Aneuploidy Screening and Genome Profiling in Couples with Spontaneous Recurrent Pregnancy Loss

Stephanie Cheung, Tyler Cozzubbo, Nigel Pereira, Queenie V. Neri, Zev Rosenwaks, and Gianpiero D. Palermo

Ronald O. Perelman and Claudia Cohen Center for Reproductive Medicine, Weill Cornell Medicine, New York, New York, USA

Abstract

Study question: To investigate whether all genome sequencing by the involvement of specific genes can supplement sperm aneuploidy studies in patients with spontaneous recurrent pregnancy loss (RPL).
Summary Answer: Gene sequencing confirmed a higher sperm aneuploidy and also evidenced mutations in a set of spermatogenesis-related genes in men that presented with RPL.
What is known already: At least 15% of infertility cases are related to genetic disorders, such as chromosomal and single-gene alterations. As a result, genetic tests are currently more frequently used as a diagnostic procedure in couples experiencing infertility or unexplained pregnancy loss. For this purpose, it is important to screen the male partner which is presently assessed by semen analysis, chromatin fragmentation, and fluorescent in situ hybridization (FISH) on spermatozoa. FISH is only capable of assessing a limited number of chromosomes, whereas all chromosomes assessment carried out by Next Generation Sequencing (NGS) may contribute to more proper profiling of the male genome.
Study design, size, duration: In a 14 month period, we assessed spermatozoa aneuploidy in 11 men with recurrent pregnancy losses. FISH analysis for chromosomes X, Y, 13, 15, 16, 17, 18, 21 and 22 was carried out. All chromosomes analysis was carried out by Next Generation Sequencing (NGS) and copy number variants (CNVs) were recorded to validate the chromosomal disomies involved. Genes with the highest CNVs were then noted for all chromosomes in each sample.
Participants/materials, setting, methods: FISH was performed on at least 1000 spermatozoa of 11 consenting men, with a threshold of >1.6% (euploid), maintaining a 2-3% error. Extracted DNA was amplified from at least 500 spermatozoa per sample using PCR-based random hexamer amplification yielding a DNA concentration of 447.8±198ng/ul and quality of 1.7±0.1nm. CNVs were recorded using CASAVA and VarScan2 software. The genes found to have the highest CNVs in each sample were selected and grouped by function.
Main results and the role of chance: A total of 11 men with an average age of 44.9±7yrs had a semen specimen concentration of 27.0 ± 34x10^6/ml, motility of 23.0 ± 26%, and normal morphology of 1.5 ± 2%. The overall average aneuploidy by 9 chromosome FISH for those patients was 0.39%. When sperm aneuploidy assessment was carried out by CNV count, the aneuploidy rate rose to 4.07% (*P*=0.0001). By NGS, we were able to detect a total of 14 putative gene mutations that were present in our study group. A few of these genes, ADAM3A, NXF2, RBMY1F, and DPY19L2, appear to be related to the development of a normal male gamete. When we assess the ability of these couples to reproduce with the help of assisted reproductive technology, only the couples treated with IVF and/or ICSI, but not IUI, were able to establish a pregnancy that resulted in a delivery of a healthy child.
Limitations, reasons for caution: Although this approach of assessing aneuploidy and gene mutations by NGS may certainly help in clarifying covert male genetic contributions involved in recurrent pregnancy loss, more cases need to be studied to further validate our findings.
Wider implications of the findings: The utilization of NGS for analysis of spermatozoa is beneficial to the assessment of aneuploidy, and detection of CNVs allow to screen for gene mutations. NGS may help identify specific genes to provide insight to underlying genetic causes of recurrent pregnancy loss, therefore clarifying the etiology of idiopathic male infertility.
Trial registration number: N/A

Background

Recurrent pregnancy losses affect greater than 1% of couples of reproductive age, and most of them remain unexplained. Fetal chromosome aberrations effect up to 25% of clinical miscarriages. Trisomy 16 and monosomy X are the most frequent, with over 70% of fetal trisomies being of maternal origin. However, a substantial number can be traced to paternal nondisjunction or premature separation of sister chromatids. Aneuploidy assessment is routinely carried out by fluorescent in situ hybridization (FISH), albeit only capable of assessing a limited number of chromosomes.

