

použili „taylor made therapy“, tedy podávání malých, kontrolovaně individuálních dávek estrogenů, následovaných po zjištění pozdní proliferace v hormonální funkční cytologii progesteronem. O úspěchu naší terapie jsme se přesvědčili po 6 měsících léčby pomocí detekce hladiny antiovariálních protilátek, kdy jsme v 40,46 % zaznamenali snížení a u 30,94% pacientek vymizení protilátek. Tento fakt je doložen i tím, že u většiny pacientek při této terapii se objektivně zlepšil stav vnitřních rodidel, sekundárních pohlavních znaků. Za zcela zásadní považujeme současně zlepšení kostní density.

## 8. SHRnutí

Výsledky naší studie a mapování výskytu sérových antiovariálních protilátek nás vede k přesvědčení, že výskyt autoprotilátek proti ovariu koresponduje s dříve či později se prezentujícími klinickými příznaky. Onemocnění může propuknout v období od puberty po celé reprodukční období. Zavedení adekvátní léčby (v naší studii se osvědčilo použití HRT) podle našich poznatků vedlo ke snížení autoimunitního poškození ovaria na minimum, k zachování jeho hormonálních funkcí a především k zachování zdravé ovariální tkáně, nezbytné pro budoucí fertilitu pacientky. Naše studie tedy potvrdila nepochybně obrovský význam včasné diagnostiky a léčby autoimunitního onemocnění ovaria. Z výsledků vyšetření hladin antiovariálních protilátek ve folikulární tekutině pacientek zařazených od IVF programu vyplývá, že při řešení neplodnosti je třeba myslet i na tuto příčinu sterility spojenou s menšími úspěchy v IVF programu.

Disertantka ve své práci vychází z grantového výzkumu, jehož byla spoluřešitelkou. Do některých souborů zařadila i poznatky z předchozích grantů se stejnou či obdobnou tematikou, zejména tam, kde to bylo vhodné pro srovnání vlastních výsledků

## 9. SUMMARY

### Introduction

Menopause usually occurs approximately at the age of 50. Premature ovarian failure (POF) is a disorder defined as a pathologic termination of menstrual cycle after puberty and before the age of 40. Frequency of this disorder is approximately 1%. Hormonal levels show hypergonadotrophic hypoestrism (FSH more than 40 IU/l). Menarche and regular menstrual cycles may be followed by menstrual cycle disorders - oligomenorrhoea or secondary amenorrhoea. Sterility or infertility at the reproductive age could be the manifestations of the

early stage of the disease. One of the possible causes of the premature ovarian failure could be an autoimmune process beginning at any time of the reproductive period.

The autoimmune damage of the ovarian hormonal production places this disease between the autoimmune endocrinopathies, characterized by direct destruction of the target cells, such as thyroiditis, insulin dependent diabetes and Addison's disease. The presence of other autoantibodies both organ specific and non-specific could be also demonstrated. Association of several endocrinopathies is frequently observed. We differentiate two main types: polyglandular syndrome I and II. POF is associated with polyglandular syndrome I in 12-17 %, and with polyglandular syndrome II in 3,5-3,6 % of the cases. It has been proved that in the case of POF are thyroideopathies the most frequent co morbidities.

Positivity of the antibodies against cells producing steroids in the early phase of the disease is associated, in experimental model, with the autoimmune oophoritis. Macroscopic cysts are present in 50% of the cases. The cysts are probably caused by the effect of increased gonadotrophin levels. Microscopic evaluation proved mononuclear infiltration in the close proximity to the follicles.

Objective of our study was to determine the occurrence of the organ specific antibodies against ovarian tissue at patients with dysfunctions of the menstrual cycle in comparison to the population of healthy females. Antibodies against germ cells and steroid producing cells were followed. Some of the antiovarian antibodies positive patients were indicated for laparoscopic biopsy of the ovarian tissue because of heavy menstrual cycle disturbances. Immunohistochemical examination of the sample documented the morphological changes. Control examination of the antiovarian antibodies levels was performed six months after the beginning of the treatment.

### Patients and methods

Patients included in the study were examined and followed up in outpatient clinic of the Department of Pediatric and Adolescent Gynecology 2<sup>nd</sup> Medical Faculty Charles University and Teaching Hospital Motol in Prague. The group consists of 110 patients. The examined group was subdivided into two subgroups: 63 patients with primary amenorrhoea (PA) and 47 with menstrual cycle disorders ranging from oligomenorrhoea to secondary amenorrhoea (OSA). All patients were screened for gonadotropin levels (FSH, LH, FSH/LH ratio) and presence of antiovarian antibodies. 30 antiovarian antibodies positive patients were indicated for laparoscopic biopsy. Bioptic samples were examined using light and electron microscopy. After the determination of the levels of gonadotropins and prevalence of

antiovarian autoantibodies, 42 patients was cured with estradiolum hemihydrate every other day followed in the second cycle phase by medroxyprogesteronum acetate every other day.

