

# Clinical, Cytogenetic and Molecular Genetic Findings in Patients with Sterility or Repeated Pregnancy Loss

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## INTRODUCTION

It has been shown that chromosomal abnormalities or specific gene diseases are often found in many couples suffering from repeated abortion or infertility.

Our work summarises the results of cytogenetic and molecular genetic analyses performed during the last two years in 305 patients with sterility or repeated pregnancy loss from the Centre of Assistant Reproduction.

Recent studies have illustrated the power of comparative genomic hybridisation (CGH) in whole genome screening for partial and whole chromosome imbalances in NB: in particular for the analysis of low stage NB for which little genetic information was available.

The aim of our study was to determine the genetic causes of infertility in the Moravian region and to propose appropriate methods of preimplantation and prenatal genetic diagnosis.

## GENETIC APPROACH

### A. GENETIC CONSULTING

### B. CYTOGENETIC EXAMINATION

- Cytogenetic studies were performed using standard method (GTG-banding of peripheral blood lymphocytes).

### C. MOLECULAR GENETIC TESTING

- DNA analysis for the microdeletion of the specific fragments sY84, sY86, sY127, sY134, sY254, sY255 (a, b, c regions of Azoospermia Factor) located in Yq11.23 in men with severe oligospermia or azoospermia.
- DNA analysis of the five most frequent mutations of CFTR gene in Czech population (dF508, dele 2,3(21kb), G542X, G551D, R553X).
- Leiden mutation G1691A in the factor V gene in women with recurrent abortions or in patients with positive family history of the thrombembolism.

#### D. ASISSTED REPRODUCTION / PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

- PGD for aneuploidy detection in blastomeres was performed by repeated fluorescent in situ hybridization using 13-, 18-, 21-, X-, and Y-chromosome probes (Sasabe et al.: J. Assist. Reprod. Genet. 16, 92-96, 1999).

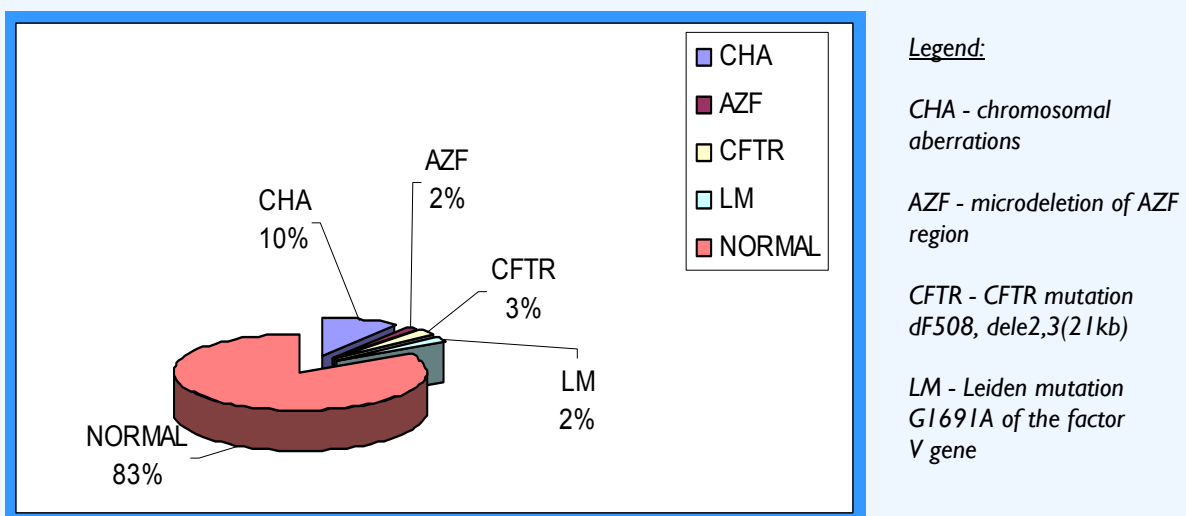
#### Digital Image Analysis:

- Fluorescence Microscope Olympus BX-60
- CCD Camera Hitachi KP-M1E/K
- Software: LUCIA CYTOGENETICS 4.61 from Laboratory Imaging s.r.o., Prague, Czech Republic