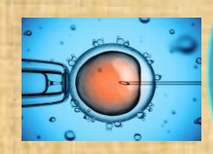
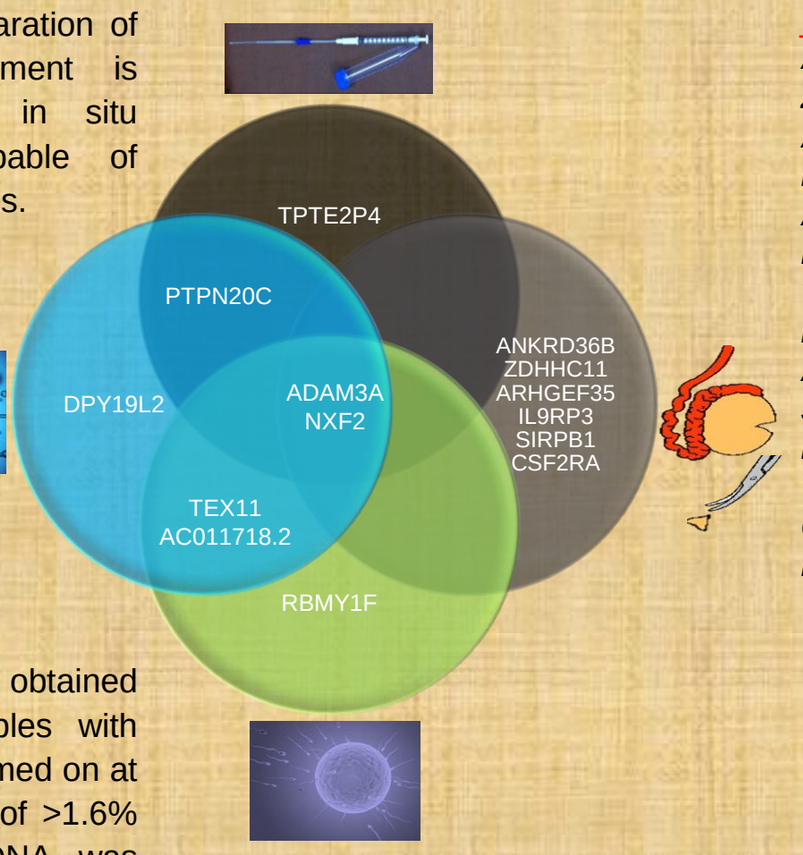


Figure 4. Venn diagram of 14 prevalent genes grouped according to insemination method.



Methods

A total of 12 ejaculated specimens were obtained from consenting male partners of couples with recurrent pregnancy loss. FISH was performed on at least 1000 spermatozoa with a threshold of >1.6% (euploid), maintaining a 2-3% error. DNA was extracted and amplified from at least 500 spermatozoa per sample with a commercial kit using PCR-based random hexamer amplification for a DNA concentration of 447.8±198 ng/ul and quality of 1.7±0.1 nm (Figure 1). CNVs were recorded using CASAVA and VarScan2 software.

	Control	Fertile	Infertile
Couples	2	3	7
Female Age (M ± SD)	42.0 ± 1	40.7 ± 1	41.3 ± 1
Male Age (M ± SD)	40.5 ± 2	43.7 ± 2	47.0 ± 5
Cycles	2	12	18
Clinical Pregnancy (+FHB)	2 (100)	9 (75.0)	2 (11.1)
Pregnancy Loss (%)	-	-	2 (100)

Results

A total of 12 men with an average age of 44.9±7yrs had a semen specimen concentration of 27.0 ± 34x10^6/ml, motility of 23.0 ± 26%, and normal morphology of 1.5 ± 2%. The average aneuploidy by 9 chromosome FISH for those patients treated with IUI was 0.18% compared to the CNV analysis at 0.82% (*P*=0.0303) (Figure 1).

