., Martini, E., Geraedts, J.P.M., Hopman, A.H.N., Lanteri, S., Conte, N., Capitanio, G.L. (1997). Comparison of gonosomal aneuploidy in spermatozoa of normal fertile men and those with severe male factor detected by in-situ hybridization. Mol Hum Reprod 3 (5): 431-438.

Bourrouillou G., Dastugue N., Colombies, P. (1985). Chromosome studies in 952 males with a sperm count below 10 million/ml. *Hum Genet* 71:366–367

Chandley A.C. (1991.) On the paternal origin of de novo mutation in man. *J Med Genet,* 28:217-223

Haines G.A., Hendry J.H., Daniel C.P., Morris I.D. (2002) Germ cell and dose-dependent DNA damage measured by the Comet assay in murine spermatozoa after testicular irradiation. *Biol Reprod* 67:854-861

Hassold T.J. (1998). Nondisjunction in the human male. *Curr Top Dev Biol*, 37:383-406

Kamische, A., Gromoll, J., Simoni, M., Behre, H.M., Nieschlag, E. (1999). Transmission of a Y chromosomal deletion involving the deleted in azoospermia (DAZ) and chromodomain (CDY1) genes from father to son through intracytoplasmatic sperm injection. *Hum Reprod.,* 14(9): 2320-2322.

Liebers I, Bonduelle, M., Van Aschee E., Devroy P., Van Steirteghem A. (1995). Sex chromosomal abnormalities after intracytoplasmic sperm injection. *Lancet,* 346:1095

Moosani, N., Pattinson, H., Carter, M., Cox, D., Rademaker, A., Martin, R. (1995). Chromosomal aalysis of sperm from men with idiopathic infertility using sperm karyopyping and fluorescence in situ hybrdization. *Fertility and Sterility,* **64**, 811-817.

Nieschlag, E. (1997). Andrology at the end of the twentieth century: from spermatology to male reproductive health. *Int J Androl.* 20:129-31

Oates, R.D., Silber, S., Brown, L.G., Page, D.C. (2002). Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. Hum Reprod,17(11):2813-2824.

Pang, M.G., Hoegerman, S.F., Cuticchia, A.J., Moon, S.Y., Doncel. G.F., Acosta, A.A., Kearns, W.G. (1999). 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 intracytoplasmatic sperm injection. Hum Reprod 14(5): 1266-1273.

Palermo, G., Joris, H., Devroey, P., Van Steirteghem, A.C. (1992). Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, 340: 17-18.

Quinghua, S., Martin, R.H. (2001). Aneuploidy in human spermatozoa FISH analysis in men with constitutional chromosomal abnormalities, and in infertile men. *Reproduction*, 121:655-66

Robbins, W.A., Baulch, J.E., Moore, D., Weier, H.U., Blakey, D., Wyrobek, A.J. (1995). Three-probe fluorescence in situ hybridisation to assess chromosome X, Y, and 8 aneuploidy in sperm of 14 men from two healthy groups: evidence for a paternal age effect on sperm aneuploidy. *Reprod Fertil Dev*, 7:799-809

Rudak E., Jacobs P.A., Yanagimachi R. (1978). Direct analysis of the chromosome constitution of human spermatozoa. *Nature*, 274:911-913

Schmid, T.E., Kamischke A., Bollwein H., Nieschlag E., Brinkworth M.H. (2002) Genetic damage in oligozoospermic patients detected by FISH, iRSM, SCSA and the Comet assay**.** *Hum Reprod* 18(7): 1474-1486

Shelby M.D., Bishop J.B., Mason J.M., Tindall K.R.(1993). Fertility, reproduction, and genetic disease: studies on the mutagenic effects of environmental agents on mammalian germ cells. *Environ Health Perspect* 100:283-91

Sloter E.D., Lowe X., Moore II D.H., Nath J., Wyrobek A.J.(2000). Multicolor FISH analysis of chromosomal breaks, duplications, deletions, and numerical abnormalities in the sperm of healthy men. *Am J Hum Genet*. 67:862-72

Van Hummelen P, Lowe XR, Wyrobek AJ. (1996). Simultaneous detection of structural and numerical chromosome abnormalities in sperm of healthy men by multicolor fluorescence in situ hybridization. *Hum Genet*, 98(5):608-15

Vegetti, W., Assche, E.V., Frias, A., Verheyen, G., Bianchi, M.M., Bonduelle, M., Liebaers, I., Steirteghem, A.V. (2000). Correlation between semen parameters and sperm aneuploidy rates investigated by fluorescence in-situ hybridization in infertile men. Hum Reprod 15(2): 351-365.