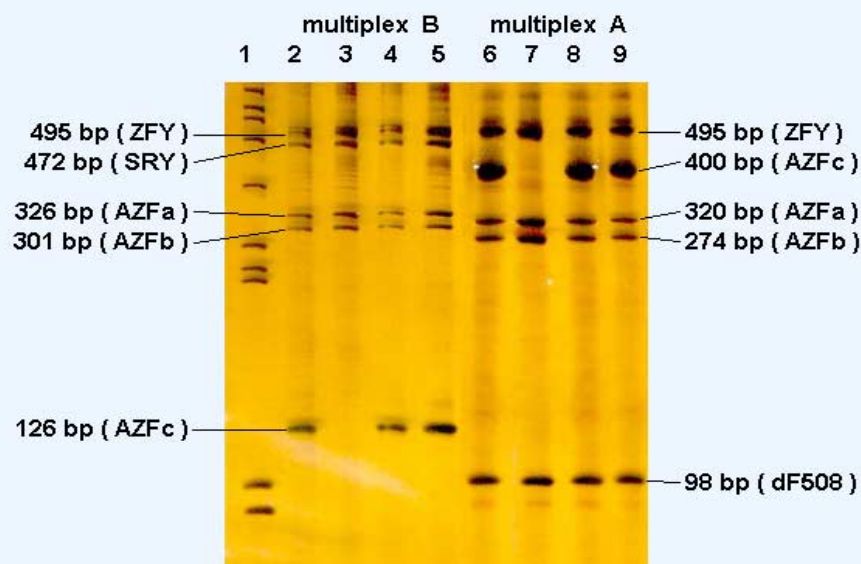
## RESULTS

The results of our complex screening programme of couples with infertility showed that:

- Chromosomal abnormalities were detected in 30 of 305 patients (in 26 patients we found aneuploidy of chromosome X, in 4 patients we could identify structural aberrations - inversions, deletions and balanced translocations).
- In 55 men with severe oligospermia or azoospermia we found 5 males carriers of microdeletion of AZF region.
- The Leiden mutation G1691A of the factor V gene we found in 7 of 62 patients.
- The mutation analysis of the five most frequent mutations in the Czech population has been performed in 163 patients and we found 8 carriers of CFTR mutation (dF508, dele2,3(21kb) and G542X).
- In 8 patients with repeated IVF failures or spontaneous abortions we performed preimplantation genetic diagnosis (PGD) of numerical abnormalities for chromosomes 13, 18, 21, X and Y in biopsied blastomeres using FISH. In 2 patients we found chromosomally abnormal (aneuploid and polyploid) embryos. In total 15 embryos were selected and transferred. Two women have become pregnant after PGD.