Figure 1. Comparison between aneuploidy detection of 9-chromosome FISH and whole genome sequencing by Next Generation Sequencing.

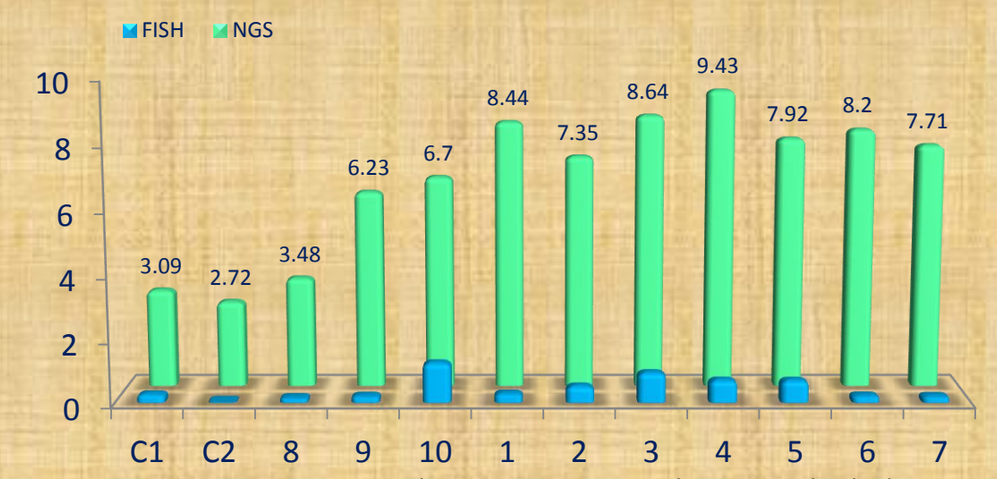


Figure 2. Comparing FISH and NGS assessment on the same individuals according to reproductive potential.

FISH aneuploidy for patients treated with IVF was 0.73%, while CNV assessment indicated 3.3% (*P*=0.2716). Patients treated with ICSI had an average FISH aneuploidy of 0.23% compared to 10.0% aneuploidy by CNV analysis (*P*=0.2993), while patients treated with ICSI and using surgically retrieved spermatozoa had an average FISH aneuploidy of 0.44% compared to 1.52% aneuploidy by CNV assessment (*P*=0.05) (Figure 2).

Table 2. Prevalent genes with high CNV

Gene	Chr	Function
ANKRD36B	2	Protein coding
ZDHHC11	5	
ARHGEF35	7	
DPY19L2	7	Sperm head elongation and acrosome formation
ADAM3A	9	Involved in sperm-egg fusion during fertilization
PTPN20C	11	Pseudogene
TPTE2P4	14	
IL9RP3	16	
AC011718.2	22	
SIRPB1	20	Recruitment of tyrosine kinase SYK
NXF2	X	mRNA export from the nucleus to the cytoplasm
TEX11	X	Regulator of crossing-over during meiosis
CSF2RA	Y	Activation of hematopoietic cells
RBMY1F	Y	Sperm development, pre-mRNA splicing in the testis

We were able to detect a total of 14 prevalent genes (Table 2) with high CNV that were present in our study group. A few of these genes, ADAM3A, NXF2, RBMY1F, and DPY19L2, appear to be functionally sperm-related (Figure 4). When separating by duplications and deletions, the infertile group had significantly higher CNVs when compared to the fertile and control group (Figure 5).