WHO (2000) World Health Organization laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Cambridge University Press, Cabridge, UK

Table 1: Results of the semen analysis

Status	Age (years)	Ejaculate volume (ml)	Sperm concentration (mill/ml)	Sperm morphology Normal forms in %	Sperm motility WHO grade a+b in %
Volunteer 1	42	8,3	35.0	25	54
Volunteer 2	24	6,5	40.0	24	55
Volunteer 3	29	4,9	42.0	29	48
Volunteer 4	41	5,9	48.0	26	48
Volunteer 5	30	3,5	49.0	20	44
Volunteer 6	28	3,5	54.5	20	50
Volunteer 7	23	2,9	57.0	30	58
Volunteer 8	32	4,3	59.0	32	60
Volunteer 9	38	5	68.0	19	66
Volunteer 10	27	3,2	73.0	20	46
Volunteer 11	37	3,5	102.0	22	53
Mean ± SD	**31 .9** ± **6.6**	**5.0** ± 1.3	**57.0** ± 18.8	**24** ± 4.5	**53** ± 6.6
Patient 1	39	4,3	4.2	14	46
Patient 2	34	3,5	5.4	5	55
Patient 3	27	3,5	5.4	11	42
Patient 4	30	3,5	6.6	15	30
Patient 5	43	3,8	11.0	9	44
Patient 6	39	3,5	11.2	9	42
Patient 7	31	3,0	13.5	14	55
Patient 8	30	6,5	15.0	17	55
Patient 9	36	3,2	17.6	9	44
Mean ± SD	**34 .3** ± 5.2	**3.7** ± 0.6	**9.9** ± 4.8	**11.4** ± 3.8	**45.8** ± 8.2

Table 2: Frequencies of sperm carrying structural and numerical chromosomal abnormalities using sperm ACM assay.

	Normozoospermic volunteers	**Oligozoospermic patients**
No. of individuals	11	9
Sperm scored	110,619	90,518
CHROMOSOME 1 CEN AND 1 PER DUPLICATIONS AND DELETIONS:		
1pter duplication	3.2 ± 2.0	5.4 ± 2.0 **
1pter deletion	2.5 ± 1.3	2.8 ± 1.3
Total 1pter dup/del	**5.7 ± 1.9**	**8.2 ± 2.2 ****
1cen duplication	1.7 ± 1.0	3.6 ± 2.0 **
1cen deletion	1.3 ± 1.2	2.9 v 1.9 *
Total 1cen dup/del	**3.0 ± 1.0**	**6.5 ± 1.6 ****
Total 1 cen and 1 per dup/del	**8.7 ± 2.9**	**14.7 ± 3.6 ****
BREAKS IN 1CEN - 1Q12 REGION		
Between 1cen and 1q12	3.0 ± 1.3	4.0 ± 1.6 *
Within 1q12 region	10.4 ± 1.2	12.4 ± 3.8 *
Total breaks	**13,4 ± 1.6**	**16.4 ± 4.3 ***
Total structural aberrations	**22.1 ± 4.1**	**31.1 ± 7.2 ****
NUMERICAL ABNORMALITIES:		
Disomy 1 or Diploidy	20.3 ± 4.2	22.1 ± 3.4
Nullisomy	0.0 ± 0.0	0.0 ± 0.0
Total numerical abnormalities	**20.3 ± 4.2**	**22.1 ± 3.4**

Frequencies Per 10^4 (Mean +SD)

* $p<0.05$ (Mann-Whitney Test)

** $p<0.01$ (Mann-Whitney-Test)

Figure 1: Correlation between sperm counts in mill/ml and frequency of sperm with breaks in 1cen - 1q12.