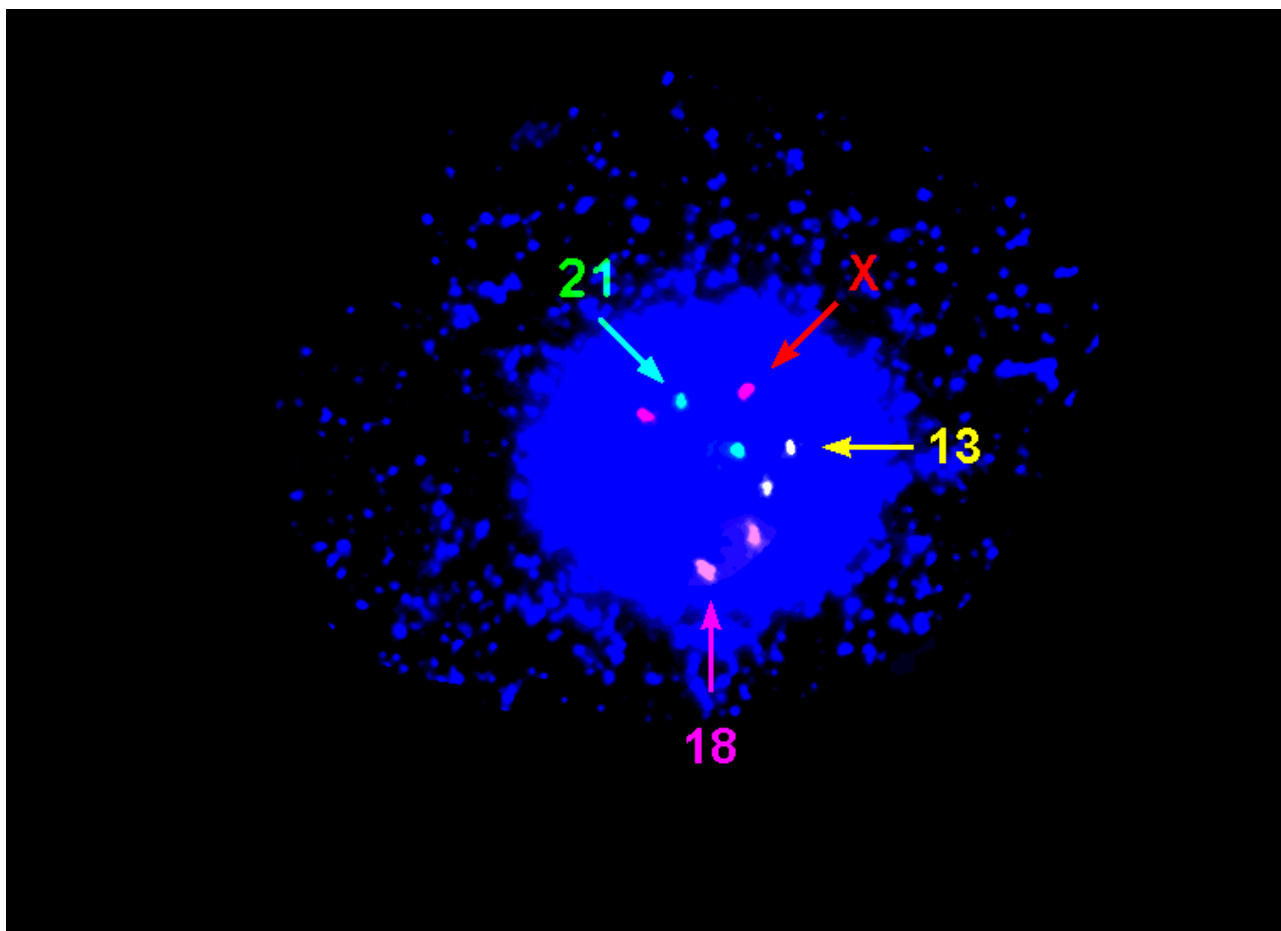
**Fig. 1** Cytogenetic and molecular genetic findings in 305 patients with sterility or repeated pregnancy loss.



Multiplex B: lane 1 - marker pBR322/AluI  
 lane 2 - DNA of normal male  
 lane 3 - DNA of AZFc deleted patient  
 lane 4,5 - DNA of normal male

Multiplex A: lane 6 - DNA of normal male  
 lane 7 - DNA of AZFc deleted patient  
 lane 8,9 - DNA of normal male

**Fig. 2** Examples of both multiplex PCR for AZFa, AZFb and AZFc regions.



**Fig. 3** Normal pattern of FISH signals in blastomere using probes specific for chromosomes X,13,18, 21. Three rounds of repeated FISH were performed using direct-labeled probes (Vysis): chromosomes X/Y (round 1), chromosomes 18/21 (round 2) and chromosome 13 (round 3). The probes were labeled with Spectrum-Green/SpectrumOrange fluophores and multicolor FISH was composed from three working images by LUCIA MFISH software.

### Chromosomal aberrations

Number of patients	305
Chromosomal aberrations in	30
Population incidence	0,6%
Our patients	9,8%

### Microdeletion of AZF

Number of patients	55
Microdeletion of AZF in	5
Incidence in infertile males	5 – 10%
Our patients	9%

### Leiden mutation of factor V

Number of patients	62
Leiden mutation of factor V in	7
Population incidence	6,3%
Our patients	11,3%

### Mutations in CFTR gene

Number of patients	163
Carriers of CFTR gene mutation	8
Population incidence	3,4 - 4%
Our patients	5%

**Fig. 4** The population incidence of the genetic abnormalities compared with our cytogenetic and molecular genetic findings.

## CONCLUSIONS

- Our results of the complex screening programme in patients with sterility or repeated pregnancy loss from the Moravian region demonstrate chromosomal and/or gene disorders in 15,4 % patients.

There are two important questions:

- Is the infertility one aspect of genetics disorder that might be transmitted?
- Will correction of infertility give an increased risk of malformations, chromosomal aberrations or genetics disorders in the offsprings?
- The determination of the genetic causes of infertility before a planned gravidity enables to propose appropriate methods of the assisted reproduction and optimal strategy of the prenatal diagnosis.

## ACKNOWLEDGEMENT

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