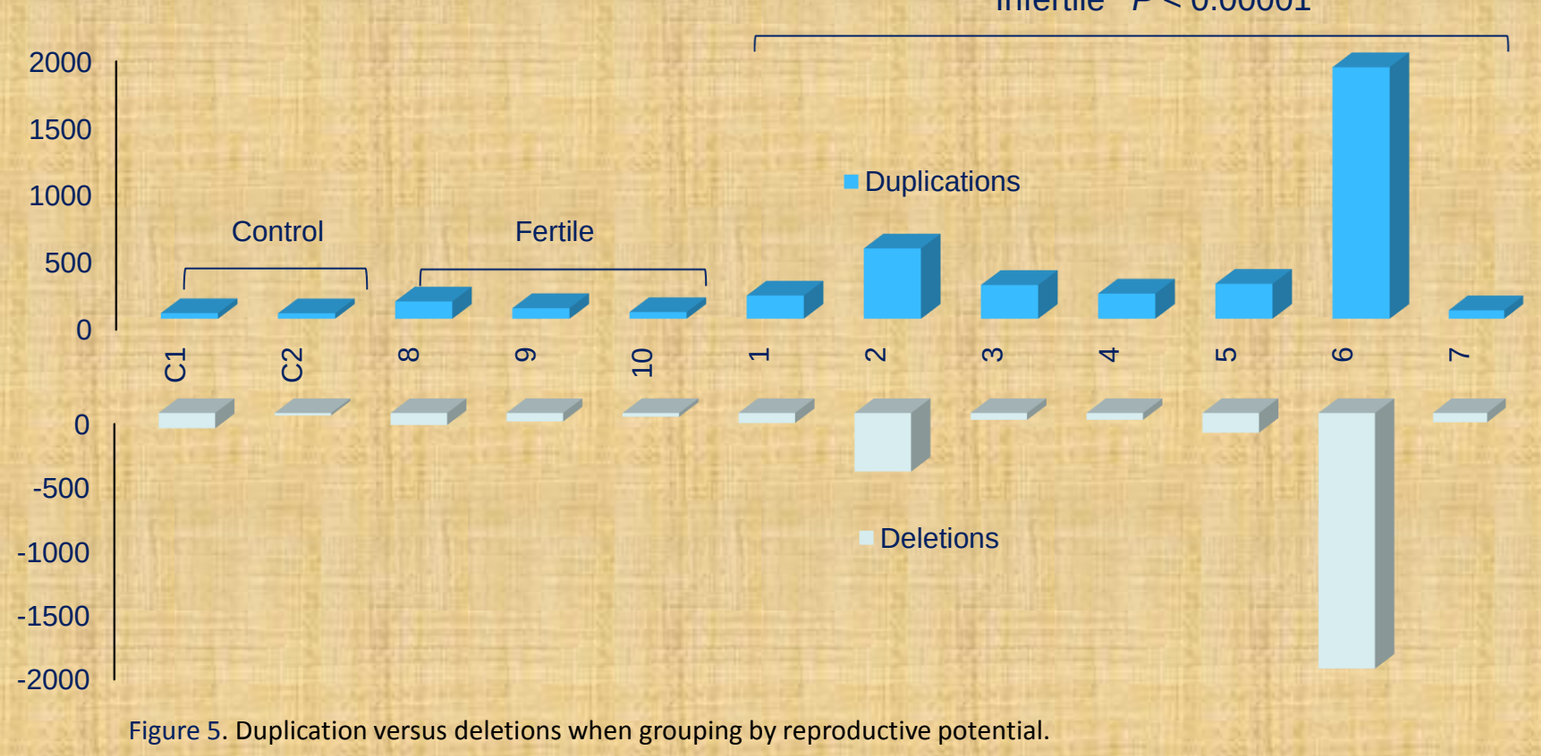


Figure 5. Duplication versus deletions when grouping by reproductive potential.

Conclusions

Among paternal meiotic errors, nondisjunction of chromosomes 15,16 and 7,8,9, as well as X or Y, are most represented in spermatozoa of men with RPL. This study evidenced the limited validity of FISH analysis in screening for sperm aneuploidy. The reliability and reproducibility of NGS on the assessment of chromosome aneuploidy has been confirmed. Although more cases need to be studied, this molecular genetic analysis may shed light on the paternal contribution to pregnancy loss.The ability to assess the male gamete by NGS may bring a better understanding of paternal contribution to embryo development. The ability to assess a limited number of spermatozoa will allow analysis of surgically retrieved specimens. CNVs for specific genes will invaluably contribute to the profiling of the paternal genome.