ABSTRACT

Title.: Utilisation of molecular cytogenetic techniques in reproductive genetics

Chromosomal abnormalities constitute one of the most important causes of birth defects, fertilization failure and/or human infertility.

Approximately, 40-50% of human conceptuses are chromosomally abnormal, 6% of the abortions during the first trimester of gestation are directly linked to chromosomal abnormality, while at term 0.6% of livebirths present with such features. Most of these abnormalities originate from the gametogenesis and arise through disturbed meiotic processes. Each gamete is a final and original product of the meiosis carrying a unique chromosomal set. Therefore, cytogenetic examination of individual gametes represents an important scientific challenge for our undrstandidng of the formation, incidence and etiology of aforementioned chromosomal abnormalities. Nonetheless, it is very technically demanding to perform efficient chromosomal investigation on gametes, hence single cells.

My Ph.D. thesis is focused on the development of new techniques for the detection of chromosomal abnormalities in gametes and embryos. We developed a PNA-based technique as an alternative to conventional FISH and PRINS-based methods for fast, efficient and robust in situ detection of chromosomal abnormalities in human gametes, polar bodies and embryos. Simple and multicolor PNA labelling procedure was performed on human oocytes, polar bodies and blastomeres using directly labelled centromere PNA probes specific for chromosomes 1, 4, 9, 16, 18, X and Y. My thesis demonstrates the efficiency of multicolour PNA procedure and shows that PNA can be a powerful alternative to FISH for in situ chromosomal examinations. This study indicates that PNA probes allow reliable chromosomal analysis in isolated human oocytes and blastomeres and thereby might provide an important adjunct to FISH for diagnostic examinations, and komplement rapidly developing arrayCGH-based methods.

The main experimental part of my thesis is focused on the study of sperm aneuploidy in carriers of germline mutations of the TP53 gene in patients with Li-Fraumeni syndrome. The rationale for this study relies on the fact that TP53, a transcriptional regulator and tumor suppressor, is functionally important in spermatogenesis. TP53 is also expressed during early embryonic development, which is associated with high chromosome instability in humans. Recently published study demonstrated that TP53 is indeed functionally important in spermatogenesis. The key regulator of the p53 pathway is MDM2. Both proteins have been functionally linked to germ cell apoptosis, which may affect human infertility. However, there is limited evidence on how common variants in these genes may influence germ cell apoptosis and the risk of male infertility. Our study demonstrated increased sperm aneuploidy, mainly concerning gonosomes when compared to normal male controls. These findings substantiate the increased risk of chromosomally aberant offsprings in carriers of germline TP53 mutations. This observation corrobates the involvement of the p53 protein in spermatogenesis and may explain two previously reported cases of Turner syndrome in families with germline TP53 mutations.

The last topics described in this work was the study of impact of the rs6836703:G>A variant of *ART3 gene* on impaired spermatogenesis within the Czech male population. Infertility affects 10 – 15% of couples in Western countries and the cause of the male infertility has not yet been determined in about 50 % of cases. Therefore, there have been many attempts to identify genetic factors associated with male infertility. We utilised the HRM method of small amplicons for genotyping, in order to evaluate its possible clinical use

and to establish medical indications targeted at the Czech infertile male population. Significant differences in allele/genotype distribution between fertile versus oligozoospermic men were found, while in azoospermic men, this difference was not observed. This study represents the first effort to evaluate in a different population the implication of rs6836703: G>A in impaired spermatogenesis. We found that the “A” allele of the rs6836703: G>A variant of *ART3* is a genetic risk factor for oligozoospermia in Czech male population.