As the control group, we have used blood samples of 94 healthy women (aged  $32 \pm 6.7$ ), who were blood donors women and had no gynecological problem and another two groups of girls and women before application of contraceptive pills.

As another group we examined 90 patients treated for infertility. They were first subdivided into two groups – if they were pregnant ( $n=27$ ) after IVF program or if not ( $n=63$ ). Then they were subdivided into four groups after examination of prevalence of antiovarian autoantibodies in follicular fluid according to success in IVF program.

Prevalence of antiovarian antibodies was studied using indirect immunofluorescence. Ovaries of sexually mature rats were used as a target organ. ELISA method was used for determination of the antibodies against ooplasm, zona pellucida, membrana granulosa cells, theca folliculi interna cells and luteal cells. Bioptic samples were processed by standard methods. After morphological microscopic examination at the electron microscope level, the sample was incubated with monoclonal antibodies against organ specific antibodies labeled mostly by fluoroscianate. The sample was then examined in the immunofluorescent microscope. Measurements of plasma luteinizing hormone and plasma follicle-stimulating hormone levels were examined by standard commercial immunoassays.

Statistical evaluation was performed by test of concordance of parameters and McNemar's test.

### Results

In patients with primary amenorhea the prevalence of antiovarian autoantibodies against germ-line cells was the highest as while as in patients with menstrual cycle dysfunction antiovarian autoantibodies against steroid-producing cells have the highest prevalence. In all patients antibodies against luteal cells have the highest prevalence, antibodies against theca folliculi interna cells were less. Levels of antibodies were examined after a period of six months. After the determination of the levels of gonadotropins and ovarian hormones, 42 patients were cured with hormonal replacement therapy as substitution of ovarian function. Then autoimmune response of the organism to the ovarian antigen determinants could be evaluated. Success of this therapy was reflected in the profile of antiovarian antibodies levels. In 40,46% of the cases marked decrease was observed, while in 30,94% of the cases were the levels undetectable at all. Results are summarized in graph 3.

In a group of 94 healthy females, nine of them had positive antiovarian antibodies screening. This surprising finding lead us to closer analysis of the group. Retrospective study

of the anamnestic data revealed that one woman had missed abortion in the first trimester and another problem with fertility. 6 women confirmed menstrual cycle disorders (4x irregular cycle, 1x oligomenorrhoea). That was why another group of 53 healthy girls and young women treated in outpatient unit of Department of Pediatric and Adolescent gynaecology for the other problem then menstrual cycle disorder, was examined for prevalence of antiovarian autoantibodies. In this group only at one girl was found antiovarian autoantibodies positivity, but the reason for this positivity was unidentified.

In infertile women is important for succes in IVF program absence of AOA against germ-line structures. Fertilisation of oocyte and future milling depend of absence of a high frequency of AOA against ooplasm.

Patients indicated for laparoscopic biopsy for heavy menstrual cycle disorder have this findings in their bioptic samples of ovarium: microscopical picture revealed results proved that ovarian autoimmunities are rarely detectable in the form of mononuclear infiltration of ovarial cortex (in two cases only - 14,3%). On the other hand atresia of follicules at different developmental stages was observed frequently. In the atretic follicles was visible delayed formation of zona pellucida. Results of prevalence of AOA in our patients correspond with the morphology of follicular atresia in the early stage of differentiation. In patients with primary amenorrhoea the depletion and alteration of follicular apparatus belonged to dominant. Corpora albicantia and corpus luteum residua were almost missing. Findings of tertiary lysosomes in the cytoplasm of follicular cells and in the ooplasm at the electronmicroscopical level were observed in primary and stimulated follicles. Frequent fibrillar aggregations were co-localized with positivity of apoptotic marker of immunohistochemical procedure in the cytoplasm of follicular cells and ooplasm as well.

### Summary

Premature ovarian failure is very serious disease that can handicap the patient. Results of our study and mapping of the antiovarian antibodies positivity in our patients supports our conviction that positivity of the antiovarian antibodies corresponds, sooner or later, with the clinical symptoms. POF can start in such patients at any time of the reproductive period. Appropriate treatment with hormonal replacement therapy as substitution of ovarian function, minimizes ovarian destruction, preserves ovarian hormonal functions and saves healthy ovarian tissue necessary for future fertility of the patient. Our study proved the importance of early diagnosis and treatment of the autoimmune ovarian damage that saves patient's fertility.